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BENCH-SCALE TREATABILITY STUDY OF A DILUTE SYNTHETIC
DAIRY WASTEWATER, BY COMBINED ANAEROBIC-AEROBIC
SYSTEMS, AT AMBIENT TEMPERATURE CONDITIONS

by

Louise S. Duguay

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A thesis

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Dr. Ronald L. Droste

in partial fulfillment of the
requirements for the degree of
Master of Applied Science

in

Civil Engineering

Department of Civil Engineering
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of the requirements of the degree of
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in the
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Eman Ergin
Chairperson of Examining Board

This thesis is dedicated to my best friend and soul mate, Daniel Melanson,

“an angel who lifts me to my feet when my wings have trouble remembering how to fly.” (Unknown)

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ABSTRACT

High-rate anaerobic treatment alternatives have reduced wastewater concentration and temperature limits for feasible process operation while emerging sequential anaerobic-aerobic treatment schemes have yet to be used to their full potential. Thus, such beneficial systems could potentially expand the applicability of anaerobic treatment to regions with moderate climates or temperature conditions, such as North America (i.e., Canada), where the domestic wastewaters produced are usually weak in strength.

This research project consisted of studying the feasibility of a combined UASB-activated sludge process configuration for treating a dilute synthetic substrate, simulating a dairy wastewater, at a laboratory temperature averaging $23.5 \pm 0.8^{\circ}\text{C}$. The bench-scale experimental work was carried-out in two parallel biotreatment systems fed in series. They consisted of relatively small-scale Upflow Anaerobic Sludge Blanket (UASB) reactors of 3.85 and 3.75 L, respectively, followed by 2 L-activated sludge polishing processes. The feed concentration applied to one of the systems consisted of the minimum influent strength recommended for successful anaerobic treatment ($\sim 1\,000\text{ mg COD}_t/\text{L}$) while the second system was of the domestic sewage-strength range ($\sim 500\text{ mg COD}_t/\text{L}$), achieved by dilution.

The mainly soluble complex feedstock used consisted of tap water and Iron Fortified Enfalac[™] Infant Formula. It was supplemented chemically to ensure that proper (N and P) nutrient requirements were met as well as to increase its buffering capacity and to adjust pH. Both systems were continuously operated during approximately 5 months, which included a short start-up/acclimation period where the anaerobic reactor organic loading rates (OLRs) were gradually increased under an initial hydraulic retention time (HRT) of approximately 15 hours.

Three experimental runs were carried out by applying increasing UASB influent flow rates of 2, 4 and 8 L/d, in both systems. Bioreactor and system performance responses were mainly assessed in terms of organic removal efficiencies and effluent quality while feed concentration and HRT were the main variables investigated. For analogous HRTs and comparable total OLRs, ranging from approximately 2 to 0.5 days and 0.5 to 2 g COD_t/L·d, respectively, the anaerobic units achieved COD removals of 90%, on average, even at ambient temperature conditions. The combined anaerobic-aerobic treatment systems were able to attain levels as high as 94 and 96%, respectively. The decreasing HRTs and increasing OLRs were not found to affect organic removal efficiencies.

However, the settling properties of the SB granules of both UASBs got progressively worse with operation time. They became inferior to those of the inoculum and showed no sign of recovery. The plaguing sludge flotation problems were so severe in AD1 that the floating bed eventually split in two distinct parts during the final run. Despite their reasonable organic removals the biogas production, methane yields and VFA levels in both units were also practically negligible throughout the course of the experiment. Methane and carbon dioxide fractions were only observed to average 44 and 4%, as well as 56 and 5%, in AD1 and AD2, respectively. Thus, the N_2 content of the biogas was very high. Also, the inhibited methane production in each unit was strangely not accompanied with a pH drop or the accumulation of VFAs.

In spite of signs of UASB reactor instability, the low-strength synthetic dairy substrate investigated was found to be amenable to anaerobic treatment, even under low applied organic loading rates and ambient temperature conditions.

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GLOSSARY

Abbreviations and Acronyms

AA	acetic acid, mg AA/L
AAT	acetoclastic (methanogenic) activity test, g AA/g VSS·d
AB	aeration basin
AD	anaerobic digester
Alk	alkalinity
APHA	American Public Health Association
ATA	anaerobic toxicity assay or modified anaerobic toxicity assay
atm	atmosphere
b	average volume of FAS used for the blank, mL
B	effluent collection bucket
BL	blank
blk	average volume of HCl required to titrate blank to pH 4.65, mL
BMP	biochemical methane potential
BOD ₅	5-day biochemical oxygen demand, mg BOD ₅ /L
C	control
cc	cubic centimeter
CFST	continuous-flow stirred-tank
CHT	constant head tank
CL	clarifier
CM	complete mixed
COD	chemical oxygen demand, mg O ₂ /L as COD
D	weight of filter, evaporation dish and dried solid residue, mg
DF	dilution factor
DO	dissolved oxygen, mg O ₂ /L
f = 1	ratio of seed in BOD diluted sample (mL) to seed in seed control bottles (mL)
F	weight of filter and evaporation dish, mg
FAS	ferrous ammonium sulfate
FDS	fixed dissolved solids, mg FDS/L
F/M (= SOLR)	food to microorganism ratio, g COD _{fed} /g VSS·d
FOG	fat, oil and grease
FS	fixed solids, % or mg FS/L
FSS	fixed suspended solids, mg FSS/L
GC	gas chromatograph
GLSS	gas/liquid/solid separator
HLR	hydraulic loading rate
HP	Hewlett Packard
HR	high-rate
HRT (or θ_d)	hydraulic retention time, d
I	weight of filter, evaporation dish and solid residue after ignition, mg
ID	interior diameter
ISA	ionic strength adjustor
ISTD	internal standard
k (U or SRR)	maximum specific substrate utilization rate, g COD _{rem} /g VSS·d
k _e	endogenous decay coefficient, d ⁻¹
KHP	potassium hydrogen phthalate
K _s	(Monod) half-velocity or affinity constant, mg AA or COD/L (or substrate concentration at which the specific growth rate equals ½ μ_{max})
LR	low-rate

M	molarity or molar, mol/L
ML	mixed liquor (or mega litre)
N	normality, equivalency/L
NA	not applicable
ND	not detected
OLR	organic loading rate, g COD _{fed} /L·d
org. N	organic nitrogen, mg N/L
PA	phenolphthalein alkalinity, mg/L as CaCO ₃
ppm	parts per million, (1 ppm = 1 mg/L)
Q	influent flow rate, L/d
Q _r	recirculation flow rate, L/d
Q _w	flow rate of solids wasted from the system, L/d
r	recycle factor or ratio (Q _r /Q)
R ²	coefficient of determination value (a regression statistic)
ROPEC	<i>Robert O. Pickard Environmental Center</i>
rpm	revolutions per minute, min ⁻¹
r _s	rate of substrate utilization or removal, g COD/L·d
RT	retention time
r _x	rate of biomass growth, g VSS/L·d
r _x '	net rate of biomass growth, g VSS/L·d
s	average volume of FAS used for the sample, mL
S	sample
SB	sludge bed (or blanket)
SBH	sludge bed height, L
SBR	sequencing batch reactor
SRB	sulfate reducing bacteria
S _e	effluent (soluble) substrate concentration, mg COD _s /L
S _o	influent substrate concentration, mg COD/L
SOLR (= F/M)	specific organic loading rate, g COD _{fed} /g VSS·d
sp	average volume of HCl required to titrate sample to pH 4.65, mL
SRR (k or U)	specific (organic) removal rate, g COD _{rem} /g VSS·d
SRT (or θ _x)	solids retention time (or sludge age), d or h
ss	steady state
SS	suspended solids, mg/L
Std	average volume of HCl required to titrate standard to pH 4.65, mL
STD	standard deviation
STP	standard temperature and pressure
t	volume of total titrant to reach pH 4.5, mL
T	temperature, °C
TA	total alkalinity, mg/L as CaCO ₃
TCMP	2-chloro-6-(trichloro methyl) pyridine
TDP	total dissolved phosphorus, mg PO ₄ ⁻³ -P/L
TDS	total dissolved solids, mg TDS/L
TKN	total kjeldahl nitrogen, mg TKN/L
TP	total phosphorus, mg PO ₄ ⁻³ -P/L
TS	total solids, % or mg TS/L
TSP	total suspended phosphorus, mg PO ₄ ⁻³ -P/L
TSS	total suspended solids, mg TSS/L
TWAS	thickened waste activated sludge
U (k or SRR)	specific substrate utilization rate, g COD _{rem} /g VSS·d
UASB	upflow anaerobic sludge blanket

UK	United Kingdom
US	United States
USB	upflow sludge bed (or blanket)
V	volume of sample, mL
V_c	clarifier volume, L
V_{50}	upflow velocity corresponding to 50% biomass washout (on a TSS basis), m/h
VDS	volatile dissolved solids, mg VDS/L
VFA (or VA)	volatile fatty acid or volatile acid, mg acid/L
V_r	volume of reactor, mL or L
VRR	volumetric (organic) removal rate, g COD _{rem} /L·d
VS	volatile solids, % or mg VS/L
VSS	volatile suspended solids, mg VSS/L
WAS	waste activated sludge
WTM	wet tip meter
X_1 - X_{10}	experimental set-up sampling locations
X_v	average biomass (or VSS) concentration in the reactor, mg VSS/L
X_{ve}	biomass in clarified effluent, mg VSS/L
X_{vss}	total biomass in reactor, g VSS
X_{vr}	biomass concentration in WAS and RAS streams, mg VSS/L
Y	cell yield factor or growth coefficient, g VSS _{prod} /g COD _{rem}
Y_{obs}	observed yield, g VSS _{prod} /g COD _{rem}
η	treatment efficiency, %
θ_d	hydraulic retention time, d or h
θ_x	solids retention time or effective sludge age, d or h
θ_x^{lim}	microorganism generation time, d
$\theta_{x\ min}$	bacterial washout time, d
μ	net specific growth rate, d ⁻¹
μ_{max}	maximum specific growth rate, d ⁻¹

Subscripts

i	initial
o (or Infl.)	influent
e (or Effl.)	effluent
f	final
prod	produced
rem	removed
s	soluble
t	total

Superscripts

c	refers to seed control
s	refers to sample

CHAPTER 1

INTRODUCTION

Although standard-rate anaerobic wastewater treatment systems were quite limited, in the past, the development of high-rate (HR) anaerobic reactors, featuring greater biomass retention capabilities and enhanced organic content removal rates, at higher loading rates (i.e., 6-20 kg COD/m³·d), has enabled the method to gain much credibility (Kennedy, 1985; McCarty and Smith, 1986; Speece, 1996). Their ability to maintain high bacteria concentrations of 30-80 g VSS/L (Speece, 1996) and solids retention times (SRTs) of 20-100 d, at hydraulic retention times (HRTs) ranging from as low as 1.3 h to 20 h (Langenhoff and Stuckey, 2000), has helped them to overcome previous process limitations and provide many successful applications (Kennedy, 1985 and 1999; Torpy, 1988). Because of their advantageous nature (Section 2.1.5.1), advanced anaerobic configurations are increasingly favoured over aerobic systems as well as some other physical/chemical options (Lettinga et al., 1987; Perle et al., 1995; Speece, 1983; Stover and Gonzalez, 1987), particularly because of their lower biological solids generation potential, which leads to considerably less operational costs and energy requirements (Choi and Burkhead, 1984; Kasapgil et al., 1994; Zitomer and Speece, 1993).

Traditionally, anaerobic digesters were the treatment of choice for the stabilization of manure, slurries and biosolids, including (primary and secondary) domestic sludges (Curi and Eckenfelder, 1976; Heertjes and van der Meer, 1979; Lettinga et al., 1980; Speece, 1983 and 1996). However, industrial and/or municipal anaerobic biological *wastewater* treatment applications are relatively modern (Bitton, 1994; Filho et al., 1996; Torpy, 1988; Wheatley, 1990). Although second generation anaerobic systems are well-established at the lab, pilot and full-scale for treating or pre-treating concentrated organic waste loads (Aulenbach and Hallock, 1972; Backman et al., 1985; Droste, 1997b; Kasapgil et al., 1994; Samson et al., 1984; Speece, 1996; Stover and Gonzalez, 1987), their purification feasibility for *dilute* liquid wastes, such as domestic sewage, has not been as extensively investigated and is still rather limited in scope (Droste et al., 1987; van Haandel and Lettinga, 1994).

Because of greater and quicker reaction rates, most anaerobic studies have also been performed at mesophilic temperatures and/or in tropical climates (Kennedy, 1999; van Haandel and Lettinga, 1994). However, in recent years, some rather encouraging investigations carried out at

low ambient to moderate temperatures and/or with domestic-strength wastewaters have become and continue to be more widespread, for various types of wastewaters and reactor configurations. Several of these studies present much potential for accommodating fairly high organic loading rates (OLRs) and short HRTs. Although still applied at the lab-scale, some even undertaken at psychrophilic temperatures have offered promising results (Rebac et al., 1999). Nevertheless, Langenhoff and Stuckey (2000) report that treatment temperatures as low as 5°C resulted in removal efficiencies ranging from 0-90% for anaerobic filters (AnFs), fluidized bed (FB), sequencing batch reactors (SBRs) and upflow anaerobic sludge blanket reactors (UASBs).

Presently, enhanced anaerobic reactors such as the latter (Section 2.1.3) are proven as fit to treat a variety of wastewaters of diverse concentrations (Andras et al., 1989; Kennedy, 1985 and 1999; Lettinga and Vinken, 1980; McCarty and Smith, 1986; Torpy, 1988), including complex wastes and some previously believed to be less appropriate for these means (Speece, 1983 and 1996; van Haandel and Lettinga, 1994; Zitomer and Speece, 1993). Conversely, certain wastes do impose particular challenges and may or may not be effectively accommodated by these systems. Yet, according to Lettinga et al. (1987), they should no longer be constrained by the strength, temperature or possible toxicity of wastewaters, especially with sufficient sludge adaptation time (Lettinga et al., 1980; Speece, 1983 and 1996). Thus, viable feedstocks can vary from mainly to partially soluble (Lettinga and Hulshoff Pol, 1991; van Haandel and Lettinga 1994), from relatively dilute (Lettinga et al., 1980; McCarty and Smith, 1986; Rittmann and McCarty, 2001 and Section 2.1.3.5) to high-strength as well as from cold to very warm. However, some of the most suitable for anaerobic biodegradation include soluble low-strength wastes (Simpson, 1971) as well as highly organic beverage and food processing-related wastewaters (Droste, 1997b; Rittmann and McCarty 2001; Speece, 1983).

Several carbohydrate-rich dairy wastewaters (Table 2.9 in Section 2.2.2.4) such as cheese whey, whey permeate and lactic acid are also among the many substrates reported to be amenable to anaerobic treatment (Kennedy, 1999; Speece, 1983; Torpy, 1988; Zitomer and Speece, 1993). Within the last 10-15 years, it has become one of the most common and attractive methods of dairy effluent pre-treatment (Page et al., 1999), from an economic (Choi and Burkhead, 1984) and operational standpoint, especially with its CH₄ recovery abilities (van den Berg and Kennedy, 1983). The latter authors had suggested that it could become a crucial waste disposal means for the dairy industry. Page et al. (1999) insist that the process is even more profitable at significant waste concentrations as well as flow rates and in the United Kingdom (UK), the milk industry is considered to be one of the food industry waste types most likely to benefit from anaerobic

treatment (Wheatley, 1990). Because it is a natural animal product and human food source, milk is alleged to be easily degradable (Brown and Pico, 1979) as well as a model substrate for bacterial growth and reproduction (Robinson, 1990).

The sub-optimal treatment temperature potential of anaerobic applications depends mostly on the waste biodegradability (Lettinga and Vinken, 1980), which is usually high for most food-processing or agro-industry effluents (Bonkoski et al., 1983; Goronszy, 1990; Speece, 1983). However, industrial dairy wastewaters and product effluents typically tend to be warm (Blanc and Navia, 1991; van den Berg and Kennedy, 1983), with higher than ambient temperatures and relatively high organic contents (Section 2.2.2.2), which makes them particularly good candidates for anaerobic digestion (Page et al., 1999; Wheatley, 1990). Although, some dairy plants produce lower temperature wastewaters of approximately 24-28°C in range (Cocci et al., 1990b; Landine et al., 1987; Monroy et al., 1995; Page et al., 1999) and satisfactory anaerobic process performance under these or even lower temperature conditions (i.e., $\leq 20^{\circ}\text{C}$) could further reduce heating and overall treatment expenses (Page et al., 1999).

Dairy wastewaters are also occasionally dilute (Section 2.2.2.2 and Table 2.9 in Section 2.2.2.4), but the majority are stronger than domestic wastewaters (Brown and Pico, 1979) and they can upset or overburden municipal treatment operations (Perle et al., 1995). Therefore, they usually require a pre-treatment of some sort, often performed on-site, before being released in a sewage system to meet effluent discharge limits (Backman et al., 1985; Blanc and Navia, 1991; Kasapgil et al., 1994; Malaspina et al., 1995; Mikula et al., 1979).

Most organic substances can be converted in either aerobic or anaerobic environments (Field et al., 1995; Speece, 1983; Zitomer and Speece, 1993) in roughly the same time per unit of sludge (Speece, 1996). Both McCarty (1964) and Speece (1983) report that, in general, wastes disposed to aerobic treatment can also be treated anaerobically. Thus, most anaerobic treatment misconceptions are actually related to the biodegradability status of the feed rather than to the type of process employed (Speece, 1996). In typical full-scale treatment trains, *more readily degradable* wastewaters arrive in head aerobic units while *more refractory* wastes that require longer HRTs (i.e., ~10-20 d) supply subsequent anaerobic ones. However, if the treatment sequence is reversed, anaerobic operations should require retention times comparable to that of the conventional aerobic processes (i.e., ~6 h), although their start-ups are still relatively lengthy to achieve methanogenesis (Speece, 1996).

Past and present-day aerobic and anaerobic means, including UASB technology (Kennedy, 1999), have been broadly applied to treat wastewaters of dairy origins (Sections 2.2.2.3 and

2.2.2.4) and those that are treatable in aerobic systems normally require long retention times (Brown and Pico, 1979). Even though anaerobic processes are generally considered as being less efficient than the former types (Filho et al., 1996; Rittmann and McCarty, 2001), under ideal circumstances, they can produce secondary standard effluents (Speece, 1996). However, most often they do not and post-treatment is usually necessary (de Sousa and Foresti, 1996; Garuti et al., 1992; Vuoriranta et al., 1987). Therefore, when these types of systems are used, anaerobic-aerobic treatment arrangements are often favoured. Even though they have mostly been exploited for enhanced biological nutrient removal (Section 2.2.1.3), they have also become the preferred process for complex wastewaters (Zitomer and Speece, 1993) as well as those containing recalcitrant and/or toxic compounds (Section 2.2.1.4). However, the latter authors report that these promising treatment schemes have yet to be employed and exploited to their full potential.

Among the various dairy treatment approaches encountered in the literature, a number of sequential anaerobic-aerobic biological systems (Table 2.9 in Section 2.2.2.4.) were reported as being the most cost-effective and extremely well-suited for (particular) wastewaters of this type to achieve required final effluent treatment levels (Cocci et al., 1990b; Kasapgil et al., 1994; Malaspina et al., 1995; Page et al., 1999). This emerging practice was also found to be the most viable way to treat a confectionary waste (Cocci et al. (1990a) in Section 2.2.1.5). Hovious (1972) reported that many potential anaerobic (Section 2.1.5.1) or anaerobic-aerobic treatment advantages exist over aerobic processes alone (Section 2.2.1.1). In fact, aerobic post-treatment is often used to avoid possible anaerobic treatment odor problems (Lettinga et al., 1983; Page et al., 1999).

de Sousa and Foresti (1996) have pointed out that combined systems generally compete well with more costly aerobic treatment alternatives (Speece, 1983) while Hovious (1972) had proclaimed that both types of systems could be optimized when jointly used and that important savings could result from doing so. The outcome of a wastewater treatment cost analysis, performed by Eckenfelder (1988), cited in Speece (1996), revealed that the anaerobic pre-treatment of industrial wastes can be economically feasible at $> 1\,000$ mg BOD/L, although this application was reported to be possibly suitable for warm lower-strength wastes with proper alkalinity and nutrient availability (Speece, 1996). Since advanced anaerobic biodegradation processes have successfully taken on primary roles in current wastewater treatment practices, they are now considered as commendable aerobic process successors (Speece, 1996; Vuoriranta et al., 1987) and their warranted use can reduce sludge disposal costs by as much as 80% (Wheatley, 1990).

Yet, even efficient aerobic or anaerobic wastewater treatment systems, inevitably produce some excess biological sludge, which causes an ongoing biomass disposal dilemma (Speece, 1996).

Despite considerable progress in microbial process understanding and in reactor design, especially over the last 3 decades, there is still a need as well as an opportunity to continue to explore and improve biosolid waste generation reduction strategies, like anaerobic-aerobic sequencing, to minimize environmental impacts and disposal expenses, which are estimated at over 60% of entire treatment costs (Tchobanoglous and Schroeder, 1985). The latter are reported to total at least half of the milk industry's annual treatment expenditures in the UK (Wheatly, 1990).

Other than cost-effectiveness and nutrient removal potentials, the hybrid biological system investigated in the present research project, consisting of an UASB reactor and an activated sludge process (with provisional recirculation of excess sludge to the anaerobic reactor), was of a nature to offer sludge reduction benefits as well as superior effluent quality in terms of secondary treatment (at least compared to that of a sole single-stage anaerobic systems of the same type). The use and operation of such a treatment process is justified provided that the behavior of the aerobic polishing step is normal when preceded by an anaerobic phase (Zitomer and Speece, 1993). Although several different anaerobic and sequential systems have been reported to effectively treat some relatively dilute wastewaters (Sections 2.1.3.5, 2.2.1.2 and 2.2.2.4), including municipal and weak food industry related wastewaters, none of the combined processes reviewed employed the abovementioned configuration for the treatment of dairy wastes. Also, based on relevant findings from the literature (Sections 2.2.2.1 and 2.2.2.4), dairy wastewaters should readily ferment to volatile acids in anaerobic reactors (Speece, 1983).

1.1 RESEARCH OBJECTIVES

The general purpose of the experimental study was to assess the start-up and performance of a continuous-mode anaerobic-aerobic biological wastewater system, under maintained sub-optimal anaerobic treatment conditions. This was achieved by monitoring basic anaerobic and some typical aerobic operational parameters (Section 3.2).

The specific objectives of the bench-scale investigation included:

- Characterization of the simulated milk wastewater employed as well as the thickened waste activated sludge (TWAS) and the anaerobic granular biomass used to inoculate the aeration basins (ABs) and the UASB reactors, respectively.
- Chemical supplementation of the substrate for adequate nutrient availability (i.e., N and P) and reactor pH.
- Evaluation of the dilute synthetic dairy feed's anaerobic treatability under ambient temperature and low OLRs.

- Assessment of the effects of wastewater concentration and HRT on UASB-activated sludge system operation, including changes in anaerobic granular biomass characteristics [i.e., specific activity, sludge inventory or solids concentration variations (on a VSS basis) as well as settleability], during maintained run conditions.
- Comparison of the joint treatment configuration when operating at roughly the minimum influent strength recommended for successful anaerobic treatment ($\sim 1\,000$ mg COD/L) and at the domestic sewage-strength range (~ 500 mg COD/L).
- Determination and comparison of individual unit process and overall treatment system efficiencies, in terms of organic matter removal (on a COD basis) and suspended solids reduction potential, under the different experimental run conditions of increasing flow rates or decreasing HRTs.
- Evaluation of the ensuing process capacity in terms of organic (OLRs) and specific organic loading rates (SOLRs) as well as volumetric (VRRs) and specific organic removal rates (SRRs).

Although HR anaerobic alternatives have reduced wastewater concentration and temperature limits for feasible process operation, dilute wastes continue to be fairly difficult to treat anaerobically (Droste, 1997b). Thus, if this could be improved upon by using sequential treatment schemes like studied herein, these systems could potentially expand the applicability of anaerobic treatment to regions with moderate climates or temperature conditions, such as North America (i.e., Canada), where the domestic wastewaters produced are usually low or weak in strength (Droste, 1997b).

1.2 THESIS OUTLINE

Chapter 2 summarizes some of the biological principles involved in anaerobic digestion and aerobic treatment, describes the main features of the UASB configuration as well as those of the activated sludge process and presents key advantages of anaerobic treatment over aerobic treatment. It also gives an overview of relevant research findings from the literature surveyed, including some for UASB reactors treating dilute wastewaters at low temperatures, various anaerobic-aerobic treatment sequences and anaerobic processes employing dairy wastes as substrate. *Chapter 3* presents the experimental set-up and operation as well as the materials, apparatus and analytical methods used in the investigation while *Chapter 4* presents, analyzes and discusses significant performance results from the bench-scale experiment. Finally, some conclusions and recommendations are presented in *Chapters 5* and *6*, respectively. The series of *Appendices* contain supplemental facts and figures that include much of the observed data, analyses details as well as extensive example calculations.

CHAPTER 2

BACKGROUND THEORY AND LITERATURE SURVEY

Anaerobic digestion (Chynoweth and Isaacson, 1987; Droste, 1997b; Eckenfelder, 1989; Grady and Lim, 1980; Kennedy, 1999; McCarty, 1964; Metcalf and Eddy, 1991; Parkin and Owen, 1986; Speece, 1983 and 1996; Tchobanoglous and Schroeder, 1985; Torpy, 1988; Van Haandel and Lettinga, 1994; WEF Manual, 1991; Wheatley, 1990), *upflow anaerobic sludge blanket reactors* (Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Lettinga and Vinken, 1980; Lettinga et al., 1980, 1984, 1987 and 1993; Van Haandel and Lettinga, 1994) and *activated sludge processes* (Droste, 1997b; Eckenfelder, 1989; Grady and Lim, 1980; Metcalf and Eddy, 1991; Stall and Sherrard, 1978; Tchobanoglous and Schroeder, 1985) are very well documented. Therefore, a general overview of their characteristics and the basic principles that govern them (Sections 2.1.2 through 2.1.4) as well as some of their advantages and drawbacks (Section 2.1.5) in biological wastewater treatment (Section 2.1.1) are presented.

Important parts of this chapter lie within Sections 2.1.3 and 2.2. They present some of the research findings found in the literature surveyed pertaining to UASB reactors, operated on dilute wastes at sub-optimal anaerobic temperatures (Section 2.1.3.5), as well as to biological anaerobic-aerobic treatment schemes (Section 2.2.1) and anaerobically treated dairy wastewaters (Section 2.2.2.4). Several of the publications reviewed had somewhat comparable environmental conditions, substrate strengths and/or nature, reactor types and process configurations or a combination thereof. A few potential problems often encountered during anaerobic treatment or associated with UASB systems (Section 2.1.2.3), are also discussed, but particularly those involving problematic compounds (Section 2.2.3).

2.1 GENERAL THEORY

2.1.1 BIOLOGICAL WASTEWATER TREATMENT

Biological treatments are primarily used to remove most of the organic matter (i.e., non-settling or dissolved) present in wastewater. Other purposes include phosphorus and nitrogen removal, sludge stabilization and/or oxidation as well as solids reduction. Several suspended-growth, fixed-film (attached-growth) or hybrid processes exist (Barber and Stuckey, 1999; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985) and they are classified as aerobic, anoxic,

anaerobic or an incorporation of these. According to Tchobanoglous and Schroeder (1985), suspended-growth systems, in which the microorganisms that consume wastewater constituents are kept in suspension, are said to produce a better quality effluent and particularly at low loading rates (Speece, 1996). They are often necessary in the case of wastes with high solids content such as food-processing wastewaters (Bernet et al., 2000). Both UASB reactors (Section 2.1.3) and activated sludge processes (Section 2.1.4) are biological methods of this type.

Until fairly recently, secondary wastewater treatment implied biological *aerobic* systems since they were predominantly used (Wheatley, 1990). However, many successful and attractive anaerobic methods have emerged and they are still growing in interest and popularity. Whether aerobic or anaerobic, these naturally occurring processes are driven by many sludge contained-microorganisms mainly of chemo-heterotrophic (or organotrophic) nature (Bitton, 1994; Chevalier, 1996). Although biological processes are more complex in anaerobic than aerobic treatment (Ferguson, 1994), similar metabolic mechanisms and treatment principles apply regardless of the biotechnology. Biodegradable macromolecules and some suspended organic matter are metabolized following hydrolysis, carried out by extra-cellular enzymes, which reduces them to small entities able to move through bacterial cell walls (Tchobanoglous and Schroeder, 1985; van Haandel and Lettinga, 1994). Organic pollutants, nutrients as well as some other inorganic matter are used for catabolism (i.e., energy production) and anabolism (i.e., cellular material synthesis).

Influent nutrient requirements are essential to wastewater treatability by biological processes. Organic carbon [usually characterized as biochemical oxygen demand (BOD), chemical oxygen demand (COD) or total organic carbon (TOC)], nutrients (i.e., N, P, S, K, Ca, Mg) as well as trace elements (and/or metals) such as Fe, Ni, Cu, Zn, Mn, Mo and Co are known to be important for microbial maintenance and cell replication (Guiot, 1992; Kennedy, 1999; Lettinga et al., 1987; Metcalf and Eddy, 1991; Parkin and Owen, 1986; Speece, 1996; Tchobanoglous and Schroeder, 1985; Wheatley, 1990). Biomass generated in biological processes is however difficult to approximate since it is a function of operation type, conditions and influent strength. Unlike water treatment or physical-chemical wastewater treatment, biological sludges do not contain the entire influent (SS) suspended solids (Droste, 1997b).

During bioprocesses, including sludge stabilization, a portion of the particulate matter is converted to gaseous and/or soluble intermediates and end products. Some dissolved components are transformed into biological solids that can be further reduced during digestion, subsequently settled and eventually wasted from the process. Other solid fractions leave the process unaltered as effluent residuals while some endogenous decayed cells are partly oxidized and the rest constitute

endogenous residue that remains in the system (van Haandel and Lettinga, 1994). Influent bioreactor solids and those produced in them should be settled in clarifiers that follow or are incorporated in biological units (i.e., anaerobic processes) and some thickening will likely occur. Physical sedimentation usually precedes biological aerobic treatment processes (i.e., activated sludge process) to remove settleable solids and suspended organics, more costly to degrade biologically. However, solids entering anaerobic processes do not normally need to be settled (Droste, 1997b). Those removed from primary clarifiers and generated in aerobic treatment processes are often solubilized and stabilized in anaerobic digesters, which can considerably reduce their amounts (Droste, 1997b).

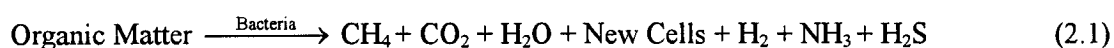
In this day and age, biological wastewater treatments are commonly achieved through exposure to sequential or alternating anaerobic and aerobic environments (Section 2.2.1). This practice is typically used for facility expansion and/or to improve wastewater treatment in order to meet stricter discharge limits (Garuti et al., 1992; Tracy and Hong, 1990). Thus, anaerobic effluent post-treatment or polishing processes are more frequently required or compulsory (de Sousa and Foresti, 1996; Garuti et al., 1992), including effluents from UASB reactors (Section 2.2.1.2), especially because their partial organic matter and poor macronutrient removals can result in the deterioration and/or eutrophication of receiving waters (Chevalier, 1996).

Many anaerobic-aerobic biological treatment schemes have been reported to be very efficient (Zitomer and Speece, 1993) and have shown to be at least equal to (Lettinga et al., 1983; Tanaka et al., 1991; Wang et al., 1997) or repeatedly outperform conventional processes such as activated sludge systems by producing lower effluent CODs. Improvements can be due to the capability of anaerobes to convert various compounds that are troublesome or that cannot be degraded aerobically (Speece, 1996; van Haandel and Lettinga, 1994; Zitomer and Speece, 1993). Some partially degraded anaerobic products (not amenable to further anaerobic biodegradation) may be more readily and rapidly biodegraded under subsequent aerobic conditions (Field et al., 1995; Lettinga et al., 1983). In many instances, combined anaerobic and aerobic biodegradation abilities have shown to be complementary despite their individual limitations (Shen et al., 1995; Zitomer and Speece, 1993). For optimal wastewater treatment, like Hovious (1972), Droste (1997b) also suggests that both types of processes should be jointly used.

2.1.2 ANAEROBIC BIODEGRADATION

Anaerobic decomposition, which occurs in oxygen-depleted environments, involves the conversion of organic compounds primarily to methane (CH_4) and carbon dioxide (CO_2) as well as

to small amounts of biosolids, inorganic matter and other trace biogases as waste stabilization end products (Curi and Eckenfelder, 1976; Eckenfelder, 1989; Kennedy, 1985 and 1999; van Haandel and Lettinga, 1994). The general concept (Eq. 2.1) is represented by the following overall reaction (Bitton, 1994; Masters, 1998), where anaerobic bacteria use some carbon from organic waste as a source of energy for growth and subsistence and most of the rest goes towards the formation of methane (Speece, 1996; Zitomer and Speece, 1993):



2.1.2.1 General Process Scheme and Microbiology

The biomechanisms and microbial interactions implicated are relatively complex and the combined effort of diverse bacterial classes (which perform distinct functions simultaneously) is required for the process to be properly carried out (Droste, 1997b; Grady and Lim, 1980; WEF manual, 1991). Figure 2.1 summarizes the series of metabolic biotransformations and the bacterial groups involved in the anaerobic digestion of complex organic substrates to the various intermediate and final by-products.

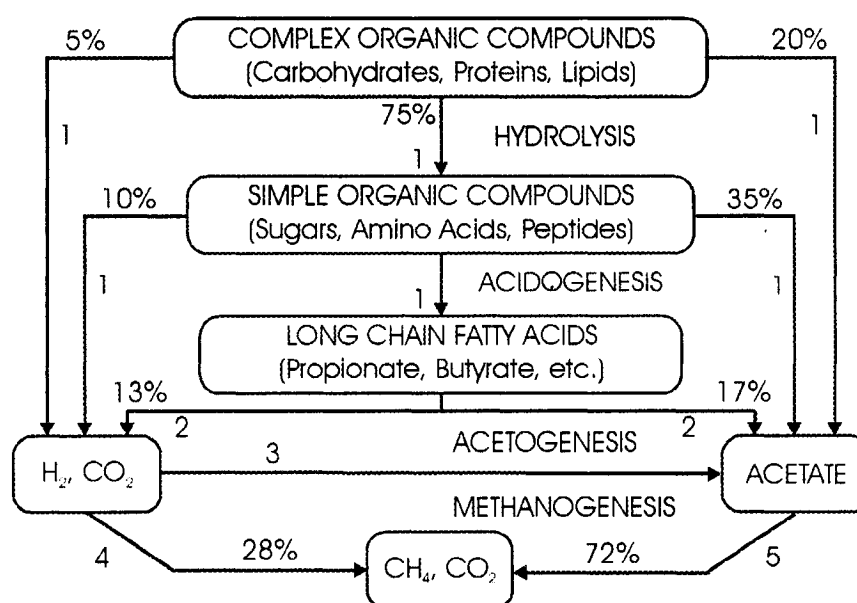


Figure 2.1: Anaerobic Biodegradation of Complex Organic Matter
[Adapted from Speece (1996)/Pham (2002), Kennedy (1985)/Droste (1997b) and Torpy (1988)]

Bacterial groups:	
1-	hydrolytic-fermentative (or acid-producing bacteria);
2-	hydrogen-producing acetogens;
3-	hydrogen-consuming (or homo-) acetogens;
4-	CO ₂ -reducing and hydrogenophilic (or hydrogenotrophic) methanogens;
5-	acetoclastic (acetophilic or acetotrophic) methanogens
Percentages (%) represent COD conversions	

Most wastewater literature presents anaerobic treatment as a 3-step transformation sequence with 3 groups of bacteria involved (Curi and Eckenfelder, 1976; Droste, 1997b, Grady and Lim, 1980; Kennedy, 1985; McCarty and Smith, 1986; Metcalf and Eddy, 1991; Obayashi and Gorgan, 1985; Parkin and Owen, 1986; Pavlostathis and Giraldo-Gomez, 1991; Speece, 1983; Tchobanoglous and Schroeder, 1985; WEF Manual, 1991) while somewhat more recent publications consider it as a 4-stage metabolic process involving 4-5 microbial trophic groupings (Bitton, 1994; Grady et al., 1999; Guiot, 1992; Guiot et al. 1992b; Kennedy, 1999; Lentz, 1996; Speece, 1996; van Haandel and Lettinga, 1994; Wheatley, 1990).

During the initial enzymatic *hydrolysis stage* (or solids liquefaction and solubilization phase), complex soluble and particulate organic materials, including carbohydrates, proteins and lipids, are broken down by heterotrophic and facultative hydrolytic bacteria into simpler soluble compounds such as sugars, amino acids and organic fatty acids.

Then, acidogenic bacteria (or acid formers) ferment the more readily degradable organic materials to alcohols, ketones, organic volatile fatty acids (VFAs), some H_2 and CO_2 . Although the products formed vary with the nature and conditions (i.e., pH and temperature) of the microbial culture (Bitton, 1994), some of the simple acids produced in the *acidogenesis* (acidification or fermentation) *step* include formic, lactic, propionic and butyric acids. Organic matter stabilization (or COD removal) does not occur during liquefaction (Eckenfelder, 1989; McCarty, 1964) and only nominal COD reduction results during acid fermentation (Curi and Eckenfelder, 1976).

In the next *acetogenesis* (or acetification) and *dehydrogenation* (or hydrogenogenesis; Grady and Lim, 1980) *phases*, hydrogen-producing acetogens, further convert (or oxidize) alcohols as well as acids containing more carbons than acetic acid (i.e., propionic and butyric acids) into CO_2 and H_2 biogases as well as acetic acid. Some less significant non-methanogens (hydrogen-consuming acetogens) also form acetate from H_2 and CO_2 (Grady et al., 1999; Guiot et al., 1992b; Kennedy, 1985).

From the acetogenic intermediate products formed, substrate-specific methanogenic bacteria (or methane formers) produce relatively insoluble methane (CH_4) by two main pathways in the final *methanogenesis* (methane fermentation or gasification) *step* (Curi and Eckenfelder, 1976; Kennedy, 1985). Acetoclastic (acetotrophic, acetophilic or acetate-splitting) methanogens transform or cleave acetate to CH_4 and CO_2 while hydrogen-utilizing (hydrogenotrophic or hydrogenophilic) methanogens reduce CO_2 gas to CH_4 (and water). Like many others, Speece (1996) reports that most CH_4 is derived from acetate (72%) and the remainder from the other route (28%), even though most methanogens can metabolize CO_2 and H_2 (Lentz, 1996; Torpy, 1988).

Yet, *Methanosaeta* (formerly known as *Methanothrix* (Guiot, 1992b; Droste, 1997b) and *Methanosarcina* (or *M. mazei*; Speece, 1996) are the only species that can generate methane from acetate (Andras, 1987; Eckenfelder, 1989; Speece, 1996; Torpy, 1988; Wheatley, 1990).

Some carbonaceous organic matter is also transformed into new cells during the last two steps (Curi and Eckenfelder, 1976). When sulfate is contained in the initial wastewater, hydrogen sulfide (H_2S) results when sulfate-reducing bacteria (SRB) outcompete methanogens in H_2 consumption (Bitton, 1994; Guiot et al., 1992b; Wheatley, 1990). This particularly occurs when little acetate is present and when $\text{COD}/\text{SO}_4^{-2}$ ratios are low (Bitton, 1994). Since the process can produce some S and N_2 gases, it is often associated with odors. According to Eckenfelder (1989) and Speece (1996) approximately 90% of wastewater COD is usually converted to CH_4 and other end products (at 10 d SRTs; Curi and Eckenfelder, 1976). Anaerobic bacteria in fullscale digesters at the ROPEC wastewater treatment plant in Ottawa (Canada) reduce 60% of the incoming organic matter to CH_4 and CO_2 . Because wastewater COD reduction is related to CH_4 production, if the process stops short of this crucial step, no substantial COD would be removed in the form of methane gas (Grady and Lim, 1980; Speece, 1996). Treatment would be minimal since the effluent would contain soluble intermediates with practically the same oxygen demand as the original organic matter (Droste, 1997b).

For instance, the conversion reactions for alcohols and VFAs to acetic acid are sensitive to H_2 partial pressure (Bitton, 1994; Guiot et al., 1992b; Tchobanoglous and Schroeder, 1985). If it exceeds 10^{-3} - 10^{-4} atm, acetate formation is reduced and organic acids tend to build-up in the liquid supernatant (Droste, 1997b; Grady et al., 1999; Gunnerson and Stuckey, 1986; Parkin and Owen 1986; Speece, 1996; Torpy, 1988). Although the process might not necessarily be affected by an increase in non-acid organics (i.e., alcohols), the degradation of propionic and butyric acids only proceeds when H_2 levels are low (Eckenfelder, 1989; Speece, 1996; Wheatley, 1990). Therefore, if organic acids are not converted to acetate at the same rate as they are produced, instability or process upset can occur due to their accumulation (Speece, 1996; van Haandel and Lettinga, 1994).

Stuck or sour anaerobic reactors are most often caused by organic overloads where methanogenic bacteria inhibition is due to the exhaustion of the system's carbonate/bicarbonate buffering capacity, which is followed by a drop in pH and a rise in the CO_2 fraction of the biogas (Eckenfelder, 1989; Grady and Lim, 1980; Speece, 1996; Wheatley, 1990). Reactor pickling, which usually results in little to no COD removal and gas production, can eventually lead to process failure by acidification, if the pH falls below 6.0-6.2 (Bitton, 1994; Curi and Eckenfelder, 1976; Droste, 1997b; McCarty, 1964; Metcalf and Eddy, 1991; Obayashi and Gorgan, 1985;

Tchobanoglous and Schroeder, 1985) and may not rapidly recover after disruption. However, most inhibition cases are reversible (Chynoweth and Isaacson, 1987).

Of all the complex phases involved in anaerobic degradation, any step may be rate-controlling. Such a condition is usually identifiable as a result of increased intermediate products prior to it. Therefore, it serves as a gauge of the metabolism speed and/or wellbeing of the conversion-bacteria implicated (Speece, 1996). Hydrolysis is usually not at fault for anaerobic biotransformation limitations of soluble wastewaters (Curi and Eckenfelder, 1976; Grady and Lim, 1980; Wheatley, 1990), but it has been reported to be the overall rate-limiting step for complex particulate substrates (Pavlostathis and Giraldo-Gomez, 1991; WEF manual, 1995). It is also often rate-controlling for wastewaters high in SS (Kennedy, 1985) as well as anaerobically treated at low operation temperatures (van Haandel and Lettinga, 1994). For some industrial wastewaters, the biodegradation of grease and lipids (fats) is said to be rate controlling (Grady and Lim, 1980; Metcalf and Eddy, 1991; Speece, 1983), particularly below 20°C (van Haandel and Lettinga, 1994) or under short retention times (Curi and Eckenfelder, 1976).

If the anaerobic bacteria cannot cope with the incoming waste, excessive organic loading can govern the process. Lettinga and Hulshoff Pol (1991) consider the OLR as the limiting factor for low-temperature anaerobic treatment of dilute wastewaters, but hydraulic loads are usually more important in reactor design, particularly for upflow systems. The latter are considered as the limiting factor in the case of extreme biomass washout (van Haandel and Lettinga, 1994). The sludge itself has also sometimes been considered rate limiting for soluble substrates (Speece 1996). However, methanogenesis is generally considered as the phase that governs the overall rate and performance of the anaerobic process (Droste, 1997b; Tchobanoglous and Schroeder, 1985; WEF Manual, 1991), especially for soluble carbohydrate-type wastes (Kennedy, 1985). These types of substrates are reported to favor the presence of acidogens by a 5:1 ratio (Speece, 1996).

H₂ and other gases formed during the process (other than CH₄ and CO₂) are generally small and rarely detected in anaerobic digesters (Curi and Eckenfelder, 1976; Droste, 1997b; Kennedy, 1985) when a favorable relation (i.e., interspecies hydrogen transfer) exists between the hydrogen-producing (acetogenic) and hydrogen-consuming (methanogenic) microorganisms (Guiot et al, 1992b; Metcalf and Eddy, 1991). Hence, a proper balance must be upheld between the two main microbial populations involved since the methanogens have slower growth rates and are much less tolerant to environmental conditions than acidogens (Droste, 1997b; Parkin and Owen, 1986; Speece, 1996; Tchobanoglous and Schroeder, 1985). The retention time (i.e., sludge age) of the former should also surpass their rate of growth (or generation time) to decrease viable microbe

washout (Curi and Eckenfelder, 1976) and to allow greater methane fermentation to occur (Bitton, 1994), especially in continuously operated reactors.

2.1.2.2 Process Performance Factors and Optimal Environmental Conditions for Methanogens

Among the many factors that affect anaerobic treatment, some are briefly presented here as well as commented on, in general, throughout Sections 2.1.3, 2.1.5 and 2.2. Process parameters commonly monitored include pH, alkalinity, volatile acids (VAs), temperature, biogas composition (CH_4/CO_2 ratio) and production rates, loading rates, COD and SS effluent quality as well as COD and SS reduction (Droste, 1997b; Kennedy, 1985; Tchobanoglous and Schroeder, 1985; Torpy, 1988). To maintain adequate anaerobic or aerobic biological system operation, proper environmental requirements should be provided. However, suitable reported values for the more sensitive methanogens vary somewhat from source to source.

2.1.2.2a Wastewater Composition and Nutrient Supply

In wastewater engineering, in order to have a non-deficiency in phosphorus (P) and nitrogen (N), a general rule of thumb is to maintain an influent COD: N: P ratio of 100: 5: 1 (the C: N: P ratio of domestic wastewater (Bitton, 1994)) to satisfy the needs of the organisms involved in conventional activated sludge processes (Droste, 1997b) or aerobic processes (Wheatley, 1990). However, for anaerobic systems, nutrient needs are lower since they produce less sludge than aerobic systems (Section 2.1.5) for the same substrate (McCarty, 1964; Speece, 1996). COD: N: P requirements of 700-750: 5: 1 (or BOD: N: P of 500: 5: 1) are usually observed for successful anaerobic treatment (Cocci, 1990; Droste, 1997b). However, a minimum ratio of 1 000: 7: 1 has been reported for low loaded systems and 350: 7: 1 for high loaded systems (Speece, 1996). Although phosphorus is usually in excess, industrial wastes often lack nitrogen (Wheatley, 1990). In addition to the many essential nutrients and elements needed for the growth and subsistence of microorganisms (presented in Section 2.1.1), some methanogens also require some sulfide, ammonia and vitamins (Andras, 1987; Speece, 1983 and 1996).

When nutrient availability is not sufficient, ammonium and/or phosphate chemical supplements should be added to the substrate (Droste, 1997b; Lettinga et al., 1987). However, their effect might only be noted after several days and the amount of nutrients thought to be adequate does not always correspond to nutrient bioavailability (Gunnerson and Stuckey, 1986; Speece, 1996; Torpy, 1988). Nutrient-deficient anaerobic systems can result in low COD removals (Lettinga and Vinken, 1980). Most wastewaters contain enough nutrients and trace elements/metals

to satisfy anaerobic bacteria nutritional needs. The latter, even in relatively low doses, are often reported to stimulate methanogenesis or bioreactor performance (Bitton, 1994; Droste, 1997b; Kennedy, 1985; Speece, 1996; Torpy, 1988).

2.1.2.2b pH, Buffer Capacity and Volatile Acids

Acid-producing anaerobic bacteria function best under a pH range of 5-6 (Filho et al., 1996), with an optimum reported at 5.6 (Droste, 1997b). However, stable near-neutral pH conditions are ideal for methanogenesis or the process as a whole (Grady and Lim, 1980; Speece, 1996; van Haandel and Lettinga, 1994; Wheatley, 1990). Bitton (1994) suggests 7-7.2. Thus, the most favorable and generally accepted functional pH range for anaerobic systems is approximately 6.5 to 7.5, to accommodate the more pH-sensitive methanogens (Chevalier, 1996; Droste, 1997b; Eckenfelder, 1989; Lettinga et al., 1987; McCarty, 1964; Tchobanoglous and Schroeder, 1985). Yet, some authors suggest broader (Curi and Eckenfelder, 1976 (6.4-7.8); Obayashi and Gorgan, 1985 (6-8); Speece, 1996 (6.5-8.2)) or narrower ranges (Bitton, 1994; Filho et al., 1996; Grady et al., 1999; Gunnerson and Stuckey, 1986; WEF Manual, 1991). The latter authors report that values outside 4-9.5 can be detrimental to microorganism growth. Those below 6.5 and above 8.5 (Tchobanoglous and Schroeder, 1985) can significantly affect methane production rates while van Haandel and Lettinga (1994) report these critical values to be 6.3 and 7.8. High pHs can cause ammonia toxicity (Grady et al., 1999). Although *Methanosarcina* acetoclasts can stand larger variations in pH, in general, methanogens from suspended-growth systems are more intolerant to low pHs than those from attached growth systems (Speece, 1996).

Propionic, butyric and acetic acids are the main VA precursors of methane (Grady and Lim, 1980). To offset significant acid production, anaerobic bioreactors require sufficient alkalinity that can be generated during methane production from NH_3 and CO_2 (Gunnerson and Stuckey, 1986; WEF Manual, 1991; Wheatley, 1990). Therefore, it actually buffers CO_2 acidity rather than VFAs (Speece, 1996). Tchobanoglous and Schroeder (1985) suggest an optimal alkalinity and VFA concentration range of 200-500 mg/L for successful process operation while Curi and Eckenfelder (1976) report a broader optimal bicarbonate alkalinity and VA range of 1 500-5 000 mg CaCO_3 /L and 50-500 mg AA/L, respectively. VA levels maintained under 100-150 mg/L are usually a sign of a well-operating process, with proper COD conversions to biogas and biomass, where acetic acid should predominate (Langenhoff and Stuckey, 2000; Samson et al., 1984).

Many industrial and carbohydrate wastewaters require control measures such as the addition of lime, sodium bicarbonate (NaHCO_3) or sodium hydroxide (NaOH), which can be added

directly to the feed, to restore pH and/or neutralize excess acid conditions (Bitton, 1994; Curi and Eckenfelder, 1976; Droste, 1997b; Parkin and Owen, 1986; WEF Manual, 1991). Speece (1996) reports an alkalinity requirement range of 1.2-1.6 g CaCO_3/g influent COD for these types of wastes. Droste (1997b) reports that alkalinity levels are usually maintained when protein-rich wastes are degraded since organic nitrogen compounds can release ammonia, which can contribute to its buffering (Speece, 1996; Wheatley, 1990). He also suggests that acceptable VA operation levels can range from more than 5 000 mg/L to less than 100 mg/L.

2.1.2.2c Temperature

Even though methanogens are quite sensitive to temperature fluctuations [Curi and Eckenfelder, 1976 ($\pm 2^\circ\text{C}/\text{day}$); Grady et al., 1999; WEF Manual, 1991 ($\pm 1^\circ\text{C}/\text{day}$)], Bitton (1994) reports that methane generation has been observed between 0-97°C while Speece (1996) reports a range of 4-100°C. Cryophilic (Obayashi and Gorgan, 1985) or psychrophilic ($< 15\text{-}20^\circ\text{C}$), mesophilic (15-40°C) and thermophilic ($> 40^\circ\text{C}$) conditions are suggested as the 3 main anaerobic operation temperature ranges (Droste, 1997b), although there is some discrepancy in the literature as to the span of these zones. For example, Curi and Eckenfelder (1976) report them as 5-25°C, 25-38°C and 50-60°C, respectively. However, the optimal temperatures of the 2 most favorable ranges for methane production are generally considered to be 35°C and 55°C, for mesophiles and thermophiles, respectively (Bitton, 1994; Chynoweth and Isaacson, 1987; Curi and Eckenfelder, 1976; Droste, 1997b; Gunnerson and Stuckey, 1986; Speece, 1996; van Haandel and Lettinga, 1994; WEF manual, 1991).

Since biological reaction rates approximately double with every 10°C rise in temperature (Barber and Stuckey, 1999; Droste, 1997b; Metcalf and Eddy, 1991; Obayashi and Gorgan, 1985; Simpson et al., 1984; Speece, 1996; Tchobanoglous and Schroeder, 1985), up to about 37°C (Curi and Eckenfelder, 1976), high operating temperatures usually improve conversion process efficiencies (Garuti et al., 1992; Rebac et al., 1999). Despite the fact that thermophilic temperatures can potentially provide greater gas production and better performance (Eckenfelder, 1989; Wheatley, 1990), most anaerobic reactors are operated under mesophilic conditions (30-40°C), with SRTs in the order of 20-40 d (Curi and Eckenfelder, 1976), since they are more stable and economical (Droste, 1997b; WEF manual, 1991). For CM systems, Rittmann and McCarty (2001) suggest a typical SRT range of 15-25 d and Grady and Lim (1980) suggest 5-30 d for lab-scale units. At 24°C, the recommended minimum and design SRTs for anaerobic digestion are reported as 8 and 20 d (Droste, 1997b; McCarty, 1964; Metcalf and Eddy, 1991).

Even though bacterial metabolic rates have been observed to be significantly slower below 30°C (Tchobanoglous and Schroeder, 1985), low treatment temperatures can be counteracted by applying longer SRTs (Grady and Lim, 1980; Obayashi and Gorgan, 1985; Speece, 1996) because the extent of conversion reactions are less affected by temperature than their rates (Wheatley, 1990). Under such conditions, dilute wastewaters also require greater amounts of biomass (or a larger reactor volume), to achieve comparable mesophilic treatment efficiencies. Smaller-sized reactors can only be used with warm wastewaters (Grady and Lim, 1980; Speece, 1996).

Although anaerobic processes are more affected by treatment temperatures reductions than aerobic processes (Speece, 1996), the literature shows that the former systems are not limited to the optimal mesophilic range (Section 2.1.3.5), although OLRs should be decreased considerably (Droste, 1997b; Speece, 1996). Kennedy (1999) reports that relatively good removal rates can be attained with most HR configurations under ambient temperature conditions, at reasonable organic loadings. This author suggests 25°C as the approximate minimum requirement for anaerobic systems while Droste (1997b) reports 20°C or even less as practicable anaerobic operating temperatures. Others have also reported that the process usually functions at ≥ 15 -20°C (Filho et al., 1996; Lettinga and Vinken, 1980) and the latter authors suggest that sewage can potentially be treated anaerobically at temperatures of 10-15°C. In any case, maintaining a stable operation temperature is considered the best approach (Droste, 1997b; Grady and Lim, 1980; Parkin and Owen, 1986; WEF Manual, 1991). Anaerobic reactors have been observed to perform consistently well when started-up and sustained at conditions lower than the ideal mesophilic temperatures (Barber and Stuckey, 1999).

Concentrated wastewaters ($> 5\,000$ mg/L) can generate large quantities of methane that can be used as a heat source (McCarty, 1964; van Haandel and Lettinga, 1994). Speece (1996) reports that feedstocks can be warmed up by as much as 3.3°C for every 1 000 mg/L COD consumed. During treatment, dilute wastewaters such as sewage do not generate a significant amount of biogas to heat the wastewater and it is uneconomical to heat them above ambient temperatures (Grady et al., 1999; McCarty, 1964; Obayashi and Gorgan, 1985; van Haandel and Lettinga, 1994). Therefore, they are generally treated at ambient conditions (with their incoming temperature) under potentially reduced reaction rates (McCarty, 1964; Speece, 1996).

2.1.2.2d Biogas Composition and Production

For anaerobic processes, the typical CH₄ fraction of biogas lies between 60-70% with the rest mainly comprising of CO₂, although trace quantities of other gases (i.e., N₂, H₂, H₂S) are

usually present (Curi and Eckenfelder, 1976; Droste, 1997b; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985; Wheatley, 1990). Typical concentration ranges for N_2 , H_2 and H_2S are respectively of 2-6%, 0.1-2% and 0.01-1% (WEF manual, 1991). The feedstock composition and SRT usually regulate the biogas quality and quantity (Curi and Eckenfelder, 1976). Complex carbohydrates have a lower COD content than proteins and lipids (WEF manual, 1991). Thus, proteinaceous wastes tend to generate biogases with high CH_4 fractions while lipid-containing wastes generally produce greater quantities of gas (Chevalier, 1996). The CH_4 content of biogases produced from carbohydrate-type wastewaters usually average 50% (Gunnerson and Stuckey, 1986).

For an undefined waste composition, the maximum theoretical methane yield can be estimated from the balanced methane oxidation reaction (Eq. 2.2). During anaerobic treatment, since COD is essentially converted to CH_4 and CO_2 end-products (and since the latter has no COD due to its completely oxidation; Gunnerson and Stuckey, 1986), the ultimate COD equivalent of methane gas can be computed as follows (Droste, 1997b; Kennedy, 1999; McCarty, 1964; Parkin and Owen, 1986; Speece, 1996):



Assuming a complete conversion of CH_4 to CO_2 and water, every mole of methane (16 g CH_4) requires 2 moles of oxygen (64 g O_2 or COD). Since 1 mole of CH_4 occupies 22.4 L at STP ($0^\circ C$ and 1 atm), 0.35 L of CH_4 should be produced per gram of COD removed from the feed (22.4 L CH_4 /64 g COD) or 0.25 g CH_4 prod/g COD_{rem} (16 g CH_4 /64 g COD). van Haandel and Lettinga (1994) report 0.39 L CH_4 prod/g COD_{rem} for $32^\circ C$ and Speece (1996) reports 0.395 L CH_4 prod/g COD_{rem} for $35^\circ C$. Observed values for anaerobic reactors range between 0.10-0.35 L CH_4 prod/g COD_{rem} (Droste, 1997b).

Similarly, from the waste oxidation equation, COD can also be correlated to the biomass. Based on an assumed anaerobic cell composition of $C_5H_7NO_2$ (Gunnerson and Stuckey, 1986; Tchobanoglous and Schroeder (1985), Droste (1997b) reports 1.42 g COD/g VSS as the COD equivalence of sludge (VSS) while Eckenfelder (1989) reports 1.21 g COD/g VSS from $C_5H_9NO_3$. Anaerobic reactors should also produce relatively constant quantities of CH_4 per unit mass of VSS reduced. Although these amounts vary with sludge characteristics, Wheatley (1990) suggests a typical value of 0.5 L CH_4 prod/g VS_{rem} and Parkin and Owen (1986) report 0.7 L CH_4 prod/g VS_{rem} for $C_{10}H_{19}O_3N$ (domestic sludge). Stable biogas production rates are indicative of well-functioning biomass (Metcalf and Eddy, 1991).

In practice, actual methane production per unit of organic matter or COD destroyed is usually lower because of partial conversions, environmental conditions, presence of toxicity or refractory organics, inaccuracies in gas measurements and COD tests, possible gas losses (including dissolved methane lost in the liquid effluent) and biomass synthesis, although a fraction of the new cells produced are destroyed by endogenous decay (Droste, 1997b; Gunnerson and Stuckey, 1986; Kennedy, 1999; van den Berg and Kennedy, 1983; Zitomer and Speece, 1993).

Based on the COD balance of continuous-flow anaerobic systems, methane production rates can be approximated as follows (Eq. 2.3), when taking cell yield into consideration (Droste, 1997b; Eckenfelder, 1989; Gunnerson and Stuckey, 1986; McCarty, 1964; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985):

$$V_{CH_4} = M[(Q(S_{t\text{ Infl.}} - S_{t\text{ Effl.}}) - 1.42r_X V_r)] \approx M(EQ S_{t\text{ Infl.}}) \quad (2.3)$$

where, V_{CH_4} = methane production rate, L/d
 M = CH_4 produced per unit of COD removed, (0.35 L CH_4 prod/g COD_{rem} at STP);
 0.2 is a conservative value (Droste, 1997b)
 Q = influent flow rate, L/d
 $S_{t\text{ Infl.}}$ = influent total COD concentration, g COD/L
 $S_{t\text{ Effl.}}$ = effluent total COD concentration, g COD/L
 E = COD treatment efficiency factor, %
 r_X = bacterial growth rate, g VSS/L·d (negligible≈0; Metcalf and Eddy, 1991)
 V_r = volume of reactor, L
 $(r_X V_r)$ = cell tissue or VSS produced, g VSS/d (often ignored; Droste, 1997b)

Biogas analyses can be used to estimate the heating and electricity generation capacity of methane. Its energy content is given as approximately 36 500 KJ/m³, at 20°C and 1 atm (Droste, 1997b; Tchobanoglous and Schroeder, 1985). For COD conversion to CH_4 , Bitton (1994) reports a corresponding calorific value of 9 000 kcal/m³ while Eckenfelder (1989), Grady and Lim (1980) and McCarty (1964) report a heating value of 960 Btu/ft³ (35 689 KJ/m³).

2.1.2.3 Some Causes of Imbalance or Toxicity and Control Measures

Sources of process upset and instability are not always apparent. In addition to H_2 and VFA accumulation as well as alkalinity insufficiency, they can include poor bioreactor mixing, rapid temperature changes, decreased SRT and the presence of toxicants (McCarty, 1964; WEF Manual, 1991). Many compounds, mostly related to industrial wastewaters, can potentially inhibit anaerobic treatment and/or be toxic to methanogens (Bitton, 1994; Curi and Eckenfelder, 1976).

Under normal operating conditions, propionic acids are usually toxic to both groups of anaerobic bacteria and their build-up is indicative of unstable processes (Grady and Lim, 1980) while acetic and butyric acids only slightly affect methanogens (Speece, 1996). To cope with acid accumulations, influent flow rates can be reduced and/or reactor internal recycling increased to re-establish adequate environmental conditions (Gunnerson and Stuckey, 1986; Speece, 1996). Acetoclastic methanogens, like *Methanothrix spp.*, can also be inhibited by long chain fatty acids (LCFAs) such as capric, lauric and oleic (Section 2.2.3) acids (Bitton, 1994). Many organic compounds (Curi and Eckenfelder, 1976; van Haandel and Lettinga, 1994; WEF Manual, 1991) and oxygen (Bitton, 1994; Gunnerson and Stuckey, 1986; Tchobanoglous and Schroeder, 1985) are well known to be extremely toxic to methanogens, even at trace levels. Unless air is introduced in the system in some way, the latter source rarely surfaces (van Haandel and Lettinga, 1994), but it would be an important safety hazard if potentially mixed with explosive biogases (Gunnerson and Stuckey, 1986). *Methanothrix* are usually the least oxygen-sensitive methanogens (Andras, 1987).

Although low concentrations of inorganic materials can enhance methane fermentation (Eckenfelder, 1989), at relatively high dosages (3 500-5 500 mg Na^+ /L, 2 500-4 500 mg K^+ /L, 2 500- 4 500 mg Ca^{+2} /L and 1 000-1 500 mg Mg^{+2} /L), they can be inhibiting (McCarty, 1964). This affect can be diminished with antagonistic cations while the reverse is also true for synergistic combinations (Curi and Eckenfelder, 1976; Eckenfelder, 1989; Grady and Lim, 1980; McCarty, 1964). Heavy metals such as Ni, Cu, Cd, Zn, Pb and Cr, mostly contained in industrial wastewaters and sludges, can be stimulatory, but in soluble form, they are toxic to anaerobic bacteria even in trace amounts (Bitton, 1994; McCarty, 1964; Tchobanoglous and Schroeder, 1985; van Haandel and Lettinga, 1994). However, it is possible to reduce or remove these metal ions by precipitation with insoluble sulfides (Eckenfelder, 1989; Grady and Lim, 1980; McCarty, 1964).

Sulfates that convert to sulfides in anaerobic conditions can cause methane production cessation since they are quite toxic to methanogens as well as to SRBs, in their soluble form and at > 150-200 mg/L (Droste, 1997b; Eckenfelder, 1989). Food-processing wastewaters are often rich in sulfates that can be precipitated out with iron salts (Curi and Eckenfelder, 1976; Droste, 1997b; WEF Manual, 1991). Acetoclastic methanogens are less affected by H_2S than hydrogen-consuming methanogens (Bitton, 1994). Dissolved ammonia (NH_3) gas is also very toxic to methanogens at concentrations greater than 80-150 mg/L while ammonium ions (NH_4^+) are inhibitory above 1 500 mg/L (Bitton, 1994; Eckenfelder, 1989; Gunnerson and Stuckey, 1986). In the anaerobic treatment of cheese industry wastes, pH-dependant ammonia toxicity has been observed (Wheatley, 1990).

However, at low pHs (< 7.2), gaseous NH_3 transforms into less toxic NH_4^+ ions (Curi and Eckenfelder, 1976; Droste, 1997b; Grady and Lim, 1980; McCarty, 1964).

The methane content of biogas and its production rates (Gunnerson and Stuckey, 1986; Metcalf and Eddy, 1991), which reflect methanogenic activity (Grady et al., 1999) as well as organic acid levels (Bargman, 1968; Curi and Eckenfelder, 1976; MaCarty, 1964) are some of the best indicators of digestion performance while alkalinity, pH (Grady et al., 1999; Wheatley, 1990) and H_2 formation are not very sensitive detection means (Speece, 1996). However, in addition to biogas composition variations, some authors consider the latter 3 parameters as good process stability indicators (Droste, 1997b; Torpy, 1988). Nevertheless, any deviation from the norm of these closely related parameters, particularly in combination, can signal potential process imbalance (Parkin and Owen, 1986; Wheatley, 1990). Although changes in the production of biogas occur quicker than effluent VSS or COD concentrations (Grady et al., 1999), the absence of gas generation does not necessarily mean that methanogenic bacteria are lacking or lifeless or that the process has failed totally (Obayashi and Gorgan, 1985; Parkin and Owen, 1986). Biomass has the ability to adapt and become more tolerant to toxicant doses (i.e., ammonia) as well as recover from upsets (McCarty, 1964; Speece, 1996). Washout will also not be as important when SRTs are longer than the acclimation time as well as the minimum SRT (Bitton, 1994; McCarty, 1964).

The VFA to alkalinity ratio (VFA/Alk) is also suggested as a good indicator of process performance (Grady et al., 1999). Parkin and Owen (1986) as well as Bitton (1994) report that total volatile acids (as AA) to total alkalinity (TA as CaCO_3) concentrations should be maintained below 0.1. When $\text{VFA/Alk} < 0.4$, Grady and Lim (1980) suggest that VA variations can be accommodated, but that ratios > 0.4 point to potential process upset and a lack of buffering capacity. At this stage ($\text{VFA/Alk} = 0.3\text{-}0.4$), the application of counteractive remedies is suggested (Droste, 1997b). VFA/Alk ratios > 0.8 usually indicate a severe system imbalance, with an important drop in pH (and high VAs) as well as the inhibition of metabolic activity and methane production (Droste, 1997b; Grady and Lim, 1980), which may continue until the bioreactor fails and the stabilization process ceases (WEF Manual, 1991). Thus, good understanding of the microbial interactions and factors involved allows for greater recognition of unstable conditions and better process control (Torpy, 1988; Wheatley, 1990).

2.1.2.4 Process Analysis and Kinetic Coefficient Estimation

Biological wastewater treatment processes transform organic substrates into new microbial mass and simpler (organic and inorganic) end products (Metcalf and Eddy, 1991). Whether aerobic

or anaerobic in nature, the complex bacterial growth and substrate uptake interaction is never easy to model. Monod (1949) formulated an empirical hyperbolic expression (Eq. 2.4) that most often adequately describes the utilization of substrate for most continuous-growth culture systems in substrate-limited conditions (Bitton, 1994; Droste, 1997b; Gunnerson and Stuckey, 1986; Jorden et al., 1971; Metcalf and Eddy, 1991; Ozturk et al., 1990; van Haandel and Lettinga, 1994). However, it presumes that the growth of a pure culture on a simple single substrate is similar to that of mixed cultures on complex organic wastes (Chynoweth and Isaacson, 1987).

$$\mu = \mu_{\max} \frac{S}{K_s + S} \quad (2.4)$$

Where, μ = specific growth rate, d^{-1}
 S = concentration of growth-limiting substrate, mg COD/L
 (Other terms as defined in Section 2.1.2.3.)

Its development is based on the fact that the rate of substrate removal (r_s) is proportional to the rate of bacterial growth (r_x ; Section 2.1.2.2d and Eq. 2.6), as well as the assumption that microorganisms essentially use substrate for growth and maintenance (Curi and Eckenfelder, 1976; Droste, 1997b; Jorden et al., 1971; Metcalf and Eddy, 1991; Ozturk et al., 1990):

$$r_x' = -Yr_s - k_e X_v \quad (2.5)$$

Where, r_x' = net bacterial growth rate, g VSS/L·d
 $-Yr_s = r_x$ = biomass or VSS production rate from substrate removal, g VSS/L·d
 $-k_e X_v$ = VSS reduction rate by endogenous decay or die off, g VSS/L·d
 r_s = rate of substrate utilization, g COD/L·d
 X_v = VSS concentration in the reactor (as the active biomass estimate), g VSS/L
 (Other terms as defined in Section 2.1.2.3.)

Net bacterial growth usually prevails although microorganisms usually face starved conditions (Droste, 1997b). However, this rate can potentially be negative under low organic matter concentrations because of maintenance rates ($-k_e X_v$). This can cause bacterial culture deterioration due to insufficient substrate availability.

Since r_x and $\mu_{\max}$ are defined as follows (Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985), Equation 2.9 results by substituting Eqs. 2.7 and 2.8 in Eq. 2.4:

$$r_x = \mu X_v, \quad (2.7)$$

$$\mu_{\max} = kY, \quad (2.8)$$

$$r_X = \mu_{\max} \frac{X_v S}{K_s + S} = Y \left(\frac{kX_v S}{K_s + S} \right) \quad (2.9)$$

The common and basic Monod equation or saturation-type function (Droste, 1997b; Ozturk et al., 1990; Speece, 1996; Tchobanoglous and Schroeder, 1985), which expresses the rate of change in substrate (organic) concentration ($-dS/dt$), is defined as follows (Eq. 2.10) by equating Eq. 2.6 to Eq. 2.9:

$$r_s = \left(-\frac{dS}{dt} \right) = -\frac{kX_v S}{K_s + S} \quad (2.10)$$

At low substrate concentrations, where S is low relative to K_s ($S \ll K_s$), the Monod ratio ($S/(S+K_s)$) reduces to a first-order or pseudo-Monod kinetic expression resulting in Eq. 2.11 and it translates to zero-order kinetics (i.e., $r_s = -kX_v$) in the reversed situation (Droste, 1997b; Kennedy, 1999; van Haandel and Lettinga, 1994). Chynoweth and Isaacson (1987) suggest that the former case is fairly reliable:

$$r_s = -kX_v S \quad (2.11)$$

Although the Monod function has been observed to adequately represent the substrate removal phenomenon in many biological processes including overall anaerobic treatment (Curi and Eckenfelder, 1976; Droste, 1997b; Gunnerson and Stuckey, 1986; Liu et al., 1996a; Speece, 1996; van Haandel and Lettinga, 1994), substrate removal rates (and specific growth rates) can be described by other expressions or Monod-type variations (Chynoweth and Isaacson, 1987; Droste, 1997b; Gunnerson and Stuckey, 1986; Metcalf and Eddy, 1991), such as Equation 2.12. Equations 2.10 and 2.11, which include the X_v term, do have some shortcomings since they use the VSS content to estimate the amount of active microbial mass contained in the sludge, but the latter usually only represents about 50-60% of VSS (Speece, 1996). Thus, these expressions are not always recommended (Chynoweth and Isaacson, 1987; Droste, 1997b; Metcalf and Eddy, 1991).

$$r_s = -\frac{kS}{K_s + S}, \quad \text{resulting in } r_s = -kS \quad (\text{first-order kinetics}) \quad (2.12)$$

Although the overall process reactions differ quite significantly for anaerobic and aerobic systems, the growth and substrate utilization mechanisms of the respective bacteria involved bear many similarities (Droste, 1997b; Obayashi and Gorgan, 1985). Therefore, the above principles as well as the following mass-balance equations, used to model process kinetics, are analogous for

activated sludge as well as anaerobic systems (i.e., UASB reactors) and are applicable to both (Chynoweth and Isaacson, 1987; Droste, 1997b; Liu et al., 1996a). For anaerobic processes, Droste (1997b) and Pavlostathis and Giraldo-Gomez (1991) report that hydrolysis usually follows first-order kinetics, although the latter suggest that it often does not exhibit Monod kinetics. However, the latter model has been observed to suitably describe the other anaerobic reaction stages and the process as a whole (Chynoweth and Isaacson, 1987; Pavlostathis and Giraldo-Gomez, 1991).

Based on Equation 2.13, CM and continuous-flow suspended-growth reactors *without sludge recycle* from a clarifier [Fig. 2.3 a) in Section 2.1.4.2], but with wasting from the reactor (Option 2) rather than the recycle line can be described with the following mass-balance analysis, when steady state conditions are assumed to exist (i.e., $dS/dt=0$ and $dX/dt=0$) and a Monod-based relation (Equation 2.10) is used (Droste, 1997b; Gunnerson and Stuckey, 1986; Jorden et al., 1971; Metcalf and Eddy, 1991; Ozturk et al., 1990). Compared to the biomass concentration in the reactor unit (Chynoweth and Isaacson, 1987), the influent microorganism mass concentration (or VSS) is considered to be negligible ($X_{V0} \approx 0$; Droste, 1997b; Gunnerson and Stuckey, 1986; Jorden et al., 1971; Liu et al., 1996a; Metcalf and Eddy, 1991), particularly for soluble industrial wastewaters. Even when substantial, it is also usually not incorporated in the solids balance if it does not contain process adapted-biomass (Droste, 1997b). Although some wasting was carried out (from the aerobic basin) for sampling purposes, the experimental wastage flow rate (Q_w) was also assumed as negligible compared to the influent flow rate (Liu et al., 1996b). Thus, the effluent flow rate (Q_e) would be considered approximately equal to Q ($Q_e \approx Q$).

$$\text{IN} - \text{OUT} \pm \text{Generation (Net Growth)} = \text{Accumulation (Net Change)} \quad (2.13)$$

2.1.2.4a Substrate Mass-Balance Around the Reactor

$$QS_o - Q_e S_e - Q_w S_e + r_s V_r = \frac{dS_e}{dt} V_r = 0 \quad (2.14)$$

$$r_s = -\frac{Q(S_o - S_e)}{V_r} - \frac{Q_w S_e}{V_r} = -\frac{(S_o - S_e)}{\theta_d} \quad (2.15)$$

When using the Monod relation (Equation 2.10),

$$\frac{kX_v S_e}{K_s + S_e} = \frac{(S_o - S_e)}{\theta_d} \quad (2.16)$$

Where, t = time
 Q = influent flow rate, L/d

$$\begin{aligned}
Q_w &= \text{liquid wastage flow rate, L/d } (\approx \text{negligible}) \\
S &= (\text{soluble}) \text{ influent substrate concentration, g COD/L} \\
S_e &= (\text{soluble}) \text{ effluent substrate concentration, g COD/L} \\
V_r &= \text{reactor volume, L} \\
\theta_d = V_r/Q &= \text{hydraulic retention time, d} \\
\text{(Other parameters as defined previously)}
\end{aligned}
\tag{2.17}$$

2.1.2.4b Solids Mass-Balance Around the Reactor

$$QX_{v_o} - Q_e X_v - Q_w X_v + r_x' V_r = \frac{dX_v}{dt} V_r = 0 \tag{2.18}$$

$$r_x' = \frac{QX_v + Q_w X_v}{V_r} = \frac{QX_v}{V_r} \tag{2.19}$$

Where,

$$\begin{aligned}
dX/dt &= \text{rate of change of microorganism concentration, g VSS/L} \cdot \text{d} \\
X_{v_o} &= \text{concentration of microorganisms in soluble influent, g VSS/L } (\approx \text{negligible}) \\
\text{(Other parameters as defined previously)}
\end{aligned}$$

At steady state, the next equation results when using Eq. 2.5, the following net specific growth rate (μ') relation (Bitton, 1994; Chynoweth and Isaacson, 1987; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985), the $1/\theta_d$ correspondence to $1/\theta_x$ (Metcalf and Eddy, 1991) and by rearranging:

$$\frac{r_x'}{X_v} = \mu' = \frac{1}{\theta_x} = -Y \frac{r_s}{X_v} - k_e \tag{2.20}$$

Equation 2.21, which results by substituting Eq. 2.15 in Eq. 2.20 and solving for X_v , can be used to predict the concentration of microorganisms in the reactor (or the reactor effluent):

$$X_v = Y \frac{(S_o - S_e)}{1 + k_e \theta_x} \tag{2.21}$$

Equation 2.20 results in an equation of linear form ($y = mx + b$) that relates $1/\theta_x$ and U :

$$\frac{1}{\theta_x} = YU - k_e \tag{2.22}$$

Where, $\mu' = \text{net specific growth rate, d}^{-1}$
 $\theta_x = \text{solids retention time (reactor bacteria mass/daily microorganism wastage), d}$
 $U = \text{specific substrate utilization rate, g COD/g VSS} \cdot \text{d}$

$$\text{Since } U = \frac{-r_s}{X_v} = \frac{(S_o - S_e)}{X_v \theta_d} = \frac{kS_e}{K_s + S_e} \quad (\text{using the Monod relation}) \quad (2.23)$$

Based on the same assumptions as the case with no recycling, a soluble substrate mass-balance performed on a biological treatment system *with recycled* sludge from a clarifier (including extended aeration systems; Metcalf and Eddy, 1991), with wasting from either the reactor or the recycle line [Fig 2.3 b) in Section 2.1.4.2], would also yield Eqs. 2.15 and 2.16 (Droste, 1997b; Ozturk et al., 1990). After algebraically manipulating Eqs. 2.22 and 2.23 and solving for S_e , the expected effluent substrate concentration (S_e) in both cases can be found with Equation 2.24 (Chynoweth and Isaacson, 1987; Metcalf and Eddy, 1991; Parkin and Owen, 1986; Tchobanoglous and Schroeder, 1985), although θ_x should be substituted for θ_d for cases with recycling (since θ_d and θ_x are never equal for systems involving recycling).

$$S_e = \frac{K_s(1 + \theta_d k_e)}{\theta_d(Yk - k_e) - 1} \quad (2.24)$$

However, differences in the microorganism mass-balance (performed around the entire system [Fig. 2.3 b) in Section 2.1.4.2] are presented below and only the reacting unit volume (V_r) is taken into consideration. For such a system, the solids retention time (as defined previously) would result in Eq. 2.25 (Metcalf and Eddy, 1991; Ozturk et al., 1990), based on the next solids balance (2.1.2.4c):

$$\theta_x = \frac{V_r X_v}{(Q - Q_w)X_{ve} + Q_w X_v} \approx \frac{V_r X_v}{Q X_{ve}} \quad (2.25)$$

2.1.2.4c Biomass Balance Around the Entire System

$$QX_{vo} - Q_e X_{ve} - Q_w X_v + r_x' V_r = \frac{dX_v}{dt} V_r = 0 \quad (2.26)$$

At steady state and $X_{vo} \approx 0$,

$$r_x' = \frac{Q_e X_{ve} + Q_w X_v}{V_r} \quad (2.27)$$

By dividing Eq. 2.27 by X_v and substituting in Eq. 2.5 as well as the inverse of Eq. 2.25, the above relation also results in Eq. 2.20 (like the no recycling case). In addition, since Eq. 2.15 still

applies for this case, where θ_d differs from θ_x , substituting it into Eq. 2.20 and solving for X_v results in the reactor microorganism concentration estimate:

$$X_v = \frac{\theta_x}{\theta_d} Y \frac{(S_o - S_e)}{(1 + k_e \theta_x)} \quad (2.28)$$

Therefore, *regardless of the case*, when S_o , S_e , X_v , θ_d and θ_x lab data are available (for more than 2 operating conditions (Droste, 1997b) and usually at least 4 system experimental runs with varying retention times (Jorden et al., 1971), Lineweaver-Burke plots can be used to estimate the kinetic coefficients (Bitton, 1994; Droste, 1997b; Metcalf and Eddy, 1991). For limited data, simple models are usually recommended (Gunnerson and Stuckey, 1986). However, for situations that involve recycling, the proper θ_x values must be used. From linear Eq. 2.22, the Y and k_e constants are determined by plotting $1/\theta_x$ (or μ') against U (or $-r_s/X_v$) and by finding, respectively, the slope and intercept of the regressed experimental data.

After substitution of a kinetic expression such as Eq. 2.10 in Eq. 2.15 and some manipulation (or taking the inverse of Eq.2.23 that also used the Monod equation), Equation 2.29 also results in a LineweaverBurke formulation that relates $1/U$ and $1/S_e$ and also *applies to both cases*:

$$\frac{X_v}{-r_s} = \frac{X_v \theta_d}{S_o - S_e} = \frac{1}{U} = \frac{K_s}{k} \frac{1}{S_e} + \frac{1}{k} \quad (2.29)$$

Similarly, the K_s and k coefficients can also be determined graphically from a linear regression by plotting $1/U$ (or $X_v/-r_s$) against $1/S_e$ and determining the intercept ($1/k$) and the slope (K_s/k). Consequently, μ_{max} can be estimated with Eq. 2.8. Such parameter estimations, which are required to predict system performance as well as for empirical modeling and design purposes (Chynoweth and Isaacson, 1987; Curi and Eckenfelder, 1976; Metcalf and Eddy, 1991), should be determined for steady state systems and for varying environmental conditions (Chynoweth and Isaacson, 1987; Gunnerson and Stuckey, 1986).

Below a minimum SRT ($\theta_{x \min}$), biological conversions do not take place (i.e., when $\theta_x < \theta_{x \min}$) since the washout of microorganisms would exceed their reproduction (Metcalf and Eddy, 1991). Thus, the biomass would not be retained within the system. Since maximum growth rates are a function of maximal substrate removal rates (Droste, 1997b), the bacterial washout time ($\theta_{x \min}$) can be estimated from the inverse of Eq. 2.22 at the maximum reactor substrate concentration ($S_e = S_o$). This results in Eq. 2.30 when applying Eq. 2.10 and in Eq. 2.31 when

$S_o \gg K_s$ (Droste, 1997b; Metcalf and Eddy, 1991) while the microorganism generation time (θ_X^{lim}) is defined as $\theta_X^{lim} = (Yk - k_e)^{-1}$ (Obayashi and Gorgan, 1985; Parkin and Owen, 1986).

$$\theta_{Xmin} = \left[Y \left(\frac{kS_o}{K_s + S_o} \right) - k_e \right]^{-1} \quad (2.30)$$

$$\theta_{Xmin} \approx Yk - k_e \quad (2.31)$$

2.1.2.5 Kinetics

Even though substrate conversion rates depend on many factors (i.e., substrate biodegradability, inoculum and reactor types, hydraulic regime and loading rate, nutrient availability, microorganism diversity as well as environmental requirements), hydrolysis, acidogenesis and acetogenesis usually proceed quickly (Bitton, 1994; Curi and Eckenfelder, 1976; Kennedy, 1985; Pavlostathis and Giraldo-Gomez, 1991; Speece, 1983) since acid forming anaerobes have very fast generation times, of a few hours. However, digesters may require relatively long retention periods for proper waste stabilization owing to the slower rate of growth of methanogens (Bitton, 1994; Metcalf and Eddy, 1991). Wheatley (1990) reports a 5-day doubling time for this type of bacteria. Their metabolism is also more inhibited at low temperatures (Speece, 1996) and Bitton (1994) reports generation times as great as 50 d, at 10°C, compared with 3 d at 35°C.

In the literature, there is quite a bit of inconsistency in reported kinetic constants, which describe anaerobic bacteria metabolism rates during treatment (Kennedy, 1999; Pavlostathis and Giraldo-Gomez, 1991). These values can be significantly affected by temperature as well as substrate composition (Pavlostathis and Giraldo-Gomez, 1991; Speece, 1996). Table 2.1 presents some typical anaerobic treatment kinetic coefficients based on the Monod model (Section 2.1.4.5), including half-velocity constants (K_s), maximum specific growth rates (μ_{max}), maximum specific substrate utilization rate constants (k), biomass growth yield coefficients (Y) and endogenous decay coefficients (k_e). Bitton (1994) has reported μ_{max} values of 1 h⁻¹ (24 d⁻¹) for acetogens and of 0.04 h⁻¹ (0.96 d⁻¹) for methanogens. For domestic wastewaters treated at 20°C, a typical Y value is 0.06 mg VSS/mg BOD₅ and 0.03 d⁻¹ for k_e , with ranges between 0.04-0.1 mg VSS/mg BOD₅ and 0.02-0.04 d⁻¹, respectively (Metcalf and Eddy, 1991). Speece (1996) reports that k values can decrease from 6.67 to 3.85 d⁻¹ and K_s values can increase from 164 to as high as 2 130 mg/L, with a temperature decrease from 35 to 20°C, respectively.

Table 2.1: Typical Monod Kinetic Constants for Anaerobic Biodegradation (at 30-40°C)

	K_s (mg COD/L)	μ_{max} (d ⁻¹)	k (mg COD/mg VSS·d)	Y (mg VSS/mg COD)	s_k (d ⁻¹)
Acidogenesis: or acidogens	200 (23 - 31 000)	2.0 -	13 (9.5 - 176)	0.15 (0.12 - 0.54)	0.5 (0.08 - 6.1)
Methanogenesis: or methanogens	50 (2 - 3 900)	0.4 -	13 (1.8 - 17)	0.03 (0.02 - 0.28)	0.02 (0.01 - 0.04)
Overall Process: or mixed-culture	^s 1 000 (200 - 4 000)	0.4 -	2 -	0.18 (0.024 - 0.21)	0.03 (0.01 - 0.04)

Presented in Droste (1997b), Pavlostathis and Giraldo-Gomez (1991) as well as van Haandel and Lettinga (1994) and adapted from ¹Henze and Harremoës (1983). Values in parentheses represent observed ranges ^s(Droste, 1997b).

Some of the most common methane-formers include *Methanosarcina* and *Methanosaeta* (or *Methanothrix*) acetoclasts. Generally, acetoclastic methanogens are considered as the weakest link of the anaerobic process since they grow slower than hydrogen-utilizing (or hydrogenophilic) methanogens (Curi and Eckenfelder, 1976; McCarty, 1964; van Haandel and Lettinga, 1994), at 3-10 d compared to 1 d in the case of the latter (Kennedy, 1999). Filamentous *Methantrix-soehngenii* is a different species than rod-type *Methanothrix* (Lettinga et al., 1987; Torpy, 1988). Although the former have a more rapid growth rate than the latter (Kennedy, 1999), *Methanosarcina* acetoclasts have an even faster metabolism (Barber and Stuckey, 1999; Droste, 1997b), higher specific activities, but a lower affinity for (acetate) substrates (Table 2.2). At low acetate concentrations (or in CSTRs), *Methanosaeta* usually predominate over *Methanosarcina* (Section 2.1.3.3) due to their greater scavenging capacity and insensitivity to acetate diffusion limitations (Barber and Stuckey, 1999; Pavlostathis and Giraldo-Gomez, 1991; Speece, 1996.)

The relatively slow growth rates and low cellular yields of acetate-consuming methanogens is due to the fact that acetate does not provide much energy (Andras, 1987). Bitton (1994) reports that methanogens also have less affinity for acetate ($K_s = 32.8$ mg AA/L) than SRBs ($K_s = 9.5$ mg AA/L). Under ideal conditions, typical doubling times for *Methanosaeta* and *Methanosarcina* are 4 d and 1.5 d, respectively (Barber and Stuckey, 1999; Droste, 1997b). Comparatively, from compiled sources, Andras (1987) reports values of 1-9 d and 1-6 d, respectively, while Kennedy (1985) reports 3-6 days for both genres. However, their affinity for acetate (K_s) and maximum specific utilization rates vary more significantly (Table 2.2). Some kinetic constants for these acetogens are reported in Table 2.2.

¹ Henze and Harremoës (1983), "Anaerobic Treatment of Wastewater in Fixed Film Reactors-A Literature Review," *Water Science and Technology*, 15, 8/9, pp. 1-101.

Table 2.2: Some Reported Biokinetic Parameters for Acetoclastic Methanogens

	K_s (mg AA/L)	k (g COD/g VSS·d)	μ_{max} (d ⁻¹)	Y (mg VSS/mg COD)	Spec. Activity (mg COD/mg VSS·d)	Reference
<i>Methanosaeta</i> :	20	2 - 4	0.1	0.017 - 0.025 ^A	2.3 ^B	1.
	30					2.
	⁴ 15 - 40					3.
	20 - 30					5.
<i>Methanosarcina</i> :	⁵ 400	6 - 10	0.3	0.03 - 0.05	2 - 12	1.
	200					2.
	⁴ 150-250					3.
Mixed Culture: (25°C)	930 ^C	5	0.25	0.05	k_e (d ⁻¹) = 0.011	6.

1. Speece (1996); 2. van Haandel and Lettinga (1994); 3. Andras (1987); 4. Kennedy (1985); 5. Barber and Stuckey (1999); 6. Pavlostathis and Giraldo-Gomez (1991); for a continuously-fed culture at 25°C

^A mg TSS/mg COD; ^B mg COD/mg TSS·d; ^C mg COD/L

2.1.3 FEATURES OF THE UPFLOW ANAEROBIC SLUDGE BLANKET SYSTEM

Tang et al. (1995) state that the earliest form of the system was introduced by Wilson and Phelps, around 1910 (Bitton, 1994). However, most researchers commend Lettinga and collaborators, from the Netherlands, for devising the UASB concept, in the 1970s (Guiot et al., 1992a and b; Gunnerson and Stuckey, 1986; Kennedy, 1999; Lettinga et al., 1980; Maat and Gorur, 1990; Obayashi and Gorgan, 1985; Rittmann and McCarty, 2001; Speece, 1996; van Haandel and Lettinga, 1994; Wheatley, 1990). Even if it is more commonly used in Europe (Chynoweth and Isaacson, 1987; Gunnerson and Stuckey, 1986; McCarty and Smith, 1986), of all the advanced anaerobic process configurations that have emerged, it is the most popular worldwide (Droste, 1997b; Lentz, 1996; Lettinga et al., 1984; van Haandel and Lettinga, 1994). This system, which can remove organic substances and some SS (de Sousa and Foresti, 1996; van Haandel and Lettinga, 1994), was first used to treat medium to high-strength, mostly soluble and non-complex (carbohydrate-type or sugar-based) food industry-related wastewaters (Bitton, 1994; Chevalier, 1996; Gunnerson and Stuckey, 1986; Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1984; van Haandel and Lettinga, 1994), at relatively low OLRs (Lettinga et al., 1987).

Because of considerable technological developments, but despite the fact that UASB systems are sensitive to waste composition (Droste, 1997b), their treatment applications presently encompass a much broader spectrum of waste strengths and operational temperatures (Section 2.1.3.5) as well as wastewater types (Andras et al., 1989; Lettinga et al., 1984 and 1987; Speece, 1996). They are becoming more feasible for the treatment of various types of industrial wastes,

including dilute sewage, in warm or mesophilic conditions as well as in subtropical and more moderate ones, to a lesser degree (Castillo et al., 1997; de Sousa and Foresti, 1996; Kennedy, 1999; Lettinga and Vinken, 1980; Lettinga et al., 1993; van Haandel and Lettinga, 1994). Nevertheless, high-rate UASB reactors are best suited for treating those rich in VFAs with a low SS content (Chevalier, 1996; Chynoweth and Isaacson, 1987; Kennedy, 1999).

Although no single HR anaerobic system is suitable for all wastewater treatment applications (Rittmann and McCarty, 2001), fixed-film (FF), sludge blanket (SB) or anaerobic contact (AnCont.) processes have been observed to be among the most efficient (Choi and Burkhead, 1984). However, whether for sludge or liquid wastewaters, several authors report the UASB as being the better, most complete and successful system (Torpy, 1988; Wheatley, 1990). Like other biological anaerobic configurations, its success for particular wastewater types and operation temperatures, is largely dependant on its biomass inventory and activity (de Zeeuw and Lettinga, 1980). This well established and simple treatment technology is well documented (Kennedy, 1999; Lettinga and Vinken, 1980; van Haandel and Lettinga, 1994; Wheatley, 1990) and some design criteria are described elsewhere [Grady and Lim, 1980; Lettinga and Hulshoff Pol, 1991; Metcalf and Eddy, 1991; van Haandel and Lettinga, 1994; WEF Manual of Practice no. 8 (CH.18), 1991].

2.1.3.1 System Description

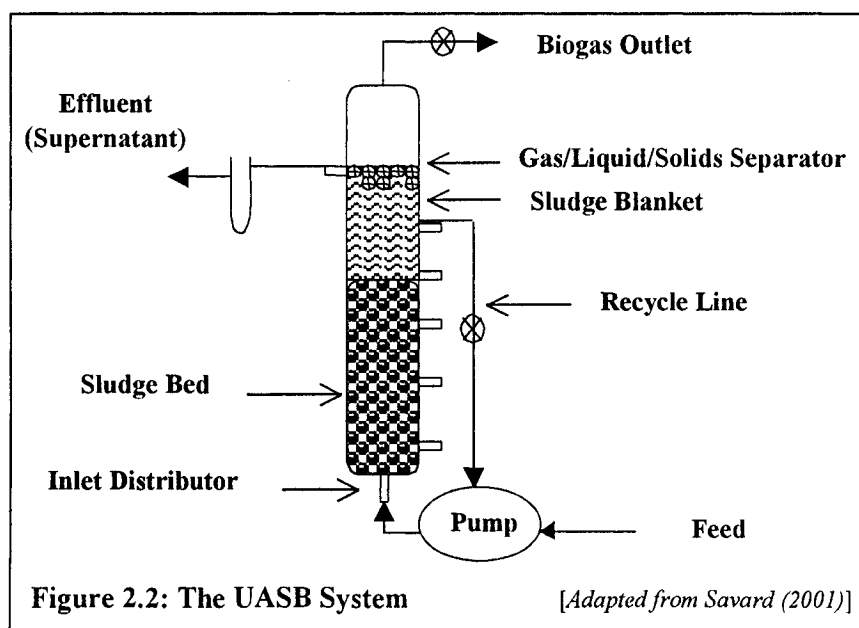
Under favorable conditions, a large amount of densely-packed, naturally flocculating and active biomass can be maintained within this distinct sludge bed-type reactor (Kennedy, 1999; Langenhoff and Stuckey, 2000; Lettinga and Vinken, 1980; Obayashi and Gorgan, 1985; Wheatley, 1990) as a result of rapid granule settling rates (Lettinga et al., 1980, 1983; Saw, 1986; Speece, 1996) compared to relatively low influent upflow velocities (Chynoweth and Isaacson, 1987; Wheatley, 1990). Thus, the sludge retention principle is based on the self-aggregation and/or maintenance of microorganisms into granular particles or flocs (Bitton, 1994; Guiot, 1992; Kennedy, 1999; Lettinga et al., 1984 and 1987; Metcalf and Eddy, 1991; Rittmann and McCarty, 2001).

In essence, treatment efficiencies rely on bacteria settling abilities and their retention within the reactor as well as their separation from the liquid overflow (McCarty and Smith 1986; Saw, 1986; Torpy, 1988; van den Berg and Kennedy, 1983). As a result, long SRTs and relatively high OLRs can be achieved even while operating under (high flow rates or) short HRTs (Andras et al., 1989; Droste, 1997b; Guiot, 1992; Kennedy, 1999; Lettinga et al., 1987 and 1993; Obayashi and

Gorgan, 1985; Speece, 1996; van Haandel and Lettinga, 1994). This is particularly critical for dilute wastewaters (Lettinga et al., 1980), in order to keep reactor volumes reasonably small (Bitton, 1994; Speece, 1996) and treatment costs viable (Langenhoff and Stuckey, 2000).

Since sedimentation rates increase with aggregate size (Barbosa and Sant'Anna Jr, 1989; Guiot, 1992; Guiot et al., 1992b), larger and heavier particles are generally easier to immobilize while voluminous suspended biomass tends to washout. In some cases, granular sludge concentrations of 100-150 g VSS/L (Droste, 1997b; Eckenfelder, 1989; Obayashi and Gorgan, 1985) and as high as 20% (Torpy, 1988) have been reported in full-scale reactors. However, well-settling flocculent or granular biomass should not compromise performance, for low to moderately loaded reactors (Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1987; van Haandel and Lettinga, 1994). Yet, a good biomass inventory of the latter type allows for greater HLRs and OLRs to be applied, especially for soluble organic liquid wastewaters (Manjunath et al., 1990), and a better SOLR to be maintained (Pham, 2002).

Unlike fixed-film reactors, this anaerobic process alternative does not require costly support media for sludge entrapment (de Sousa and Foresti, 1996; Guiot, 1992; Guiot et al., 1992b; Gunnerson and Stuckey, 1986; Monroy et al., 2000; Speece, 1996; van Haandel and Lettinga, 1994). Nevertheless, inert flexirings are occasionally used in novel, hybrid UASB-AF or UBF systems (Elmitwalli et al., 1999; Guiot et al., 1992b; Kennedy, 1999; Monroy et al., 2000) to promote biofilm development and reactor solids withholding (Droste et al., 1997b). Since substrate and product are respectively dispersed in and out of flocs, McCarty and Smith (1986) suggest that UASBs respond to some extent like beneficial biofilm-based processes. Guiot (1992) states that granules (Section 2.1.3.3) are a specific type of biofilm and are very similar, at the microscopic stage, since they both involve microbial interactions.



Although Figure 3.4 (Section 3.1.1.1) illustrates, in detail, the experimental units used, Figure 2.2 presents the most important components of a typical (bench-scale) UASB system. The latter is usually characterized by 3 sections, where acidification and methanation take place within the single-stage unit (Lettinga and Vinken, 1980). The *Capetown group*, referred to in Speece (1996), consider these as lower and upper active zones followed by an upper inactive one while most of the literature classifies them as the *sludge bed* (digestion or biological reaction region), the *sludge blanket* (transitional or upper liquid level region) and the *upper settling/biogas separation zones* (Chynoweth and Isaacson, 1987; Obayashi and Gorgan, 1985). The blanket (liquid supernatant) and (biomass) bed terms are often synonymously used in the literature.

Influent wastewater enters at the base of UASBs and reaches the effluent outlet in an upward mode (by pumping), like the configuration name implies. As it passes through the lower anaerobic sludge bed, where most biochemical conversions occur, the incoming organic matter is broken down and transformed into biological solids as well as biogas (Castillo et al., 1997; Chynoweth and Isaacson, 1987; Droste, 1997b; Eckenfelder, 1989; Lettinga and Hulshoff Pol, 1991; Metcalf and Eddy, 1991; Obayashi and Gorgan, 1985; Torpy, 1988). The latter is then collected at the top of the reactor (Bitton, 1994; Ulrix, 1994; Wheatley, 1990). Thus, the incoming waste should be circulated as evenly as possible, via a feed inlet distribution system (Lettinga and Vinken, 1980; Lettinga et al., 1984), to provide optimal bacteria-substrate contact (Kennedy, 1999; Lettinga et al., 1993; van Haandel and Lettinga, 1994) and minimize the risk of SB channelling and/or dead spaces/short circuiting (Garuti et al., 1992; Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1987; Obayashi and Gorgan, 1985).

UASB feed inlet point guidelines vary somewhat in the literature. However, Lettinga et al. (1987) generalize and state that < 1 point per m^2 is sufficient for systems with granular or flocculent biomass with > 2 g COD/L·d loads. A more uniform waste distribution is usually recommended for low-strength wastewaters and/or for sub-optimal temperature conditions (Section 2.1.3.5) as well as for low-loaded reactors (< 1 -2 g COD/L·d), with thick biomass, which can lack substrate biodegradability (Lettinga et al., 1983, 1984, 1987 and 1993; Torpy, 1988). Lettinga and Vinken (1980) advise the use of > 1 point per 10 m^2 of bed surface area (for < 5 g COD/L·d) while Torpy (1988) suggests > 1 point each 5 - 8 m^2 (for < 6 g COD/L·d). A large number of inlet points can also cut down UASB start-up periods (Section 2.1.3.2) since the production of biogas is typically low during this phase (Torpy, 1988).

The latter author states that UASBs do not require mechanical mixing or sludge recirculation while other authors suggest keeping them at least to a minimum (Lettinga and Vinken, 1980;

Obayashi and Gorgan, 1985). Although UASB effluent recycling, which is primarily used to attenuate influent waste concentrations, shock loads or toxicity and reactor sub-optimal pH problems (Barber and Stuckey, 1999; Lettinga and Hulshoff Pol, 1991), is not always recommended (Droste, 1997b; Lettinga et al., 1984), it can promote mixing in low-loaded reactors (with produce little biogas) or for ambient temperature operation (Kennedy, 1999). Its application is also suggested for high fat concentration-wastes (Lettinga and Hulshoff Pol, 1991). However, it can also negatively impact process performance because greater hydraulic loading rates tend to cause more dead space and solids loss (Barber and Stuckey, 1999; Speece, 1996).

In any case, UASBs call for some degree of mixing (Lettinga et al., 1987), whether by mechanical means, liquid/sludge recirculation and/or gas evolution (Rittmann and McCarty, 2001), to promote interaction between the substrate utilising bacteria and the wastewater (Castillo et al., 1997; Guiot, 1992; Kennedy, 1999; Torpy, 1988). The extent of UASB internal mixing, which influences the reactor's treatment efficiency, usually depends on the upflow velocity (influent fluid flow and recycling conditions) and the biogas flux (Chynoweth and Isaacson, 1987; Guiot, 1992; Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Ulrix, 1994; Wheatley, 1990). The latter is said to provide significant SB mixing (Droste, 1997b; Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Obayashi and Gorgan, 1985) although Lettinga and Vinken (1980) report that biogas production only contributes slightly to it. Yet, if the agitation and upward velocities are too intense, biomass dispersion and loss (particularly of fine flocs) as well as aggregate break-up may occur, due to shearing (Guiot, 1992; Kennedy, 1999; Speece, 1983 and 1996). However, contrary to Kennedy (1999), Guiot et al. (1992a) state that a high ascending throughput (Section 2.1.3.4) does not always result in smaller-sized granules and/or greater biomass washout.

Continuous feeding modes are preferable (Grady and Lim, 1980; Wheatley, 1990), although intermittent (Lettinga and Hulshoff Pol, 1991) and pulse (Kennedy, 1999) approaches are reported to provide improved mixing conditions, especially for low gas producing reactors. With tracer experiments, the internal mixing regime of both the sludge bed and blanket of a full-scale UASB has been observed to be completely mixed (CM) (with 2.5% dead space), with an upper PF settling section (Kennedy, 1999). Obayashi and Gorgan (1985) also state that both zones are regarded as ideally mixed. For a pilot-scale system, with a 10:1 recirculation ratio, the process has been reported in Kennedy (1999), as an ideal CSTR with less than 3% of influent bypass. For $> 25^{\circ}\text{C}$, wastewater strengths $> 1\,000\text{ mg COD/L}$ and OLRs $> 5\text{ g COD/L}\cdot\text{d}$, Lettinga and Vinken (1980) report that UASB digester biogas production should result in mixing conditions approaching CM while Torpy (1988) reports that $> 6\text{ g COD/L}\cdot\text{d}$ OLR are required. However, some influent fraction

is bound to bypass the main bacteria mass due to cracks that form in the bed (Chynoweth and Isaacson, 1987; Ulrix, 1994). Therefore, the system can never quite be considered as perfectly mixed (Obayashi and Gorgan, 1985).

Mixing also contributes to the suspension as well as the expansion of the SB and flocculated blanket (Castillo et al., 1997; Chynoweth and Isaacson, 1987; Metcalf and Eddy, 1991; Obayashi and Gorgan, 1985). Therefore, they can also result from gassing and applied upflow fluid velocities or hydraulic loads (Lettinga et al., 1984, 1987 and 1993; Wheatley, 1990). UASB operation only requires minimal SB expansion (Guiot et al., 1992a), which can also be influenced by microbial growth and granule composition (Kennedy, 1985 and 1999) as well as effluent recycle means (Chynoweth and Isaacson, 1987) and/or the use of tall reactors (Lettinga et al., 1993). However, significant blanket expansion into the settling region of UASBs and flocculent bacteria washout are not necessarily detrimental (Lettinga et al., 1984 and 1987). They are tolerated and can even be beneficial (Section 2.1.3.2), as long as the CH_4 generation does not decrease considerably (Chynoweth and Isaacson, 1987; Lettinga et al., 1987).

Active bacteria and even large aggregates can float and escape from UASB reactors (Speece, 1996), which have SS removal limitations due to their upflow loading conditions (Garuti et al., 1992). Therefore, an internal GLS (Gas/Liquid/Solids) separator device, even of simple form (Lettinga and Hulshoff Pol, 1991; Lettinga and Vinken, 1980; Lettinga et al., 1993), is essential in the upper quiescent part of the reactor to minimize sludge losses and excessive sludge blanket expansion (Lettinga and Hulshoff Pol, 1991; Rittmann and McCarty, 2001; van Haandel and Lettinga, 1994). It is usually employed, in conjunction with an (inverted) internal settler, to separate buoyant SB and blanket biosolids from the upflowing and/or attached biogas bubbles and the treated liquid effluent (Chynoweth and Isaacson, 1987; Dröste, 1997b; Gunnerson and Stuckey, 1986; Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Lettinga and Vinken, 1980; Lettinga et al., 1987; Metcalf and Eddy, 1991; Obayashi and Gorgan, 1985). The primary functions of the 3-phase separation system are, however, to promote the settling of viable biomass and retain it within the SB digestion zone (Chynoweth and Isaacson, 1987; Lettinga et al., 1987; Metcalf and Eddy, 1991; Rittmann and McCarty, 2001; Speece, 1996; Torpy, 1988; Wheatley, 1990).

2.1.3.2 Seed Sludge Inventory and Start-Up Considerations

Although no set UASB reactor inoculum quantity to reactor volume ratio has been established (Kennedy, 1999), according to Gunnerson and Stuckey (1986), the SB should occupy approximately 50% of the digester volume. However, Obayashi and Gorgan (1985) as well as

Torpy (1988) report a 30% value and Kennedy (1999) states that 10-30% of the total reactor volume constitutes a reasonable amount of seed sludge. Wheatley (1990) reports that SBs have been observed to reach 0.5-0.67 of the reactor height while a upflow sludge blanket (USB) reactor, treating dairy wastewater, was inoculated with granular biomass representing 29% of the unit's volume (Samson et al., 1984).

UASB digester start-up times also vary greatly and like for other anaerobic reactor types this critical, sometimes difficult and often time-consuming phase (de Zeeuw and Lettinga, 1980; Droste, 1997b; Garuti et al., 1992; Gunnerson and Stuckey, 1986; Kennedy, 1985 and 1999) is influenced by substrate composition and biodegradability, seeding material nature, quality and quantity as well as operational conditions (i.e., OLRs), bacteria growth rates and reactor retention efficiency (Chynoweth and Isaacson, 1987; Kennedy, 1999; Speece, 1996; Torpy, 1988; van Haandel and Lettinga, 1994). Among the many anaerobic dairy waste treatment investigations surveyed in this review, several successful acclimation periods were referred to in Section 2.2.2.4, where some pertained to UASB reactors of various scales.

Also, Lettinga et al. (1984) report 4-16 weeks as typical start-up durations for USB reactors. Speece (1996) advises that steady state might only be reached after 2-12 months for highly loaded systems ($5\text{-}25\text{ kg/m}^3\cdot\text{d}$) although LR reactors ($1\text{-}5\text{ kg/m}^3\cdot\text{d}$) need less time to adapt. Lettinga et al. (1987) and Torpy (1988) state that approximately 2 months are required to acclimatize digested sewage seed sludge and improve its characteristics as well as for it to be able to sustain OLRs greater than $5\text{ kg COD/m}^3\cdot\text{d}$ (Lettinga et al., 1984). Although, Lettinga and Vinken (1980) state that good biomass activity can be achieved within 6 weeks and that only 2-3 weeks might be required, when at least some adapted sludge is used as seed.

Start-up times can also be reduced, to < 1 month (Torpy, 1988), when employing available anaerobic granular inoculum (Section 2.1.3.3) from operating UASBs (Droste, 1997b; Guiot, 1992; Kennedy, 1999; Torpy, 1988; Wheatley, 1990). Generally, acclimation periods can be shortened with superior quality as well as large amounts of seed (de Zeeuw and Lettinga, 1980; Speece, 1996; van Haandel and Lettinga, 1994) and higher OLRs can quickly be applied (Kennedy, 1999). However, Obayashi and Gorgan (1985) report that there is more influent bypass in SBs with larger volumes of biomass. Lettinga et al. (1987) also mention that the occurrence of channelling increased with greater sludge hold-up. Yet, Lettinga et al. (1984 and 1993) report that this risk is higher with lower reactor SBHs.

Still little information is available in the literature in terms of anaerobic reactor start-up procedures. However, Lettinga et al. (1984) as well as Kennedy (1985) present a few general

recommendations for the development of UASB granular biomass (from digested sewage). They suggest using specific sludge loading rates of 0.05-0.1 kg COD/kg VSS·d and that VFAs should be greatly reduced (by $\geq 80\%$) before applying stepwise increases in OLRs. This strategy promotes balanced microbial population growth. The authors also suggest the use of typical seed sludge amounts ranging from about 10-20 kg VSS/m³ while Wheatley (1990) recommends the lowest value to minimize inoculum adaptation time. Lettinga et al. (1987) suggest employing seed concentrations up to 12-15 g VSS/L, to avoid excessive expansion washout. However, Speece (1996) only reports a fairly low 2-10 g/L requirement for LR anaerobic reactors. He advises that a medium influent strength ($\sim 1\,000$ mg COD/L) should be used for start-up (or $> 2\,000$ mg COD/L in the presence of granules) and that HRTs should be lowered with increasing OLRs.

Fine, dispersed biomass is expected to washout and continues to do so as the SB expands (Kennedy, 1985 and 1999; Lettinga et al., 1984 and 1987; van Haandel and Lettinga, 1994). Lettinga et al. (1987) report that considerable flocculent sludge removal improves bacterial aggregation and discourages voluminous sludge production. Guiot (1992) considers it best to use active inoculum (≥ 0.6 g COD/g VSS·d) and Kennedy (1999) recommends applying relatively high specific COD loads of 0.3-0.5 kg COD/kg VSS·d, early on, to stimulate and enhance granulation (Section 2.1.3.3). However, Lettinga et al. (1987) state that start-up rates depend more on the retention efficiency of produced microbes than on the activity of the initial seed material. During start-up, Torpy (1988) and van Haandel and Lettinga (1994) also suggest minimizing reactor shutdowns and exercising continuous recycling while Lettinga et al. (1993) recommend employing feed interruptions to promote methanogenic biomass growth and increase start-up rates.

Kennedy (1985) reports that continual substrate underloading and/or overloading can be unfavorable. The former can promote the development of dispersed biomass (Guiot, 1992) that can easily be expelled with the effluent while the latter can cause settling problems, due to increased biogas production, as well as reactor souring (van Haandel and Lettinga, 1994). Kennedy (1999) considers the start-up phase complete when VRRs of 3-5 kg COD/m³·d are maintained, for 15-20°C temperatures, while Lettinga et al. (1993) regard it as finished, for 30°C, when stabilized biomass, with at least 0.1 kg CH₄-COD/kg VSS·d methanogenic activity fills the system. van Haandel and Lettinga (1994) consider it done when effluent quality as well as reactor biomass quality and quantity are reasonably constant. However, the latter present the case of 2 full-scale UASBs at a sewage treatment plant, where a faulty GLS separator prohibited steady state from being reached, even after a 16-weeks of operation, during which significant sludge wash-out took place.

2.1.3.3 The Granulation Process and Anaerobic Granular Biomass

Complex mechanisms of aggregation are not entirely understood (Droste, 1997b; Kennedy, 1999). However, this natural and characteristic phenomenon of UASB-type reactors (Guiot, 1992; Wheatley, 1990) is particularly influenced by (first) start-up conditions as well as by numerous physical, chemical and biological factors (Andras et al., 1989; Lettinga et al., 1987; Torpy, 1988), which also benefit anaerobic bacterial growth. Some of these include pH, temperature, nutrient bioavailability (environmental conditions) as well as operational and hydrodynamic conditions, such as solids, hydraulic and biogas loading rates (Guiot, 1992; Bitton, 1994) in addition to the nature, composition and properties of the inoculum and of the substrate (Bitton, 1994; Guiot, 1992; Kennedy, 1999; Lentz, 1996; Lettinga et al., 1987; Rittmann and McCarty, 2001; Wheatley, 1990).

Granules, with differing compositions and properties (Andras et al., 1989), develop with various types of wastewaters and start-up conditions (Kennedy, 1999; Torpy, 1988). Sugar, VFA, ethanol and propionate-based substrates (Guiot, 1992) as well as mainly soluble wastes (Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1987; Speece, 1996) usually induce and/or stimulate granulation. Its rate (as well as that of biomass growth) depends mostly on waste concentration and composition (Kennedy, 1999; Lettinga et al., 1987). It is reported to be greater with dilute soluble (carbohydrate) wastewaters (Bitton, 1994; Chynoweth and Isaacson, 1987). Superior sludge blankets have also been observed with low-strength wastes having < 1-2 g TSS/L (Droste, 1997b) and large, but low density aggregates usually result from carbohydrate-rich substrates (Kennedy, 1999).

The stability of granules can be affected by important changes in conditions (Lettinga et al., 1987), such as shock loads and temperature fluctuations (Kennedy, 1999; Speece, 1996). Aggregates are also relatively sensitive to drastic changes in wastewater nature and composition or to significant differences between the feed being treated and the one the granular inoculum was cultivated on (Kennedy, 1999; Torpy, 1988). This is especially the case for those initially developed on acidified wastes. Such feed substitutions can cause granules to settle poorly and eventually washout after becoming gelatinous or flocculent (Torpy, 1988). In lab or full-scale UASBs, active and good-settling granular sludge does not always maintain its form and properties (particularly those treating wastes containing dispersed solids or refractory compounds) and in some cases, granule formation may simply be inhibited (Guiot, 1992; Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1987; Torpy, 1988). Wheatley (1990) reports that an unsatisfactory UASB reactor sludge blanket developed from a dairy effluent. Torpy (1988) also states that granulation was unsuccessful with a conditioning liquor, regardless of GLSS

improvements, reactor re-seeding and anaerobic contact mode operation, with effluent solids recirculation and settling.

Carbohydrate-fed granules can be unstable under acidified and starved conditions (Guiot, 1992). However, Lettinga et al. (1987) report that un-acidified soluble wastewaters can contribute to granular inoculum deterioration while partially acidified wastes typically do not. In general, granular biomass is maintained when some form of waste pre-acidification is applied, even without complete phase separation (Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1987), but Kennedy (1999) reports that this preliminary step is not required for wastes with low carbohydrate content. Its application is also favorable with partly insoluble wastes as well as to avoid the development of bulking sludge due to filamentous bacteria growth (Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1987). Thus, it is key to observe appropriate start-up procedures and to allow a balanced anaerobic microbial consortium to establish itself or the granular culture to adapt to any new conditions over time (Speece, 1996), especially for one-step systems (Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1987).

Granular sludge formation, composition, activity, settling properties (Kennedy, 1999) and their bioconversion capabilities are significantly affected by the availability of certain trace elements and metals as well as micronutrients (Section 2.1.1) and vitamins in wastewaters (Speece, 1996; Torpy, 1988). In general, polyvalent cations and inorganic materials such as calcium (below 200-300 mg/L) tend to enhance the granulation process (Eckenfelder, 1989; Guiot, 1992; Kennedy, 1999; Lettinga and Vinken, 1980; Lettinga et al., 1980; Torpy, 1988; Wheatley, 1990).

Rittmann and McCarty (2001) give 10% as the approximate mineral or ash fraction of granules. However, Kennedy (1999) and Guiot (1992) report variable and more significant estimates of 19-46% (for industrial wastewaters) and 11-55%, respectively. A low 12-16% range is also reported for synthetically fed lab-scale UASBs because of typically low Ca, Fe and/or FSS containing-feeds (Kennedy, 1999). The latter also states that the ash content of seeding material is usually much greater than that of granular biomass. In general, the FSS are known to be more concentrated at the base of SB reactors (Torpy, 1988; van Haandel and Lettinga, 1994), where it also contains the most inert solid fraction (Kennedy, 1999). Rittmann and McCarty (2001) report 10-20 g/L (1-2%) as common settled sludge concentrations.

Although many types of seeding materials are available (i.e., waste activated sludge (WAS), digested sludge, sewage or manure), granular biomass is the best and the recommended inoculum source for the start-up of UASBs (Guiot, 1992; Kennedy, 1999; Lettinga et al., 1983 and 1987; Wheatley, 1990). However, they can still operate well with non-granular sludges (Kennedy,

1999; Lettinga et al., 1987) or even without being inoculated at all (Barbosa and Sant'Anna, 1989; Lettinga et al., 1993; van Haandel and Lettinga, 1994). Particles may also result from flocculent biomass with various types of wastewaters under ambient, mesophilic and thermophilic temperatures (Kennedy, 1999). However, he reports that granular inocula should retain their structure when treating cheese whey wastes, although granules would not form if non-granular biomass were used. This is the case for most food-processing wastewaters, where aggregates can tolerate and usually adapt to differing substrates (Kennedy, 1999).

Wheatley (1990), report that 50 days are usually needed for ordinary seed to form rapid settling granules. With digested sewage and a VFA substrate mixture, Lettinga et al. (1984) and (1987) state that the granulation process requires about 40 days or 5-8 weeks, respectively. However, Kennedy (1999) reports that aggregate development from non-granular inoculum can take up to 10-12 months, under ambient temperature conditions, and 2-6 months under mesophilic ones. Flocculent or poorly converting granular biomass enriched with even small quantities of active but un-acclimated, poor settling and disintegrated or crushed granular inoculum can enhance aggregation and waste biodegradation, as long as the sludge is retained in a properly operated reactor (Guiot, 1992; Kennedy, 1999; Lettinga et al., 1987). From a non-inoculated and sewage treating-UASB, 1 mm-granules formed within a month, although adaptation was only complete after 4 months of continuous operation, and the granules eventually reached 8 mm (Barbosa and Sant'Anna Jr, 1989).

Normally, methane-forming anaerobic granular biomass is black (Heertjes and van der Meer, 1979; Lentz, 1996), even though Rittmann and McCarty (2001) as well as de Zeeuw and Lettinga (1980) describe it as grey-white. However, acid-forming sludge is white and a shift towards this colour generally indicates a reduction in the methanogen fraction or a predominance of acidogens (Heertjes and van der Meer, 1979). Usually, granular sludge also has a high VSS content (Bitton, 1994) and is highly active (Andras et al., 1989; Kennedy, 1999; Lentz, 1996). Metabolic biomass activities (Section 2.1.3.4), which are influenced by the composition of the feed and the granules as well as the latter's growth conditions (Kennedy, 1999), essentially represent the quantity of viable organisms in the sludge as it is treating a particular substrate (Guiot et al., 1992a). Compared to flocculent-natured sludge, (specific) acetoclastic activities are usually greater in dense granular biomass and large-sized granules (Guiot, 1992; Guiot et al., 1992a; Kennedy, 1999).

The shapes of granules vary but they are usually compact, well defined and nearly spherical (Guiot, 1992 and 1992b; Lentz, 1996; McCarty and Smith, 1986). Most literature sources

report an average diameter range of 1-5 mm (Bitton, 1994; Chevalier, 1996; McCarty and Smith, 1986; Speece, 1996), although Guiot et al. (1992a) report 1-3 mm and Kennedy (1999) reports 0.1-8 mm. The size and distribution of aggregates depend on their composition as well as that of the substrate in addition to the reactor's operating conditions, including biogas and hydraulic loading rates (Guiot et al., 1992a; Kennedy, 1999; Lentz, 1996). Granular flocs usually develop in a 6-7.5 pH range, in the case of lab-scale UASBs (Kennedy, 1999), although slower developing and pH-resistant ones can be grown at pH values as low as 5.8-6 (Lettinga et al., 1987). However, UASBs operate best between 6.7-7.5 (Kennedy, 1999; Lettinga et al., 1987).

The co-existing and closely dependant groups of bacteria (i.e., hydrolytic-fermentative, syntrophic acetogenic, methanogenic and sulfate reducing) that are involved in the anaerobic digestion process (Section 2.1.2) are also contained within UASB reactor granules (Kennedy, 1999). Their microbial composition can be significantly affected by the nature of the inoculum (Lettinga et al., 1984 and 1987) and of the substrate treated (Bitton, 1994; Lentz, 1996), although (acetate-utilising) methanogens typically predominate (Guiot, 1992; Kennedy, 1999). In the case of carbohydrate-rich feeds, the next most common anaerobes are usually of the hydrolytic-fermentative type (Kennedy, 1999). Yet, like *Methanosaeta*, syntrophic bacteria contribute significantly to aggregation as well as to the stability and strength of the granule structure (Bitton, 1994; Guiot, 1992; Kennedy, 1999). During anaerobic wastewater treatment, *Methanosaeta* (or *Methanothrix*) are generally the most prevalent organisms in granular biomass (Droste, 1997b; Kennedy, 1999; Lentz, 1996; Speece, 1996), particularly at mesophilic conditions (Torpy, 1988).

Some wastewater community scientists believe that anaerobic sludge granules have a random microbial composition. However, recent findings based on microscopic observations suggest that their bacteria population has a structured distribution, which can be viewed as 3 concentric zones (Bitton, 1994; Guiot, 1992; Guiot et al., 1992a and 1992b). This natural spatial organization, which results from substrate and product diffusion limitations with granule depth (Guiot, 1992), optimises the cooperation and the nutritional environments of the various anaerobes involved in the metabolic process (Guiot et al., 1992a and 1992b; Speece, 1996).

Rod-shaped (or filamentous) *Methanosaeta* aceticlasts preferentially colonized the nucleus (Bitton, 1994; Guiot, 1992; Guiot et al., 1992a and 1992b; Speece 1996) while rods (H_2 -utilizing and producing (acetogenic) microbes) and cocci make-up most of the mid-layer (Bitton, 1994; Guiot, 1992). Propionate degraders also develop within the latter section (Guiot et al., 1992a). Finally, the heterogeneous periphery usually contains the same bacterial population as the sub-surface, in addition to filamentous (i.e., *Methanococcales* and *Methanospirillum*) and fermentative

organisms, but with a predominance of acidogens and some hydrogenotrophic methanogens (Bitton, 1994; Guiot, 1992; Guiot et al., 1992a and 1992b; Speece, 1996). Coccoid *Methanosarcina* species (or *M. mazei*) and SRB can also be located at the floc surface (Guiot et al., 1992a and 1992b; Speece, 1996). Thus, primary substrate consumers (i.e., fermenters and acidogens) occupy the outskirts of the granule while acetogens and methanogens are contained within the inner layers (Guiot et al., 1992a) to minimise interspecies metabolite transfer distances (Guiot, 1992; Guiot et al., 1992b).

Granules dominated by the *Methanosaeta* genus, which develop under low acetate concentrations, are usually active and offer good treatment efficiency (Barber and Stuckey, 1999; Lettinga et al., 1987; Speece, 1996). The former authors report « 500 mg/L as the acetate limit for this condition while Guiot (1992) suggests < 200 mg/L, Speece (1996) reports < 70 mg/L and van Haandel and Lettinga (1994) suggest < 55 mg/L. But, greater acetic acid availability can be tolerated (Lettinga et al., 1987; Speece, 1996). Wheatley (1990) states that filamentous *Methanothrix soehngenii*-enriched sludge also tends to form when it is exposed to highly soluble substrates. Rod-type *Methanosaeta* develop on high acetate concentrations and are also sustained on little acetate (Guiot, 1992). Smaller *Methanosarcina*-enriched granules grow when exposed to and maintained in high acetate environments (Barber and Stuckey, 1999; Guiot, 1992; Kennedy, 1999; Lettinga et al., 1987; Speece, 1996). They are prone to washout as well as exhibit poor performance under low acetate concentrations, unless low SLRs are applied (Lettinga et al., 1987; Torpy, 1988). However, if the reactor pH is kept above 6.5-7 and the acetic acid strength is maintained below 1 000 mg/L, they usually will not form in UASBs (Lettinga et al., 1987).

2.1.3.4 Reported Operation Conditions

Droste (1997b) reports that the UASB system typically operates at ≤ 1 d HRT, with OLRs between 0.5-40 kg COD/m³·d, but Gunnerson and Stuckey (1986) affirm that this upper load has been reached at HRTs as low as 3.5 h for a mainly soluble potato waste. Other characteristic UASB operation and performance data presented in the literature include the treatment of 5 000-15 000 mg COD/L waste strengths at 4-12 h HRTs and 4-12 kg COD/m³·d OLRs with 75-85% COD removals (Metcalf and Eddy, 1991) as well as 70-90% COD conversions for 2-15 kg COD/m³·d OLRs, under 8-50 h HRTs (Wheatley, 1990). Rittmann and McCarty (2001) state that 4-15 kg COD/m³·d is considered as a common process design LR range while Torpy (1988) reports that OLRs of 5-20 kg COD/m³·d can be reached with full-scale dairy waste treating-UASBs under normal operation. Depending on seasonal temperature conditions COD removals in UASB reactors

usually range from 40-80%, although 80-90% can be reached for degradable food-processing wastewaters under mesophilic conditions (Kennedy, 1999). The latter also reports typical SS removals of 70-90% while the WEF Manual (1991) reports 40-65% as good anaerobic digestion VSS destruction.

Guiot (1992) reports that granular SB SRTs of 100 days or more are not atypical and that some greater than 1 000 days have been encountered. Manjunath et al. (1990) report 200-300 d SRTs while SRTs of > 500 days were reported for a 3.84 L-UASB (8 h HRT), with small-sized granules, treating domestic sewage under a low 0.075 m/h upflow velocity, at 13°C (Elmitwalli et al., 1999). According to Lettinga and Hulshoff Pol (1991), under 20°C conditions, they commonly average 80-120 days for liquefying reactors. Lettinga et al. (1993) report 30-50 d SRTs, for average operational conditions corresponding to a 6 d HRT and a 30-40 g TSS/L_{Reactor} sludge hold up while Ándras et al. (1989) report 15-100 d SRTs for 0.4-4 d HRTs and 20-80 g VSS/L biomass concentrations. Furthermore, Rittmann and McCarty (2001) report 1-2 kg COD/kg VSS·d as a typical specific anaerobic sludge activity range while Torpy (1988) and Kennedy (1999) report 0.5-2 kg COD/kg VSS·d and 0.5-1 kg COD/kg VSS·d, respectively, for full-scale UASB reactors treating complex wastes. Eckenfelder (1989) also reports 1 kg COD/kg VSS·d as being usual for anaerobically treated soluble industrial wastewaters.

Like Metcalf and Eddy (1991), which give 0.6-0.9 m/h as common SB reactor upward velocities, both van Haandel and Lettinga (1994) and Lettinga and Hulshoff Pol (1991) suggest that UASB treatment should also be accomplished at upflow rates < 1 m/h. Although these reactors can endure greater rates, to avoid aggregate degeneration, De Zeew (1988), cited in Guiot et al. (1992a), agrees with this and suggests 0.02 m/h as an adequate initial start-up rate. For mesophilic reactor start-ups, the best initial range was observed to be 0.03-0.04 m/h (Speece, 1996). Lettinga and Hulshoff Pol (1991) give 3 m/h and 1-1.25 m/h as acceptable daily superficial velocities for granular sludge UASB reactors treating soluble and partly soluble wastewaters, respectively. However, Wheatley (1990) reports 1-1.5 m/h as usual gas and liquid velocities, which are similar to the 1-2 m/h range reported in Droste (1997b) and in van Haandel and Lettinga (1994).

These authors affirm that a beneficial expanded granular bed state, resembling that of FB configurations, can be achieved under 6-12 m/h while Guiot et al. (1992a) report a 10 m/h rate. Guiot (1992) suggests that upflow velocities up to 7 m/h are advantageous as far as specific activity and particle size are concerned. He also reports that UASB technology operated under fluidization rates (i.e., 2-6 m/h) can be profitable for granulation as well as for mixing and substrate diffusion, due to less mass transfer resistance (Guiot et al., 1992a). Larger granules also have a tendency to

form at upflow velocities of about 2 m/h, which is considered the minimum rate to fluidize carbohydrate-fed granular beds (Guiot et al., 1992a).

UASB reactor sludge settling velocities as high as 90 m/h (Wheatley, 1990) and 108 m/h (Andras et al., 1989) have been reported. Guiot et al. (1992a) give 2-90 m/h as a typical range while a minimum rate of 10 m/h is recommended to sustain the effectiveness of the process (Wheatley, 1990). Torpy (1988) reports values of 25-54 m/h for a pilot-scale UASB that treats a high-strength cheese factory wastewater, in Wisconsin (USA), and that was seeded with good-quality granular biomass.

2.1.3.5 Dilute Wastewaters Under Low Temperature Conditions

UASB configurations have been used to some extent in treating low organic strength wastewaters at ambient (or greater than ambient) temperature conditions and they are relatively efficient (Kennedy, 1999; Rittmann and McCarty, 2001). Table 2.3 presents a few documented cases conducted at even lower operating temperatures. In general, most of the investigations with regards to these conditions involve the treatment of domestic wastewater/municipal sewage.

Table 2.3: UASB Studies Treating Low-Strength Wastewaters at Sub-Optimal Temperatures

Reference	Type of Waste	Treatment Arrangement	Influent (mg COD/L)	Temperature (°C)	%COD Removal	HRT (h)
Lettinga et al. (1983)	Raw Domestic Sewage	UASB	322-948	8-20	50-85	8-17
Barbosa and Sant'Anna (1989)	Raw Domestic Sewage	UASB	627	18-28	74	4
Vieira and Garcia (1992)	Domestic Sewage	UASB	188-459	18-28	60	5-15
Sayed and Fergala (1995)	Raw Domestic Sewage	Two-Stage UASB	200-700	18-20	75, 84	10, 6
Tang et al. (1995)	Raw Sewage + Brewery Waste	UASB + 2 upflow packed columns	536	20	80	6-72
Elmitwalli et al. (1999)	Raw + Pre-settled Sewage	UASB and Anaerobic Hybrid	339-456	13	60 64	8 8

Although certain reactors are more appropriate for certain types of wastes, other proven anaerobic methods used to treat weak-strength wastewaters (and at sub-mesophilic temperatures) include Anaerobic Contact Processes (Pretorius, 1971; Saw, 1986; Simpson, 1971), Biofilm/Anaerobic (Packed Bed) Filter or DSFF Reactors (Droste et al., 1987; Kobayashi et al., 1983; Metcalf and Eddy, 1991; Saw, 1986; van den Berg and Kennedy (1983), Anaerobic Fluidized Bed (Droste, 1997b; Heertjes and van der Meer, 1979; Maragno and Campos, 1992; Sanz and Fdz-Polanco, 1990; Speece, 1983; van der Last and Lettinga, 1992), Expanded Granular

Sludge Bed (Bitton, 1994; Droste, 1997b; Heertjes and van der Meer, 1979; Lettinga, 1996; Rebac et al., 1999; Speece, 1983; van der Last and Lettinga, 1992), Anaerobic Sequencing Batch Reactors [Ndon and Dague (1994) cited in Speece (1996)] as well as Anaerobic Baffled Reactors (Barber and Stuckey; Langenhoff and Stuckey, 2000).

Although anaerobic treatment is not limited to highly concentrated wastewaters and can be successful under sub-optimal conditions, influent strengths of at least 1 000 mg COD/L have been recommended for it to work well (Droste, 1997b). Yet, Speece (1996) states that the process is not restricted by practical minimum organic (BOD) considerations, but rather by the following fundamental design criteria:

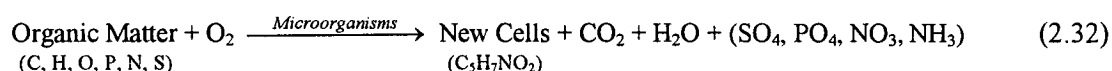
- Organic Loading Rates
- Biodegradability of the Pollutant
- Buffering Capacity or Inherent Alkalinity
- Required Effluent Quality or Treatment Objectives
- Reactor Configuration
- Method of Sludge Immobilization
- Sludge Inventory Concentration and Substrate Consumption Rates
- Influent Wastewater Temperature and Economic Concerns

Since some of the implications and effects of dilute wastes and/or moderate/low temperatures on anaerobic processes are presented throughout the document, but especially in this chapter, they will not be further discussed.

2.1.4 AEROBIC DECOMPOSITION AND THE ACTIVATED SLUDGE PROCESS

2.1.4.1 Aerobic Biodegradation and Microbiology

During oxidative metabolism, aerobic microorganisms essentially assimilate biodegradable organic waste materials and convert them into new bacterial mass as well as various mineral/inorganic end products, in the presence of oxygen (Curi and Eckenfelder, 1976; Droste, 1997b; Speece, 1996; van Haandel and Lettinga, 1994). The degradation and synthesis that take place in aerobic systems (such as in aeration basins of activated sludge processes) can be represented by the following general biological reaction (Bitton, 1994; Masters, 1998; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985):



While anaerobic processes (Speece, 1996) are driven mostly by bacteria, several different types of microbes are involved in aerobic operations and their interactions are less complex (Droste 1997b; van Haandel and Lettinga, 1994). Aerobic microbial populations usually observed in activated sludge flocs include active and prolific bacteria (i.e., *Pseudomonas*, *Zooglea*, *Achromobacter* and *Flavobacterium*), nitrifiers (i.e., *Nitrosomonas* and *Nitrobacter*), protozoans and rotifers as well as yeast and less desirable fungi (Bitton, 1994; Chevalier, 1996; Droste, 1997b; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985). Although ciliated protozoans and rotifers play a less significant role than bacteria and mostly act as aerobic effluent polishers, their presence in the aerobic biological units is a sign of a well-functioning process (Bitton, 1994; Droste, 1997b; Tchobanoglous and Schroeder, 1985). Protozoa, which generally consume non-flocculated bacteria, grow best at high DO but low soluble organic matter concentrations (Droste, 1997b) and nitrifying bacteria metabolize inorganic nitrogen compounds (Chevalier, 1996).

2.1.4.2 The Activated Sludge Process

This suspended-growth aerobic system is the most widely used secondary or biological method for municipal wastewater treatment (Bitton, 1994; Metcalf and Eddy, 1991; Tang et al., 1995). Although Arden and Lockett developed its original batch process form in England, in 1914 (Chevalier, 1996; Droste, 1997b; Metcalf and Eddy, 1991), many activated sludge process variations exist nowadays (Bitton, 1994; Droste, 1997b; Eckenfelder 1989; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985).

In a typical continuous flow and CM configuration (Figure 2.3), some insoluble waste compounds as well as non-settleable colloidal and dissolved organics are metabolized by microorganisms in the aeration basin (AB) and transformed into settleable (gelatinous) microbial flocs (Chevalier, 1996), which are subsequently separated and removed from the clarified wastewater in a gravity settling tank (Curi and Eckenfelder, 1976; Droste, 1997b; Eckenfelder, 1989; Pipes, 1977; Speece, 1996; Tang et al., 1995). Flocculated sludge quality is established by aerobic reactor conditions, but the overall effluent quality depends largely on the extent of clarification in the sedimentation basin (Pipes, 1977). Although biological waste stabilization (or substrate uptake) is assumed not to take place in this unit (Chynoweth and Isaacson, 1987; Droste, 1997b; Metcalf and Eddy, 1991), the sludge does stay active and much entrapped nitrogen gas can cause some of it to rise and washout, especially at temperature exceeding 20°C (Droste, 1997b; Metcalf and Eddy, 1991).

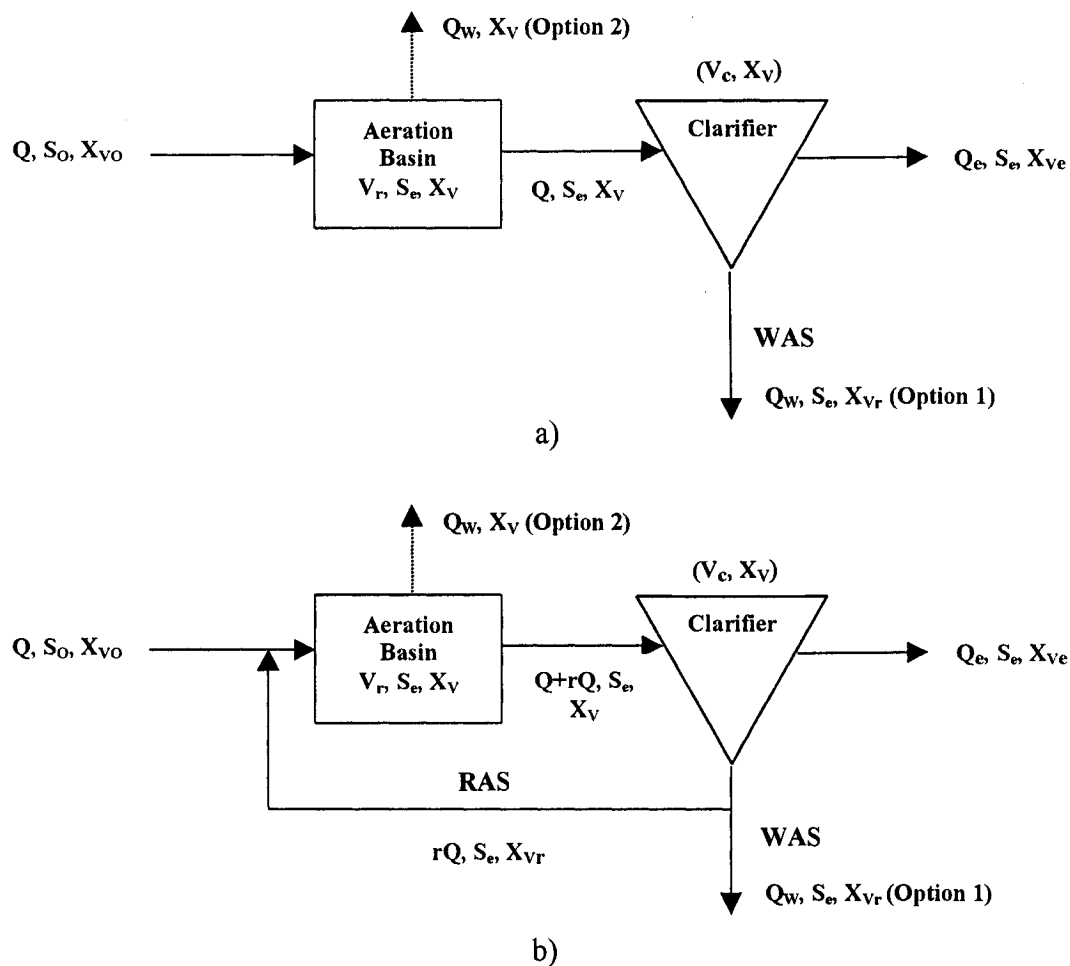


Figure 2.3: Completely-Mixed Activated Sludge Process a) Without Sludge Recycle and b) With Sludge Recycle

It is referred to as aerobic activated sludge since the microbial mass involved is *activated* and kept in suspension by a constant (or semi-continuous) input of air (or oxygen). It is most commonly supplied by diffusers at the bottom of the AB (i.e., compressed air) or via mechanical aeration means (Bitton, 1994; Chevalier, 1996; Droste, 1997b; Eckenfelder, 1989; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985). This provides intensive agitation and good bacteria-wastewater contact, under aerobic conditions. The mixed liquor (ML) refers to the entire AB contents while the activated sludge or mixed liquor suspended solids (MLSS) encompass all the flocculent biological matter (organic and bacterial) contained in the liquid wastewater (Bitton, 1994; Chevalier, 1996; Droste, 1997b; Metcalf and Eddy, 1991). Homogeneous aeration, MLSS and OLR conditions are sustained throughout CM aerobic bioreactors (Metcalf and Eddy, 1991).

Some settled sludge, withdrawn from the clarifier underflow and referred to as return activated sludge (RAS), is usually recirculated back to the aerobic reactor and mixed with the incoming wastewater [Figure 2.3 b)] to maintain microorganism supply and MLSS concentration levels (Bitton, 1994; Metcalf and Eddy, 1991). Surplus solids produced by the process are also usually discarded as waste activated sludge (WAS) and/or are further processed and stabilized (i.e., by anaerobic digestion) prior to ultimate disposal (Bitton, 1994; Droste, 1997b; Metcalf and Eddy, 1991). Sludge recycling allows for the microbial RT to be independent from the HRT (Chevalier, 1996; Tchobanoglous and Schroeder, 1985), where SRTs are generally controlled by sludge wastage flow rates (Q_w).

More energy is extracted from the oxidation of organic substrates during aerobic metabolism compared to anaerobic metabolism. As a result, aerobic systems have higher synthesis capabilities and generate greater amounts of nutrient-rich sludge (Metcalf and Eddy, 1991). Their aeration requirements, which constitute a major operating cost, make these processes very energy-intensive, especially for relatively strong wastes (Droste, 1997a and b; Tchobanoglous and Schroeder, 1985). Although toxic and shock loads can be tolerated (Bitton, 1994; Metcalf and Eddy, 1991), dilute wastewaters (i.e. sewage) are easily treatable in the activated sludge process (Wheatley, 1990).

Aeration or aerobic processes of this type are often used for odor control and may be employed to polish the effluents of other treatment methods (Droste, 1997b). In this case, the mass of excess sludge produced should be significantly reduced and quite low, unless the performance of the pre-treatment unit is poor (Lettinga and Hulshoff Pol, 1991). Other than biodegradable TSS and organic pollutants, phosphorus is usually only partially removed (Droste, 1997b) in the AB, although modified versions of the activated sludge process can be used to provide tertiary treatment (Section 2.2.1.3).

2.1.4.3 Some Important Process Operating Parameters

Since they are constrained by oxygen transfer and clarifier operation, activated sludge processes are usually designed for low OLRs, with relatively low AB biomass concentrations that are about 5-10 times smaller than those of anaerobic processes (Speece, 1996). He reports that feasible aerobic system OLRs commonly vary between 0.5-3.2 g/L·d, although typical values usually range from 0.5-1 g/L·d, which are over 10 times less than anaerobic systems. Table 2.4 presents other reported activated sludge process OLRs and operating parameter values, including suggested AB MLSS concentrations. Metcalf and Eddy (1991) also suggest that CM systems typically result in 85-95% BOD removals.

Table 2.4: Typical Air-Activated Sludge Process Operational Parameters

HRT (h)	OLR (g BOD ₅ /L·d)	SRT (d)	F/M or SOLR (g BOD ₅ /g MLVSS·d)	MLSS (mg TSS/L)	r (Q ₁ /Q)	Reference
3 - 5 3 - 8	0.8 - 1.9 < 2 ^A	5 - 15 4 - 10	0.2 - 0.6	2 500 - 6 500 1 500 - 3 500 700 - 5 000 ^B	0.25 - 1	1. CM 2. CFST 3.
18 - 36 24 - 48	0.2 - 0.4	20 - 30	0.05 - 0.15	1 500 - 5 000	0.5 - 1.5	1. Ext. Aeration 4. Ext. Aeration

1. Metcalf and Eddy (1991); 2. Tchobanoglous and Schroeder (1985); 3. Droste (1997b); 4. Chevalier (1996)

^A g COD/L·d, ^B mg VSS/L, Ext. Aeration: extended aeration configurations

While the process can typically function between 500-5 000 mg MLSS/L, Grady et al. (1999) report that it is the mass rather than the concentration of MLSS that controls the efficiency of the process. Operation under the suggested minimum range of 500-1 000 mg MLSS/L can result in non-flocculent and poor settling sludge as well as poor overall treatment. Although MLVSS/MLTSS ratios depend on wastewater properties and reactor operating conditions, Bitton (1994) reports a typical value of 0.65-0.75 while Droste (1997b) and Tchobanoglous and Schroeder (1985) report that aerobic biomass is typically composed of 0.75-0.8 and 0.7-0.9 MLVSS/MLTSS, respectively. MLVSS, which represent the organic portion of MLSS, are often used as bacteria concentration estimates (Bitton, 1994; Droste, 1997b; Tchobanoglous and Schroeder, 1985).

Food/microorganism ratios (F/M), which characterize microbial food availability, are less for aerobic processes than for anaerobic processes since the former have much smaller K_s values or a much higher affinity for a given wastewater (Speece, 1996). The latter author reports a common range of 0.1-0.5 g BOD/g VSS·d for aerobic systems. High F/M ratios, which can result from insufficient RAS, can lead to floc dispersion while low F/Ms (associated with high MLSS) indicate potential microorganism undernourishment. At low substrate concentrations (highly starved conditions) and low bacterial growth rates, effluents can become turbid and contain small difficult to settle pinpoint flocs (Droste, 1997b; Tchobanoglous and Schroeder, 1985). Although successful biological treatment systems require a balance between bacteria quantities and substrate OLRs, settling and organic matter removals are generally better under starved conditions or when the biomass is in a slight endogenous decay state (Bitton, 1994; Droste, 1997b). As for SRTs, F/M ratios are usually controlled by the wastage rate of WAS (Bitton, 1994).

Aerobic processes usually operate in relatively low DO environments (Droste, 1997b) since excessive aeration can airstrip sulfide rather than promote biological degradation (Page et al.,

1999). Bioreactor performance and DO requirements depend mostly on the system's specific oxygen uptake and applied loading (Grady et al., 1999; Metcalf and Eddy, 1991). Most of the literature states that, under normal loading conditions, the air flow rates injected in AB's must be adjusted to maintain DO concentrations of at least 2 mg O₂/L (Chevalier, 1996; Kettunen et al., 1996; Metcalf and Eddy, 1991; Page et al., 1999), especially to avoid filamentous bacteria growth (Bitton, 1994). However, this value might be excessive or insufficient in some cases (Grady et al., 1999). Hoover and Porges (1952) report that oxygen supply rate difficulties have been encountered during the aerobic treatment of dairy wastes. To prevent oxygen diffusion limitations, Droste (1997b) reports that 0.2-2 mg O₂/L concentrations are typical and increased oxygenation, by pure oxygen addition as opposed to air, can be used to yield adequate aerobic conditions, at higher cost. Although expensive extended aeration systems require a high DO content, high SRTs and HRTs as well as operate at lower F/M ratios and OLRs, they are usually very efficient and minimize sludge wasting (Bitton, 1994; Chevalier, 1996; Droste, 1997b; Eckenfelder, 1989).

The adjustment of pH is usually not required for aerobic processes (Droste, 1997b; Eckenfelder, 1989), although relatively uncommon filamentous fungi growth is favored at low pHs (Bitton, 1994; Grady et al., 1999). However, Droste (1997b) suggests values of 7-8 as the best aerobic pH range while Bitton (1994) reports an optimal range of 7-7.5. Aerobic systems are normally operated at ambient temperature since it is more economical, but this condition is not necessarily the most favorable for aerobes (Droste, 1997b). Reductions in AB operation temperatures tend to decrease the treatment efficiencies of soluble organics and increase effluent SS. For typical activated sludge systems, mesophilic operation temperatures would be acceptable (Grady et al., 1999). Although trace amounts of iron can benefit aerobic processes (Speece, 1996), their addition has also been observed to deteriorate settling and cause filamentous growth in phosphorus-limited conditions (Droste, 1997b).

2.1.4.4 Common Operational Problems Associated with Activated Sludge Treatment Processes

The biggest concern in activated sludge processes is the overabundance of filamentous microorganisms in the ML, which may cause bulking sludge (Chevalier, 1996; Droste, 1997b; Tchobanoglous and Schroeder, 1985). Filamentous bulking is observed when fluffy or voluminous flocs, which do not settle well and yield a high sludge volume index (SVI), develop under adverse conditions (Bitton, 1994). It can cause important settling problems and poor quality effluents (Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985).

Slow-growing filamentous microorganisms, which have lower K_s and $\mu_{\max}$ values than floc-forming bacteria (Grady et al., 1999), have been observed to proliferate at low DO and low organic substrate concentrations (i.e., low F/M or low OLR) as well as under low pH, low RAS (or low SRT) and nutrient deficient (i.e., N, P, Fe) conditions (Bitton, 1994; Chevalier, 1996; Droste, 1997b; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985). Readily degradable wastewaters from the food processing industry (Eckenfelder, 1989) and those high in carbohydrates also stimulate their growth and cause poor settling sludge (Bitton, 1994), especially when treated at low concentrations in CM systems (Chevalier, 1996; Droste, 1997b; Metcalf and Eddy, 1991).

Depending on the cause, some typical bulking control measures include the adjustment of F/M ratios and DO concentrations, the application of disinfectants (chlorine or other biocides) and flocculants, the addition of nutrients, an increase in sludge wastage and the use of small CM selectors with short contact times placed prior to the aeration unit (Bitton, 1994; Droste, 1997b; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985). To maintain a balance between floc-forming and filamentous bacteria, Grady et al. (1999) suggest the use of 3-15 d SRTs and cell retention times are often at least an order of magnitude greater than HRTs (Droste, 1997b). However, after the application of remedial means, process recovery can take some time (Droste, 1997b).

Sludge foaming (or scum formation) is another problem commonly associated with the process (Droste, 1997b; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985). Among other things, it can result in odors as well as in the deterioration of BOD and SS effluent levels (Bitton, 1994; Pipes, 1977). Some potential reasons for scum formation include low F/Ms, insufficient sludge wastage, accumulation of MLSS and high SRTs (Droste, 1997b). This phenomenon can be more frequent in warm and high fat-containing wastewaters and is usually due to *Nocardia* organisms (Bitton, 1994; Droste, 1997b). Since many of the same problem-causing bacteria are responsible for sludge bulking as well as foaming, many similar control measures apply (Droste, 1997b). Although difficult to overcome, foaming is most commonly controlled by reducing the SRT, physically removing the foam or chlorinating the RAS line (Bitton, 1994; Droste, 1997b).

Viscous or non-filamentous sludge bulking (also known as “zooglear bulking”), which causes reduced sludge settling, can also occur on occasion when microbes produce excessive exopolysaccharides or slime (Bitton, 1994). It can result from overaeration or inadequate loading rates (Metcalf and Eddy, 1991) and has been observed in oleic acid-rich substrates (Droste, 1997b).

2.1.4.5 Kinetic Constants

Since considerably more energy is available for bacteria during aerobic treatment processes (Section 2.1.4.2), the excess biomass produced is usually 3-20 times more than that of anaerobic systems for the same substrate (Bitton, 1994; Droste, 1997b; Speece 1996). Only about 4-10% of influent organic matter (as COD) is converted to new microbial cells during the latter process (Parkin and Owen, 1986) because most of the energy is found in the final CH_4 product (Chevalier, 1996). However, under aerobic conditions, approximately 50% of degradable organic carbon or COD is typically transformed into biomass (Bitton, 1994; Curi and Eckenfelder, 1976; Speece, 1996; Tchobanoglous and Schroeder, 1985).

Chevalier (1996) reports a typical Y value range of 0.4-0.8 mg VSS/mg COD for aerobic systems and 0.08-0.2 mg VSS/mg COD for anaerobic applications while Droste (1997b) suggests representative Y and k_e coefficients of 0.5 mg VSS/mg COD and 0.06 d^{-1} as well as 0.15 mg VSS/mg COD and 0.03 d^{-1} for aerobic and anaerobic processes, respectively. Kennedy (1999) suggests 10-15 mg/L as a common K_s range for the former systems and Speece (1996) suggests typical k and K_s values of 8 and 6.6 d^{-1} as well as 22 and 45 mg/L, for 20 and 10°C , respectively. Table 2.5 presents typical activated sludge process kinetic values.

Table 2.5: Typical Activated Sludge Process Kinetic Coefficients

K_s (mg COD/L)	k (g BOD_5 /g VSS·d)	Y (mg VSS/mg BOD_5)	k_e (d^{-1})	Reference
40 (15 - 70)	5 (2 - 10)	0.6 (0.4 - 0.8)	0.06 (0.025- 0.075)	1. (sewage at 20°C)
10 - 40 ^B	2 - 4	0.3 - 0.65	0.04 - 0.06	2. (CFST, 13-18°C)
		0.4 (0.24-0.4) ^A	0.06 (0.004 - 0.1)	3.
100 ^B	5.1	0.48	0.045	3a. (skim milk)
10 - 180	4.3 - 28.7 ^C	0.46 - 0.69 ^A		3b. (sewage at 20°C)

1. Metcalf and Eddy (1991); 2. Tchobanoglous and Schroeder (1985); 3. Droste (1997b); a. Lawrence and McCarty (1970), b. Henze et al. (1987); ^A g VSS/g COD; ^B mg BOD_5 /L; ^C g COD/g VSS·d; Values in parentheses are ranges.

2.1.5 MERITS AND LIMITS OF ANAEROBIC OVER AEROBIC PROCESSES

The following sub-sections summarize some of the main anaerobic process advantages compared to conventional aerobic methods of the activated sludge-type as well as some of its most common drawbacks cited in the literature (Bitton, 1994; Curi and Eckenfelder, 1976; Droste, 1997b; Grady and Lim, 1980; Hovious, 1972; Kennedy, 1985; Lettinga, 1996; Lettinga et al., 1980, 1984 and 1993; Lettinga and Vinken, 1980; McCarty, 1964; Obayashi and Gorgan, 1985; Speece, 1996; WEF Manual, 1991; Zitomer and Speece, 1993) and Table 2.6 presents some key features of both biotechnologies.

Table 2.6: Anaerobic vs. Aerobic Process Characteristics

	Anaerobic	Aerobic
Net excess microbial cell production:	20-150 kg	400-600 kg
Electrical power (requirements):	-	500-2 000 kWh
Methane gas production:	12 x 10 ⁶ BTU	-

[Based on 1 ton of COD destroyed; adapted from Speece (1983 and 1996)]

2.1.5.1 Benefits

Among the many well-known advantages of biological anaerobic wastewater treatment, the most relevant include reduced energy needs and less biomass production. The latter results from low cellular yields, which requires less nutrient demand (Eckenfelder, 1989; Grady and Lim, 1980) and minimizes difficult to dispose of excess sludge. Anaerobic sludges, which have good dewatering and thickening characteristics, can be used for agricultural purposes (i.e., fertilizer or soil conditioners), while treated sewage effluents are suitable for irrigation (Elmitwalli et al., 1999; Lettinga and Vinken, 1980; Lettinga et al., 1987). Although their nutrient content is similar to aerobic sludges (Droste, 1997b), they are inoffensive and less odorous products.

This type of process provides much stabilization, from concentrated industrial to dilute wastewaters and under favorable conditions it offers better organic pollutant removals at higher organic load rates. In some cases, anaerobic digestion may biodegrade conventional aerobic treatment non-biodegradables and can successfully process some quite recalcitrant toxic compounds (Speece, 1996).

The method can be applied practically anywhere from very small to very large scales (Lettinga, 1996; Lettinga et al., 1980, 1987 and 1993). Thus, modern HR anaerobic systems require relatively less space, reducing land demand as well as associated costs. Treatment rates are also not limited by oxygen transfer between gas and liquid phases (Perle et al., 1995; Speece, 1996). Since aeration equipment and oxygen supply are not required, the process also presents major energy consumption and operating cost savings (Lentz, 1996) that are further enhanced by its considerable methane gas generation and heat recovery potential.

Anaerobic sludge can be stored and withstand prolonged starvation periods without significant adverse effects on activity or other sludge properties. Lettinga and Vinken (1980) suggest that it is best kept below 15°C while Kennedy (1999) recommends storing granular biomass under ambient temperature conditions of 15-25°C. This biomass viability maintenance trait is due to drastically reduced endogenous decay during starvation (Kennedy, 1985; Speece, 1996). Active anaerobic organisms can also recover relatively quickly from upsets and following

re-starts, after long reactor downtime or dormancy periods (Droste, 1997b; Kennedy, 1985 and 1999). Seasonal treatment can thus also be realized with this process. In principle, almost complete treatment can be accomplished by combining it (Section 2.2.1) with an aerobic process (Droste, 1997b; Hovious, 1972; Lettinga, 1993).

2.1.5.2 Downfalls

Anaerobic treatment alone is rarely sufficient to achieve secondary discharge limits and is essentially considered a pre-treatment step (Filho et al., 1996; Vuoriranta et al., 1987). Its effluent normally requires further polishing, often in the form of aerobic post-treatment, to remove residual biodegradable organic matter, suspended solids, ammonia and nutrients (van Haandel and Lettinga, 1994).

The process, which requires much operational monitoring (Droste, 1997b), has been regarded as unstable and subject to occasional upset or failure, although its sensitivity has been attributed to the complex anaerobe interactions involved. It may also be more vulnerable at low ambient temperatures and to certain chemical substances (Lettinga et al., 1980 and 1993). Acetogens and particularly methanogens are susceptible to inhibition by several xenobiotic compounds (Lettinga et al., 1984; Lettinga, 1996) although some organic contaminants (i.e. aromatics) are resistant to anaerobic degradation (Zitomer and Speece, 1995).

The process is also slower than aerobic digestion because anaerobic kinetic growth rates (i.e., microbial doubling times) are low (mainly those of methane producing bacteria at sub-optimal mesophilic temperatures). These very slow rates can limit the adaptability of the process to environmental condition changes and consequently, digester souring or acidification occasionally takes place. Even at elevated temperatures, the process generally requires a longer SRT and HRT which entailed much larger reactor volumes prior to the development of advanced anaerobic configurations. When active seed sludge from an existing installation is not available, several weeks can be required for the initial process start-up in order to accumulate sufficient biomass inventory. The beneficial low synthesis rates can also be seen as disadvantageous in the event of toxic shock loadings and large biomass losses (Wheatley, 1990).

Since the process produces flammable and explosive methane gas, it requires proper safety countermeasures. Insufficient methane productions commonly result from dilute or low COD wastewaters and external heating source are often needed since optimal operation is known to require relatively high temperatures (Eckenfelder, 1989). Nitrification is also not realizable while odor generation can result from the nature of the process (Lettinga and Hulshoff Pol, 1991; Tang et

al., 1995). Finally, although they are becoming more common, there are still relatively few full-scale applications and they call for high initial capital investment costs (Kasapgil et al., 1994).

For high-strength wastes amenable to anaerobic treatment, the considerable benefits associated with the method generally compensate for the few minor drawbacks while for less concentrated wastes, the latter may prevail and limit process applicability, except for relatively warm dilute wastes (Curi and Eckenfelder, 1976; McCarty, 1964; Speece, 1996). In spite of its merits, Speece (1996) cautions that anaerobic treatment may not be the preferred method for: carbohydrate or low-strength wastewaters lacking inherent alkalinity generation potential, low temperature conditions, dilute wastes or for effluent quality to have to attain final organic matter emission standards. This author also suggests that methanogenic biodegradation is not necessarily the appropriate choice for small treatment facilities with low pollutant loads.

2.2 LITERATURE REVIEW

The following sub-sections summarize a number of anaerobic-aerobic treatment applications and highlight their superior performance potential for wastewater purification. However, Zitomer and Speece (1993) give an insightful and critical review of sequential environments for aqueous contaminants.

2.2.1 ANAEROBIC-AEROBIC WASTEWATER TREATMENT SYSTEMS

The overall outcome of multistage wastewater treatment systems depends on the performance of the various unit operations and/or processes that make them up and particularly on the effectiveness of the head systems of the treatment train (Droste, 1997b; Vuoriranta et al., 1987).

2.2.1.1 Advantages of Sequential Treatment Processes

Anaerobic-aerobic treatment sequences offer the same attractive advantages as purely anaerobic processes (Section 2.1.5) in addition to other cost-efficiency related benefits (Cocci et al., 1990a and 1990b; Huster and Möbius, 1990; Landine et al., 1987; Page et al., 1999). Aerobic post-treatment processes typically serve to remove residual SS and organic compounds from anaerobic pre-treatment stages, although they also have some pathogen and macronutrient reduction potential (de Sousa and Foresti, 1996; van Haandel and Lettinga, 1994). Therefore, aerobically treated anaerobic effluents demonstrate better overall quality, are less odorous (Lettinga et al., 1983) and tend to reduce pre-treatment effluent variability (Bonkoski et al., 1983). In the

case of pulp and paper wastewaters, both Vuoriranta et al. (1987) and Zitomer and Speece (1993) state that when the treatment efficiency of the anaerobic stage was temporarily impaired, the remaining organic load was adequately dealt with in the second aerobic stage, yielding stable effluent quality.

When excess aerobic sludge is recirculated from an activated sludge step to an anaerobic stage for digestion it can eliminate the necessity for a subsequent stabilization system and increase the production of biogas (Bonkoski et al., 1983). Theoretically, methane recovery and utilization potentials could also make the configuration self-sufficient, especially in areas with warm climates (van Haandel and Lettinga, 1994). Sludge return practices of such systems can also decrease the need for and thus reduce the cost of chemical supplements (for industrial wastes in particular) since the nutrient requirements of aerobic biomass (Section 2.1.4) are greater than those of its anaerobic counterpart (Section 2.1.2).

Because substantial treatment of undesirable suspended and dissolved organic compounds can be achieved in a preceding anaerobic stage, significantly less sludge generation results from a subsequent activated sludge system (Kettunen et al., 1996; Vuoriranta, 1987) as well as from the entire system, compared to most strict aerobic (Castillo et al., 1997) or even anaerobic processes (de Sousa and Foresti, 1996). Consequently, combined anaerobic-aerobic treatment processes with surplus aerobic sludge recycling can be realized in reactors representing less than half of the volume required for aerobic systems alone, including a thickener and post-digester (van Haandel and Lettinga, 1994). Thus, dual systems reduce sludge processing and waste disposal expenses as well as the energy consumption required for aeration (Kettunen et al., 1996; Tanaka et al., 1991). Therefore, this results in considerable construction and operational cost savings.

In addition to influencing the rate and extent of biodegradation in wastewater treatment (Kennedy, 1999; Wang et al., 1997), beneficial anaerobic (and anoxic) suspended-growth selectors, used more and more frequently upstream of activated sludge aerobic tanks (Section 2.2.1.3), have been reported to provide better aerobic sludge settling properties (Speece, 1996; Vuoriranta et al., 1987; Zitomer and Speece, 1993), resulting in better control of filamentous bulking (Kennedy, 1999; Tracy and Hong, 1990), even at sewage plants with problematic flocculation tendencies (Huster and Möbius, 1990; Tanaka et al., 1991).

Even though there is still limited research experience pertaining to combined anaerobic-aerobic treatment strategies (Zitomer and Speece, 1993), this biotechnology concept has been proven to be particularly effective in enhancing final effluent quality and reducing sludge production as well as operational treatment costs (Section 2.2.1.5). In this view, Tracy and Hong

(1990) as well as van Haandel and Lettinga (1994) state that it is an oversight not to consider anaerobic pre-treatment, especially for sewage treatment in hot climate regions.

2.2.1.2 Some Applications

Effluent polishing of anaerobically treated wastewaters can include biological, physical and chemical means or a combination of these (van Haandel and Lettinga, 1994); many of which can be successfully applied to UASB effluents. In some of the literature surveyed, anaerobic-aerobic biotreatment schemes such as UASB+Extended Aeration Act. Sludge with selector (Çiftçi and Öztürk, 1995), UASB+Act. Sludge (Kettunen et al., 1996; Vuoriranta et al., 1987), UASB+RBC (Castillo et al., 1997), UASB+ASBR (de Sousa and Foresti, 1996), UASB+Stabilization Ponds (Van der Steen et al., 1999; van Haandel and Lettinga, 1994), UASB+Lagoon (Lettinga et al., 1993), UASB+AFB (Collivignarelli et al., 1990), AnF and UASB+Act. Sludge and RBC (Mennerich, 1987), An-A Act. Sludge Process (Tanaka et al., 1991), AnCont.+Act. Sludge (*ANAMET*[™]: Bonkonski, 1983; Liu et al., 1996a and b) and AnSBR+ASBR (Bernet et al., 2000) have been used to treat various types of wastewaters, at bench-scale, pilot-scale and full-scale levels.

Including dairy wastes (Table 2.9), some other types of wastewaters treated by anaerobic-aerobic sequences include food-processing industry (*ANAMET*[™]: Bonkonski, 1983; Oleszkiewicz and Hutchison, 1990), confectionary (Cocci et al., 1990a), fermentation industry (Çiftçi and Öztürk, 1995), petrochemical (Hovious, 1972), landfill leachate (Kettunen et al., 1996; Mennerich, 1987; Thirumurthi et al., 1986), domestic sewage (Castillo et al., 1997; Collivignarelli et al., 1990; de Sousa and Foresti, 1996; *ANANOX*[®] (*ANAerobic-ANoxic-OXic*): Garuti et al., 1992; Lettinga et al., 1993; Tanaka et al., 1991), tannery (Reemtsma and Jekel, 1997), piggery (Bernet et al., 2000), pulp and paper (Ferguson, 1994; Huster and Möbius, 1990; Liu et al., 1996a and b; Vuoriranta et al., 1987; Wang et al., 1997) as well as high-strength and industrial wastes (Abeling and Seyfried, 1992; *ANAMET*[™]: Bonkonski, 1983; Fox and Venkatasubbiah, 1996; Haberl et al., 1999; Ulrich, 1994).

UASB-Upflow Anaerobic Sludge Blanket, An-Anaerobic, A-Aerobic, Act. Sludge-Activated Sludge Process, RBC-Rotating Biological Contactor, SBR-Sequencing Batch Reactor, AFB-Aerobic Fixed-Bed, AnF-Anaerobic Filter, AnCont.-Anaerobic Contact

2.2.1.3 Enhanced Biological Nutrient Removal

Although *not addressed in this research project*, it is known that an anaerobic phase followed by an aerobic (i.e., oxic) one induces enhanced biological phosphorus removal (Carucci et al., 1995; de Sousa and Foresti, 1996; Droste, 1997b; Tanaka et al., 1991), while anoxic conditions succeeding aerobic ones favor nitrogen removal (Abeling and Seyfried, 1992; Bernet et al., 2000; Garuti et al., 1992; Tchobanoglous and Schroeder, 1985; Tracy and Hong, 1990; Ulrix, 1994) in modified activated sludge processes. Some phosphorus-removing proprietary systems include the *Phostrip*[™], the *A/O*[™] and the *Bardenpho*[™] process. The latter is also used for nitrogen removal (i.e., *5-stage Bardenpho*[™]), where nitrified effluent is recycled to an intermediate anoxic stage (oxygen absence and nitrate or nitrite presence) near the head of the treatment process, while the *A/O*[™] process (Garuti et al., 1992; Tracy and Hong, 1990) directly applies anaerobic/aerobic sequences that create a favorable environment for polyphosphate storage.

Many other enhanced pollutant removal schemes (i.e., *UCT*[™], *A²/O*[™], *VIP*[™]) and alternatives exist. They usually involve series of basins that apply synchronous anaerobic-anoxic-aerobic phases of operation, with return sludge streams, that favor the metabolism and growth of organisms responsible for combined nutrient removal (Tracy and Hong, 1990). Analogous organic (i.e., BOD), N and/or P removal can now be performed in single-sludge (or “*single-stage*”) systems (Metcalf and Eddy, 1991; Zitomer and Speece, 1993) that exploit these sequential environments or by cyclic sequencing batch reactor (SBR) biotreatment (Bernet et al., 2000), even within a single bioreactor (Carucci et al., 1995).

Biological transformation reactions, treatment process configurations and biomechanisms involved in release-luxury uptake or dephosphatation and nitrification-denitrification, for phosphorus and nitrogen control, respectively, are extensively described elsewhere (Chevalier et al., 1996; Droste, 1997b; Metcalf and Eddy, 1991; Tchobanoglous and Schroeder, 1985).

2.2.1.4 Refractory Compounds and Toxicants

Although *outside the context of the present study*, the literature also reports that separate anaerobic and aerobic biodegradation environments in sequence or coexisting within a sole unit during successive periods (Field et al., 1995; Shen et al., 1995; Zitomer and Speece, 1993), are sometimes the most successful treatment options for wastewaters containing certain recalcitrant

A/O- Anaerobic-oxic, UCT-University of Cape Town, A²/O-Modified anaerobic-oxic process (with anoxic stage), VIP-Virginia Initiative Plant

and/or toxic organic compounds such as xenobiotic aromatics and azo dyes (Ferguson, 1994; Field et al., 1995; Kennedy, 1999; Metcalf and Eddy, 1991; Speece, 1996; Zitomer and Speece, 1993). These substances often persist within single conventional biological processes and usually require reductive and oxidative treatment steps or detoxification (Barber and Stuckey, 1999). Speece (1996) states that wastewaters from milk and ice-cream manufacturing, with elevated fat contents, have demonstrated some substrate toxicity potential.

A few studies have confirmed that anaerobic zones, containing methanogens, can even develop and be sustained in aerobic microenvironments such as biofilms and sludge granules since some anaerobes can tolerate some oxygen exposure, especially when it is not excessive (Field et al., 1995; Barber and Stuckey, 1999; Shen et al., 1995). While satisfactory treatment can be achieved with one-step or single unit treatment concepts, toxicity and inhibition problems have led to the development of phase separation or reactor staging (Bitton, 1994; Droste, 1997b; Haberl et al., 1990; Kennedy, 1999; Lettinga, 1996; Lettinga et al., 1987), which provide process efficiency and stability improvements, mainly with separate solids hydrolysis and biological aerobic polishing (Lettinga et al., 1993; Speece, 1996; Wheatley, 1990).

2.2.1.5 Some Research Findings

As listed and mentioned previously, several anaerobic-aerobic sequences have been investigated around the world and used in a number of wastewater treatment applications, at various scales and for various purposes. Some pertaining explicitly to dairy wastewaters are presented in Section 2.2.2.4. These types of hybrid systems were originally used in the food-processing sector, but they are now being extensively applied to wastewaters containing complex and toxic substances such as those from the pulp and paper industry (i.e., highly chlorinated compounds), including its bleaching processes (Ferguson, 1994; Wang et al., 1997; Zitomer and Speece, 1993).

Bonkoski et al. (1983) report that this practice, of using the best of both biomechanisms, has been successfully demonstrated on several pulp mill substrates. In a comparative 2 year bleaching wastewater treatment study by Ferguson (1994), a 20% higher AOX (adsorbable organic halide) removal (a biological treatment effectiveness measure often used when wastewater toxicants are involved) was observed when applying anaerobic pre-treatment (i.e., either UASB or FB/EGSB units) to aerobic biological reactors simulating activated sludge treatment, under sequencing batch mode, compared to operating the aerobic step alone.

Zitomer and Speece (1993) have reported that the overall sludge production from an anaerobic-aerobic sequence treating white water from thermochemical pulp processing was approximately $\frac{1}{3}$ of that generated from a conventional activated sludge process. With the help of an ASCAM model simulation, Garuti et al. (1992) state that the *ANANOX*[®] system, consisting of a 3-chamber ABR with an anoxic selector followed by a suspended-growth aeration tank with settler, produces about 50% less sludge than customary nitrification and denitrification processes. Overall, the anaerobic-anoxic-oxic municipal wastewater treatment process proved to be efficient with 89.6% COD and 89.2% TSS reductions as well as demonstrating about 82% total nitrogen removal.

In an investigation by Bonkonski et al. (1983), a full-scale proprietary *ANAMET*[™] process (consisting of an anaerobic contact and aerobic activated sludge process with WAS recirculation to both units) was used to investigate the treatment of a high-strength wheat starch processing wastewater. The low overall sludge yield across the system ranged from 0.03-0.1 kg VSS/kg COD_d and Garuti et al. (1992) have reported sludge productions of 0.2 kg TSS/kg COD_{rem} with the *ANANOX*[®] process.

Excess sludge production from a bench-scale UASB reactor followed by 2 identical aerobic SBR systems, fed with synthetic substrate simulating domestic sewage of 422 mg COD/L on average, was found to represent only 4% of the total influent COD (de Sousa and Foresti, 1996). The coupled system removed over 95% COD and 96% TSS while the UASB alone, which received excess aerobic sludge from the SBRs and was operated with a 4 hour HRT at 30°C, removed at least 86% COD.

During a 121-day pilot plant study, for the treatment of a low pH and concentrated confectionary wastewater (combined with a cooling and sanitary waste stream), averaging 10 560 mg COD/L, Cocci et al. (1990a) observed average removals of 97, 74 and 96 for COD, SS and FOG (fat, oil and grease), respectively, when using a 6.4 L low-rate anaerobic *ADI-BVF*[®] reactor that maintained a pH near neutrality. It was seeded with anaerobic municipal sludge and operated at 31°C with a 9.1 d HRT, a 1:1 sludge recycle ratio as well as a 1.1 kg COD/m³·d loading rate. Shortly after the 55-day start up period of the pH and nutrient controlled study, the BOD: N: P ratio was lowered from 500: 5: 1 to a less conservative 750: 5: 1 proportion without affecting the process. Except for SS, all sewer effluent discharge criteria could be met solely by anaerobic treatment and an important CH₄ fraction (81%) was found in the biogas.

Consequently, a similarly configured full-scale treatment plant was implemented with a 4.2 mega litre (ML) *ADI-BVF*[®] unit, operated at a 14.5 d HRT and a 0.31 kg BOD/m³·d OLR (from 27°C to 43°C without performance disturbance), followed by a once-through aeration system,

consisting of an aeration tank and 60° conical bottom *Dortmund*TM tank clarifier with possible activated sludge operation. Both influent pH (soda ash and Na₂CO₃) and nutrient content (urea) were supplemented. Also, no WAS was recycled to the aeration basin or deliberately wasted from the system, since all of it was directed to the anaerobic reactor, and digested sludge wasting was only scheduled after 2.5 years of operation. The 0.95 ML aeration tank MLVSS varied from 750-1 000 mg/L while the *ADI-BVF*[®] system sustained 300-500 mg VAs/L, 1 100-1 200 mg TA/L and a pH of 6.5-6.6 as well as intermittent slow mixing and recycling. The latter generally yielded 90-95% BOD and 75-85% SS reductions whereas overall system removals ranged from 98-99% for BOD and 77-95% for SS. From sludge bed profile data, SS were found to vary from 1% (top) to 4% (bottom) and the *BVF*[®] reactor bed height was found to fluctuate with gas production and loading (Cocci et al., 1990a).

Kettunen et al. (1996) studied the anaerobic-aerobic continuous flow treatment of oil shale ash leachate in a UASB reactor followed by an aeration and separate settling unit. Although higher average COD effluent concentrations resulted at 10°C than at 20°C, the overall process COD removals were of the same order (67-73%) for both temperatures and relatively stable effluent quality was produced regardless of the feed fluctuations. However, poor COD removals of less than 30% were observed in the anaerobic reactor particularly at the lowest temperature.

Tanaka et al. (1991) compared the performance of three municipal sewage plants, modified to allow for phosphorus removal, to that of a conventional treatment process. In addition to efficient P removal (80-90%) and improved sludge settleability, the findings of the comparative study revealed that the anaerobic-aerobic activated sludge process required approximately 20% less aeration and used about 12% less power, for similar DO concentrations in the two treatment schemes studied. However, except for the P removals, contrary to most anaerobic-aerobic study results, sludge generation, organic and SS removal efficiencies as well as general effluent quality were reported to be almost equivalent to conventional processes.

2.2.2 DAIRY WASTEWATERS

2.2.2.1 Composition and Characteristics

According to Blanc and Navia (1991) and Goronszy (1990), milk (or whole milk) is mainly composed of 87-88% water, but typically contains (butter fats or) fats (3.7-4%), proteins [i.e., casein (3%) and albumin] and carbohydrates [i.e., lactose (5%)]. The last 3 constituents are the main contributors of milk waste organics and their proportions vary owing to its nature and type of production process (Brown and Pico, 1979; Kasapgil et al., 1994; Perle et al., 1995). Although

regarded as complex multi-component organic mixtures (Langenhoff and Stuckey, 2000; Lettinga and Vinken, 1980), milk wastewaters are, in essence, easily degradable by nature of their origin (Brown and Pico, 1979; Goronszy, 1990; Huster and Möbius, 1990; Malaspina et al., 1995; Monroy et al., 1995). Such is also the case for their constituents, their breakdown products and for dairy waste organic loads as well as for many food industry wastewaters (Eckenfelder, 1989; Lettinga and Vinken, 1980).

Skim milk has often been employed in activated sludge experimental studies (Pipes, 1977) as well as anaerobic treatment research (Langenhoff and Stuckey, 2000; Lettinga and Vinken, 1980; van den Berg and Kennedy, 1983). Casein and lactose are its core components (Hoover et al., 1951) and according to Blanc and Navia (1991), the latter is a soluble sugar that accounts for approximately 40% of milk solids. It is attacked quickly by bacteria (Buswell et al., 1932) and is readily converted to lactic acid under oxygen-limited conditions (Goronszy, 1990). Casein often represents milk proteins in laboratory investigations since it can correspond to 80% of proteins in milk and dairy plant effluents (Perle et al., 1995). It can also contain up to 7% of inorganic milk material and even though particles of this substance are relatively large, they tend to act as colloids (Blanc and Navia, 1991). Furthermore, methanogenic bacteria are usually not negatively affected by this non-soluble protein compound or its derivatives (van den Berg and Kennedy, 1983), especially not when adapted to it (Perle et al., 1995).

Nevertheless, other than milk, wastes from dairy product manufacturing plants can provide added organic pollutants (Blanc and Navia, 1991). These often result from machinery malfunctions, accidental product discharge, by-product wasting, residues from processing operations, milk dilutions and wash waters containing cleaning and sanitizing materials in the form of detergents, chemicals and lubricants (Blanc and Navia, 1991; Brown and Pico, 1979; Goronszy, 1990; Hamza, 1982; Kasapgil et al., 1994; Page et al., 1999; Samsom et al., 1984; Viraraghavan and Kikkeri, 1990). Dairy plant equipment sanitizers and cleaners can influence dairy effluent pH since they contribute alkalinity or acidity and can contain phosphates (Aulenbach and Hallock, 1972; Brown and Pico, 1979; Goronszy, 1990; Kasapgil et al., 1994; Monroy et al., 1995). They are able to impact biological systems considerably even though they do not affect the organic loading very much (Goronszy, 1990). When dairy wastewaters are alkaline, they can help emulsify greases and buffer biological processes (Brown and Pico, 1979).

Large quantities of water are commonly employed in dairy processing operations (i.e., cleaning waters) and can constitute a good portion of the total wastewater flow (Hamza, 1982; Kasapgil et al., 1994). In addition to Brown and Pico (1979) as well as Buswell et al. (1932),

mentioned in Section 2.2.2.3, Malaspina et al. (1995) have observed that dilution positively impacts effluent quality. For the anaerobic treatment of whey, a concentrated organic waste usually derived from cheese product manufacturing (Brown and Pico, 1979), most investigators employ a diluted form, which is much simpler to treat (Malaspina et al., 1995).

2.2.2.2 Typical Strengths and Parameter Characterization Reported in the Literature

Dairy industry and food-processing waste strengths (Kasapgil et al., 1994) as well as hydraulic and organic loadings are characterized by high variability (Brown and Pico, 1979; Goronszy, 1990; Page et al., 1999; Viraraghavan and Kikkeri, 1990) that can often affect the efficiency of treatment processes (Mikula et al., 1979). BOD and alternative COD tests are the usual parameters employed to classify and monitor dairy effluent strengths. As is the case for other waste types, dairy wastewater BOD values are always smaller than COD values (Brown and Pico, 1979).

Torpy (1988) affirms that dairy effluent COD can span from just about nothing in process cooling waters to values of several hundred thousand for involuntary raw product spills. Dairy plant wastes can range from as low as 80 to 95 000 mg COD/L, in the US (Brown and Pico, 1979) and from 180 to 23 000 mg COD/L, in New Zealand (Viraraghavan and Kikkeri, 1990). The latter authors report a typical dairy effluent strength to be of 4 000 mg COD/L and of 3 390 mg COD/L, on average, in Canada.

Choi and Burkhead (1984) state that wastewater COD concentrations can vary from less than 100 mg/L to over 20 000 mg/L, depending on the dairy product. Wheatley (1990) also reports the same lower limit and up to 2 250 mg COD/L, with a low typical dairy industry strength of 500-750 mg BOD/L for the UK. Kasapgil et al. (1994) maintain that UK dairy plant effluents are of medium strength and usually generate substantial waste loads.

In Mexico, milk industry wastewaters contribute 1 000-4 000 mg BOD₅/L, on average, and the cheese production industry generates 1 800-2 000 mg BOD₅/L of milk while the dairy industry generates a daily average of 500-2 000 mg BOD₅/L with a 1-50 000 mg BOD₅/L range (Monroy et al., 1995). Goronszy (1990) reports 800-8 000 mg COD/L, 110-5 400 mg BOD/L and 80-2 600 mg TSS/L as typical parameter values for milk manufacturing. Although dairy plant effluent SS data are relatively scarce (Brown and Pico, 1979), Blanc and Navia (1991) report that dairy wastewaters can commonly have up to 2 000 mg SS/L and are mainly organic in nature. Page et al. (1999) also state that other than COD and BOD, these wastes can have high levels of FOG as well

as SS. In addition to the latter type of solids, industrial dairy wastewaters contain soluble and colloidal solids (Blanc and Navia, 1991; Kasapgil et al., 1994).

The following table summarizes the average values and broad variation of characteristic dairy wastewater parameters reported in a review paper by Brown and Pico (1979). The data presented originate in part from a number of Kraft dairy processing plant effluents (Kraft Dairy Plant Averages) including *milk*, Swiss cheese, ice cream, cottage cheese, process cheese, Swiss cheese plus 50% whey, parmesan and cheese powder, as well as from a few different dairy plant studies found in the literature and considered by these authors. Some of the values possibly contain raw whey, which is stronger than average dairy wastes but similar in composition (Brown and Pico, 1979).

Table 2.7: Summary of Various Dairy Waste Effluent Characteristics

Parameter	Kraft Milk Plants		*Kraft Dairy Plant Averages		‡Dairy Literature Data (from various plants)	
	Range (mg/L)	Mean (mg/L)	Range (mg/L)	Mean (mg/L)	Range (mg/L)	Mean (mg/L)
BOD₅	560-1 420 †(700-2 200)	1 180 †(1 482)	240-5 400 †(168-5 700)	1 490 †(2 011)	#40-4 800	#2 300
COD	1 500-2 430	2 040	340-9 170	2 580	†80-95 000	†4 500
SS	410-540	450	40-1 600	560	#24-4500	&2 000
pH	7.0-10.6	9.6	2.8-11.6	7.2	#4.5-9.2	#7.2
FOG	150-300	260	15-1 320	250	§35-500	§209
P	15-40	27	14-200	54	§11-160	§50

[Adapted from Brown and Pico (1979) and *Blanc and Navia (1991); FOG-Fat, oil and grease]

BOD₅/COD ratios of 0.5 or more can be a sign of good biodegradability and consequently of treatability, mostly for wastes of relatively constant composition (Brown and Pico, 1979; McCarty et al., 1972). The average ratio for the Kraft dairy plant data was 0.57 ($\pm$ 0.06) while 0.53 was reported for the dairy literature sources surveyed in Brown and Pico (1979). Among the various kinds of dairy plants, butterfat and ice cream producing facilities have been found to generate the greatest FOG-containing effluents (Brown and Pico, 1979).

Goronszy (1990) reports that dairy wastes usually have a soluble BOD fraction of over 80% and that 1% milk waste can contain about 12 mg P/L as well as about 55 mg N/L, whereas Brown and Pico (1979) report 10 mg P/L for the same milk percentage. Thus, a significant portion of phosphorus in dairy plant effluents can originate from milk waste although some of it can also

*Kraft milk plant wastes are included in these range and mean values. †Adapted from a series of selected dairy industry plant effluent data (i.e., milk, cheese, ice cream and cottage cheese) reported by Harper et al. (1971), cited in Brown and Pico (1979). §Various authors cited in Brown and Pico (1979) including †Harper et al. (1971), #Harper (1973) and §Jones (1974).

come from product spillage and cleaning agents (Blanc and Navia, 1991; Brown and Pico, 1979). Most of that contained in raw milk is found in the orthophosphate form (Brown and Pico, 1979).

2.2.2.3 Treatment Alternatives

Over the years, diverse means (Table 2.8), mostly of the biological type, have been and continue to be employed to treat or pre-treat dairy food wastewaters (Blanc and Navia, 1991; Brown and Pico, 1979; Mikula et al., 1979). Activated sludge package plants were often considered as the distinctive and suitable treatment systems for milk and milk truck washing wastes (Aulenbach and Hallock, 1972). Hamza (1982) as well as Backman et al. (1985) have also recognized classic aerobic biological processes as successful treatment methods for organic pollutants in a variety of food-processing effluents, including those of dairy industry wastes. However, experimental work by Spaulding (1948) suggests that the anaerobic digestion of milk wastes dates back to the early to mid 1900s. The author states that the method was practicable for the destruction of milk solids, in some cases, although it was much criticized, due to poor reliability and operational problems. Aerobic upstream or downstream treatment processes were also already being considered since Buswell et al. (1932) recognized some benefits from aerobic filtration following anaerobic fermentation.

According to Samson et al. (1984), Blanc and Navia (1991) and Perle et al. (1995), some dairy treatment alternatives consist of activated sludge, trickling filters (Buswell et al., 1932), rotating biological contactors (Mikula et al., 1979), oxidation ditches, lagooning or aeration ponds, land applications (particularly for whey waste) or irrigation (Brown and Pico, 1979; Buswell et al., 1932; Eckenfelder, 1989) and anaerobic reactors. Applicable dairy waste treatment technology of the latter type, include upflow filters (Kasapgil et al., 1994), upflow sludge blanket (USB), fixed-film (FF), fluidized bed (FB), expanded bed reactors (EBR) and anaerobic contact (AnCont.) processes (Lettinga et al., 1980; Samson et al., 1984; van den berg and Kennedy, 1983). Some of these as well as others have been proven to be successful in treating relatively dilute dairy wastewaters (Table 2.9 in Section 2.2.2.4).

Past dairy industry experiences with advanced anaerobic technology reveal that 60-95% COD conversion efficiencies are achievable at HRTs of 5-25 h and OLRs of 0.5-18 kg COD/m³·d (Monroy et al., 1995). Samson et al. (1984) also reported comparable organic removals for even greater OLRs (i.e., 7-30 kg COD/m³·d) while high-rate UASB reactors have been shown to treat dairy effluents reaching 10 kg/m³·d with 80% efficiency (Monroy et al., 1995).

Table 2.8: Efficiencies of Various Dairy Wastewater Treatment Methods

Process	Typical BOD ₅ Reduction (%)
Activated Sludge	24-99
Aerated Lagoons	70-99
Oxidation Ditches	74-99
Septic Tanks and Anaerobic Digesters	50-87
Trickling Filters	21-99 (80% per stage)

[Adapted from Brown and Pico (1979) and Blanc and Navia (1991)]

Properly operated aerobic activated sludge, lagoon and oxidation ditch systems treating Kraft dairy wastes (Brown and Pico, 1979) were found to sustain 90% treatment efficiencies, or more (Table 2.8). Samson et al. (1984) have designated the latter type as the better treatment means for dairy wastewaters even though they produce large amounts of excess biomass. Most secondary biological systems mentioned by Brown and Pico (1979) can also reduce other dairy waste parameters such as SS, pH and FOG as well as P to some extent (10-20%). They also affirm that mainly soluble and carbohydrate-type dairy waste effluents do not impede biological systems, that they can be readily and well treated in municipal treatment works and that, contrary to popular belief (Chapter 1; Introduction), their pre-treatment is normally not called for. However, as is also the case with anaerobic technologies, some authors disagree with this statement.

Early studies reported in Buswell et al. (1932), revealed that milk waste treatment by activated sludge was not viable, but when diluted, this type of waste could be properly treated with sewage treatment methods of the day. Problems involved in meeting aeration rate requirements could also have limited the aerobic handling of high-strength dairy wastes (Aulenbach and Hallock, 1972; Hoover and Porges, 1952). Yet, Pipes (1977) has reported that numerous dairy treatment facilities have employed activated sludge-type systems (or have discharged their effluents in treatment works that make use of this process), even though dairy wastewaters can bring about operational difficulties such as reduced biomass settling.

Stover and Gonzalez (1987) suggested that aerobic biological methods are frequently constrained by the strength (Aulenbach and Hallock, 1972) and the inconsistency of industrial wastewaters. Dairy wastes greater than 2 000 mg BOD/L have been observed to affect the treatment effectiveness of activated sludge installations via sludge bulking (Goronszy, 1990). Furthermore, Kasapgil et al. (1994) reported that dairy industry effluents in the UK were often inadequately handled by aerobic treatment facilities and failed to meet present-day dairy effluent sewer consent conditions.

Although dairy wastewater chemical pre-treatment is not very common, coagulation applied prior to dairy waste biological treatment has been demonstrated to provide high-quality effluent, by reducing milk solids, BOD, and COD as well as reducing the size of treatment units, and should be considered according to Blanc and Navia (1991). This type of process is most economically feasible when used to diminish disposal surcharge costs in urban regions. However, Langenhoff and Stuckey (2000) report that biological processes are generally favored over more expensive chemical means for the treatment of domestic and industrial wastewaters.

2.2.2.4 Survey of Dairy Wastewater Treatment Investigations with Advanced Anaerobic Reactors

Although biological dairy wastewater treatment is relatively common and its effectiveness has been the subject of many studies, Table 2.9 summarizes the key average operational conditions and process parameters as well as steady state performance results from relevant anaerobic dairy treatment investigations that were encountered and reviewed. Some of the case studies had organic pollution levels of the same range as the present study and a number of them (i.e., Cocci et al., 1990b; Filho et al., 1996; Landine et al., 1987; Malaspina et al., 1995; Monroy et al., 1995; Page et al., 1999; Samson et al., 1984) included sequential anaerobic-aerobic biodegradation systems (Section 2.2.1), where the majority of the anaerobic effluents overflowed to the aerobic units by gravity. Many of the influent concentrations used in the investigations were chosen to simulate or exceed raw dairy wastewater strengths expected in full-scale systems and most studies applied different chemical supplements, in varying amounts, to improve alkalinity and pH as well as micro and macronutrients for optimal reactor operation. For start-ups, which varied in length from system to system, most reactors were inoculated with some kind of seed sludge. Furthermore, several practical and operational problems discussed in Section 2.2.3 were observed in some of the technical publications surveyed.

Due to skim milk build-up and a delayed hydrolysis rate, during some pilot study overload conditions, Lettinga and Vinken (1980) observed sludge yields as high as 0.5 kg sludge/kg COD_{rem} (which could significantly diminish long term digestion efficiency) in a periodically mixed UASB digester, seeded with domestic sewage and supplemented with NaHCO₃. However, after 30 days of acclimation, for OLRs less than 6 kg COD/m³·d and a pH > 6, it had maintained 0.18 kg sludge/kg COD_{rem} under steady state. To alleviate possible active biomass boil over, mechanical mixing of the reactor's bottom portion was also recommended while a polishing step was suggested as a means to further reduce BOD, PO₄⁻³-P, NH₄-N and odors. Like the experimental configurations used by Kasapgil et al. (1994) and Malaspina et al. (1995), with separate pre-acidification and

methanogenic zones, Lettinga and Vinken (1980) advise that anaerobic technologies with 2 or 3 stages may be very beneficial for such mainly soluble complex wastes and mostly those containing high solids. However, single-step systems can still be useful in these cases as well as be able to accommodate granule formation (Lettinga et al., 1987).

In a comparative dairy waste study by van den berg and Kennedy (1983), a 66 000 mg COD/L cheese whey waste was treated in a needle punch polyester (NPP) and 2 fired potters clay media-downflow stationary fixed-film (DSFF) reactors while a similar clay-type support material DSFF unit was used to treat a 4 000 mg COD/L commercial instant skim milk powder substrate. A 4 000 mg COD/L cheese factory dilute dairy waste (containing whey and alkaline wash water) was also treated in both a UASB and DSFF reactor with NPP-type media. The former type of reactor was equipped with an improvised solids settling vessel made from an overturned 2 L-Erlenmeyer flask that allowed washed-out biomass to slip back to the SB, while all the SS in the DSFF were adhered to the media. Every mature experimental reactor used had received other types of substrates and they were gradually loaded with the respective wastes to which NaOH was added for enhanced buffering capacity.

For the OLRs tested, the soluble skim milk powder waste was not as readily treatable as the two others. However, its average COD removal (72%) was independent of OLRs (5-10 kg COD/m³·d) and for a comparable waste strength and OLRs, it was a bit less than the average 75% reduction by the clay media-DSFF unit treating dilute dairy waste. Possible differences in waste composition (i.e., casein content and/or trace element accumulation) were suggested as reasons for the slightly better performance. For dilute dairy waste, some treatment efficiency disparities were observed between the UASB and DSFF reactors although both systems were limited to an OLR of 15 kg COD/m³·d. The formers average effluent suspended COD content (35%) was lower than that of the latter's (40%), yet its COD reductions were superior, in general, particularly at the lowest 5 kg COD/m³·d OLR (82-92%). For the concentrated cheese whey waste, the treatment outcome was similar for both types of DSFFs. COD removals were very good (92%), even up to OLRs of 22 kg COD/m³·d, and were generally much greater than those observed for the dilute dairy and skim milk wastes in a similar type of reactor and at similar OLRs. Cheese whey effluent COD was mostly in the SS form. About 1/3 of its effluent soluble COD consisted of VFAs and 1/4 to 1/3 in the case of the dilute dairy waste. Effluent to feed recycle (4:1) was not observed to improve reactor performance and was discontinued. The CH₄ yields of all the reactors under study reached 0.32 m³/kg COD_{rem}, on average, although the whey treating-DSFFs produced

3-6 $\text{m}^3 \text{CH}_4/\text{m}^3_{\text{reactor void vol.}} \cdot \text{d}$, at STP, while the reactors treating dilute dairy waste produced 1-3 $\text{m}^3 \text{CH}_4/\text{m}^3_{\text{reactor void vol.}} \cdot \text{d}$ (van den berg and Kennedy, 1983).

Choi and Burkhead (1984) used 1.5 L batch FF (1.6 cm Flexirings) and SB units, with 5 d HRTs, as well as a 38°C-9 L continuously fed upflow FF reactor (3.8 cm Flexirings), to compare the effects of feeding methods, plastic media use and operation temperatures on anaerobic system performance. The effluent recycle rate of the larger lab unit, seeded with anaerobically digested primary and secondary sludge, was fairly high at 43.2 L/d. The variable-strength synthetic dairy substrate employed, made up of diluted low fat milk and micronutrient-rich primary effluent, was composed of 55% carbohydrates, 5% fat and 40% protein. At 37°C, SRTs decreased from 78-30 days and from 109-94 days while MLVS varied from 4 500-14 000 mg/L and 14 008-22 000 mg/L, at increasing OLRs, for the FF and SB batch reactors and their respective maximum OLR was found to be 5 and 7 kg COD/ $\text{m}^3 \cdot \text{d}$. The without media-batch SB unit was more efficient than both FFs and it maintained high COD removals although it was operated at a 5 d HRT like the batch FF unit. These results indicated that both types of systems could treat a 2 000-4 000 mg COD/L influent concentration range, at 1 d HRT, with more than 90% efficiency.

At low OLRs, settling characteristics were good, but effluent SS increased with OLRs, for all units. Poor settling filamentous bacteria were encountered in the continuous FF unit, which had a slight suspended growth inclination. Both the anaerobic biogas production and CH_4 content had a tendency to decrease with increasing OLRs while gas production rates and reactor pH were inclined to increase. On average, the USB had the greatest pH (7.1) and the continuous FF unit, the greatest CH_4 fraction (71%). For this waste, the growth constant, endogenous respiration and net biomass production rates were estimated to be 0.165 g VSS/g COD_{rem} , 0.0256 d^{-1} and 0.11-0.12 kg sludge/kg COD_{rem} . For suitable batch reactor performances, the observed F/M ratio range was approximately 0.25-0.42 kg $\text{COD}_{\text{rem}}/\text{kg MLVS} \cdot \text{d}$ and it reached 0.56 kg $\text{COD}_{\text{rem}}/\text{kg MLVS} \cdot \text{d}$ for the continuous FF unit. At 25-28°C, the batch FF reactor was observed to be less efficient, had a lower CH_4 content, was limited to an OLR of 1.3 kg COD/ $\text{m}^3 \cdot \text{d}$ and only had an F/M ratio of 0.11-0.15 kg $\text{COD}_{\text{applied}}/\text{kg MLVS} \cdot \text{d}$. Compared to its performance at 37°C, the thermophilic batch FF unit achieved greater removals, attained a 0.44 kg $\text{COD}_{\text{applied}}/\text{kg MLVS} \cdot \text{d}$ F/M ratio, had a smaller CH_4 fraction and, although VFAs accumulated, it had a higher pH (Choi and Burkhead, 1984).

Samson et al. (1984) present the results of the first 12 months of operation of a wastewater plant (0.8 ML equalization tank-0.4 ML DSFF-0.4 ML USB + 0.193 ML settler-2 aeration and 2 sedimentation ponds) that treated a dilute cheese factory effluent of 2 800 mg COD/L, on average, at 30-31°C. In 1984, approximately 20% of the milk produced in Canada was processed at the

Agropur plant, which manufactures milk powder as well as cheddar cheese. The aerated ponds, with 7 and 8 d HRTs, were both divided with baffles to retain SS more efficiently in their front compartments. A fabric baffle was used in the 2nd aeration lagoon and the final settling ponds each had 1.5 d HRTs. A wash water stream (containing nitric acid and concentrated caustic soda) was directed in the 12 h HRT head buffer tank, to even out wastewater pH variations and keep them between 5.8-7, using hydrochloric acid and hydrated lime. The overall effluent COD reductions were predicted to reach 95%. The fired clay block-DSFF reactor (similar to a few lab-scale reactors studied by van den berg and Kennedy (1983)) was seeded with 0.189 ML of 3% SS sludge from the acid-fermentation stage of a municipal AD in Cornwall, Ontario (Canada) while the USB unit was inoculated with 0.116 ML of granular sludge, from Holland, which maintained a very good methanogenic activity of 4 g AA_{rem}/L·d.

As a result of its low initial volumetric activity of 0.8 g AA_{rem}/L·d, the DSFF was reinoculated with 0.07 ML of 1.17% SS active biomass from the USB (with 6.5 g AA_{rem}/L·d and 0.7 g AA_{rem}/g VSS·d specific activity at the time), after demonstrating 5 months of poor performance, where it had been operated in an upflow mode due to recirculation pump failure. It maintained a 75% CH₄ content and a pH of 7.4 with a 1 220 mg TA/L and during proper operation. It reduced CODs by 66% at a 5 kg COD/m³·d OLR and 0.58 d HRT, on average. The biogas production rate and CH₄ yield were respectively only 1 m³ biogas/m³·d and 0.21 m³ CH₄/kg COD_{rem}, due to leakage. On average, after the first 2 months of operation, the USB achieved COD removals of 82% at a 1.2 d HRT and a 3 kg COD/m³·d OLR while its CH₄ content was 80%. The biomass concentration was observed to decrease throughout the bed height while its average production rate (Y) was of 0.015 kg VSS/kg COD_{rem}. Even though they did not upset the DSFF reactor, the high wastewater fat content and occasional overloading were considered to cause granular biomass flotation problems in the USB, with subsequent sludge loss and obstruction difficulties. Thus, the USB was also reinoculated with some active settling tank sludge. However, it was observed to be more efficient than the DSFF, with a greater biogas production rate (2.5 m³ biogas/m³·d) and CH₄ yield (0.23 m³ CH₄/kg COD_{rem}).

In a bench-scale study by Backman et al. (1985), a biodegradable synthetic whole milk waste was treated at 35°C in a series of 15.172 L continuously operated upflow packed bed reactors (UPBRs), with arbitrarily distributed 5.1 cm-pall rings. The feed contained enough nutrients, but to sustain a 6.8-7.2 reactor pH, supplemented NaHCO₃ yielded 1 500 mg TA/L. The influent concentrations investigated were 1 000 and 3 000 mg COD/L, over the same 0.31-3.6 d HRT range. For corresponding OLR increases from 0.22-2.85 and 0.80-7.64 kg COD/m³·d as well as 0.09-1.39 and 0.37-3.34 kg COD_s/m³·d, respectively for the former and latter waste strengths,

COD_i removals ranged from 94-81% and 90-61% while slightly greater COD_s removals ranged from 94-76% and 93-74%. VSS loading and removal rates resulted in 0.07-0.84 and 0.17-1.84 kg VSS/m³·d as well as 0.06-0.30 and 0.14-0.37 kg VSS/m³·d, with associated VSS removal efficiencies of 90-36% and 82-21%, respectively. The latter were greater than the TSS removals for the lower strength waste (89-31%), but they were slightly lower in the case of the higher strength waste (83-22%). Biogas started to evolve after 2 weeks of operation. In the 1 000 and 3 000 mg COD_i/L-fed reactors, respectively, CH₄ production was observed to range from 1.1-10.1 L/d and 3.6-21.7 L/d with increasing OLRs. With the latter, specific CH₄ yields ranged from 0.432-0.339 and 0.621-0.349 m³/kg VSS, respectively, while the UPBRs average CH₄ yield was 0.3 m³ CH₄/kg COD_{i rem} and their CH₄ content varied from 70-85%. The findings of Backman et al. (1985) suggested that substrate strength and HRT affect colloidal waste treatment efficiencies the most.

Effluent TSS and VSS increased with OLRs and (similarly to the anaerobic *BVF*[®] investigation by Cocci et al. (1990a) and the USB reactor one by Samson et al. (1984)) biomass concentrations were greater at the bottom of UPBR reactors. This possibly increased fluid upflow velocities and caused some SS washout, which was estimated at 3.5 g/d. The total VSS found in the UPBR treating 1 000 mg COD_i/L at a 12.2 h HRT amounted to 252 g VSS (71% were fixed to the filter media). Consequently, a 71 d SRT resulted. For reactors with comparable conditions, this value is reasonable (Backman et al., 1985). A 52% inactive reactor area, indicative of poor substrate-biomass contact, was established from tracer tests while a settling rate of 0.96 m/h (1.6 cm/min) and an upflow velocity of 0.0012 m/h (0.002 cm/min) were obtained from sludge settleability assessments. Thus, mixing and biogas production were thought to be the causes of washout.

An anaerobic biodegradation step analysis was also performed, according to the van Velson method (1977) (Backman et al., 1985), with both the 1 000 mg COD_i/L and 3 000 mg COD_i/L waste solutions, at their associated OLRs ranging from 0.22-2.85 and 0.80-7.64 kg COD/m³·d. The former concentration was found to be limited by acidification while the latter was rate limited by both the hydrolysis and acid formation steps. The researchers believe that hydrolysis is the limiting step when the methanogen replication time is less than the HRT, but that either acidification or methane formation can be rate limiting in the opposite situation. The maximum OLR for proper UPBR operation was observed to be 5 kg COD/m³·d, similarly to the FF reactors in the study by Choi and Burkhead (1984). In the latter case, only about 1 month was required to reach quasi-steady state conditions while the UPBR necessitated 80 days or more to realize a successful loading rate change and only biogas production and effluent COD_i attained

stability (Backman et al., 1985). However, Speece (1996) reports that a DSFF reactor treating casein and a cheese processing plant effluent took as long as 5-8 months to start-up.

Analogous to the investigations carried out by Cocci et al. (1990a) for the treatment of a candy bar manufacturing waste (containing peanuts, corn syrup, chocolate, condensed milk and fat residues), referred to in Section 2.2.1.5, three comparable pilot-scale dairy treatability studies, by Landine et al. (1987), Cocci et al. (1990b) and Page et al. (1999), lead to somewhat similar full-scale sequential treatment processes with common low-rate anaerobic *ADI-BVF*[®] technology and differing forms of aerobic post-treatment for the pre-treatment upgrade of 3 independent dairies. Since many dairy wastewaters contain high concentrations of SS and FOG as well as COD and BOD, the above mentioned anaerobic system was selected because it can treat wastes with these characteristics (Page et al., 1999).

Landine et al. (1987) present the results of a new and improved aerobic system put in place to treat an odor producing-complex dairy waste of 3 910 mg COD/L at a plant that processes butter, condensed milk, baby formula, dried and condensed whey as well as cheeses. The activated sludge-type system, including an aeration basin, a contact pit and trickling filter, a solids contact clarifier as well as an aerobic sludge digester and decant tank, recirculated clarifier settled sludge to the aeration tank, with a F/M ratio < 0.1. The aerobic processes were preceded by a 11.4 ML *ADI-BVF*[®] fermenter (with internal recycling) after a successful on-site pilot study was conducted at a 0.54 kg COD/m³·d OLR, in an 8 L anaerobic unit of this type (Table 2.9). To lessen the clarifier solids inventory, WAS was recycled to the aerobic and anaerobic digester and some of it was combined to the incoming raw waste (with an approximate pH of 7.4 after NaOH addition) along with trickling filter sludge. The full-scale anaerobic unit, with slow-speed intermittent mixing, was seeded with 1.14 ML of municipal digested sludge (i.e., 10% of the *BVF*[®] volume) and gradually adapted over 12 weeks. On average, its CH₄ content was 61% while its effluent had 40% of influent SS (i.e., ~200 mg/L), 1 257 mg TA/L and a pH of 6.6. Over the 1st year of operation, both the anaerobic and aerobic phases provided COD reductions of 84%, on average. Performance-wise, the total system worked well, with a final effluent pH ≥ 8 and COD removals reaching over 97% (Landine et al., 1987).

In addition to their satisfactory (Table 2.9) pilot investigation (similar in many respects to that of Cocci et al. (1990a) and with comparable removals to that of Landine et al. (1987) for a much stronger wastewater at over twice the organic loading rate), Cocci et al. (1990b) discuss the subsequent full-scale treatment of a dairy plant waste derived from the manufacturing of cottage cheese, sour cream, yoghurt and ice cream as well as whey and process wash water. At some point

in the treatability study, the 2 g NaHCO_3/L influent alkalinity supplements were reduced to 0.25 g NaHCO_3/L without any harm [as was also the case for Cocci et al. (1990a)] and since enough nutrients were available, chemical additions stopped prior to the end of the 56-day acclimation period.

The odor-free full-size process involved a *Davis*[™] extended aeration, activated sludge system, with a 1.3 d HRT and a 0.15 F/M ratio, which was preceded by a 9.5 ML *ADI-BVF*[®] unit, operating at a 13.9 d HRT, under an average 0.6 kg COD/ $\text{m}^3\cdot\text{d}$ OLR and 21°C design temperature, with low-speed mixing and internal recycling. Municipal anaerobic digester sludge (1.9 ML), containing 1-2% SS, was used to seed this reactor and its 30°C start-up took about 16 weeks, with gradual whey addition, although 0.04 ML of it was initially mixed in with the inoculum. Clarifier sludge was recirculated to the aeration tank and, like in the Cocci et al. (1990a) study, all WAS was wasted to the *BVF*[®] digester also equipped with 2 gas/liquid/solid separators (GLSSs). The latter reactor achieved 95% COD and 85% SS removals and had a typical methane content of 65% while its effluent pH and total alkalinity averaged 6.7 (or greater) and 1 700 mg TA/L, respectively. The packaged aerobic system reduced COD by 66% and SS by 72% although overall system removals were of 99% for COD and 95% for SS, on average. Nutrient addition was not required and influent pH adjustments (with Na_2CO_3 during start-up as well as with soda ash and lime) were discontinued after the first 10 months possibly because of elevated TKN concentrations in the raw wastewater (Cocci et al., 1990b).

An 18-week anaerobic-aerobic pilot-scale study that investigated the treatment of an orange juice, milk and cream manufacturing plant effluent is reported by Page et al. (1999). The low temperature and 4 300 mg COD/L (or greater) liquid food waste was treated in an automated 50 L *ADI-BVF*[®]-10 L aerated tank system (with no clarifier) and when required, it was supplemented with fat-free or skim milk to maintain its strength. Based on previous dairy effluent treatment experiences, *ADI International* (previously *ADI Systems Inc.*) collaborators believed that this low-rate anaerobic technology would perform well at the dairy wastewater's mean $27 \pm 2^\circ\text{C}$ temperature. However, when wastes of this type are produced, they are usually much warmer (Page et al., 1999; van den berg and Kennedy, 1983). The anaerobic reactor, equipped with a GLSS, was seeded with $1/5$ of similar pilot plant anaerobic sludge of 3% VS and similarly to Lettinga and Vinken (1980), full-strength waste was only applied after 6 weeks of acclimation. After a couple of weeks, the 3 g bicarbonate/L alkalinity addition could also be reduced to nothing. Periodic internal recycle accompanied the anaerobic unit's interval feeding while daily hand mixing gave more fluidity to its SB.

The HRTs investigated were 7, 6 and 5 days with corresponding mean OLRs of 0.64, 0.77 and 0.90 kg COD/m³·d as well as solids loading rates of 0.14-0.18 kg TSS/m³·d. During start-up, at the first HRT and average daily load, the reactor's estimated F/M ratio was 0.1 d⁻¹. The 2 mg O₂/L and CM diffused air-aerobic polishing tank, which was continuously operated at a 1 d HRT and at room temperature (15.5-21.1°C), was mostly used for odor problem prevention (i.e., sulfide oxidation). Even though it was not inoculated, it was started at the same time as the anaerobic unit and some biomass eventually built up from overflowing *BVF*[®] reactor seed. Two-day shock load tests carried out during the study had little effect on the performance, recovery time and ultimate effluent quality of the sequential treatment system. However, load doubling resulted in a considerable biogas production increase. Throughout the various operating conditions, on average, 90% COD and 83% TSS removals were attained in the anaerobic *BVF*[®] unit, regardless of the sub-mesophilic operation temperature. The aerobic reactor further reduced COD by roughly 200 mg/L, yielding 50% COD removals while effluent TSS concentrations almost doubled through the aeration tank. The entire pilot plant system achieved overall removal efficiencies of 95% for COD and 79% for TSS with final aerobic effluent concentrations of 215 mg COD/L and 131 mg TSS/L, which were both well within the sewer discharge limits. Consequently, a full-scale system consisting of 15.9 ML low-rate anaerobic unit followed by a 2.3 ML aerobic polishing reactor was being built (Page et al., 1999).

In a 2-Phase study by Stover and Gonzalez (1987), a series of 11 L CM and continuous flow anaerobic contact units (7.5 L reactors + 3.5 L settlers), operated under mesophilic temperature ranges, were used to treat a concentrated cheese processing waste (including wash water and whey), at a few differing strengths. A control reactor (i.e., full-scale conditions) accompanied each phase. The various environmental and loading conditions investigated included half loadings (R2), micronutrient supplements (R3) and higher ML pH (R4), in Phase I (P1), as well as cultured bacteria addition (R1), ultrafiltration permeate addition instead of whey (R2), micronutrient supplements (R3) and increased wash water flow (R4), in Phase II (P2). At the respective influent COD concentrations, HRTs and OLRs (Table 2.9), the average F/M ratios, observed yields and pHs, for all the reactor conditions assessed, ranged from 0.297-0.572 and 0.421-0.565 kg COD/kg MLVSS, 0.050-0.069 and 0.053-0.125 as well as 6.7-7.4 and 6.6-7.2, during P1 and P2, respectively. The average gas quality (55-60%) was similar for all test units although biogas production rates and methane yields differed. In both phases, the latter were considerably greater for those receiving 0.1 mg/L of (Ni, Co, Cu and Mo) micronutrient supplements, but had no effect on settling or kinetic rates. The best biomass settling characteristics were encountered in the low-load reactor, which had fine compact flocs, while the cultured bacteria

unit that developed slimy sludge exhibited some of the worst settleability. However, a slight potential floating tendency was observed during zone settling velocity (ZSV) and sludge volume index (SVI) settling tests, even for good settling sludges. It was also demonstrated that a reactor pH < 6.8 was unfavorable to biological kinetics as well as to CH₄ yields and that all reactor conditions studied resulted in excellent COD removals, with greater than 94%, on average. However, high effluent TSS concentrations were observed. The results of a biological kinetic analysis, performed with the Stover-Kincannon activated sludge method, suggested that the waste was biodegradable and anaerobically treatable.

Similarly to the treatment improvement investigation by Filho et al. (1996), Kasapgil et al. (1994) demonstrated (with a comparative pilot study) that an upflow anaerobic filter (UFAnF) system was much more efficient than a physical/chemical dissolved air flotation (DAF) aerobic process, in pre-treating a similar strength dairy processing wastewater, for the upgrade of a non-consenting secondary aerobic biological treatment system with 2 settling tanks. The milk plant flow rate was approximately of 700 m³/d. Some waste characteristics included a pH of 8-11, 150-300 mg/L alkalinity, 300-500 mg/L total fatty matter and 350-1 000 mg TSS/L. Throughout the 3-month experiment, the 5 cm polypropylene plastic media-UFAnF was operated with a 20 h HRT, a 6 kg COD/m³·d and a total upflow velocity of 19-20 m/d (0.81 m/h). Its chemically adjusted pH varied from 6.8-7.2 and its CH₄ content was high (75-85%). Although fatty matter and load reducing-DAF units correspond to much less capital expenses, cost-analysis estimations revealed that UFAnFs have significantly lower operational costs, which are advantageous over time. At the full-scale, because of odor and land considerations, an activated sludge process was recommended to improve on the former plastic media aerobic filter post-treatment. The final anaerobic-aerobic treatment effluent met the UK's present-day river discharge limit of less than 125 mg COD/L. With a two-stage CSTR+UFAnF (2.5 cm pall rings), an additional 14-month lab-scale study proved that the process was as efficient as the single-stage UFAnF, under an OLR of 10 kg COD/m³·d, since the separate pre-acidogenic step (10 L CSTR reactor) showed resilience and improved effluent quality.

Malaspina et al. (1995) present 9 months of laboratory results for an anaerobic-aerobic biological process with a novel, two chamber downflow-upflow hybrid reactor (DUHR) technology, developed for the treatment of concentrated raw cheese whey that is customarily disposed of, in Italy, as pig feed liquid fractions. The 51 L anaerobic DUHR, seeded with pig waste digester sludge and acclimated over 40 days, had a 1:5 CM downflow pre-acidification to PF methanogenic upflow stages (with polyurethane filter support material) that respectively

maintained pHs of 6.5-6.7 and 7.5, without any alkalinity supplementation. However, the latter chamber's pH dropped somewhat with increasing OLRs. The DUHR also had 1:4 to 1:2.5 recycle ratios, a persistent viscous ML and a low 53% average CH_4 content, which did not vary with OLRs. Biogas productions reached $10 \text{ m}^3 \text{ gas/m}^3_{\text{reactor}} \cdot \text{d}$, at $10 \text{ kg COD/m}^3 \cdot \text{d}$, and due to such high rates, upflow velocities were observed to increase by up to 2-fold with respect to the liquid velocities.

Although the DUHR supernatant was clear, the inefficient GLSS device led to biomass washout and caused effluent COD_i and SS to be relatively high. The latter were even greater than those of the influent and the effluent COD_s remained over $1\,000 \text{ mg/L}$. However, the DUHR provided greater than 90% COD removals for all conditions, with no OLR dependency. Over the mean $8.75 \text{ kg COD/m}^3 \cdot \text{d}$ OLR investigated, low sludge yields of $0.075 \text{ g VSS/g COD}_{\text{rem}} (Y_{\text{obs}})$ as well as average DUHR biological kinetic constants and process parameters of $0.63 \text{ g COD/g VSS} \cdot \text{d}$ (F/M), $0.55 \text{ g COD}_{\text{rem}}/\text{g VSS} \cdot \text{d}$ (U_{specific}) and 0.04 d^{-1} (μ) were observed. A process wash water stream with a high organic content (C/N ratio) was added to the anaerobic effluent (at 2:1) to post-treat it in a 5 L SBR with good digested whey residual nutrient and organic treatment potential. Several nitrification/denitrification cycles, anaerobic-aerobic-oxic phase lengths and HRTs combinations were tested. These resulted in various COD removals ranging from 90.7-58.6%, for OLRs of $0.93\text{-}1.28 \text{ kg COD/m}^3 \cdot \text{d}$, and associated F/M ratios varied from $0.13\text{-}0.27 \text{ g COD/g VSS} \cdot \text{d}$. Some conditions resulted in filamentous bulking problems and poor settling (high SVIs). Tertiary chemical treatment (coagulation and flocculation) and precipitation were proposed to reduce un-biodegradable COD caused by residual colloidal and soluble organic matter.

Sludge washout and decreased settleability can be caused by overproduced gelatinous microbial exopolymeric materials (or EPS-exopolysaccharides) contained in raw cheese whey and although very degradable, low alkalinity concentrated whey, which acidifies quickly, is not easy to digest anaerobically at high loads. UASBs are therefore less suitable as treatment options since granulation is generally not obtained with this type of substrate (Malaspina et al., 1995). In a comparative anaerobic treatment study reported in Speece (1996), the formation of granules did not occur, over 3 years, in a highly loaded UASB treating a $5\,800 \text{ mg/L}$ ice cream wastewater with a 610 mg/L lipid content, whether it was supplemented with trace metals or not. However, a two-stage process increased COD removals and trace metals as well as well-formed granule seeding improved general reactor operation. Some granulation problems can also result from surplus phosphorus (Monroy et al., 1995).

Monroy et al. (1995) present the results of a low-cost pond system upgrade at the non-consenting *El Sauz* processing plant, in Mexico, which used national technology to treat cheese wastewater produced at $500 \text{ m}^3/\text{d}$. It contained 754 mg/L of oil and grease, on average, 70% of it

was rid by flotation in the degreasing tank while the rest was inclined to gather on the surface of the following UASB-type pond. A cleaning water stream, containing NaOH and H_3PO_4 , was recirculated within the plant and the influent COD: N: P ratio was estimated to be 700: 2.4: 2.21. The 0.2 kg COD/kg MLSS·d aerobic pond F/M ratio resulted from average 1.2 g/L MLSS and 2 mg O_2 /L concentrations. Effluent TSS increased in this stage although the tertiary biological water hyacinth treatment pond (WHP) accomplished considerable solid polishing (93%). For further stabilization, aerated pond MLSS were intermittently recycled back to the upflow anaerobic pond that managed to reduce soluble COD by 63%, even if it was not equipped with a GLSS. The average pH of the influent, aerated lagoon and WHP was 7.3 while that of the UASB pond was 6.4. Despite the relatively inefficient physical-anaerobic-aerobic-WHP treatment process units and a low 0.55 kg COD/ $\text{m}^3\cdot\text{d}$ OLR, suitable overall system COD removals prevailed at 84-96% while ultimate oil and grease reductions amounted to 99.8%.

Filho et al. (1996) present the results of an anaerobic upgrade applied to an existing aerobic treatment facility (including an aeration-settling pond post-treatment system) used to pre-treated a dairy liquid effluent of 6 318 mg COD/L, on average. To accommodate greater industrial production and enhance organic removal efficiency, the existing head DAF unit was modified into a 2 chamber-0.051 ML upflow anaerobic reactor, which was seeded with septic tank sludge, manure and water. An equalization tank for grease removal preceded the improved anaerobic-aerobic system and the anaerobic unit was started-up 1 year prior to the experimental period. Throughout the study, the dairy influent that included residual processing water and whey had an estimated COD: N: P ratio of 100: 3: 1, a BOD/COD relationship varying from 0.55 to 0.83 and its VSS accounted for approximately 85-90% of the solids. For the different digester HRTs investigated (1.2, 0.6, 0.4 and 0.3 d), over a period of 3 months each, the OLRs applied ranged from 2.6 to 31.9 kg COD/ $\text{m}^3\cdot\text{d}$ while the average was 10.8 kg COD/ $\text{m}^3\cdot\text{d}$. Only passable treatment efficiencies of 53.4% COD and 46% TS were achieved, on average. However, the first 0.03 ML compartment gave average COD reductions of 47%, which makes up about 88% of the anaerobic unit's removal. The feed had a high average pH of 8.6 although it decreased significantly in the anaerobic digester, which had an average effluent value of 5.9. COD removals were found to rely more on concentration than on flow rate and an unusual and slight OLR-dependent tendency was observed. Slimy floating solids, with a low functioning potential, were periodically encountered and problematic pipe obstructions were frequent. Treatment was also not odor-free.

Finally, in a comparative study by Langenhoff and Stuckey (2000), an 8-compartment and seeded 10 L anaerobic baffle reactor (ABR) was used to treat a low-strength semi-skim milk waste

of 500 mg COD/L, at 3 decreasing temperatures. At a mean 1.2 kg COD/m³·d OLR and 10 h (or 0.42 d) HRT, a less than expected 25% reduction in COD removal efficiency accompanied the operating temperature drop from 35 to 20°C and only an additional 10% decrease was observed from 20 to 10°C. VFAs, mainly composed of acetate, started to accumulate slightly in the ABR, after both temperature reductions. At 20 and 10°C, they accounted for much of the effluent COD and corresponded to 75% for the former and 90-25% for the latter. However, over time at 10°C, soluble microbial product fractions increased (75%) in the average 200 mg COD/L effluent. Aerobic post-treatment was therefore suggested as a way to promote their residual biodegradation.

At the decreased temperatures, the poor substrate-biomass contact affected reactor performances owing to less mixing and biogas yield as well as propionic acid production. Although the mesophilic biomass developed lower metabolism rates, it was not excessively susceptible to temperature shocks and the results demonstrated a low temperature treatment potential for dilute wastes, at relatively low HRTs, when enough active biomass was available. With experimental batch bioassays, the sludge was observed to maintain its initial specific acetogenic and methanogenic activities, even after psychrophilic temperature exposure, but it was inclined to produce propionate. It was concluded that methanogens (acetate degraders) are less distraught by temperature decreases than syntrophs (propionate degraders) and that low temperatures do not appear to influence LCFA and butyrate biodegradation rates. This was also observed in expanded granular sludge bed (EGSB) reactors (Langenhoff and Stuckey, 2000).

2.2.3 POTENTIAL ANAEROBIC TREATMENT DIFFICULTIES

2.2.3.1 Biomass Flotation and Washout

Some of the most common problems encountered in UASB operation include reactor start-up, fixed solids accumulation and SB scaling, the loss of granulation and settling properties, sludge washout as well as granule flotation (Kennedy, 1999; Rittmann and McCarty, 2001; Speece, 1996). The latter typically happens when hydraulic and/or organic shock loads are applied (Lettinga, 1993), when sudden temperature variations occur or when sludge particles are fed after being deprived of substrate (Kennedy, 1999). Thus, a lack of substrate can result in granule deterioration and buoyancy due to the hollowing of their inner core, as a consequence of endogenous decay (Guiot, 1992b; Lettinga and Hulshoff Pol, 1991). Although floating sludge and consequent washout can take place because of important biogas evolution and high liquid velocities (Barber and Stuckey, 2001; Guiot, 1992; Kennedy, 1999), it is possible for relatively large aggregates to float,

even under low gas production rates, especially if the biomass retention times are low (Lettinga et al., 1987; Speece, 1996).

Other factors such as high influent Ca^{+2} (de Zeeuw and Lettinga, 1980) and SS concentrations as well as their accumulation in the SB can induce the heavy washout of granules in UASB reactors (Guiot, 1992; Kennedy, 1999; Lettinga and Hulshoff Pol, 1991; Maat and Gorur, 1990; Sayed and Fergala, 1995). The prolonged entrapment of voluminous SS in granular sludge beds as well as the coating of bacteria aggregates with fats has been reported to lead to sudden and massive SB flotation along with washout (Guiot, 1992; Lettinga and Hulshoff Pol, 1991).

As reported by Lettinga et al. (1987), the main causes of sludge flotation in UASB reactors are:

- Adherent and/or entrapped biogas which usually arises from high specific gas production rates due to high biomass loading rates. Thus, gas generation surpasses the rate of degassification of the biomass aggregates.
- Adsorption of lipids in or on granules, and/or the incorporation of sludge in fat agglomerates, thus in compounds that float easily. This can take place with relatively high lipid (or fat)-containing wastewaters, such as edible oil, dairy or meat processing wastes [Hwu (1997) in IAWQ Newsletter (January 1999)].
- Adsorption of slowly or poorly degrading proteins (Barber and Stuckey, 1999; Perle et al., 1995) that can impede biogas bubble release from the sludge particles. Amino acid or protein-bearing wastes tend to cause foaming and scumming (Lettinga and Hulshoff, 1991; Lettinga et al., 1987 and 1993) as well as granules deterioration (Perle et al., 1995; Speece, 1996).

In the study by Elmitwalli et al. (1999) presented in Table 2.3, important SB flotation problems were encountered due to a high influent COD_{ss} content while the UASB treating domestic sewage, in the study by Vieira and Garcia (1992), suffered from severe sludge loss when operated at low HRTs of approximately 5 h. Although many investigations have demonstrated the good performance of UASBs in treating soluble food-processing wastes, problems including losses of fine solids, impaired settleability and sludge retention are quite common with dairy and slaughterhouse wastewater applications (Wheatley, 1990).

2.2.3.2 Challenging Compounds

Perle et al. (1995) report that proteins and fats are often responsible for common digestion difficulties encountered when typical dairy wastewater effluents are anaerobically treated.

However, their impact is frequently overlooked in research studies that use comparable substrates since their concentrations are usually low compared to other milk constituents.

In a study by Tor et al. (1998)², *In Vitro Digestion of Proteins in Human Milk Fortifiers* (Enfamil™ and Presemp™) and in Preterm Formula (Similac™ and Enfalac™), casein, was found to be the most rapidly degraded protein in all preterm infant and adult products tested (including human milk and whey protein concentrates). However, the hydrolysis of bovine whey proteins in human milk fortifiers and in preterm formulas was concluded to proceed at a relatively slow rate. Throughout the literature examined, this was the only publication that was found to directly use and give some information on the digestion rate of the actual product employed as feed (i.e., *Enfalac™ Infant Formula* by Mead Johnson Nutritionals™) in this research project.

As an aside, McCarty et al. (1972) used vanilla flavored *Metrecal*, a dietary food product, as synthetic sewage for their activated sludge study, but at a much higher organic strength. Huang and Bates (1979) used a diluted commercial dry milk powder, as a synthetic milk waste, in their rotating biological contactor study while Hanaki et al. (1987) also used a powdered whole milk substrate to investigate the inhibitory effects of LCFAs during anaerobic treatment (Section 2.2.3.3).

Besides proteins, high concentrations of lipids are also known to stimulate scum and foam production (Lettinga and Hulshoff Pol, 1991; Speece, 1996) and their anaerobic breakdown can be slow (Grady and Lim, 1980). In addition to undesirable sludge flotation/washout, Hwu (1997), cited in the IAWQ Newsletter (January 1999), reports that aerobic bacteria inhibition, by means of LCFAs (Koster and Kramer, 1987), can occur during the anaerobic treatment of lipid-containing wastewaters. Based on his experimental findings, the author recommends the use of granular sludge as seed, the acclimation of the biomass to LCFAs, the application of thermophilic conditions, the avoidance of excessive sorption as well as the recirculation of the biomass carried out in the effluent as measures to alleviate these potential difficulties.

When fat-rich wastes are treated, others have also suggested the use of effluent recycle, pre-treatment steps (Brown and Pico, 1979) as well as prolonged biomass adaptation times to overcome granule-floating tendencies (Lettinga and Hulshoff Pol, 1991; Maat and Gorur, 1990). Thus, some authors stress the importance of efficient gas/solid/liquid separators when UASB reactors are employed as well as for external solids capture devices to be incorporated to the

² *Journal of Pediatric Gastroenterology & Nutrition*, 27, 1, pp. 30-36. (Only the abstract could be consulted.)

treatment system in order to retain and prevent extreme washout, if granule flotation occurs (Lettinga et al., 1987; Speece, 1996).

Ice cream and milk processing operations generate wastewaters with high fat and fatty acid content that can manifest significant toxicity (Speece, 1996). Wheatley (1990) as well as Zitomer and Speece (1993) also state that fat bearing and dairy wastes have commonly demonstrated negligible anaerobic gas production. However, biomass activity inhibition in the presence of milk fat in anaerobic digester feed is assumed to be mainly caused by resulting LCFAs (Perle et. al, 1995). The latter authors examined the inhibition mechanisms of dairy wastewater compounds (casein, amino acids, milk fat) and their breakdown derivatives (glycerol and oleic acid) on anaerobic process efficiency rates. Based on their results, they recommend that milk fat concentrations should be limited to < 100 mg/L since this strength was observed to reduce biogas generation by as much as 71% of that of the controls. Since the consumption rates of casein were also found to be slow, but not inhibitory, adequate biomass acclimation was also recommended when sludge is to be exposed to wastes containing this compound.

2.2.3.3 Methanogenesis Inhibition by LCFAs

Several authors have investigated the degradation and potential inhibitory effects of LCFAs on the anaerobic digestion process (Hanaki et al., 1981; Koster and Cramer, 1987; Lalman and Bagley, 2000 and 2001; Rinzema et al., 1987). These compounds form when FOGs are anaerobically hydrolysed and can subsequently undergo slow degradation to SCFA. Yet, they have been observed to inhibit the activity of anaerobic organisms and particularly of the acetoclastic methanogens (Hanaki et al., 1981; Koster and Kramer, 1987; Lalman and Bagley, 2000 and 2001). Although Koster and Cramer (1987) have reported that the adsorption of inhibitors by bacteria can be a potential inhibition mechanism, like Rinzema et al. (1994), they state that inhibition caused by long-chain acids is primarily related to their concentration.

Linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acids, which are both unsaturated 18 carbon acids with two double bonds and one double bond, respectively, are commonly found in most fats and many vegetable oils (Koster and Kramer, 1987; Lalman and Bagley, 2000 and 2001). Although milk fats are complex and are said to have slightly different properties than other types of fats, they are mostly made up of saturated and unsaturated fatty acids (Harper and Hall, 1976).

According to Hanaki et al. (1987), the fat contained in the whole milk substrate used during their study hydrolyzed easily to LCFAs, but subsequently inhibited the anaerobic digestion process. McCarty (1964) also reports similar inhibition effects by oleate, a typical LCFA. At 21°C

and concentrations of 30 mg/L or more, linoleic (Lalman and Bagley, 2000) as well as oleic (Lalman and Bagley, 2001) acids were observed to significantly restrict the activity of aceticlastic methanogens as well as methane production rates. Although both acids were found to only slightly impact hydrogenotrophic methanogenesis (Lalman and Bagley, 2000 and 2001), at this temperature, the linoleic acid fully inhibited aceticlastic methanogenesis.

Thus, vegetable oil-containing wastewaters with important linoleic acid concentrations can be challenging to treat anaerobically and reduced temperatures can intensify their inhibitory effects as well as those of their degradation by-products, such as oleic acids (Lalman and Bagley, 2000). Therefore, to minimize their inhibition and that of other LCFAs contained in wastewaters, the latter authors suggest the use of phase separation. They also report that the inhibitory effects of linoleic acids can be decreased with acclimation.

CHAPTER 3

MATERIALS AND METHODS OF ANALYSES

The sections in this chapter present the laboratory-scale suspended-growth biological treatment system employed in the investigation, the materials used to feed and seed all the reactors [synthetic dairy waste, anaerobic granular biomass and thickened waste activated sludge (TWAS)] as well as a summary of the operating conditions, the testing regime and all the analytical techniques performed throughout the study.

3.1 EXPERIMENTAL DESIGN, APPARATUS AND OPERATION

The experimental set-up (Fig. 3.1) used to treat a low concentration feedstock was assembled and operated in the Water Pollution Lab at the University of Ottawa. Its small-scale design was based on preliminary calculations presented in *Appendix A*. Prior to conducting the actual study, much time was dedicated to preliminary operations: the assembly of the set-up, the characterization of the sludges and wastewater (Section 4.1, CH.4) and the acquaintance with the analytical apparatus and methods (Section 3.2) since all analyses were performed by the student on site.

3.1.1 DESCRIPTION OF THE EXPERIMENTAL SET-UP

A dilute simulated dairy wastewater was treated, at ambient temperature, with a combined anaerobic-aerobic treatment process operating in a continuous mode. The bench-scale system consisted of two sets of *low-rate* single-stage UASB reactors followed by continuous-flow stirred-tank (CFST) activated sludge processes acting as aerobic polishing. The two practically identical systems were fed in series, but operated in parallel. Although they were fed from the same ($\sim 1\,000$ mg COD_t/L) feed tank, at the same desired flow rates, the influent feed concentration of one of the anaerobic digesters (ADs) was to be half of that of the other (~ 500 mg COD_t/L in AD2), for performance comparison purposes. An external tap water dilution line was therefore inserted in its feed line prior to the T-inlet liquid distributor. All reactor volumes were to remain constant during the experiment. A schematic representation of the initial experimental system used in the study (Days 1 to 45) is provided in Fig. 3.1 and a picture of the actual set-up is shown in Fig. 3.2.

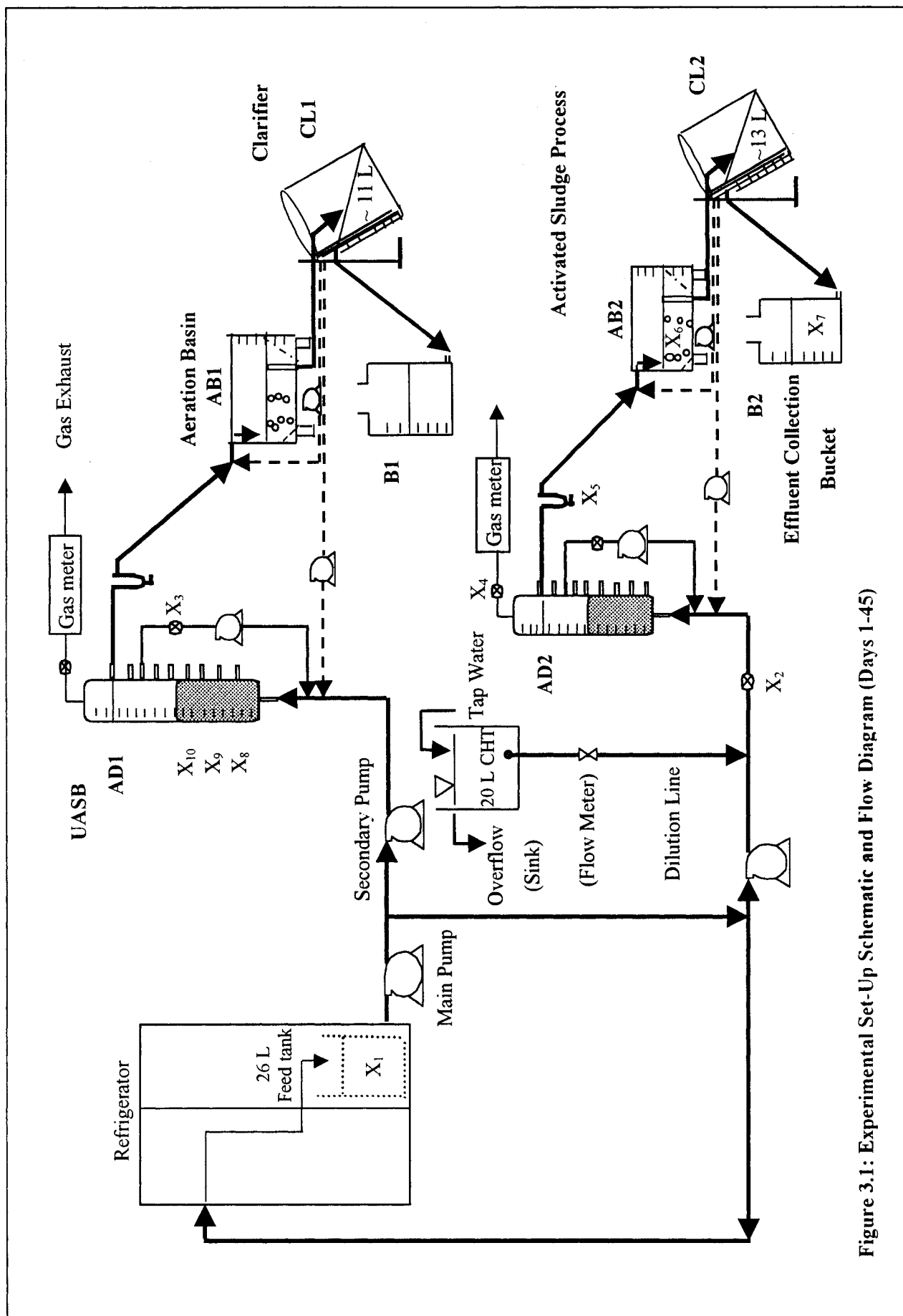


Figure 3.1: Experimental Set-Up Schematic and Flow Diagram (Days 1-45)

The variable speed Cole-Parmer (6-600 rpm) peristaltic main feed pump, with Masterflex® Easy Load head, circulated dilute Enfalac™ wastewater feed (Section 3.1.2.1) within a 0.79 cm ($\frac{5}{16}$ in.) ID Nalgene tubing main feed line, from the bottom of a 26 L capacity refrigerated feed storage tank (Fig. 3.3), at a relatively fast and constant rate. The feedstock, prepared without pre-treatment, was maintained at a low temperature to minimize biological degradation during its storage. Some of the feed was withdrawn from this insulated recycle loop by 2 secondary feed pumps tapped into the main feed circulation line. It was then distributed to both anaerobic digesters (AD1 and AD2), in an upflow mode with controlled influent flow rates, to achieve desired reactor HRTs. Both AD feed lines were also insulated up to the inlet port.

The remainder of the feed in the main feed line was recycled back to the tank, through the freezer compartment of the refrigerator. The content of the feed tank was recycled to maintain consistency in its quality, to give it some degree of mixing and to have enough flow for more than one set-up to be fed, while avoiding air pockets. A few different types of pumps acted as secondary pumps for both reactors since some of them broke down, especially during the start-up period. For most of the experiment, a Harvard Apparatus® peristaltic pump was used as the influent feed pump for AD1 while a peristaltic Masterflex® Pump Controller, by Cole-Parmer, was used to feed AD2.

An elevated 20 L constant head tank (CHT), supplied by a water tap and having an overflow to a nearby sink, was used to dilute AD2's feed line by half, via gravity. A simple pre-calibrated stopcock and then a flow meter (Section 3.2.1.3.b), inserted within the dilution line, were used to control the tap water input. However, the former's accuracy was questionable and the latter's regulation was problematic. The volume of biogas (Section 3.2.1.1e), generated by the UASB reactors during the anaerobic digestion process, was recorded with calibrated wet tip gas meters and exhausted to the atmosphere outside the lab, through a fume hood ventilation system.

The UASB reactor effluents continuously flowed, by gravity, from the outlet ports through U-shaped glass manometer tubes, with which the pressure state inside the reactors could visually be evaluated. Also by gravity, air activated sludge-type reactors [aeration basins (ABs) plus clarifiers (CLs)], continued to treat the anaerobic effluents. Two 20 L containers, roughly inclined by 54°, acted as CLs of approximately 11 L (CL1) and 13 L (CL2). Effluents from the activated sludge systems were then collected in two other 20 L capacity effluent collection buckets (B1 and B2). Both CLs, that were empty at the start of the experiment, started overflowing into them on Days 2 and 3 for AD1 and AD2, respectively [Figure 3.5 b)].

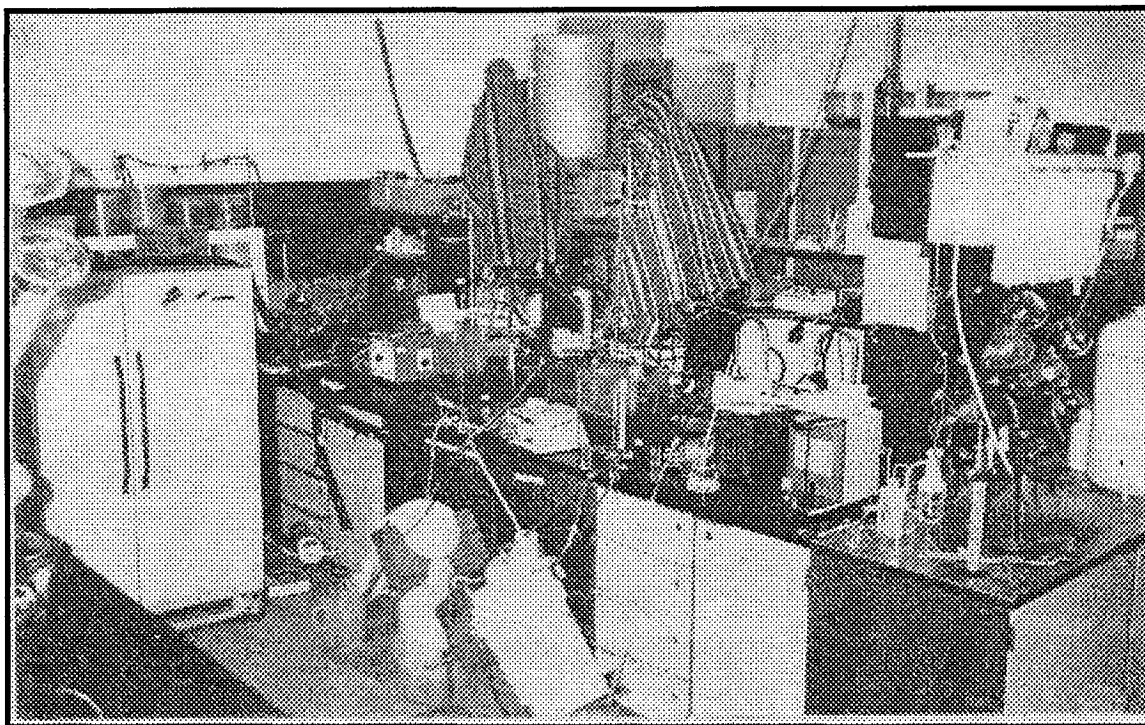


Figure 3.2: Combined Anaerobic-Aerobic Treatment System

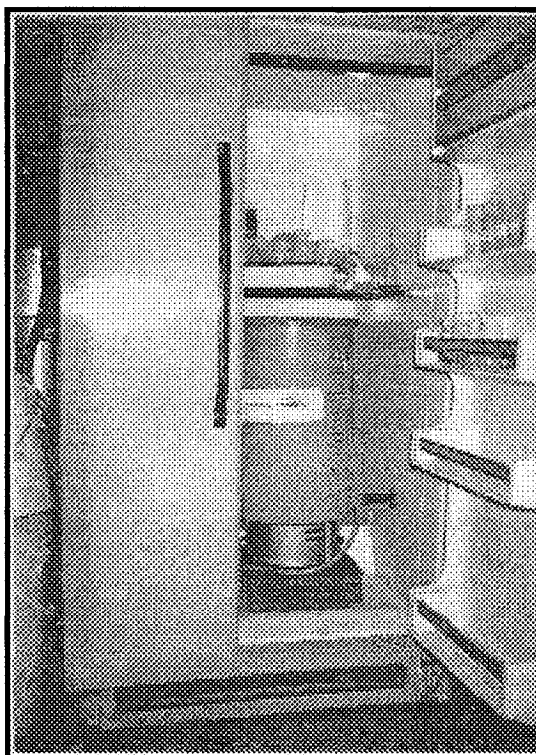


Figure 3.3: 26 L Refrigerated Feed Storage Tank

Once this occurred, the continuous recycling of TWAS, from the bottom of the clarifier buckets back to each of the UASB reactors for digestion (using variable speed Cole-Parmer (6-600 rpm) pump drives with Easy load II Masterflex® L/S™ heads) and to the head of each of the ABs, was initiated with 2 variable speed Cole-Parmer (6-600 rpm) pump drives [one of which had an Easy load II Masterflex® L/S™ head (AB1) and the other a Masterflex® standard pump head (AB2)]. The recirculation-to-feed flow ratio to both UASBs and ABs was approximately 2:1 (Grady and Lim, 1980; McCarty, 1964) and 0.5:1, respectively (Metcalf and Eddy, 1991; van Haandel et al., 1994). Most of the tubing used within the experimental set-up was the same size transparent Nalgene tubing as the main feed recycling line [0.79 cm ($\frac{5}{16}$ in.) ID]. This allowed observation of any blockages or flow interruptions. Short lengths of more durable 0.16 cm ($\frac{1}{16}$ in.) thick tubing were however used within all peristaltic pump heads. The yellow and easily replaceable pieces of Norprene® tubing, by Masterflex®, for the secondary feed lines had a 0.32 cm ($\frac{1}{8}$ in.) ID while Norton tubing used in the main feed pump head had a 0.95 cm ($\frac{3}{8}$ in.) ID.

Both UASB reactors also had continuous internal recycle lines (4:1) with draw-offs located below their effluent ports. They recycled sludge blanket supernatant back to each anaerobic reactor secondary feed lines, just prior to the inlet distribution system, using variable speed Cole-Parmer (6-600 rpm) pump drives; one of which had an Easy load II Masterflex® L/S™ head (AD2) and the other a Masterflex® standard pump head (AD1). Black Norprene® tubing of 0.79 cm ($\frac{5}{16}$ in.) ID was used in all recycle line pump heads. Influent feed was therefore introduced at the bottom of the UASBs through T-shaped liquid distributor inlets, to minimize channeling; with inputs from both the internal recycle lines and the lines recycling activated sludge back to the digesters from the CLs (Days 1-45). Except for sampling and discarding the daily aerobic supernatant effluent collected, activated sludge was not externally wasted from the system.

During continuous feeding, sludge granules from the anaerobic sludge beds (Section 3.1.2.2) often fell and accumulated in the AD influent feed lines despite their T-shaped inlets and upflow feed mode. Over time, Enfalac™ precipitates also formed and seemed to adhere to the main feed tubing line. To control build-up, main and secondary pump speeds were regularly quickly increased and returned to their operation settings. Once in a while, tubing was manipulated manually from the outside to dislodge attached materials. The agglomerates were however worst in the plastic connectors used within the tubing system. Sometimes, the feeding was shut-off for a brief time to clean the inside of the tubes and/or connectors with a brush or replace them with new ones. General set-up maintenance practices were also regularly done (i.e., aeration basin, feed storage tank and effluent collection bucket walls).

All the identical gas, liquid and solid sampling locations used within the 2 parallel set-ups during the study are identified on Fig. 3.1. The feed parameters listed in Table 3.3 (Section 3.2) were sampled, at X_1 , from the main feed line looping back to the refrigerated tank. This sampling location was assumed to better represent the state of the feed entering the UASBs. However, when a batch of fresh feed was added, it was sampled from well-mixed samples taken directly from the tank, to quantify its strength (or COD). For AD2, influent total and soluble COD feed samples (COD_t and COD_s) were taken at X_2 , from a T-shaped connector set after the dilution and feed line mixing point, but fairly close to the reactor inlet, to monitor the effect of the dilution. The pH and alkalinity were however not tested at this location and the influent suspended solids (SS) were taken as that of the feed tank samples, assuming they were equal or lower, to reduce the analyses load.

For both ADs, samples were withdrawn from the internal recycling line sampling port (X_3) for VFA analysis while they were taken from the off-gas line port (X_4) to determine the biogas composition. These two glass T-shaped sampling ports were directly inserted in the tubing and fitted with rubber septa to allow for syringe sample withdrawals. Anaerobic reactor effluent parameters (Table 3.3) were sampled from the bottom of the U-tubes at X_5 . This was done carefully to maintain a small amount of effluent liquid on either side of the tube at all times. Although distilled water was quickly added when needed, the tube did run dry in some instances allowing some air to enter the reactors for a brief moment, disturbing the anaerobic conditions.

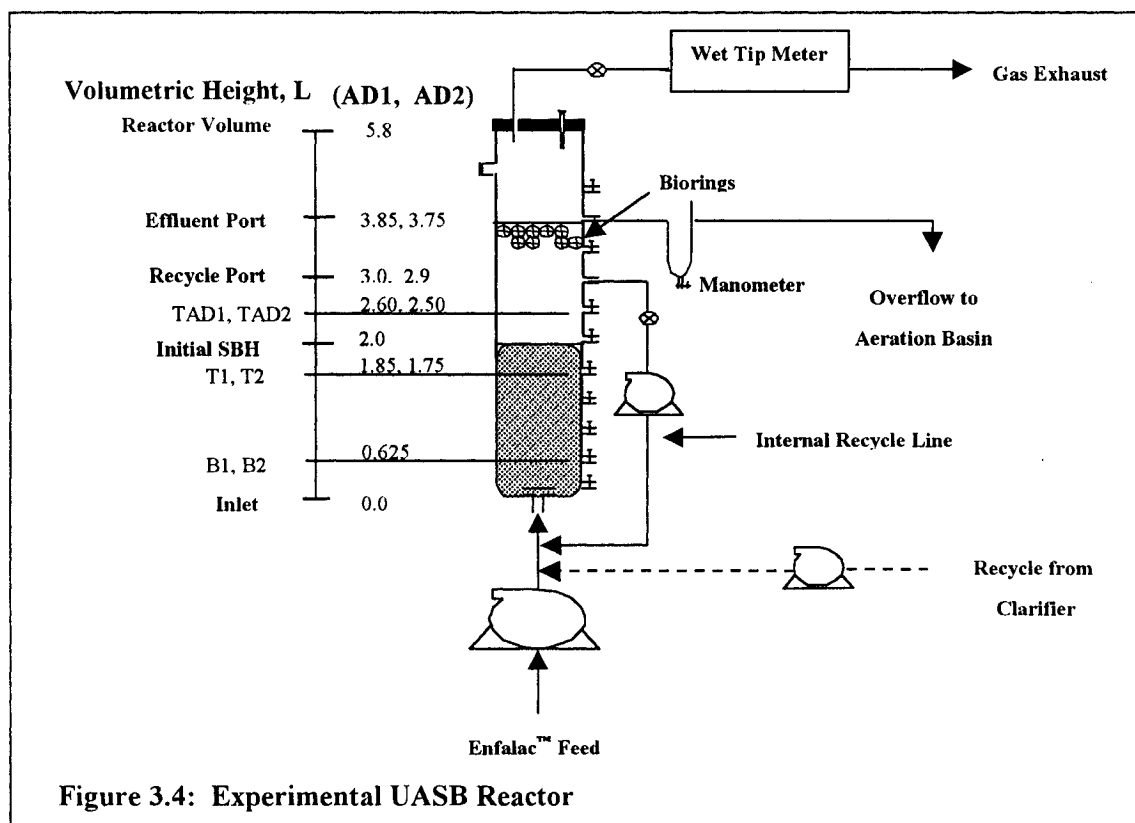
AB temperature and dissolved oxygen (DO) measurements as well as small sample volumes for pH, total alkalinity (TA) and SS were taken directly from the ABs (X_6). This was also done carefully to minimize disturbance of the basin overflows. Activated sludge effluent samples were taken from the effluent collection buckets (X_7), after measuring the volume collected to quantify daily flow rates. Finally, X_8 (B1 and B2), X_9 (T1 and T2) and X_{10} (TAD1 and TAD2), were the sampling ports used to collect anaerobic granular biomass profile data (Sections 3.1.1.1 and 3.2.3).

All reactors were kept on lab benches and operated at ambient temperature for the duration of the investigation. With no temperature control, the granular sludge was assumed to be at room temperature. However, the laboratory and refrigerator temperatures were monitored almost daily. The numerous peristaltic pumps used were calibrated before the start of the experiment, with the tubing size to be used. They were all equipped with built-in or external speed controllers for adjusting flow settings. The short lengths of tubing within the pump heads were also changed regularly to avoid overuse, causing leaks.

3.1.1.1 UASB Reactors

The cylindrical UASB reactors used in the laboratory study were made of glass and fitted with a rubber lid that was sealed tightly with the aid of vacuum grease and electrical tape (to create and maintain oxygen-free environments). The lids had 2 openings each; one connected the gas outlet to the wet tip gas meter, via Tygon tubing, while the other was clamped and sealed to allow access to the reactor if required.

Both reactors had similar configurations and a total volume of about 5.8 L. AD1 was 81 cm in height, 32 cm in circumference [10.18 cm (4 in.) ID] and had an approximate working volume of 3.85 L, taking the inoculum into account. Closely matched, AD2 had an approximate working volume of 3.75 L, a height of 82 cm and a 31 cm circumference [9.87 cm (3.9 in.) ID]. Large headspaces were therefore left in the reactors above the supernatant blankets. A schematic diagram of one of them is presented in Fig. 3.4.



Once vertically mounted to the laboratory benches with supports, both reactors were calibrated with water, prior to inoculation. A long piece of tape placed along the outside wall served as a graduated volumetric reference for the reactor contents. Initially, each reactor was given a settled granular sludge bed height (SBH) of 2 L, which represented approximately $\frac{1}{2}$ of

its operating volume and about $\frac{1}{3}$ of the total reactor volume. Both UASBs were equipped with 12 sampling ports throughout their height. Five of them were used and the others were sealed and tightly clamped with stopcocks. At the heights indicated, the 2nd and 5th ones from the bottom on Figure 3.4 were used to extract samples from the bottom (B1 and B2) and top (T1 and T2) of the beds in order to gather sludge profile data. When the sludge beds grew (after Day 113), the ports at TAD1 and TAD2 were used as the new sampling locations for the top of the beds, with T1 and T2 becoming intermediate ones. Constant efforts were made to keep the anaerobic reactors sealed as airtight as possible.

The highest ports, set at volumetric heights of 3.85 L for AD1 and 3.75 L for AD2, were used as the anaerobic reactor effluent outlets. These were fitted with U-tube manometers that acted as a liquid seal to prevent air from entering the anaerobic units. They had an opening to the atmosphere at the top (on one side) and were used as indicators of the reactor inner pressure. It should always be greater than atmospheric pressure, if biogas is being produced and the reactors have no leaks. Positive pressure could therefore be confirmed when the liquid level within a tube was uneven and lower on the reactor-side, usually by a few cm.

As mentioned in Section 3.1.1, an internal liquid circulating line was positioned at approximately $\frac{3}{4}$ of the working volumetric height of each reactor. Its main purpose was to provide mixing, to maintain better contact between the bacteria and to provide a more uniform environment within the bed. Therefore, the UASBs were considered as completely mixed (CM) bioreactors. In both ADs, a fairly high internal recycle to influent feed flow ratio of approximately 4:1 (Malaspina et al., 1995; van den Berg and Kennedy, 1983) was used initially for the 6 L/d (HRT $\approx$ 15 h) start-up flow.

Some channeling and floating sludge bed problems (i.e., significant cracks and SB expansion) were encountered very early. Therefore, instead of varying the UASB internal recycle flow rates proportionally with varying influent flows (by the above mentioned ratio), they were simply maintained to a constant less severe rate of approximately 8 L/d [Figures 3.5 c) – e)] for the remainder of the experiment. During the start-up period of large fluidized bed systems, initially operated at 24 m/h, van der Last and Lettinga (1992) had to reduce the superficial liquid velocities, at least by half, in order for the particles to remain within the reactors.

The small and numerous (1.5 cm ID) plastic biorings or flexirings floating at the surface of the sludge blanket, just below the effluent overflow level in Fig. 3.4, were used as a GLS separator device. They were put in place to retain biomass particles, by deflection, and acted as somewhat of a barrier in case of sludge bed expansion. Large pieces of cloth were eventually

used (Day 30) to cover the UASB reactors to prevent photo degradation (Kettunen et al., 1996) and further algae growth, which could produce oxygen, by photosynthesis (Chevalier, 1996). Up until then, they had unfortunately constantly been exposed to most of the laboratory lights, which could not be completely shut off, as well as sunlight from many nearby windows with no curtains.

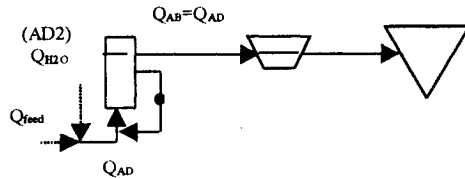


Figure 3.5 a): Day 1 to 2 – Start-Up (CLs filling; 11 L-CL1 started overflowing on Day 2; $Q_{AD} = Q_{feed} + Q_{H2O}$ for AD2)

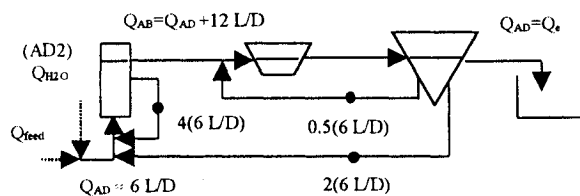


Figure 3.5 b): Days 2/3 to 17 - Acclimation (Recycles to ADs and 2 L-ABs on; Started on Day 3 for AD2 when 13 L-CL2 started overflowing)

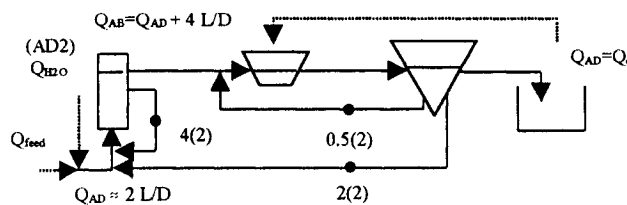


Figure 3.5 c): Reduced internal recycling rates to set 2 L/D flow for remainder of experiment because of cracks observed in sludge beds (floating beds). Days 18 to 45: Start of Run 1 (Day 23; tried out flow indicator in AD2 dilution line. Day 24; installed flow meter in AD1 dilution line for monitoring of CHT tap water input. Day 36; Started frequent manual batch addition of effluent bucket solids (usually settled overnight) to 2 L-ABs to maintain MLSS.

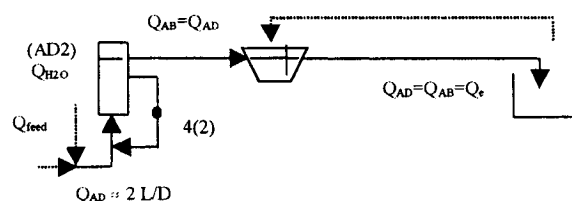


Figure 3.5 d): Day 45; Modified Activated Sludge Processes to 3.5 L-combined aeration + settling chambers separated by an adjustable baffle and continued effluent solids batch recycle to deeper 2.8 L-ABs. Day 51; Added fresh TWAS batch to ABs (aerated until Day 61 before Run 2).

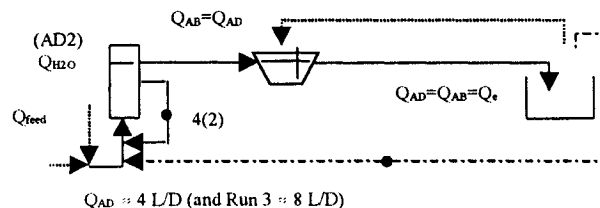


Figure 3.5 e): Day 62 – Start of Run 2 (Day 70; Started frequent batch recycle of effluent bucket solids to ADs). Day 114 – Start of Run 3 (~8 L/D). Day 131; AD1 U-tube removed after 3rd blockage problem due to floating sludge bed. AD1 effluent line connected directly to AB1 for remainder of Run 3 and until end of experiment (Day 146)

3.1.1.2 Activated Sludge Processes

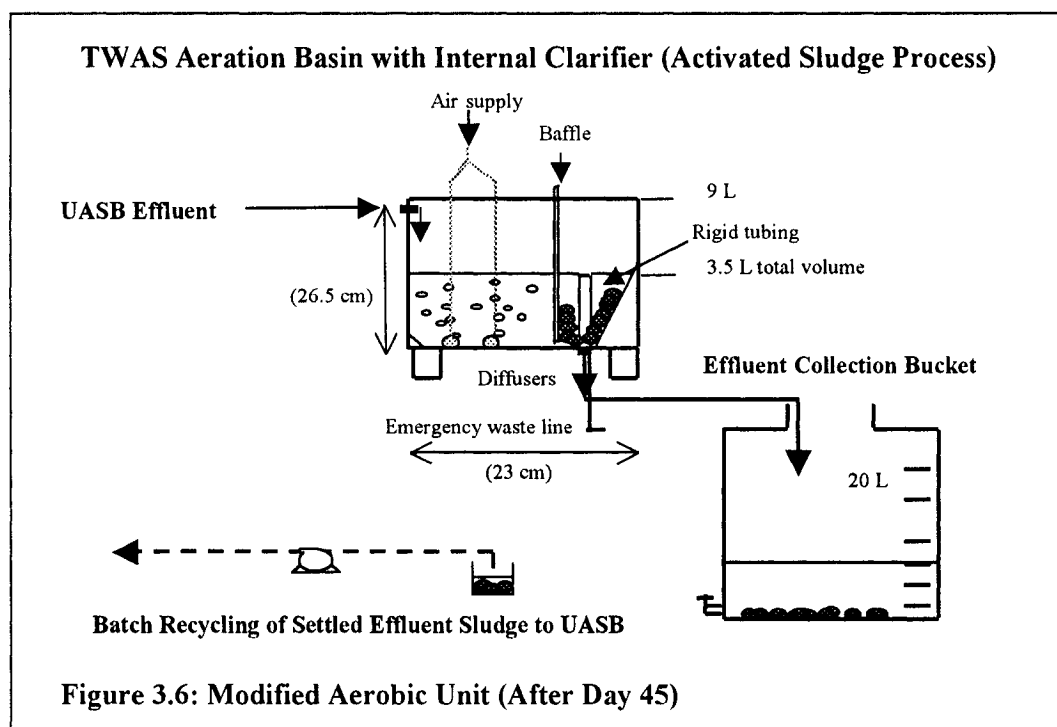
From Days 1 to 45, aerobic reactors used in the study to simulate a conventional activated sludge treatment consisted of separate aeration basins (AB1 and AB2) and clarifier units (Fig. 3.1, Section 3.1.1). The ABs, continuously fed from the UASB effluents, were made of Plexiglas. They were practically rectangular, except for a small slant at the bottom of the reactor on the influent side as well as a more important one on the effluent side. The settlers of the activated sludge systems were improvised from inclined and supported, standard 20 L plastic buckets yielding approximate volumes of 11 L and 13 L for CL1 and CL2, respectively. Prior to start-up, all the above containers were calibrated with water. The CLs, lightly covered with plastic lids, each had a piece of tubing attached at the bottom that ran parallel to them, on the outside, and acted as a graduated volumetric reference for their contents.

Both identical ABs were open to the atmosphere and had an approximate total volume of 9 L with approximately 23 cm in length, 26.5 cm in height and 16 cm in width. Since there was no port in place to accommodate the effluent, at a smaller volumetric height, a piece of 0.95 cm ($\frac{3}{8}$ in.) ID rigid plastic tubing, of the proper length, was inserted on a connector at the bottom of each basin to yield a 2 L working volume (Days 1-45). The important slant in the reactor wall, located close to it, somewhat promoted sludge settling and accumulation. The basins were initially seeded with 2 L of diluted TWAS, acting as mixed liquor (ML) (Section 3.1.2.3 and Table 4.3), which was aerated for about 3 days before the experiment start-up. The rod allowed TWAS supernatant from the surface to exit at the bottom and find its way to the bottom-fed effluent buckets, by gravity, once the CLs started overflowing (Section 3.1.1 and Figure 3.1).

Air introduced in the ABs, through split tubing lines attached to a single bench mounted air outlet valve, was distributed by 2 submerged diffuser stones per basin. In addition to aerobic conditions, it also supplied mixing inside the tanks to keep the ML solids in suspension. DO levels in the basins were periodically monitored with a DO meter (Section 3.2.1.2a). Although the airflow rate was not quantified, at least 2 mg O_2 /L were maintained in the basins at all times (Chevalier, 1996; Droste, 1997b; Langenhoff and Stuckey, 2000). Reactor walls were also frequently cleaned with a brush to prevent biofilm (or attached) growth. On Day 22, numerous mosquito larvae were found in CL1; some of which were swimming around in it. They were carefully strained and removed.

The TWAS concentration and the suspension of solids in both ABs quickly deteriorated, regardless of airflow vigor adjustments and other modifications performed (i.e., less or more diffuser stones and their placement in the basin). Subsequently to Day 36 (Aug. 28), after

measuring the volume of effluent collected and discarding the supernatant, solids from the collection buckets, left to settle in large containers, were manually poured into the aerated portion of the basins practically every day to maintain their solids content. However, after Day 45, due to washout problems (*Appendix L.4*), the activated sludge process configuration was altered to try to improve the situation. A schematic diagram depicting the modified process is presented in Fig. 3.6. The emergency waste line was only used to empty the basins.



The large separate clarifier units were abandoned and a 6 mm solid Plexiglas baffle wall was inserted in each basin. With longer rigid rods put in place, the new AB and internal settler volumes were approximately 2.8 L and 0.7 L, respectively, yielding an increased total basin volume of 3.5 L. Thus, each activated sludge process became a joint aeration-clarifier unit or an “extended aeration” system with internal clarifier. The mixing within the AB was to recycle solids from the clarifier chamber (Droste, 1997b). The basins were temporarily refilled with their respective contents before modifications (until Day 52) in addition to some settled solids from the larger abandoned CLs to increase their concentrations.

From then (Day 45), the aerobic effluents flowed directly into the effluent collection buckets and the continuous activated sludge recycle lines back to the ADs as well as the ones to the head of the ABs were abandoned [Figure 3.5 d)]. If continuous feeding had to be interrupted, for a day or more, for operational or other reasons, evaporation was also noticeable in the ABs. Thus, before restarting the treatment systems, the basins were filled to the AB overflow levels

with well-mixed settled effluent from the collection buckets. On Day 52, a new batch of TWAS, of about the same initial diluted concentration (Table 4.3), was used to seed the deeper 3.5 L basins. It was left to aerate for several days, prior to the start of Run 2 (Day 62) affected by a flow rate increase (~ 4 L/d). Although slight overall improvements were noticed after the various changes, ML concentrations returned to fairly dilute levels. Minor airflow rate and baffle height adjustment attempts were still made over time to better maintain the solid suspension in the basins.

From Day 70 onward, activated sludge from the effluent collection buckets, usually settled overnight, started to be measured out, sampled and recycled back to the UASB reactors in *batch mode* [Figure 3.5 e)]. This was performed in a few minutes, about 3 times a week, using the previous continuous mode recycling pumps (Section 3.1.1). Initially, the volume of sludge recycled (*Appendix L.5*) was based on 2% of the influent flow rate and then on 10% of the reactor volume (~ 350 mL). The rest of the effluent bucket settled solids was still regularly added to the ABs, since their solids concentration remained very low. No substantial amount of sludge was otherwise wasted from the system externally.

3.1.2 MATERIALS USED TO FEED AND SEED ALL REACTORS

3.1.2.1 Enfalac™ Feed Mixture

Iron Fortified Enfalac™ infant formula, manufactured by *Mead Johnson Nutritionals™* [a division of Bristol-Myers Canada Inc. (Ottawa)], was the main component of the feed used in this experiment to simulate low-strength dairy wastewater. Expired products (17 g Easy One powder packets) were generously supplied by the company's regional office, located in Ottawa.

The carbohydrate-rich feedstock used was believed to be an easily degradable but complex waste that could provide a satisfactory substrate for the experimental purposes. Because of its advantageous powder form, it could be manipulated easily and weighed with precision for the repetitious preparations. Feed was also only made when required to keep it as fresh as possible. It was hoped that its form and nature as well as its refrigeration would maintain its composition and desired strength relatively constant.

The following table (Table 3.1) as well as the ingredient, mineral and vitamin listing, reproduced directly from the *Mead Johnson Nutritionals™* product packaging, gives the content description of the 17 g Iron Fortified Enfalac™ infant formula packets (used to prepare standard 120 mL baby bottles).

Table 3.1: Composition of the Iron Fortified Enfalac™ Infant Formula

<i>*Iron Fortified Enfalac™</i>	Units	Per 100 g	Normal Dilution (100 mL)
Protein	g	11.2	1.43
Carbohydrate	g	54	6.9
Fat	g	29.4	3.8
Linoleic Acid	g	4.76	0.61
Linolenic Acid	g	0.5	0.06
Ash	g	2.3	0.3
Energy	Kcal	525	67
	KJ	2194	281
Vitamin A	I.U.	1560	200
Vitamin D	I.U.	312	40
Vitamin E	I.U.	7.8	1.0
Vitamin C	mg	43	5.5
Vitamin B ₁	mg	0.39	0.05
Vitamin B ₂	mg	0.47	0.06
Niacin	mg	6.2	0.8
Vitamin B ₆	mg	0.31	0.04
Folic Acid	mg	0.039	0.005
d-Pantothenic acid	mg	2.3	0.3
Vitamin B ₁₂	mcg	1.2	0.15
Vitamin K	mg	0.055	0.007
Biotin	mg	0.012	0.002
Sodium	mg	125	16
Potassium	mg	540	69
Chloride	mg	330	42
Calcium	mg	350	45
Phosphorus	mg	233	30
Magnesium	mg	35	4.5
Iron	mg	5.5	0.7
Iodine	mg	0.035	0.004
Copper	mg	0.32	0.04
Zinc	mg	3	0.4
Manganese	mg	0.027	0.003
Choline	mg	63	8.1
Taurine	mg	31	4.0
Nucleotides	mg	22	2.8

**Mead Johnson Nutritionals™*

Ingredients: lactose, skim milk powder, palm olein oil, whey protein concentrate, soy oil, coconut oil, sunflower oil, corn syrup solids, maltodextrin, taurine, choline chloride, ascorbyl palmitate, cytidine-5'-monophosphate, disodium guanosine 5'-monophosphate, disodium uridine 5'-monophosphate, adenosine 5'-monophosphate,

Minerals (calcium chloride, calcium hydroxide, cupric sulfate, ferrous sulfate, magnesium chloride, manganese sulfate, potassium bicarbonate, potassium citrate, sodium iodide, sodium citrate and zinc sulfate),

Vitamins (ascorbic acid, biotin, calcium d-pantothenate, cholecalciferol, dl-alpha-tocopheryl acetate, folic acid, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, vitamin A palmitate, vitamin B₁₂ and vitamin K₁), and soybean lecithin.

Based on average experimental results, Table 4.1 (Section 4.1.1) further characterizes the un-supplemented composition of the simulated dairy substrate prepared to 1 000 mg COD_L.

During the entire course of the experiment, a 20 L bucket was used to prepare and mix the dairy waste. It was made up of Enfalac™ powder dissolved in tap water and the appropriate amounts of N and P additives (*Appendix B*) to maintain a COD: N: P ratio of 100: 5: 1 (Section 4.1.1). Contrary to the nutrient supplements, sodium bicarbonate (NaHCO₃) was *not* always used to provide additional alkalinity to the feed. It was only added, on an as needed basis, to maintain UASB effluent pH near neutral values.

The proper amounts of Enfalac™ (Section 4.1.1) were weighed with a Sartorius analytical balance (capable of weighing to 0.01 mg). Nutrient chemical supplements were weighed with a Mettler AJ150 analytical balance (0.1 mg sensitivity) while a Mettler P1210 analytical balance (10 mg sensitivity) was used to weigh the NaHCO₃ supplement. Weighing dishes and beakers were always rinsed with distilled water to ensure complete addition of the reagents. Once all ingredients were added, the bucket content was stirred before and after transferring it to the refrigerated feed storage tank, although the faucet pressure provided good preliminary mixing. When a 26 L feed batch was prepared, an additional 6 L of tap water was added to the container to fill it completely. The quantity of fresh feed, the day and time at which it was added as well as the amount of NaHCO₃ and nutrient supplements added were always recorded.

Feed changes were most often done during SBH measurements (Section 3.2.1.1g), already requiring an imposed feeding shutdown. Once a new mixture was prepared, feed tank replenishment usually only took about 10 minutes to complete. The process consisted of emptying the small amount of feed left in the container, rinsing it with distilled water, cleaning its walls with a brush, refilling it with fresh feed, stirring it and finally, putting the reactors back into operation. The secondary feed pumps were always temporarily shut down, along with the main feed pump, while the anaerobic internal recycle lines were usually left on. Because the 2 systems were fed from the same relatively small-sized tank (26 L), with increasing flow rate conditions, the feed was rarely left in the tank for over 3 days. Feed replacement was scheduled so that the refrigerated storage tank never ran dry, except for the odd mishap. Samples were usually collected immediately and the process was frequently repeated as consistently as possible.

It was difficult to maintain the homogeneity of the slightly white, milky and mainly soluble substrate solution. Once transferred in the feed tank, Enfalac™ feed particles would often settle at the bottom of the tank or accumulate within the main feed line tubing. In addition to the initial faucet pressure, the looping of the feed back into the refrigerator and some manual stirring, other

efforts were exerted to try to insure complete mixing of the feed tank. Before filling it, on Day 36, the entire volume of fresh feed was passed through a Waring commercial blender, in small portions, to investigate any benefits. This did not seem to prevent precipitation. Finally, on Day 39, a large Mag-Mix GCA/Precision Scientific magnetic stirrer (Fig. 3.3) and large 12.7 x 1.59 cm (5 x 5/8 in.) Spinbar® stirring bar were placed under and inside the tank, respectively. Although still not ideal, this medium speed continuous mixing technique made some improvement and was maintained thereafter.

3.1.2.2 Anaerobic Granular Sludge Inoculum

Active anaerobic granular sludge from an existing full-scale mesophilic digester unit at the *Lake Utopia Paper* chemical/thermal/mechanical pulp treatment plant (an Irving Forest Products Company), located in St-George, New Brunswick (Canada), was used to inoculate both experimental UASB reactors. This easily obtainable material has been employed frequently and successfully, as an inoculum source, in many anaerobic reactor experiments (University of Ottawa). It was therefore assumed to possess microorganisms that could degrade the organic compounds in the synthetic dairy wastewater and to give good COD removal rates, in a relatively short start-up period, due to its granular state.

A fresh anaerobic granular sludge shipment was received on April 1, 2000, and stored at room temperature until needed. It underwent a few initial batch tests (*Appendix L.10, L.11 and L.12*) and some degree of characterization prior to start-up and operation to assess its initial state (Table 4.2 in Section 4.1.1). As mentioned in Section 3.1.1.1, 2 L of settled granular sludge, not acclimated to the feed, were used to seed each UASB reactor. Initially, the soft biomass granules were gray to dark gray and had dark brown supernatant. An acclimation period of approximately 17 days was used to adapt the granules to the new wastewater (Section 3.1.3.1).

3.1.2.3 Thickened Waste Activated Sludge

TWAS from the *Robert O. Pickard Environmental Center* (ROPEC) located in Gloucester (Canada), was used as ML to seed the ABs of the activated sludge processes. On March 3, 2000, approximately 20 L of it were collected at this municipal wastewater treatment facility. It was sampled prior to the anaerobic digestion of the municipal sewage, but after the centrifuging process, where WAS from the bottom of the secondary clarifiers had been thickened.

The sample was brought to the environmental lab at the University of Ottawa, stored in a 4°C cold room until needed and somewhat characterized prior to start-up (Table 4.3 in Section

4.1.1). The raw TWAS was diluted with tap water to achieve a desired ML concentration of approximately 1 500 mg TSS/L (Droste, 1997b; Tchobanoglous and Schroeder, 1985). Also, in a study conducted by Cocci et al. (1990a) with a full-scale low-rate ADI-BVF digester followed by an aerobic system treating a confectionary waste, the aeration tank MLSS typically ranged from 750 to 1 000 mg/L.

3.1.3 *SYSTEM OPERATING CONDITIONS*

The entire laboratory investigation, including the UASB acclimation period, was conducted at ambient temperature, over approximately 5 months (146 days), from July 24 to December 16, 2000. The anaerobic reactors were always fed the dilute synthetic dairy wastewater described in Section 3.1.2.1. All reactors were operated in a continuous mode throughout the study and downtime tried to be minimized, even between runs. However, several shutdown periods were unavoidable because of feed replenishment and SBH measurements while others took place due to set-up maintenance and operational problems in addition to a few general building power shut-offs [Aug. 3 (11 pm-6 am) and Sept. 1 (8 am-8 pm)]. Also, a few pump breakdowns occurred early on. Therefore, the systems had some unforeseen feedless periods during the experimental time course (*Appendix L.2 and L.5*).

The bench-scale study was initiated with an acclimation period, prior to the application of the full-strength waste, to allow the biomass to adapt to the dairy substrate being treated. It was followed by three different periods of maintained operating conditions, where feed flow rates were progressively increased. A number of routine anaerobic and aerobic process parameters (Section 3.2) were monitored throughout the runs to assess general reactor performance and response to condition changes. Among the former, reactor pH and VFAs were closely monitored in order to detect potential system upsets.

3.1.3.1 Reactor Acclimation and Start-Up

Following the characterization of the biomaterials used (Section 4.1.1, CH. 4) and reactor inoculation with seed sludges (Sections 3.1.2.2 and 3.1.2.3), the UASBs underwent a conditioning period during the first 17 days of operation, which was considered reasonable given the low influent waste strengths and applied OLRs targeted (Tables 3.2 and 4.4). Before feeding was instigated, the granular sludge was left to settle (with intermittent internal recycle) and the TWAS was allowed to aerate for about 3 days.

During the start-up process, the ADs were to operate at a 6 L/d influent flow rate, resulting in an initial HRT of approximately 15 h. Thus (after Days 2 and 3), the ABs would have an initial HRT ($= V_{AB}/Q_{AD\text{ Effl.}}$) of about 2.7 h [Fig. 3.5 b)]. However, the incremental influent feed strength increases were aimed to range from 100 to the desired 1 000 mg COD_i/L concentration [Tables L.3 a i) in *Appendix L.3*] for AD1 and ideally, from 50 to the intended 500 mg COD_i/L target for AD2. Thus, the corresponding low OLRs applied initially to the UASBs were expected to range from approximately 0.16-1.6 g COD_i/L·d for AD1 and 0.08-0.8 g COD_i/L·d for the more dilute AD2 reactor [Figures 4.2 a) and b)].

Although not presented herein, two start-ups conducted with the same dilute dairy waste (in March and April 2000) failed prior to the 5-month study. Both were halted within the first 10 days due to major sludge bed buoyancy as well as biomass flotation and washout problems. The granules were observed to have become gelatinous and much lighter in color (Heertjes and van der Meer, 1979) with much Enfalac™ particles entrapped within the bed, especially at the bottom. The beds rose as a unit with much biomass going into the aerobic basins. Solids washout increased the total effluent COD concentrations to values considerably higher than that of the influent. Due to these operational failures, the reactors were shut down and the experiment brought to an end.

Smaller scale (~1.5 L) and smaller diameter units (adapted from fluidized bed reactors), older granular seed sludge from the same source (left over in the lab for some time), shallower SBHs (~0.5 L) and higher influent feed flow rates (Droste et al., 1987), in the 20 L/d range, might all have been contributing factors to the malfunctions encountered. The second unsatisfactory start-up was conducted using a different batch of lab-maintained granular sludge (from the same source) that was batch fed and acclimatized on aircraft deicing fluid at the University of Ottawa. High flow rates (or upflow velocities) were dismissed as a cause for the floating sludge in the UASBs since it even occurred after overnight settling, when pumping and internal recycling were completely shut off. Therefore, prior to the start of the official study, an ATA test (Section 4.1.2) was performed to assess potential inhibition effects of the experimental substrate on a fresh batch of un-acclimated granular biomass, which was eventually used to re-inoculate the larger sized UASB reactors used.

3.1.3.2 Maintained Influent Flow Rate Operation

Once the target 1 g COD_r/L influent substrate concentration was reached, contrary to the start-up, the intention was to keep it constant throughout the remainder of the experiment, to progressively reduce the HRTs and to determine UASB and overall system effluent characteristics, at the different influent organic loading rates. Chemical supplements were added to the feed for anaerobic reactor pH and nutrient control (Sections 3.1.2.1, 4.1.1 and *Appendix B*). In the case of the former chemical additions, they were adjusted, as required, throughout the study (*Appendix L.2*).

Feed concentration and HRT were the main focus points of the research project. Two different feed concentrations [100% feed strength (AD1) and 50% feed strength (AD2)] could be examined at the same time by using two parallel treatment systems fed in series. For the duration of the study, a 1 000 mg COD_r/L concentration was to be maintained in AD1 while 500 mg COD_r/L was aimed for in AD2, by dilution. Although they were held for reasonable time intervals, only a few anaerobic and aerobic reactor HRTs were investigated mainly due to the operational problems encountered. The target feed concentration and similar HRT conditions of the two treatment systems during the 3 experimental runs carried out are summarized in Table 3.2.

Table 3.2: Targeted Operating Conditions for the Continuously Fed Anaerobic-Aerobic Systems

Anaerobic/Aerobic Systems	Acclimation/Start-Up Days 1-17 (~6 L/d)	*Run 1: Days 18-61 (~2 L/d)	Run 2: Days 62-113 (~4 L/d)	Run 3: Days 114 -146 (~8 L/d)
AD1-AB1 (100% feed strength)	~100-1 000 mg COD _r /L $\theta_{d AD1} = 0.64$ d $\theta_{d AB1} = ^s0.11$ d	1 000 mg COD _r /L $\theta_{d AD1} = 1.93$ d $\theta_{d AB1} = ^s0.33$ d; 1.4 d	1 000 mg COD _r /L $\theta_{d AD1} = 0.96$ d $\theta_{d AB1} = 0.70$ d	1 000 mg COD _r /L $\theta_{d AD1} = 0.48$ d $\theta_{d AB1} = 0.35$ d
Total System 1	$\theta_{d AD1-AB1} = 0.75$ d	$\theta_{d AD1-AB1} = 2.26$ d, 3.33 d	$\theta_{d AD1-AB1} = 1.66$ d	$\theta_{d AD1-AB1} = 0.83$ d
AD2-AB2 (50% feed strength)	~50-500 mg COD _r /L $\theta_{d AD2} = 0.63$ d $\theta_{d AB2} = ^s0.11$ d	500 mg COD _r /L $\theta_{d AD2} = 1.88$ d $\theta_{d AB2} = ^s0.33$ d; 1.4 d	500 mg COD _r /L $\theta_{d AD2} = 0.94$ d $\theta_{d AB2} = 0.70$ d	500 mg COD _r /L $\theta_{d AD2} = 0.47$ d $\theta_{d AB2} = 0.35$ d
Total System 2	$\theta_{d AD2-AB2} = 0.74$ d	$\theta_{d AD2-AB2} = 2.21$ d, 3.28 d	$\theta_{d AD2-AB2} = 1.64$ d	$\theta_{d AD2-AB2} = 0.82$ d

*During Run 1, the 2nd θ_d listed for the ABs is when the aerobic basin volume was increased to ~2.8 L, on Day 45 (θ_d =Nominal HRT).

^sHRT calculations for the ABs during Acclimation and Run 1 take the recycle flow rate from the CLs to the UASBs into account.

From fear of increasing the channeling and biomass flotation problems encountered in the UASBs during the acclimation period, it was thought best to apply less severe influent hydraulic conditions over the subsequent runs (Section 4.3.3). As a result, successively increasing influent flow rates corresponded to 2, 4 and 8 L/d for Runs 1-3, respectively. However, transitional flows led to the desired flow rates. Thus, the anaerobic reactor HRTs were decreased from approximately 2 days to 0.5 day (12 h) with corresponding OLRs ranging between approximately 0.5-2 g COD/L·d and 0.25-1 g COD/L·d for AD1 and AD2, respectively [Table 4.4 and Figures 4.2 a) and b)]. However, the continuous or batch-recycled return activated sludge from the aerobic processes [Figures 3.5 b) – e)] are not included in the anaerobic loading estimate ranges reported for the acclimation or maintained condition phases. In addition, the constant state flow periods (that allowed somewhat of a stable operating performance to develop within the reactors) were held for a different amount of days during the various run lengths (*Appendix L.5* and Section 4.2).

Over the experiment time course, a few changes were made in terms of recycling as well as with respect to the aerobic portion of the set-up [Figures 3.5 a) – e)]. Two values are reported for the ABs in Table 3.2 since the modifications impacted their HRTs. The experimental approach also did not include run replicates due to time constraints. It rather focused on observing the behavior and the performance of the systems and to compare them over the range of operating conditions. The anaerobic reactor biomass analyses (Section 3.2.3), excluding the ATA, were performed at the end of each run, but not at the end of the acclimation period. However, they were conducted during the initial characterization of the granular sludge, prior to start-up.

Although larger scale and larger diameter reactors, fresh granular sludge as well as decreased flows and greater SBHs were used (~50% of the total operating volume) in the 5-month study, some problematic sludge behavior still occurred within the UASBs, to a lesser extent and much later into the experiment, compared to the previous failed start-up attempts. Flow rates effects were also ruled out, for the same reason stated in Section 3.1.3.1. Overall, AD1 was the more affected of the two anaerobic reactors. The bed developed important cracks throughout the course of the study (including the start-up period), eventually split and separated from the bottom portion during Run 3. Due to floating sludge, on Day 131, its U-tube had to be completely removed after persistent blockages caused continual spillage. Therefore, AD1's effluent overflow was directly connected to AB1 until the final days of operation, which were quick to follow because of uncontrollable and further reactor performance deterioration.

3.2 EXPERIMENTAL ANALYSES

The following sections describe the testing regime and all the analytical techniques conducted prior to and during the study. Sampling and analyses were performed according to a 7-day week throughout the continuous operation of the experiment. Results are presented in Chapter 4 and most experimental data are in *Appendix L*. Table 3.3 shows the approximate sampling and testing schedule followed throughout the study on all components of the experimental system. However, analysis frequencies deviated somewhat from this, mostly during the start-up/acclimation period, transient periods and the last experimental run (Run 3).

Table 3.3: Testing Schedule of the Continuously Operated Anaerobic-Aerobic Systems

Parameter	* Feed	Anaerobic Reactor	Anaerobic Reactor Effluent (Aeration Tank Influent)	AB Content / Activated Sludge Effluent
pH	~ Every 2 days	*	~ [§] Every 2 days	(Spot checks)
TA	2-3 times/week	*	2-3 times/week	(Spot checks)
COD _t and COD _s	2-3 times/week	*	2-3 times/week	2-3 times/week
TSS/VSS	2-3 times/week	*	2-3 times/week	Basin: ~3 times/week Effl.: ~3 times/week
VFAs	(tested once)	3-5 times/week	-	-
Biogas Production	-	Daily	-	-
Biogas Quality	-	3 times/week	-	-
SBH (during settling)	-	Weekly	-	-
DO/AB Temperature	-	-	-	Checks, 3 times/week
Temperature (refrigerator & room)	Daily	-	[†] Daily	-
Flow Rates	-	Daily (from activated sludge effluents)	-	Daily
AAT Profile	-	After each constant-state period	-	-
SS Profile	-	After each constant-state period	-	-
Settleability Profile	-	After each constant-state period	-	-

* Nutrient supplemented and pH adjusted feed, *During characterization, on initial supernatant and/or biomass (Chapter 4).

[§] Assumed to represent the pH within the UASB reactors, Daily ≈ 7 times/week, [†] Ambient laboratory temperature

3.2.1 ANALYTICAL TECHNIQUES PERFORMED ON REACTOR CONTENTS/EFFLUENT SAMPLES

All samples were analyzed with equipment available in the Water Pollution Laboratories at the University of Ottawa. Care was put into the sampling, preparation of solutions and standards, storage of samples and solutions as well as the cleanliness of the glassware used. Preservation techniques and procedures were followed as closely as possible for all parameters. Refrigeration was however the main preservation method used.

Table 3.4 summarizes all the parameters monitored regularly from influent feed and effluent samples of the combined anaerobic-aerobic systems during the experiment, as well as a few parameters testing the contents of the reactors. Sampling locations and details of the various parameters tested, with respect to the different type of reactors are discussed in Section 3.1.1, Table 3.3, Section 3.1.1.1 (UASB Reactors), Section 3.1.1.2 (Aerobic Reactors) and Section 3.2.3 (Analyses Performed on the Anaerobic Granular Biomass). Sample volumes of all parameters analyzed were kept to a minimum throughout to cause as little disturbance as possible to the small-scaled treatment systems.

In addition to the first 6 parameters listed in Table 3.4, the parameters summarized in Table 3.5 were only performed on representative feed samples prior to the beginning of the experiment. Extensive preliminary tests were conducted to characterize the composition of the 1 000 mg COD/L Enfalac™ feed mixture, when no chemical supplements were added, either for nutrient or pH adjustments. The amount of nutrient chemical supplements to be added to the feed (*Appendix B*) to maintain an adequate COD: N: P ratio (Section 4.1.1) was calculated from the characterization results presented in Table 4.1 (Chapter 4).

Table 3.4: Summary of the Analytical Techniques Performed on Influent, Reactor Content and Effluent Samples During Continuous Operation

Parameter	Method ³	Specific Apparatus	V (mL)
pH	4500-H ⁺ B. Electrometric	Fisher Accumet® Model 320 pH Meter and Fisher Scientific Flexa-Mix magnetic stirrer	12-40
TA	2320 B. Titration	Fisher Accumet® Model 320 pH Meter, 25 mL buret and Fisher Scientific Flexa-Mix magnetic stirrer	12-40
COD _t (*COD _s)	5220 C. Titrimetric *Closed Reflux	Blue M Electric Company Stabil-Therm Gravity 150°C oven, COD digestion tubes	0.5-5 (dilutions)
COD _s (*COD _t)	5220 B. 4.b. *Low-Level	Same as above plus International Equipment Co. Model B-20 High Speed Refrigerated Centrifuge	0.5-5 (dilutions)
TSS	2540 D. (103-105°C)	1.2 µm GF/C glass fiber filters, filtration apparatus, aluminum dishes, 105°C Fisher Isotemp® drying oven, dessicators, Sartorius analytical scale (0.01 mg)	5-100
VSS	2540 E. (550°C)	Same as TSS except Model 186A 550°C Fisher Scientific Isotemp® muffle furnace	5-100
VFAs	Ackman (1972), C. M. Elliott (1989)	HP 5840A Capillary column GC and terminal integrator, HP 7672A Autosampler, Model 4700 Eppendorf ejector pipet, Brinkmann Model 5415 mini-centrifuge	0.5
Biogas Production	NA	Wet tip meters and counters	NA (mL/tip)
Biogas Composition	van Huyssteen (1967), C. M. Elliott (1989)	HP 5710A GC and HP 3396A Integrator, 1 mL disposable syringe	0.5
SBH	Visual estimate	Calibrated volumetric graduations	NA
DO and Temperature	4500-O G. Membrane Electrode	ORION Model 860 DO meter and probe	(in ABs)
*Room & Refrigerator Temp.	2550 B. 1. Laboratory	Mercury-filled Celsius thermometer	NA
*Flow Rates	Volumetric graduation	Graduated cylinders	-
ATA	L. Y. Young in K. J. Kennedy (1999)	160 mL serum bottles, rubber septa, aluminum crimp caps, Lab Line instruments Junior Orbit shaker, manometric biogas measurement device	20
AAT	K. J. Kennedy (1999)	Same as VFA	10
SS	2540 D. and 2540 E.	Same as TSS and VSS	10
Settling Test	K. J. Kennedy (1999), E. Andras et al. (1989)	Filtration apparatus, 19 mm dia. glass tube, Whatman® 185 mm dia/Grade 1 filter papers (11 µm pore), 105°C Fisher Isotemp® drying oven, dessicators, Sartorius analytical scale (0.01 mg)	10

NA–Not Applicable, *Classified as other parameters in Section 3.2.1

³ *Standard Methods for the Examination of Water and Wastewater* (1995), 19th ed., American Public Health Association, Washington, DC. (unless stated otherwise)

Table 3.5: Summary of the Additional Analytical Techniques Only Performed on the Feed During its Characterization

Parameter	Method ³	Specific Apparatus	V (mL)
TDS/VDS	2540 C. and 2540 E.	Same as TSS and VSS, except for porcelain evaporating dishes and 180°C Blue M Electric Company Stabil-Therm Gravity oven	50
TS/VS	2540 B. and 2540 E.	Porcelain evaporating dishes, 105°C Fisher Isotemp® drying oven, dessicators, Model 186A 550°C Fisher Scientific Isotemp® muffle furnace, Sartorius analytical balance (0.01 mg)	100
(%) TS/VS in solid samples	2540 G. 3.b.	Same as TS/VS except for Mettler P1210 analytical balance	(25 g)
TP	4500-P B. 5.c. Persulfate Digestion and 4500-P D. Stannous Chloride	125 mL Erlenmeyers, 100 mL volumetric flasks Market Forge Sterilmatic Autoclave, Bausch and Lomb Spectronic 70 Spectrophotometer	50 (dilutions)
TDP	4500-P B. 5.c. Persulfate Digestion and 4500-P D. Stannous Chloride	1.2 µm GF/C glass fiber filters, 0.45 µm membrane filters, filtration apparatus (glass membrane filter funnel, fritted glass support), 125 mL Erlenmeyers, Market Forge Sterilmatic Autoclave, Bausch and Lomb Spectronic 70 Spectrophotometer	50 (dilutions)
BOD ₅	5210 B. 5-Day BOD Test and 4500-O G. Membrane Electrode	Drummond Scientific pipet-aid®, 300 mL BOD bottles, VWR Scientific Model 2020 (20 ± 1°C) incubator, ORION Model 860 DO Meter and electrode (overflow funnel, fixing ring, rotor stirring accessory), Fisher Scientific Flexa-mix magnetic stirrer	0.5, 1, 2, 3, 6, 10
TKN	4500-N _{org} C. Semi-Micro Kjeldahl	BÜCHI 430 Digestor, BÜCHI 342 Control unit, BÜCHI 322 Distillation unit, METROHM 614 Impulsomat, 300 mL BÜCHI digestion vessels, METROHM 632 pH meter and METROHM 655 Dosimat	50
org. N	4500-N _{org} C. 4.b. Semi-Micro Kjeldahl	Same as TKN plus Fisher Accumet® Model 320 pH Meter (pH adjusted to 9.5)	50
NH ₃ /NH ₃ -N	1- 4500-NH ₃ B. and C. (Direct distillation and titration) and 2- 4500-NH ₃ D. Ammonia-Selective Electrode by Direct Calibration and Low-Level	Same as TKN less BÜCHI 430 Digestor and ORION Model 95-12 Ammonia Electrode, Fisher Accumet Model 750 pH/Ion Meter, American Dade Tek-Pro® Tek-Stir® Magnetic Stirrer	25 50 (50:1 ratio)
NO ₃ /NO ₃ -N	4500-NO ₃ ⁻ D. Nitrate Electrode by Direct Measurement and Low-Level	Same as NH ₃ , except for ORION Model 93-07 Nitrate Electrode, ORION Model 90-02 Double Junction Reference Electrode	25, 50 (50:1 ratio)

³ *Standard Methods for the Examination of Water and Wastewater* (1995), 19th ed., American Public Health Association, Washington, DC. (unless stated otherwise)

3.2.1.1 UASB Reactors (Contents and Effluents)

VFAs, biogas production and composition, pH, TA as well as settled SBHs were used to monitor the content and state of the ADs while UASB effluent quality was determined from COD_t and COD_s and TSS and VSS.

During operation, samples for pH, TA and the last parameters mentioned were collected from X₅, the port located at the bottom of the effluent-filled U-tubes (Fig. 3.1). It was assumed that pH and TA samples, taken from this sampling location, represented the sludge blanket liquid within the anaerobic reactors. Considering set-up limitations, this was one of the only ways to sample the anaerobic reactor contents/effluents while preventing oxygen from entering. Thus, it was done carefully for the tube to maintain somewhat of a liquid seal on both sides. However, it did run dry in some instances, as mentioned previously.

3.2.1.1a pH and Total Alkalinity

The routine pH analyses of all grab samples were determined to the nearest 0.1 pH unit by Method 4500-H⁺ B., according to *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, (APHA, 1995), with a Fisher Accumet[®] Model 320 Expanded Scale Research combination electrode pH Meter. Prior to initial use, the instrument was calibrated with both VWR Scientific pH 4.00 (red) and pH 7.00 (yellow) standard buffer solutions. The buffers and samples, collected in small glass beakers (≤ 100 mL) in volumes large enough to accommodate the immersion of the electrode, were brought approximately to room temperature prior to analysis ($20^\circ\text{C} < T < 25^\circ\text{C}$).

Following pH measurements, TA determinations were usually performed on the same samples using ready to use VWR Scientific 0.02 N sulfuric acid (H_2SO_4) solution as the titrant. Sample volumes were measured with 50 mL graduated cylinders. Most samples were titrated without undue delay, with a 25 mL buret, by Titration Method 2320 B. 4c, in APHA (1995), to a pre-selected 4.5-pH end-point (TABLE 2320:I)³. Since the samples analyzed had interfering color and/or turbidity, no color indicators were used. Instead, the properly calibrated pH meter described previously was used to make the end-points distinguishable. The Low-alkalinity method (2320 B. 4c) was also not employed on samples found to have little alkalinity.

³ *Standard Methods for the Examination of Water and Wastewater* (1995), 19th ed., American Public Health Association, Washington, DC.

3.2.1.1b Total and Soluble Chemical Oxygen Demand

For samples with *more than 50 mg COD/L*, the CODs were determined by the 5220 C. Closed Reflux Titrimetric Method (APHA, 1995). However, for samples containing *less than 50 mg COD/L*, the alternate Low-Level COD procedure (5220 B. 4b) was used to improve accuracy.

The digestion vessels used for all analyses were KIMAX[®] and PYREX[®] borosilicate culture tubes (20 x 150 mm) with Teflon-lined screw caps. Before the first use, the reusable culture tubes and caps were washed with dilute acid (20% H₂SO₄). According to TABLE 5220:I (APHA, 1995), 5 mL of well-mixed samples were pipetted into the COD tubes and proper reagent volumes were added with calibrated dispensers. All reagents used were prepared as directed in Method 5220 B.3 (no sulfamic acid was used). A Blue M Electric Company Stabil-Therm Gravity oven, pre-heated to 150 ± 2°C, was used for the 2 h digestion. One drop (0.05 mL) of commercially prepared ferroin (phenanthroline ferrous sulfate) indicator solution was added to all culture tubes, prior to titrating the excess dichromate. Titrations were performed with a 50 mL buret, directly in each vial, with either 0.10 M (High-Level) or 0.025 M (Low-Level) ferrous ammonium sulfate (FAS) titrant and their contents were swirled manually.

Since the COD test is known to be time consuming and required frequent analysis of many samples, it was performed on large sample batches, collected at various sampling locations over a week (Section 3.1.1, Fig. 3.1 and Section 3.2, Table 3.3). After collection, these samples were preserved by refrigeration instead of acidification since the remaining sample volumes were used for the SS tests (Section 3.2.1.1c). Duplicate blanks, FAS molarity checks and KHP standards were prepared and analyzed with each sample batch, for both high and Low-Level COD procedures. The COD analysis of blanks, containing the reagents and 5 mL of distilled water, was similar to the one performed on the samples. For the molarity checks on the FAS solution, the sample volume was substituted by distilled water. After reagent addition, these 2 vials were *not* digested, but simply cooled to room temperature before titration.

The 0.10 M and/or dilute 0.025 M FAS molarities were measured at each use. They were standardized against 0.0167 M or 0.00417 M potassium dichromate (K₂Cr₂O₇) digestion solution, respectively by dividing the volume of K₂Cr₂O₇ solution titrated (3 mL) by the volume of FAS used in the titration (mL) and multiplying the result by either 0.10 or 0.025. Since FAS is very sensitive to light, to prevent degradation, it was always kept in an amber bottle and stored in darkness.

According to Pitwell (1983)⁴, referenced in Standard Method 5220 B. (APHA, 1995), potassium hydrogen phthalate (KHP or $\text{HOOC}\text{C}_6\text{H}_4\text{COOK}$) has a theoretical COD of 1.176 mg O_2 /mg. Technique and reagent quality were therefore assessed by conducting the analysis with KHP standard solution of 500 mg O_2 /L (500 μg O_2 /mL) theoretical COD value.

Most often, batch analyses had to be performed over a few days. Customarily, all sample dilution tubes were prepared, capped and stored in a refrigerator until the next day where they were brought to room temperature, usually digested and sometimes titrated. If not, titrations were performed on the day following the digestion.

COD_s concentrations were determined along with and similarly to COD_t concentrations by centrifuging the samples, in an International Equipment Co. (IEC) Model B-20 High Speed Refrigerated Centrifuge, for a minimum of 8 min at 10 000 rpm. Their supernatants were then used in the procedure. Exceptionally, for samples taken from Sept. 3-9, soluble CODs were alternately performed on SS analyses filtrates since the centrifuge was out of order. Dilution factors employed (not presented herein) were always factored in COD_t and COD_s calculations (*Appendix M*) with the appropriate average FAS molarity and blank values.

3.2.1.1c Total, Volatile and Fixed Suspended Solids

Method 2540 D. was used to determine the TSS of samples and the dry solid residue was ashed for 20 min at 550°C, according to Method 2540 E., to determine VSS (APHA, 1995). The remaining FSS were determined mathematically from the difference between the two SS results. Whatman[®] 70 mm diameter glass-micro fiber GF/C grade filter disks, of 1.2 μm pore size, were used to filter all well-mixed samples, under applied suction. The filtration apparatus consisted of two-piece polypropylene Büchner funnels and a vacuum pump with a side arm Erlenmeyer flask water trap.

All VWR Scientific 70 mm aluminum weighing dishes and filters used were conditioned prior to analysis, using distilled water, according to procedure 2540 D. 3b. They were always pre-ignited at 550°C for 15 min, in a Model 186A Fisher Scientific Isotemp[®] muffle furnace, since VSS analyses were always subsequently performed. They were then stored in desiccators for at least 30 min (to cool to balance temperature) or until needed and tared immediately before use.

⁴ Pitwell, L. R. (1983), "Standard COD", *Chem. Brit.*, 19, p. 907

For TSS determinations, dish, filter and sample combinations were evaporated for at least 1 hour, but usually overnight, in a Fisher Isotemp[®] drying oven, pre-heated to 103-105°C.

At the beginning of the study, SS tests were performed in duplicate. Over time, this practice was discontinued even though a few results did not agree within 5% of their average. Although the procedure calls for duplicate sample analysis, these proved to be time consuming and costly when considering glass fiber filter costs as well as analysis frequency. This way, the systems were also less disturbed. Similarly to the COD analysis, SS tests were usually conducted in large batches, over a few days. Because of the numerous sampling locations as well as the lengthy and frequent analyses, multiple weighing, drying/igniting, desiccating, and final weighing cycles [with suggested weight changes within less than 4% or 0.5 mg (APHA, 1995)] were not performed for each determination.

In most cases, these analyses were performed with the remaining COD test grab sample volumes since sampling locations were to be the same. Samples were preserved, at about 4°C, until analyzed to minimize the microbiological decomposition of the solids, but they were brought to room temperature prior to analysis. The calcium sulfate (CaSO₄) desiccant was also regenerated regularly at 275°C.

3.2.1.1d Volatile Fatty Acids Determination

The procedure described by C. M. Elliott (University of Ottawa Water Pollution Laboratory, 1989), based on the method of Ackman (1972), was used to determine VFA concentrations in AD samples. They were collected from the internal recycle line sampling ports (Fig. 3.1, port X₃) with a 1 mL disposable syringe. The acids monitored included acetic, propionic and butyric.

The analyses were routinely performed with an HP 5840A Capillary Column GC, set to a 5% retention time window, and a 5840 GC Terminal Integrator. The GC was equipped with a 250°C injection port, a 350°C hydrogen-air flame ionization detector, a Chromosorb 101 (styrene divinyl benzene polymer) packed column held at 180°C and an HP 7672A Automatic Sampler. The 15 mL/min carrier gas used was 99.9% pure helium, which was saturated with formic acid to suppress peak tailing and other absorption effects. The effective range of the GC apparatus was 0 - 2 000 mg acid/L ($\pm$ 5 mg/L). Because of resulting sampling and injection perforations, the sampling and GC injection port rubber septum were changed every so often for them to be maintained as airtight as possible. The latter was replaced after each 100 samples analyzed. The same applied to the septa used in the biogas composition analyses (Section 3.2.1.1f).

Prior to analysis, samples collected in VWR Scientific 1.7 mL plastic disposable micro-centrifuge tubes were centrifuged, for at least 4 minutes at 7 000 rpm, in a Brinkmann Eppendorf model 5415 mini-centrifuge. Each sample was prepared by extracting 0.5 mL of supernatant with a 500 μ L model 4700 Eppendorf (Tri-Volume Tip) Ejector Pipetter and adding it to 0.5 mL of Internal Standard (ISTD), in a clean auto sampler vial. The working ISTD solution (*Appendix D*) contained 0.2% isobutyric acid (2 000 mg/L) while the working VFA standard mixture (*Appendix D*) used was made up of 0.2% of each acetic, propionic and butyric acids (2 000 mg/L). To reduce instrumental errors, the GC was standardized at least once, each day it was used, with a standard containing 0.5 mL ISTD (1 000 mg/L) and 0.5 mL VFA standard mixture (1 000 mg/L). A dilution factor of 2 was always incorporated in the machine and this ideally yielded $2\,000 \pm 5$ mg/L for each VFA. If this was not the case, before proceeding to any sample analysis, the GC was calibrated until a concentration within this range was achieved.

The volume of sample injected into the apparatus was 1 μ L. To avoid contamination, every 2 successive samples analyzed in the GC were both preceded and succeeded by a distilled water-filled vial to clean the plunger injection needle. The VFA concentrations contained in the samples were obtained directly on the integrator response curve, from the areas under the chromatographic constituent peaks. Samples were usually collected and analyzed immediately. If analysis was delayed, either the micro-centrifuge tube contents or the prepared sample vials were refrigerated and covered with Parafilm[®] M laboratory film.

3.2.1.1e Biogas Production

Wet tip gas meters from Wet Tip Inc., with a submerged internal drum, were used to determine the volumetric biogas production in each reactor. The number of tip readings over time, recorded with either an Omron H7EC digital counter (AD2) or battery operated counter (AD1), could be converted to a rate of biogas produced, based on the meter calibration results.

They were first checked with a large 100 cc glass syringe and then more accurately with a 0.32 cm ($\frac{1}{8}$ in.) stainless steel float Fisher Scientific Lab-Crest Mark III flow meter (tube cat. no. 448-118). The latter's appropriate calibration curve gave the corresponding airflow (mL air/min @ STP) for a chosen low scale reading (similarly to the flow meter calibration curve for liquid flow, presented in *Appendix E*). By taking the time over which the gas meter was calibrated into consideration, this flow could be converted into a volume per tip. The daily gas discharge could therefore be calculated by multiplying the resulting number of tips over time by the volume per tip found for each meter during calibration.

3.2.1.1f Biogas Composition

The procedure described by C. M. Elliott (University of Ottawa Water Pollution Laboratory, 1989), based on the technique described by van Huyssteen (1967), was used to routinely quantify the percent composition (%N₂, %CH₄ and %CO₂) of the anaerobic reactor biogas samples, with a Hewlett Packard (HP) model 5710A Gas Chromatograph (GC), set to a 10% retention time window, and a HP model 3396A Integrator. The GC was equipped with a Porapak type T porous polymer packed column held at 75°C, a 150°C thermal conductivity detector and an injection port set at 100°C. The 99.9% pure helium carrier gas flowed at 25 mL/min. The instrument was calibrated regularly by the laboratory technician with an 80% N₂ and 20% CO₂ gas standard.

Approximately 1 mL samples were collected for analysis through the gas sampling port rubber septum, (Fig. 3.1, port X₄) with a disposable plastic syringe, fitted with a special 2-way closing valve. It was filled and discarded once before taking the actual sample. After inserting the needle, the plunger was then moved up and down several times to make sure the samples were well mixed. Gas was extracted slowly and uniformly before closing the valve. The needle tip was removed and capped with a rubber stopper for transportation purposes and 0.5 mL of sample was manually injected in the instrument's injection port for analysis.

The percentage of each biogas present in a sample could be determined from the chromatogram printouts by dividing individual gas amounts (mL) by the sum of all three biogas fractions (mL) and multiplying by 100. The total was to lie within 0.5 ± 0.025 mL. If not, additional injections were made until it did.

3.2.1.1g Sludge Bed Height

The settled granular SBH of both UASB reactors was visually evaluated, usually on a weekly basis. The SBHs were estimated by comparing them to the volumetric graduations taped along the UASB column heights. Before recording bed measurements, feed and internal recycle line flows were stopped completely, for 1.5 hours, on average. The reactors themselves were shaken to allow the granular sludge to settle to the true bed heights, as opposed to expanded ones due to pumping. This usually made the top of the beds somewhat more horizontal and more accurate to read although they were still slanted sometimes. While in operation, the reactors expanded bed heights were occasionally evaluated for comparison.

3.2.1.2 Aerobic Reactors (Contents and Effluents)

The diluted TWAS, acting as ML and used to fill the ABs of the aerobic systems, was characterized (Table 4.3 in CH. 4) and left to aerate for a few days prior to start-up. The initial basin contents were analyzed for pH, DO and temperature as well as TSS and VSS. The DO levels and temperatures of the ABs were periodically measured during the study to insure that the ML DO concentrations were always $> 2 \text{ mg O}_2/\text{L}$ (Section 3.1.1.2). The SS of the ML were also frequently analyzed while its pH and alkalinity values were only measured occasionally.

The first four parameters described in Section 3.2.1.1 for the UASB reactors (pH, TA, COD/COD_s and TSS/VSS), were also regularly monitored for the activated sludge process effluents. They were determined from collection bucket samples during the ongoing experiment. When the large CLs of approximately 12 L each were in place, their pH and COD/COD_s were only analyzed on rare occasions.

3.2.1.2a Aeration Basin Dissolved Oxygen and Temperature

A digital bench top ORION Model 860 DO Meter and a polarographic Clark-type oxygen-permeable membrane electrode were used for periodic DO measurements of the ML. The readings were taken directly in the ABs, according to the manufacturer's *Calibration and Measurement Procedure* and based on the 4500-O G. Membrane Electrode Method described in APHA (1995). The temperature of the solution in which the probe was immersed was also displayed automatically and recorded in °C. The meter was calibrated before each use, by performing a slope check on the membrane electrode system, in a calibration sleeve, with distilled water-saturated air as the standard.

3.2.1.3 Other Parameters Monitored

3.2.1.3a Room and Refrigerator Temperatures

The ambient laboratory temperature was monitored, on a daily basis, near the set-up, with a mercury-filled thermometer partially immersed in an Erlenmeyer flask, according to Method 2550 B. 1. (APHA, 1995). In a similar fashion, the refrigerator temperature was also recorded daily to monitor the feed tank temperature. The feed temperature was kept around 4°C in order to minimize biological activity and microbial growth in temporarily stored samples, solutions as well as the contained feed. Robinson (1990) reports that low cooling and storing temperatures ($\leq 4^\circ\text{C}$) usually prevents bacterial multiplication in milk for at least 1 day.

3.2.1.3b Flow Rates

The experimental set-up operation was tested, before inoculation, and all the peristaltic pumps within the system were calibrated with water, for the flows considered.

During the experiment, UASB reactor influent feed flow rates were quantified indirectly by measuring the daily effluent volumes collected from each activated sludge process, since all reactor volumes were held relatively constant. Before discarding effluents, flow rates were calculated by dividing the volume of liquid accumulated in each effluent bucket by the amount of hours over which they were collected. Approximate liquid volumes were measured with large graduated cylinders. Both activated sludge processes were gravity-fed from the UASB effluents and the total system effluent flows were assumed to equal the UASB influent feed flows, regardless of the recycling occurring within the systems.

Initially, a calibrated stopcock was inserted in AD2's gravity dilution line to control the water-input rate from the 20 L CHT, in order to dilute its feed concentration by half. It proved to be a very poor means since it was very difficult to regulate and to see if water was in fact flowing into the reactor's pumped feed line. A Bel-Art Scienceware[®] water flow monitor, made of clear plastic, was temporarily placed following it (Day 18) to give a visual indication of water flow. At the time, the set flow was possibly too small to make the ball rotate around its circular channel.

After Day 24, the simple stopcock-flow indicator combination was replaced by a Fisher Scientific Lab-Crest Mark III flow meter [tube cat. no. 448-118 with a 0.32 cm ($\frac{1}{8}$ in.) sapphire float]. It could regulate liquid input rates between 0.72 and 33.13 L/d. Although its scale reading and calibration curve (*Appendix E*, Flow Meter Calibration Curve) gave more precision, the flow meter's float was however very sensitive to debris. Over time, it often did not maintain itself at the desired flow rate setting and sometimes fell to the zero scale reading. The problem was dealt with when it was noticed.

When the final effluent flow volumes, collected over a certain time, were measured, the amount of feed left in the feed tank at that time was always recorded. This information allowed for dilution line contribution estimates, to a certain extent (especially when the continuous recycle lines back to the ADs and the ABs were abandoned, after Day 45), and was particularly useful when the flow meter was malfunctioning.

3.2.2 TESTS AND ANALYSES PERFORMED ON THE IRON FORTIFIED ENFALAC™ FEED

After the acclimation period, analyses regularly performed on samples from the proposed 1 000 mg COD_i/L Iron Fortified Enfalac™ feed mixture during the entire investigation were pH, TA, COD_i and COD_s as well as TSS and VSS. The temperature of the refrigerator, in which the feed storage tank was kept, was monitored almost daily (Section 3.2.1.3a) while VFA concentrations were only measured once, from one of the many feedstock batches (Oct. 25).

These parameters were analyzed on nutrient-supplemented and pH-adjusted feed mixtures (when pH was chemically supplemented). On occasion, pH and TA were analyzed prior to chemical addition for comparative purposes. In addition to the above mentioned parameters, TDS/VDS, TS/VS, Percent TS/VS (Enfalac™ powder), BOD₅, TP/TDP, TKN/org. N, NH₃ (NH₃-N) and NO₃ (NO₃-N) analyses were also conducted on feed mixture samples, from fresh and un-supplemented 1 L batches, during the preliminary material characterization tests. The procedures used for these particular analyses are described in *Appendix C* while the complete feed characteristic results are presented in Table 4.1 (Section 4.1.1 in Chapter 4).

3.2.3 ANALYSES PERFORMED ON THE ANAEROBIC GRANULAR BIOMASS

Analysis performed on the initial raw anaerobic granular sludge supernatant during the characterization of the granular biomass included pH, TA, COD_i and VFAs. Prior to start-up, a batch anaerobic toxicity assays (ATA), acetoclastic activity tests (AATs) and settling tests (all described below) as well as TSS/VSS determinations (Section 3.2.1.1c) were also conducted, at least in duplicate, on the granular sludge inoculum initially un-acclimated to the Enfalac™ feedstock. Characterization results are summarized in Table 4.2 (CH. 4).

The last three types of tests were also conducted on acclimatized granular sludge from each UASB reactor, at the end of each experimental run. Representative anaerobic biomass samples were withdrawn from desired sludge bed height sampling ports with a 10 mL wide-mouth disposable syringe (fitted with a connector and a piece of tubing instead of a needle). This allowed to gather sludge profile data for particular operational times (Section 4.3 in CH.4).

3.2.3.1 Modified Anaerobic Toxicity Assay

Prior to system start-up, a modified ATA (or inhibition test), based on the analysis procedure described by L. Y. Young (unknown date) in the *Anaerobic Methods* manual (Kennedy, 1999), was performed using un-acclimated anaerobic granular inoculum.

Three different Enfalac™ wastewater feed sample dilutions and a positive control bottle, containing 20 mL of sludge and 20 mL of defined medium (*Appendix K*) each, were prepared from a 4 000 mg COD_l/L feed stock mixture to yield the concentrations found in Table 3.6, using total volumes of 80 mL within closed 160 mL serum bottles. The test was therefore performed on triplicate feed concentrations with duplicate sets of bottles:

Table 3.6: ATA Test Conditions

Enfalac Concentrations (mg COD _l /L)	Sludge (mL)	Defined Medium (mL)	Distilled H ₂ O (mL)	4 000 mg COD _l /L Stock Solution (mL)	Total Volume (mL)	Buffer Medium (%)	Enfalac Feed (%)
0 (control)	20	20	40	0	80	33.3	0
250	20	20	35	5	80	33.3	8.3
500	20	20	30	10	80	33.3	16.7
1 000	20	20	20	20	80	33.3	33.3

The medium was anaerobically dispensed and inoculated with sludge in 8 nitrogen (N₂) gassed serum bottles. They were sealed and capped, with rubber septa and aluminum crimp caps, as well as shaken overnight at *room temperature*, until clear, at approximately 60 rpm on a Lab-Line instruments Inc. Junior Orbit shaker. Then, the wastewater was added to each bottle, in the intended proportions, before being re-capped. These were shaken again at *room temperature*, for 30 minutes more, after being flushed and over pressurized with N₂ gas. They were finally spiked with 0.3 mL of acetate stock solutions A and B (*Appendix K*). The assay was initiated (t=0) when the headspace of all bottles was adjusted to 1 atm, by releasing the excess gas with the insertion of a manometer needle. The methane fractions of the biogas produced were not sampled.

Cumulative biogas production in each serum bottle was monitored over time, for just over 30 days (Droste, 1997b), with a hypodermic needle connected to a calibrated fluid reservoir (Speece, 1996), which was located in a (33 ± 2°C) temperature-controlled room. Readings were taken after 24 hours and monitored daily, for the first 5 days. Subsequent measurements were made about 3 times a week for the next 2 weeks, with 2 final weekly measurements since gas production had slowed down but not quite ceased. A background control was not considered.

3.2.3.2 Acetoclastic Activity Test

The AATs were performed on 10 mL sludge samples by monitoring the disappearance of acetate in anaerobic serum vials with time, according to the procedure described in the *Anaerobic Methods* manual (Kennedy, 1999).

Sample test bottles were prepared by anaerobically dispensing the biomass and 40 mL of defined medium (*Appendix K*) in 160 mL serum bottles. Blanks, containing 50 mL of medium (but no biomass), and control bottles, with the same contents as the sample bottles, were employed. All bottles were continuously purged with N₂ gas until sealed and capped with rubber septa and aluminum crimp caps. They were also usually shaken at 60 rpm and *room temperature*, overnight, on the same Lab-Line instruments Inc. Junior Orbit shaker.

The assay was initiated by spiking the sample and blank bottles with 0.15 mL of each of the two acetate stock solutions (*Appendix K*), to provide initial acetic acid concentrations of about 1 600 mg/L or less (*Appendix L.10* at $t=0$), while 0.3 mL of phosphate buffer (*Appendix K*) was injected in the controls. The bottles were re-shaken and 1 h later ($t=0$), less than 1 mL of supernatant was withdrawn from the inverted bottles, with a 1 mL syringe, to determine initial acetic acid (AA) concentrations of all bottles.

Micro-centrifuged samples were analyzed according to the VFA GC analysis (Section 3.2.1.1d), with a shorter retention time (~ 6.5 min) to measure only acetic acid. Bottles were then sampled at various time increments, of 1.5-2 hours, on average, although the sampling schedule depended on the microbial activity of the sludge. This was repeated over time to record at least five decreasing concentrations, resulting in monitoring periods of approximately 1 day or more.

3.2.3.3 Total, Volatile and Fixed Suspended Solids

Although they could have been performed on the biomass content of the AAT serum bottles, additional 10 mL sludge grab samples were removed at each of the chosen SBH ports to quantify UASB granular biomass TSS and VSS (Section 3.2.1.1c). In addition to these granular biomass inventory estimates, TSS determinations were also involved and combined with the following test.

3.2.3.4 Settleability Assessment (Settling Velocity Test)

Settling velocity tests were conducted on 10 mL of anaerobic granular sludge, directly after sampling. The analysis was based on the procedure described by Andras et al. (1989) in the *Anaerobic Methods* manual (Kennedy, 1999). The test apparatus consisted of a water pail, a feed pump and controller, a 19 mm ID vertical glass tube, pre-weighed Whatman[®] 185 mm diameter Grade 1 Qualitative filter papers of 11 μ m pore size as well as a large Büchner funnel and Erlenmeyer collection flask, placed under the upflow velocity tube's overflow port and connected to a vacuum pump.

Each biomass sample transferred to the water-filled tube was subjected to five increasing upflow velocities, maintained for 5 min each, after its content was stirred to create a vortex just prior to filtration. Under applied suction and while 21 to 24°C-tap water was pumped through the system, exiting granule fractions were retained on fresh filter papers, at each velocity setting used. The volume of water accumulated in the flask, over time, was also measured. The solids collected on the Whatman[®] filter papers were dried and weighed to obtain the TSS of the particular fractions. The distribution of the sludge mass with respect to the various upflow velocities applied helps to provide information on sludge settleability (*Appendix L.12*).

Numbered filters were always pre-conditioned and were ready for use at the start of an analysis. They were rinsed with distilled water, dried overnight in a 105°C Fisher Isotemp[®] drying oven, stored in desiccators (for at least 2 hours or until needed) and rapidly tared just prior to use. The Sartorius analytical balance used for TSS/VSS determinations was used for all weighings. Since the large filters used were extremely hygroscopic, their weight was taken as the first stable reading. The drying and weighing routine for clean and granule-carrying filters was repeated as consistently as possible, including the time spent in the oven and desiccators as well as the way the readings were taken. Any sludge left in the tube after the final velocity setting was tested was accounted for in the last TSS fraction.

CHAPTER 4

RESULTS, DATA ANALYSIS AND DISCUSSION

The following sections of this chapter present key quantitative characteristics of the dilute synthetic dairy feed and the reactor inocula (anaerobic granular biomass and TWAS) as well as major results from the 146-day anaerobic-aerobic treatability investigation. On some occasions, due to set-up maintenance or operational problems, the reactors could not be fed with incoming substrate. Most collected data are reported in *Appendix L* and extensive parameter example calculations are presented in *Appendix M*. Based on wastewater treatment plant performance studies, Tchobanoglous and Schroeder (1985) report that standard deviation (STD) gives the best process stability (or performance variability) estimate. Therefore, this statistical parameter accompanies most experimental results. The percent COD removals (organic content reduction) achieved by the anaerobic and aerobic units as well as the overall systems were the main measures used to evaluate process performance.

As previously stated, the study was essentially divided into 3 phases. These included the preliminary assessment of the substrate and sludge characteristics (Section 4.1), the short start-up and progressive acclimation of the biological systems to the experimental feed as well as their operation under maintained feeding conditions (Section 4.2). During the latter time period, the two treatment systems of differing influent strengths were subjected to varying OLRs by applying a few different influent flow rates. All references to “Days” are always with respect to reactor inoculation, which was considered as the beginning of the start-up phase. Thus, the 17-day acclimation period was considered as an integral part of the 146-day lab study.

4.1 PRELIMINARY CHARACTERIZATIONS

Table 4.1 summarizes the Enfalac™ feed solution characteristics, without any applied pH control or nutrient addition. A general characterization of the anaerobic granular biomass and of the initial TWAS, used to seed the UASB reactors and the aeration basins, is also given in Tables 4.2 and 4.3, respectively. The results reported are the averages, STDs and ranges obtained from the number of samples and/or duplicates tested. Some of the trial results used to compute the average material characteristics can be found in *Appendix M*, where calculation examples are presented.

4.1.1 CHARACTERIZATION RESULTS OF MATERIALS USED TO FEED/SEED ALL REACTORS

The parameters reported in Table 4.1 were tested from well-mixed samples taken from freshly made 1 L-feed batch mixtures to characterize the un-supplemented synthetic milk wastewater prior to the start of the experiment.

Table 4.1: Average Un-supplemented 1 000 mg COD_L/L Fortified Iron Enfalac™ Feed Characteristics

Parameter	Units	Average	STD (±)	Range	# Sample	V (mL)
COD Total	mg/L	‡ 1 059	‡ 65	‡ (994 & 1 125)	‡ 2 (dupl.)	5
Soluble (centrifuged)	mg/L	‡ 756	‡ 13	‡ (743 & 769)	‡ 2 (dupl.)	5
BOD ₅	mg/L	‡ 665	‡ 5	‡ (660 & 670)	‡ 1 (dupl.)	0.5, 1, 2; 3, 6, 10
pH	-	6.2	0.2	(6.0 - 6.4)	3	50
Total Alkalinity, as CaCO ₃	mg/L	29.5	1.5	(28.0 & 31.0)	2	50
Total VFA (0.6g NaHCO ₃ /L)	mg/L	0.937 (AA) + 1.522 (Pr) = 2.459	NA	NA	1 (Oct.25)	0.5
TSS	mg/L	123	3	(120 & 126)	1 (dupl.)	100
VSS	mg/L	112	9	(104 & 121)	1 (dupl.)	100
FSS	mg/L	11	5	(16 & 5)	1 (dupl.)	100
TDS (filtered sample)	mg/L	391	7	(384 & 398)	1 (dupl.)	50
VDS	mg/L	325	5	(320 & 330)	1 (dupl.)	50
FDS	mg/L	66	12	(54 & 78)	1 (dupl.)	50
TS (liquid sample)	mg/L	759	2	(757 & 761)	1 (dupl.)	100
VS	mg/L	684	3	(682 & 687)	1 (dupl.)	100
FS	mg/L	75	0.5	(74 & 75)	1 (dupl.)	100
TS (powder sample)	%	97.83	0.07	(97.76 & 97.90)	1 (dupl.)	25 (g)
VS	%	97.41	0.01	(97.42 & 97.40)	1 (dupl.)	25 (g)
FS	%	2.59	0.01	(2.58 & 2.60)	1 (dupl.)	25 (g)
PO ₄ ⁻³ -P Total	mg/L	2.74	0.68	(2.02 - 3.46)	4	5
Dissolved	mg/L	0.73	0.22	(0.51 & 0.94)	2	5
TKN-N	mg/L	~18.5 if 100% recovery (13.91 @75%)	0.41	(13.50 & 14.31)	1 (dupl.)	5
org. N	mg/L	~14.7 if 100% recovery (10.31 @70%)	0.30	(10.00 & 10.61)	1 (dupl.)	5
NH ₃ -N (4500-NH ₃ B. & C.)	mg/L	~3.2 if 100% recovery (2.69 @82%)	0.60	(2.09 & 3.28)	1 (dupl.)	25
Electrodes:	(Direct); Low-Level Method					
*NH ₃ (feed sample)	mg/L	(0.045); < 0.017 or < 9.8 x 10 ⁻⁷ M	♦ NA	♦ (NA)	(2), 1	5
*NH ₃ -N	mg/L	(0.037); < 0.014 or < 9.8 x 10 ⁻⁷ M	♦ NA	♦ (NA)	(2), 1	5
*NH ₃ (tap water)	mg/L	(«10 ⁻⁸ M); < 0.017 or < 9.8 x 10 ⁻⁷ M	♦ NA	♦ (NA)	(2), 1	5
*NH ₃ -N	mg/L	(«10 ⁻⁸ M); < 0.014 or < 9.8 x 10 ⁻⁷ M	♦ NA	♦ (NA)	(2), 1	5
*NH ₃ (distilled water)	mg/L	(≤ 10 ⁻⁸ M); < 0.017 or < 9.8 x 10 ⁻⁷ M	♦ NA	♦ (NA)	(2), 1	5
*NH ₃ -N	mg/L	(≤ 10 ⁻⁸ M); < 0.014 or < 9.8 x 10 ⁻⁷ M	♦ NA	♦ (NA)	(2), 1	5
*NO ₃ (feed sample)	mg/L	(3.85), 2.37	♦ 0.06	♦ (2.31; 2.43)	(2), 1	25 A
*NO ₃ -N	mg/L	(0.87), 0.54	♦ 0.02	♦ (0.52; 0.55)	(2), 1	25 A
*NO ₃ (tap water)	mg/L	(2.44), 1.96	♦ 0.07	♦ (2.03; 1.88)	(1), 1	25 A
*NO ₃ -N	mg/L	(0.55), 0.44	♦ 0.02	♦ (0.46; 0.42)	(1), 1	25 A
*NO ₃ (distilled water)	mg/L	(1.54), < 0.062 (< 10 ⁻⁶ M)	♦ (NA)	♦ (NA)	(1), 1	25 A
*NO ₃ -N	mg/L	(0.35), < 0.014 (< 10 ⁻⁶ M)	♦ (NA)	♦ (NA)	(1), 1	25 A
COD: N: P Ratio		1 059: 19.1: 2.74 or ~106: ~2: ~0.3	Adjusted COD: N: P		~110: 5.4: 1	

§ Boric Acid Preservation Solution added (no Interference Suppressor); ‡ Dilution duplicates; dupl.: Duplicate; NA – Not Applicable;

♦ Ranges and STDs between *Excel* and *MATLAB* results (for Low-Level trials only); AA-Acetic acid; Pr-Propionic acid

* Test Limits for Direct Measurement Method: For NH₃ [1 M to 5 x 10⁻⁷ M or 0.01 - 17 000 ppm NH₃ (0.01 - 14 000 ppm NH₃-N)];
For NO₃ [1 M to 7 x 10⁻⁶ M or 0.4 - 62 000 ppm NO₃ (0.1 - 14 000 ppm NO₃-N)];

* Low-Level Method: For NH₃ [for < 4 x 10⁻⁶ M or 0.07 ppm NH₃ (0.06 ppm as NH₃-N)];
For NO₃ [for < 10⁻⁴ M or 6.2 ppm NO₃ (1.4 ppm as NO₃-N)];

Extensive trial and error COD tests (Section 3.2.1.1.b) were conducted to determine the amount of dry Enfalac™ powder required to obtain the desired 1 000 mg COD_L/L concentration.

Although the trials were numerous and are not presented herein, 725 mg of Enfalac™ per liter of tap water was found to yield $1\,059 \pm 65$ mg COD_t/L, on average. Therefore, during the experiment, the feed mixtures were always prepared with these proportions of powder and water, either in 20 L (14.5 g of Enfalac™) or 26 L (18.85 g of Enfalac™) batches, on an as needed basis. According to Metcalf and Eddy (1991), a 1 000 mg/L influent would be classified as a strong (untreated) domestic wastewater. However, it is considered as a low-strength feed for anaerobic treatment since it is regarded as the minimum concentration required for a biological process of this type to be successful (Section 2.1.3.5) under practical operating conditions (Droste, 1997b).

Based on the results in Table 4.1, the experimental feed used was considered as a mainly soluble substrate since the average COD_s fraction corresponded to over 70% of COD_t. Its BOD₅ was found to be approximately 1.6 times less than its COD_t (Table M.4 in *Appendix M*), which is comparable to typical conversion factors reported in the literature to transform organic matter from a COD to a BOD₅ basis (Droste, 1997b). On average, the Enfalac™ mixture had a very low inherent TA, at 29.5 ± 1.5 mg/L as CaCO₃, and a somewhat acidic pH of 6.2 (± 0.2), which is common for milk substrates (Section 2.2.2). The feed's solids content was also mostly of the dissolved form when compared with the SS fraction (3:1 or ~75%) and the latter was considered to be relatively low (~100 mg/L range). The synthetic feed had little FSS since the VSS/TSS ratio was slightly above 0.9 and it was assumed to have a high organic content (Droste, 1997a), which is common for food-related wastewaters. Hence, it was also assumed to be a readily degradable material.

Although both solid forms did not add up to the observed TS (or VS) concentration, the TKN-N approximately totalled the sum of the org. N and NH₃-N concentrations [Method 4500-NH₃ B. and C. (APHA, 1995)], as it should, especially when adjusted for 100% N recovery (*Appendix M*). When considering the problems encountered during TKN and org. N determinations (*Appendix G*), the average recoveries obtained were deemed as reasonable even though they were somewhat low compared to those reported in APHA (1995). However, both techniques used to evaluate the NH₃-N content did not yield analogous results and they differed by at least 100-fold (Table 4.1). Trace amounts were found when using an ammonia electrode [Method 4500-NH₃ D. (APHA, 1995)], whether with the Direct (0.037 mg NH₃-N/L) or Low-Level method (< 0.014 mg NH₃-N/L), while direct distillation followed by titration [Method 4500-NH₃ B. and C. (APHA, 1995)] resulted in 2.69 ± 0.60 mg NH₃-N/L for an 82% N recovery. The latter method should be employed for concentrations > 5 mg NH₃-N/L and a larger test sample volume (~250 mL) should have been used (APHA, 1995), if the test material's N concentration range would have been known.

All NH_3 and NO_3^- electrode concentration measurements obtained in the molar form from calibration curve regression equations (*Appendix I* and *J*) were transformed in the N form with the conversion factors presented in *Appendix M*. Direct methods were performed first since the amount of these compounds within the proposed feed solution was not known. Because the concentrations obtained were found to lie at the lower-end of this method's test limits and within the range of the Low-Level methods, these were also applied to obtain more accurate estimates. The first and second values reported in Table 4.1 are those found by the Direct and Low-Level methods, respectively. The NH_3 and NO_3^- contributions in tap and distilled water were quantified since they were used to prepare the feed as well as standards and Low-Level calibration curve dilutions (i.e., NH_3 and NO_3^- -free water was unavailable), respectively. All the results were relatively insignificant and particularly those for NH_3 . NO_2^- -N concentrations were not determined.

By disregarding the NH_3 -N results found with the ammonia electrode method, the average total nitrogen content in the synthetic feed was estimated as roughly 20 mg N/L [18.55 ± 0.41 mg TKN-N/L + 0.54 ± 0.02 mg NO_3^- -N/L (Low-Level Method) = 19.09 ± 0.43 N mg/L] while the total phosphorus content was approximated at 2.74 ± 0.68 mg PO_4^{3-} -P/L (*Appendix M*), on average. Thus, the preliminary feed characterization revealed that the substrate mixture had an average COD: Nitrogen: Phosphorus composition of 1 059: 19.09: 2.74 or approximately 106: ~2: ~0.3 without any chemical addition (Table 4.1).

This estimate yielded a N: P ratio just shy of typically accepted proportions for aerobically or anaerobically treated wastewaters (i.e., P content $\approx 1/5$ of the N content). Even though the feed composition should have been adequate to support the N and P nutrient needs of anaerobic microorganisms with respect to available COD (500: 5: 1); aerobic bacteria nutrient requirements would not have been sufficient, especially for P. Since the experimental treatment systems consisted of both biological processes, N and P chemical supplements were therefore always supplied to the feed during its preparation (*Appendix B*), over the entire investigation period (including reactor start-up and acclimation), to meet the more conservative aerobic ratio. To ensure the presence of sufficient nutrients, a 10% surplus was also taken into consideration (100: 5: 1 +10% = 110: 5.5: 1.1). Vitamins and trace elements (i.e., K, Ca, Na, Fe) were assumed to be sufficiently available (Table 3.1).

Most parameters were tested at least in duplicate and except for a few parameters [FSS, FDS, NH_3 -N (Method 4500- NH_3 B. and C.), TP and TDP], STDs generally represented much less than 8% of the result averages. The STDs reported for Low-Level NO_3^- determinations represent the deviations between the results obtained by *Microsoft Excel's* best-fit trend line and *MATLAB's*

Polyfit function (*Appendix M*) since only one trial was conducted and the measured concentrations fell in non-linear regions of the calibration curves. All other NO_3^- and NH_3 STDs and ranges were reported as not applicable (NA) since the results fell below the lowest test limits. Many of the parameters measured during this initial phase were not monitored afterwards. They were simply quantified to get a better feel for the composition of the proposed experimental substrate mixture.

The parameters reported in Table 4.2 were tested to characterize the original granular biomass inoculum. Additional details on its initial properties are presented in Section 4.3. The anaerobic seed sludge was found to have relatively little FSS, a SS content of approximately 31 ± 3 g VSS/L and a high VSS/TSS ratio of 0.88, on average (*Appendix L.11*). Thus, it was considered to have a high initial organic content and excellent initial settling properties (Section 4.3), resulting in an average upflow velocity (V_{50}) of 66.9 ± 0.6 m/h when evaluated at 50% TSS exited or granule washout (*Appendix L.12*). However, based on the AAT results, the average initial specific acetoclastic activity of the un-acclimated and raw biomass was unusually low at 0.101 ± 0.004 g AA/g VSS·d (*Appendix L.10*). When this value was evaluated, the anaerobic granular sludge had been sitting at room temperature and had been unfed for about 2 months.

Table 4.2: Average Anaerobic Granular Biomass Inoculum Characteristics

Parameter	Units	Average	STD ($\pm$)	Range	# Sample	V (mL)
Anaerobic Granular Sludge Inoculum (<i>Appendices L.10, L.11 and L.12</i>):						
Average Specific Acetoclastic Activity	g AA/g VSS·d	0.101	0.004	0.097 - 0.104	2	10
TSS	mg/L	35 360	3 689	(32 499 - 40 468)	2 (duplicates)	10
VSS	mg/L	31 275	3 446	(28 502 - 35 950)	2 (duplicates)	10
FSS	mg/L	4 085	293	(3 873 - 4 518)	2 (duplicates)	10
Initial Ave. Upflow Velocity (V_{50} for 50% TSS Exited)	m/h	66.9	0.6	(67.5 - 66.3)	2	10
Raw Anaerobic Granular Sludge Supernatant:						
pH	-	7.8	0.2	(7.5 - 7.9)	4	50
Total Alkalinity, as CaCO_3	mg/L	4 585	65	(4 520 & 4 650)	2	30 & 20
COD Total	mg/L	♦ 5 809	NA	NA	♦ 1	5
Soluble (centrifuged)	mg/L	♦ 4 648	NA	NA	♦ 1	5
Total VFAs	mg/L	~3 (Ave. AA)	3	(1 - 6)	3	0.5
		+ ~2 (Ave. Pr)	2	(1 - 4)		
		~5 (Total VFAs)	5	(2 - 11)		

STD--Standard deviation, NA--Not Applicable, ♦ One of the dilution trials had to be rejected; AA-Acetic acid; Pr-Propionic acid

The raw supernatant had a moderate organic strength (estimated as 5 809 mg COD_L/L) and was mostly soluble in nature (COD_s represented 80% of COD_L). It had a relatively high average pH

(7.8 ± 0.2) and total alkalinity ($4\,585 \pm 65$ mg TA/L as CaCO_3), which corresponded to the upper-end of reported ideal ranges for anaerobic systems (Section 2.1.2.2b). Thus, the UASB reactors should have a good initial buffering capacity. However, the TA concentration of the proposed influent wastewater is much smaller by at least 100-fold. Like the feed sample (Table 4.1) tested towards the end of the 2nd run [on Day 94 (Oct. 25)], when the feedstock contained the usual nutrient supplements and approximately 600 mg NaHCO_3 /L [Table L.2 c ii) in *Appendix L.2*], butyric acid was also undetected in the raw anaerobic sludge supernatant. However, the total VFA content in both liquid solutions was considered negligible. The anaerobic granular inoculum parameters were all tested at least in duplicate and except for total VFAs, the STDs represented less than 10% of the result averages.

Table 4.3: Average TWAS Characteristics

Parameter	Units	Average	STD ($\pm$)	Range	# Sample	V (mL)
Raw TWAS from ROPEC:						
TSS	mg/L	63 537	3 754	(60 163 - 69 214)	4 (duplicates)	5 & 10
VSS	mg/L	43 694	3 234	(39 720 - 47 642)	4 (duplicates)	5 & 10
FSS	mg/L	19 843	1 715	(16 640 - 21 776)	4 (duplicates)	5 & 10
VSS/TSS	-	0.69	-	-	-	-
Initial Diluted TWAS Used to Seed the ABs (July 21): (DF = 365 mL TWAS/12 000 mL Tap Water)						
TSS	mg/L	1 449	51	(1 398 & 1 500)	1 (duplicates)	10
VSS	mg/L	974	32	(942 & 1 006)	1 (duplicates)	10
FSS	mg/L	475	19	(456 & 494)	1 (duplicates)	10
VSS/TSS	-	0.67	-	-	-	-
Initial Average Diluted TWAS (July 21-24):						
pH	-	7.8	0.3	(7.5 - 8.1)	4	30
DO	mg/L	7.8	0.4	(7.2 - 8.2)	4	NA
Temperature	°C	19.3	0.5	(18.7 - 19.8)	4	NA
Average Diluted TWAS Used to Re-Seed ABs on Day 52 (same DF as above): [Aerated from Sept. 13 – 23, until start of Run 2 ($Q_{in} \approx 4$ L/d)]						
TSS	mg/L	1 498	410	(1 126 - 1 862)	2 (duplicates)	10
VSS	mg/L	992	279	(746 - 1 240)	2 (duplicates)	10
FSS	mg/L	506	131	(380 - 622)	2 (duplicates)	10
VSS/TSS	-	0.66	-	-	-	-
Average Diluted TWAS (Sept. 22 – 24):						
pH	-	7.6	0.1	(7.5 & 7.6)	2	30
Total Alkalinity, as CaCO_3	mg/L	112.5	14	(98 & 127)	2	30
DO	mg/L	7.2	0.1	(7.1 & 7.2)	2	NA
Temperature	°C	21.0	0.1	(20.9 & 21.1)	2	NA

STD-Standard deviation, NA-Not Applicable

Both aeration basins were initially inoculated with average diluted TWAS (Table 4.3) of $1\,449 \pm 51$ mg TSS/L (974 ± 32 mg VSS/L). To achieve a desired aerobic mixed liquor (ML) concentration of approximately 1 500 mg TSS/L (Section 2.1.4.3 and 3.1.2.3), raw TWAS un-acclimated to the experimental feed was watered down, by a DF of about 1.52:50 (or 0.365 L TWAS to 12 L of tap water). In its raw form, the thickened waste obtained from ROPEC (Section 3.1.2.3) was observed to have an even greater SS content than the anaerobic granular biomass (64 g TSS/L and 44 g VSS/L, on average) as well as a larger FSS fraction.

On Day 52 (Sept. 13), after the 2 L ABs were transformed into joint 3.5 L aeration-clarifier units, the ML was replaced with a new batch of diluted TWAS, which had a SS concentration ($1\,498 \pm 410$ mg TSS/L and 992 ± 279 mg VSS/L, on average) and characteristics comparable to that of the original ML. These steps were undertaken particularly because the solids content of the aerobic units had been greatly reduced by washout (*Appendix L.4*).

The VSS/TSS ratio of the raw TWAS was less significant than that of the anaerobic sludge (and the feed) and basically remained unchanged after dilution. The average pH of both TWAS mixtures also corresponded well with typical values for aerobic reactors (Section 2.1.4.3). The DO and temperature of the ML was measured directly in the ABs once they were inoculated. Initial aerobic seeding materials were well aerated since average DO concentrations were well in excess of 2 mg O₂/L, in both instances. However, their organic content (or COD) was not measured.

Although the SS content of the second diluted TWAS batch was evaluated with more replicates than the first seeding batch, the results yielded greater inaccuracy (STDs represented ~27% of result averages). All parameters were tested at least in duplicate and except for the above-mentioned parameters, the STDs represented much less than 12% of the result averages.

More trials could have been performed to increase the precision of the material composition results, but the experimental time frame did not allow it. However, the STDs obtained for most parameters were considered to be reasonable when compared to those reported in APHA (1995), especially for the intent of this initial phase. It should also be noted that it was impossible to perform the feed characterization tests and replicates for all parameters from the same feed mixture. However, all batches used were prepared in an identical fashion.

4.1.2 MODIFIED ANAEROBIC TOXICITY ASSAY PERFORMED ON GRANULAR SLUDGE INOCULUM

Because 2 system start-up attempts were unsuccessful [mostly due to biomass and sludge bed flotation (Section 3.1.3.1)], an anaerobic toxicity assay (ATA) was conducted (from June 4 –

July 6) during the pre-experiment phase to evaluate the anaerobic biodegradability potential of the low-strength Enfalac™ feed as well as to assess its possible inhibition and/or toxicity effects on the un-acclimated anaerobic granular biomass (or methanogenic bacteria source).

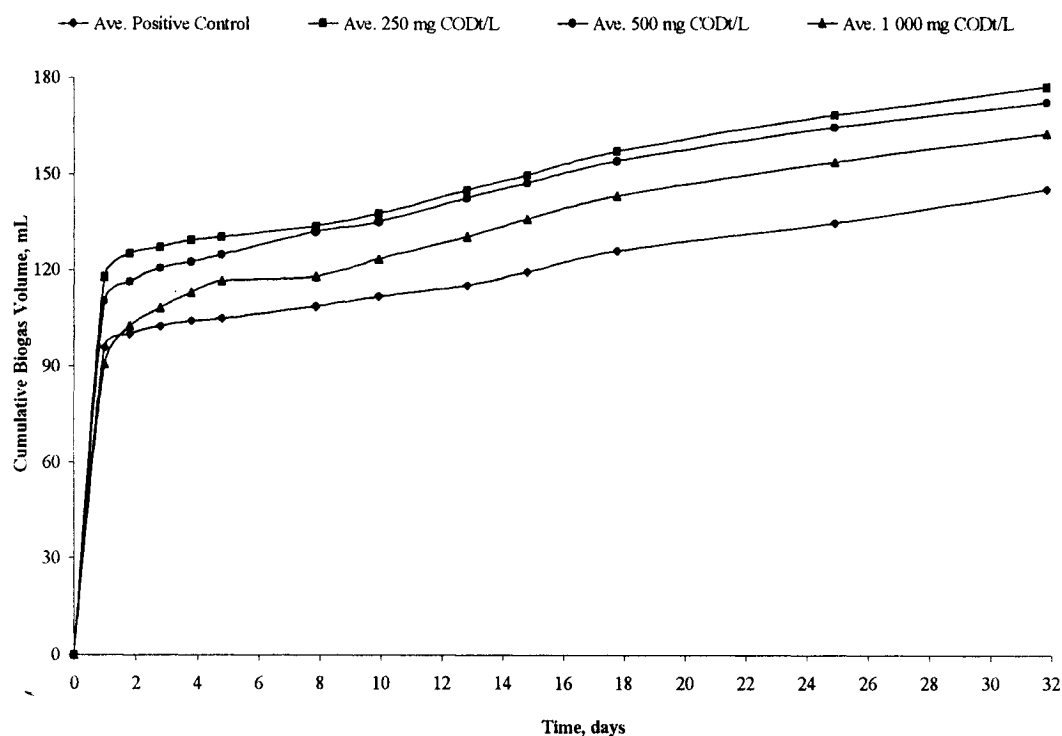


Figure 4.1: Average Room Temperature-ATA Carried Out on Un-acclimated Granular Biomass Inoculum Exposed to the Proposed Feed (June 4 – July 6, 2000)

Figure 4.1 presents the average cumulative biogas generated during the batch test while the tabulated experimental data recorded are presented in *Appendix L* [Tables L.1 a) and b)]. The plot shows that the majority of the gas was produced within the first day for all feed doses examined. Their initial and overall biogas production rates were comparable to each other (i.e., similar initial slopes and somewhat parallel lines) and matched that of the uninhibited active positive control (~96 mL/d), which did not contain the candidate feed mixture. Young and Irwin (1999) report that such a condition represents a potentially complete biodegradation of the easily degradable organic matter (i.e., acetic acid) in the test wastewater.

However, the initial rate (~90 mL/d on Day 1) of the 1 000 mg COD_L/L dilution was slightly less than that of the control (*Appendix L.1*). The approximate 6% reduction was possibly due to early inhibition of the inoculum's methanogenic activity. Its biogas production rate recovered beyond this point and was generally greater than that of the other concentrations tested.

Also, the total amounts of gas produced were not related to the strength of the test substrate. The most concentrated sample (i.e., 1 000 mg COD_i/L) generated the least amount of gas, at approximately 162 mL over the 32 day-test period while the least concentrated one (250 mg COD_i/L) generated approximately 177 mL. The control produced roughly 145 mL of biogas in total. The slight decrease in gas production with increasing feedstock concentration is evidence of some form of inhibition.

However, the wastewater constituents showed no other observable toxicity effects. They were considered as biodegradable or at least to offer some stimulation to the methanogenic bacteria since the total gas outputs of the 3 concentration levels tested were greater than that of the average control. This was somewhat predictable due to the nature of the synthetic wastewater. Even though daily biogas production increments of the samples slowed down considerably after the first reading, positive biogas measurements were observed throughout the course of the bioassay and they were still occurring at the end of the test. However, the results suggest that the feed's gas generation and degradation potential was especially suppressed at 1 000 mg COD_i/L. The inhibition signs were not considered as sufficient to disregard the anaerobic use of the experimental feed over the range of dilutions examined, particularly with proper acclimation of the UASB reactors.

Since the ATA was only performed with an acetic acid spike (Obayashi and Gorgan, 1985) which acted as the readily degradable substrate surplus (or main carbon source), the aforementioned interpretations mainly referred to the response of the anaerobic bacteria that primarily use acetate to produce methane. The conversion capacity of methanogens is considered as the rate-limiting step in the anaerobic process, although acetoclastic methanogens are responsible for over 70% of the biogas produced (Section 2.1.2.1). The latter are also most sensitive to inhibition/toxicity (Speece, 1996).

4.2 PERFORMANCE OF THE ANAEROBIC-AEROBIC TREATMENT SYSTEMS

Although theoretical steady state conditions imply that all treatment system parameters should be constant over a period of time, at any given location (Droste, 1997), they have been described by a variety of different criteria in the literature. Some authors report that reactor effluent COD concentrations should not change with time for an invariable influent waste strength and loading rate (Backman et al., 1985; Elmitwalli, 1999) or that COD removal efficiencies should be maintained during a definite time period. Others have suggested sustaining a minimum operational

time (i.e., 2 weeks) of at least a few times as long as the reactor's HRT or limiting the parameter variations to within 10% of their mean (Elmitwalli, 1999; Mikula et al., 1979). Curi and Eckenfelder (1976) have suggested that a steady state is reached when methane-forming organisms break down acetate at the same rate at which it is produced. Many sources also state that flow and loading rates should only be increased if reactors are stable; that is if effluent VFAs are maintained below a certain level and the reactor pH is steady.

During the present study, the anaerobic bacteria seemed to suffer from some form of inhibition [i.e., low VFAs (Section 4.2.3.3) and gas production (Section 4.2.3.4)]. Due to granular sludge flotation problems and solids washout encountered in both types of reactors, effluent SS were particularly variable [Figures 4.5 a) and b)], even after relatively long run times, under maintained operation conditions. Therefore, it is difficult to tell if and when the treatment systems reached this condition. Thus, unless stated otherwise, all average run results (and STDs), reported in this chapter, were based on bioreactor data that corresponded to the most stable influent flow rate conditions for each particular run. The data analyzed were also limited to those within deviations of approximately 30% (or less) of parameter averages and this outlier cut-off criterion was consistently applied over the various parameter calculations.

Although Run 1 (44 days) was considered to span from Days 18–61, Run 2 (52 days) from Days 62–113 and Run 3 (33 days) from Days 114–146, for both treatment systems, the *maintained influent flow rate period* of the respective runs was considered as Days 30–44 (Run 1), Days 72–113 (Run 2) and Days 137–146 (Run 3) for the case of AD1-AB1 (*Appendix L.5* and *L.6*). However, for the AD2-AB2 system, it was considered as Days 31–44 for Run 1 [Tables *L.5* and *L.6* b ii) in *Appendix L.5* and *L.6*]. Although the shaded areas presented in the various data tables of the appendices were not considered in parameter average and STD calculations, the more constant data that was used corresponded to run periods of at least 7 times the HRTs, whether for the ADs or the ABs.

4.2.1 BIOREACTOR OPERATING CONDITIONS AND ORGANIC LOADINGS

Although various targeted values were previously presented in Section 3.1.3, the intended and actual operational run conditions of both UASB reactors, in terms of influent feed concentrations (*Appendix L.3*) and flow rates as well as HRTs and OLRs, are summarized in Table 4.4. The daily and average run results for the latter 3 parameters can also be found in Tables *L.5* a i) – d ii) of *Appendix L.5*.

Table 4.4: Average UASB Operating Conditions

Intended Values					Actual Results			
AD1	Q_{in} (L/d)	HRT (d)	COD_t (mg/L)	OLR_t (g COD_t /L·d)	Q_{in} (L/d)	HRT (d)	COD_t (mg/L)	OLR_t (g COD_t /L·d)
Run 1	2	1.93	~1 000	0.52	2.0 ± 0.3	2.1 ± 0.4	777 ± 380	0.5 ± 0.2
Run 2	4	0.96	~1 000	1.04	4.3 ± 0.3	0.91 ± 0.06	794 ± 195	0.9 ± 0.2
Run 3	8	0.48	~1 000	2.08	8.8 ± 0.4	0.44 ± 0.02	829 ± 180	1.9 ± 0.4
AD2	Q_{in} (L/d)	HRT (d)	COD_t (mg/L)	OLR_t (g COD_t /L·d)	Q_{in} (L/d)	HRT (d)	COD_t (mg/L)	OLR_t (g COD_t /L·d)
Run 1	2	1.88	~500	0.27	2.2 ± 0.6	1.9 ± 0.5	765 ± 119	0.6 ± 0.2
Run 2	4	0.94	~500	0.53	4 ± 1	1.0 ± 0.3	1076 ± 247	1.1 ± 0.4
Run 3	8	0.47	~500	1.07	8.4 ± 0.5	0.45 ± 0.02	1052 ± 300	2.067 ± 0.002

(The OLR_t estimates do not include the solids recirculated from the aerobic processes.)

Except for influent total COD_t s, on average, the actual operating conditions resulted in values similar to those intended and they generally had sensible STDs. However, constant flow rates were not easy to achieve and to maintain since the numerous pumps and controllers employed were not equipped with digital read-outs. Since flow rate settings had to be calibrated and adjusted manually, their control was limited. On a few occasions, some pumps also failed and required replacement. As discussed in Section 3.2.1.3b, UASB reactor influent flow rates and corresponding HRTs were based on the daily volumes of final effluent collected from the treatment systems. The average HRTs in AD1 (which had a somewhat greater reactor volume) were slightly smaller than those of AD2 during Run 2 and 3 because their influent flow estimates were generally higher in AD1, on average.

The fluctuations and deviations in the feed COD_t concentrations as well as the considerable discrepancy observed between AD1's desired and actual feed strength was largely due to the significant and unexpected degradation that seemed to be occurring in the $6.2 \pm 1.6^\circ\text{C}$ refrigeration unit, even over only a few days of storage [Table L.3 in *Appendix L.3* and Figure 4.3 a)]. Its door did have to be opened often for solution and sample storage, feed sampling and replenishment. To reduce the effects, the Enfalac™ wastewater was frequently replaced with fresh batches, made every few days (whether some feed was left in storage tank or not). Also, despite the mixing technique implemented on Day 39 to keep un-dissolved milk solids in suspension (Section 3.1.2.1), they still precipitated. This could explain the reduction in the organic strength of the substrate samples with

time as well as their lower average SS and COD concentrations compared to the estimates of the characterization phase (Table 4.1). However, feed mixtures volumes were smaller and most likely more homogeneous.

In addition, the higher average influent COD_i concentrations found in the continuously diluted UASB reactor compared to those of AD1 (fed directly from the feed tank) are completely unrealistic (Table 4.4). The organic strength of the feed averaged 795(±232) mg COD_i/L, on the whole, while that of AD2's influent averaged 946(±255) mg COD_i/L (Table 4.6). The latter result was thought to be caused by the build-up of Enfalac™ residue in the Y-shaped connector that was located within the main feed recirculation line and led to AD2's inlet. It was also situated very close to the meeting point of the reactor's feed and dilution lines as well as to its influent sampling port (Figure 3.1). Although an input of water was always perceptible in the dilution line, the flow meter installed on Day 24 also experienced some operational difficulties. It might not always have maintained its intended setting since its sapphire float was often observed to decrease over time (Section 3.2.1.3b).

Thus, AD2's high influent strengths (Table 4.4 and *Appendix L.3*), that were much greater than the desired 500 mg COD_i/L, resulted in total OLRs greater than those of AD1, on average [Figure 4.2 b)]. However, the influent COD_s and OLR_s results [Figure 4.3 b) as well as Tables 4.6 and 4.7, respectively] clearly show that AD2 had a much smaller soluble organic load, when compared to AD1, and they reflect the dilution being applied to AD2's influent. The low organic loadings imposed on each UASB (*Appendix L.5*) over the entire experiment (including the acclimation period) as well as those resulting in the aerobic bioreactors (*Appendix L.6*) are presented in Figures 4.2 a) and b), in terms of total COD.

Similarly to those of the UASB reactors, the actual operating conditions of the ABs can be found in Tables L.6 a i) – d ii) of *Appendix L.6*. Average run results are also summarized in Tables 4.5 a) and b), for AB1 and AB2, respectively. Their influent concentrations and OLRs could not be predicted since they were dependant on the removal efficiencies attained by the ADs. Also, the influent flow rates and HRTs of the aeration basins could not be independently controlled since the AD effluents flowed into them by gravity.

It should be noted that Figures 4.2, 4.3 and 4.5 do not depict all the data presented in *Appendices L.5, L.6, L.3 and L.4*. Some outliers are not shown [particularly in Figures 4.5 a) and b)] since the y axis scales were limited to allow for a more comprehensible outlook on the bulk of the data points.

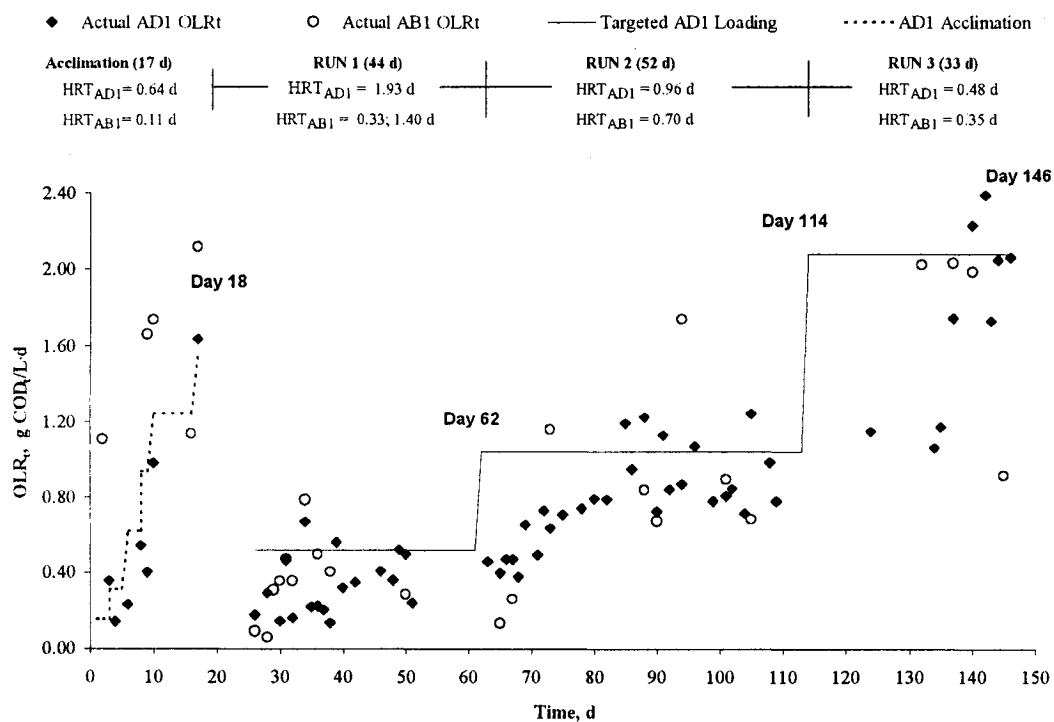


Figure 4.2 a): AD1's Target ($S_0 \approx 1\,000\text{ mg COD}_t/\text{L}$) and Actual Organic Loading

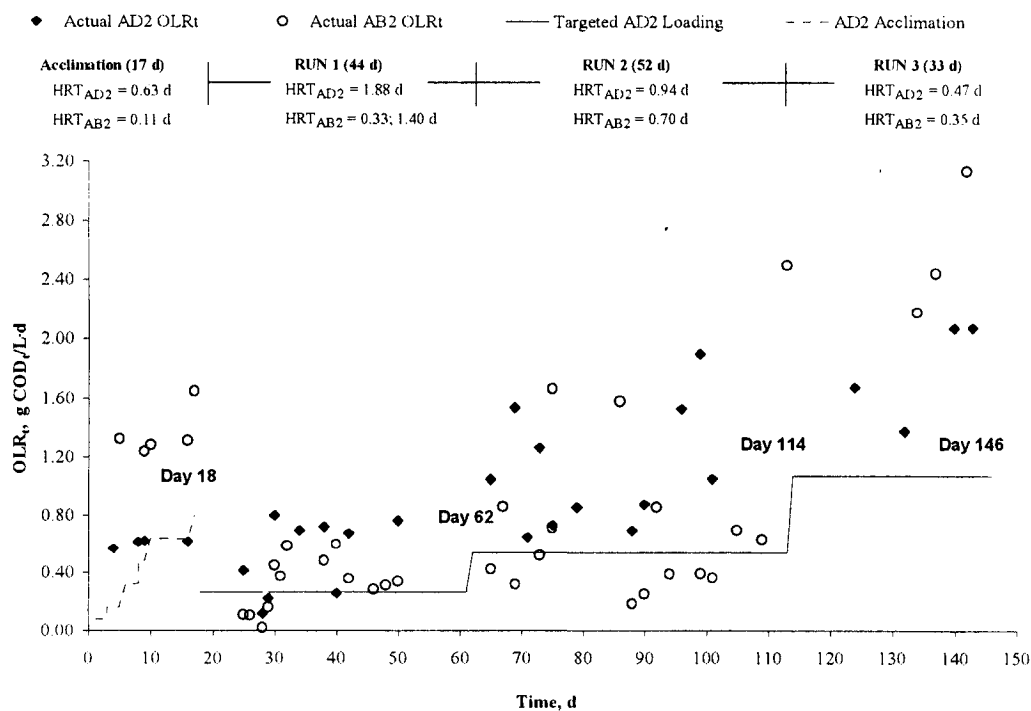


Figure 4.2 b): AD2's Target ($S_0 \approx 500\text{ mg COD}_t/\text{L}$) and Actual Organic Loading

4.2.2 EFFICIENCIES OF THE BIOREACTORS AND OF THE COMBINED TREATMENT SYSTEMS

Figures 4.3 a) and b) as well as Figures 4.5 a) and b), respectively, present the variations in the influent characteristics and effluent qualities of both treatment systems, throughout the entire experimental time course, in terms of COD_s and TSS. However, the influent properties of each UASB reactor were presented in terms of TSS and VSS as well as COD_t and COD_s concentrations.

To minimize the use of fiber filters, the SS estimates for the synthetic substrate were used as AD2's influent SS concentrations [Appendix L.4, Figures 4.5 a) and b) as well as Table 4.6]. Therefore, they were assumed to be smaller or equal to those of the feed since AD2's influent was being continuously diluted. However, the latter unit's influent CODs were determined on samples taken prior to its inlet, but past the mixing point of the dilution and feed lines (Section 4.2.1).

Also, the total effluent CODs as well as the TSS and VSS concentrations of both UASBs were probably overestimated since the samples had to be collected at the bottom of the U-tubes to be obtained. Thus, instead of flowing into the ABs, the anaerobic granules that washed out had a tendency to build-up in this area and were unavoidably sampled with the effluent liquid overflow. Since effluents were generally observed to be high in particulate matter, percent COD removals were estimated on a $COD_{t\ Infl.} - COD_{s\ Eff.}$ basis. Like every other calculated result, the %COD and %SS reductions were evaluated in terms of influent and effluent parameter monitoring on the same day. Thus, general process and system performances were assessed by way of the average effluent quality and treatment efficiencies achieved during each run. These results are reported in Tables 4.5 a) and b) while Table 4.6 sums up the overall average results of the entire study.

4.2.2.1 COD Effluent Qualities

Tables L.3 a i) – d ii) of Appendix L.3 present the daily and average COD_t and COD_s concentrations of all bioreactor influents and effluents. The percent COD removals realized by the individual units and the entire systems during each run are also presented in these tables. As pointed out in Section 4.2.1, the COD concentrations of the synthetic wastewater were observed to be quite variable throughout the study period [Figure 4.3 a)]. In spite of these important fluctuations, the feed maintained a high soluble fraction of approximately 60% [Table 4.5 a)], which was comparable to the ratio presented in Table 4.1. However, an experimental error surely occurred on Days 40 and 82 since the COD_s of the feed was higher than its COD_t . Nevertheless, the residual CODs in the anaerobic and aerobic reactor effluents had significantly greater particulate fractions, which means that the microorganisms consumed soluble substrate.

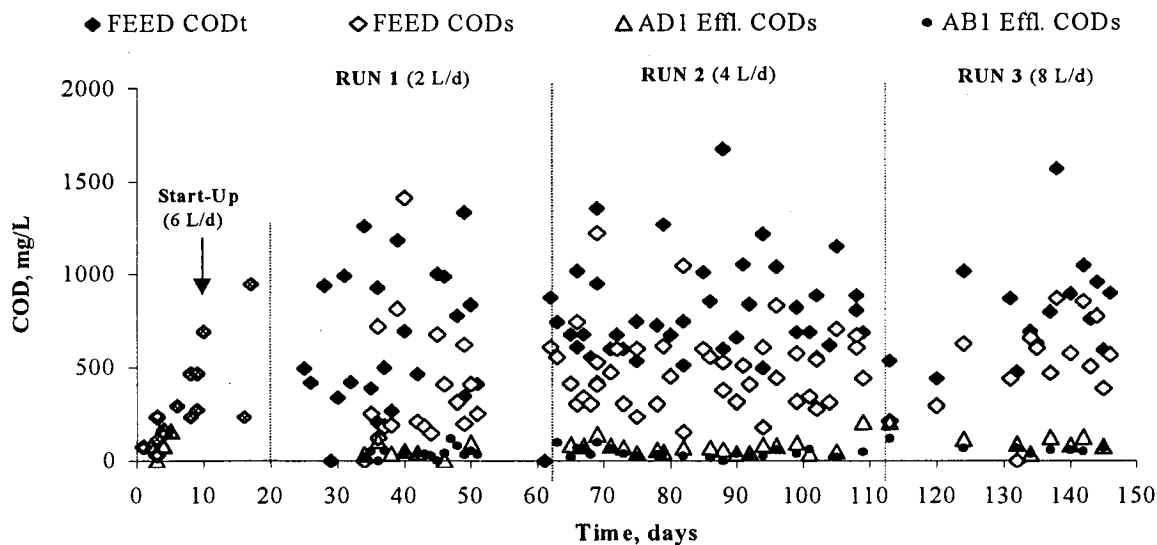


Figure 4.3 a): Feed-AD1 Effl.-AB1 Effl. CODs

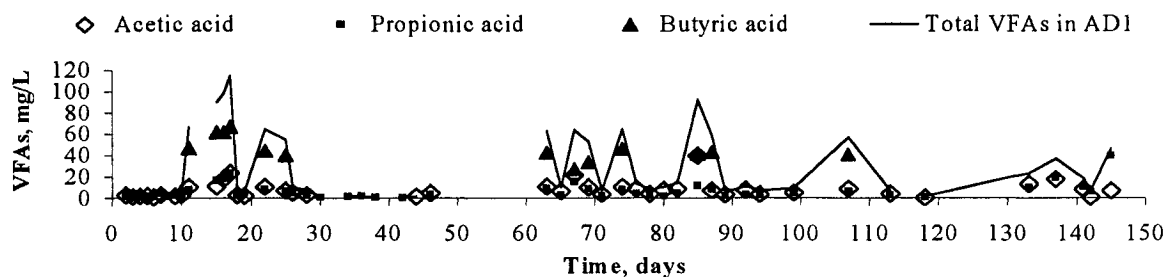


Figure 4.4 a): VFAs in AD1

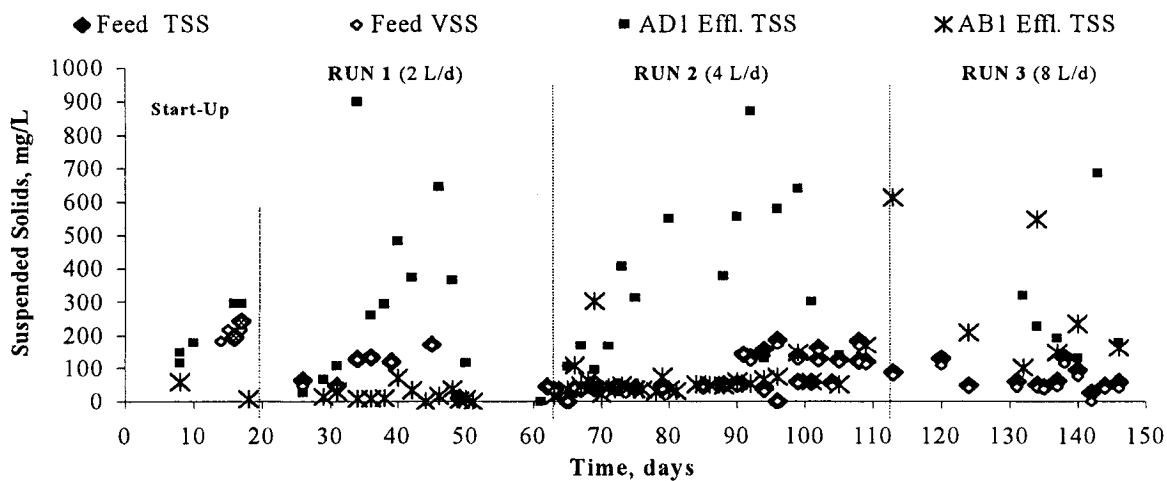


Figure 4.5 a): Feed-AD1 Effl.-AB1 Effl. SS

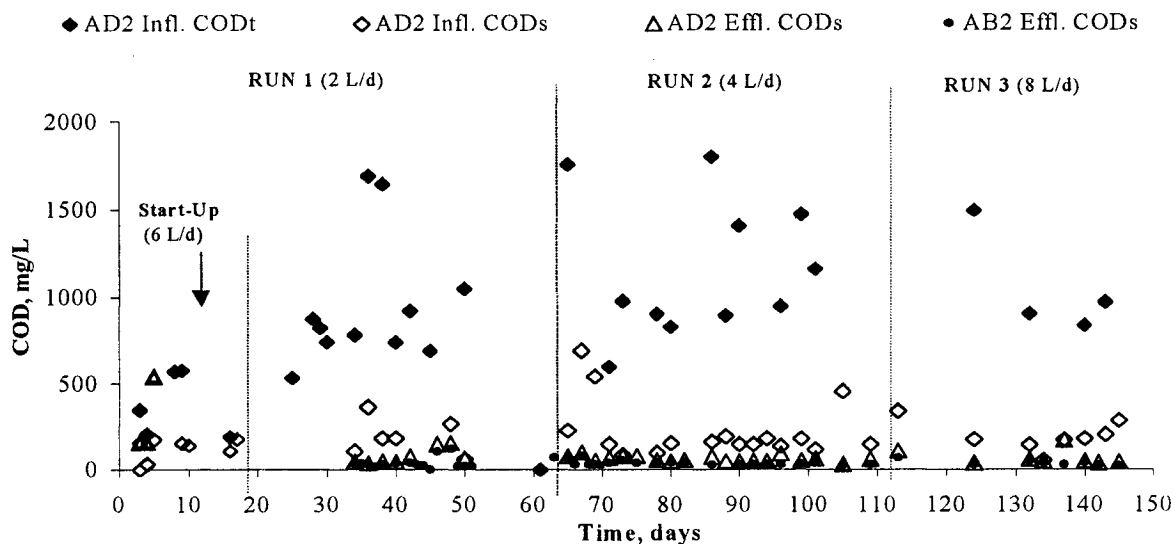


Figure 4.3 b): AD2 Infl.-AD2 Effl.-AB2 Effl. CODs

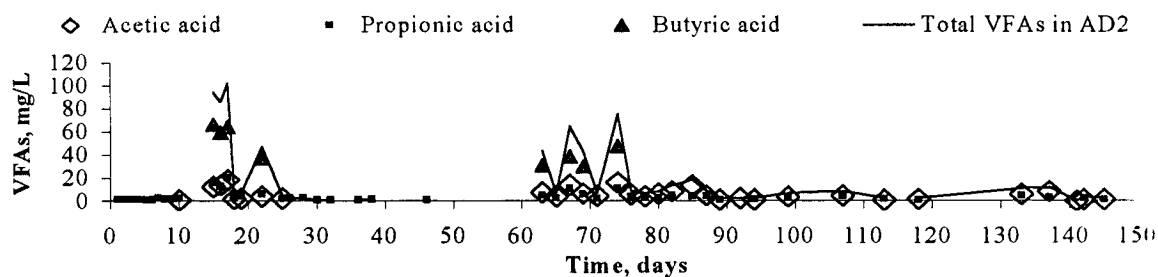


Figure 4.4 b): VFAs in AD2

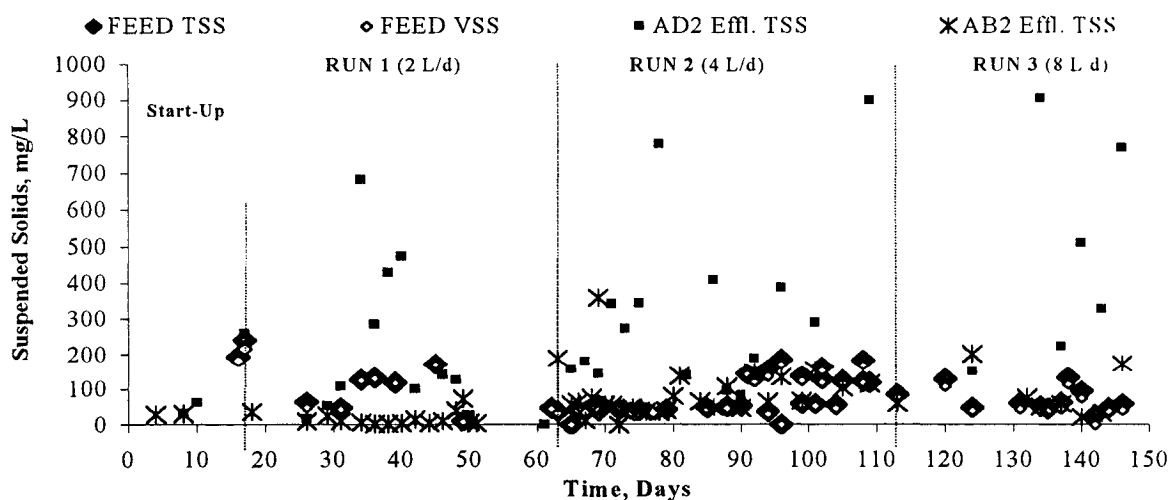


Figure 4.5 b): Feed-AD2 Effl.-AB2 Effl. SS

The average COD_t concentrations of both UASB effluents were high and quite variable [S_0 for ABs in Tables 4.5 a) and b)]. This was especially the case for AD2 [487($\pm$ 538) mg COD_t/L in Table 4.6] since an extremely large mean value of 1 513($\pm$ 405) mg COD_t/L resulted during Run 3. Over this period, overflowing granules caused a noticeable increase in its average washout rate (i.e., decrease in mean SRT) as well as in AB2's average SS inventory and influent OLR_t [i.e., 4.7($\pm$ 1.6) g $\text{COD}_t/\text{L}\cdot\text{d}$], on account of the highest applied flow rate. However, the final effluent quality of the system did not suffer and was quite low in terms of TSS and COD_s . To a much lesser extent [i.e., 564($\pm$ 141) mg COD_t/L], AD1's effluent COD_t concentrations peaked during Run 2.

In contrast, effluent COD_s concentrations sustained relatively constant levels throughout the runs, whether for the ADs or the ABs of each treatment system [Figures 4.3 a) and b) as well as Tables 4.5 a) and b)]. However, average concentrations were found to rise slightly in AD1 and AB1, with increasing OLR_s . The important settling deterioration, flotation problems and loss of sludge encountered in the former unit during the last run (Section 4.3.3) yielded a significant increase in AB1's average MLVSS concentration [i.e., 2.4($\pm$ 0.9) g VSS/L]. Owing to the considerable granule spill over into this aeration tank and to its highest average VSS washout rate, the quality of the final effluent drastically declined in terms of TSS [i.e., 182($\pm$ 47) mg/L], but particularly in terms of COD_t [i.e., 1 690($\pm$ 224) mg/L in Table L.3 d i) of *Appendix L.3*]. Both final effluent parameters resulted in concentrations that were even higher than that of AB1's influent or AD1's effluent [Table 4.5 a) and *Appendix L.3*]. However, over the runs, the soluble organic strength of the final effluent discharged from both treatment systems were quite low and ranged from 37–60 mg COD_s/L , in AD1-AB1, and from 27–34 mg COD_s/L , in AD2-AB2.

For the various operational conditions and contact times reported in Tables 4.5 a) and b), both the UASB and AB units maintained reasonably good %COD removals. This implies that the reactor biomass was not stressed organically and that it adapted to the progressive increases in the low loading rates applied. Although a slight decrease in COD reduction was detected in AD1 and AB1, during the highest hydraulic and organic loading conditions, in essence, neither type of reactor or treatment system was observed to be affected by the increasing OLR_s and decreasing HRTs. The average COD removals achieved by AD1 and AD2 varied from 87–92% and 93–94% while those of aerobic processes averaged 86–93% in AB1 and 83–98% in AB2, respectively. Thus, the lower loaded system achieved slightly superior organic reductions since anaerobic-aerobic treatment efficiencies ranged from 90–95% and 95–96%, on average, in AD1-AB1 and AD2-AB2, respectively. Under comparable conditions, these results were similar to those of a few

HR anaerobic systems presented in Table 2.9 (Section 2.2.2.4) that treated different types of dairy wastewaters, although most of them had higher influent organic strengths.

4.2.2.2 Suspended Solid Effluent Qualities

The influent and effluent SS concentrations of the treatment system bioreactors as well as the SS concentrations contained within the ABs are presented in Tables L.4 a i) – d ii) of *Appendix L.4*. Daily and average removals achieved by the UASBs, ABs and the combined configurations, during each run, are also presented in these tables (on a TSS basis). As depicted in Figures 4.5 a) and b), the effluent TSS data were quite erratic in both treatment systems. However, this was more pronounced in the ADs, which had particularly high concentrations and experienced considerable washout rates compared to the ABs.

As reported in Tables 4.5 a) and b), no solids removal took place by way of these treatment units since the SS contained in their effluents were always much greater than those of their influents. On average, the former ranged from 168–510 mg TSS/L, in AD1, and from 316–418 mg TSS/L in AD2, for the HRT and flow rate conditions investigated during the three runs. While SB expansion and floating granules were experienced over time in both anaerobic reactors, the simple bioring deflectors used were believed to have poor solids retention capabilities.

Although no trend was explicitly detected in either UASB effluent, a significant increase in AD1's average effluent TSS concentration was noted over Run 2 as well as during Run 3 in AD2. Both cases were found to correspond to the greatest average effluent COD_t concentrations and washout rate of the reactors [Tables 4.5 a) and b)]. Due to significant sludge flotation problems, AD1's SB had actually split in two distinct parts, by Run 3, and suffered from a considerable loss in SS. However, some of it was due to an important sampling mishap. Thus, the less significant average concentration that resulted in AD1's effluent [i.e., 168(±33) mg TSS/L] was probably due to the fact that less SS were present in the reactor, especially closer to the overflow vicinity compared to the previous run.

Throughout the experiment, the aerobic MLSS also proved to be very hard to retain in each aeration tank [Tables 4.5 a) and b)]. Since VSS concentrations dropped dramatically from the initial seeding concentration (*Appendix L.4*), manual batch recycling of all settled effluent bucket solids to the ABs was initiated on Day 36. This was done to try and build-up or at least sustain the SS inventories until basin modifications (Day 45) and re-seeding (Day 52) were performed. The second TWAS inoculation (Table 4.3) as well as the insertion of a baffle in the tanks, which created deeper aeration basins and joint AB-clarifier units [Figure 3.5 c)], were used to try to

contain the MLVSS. However, the aerobic batch solids recycling was performed until the end of the study since the other efforts were clearly ineffective. Subsequently to the elimination of the oversized clarifiers, the average final effluent concentrations of both systems increased slightly. Yet, the few daily peaks observed in the final effluents, shortly after the re-seeding (start of Run 2), may have been due to customary solids washout following inoculation.

Although final effluent TSS concentrations were generally low and fairly stable, VSS inventories in the ABs were drastically low (≤ 200 mg VSS/L), particularly during Run 1. The final effluent TSS were observed to increase from 9–182 mg TSS/L, on average, in AD1-AB1, while those AD2-AB2 ranged from 4–51 mg TSS/L, over the study period. Unlike for COD, the overall %TSS removals achieved in both systems were much smaller (Table 4.6) and were found to decrease significantly, over the runs, with the hydraulic and organic loading rate increases [Tables 4.5 a) and b)]. The average SS removal efficiency of the AD1-AB1 system ranged from 93%TSS to no removal and that of the AD2-AB2 system ranged from 96 to 7%TSS while that of the aeration units ranged from approximately 98 to a low 16%, in AB1, and from 99 to 88% in AB2.

On average, the greatest bioreactor effluent washout rates ($Q_{\text{eff}} \cdot X_e$) corresponded to their smallest SRT estimate, but anaerobic SRTs were always above the 20 d value recommended for design at 24°C. However, in general, the greatest TSS and COD removals realized in each unit process did not necessarily correspond to the instance when they contained the most SS or when their HRT was largest. Nonetheless, since treatment capacities of biological systems are largely attributed to the mass of active bacteria (or VSS) present in them (Droste, 1997b), the lowest organic removal achieved in AB2 (i.e., 83%), during Run 1, was probably due to the unusually small amount of SS that it was estimated to contain (i.e., 168 mg VSS in *Appendix L.4*).

4.2.3 PERFORMANCE OVERVIEW OF THE BIOLOGICAL UNITS AND TREATMENT SYSTEMS

Tables 4.5 a) and b) respectively summarize the average operational conditions and performance of each biological treatment unit and combined UASB-activated sludge system studied during the three experimental runs conducted. These exclude start-up data and were based on the parameter values observed during the selected periods of stable influent flow rates, during each particular run (Section 4.2). In addition, the values reported in parentheses represent the resulting STDs of the average parameters. Most of the parameters presented in these tables have been or will be discussed independently and/or in relation to other performance variable results, in previous or subsequent parts of subsection 4.2. However, the changes experienced over time in the UASB granular biomass characteristics are presented separately in Section 4.3.

Table 4.5 a): Average Performance Results of the AD1-AB1 Treatment System (Under Maintained Influent Flow Conditions)

AD1 (3.85 L)	Temp. (°C)	Q _{in} (L/d)	IIRT (d)	¹ S _e (mg/L)	COD _e (mg/L)	² OLR _e (g VSS/L)	X _v (g VSS/L)	X _e (mgTSS/L)	³ S _e (%)	⁴ COD (%)	TSS (%)	⁵ Washout (g VSS/d)	SRT (d)	pH	TA (mg/L)	VFA _{tot} (mg/L)	Biogas (L/d)	CH ₄ , CO ₂ (%) (%)	⁶ Yield _{CH₄}			
Run 1 (24 d)	23.4 (1.0)	2.0 (0.3)	2.1 (0.4)	777 (380)	412 (238)	0.5 (0.2)	20.9 (NA)	352 (99)	56 (9)	89 (3)	NR	1.8 (0.6)	49 (16)	7.7 (0.2)	421 (244)	2 (3)	0.02	24, 2 (17), (1)	0.009			
Run 2 (36 d)	23.8 (0.5)	4.3 (0.3)	0.91 (0.06)	794 (195)	472 (159)	0.9 (0.2)	25.5 (NA)	510 (183)	61 (19)	92 (4)	NR	1.8 (0.4)	58 (13)	7.4 (0.2)	505 (123)	28 (29)	0.2	64, 6 (10), (2)	0.02			
Run 3 (12 d)	22.6 (0.4)	8.8 (0.4)	0.44 (0.02)	829 (180)	576 (154)	1.9 (0.4)	17.3 (NA)	168 (33)	97 (27)	87 (3)	NR	0.8 (0.2)	89 (24)	7.8 (0.3)	643 (34)	28 (18)	0	23, 0.9 (3), (0.6)	0			
AB1 (2/2.8 L)	Temp. (°C)	Q _{in} (L/d)	IIRT (d)	¹ S _e (mg/L)	COD _e (mg/L)	² OLR _e (g VSS/L)	X _v (gMLVSS/L)	X _e (mgTSS/L)	³ S _e (%)	⁴ COD (%)	TSS (%)	⁵ Washout (g VSS/d)	SRT (d)	pH	TA (mg/L)	DO (mgO ₂ /L)						
Run 1 (24 d)	20.6 (1.3)	6.0/(2.1) (0.3)/(0.3)	0.34/(1.4) (0.02)/(0.2)	162 (51)	56 (9)	0.5 (0.2)	0.17 (0.06)	9 (1)	37 (11)	92 (1)	98 (1)	0.014 (0.002)	18 (0.06)	8.5 (0.3)	445 (208)	8.0 (0.3)						
Run 2 (36 d)	20.3 (0.6)	4.3 (0.3)	0.66 (0.04)	564 (141)	61 (19)	0.9 (0.2)	0.19 (0.09)	50 (14)	40 (12)	93 (3)	89 (4)	0.11 (0.04)	4 (2)	7.8 (NA)	614 (NA)	8.2 (0.3)						
Run 3 (12 d)	18.7 (0.7)	8.8 (0.4)	0.32 (0.01)	434 (205)	97 (27)	1.7 (0.6)	2.4 (0.9)	182 (47)	60 (7)	86 (9)	16 (7)	1.3 (0.5)	4 (1)	7.7 (0.05)	802 (82)	8.2						
Overall System	IIRT (d)						COD (%)			TSS (%)			NA-Not Applicable, NIR-No Removal VFA _{tot} =Ac + Bu + Pr acids %N ₂ = 100% - %CH ₄ - %CO ₂ ¹ mg COD/L ² mg COD/L·d ³ mg COD/L ⁴ %COD removals on COD _i - COD _e basis ⁵ Washout = Q _{in} /X _e (g VSS/d) ⁶ 1 mg COD _i /g COD _e									
Run 1 (24 d)	2.4/(3.5) (0.4)/(0.6)									90 (3)			93 (1)									
Run 2 (36 d)	1.6 (0.1)									95 (2)			41 (22)									
Run 3 (12 d)	0.76 (0.03)									92 (3)			NR									

The number of days represents the amount of feeding days per run (Appendix L.2, L.5 and L.6).

The values reported in parentheses, (), represent the STDs of the average parameters.

The IIRTs and flow rates in brackets, { }, correspond to the conditions once the ABIs were modified.

NA-Not Applicable, NR-No Removal

VFA_{tot}=Ac + Bu + Pr acids

%N₂ = 100% - %CH₄ - %CO₂

¹ mg COD/L

² mg COD/L/d

³ mg COD/L

⁴ %COD removals on COD_i - COD_e basis

⁵ Washout = Q_{effluent} (L/d) · X_e (g VSS/L)

⁶ L_{CH₄}/g COD_i rem

Table 4.5 b): Average Performance Results of the AD2-AB2 Treatment System (Under Maintained Influent Flow Conditions)

AD2 (3.75 L)	Temp. (°C)	Q _{in} (L/d)	HRT (d)	¹ S _e (mg/L)	COD _e (mg/L)	² OLR _i (g VSS/L)	X _v (g VSS/L)	X _s (mgTSS/L)	³ S _e	⁴ COD (%)	TSS (%)	S _{Washout} (g VSS/d)	SRT (d)	pH	TA (mg/L)	VFA _{tot} (mg/L)	Biogas (L/d)	CH ₄ ,CO ₂ (%) (%)	⁶ Yield _{CH₄}
Run 1 (24 d)	23.4 (1.0)	2.2 (0.6)	1.9 (0.5)	765 (119)	146 (54)	0.6 (0.2)	21.3 (NA)	397 (98)	49 (16)	93 (1)	NR	2.5 (0.9)	34 (9)	7.4 (0.4)	190 (94)	1.2 (0.3)	0.2	40, 3 (13), (3)	0.08
Run 2 (36 d)	23.8 (0.5)	4 (1)	1.0 (0.3)	1076 (247)	146 (32)	1.1 (0.4)	22.9 (NA)	316 (81)	59 (16)	94 (2)	NR	1.2 (0.4)	78 (22)	7.1 (0.3)	447 (124)	13 (19)	0.001	66, 6 (7), (1)	0.0005
Run 3 (12 d)	22.6 (0.4)	8.4 (0.5)	0.45 (0.02)	1052 (300)	195 (49)	2.067 (0.002)	18.9 (NA)	418 (90)	43 (6)	94 (NA)	NR	3 (0.7)	28 (7)	7.8 (0.3)	625 (122)	4 (3)	0.06	57, 6 (9), (1)	0.004
AB2 (2/2.8 L)	Temp. (°C)	Q _{in} (L/d)	HRT (d)	¹ S _e (mg/L)	COD _e (mg/L)	² OLR _i (g VSS/L)	X _v (gMLVSS/L)	X _s (mgTSS/L)	³ S _e	⁴ COD (%)	TSS (%)	S _{Washout} (g VSS/d)	SRT (d)	pH	TA (mg/L)	DO (mgO ₂ /L)			
Run 1 (24 d)	20.0 (1.3)	6.2/(2.5) (0.8)/(0.5)	0.33/(1.1) (0.04)/(0.2)	161 (48)	49 (16)	0.5 (0.1)	0.084 (0.009)	4 (2)	32 (5)	83 (1)	99 (0.3)	0.003 (0.001)	65 (18)	8.2 (0.5)	186 (87)	8.2 (0.2)			
Run 2 (36 d)	20.2 (0.6)	4 (1)	0.8 (0.2)	305 (85)	59 (16)	0.5 (0.2)	0.2 (0.1)	51 (15)	34 (8)	89 (5)	88 (1)	0.14 (0.05)	4 (2)	7.7 (NA)	540 (NA)	8.3 (0.4)			
Run 3 (12 d)	19.8 (0.5)	8.4 (0.5)	0.34 (0.02)	1513 (405)	43 (6)	4.7 (1.6)	0.7 (0.3)	26 (5)	27 (9)	98 (1)	93 (3)	0.11 (0.02)	13 (2)	7.3 (0.4)	656 (NA)	7.8 (0.2)			
Overall System				HRT (d)							COD (%)	TSS (%)	NA=Not Applicable, NR=No Removal VFA _{tot} =Ac + Bu + Pr acids %N ₂ = 100% - %CH ₄ - %CO ₂ ¹ mg COD/L ² mg COD/L/d ³ mg COD/L ⁴ %COD removals on COD _i - COD _e basis ⁵ Washout = Q _{Effluent} (L/d) · X _e (g VSS/L) ⁶ %CH ₄ in COD						
Run 1 (24 d)				2.2/(3.0) (0.5)/(0.7)							95 (0.3)	96 (1)							
Run 2 (36 d)				1.8 (0.5)							96 (1)	25 (9)							
Run 3 (12 d)				0.79 (0.04)							95 (NA)	7 (NA)							

NA=Not Applicable, NR=No Removal

VFA_{tot}=Ac + Bu + Pr acids%N₂ = 100% - %CH₄ - %CO₂¹ mg COD_i/L² mg COD_i/L-d³ mg COD_i/L⁴ %COD removals on COD_i - COD_e basis⁵ Washout = Q Effluent (L/d) · X_e (g VSS/L)⁶ L_{CH4}/g COD_i rem

The number of days represents the amount of feeding days per run (Appendix L.2, L.5 and L.6)
 The values reported in parentheses, (), represent the STDs of the average parameters.
 The HRTs and flow rates in brackets, { }, correspond to the conditions once the ABs were modified.

Table 4.6 summarizes the average results of the pooled stable flow data in each system, in terms of COD as well as SS. It also reports the removal efficiencies estimated for the individual units as well as for the complete treatment systems for the entire study period.

Table 4.6: Overall Average COD and SS Results of Both Treatment Systems

	System 1			System 2		
	Feed	AD1 _{EM}	Final _{EM}	Influent	AD2 _{EM}	Final _{EM}
COD_i (mg/L)	795 ± 232	381 ± 230	308 ± 541	946 ± 255	487 ± 538	88 ± 47
COD_s (mg/L)	476 ± 181	63 ± 27	44 ± 13	160 ± 44	55 ± 16	33 ± 8
TSS (mg/L)	88 ± 48	406 ± 196	61 ± 52	88 ± 48	357 ± 95	37 ± 24
VSS (mg/L)	79 ± 47	311 ± 169	37 ± 43	79 ± 47	283 ± 76	25 ± 18
%Removals	AD1	AB1	Overall	AD2	AB2	Overall
%TSS	NR	79 ± 29	54 ± 31	NR	93 ± 5	32 ± 28
%COD	90 ± 4	90 ± 6	94 ± 3	94 ± 2	90 ± 6	96 ± 1

Percent COD removals were calculated on a $COD_{i\text{ infl}} - COD_{s\text{ EM}}$ basis. NR—No Removal

As explained in Section 4.2.1, AD2's average influent COD_i concentration was unfortunately found to be significantly greater than AD1's, but the former reactor visibly had the lowest influent soluble CODs (Table 4.6). Since AD2 also produced a lower overall effluent COD_s, on average, at 55(±16) mg/L compared with 63(±27) mg/L in AD1, its mean COD removal efficiency was slightly greater over the entire monitoring period [i.e., 94(±2)% vs. 90(±4)%]. However, despite the significant sludge flotation, losses and washout problems observed in AD1 (i.e., Run 3), its parted granular bed achieved removals comparable to those of AD2. Yet, due to granule washout, the latter produced an extremely high average effluent COD_i concentration during Run 3.

However, nearly all of the organic matter was observed to be removed by the UASB reactors, given that the overall average COD removals of the combined AD1-AB1 and AD2-AB2 systems resulted in 94(±3)% and 96(±1)%, respectively. Consequently, the organic contents decreased by an additional 4% (AB1) and 2% (AB2) through the activated sludge process polishing units, in spite of the extremely low SS inventories in both ABs [(Tables 4.5 a) and b)]. The average final COD_i effluent concentrations were observed to be rather high, on the whole, but particularly in AD1 because of Run 3's average result of 1.69 g COD_i/L, owing to granule washout. Although

the influent COD strengths of the ADs were weak, overall average effluent CODs resulted in values of $44(\pm 13)$ mg/L in AD1-AB1 and $33(\pm 8)$ mg /L in AD2-AB2, in terms of COD_s .

While both ABs produced analogous COD removals of $90(\pm 6)\%$, their overall average TSS removal efficiencies differed and resulted in $79(\pm 29)\%$, for AB1, and in $93(\pm 5)\%$, for AB2. However, the STD of the result for the former treatment unit was much larger because it achieved a much lower average TSS removal (or much greater final effluent TSS concentration) during the last run compared to the two previous ones. As stated in Section 4.2.2.2 and reported in Table 4.6, both UASBs did not removed any influent SS since their effluent TSS concentrations were much greater than the low values of the synthetic feed.

Thus, contrary to the COD reductions, the activated sludge processes were, in essence, responsible for reducing the final effluent qualities to average concentrations of $61(\pm 52)$ mg TSS/L and $37(\pm 24)$ mg TSS/L, via AB1 and AB2. On the whole, the combined systems were estimated to yield TSS reductions of $54(\pm 31)\%$ in AD1-AB1 and $32(\pm 28)\%$ in AD2-AB2, on average. Also, the greater variability observed in the mean %TSS removals compared with those of the %COD removals is simply due to the greater effluent TSS concentration fluctuations in all bioreactors (Figures 4.3 and 4.5).

Table 4.7 reports the average bioreactor performance in treating the synthetic dairy feed, in terms of volumetric (OLR) and specific organic loading rates (SOLR) as well as in terms of volumetric (VRR) and specific removal rates (SRR), on a soluble substrate basis. The two former loading rates reflect the COD applied to the bioreactors while the latter two rates reflect the organic content removed from them. In addition, the specific loading and removal rates are given in terms of the estimated microorganism mass (g VSS) present in each particular reactor during each run. The OLR and VRR results of the ADs and ABS, in terms of COD_t as well as COD_s , can be found in *Appendices L.5* and *L.6*, respectively. Although not presented, the SOLRs and SRRs in terms of COD_t would obviously be greater than those presented in Table 4.7, for all reactors, since total OLRs were always significantly greater than soluble OLRs.

Of the two UASB reactors, AD1 always had the highest soluble OLRs. Thus, its SOLRs and consequently its SRRs (and VRRs) were also greater during the study. Because the extent of their organic removal was reasonably high, on average, at $> 83\%$ in each reactor type, over the various runs [Tables 4.5 a) and b) as well as Table 4.6], the VRRs and SRRs achieved were always very similar to the OLR and SOLR values, respectively (Table 4.7). Like total OLRs [Tables 4.5 a) and b)], soluble OLRs and VRRs as well as soluble SOLRs and SRRs increased with time in the ADs essentially because of the increase in their influent flow rates from run to run.

Table 4.7: Average Organic (and Specific) Loading and Removal Rates

AD1	OLR _s	VRR _s	SOLR _s	SRR _s	*Ave. AAT _{AD1} (Section 4.3)	
	g COD _s /L·d	g COD _{s,rem} /L·d	g COD _s /g VSS·d	g COD _{s,rem} /g VSS·d	g COD _{rem} /L·d	g COD _{rem} /g VSS·d
Run 1	0.12 ± 0.04	0.10 ± 0.04	0.006 ± 0.002	0.005 ± 0.001	2.6 ± 0.2	0.36 ± 0.03
Run 2	0.5 ± 0.2	0.5 ± 0.2	0.020 ± 0.007	0.019 ± 0.006	2.0 ± 0.2	0.34 ± 0.04
Run 3	1.3 ± 0.4	1.2 ± 0.4	0.08 ± 0.02	0.07 ± 0.02	0.58 ± 0.04	0.12 ± 0.01
AD2	OLR _s	VRR _s	SOLR _s	SRR _s	*Ave. AAT _{AD2} (Section 4.3)	
	g COD _s /L·d	g COD _{s,rem} /L·d	g COD _s /g VSS·d	g COD _{s,rem} /g VSS·d	g COD _{rem} /L·d	g COD _{rem} /g VSS·d
Run 1	0.08 ± 0.02	0.08 ± 0.02	0.0037 ± 0.0008	0.0035 ± 0.0007	2.3 ± 0.2	0.32 ± 0.02
Run 2	0.16 ± 0.06	0.14 ± 0.06	0.007 ± 0.003	0.006 ± 0.002	2.0 ± 0.1	0.37 ± 0.02
Run 3	0.48 ± 0.09	0.4	0.026 ± 0.005	0.024 ± 0.005	1.36 ± 0.07	0.24 ± 0.01
AB1	OLR _s	VRR _s	SOLR _s	SRR _s	*Tests run at room temperature.	
	g COD _s /L·d	g COD _{s,rem} /L·d	g COD _s /g VSS·d	g COD _{s,rem} /g VSS·d		
Run 1	0.13 ± 0.03	0.12 ± 0.02	0.8 ± 0.1	0.8		
Run 2	0.09 ± 0.03	0.07 ± 0.02	0.5 ± 0.2	0.4 ± 0.4		
Run 3	0.33 ± 0.08	0.27 ± 0.08	0.16 ± 0.07	0.14 ± 0.06		
AB2	OLR _s	VRR _s	SOLR _s	SRR _s		
	g COD _s /L·d	g COD _{s,rem} /L·d	g COD _s /g VSS·d	g COD _{s,rem} /g VSS·d		
Run 1	0.16 ± 0.06	0.103 ± 0.005	2.1 ± 0.9	1.31 ± 0.03		
Run 2	0.08 ± 0.03	0.08 ± 0.03	0.4 ± 0.3	0.3 ± 0.13		
Run 3	0.13 ± 0.03	0.13 ± 0.03	0.235	0.230		

SOLRs are estimates for F/M (g COD_{s, fed}/g VSS·d) while SRRs are estimates for U or k (g COD_{s, rem}/g VSS·d).

However, this was not the case for the ABs. The applied loading rates were slightly higher during Run 1 than during Run 2 due to the aerobic sludge recycling from the clarifier to the ADs (Days 18–45) that resulted in greater influent flow rates (i.e., ~6 L/d vs. ~2 L/d). The changes made to the AB configurations after Day 45, which increased their volume from 2 to 2.8 L [Fig. 3.5 d)], also contributed to some extent to the reduction in their soluble OLRs from Run 1 to 2. Nonetheless, for similar influent flow rates and basin sizes, both ABs had analogous soluble OLRs. Interestingly, this occurred since both ADs produced comparable effluent soluble CODs (except in Run 3), but despite the fact that AD1 had much greater influent soluble CODs, on average [Tables 4.5 a) and b)].

Contrary to the trend observed in the UASB reactors, soluble SOLRs (as well as SRRs) decreased with time in the ABs mostly because their average SS inventories (g VSS) increased over the runs [Tables 4.5 a) and b)]. This was probably due to the daily return of settled effluent solids to the aerobic basins (after Day 36) and/or to the overflow of anaerobic granules into the aeration tanks. The SOLRs were also observed to be much smaller in the ADs than in the ABs

since anaerobic granular biomass concentrations were always extremely high compared to those of the MLVSS.

In spite of the fact that the SS content in each aeration tank was very poor, the aerobic F/M ratios (or SOLRs) were similar to values suggested in the literature (Section 2.1.4.3), except for Run 1. Thus, filamentous bulking was ruled out as a cause for the solids washout encountered in the ABs since their SOLRs as well as the aerobic OLRs were reasonable. However, their average SRTs were observed to decrease considerably throughout the runs, especially in AB1, where the average values were estimated to be at the lower end of the suggested range (Section 2.1.4.3) during Runs 2 and 3 [Tables 4.5 a) and b)]. SRT results were based solely on the solids washout ($Q_{\text{eff}} \cdot X_e$; *Appendix M*) since sampling wastage was considered to be negligible.

The average volumetric and specific acetoclastic activity rates reported in Table 4.7, obtained from granular biomass samples collected from the anaerobic reactor SBs, are also presented in Section 4.3.1. However, the AATs that were performed at room temperature were only used as a means to estimate the substrate utilization rate of the biomass (k or U).

Actual SB activities or SRRs were greatest in both anaerobic units, during the final run, when they were submitted to the greatest applied loadings. This was still the case for AD1 even though it encountered an important decrease in its SBH and SS content over the latter part of Run 3. However, the relatively low degradation rates (SRRs) of the continuously fed UASB reactors mainly reflect the very low applied OLRs and SOLRs since the organic removal efficiencies they achieved during their operation were reasonable.

A few potential causes for low biomass activities and/or inhibition have been pointed out in the closing paragraphs of Section 4.2.3.5.

4.2.3.1 Reactor Temperatures and DO Concentrations in the Aeration Basins

The refrigerator and laboratory temperatures as well as the DO concentrations and corresponding temperatures in both aeration basins were regularly monitored throughout the study. When measured, the first two temperatures were taken at about the same time and usually early in the morning. These readings are presented in *Appendix L.8* [Tables L.8 a) – d)] while those pertaining to the ABs are presented in *Appendix L.2* [Tables L.2 a) – d)]. The latter were essentially recorded at the same time as their pH and alkalinity were evaluated. Because the contents of the UASBs were not easily accessible, since the reactors were sealed to maintain anaerobic conditions, their temperatures were assumed to be the same as that of the ambient room temperature. Due to

this limitation, the supernatant effluent samples, taken at the bottom of the U-tubes for convenience, were also assumed to represent the pH and alkalinity (as well as the SS and COD) conditions found within the reactor.

The average temperature of the feed refrigeration unit over the different runs is presented in Table 4.8 while that of the two sets of ADs and ABS is summarized in Tables 4.5 a) and b). The mean DO concentrations recorded in both aeration tanks during the three runs are also summarized in the latter tables. Over time, there was an apparent deterioration in the storage conditions of the feedstock, samples and solutions since the refrigeration temperature progressively digressed from 4°C, which is considered best for preservation. This was mostly due to the malfunctioning of the heat exchanger of the old second-hand refrigeration unit employed that was noticed during the second experimental run. However, as the flow rates increased, the refrigerator door also had to be opened more often in order to replenish the relatively small feed tank.

Table 4.8: Average Feed Refrigeration Temperature

Operational Time	Refrigerator Temperature (°C)
Start-Up (<i>Days 1-17</i>)	4.7 ± 1.3
Run 1 (<i>Days 18-61</i>)	5.4 ± 2.2
Run 2 (<i>Days 62-113</i>)	6.7 ± 0.8
Run 3 (<i>Days 114-146</i>)	7.3 ± 1.2
Overall (<i>Days 1-146</i>):	6.2 ± 1.6

Conversely, the un-controlled temperature conditions of the laboratory in which the experiment was conducted decreased slightly, on average, but mostly over the last run (Table 4.5). They were unsurprisingly warmer at the beginning of the official study period (i.e., $24.1 \pm 0.2^\circ\text{C}$ during acclimation) than at the end since it ran from summer to winter months (late July to mid-December). However, the overall feed refrigeration, laboratory (or AD) and AB temperatures for the entire experimental time course (Days 1–146) were respectively of $6.2 \pm 1.6^\circ\text{C}$, $23.5 \pm 0.8^\circ\text{C}$, $20.2 \pm 1.2^\circ\text{C}$ (in AB1) and $20.1 \pm 1.0^\circ\text{C}$ (in AB2), on average. In general, the various temperatures recorded were relatively stable during a run and from run to run. However, more significant temperature variability was observed in the case of the refrigeration unit.

During most of the investigation, each AB contained 2 diffuser stones that aerated and kept their MLSS in suspension. However, due to concern that the mixing was too intense and that it made the solids washout even worse, one was removed in some instances. Airflow rates, which were not quantified, were also sometimes increased or decreased, accordingly. Nonetheless, aerobic conditions were maintained in the ABs at all times since all DO readings were well over 2 mg O₂/L (Section 2.1.4.3). The constant input of air also kept the temperature of the MLSS below ambient temperature, by roughly 3 degrees, on average. DO concentrations and temperatures also did not vary much in each basin over a run or from run to run and peculiarly, DO concentration readings did not always decrease with a rise in AB temperature. Throughout the study, the values of these parameters were also very similar from basin to basin, although a slightly greater discrepancy was observed during the last run. When excluding the start-up period and for stable (liquid) influent flow conditions, the overall average DO concentration was found to be 8.1 ± 0.3 mg O₂/L in AB1 and 8.3 ± 0.3 mg O₂/L in AB2.

4.2.3.2 pH and Total Alkalinity

The pH and total alkalinity (TA) of the feed, anaerobic and aerobic reactors as well as a few spot checks on final effluent samples during the continuous operation of the dual treatment systems are presented in Tables L.2 a) – d) of *Appendix L.2*. The average reactor content estimates (and STDs) over the different runs are summarized in Tables 4.5 a) and b). An example calculation for TA is shown in *Appendix M*.

After 9 days into the start-up of the experiment and the reactor acclimation period, a gradual drop in pH was observed in both UASBs, from their initial high values to near or below neutral values. Over the first week of operation (Days 1–7) and even before the desired influent feed strength was applied, they had also already lost much of their buffering capacity, which was estimated as approximately 4 500 mg CaCO₃/L (Table 4.2) in the original anaerobic supernatant. At this point, alkalinity concentrations were down to roughly 200 mg CaCO₃/L in both ADs, which corresponded to lower levels of reported TA ranges for anaerobic digestion (Section 2.1.2.2b).

Therefore, sodium bicarbonate (NaHCO₃) was added directly to the 1 000 mg COD_L/L nutrient-supplemented feedstock [which was found to have a low inherent pH and very little TA prior to any chemical supplementation (Table 4.1)], from Days 9–18, on a trial and error basis. This was done to increase the buffering capacity and alkalinity of the digesters as well as to maintain their pH levels to slightly above neutrality, for greater process stability. The additions of 1.9 g NaHCO₃/L, on average (*Appendix L.2*), resulted in higher feed pH and significantly greater

alkalinity content (i.e., 7.8 and 1 000 mg CaCO_3 mg/L, respectively on average) as well as in improved environmental conditions in the anaerobic reactors. By the end of the start-up period, the latter reached values that were closer to the optimal anaerobic ones suggested in the literature.

Although UASB reactor alkalinities were more reasonable (and continued to show improvements) up to Day 25, the pH values of the AD and AB units of both treatment systems seemed to be headed beyond acceptable upper limit levels for anaerobic and aerobic processes. This was especially the case in AD1 and AB1. Therefore, during the remainder of the first experimental run (Days 28–61), the pH adjustment of the anaerobic units (by NaHCO_3 addition in the feed) was discontinued. However, despite the fact that both anaerobic digesters as well as both aeration tanks suffered an apparent and relatively rapid drop in alkalinity (or buffer capacity), after the fact, more adequate operational pH levels were maintained.

Since AD and AB alkalinity concentrations reached critically low levels by the end of Run 1, NaHCO_3 additions were resumed at the beginning of the second run (Day 62) and maintained until the end of the experiment. This was done to reduce the alkalinity deficiencies encountered in the biological reactors, especially since the treatment systems were showing other signs of instability. The buffering agent dosages added to new feedstock batches over this period and the final run averaged 0.7 g NaHCO_3 /L and 1.0 g NaHCO_3 /L, respectively (*Appendix L.2*). The amounts of NaHCO_3 employed basically depended on the resulting day-to-day pH measurements found within the ADs. Less of the chemical was used than during the last part of the acclimation period to try to sustain pH levels at near neutral values (i.e. 6.5–7.8). However, the resulting alkalinity concentrations in the UASBs were relatively low but, on average, they maintained levels that corresponded to the lower end of suggested values for anaerobic processes (i.e., 500 mg CaCO_3 /L).

During the first 45 days of operation, the buffering capacity of the AD and AB units of each treatment system were observed to be of the same order of magnitude (even though the alkalinity of the original AD supernatant (Table 4.2) was estimated to be about 40 times more concentrated than that of the raw diluted TWAS (Table 4.3). Also, practically until the end of Run 1, the alkalinity levels in both types of biological units of the greater loaded system (AD1-AB1) were approximately double those of the system being fed diluted substrate (AD2-AB2). This trend was not apparent during the subsequent runs, although alkalinity and pH measurements were very limited in both ABs after this point.

In general, both the pH and alkalinity values of AD1 and AB1 continued to be slightly greater or equal to those of AD2 and AB2, respectively. With very little exceptions in both

treatment systems, the pHs of the ABs were greater than in ADs, as was expected. However, the few alkalinity measurements taken in both ABs during the last run were greater than the corresponding values of the ADs and, in these instances, the pHs of the ABs were smaller or equal to those of the ADs. Also, the alkalinity and pH values of the final effluent samples (B1 and B2) were found to be more comparable to those of the ABs prior to the changes in the configuration of the activated sludge processes (Day 45) than when the aerobic effluents flowed directly from the combined basins into the final effluent buckets.

Over the course of the investigation, relatively large variations were observed in the alkalinity results, whether NaHCO_3 was supplemented in the feed or not [Tables 4.5 a) and b)]. However, reactor pHs remained relatively constant, with low STDs, on average, and they usually laid within typical anaerobic (Section 2.1.2.2b) and aerobic (Section 2.1.4.3) ranges. The average pH in AD1 and AD2 ranged from 7.4–7.8 and from 7.1–7.8, respectively, while that of AB1 and AB2 ranged from 7.7–8.5 and 7.3–8.2, respectively. Strangely, the pHs were highest in the ABs when no buffering agent was added to the feed, during the first run. This was possibly due to a delay in the effects of the NaHCO_3 addition during start-up. The overall average pHs and TAs observed in the biological units during most of the study (excluding start-up and Run 1) are presented in Table 4.9, for the periods when the feedstock was regularly supplemented with alkalinity.

During Run 1, the average pH and TA of the nutrient-supplemented feed (which was also supplemented with some NaHCO_3 from Days 9–25) were $4.8(\pm 1.1)$ and $11(\pm 13)$ mg CaCO_3/L , respectively. These values were found to be much lower than those estimated on un-supplemented substrate samples (prepared in 1 L batches) during preliminary characterization tests (Table 4.1). On a few occasions, the 1 to 2-day old feedstock was assumed to have no buffering capacity since its acidic pH was already smaller than the selected titration end-point pH of 4.5 of the TA tests. For most samples tested throughout the experiment, pHs before titration were less than 8.3. In these instances, the bicarbonate (HCO_3^-) specie was assumed to be the main contributor of alkalinity [TABLE 2320:II in APHA (1995)]. Even though this was not the case for all samples (i.e., AB mixed liquors and effluents), phenolphthalein alkalinity (PA) was never determined and alkalinities were solely evaluated in terms of TA concentrations. Since the volatile fatty acids (VFAs) produced in both UASBs were practically negligible during stable influent flow conditions (Section 4.2.3.3), their TA results could also be assumed to equal bicarbonate alkalinity (Kennedy, 1999).

Table 4.9: Average pH and Alkalinity During Maintained Flow Conditions of Runs 2 and 3

RUN 1 (NO Alk)	NaHCO₃ (g/L)	Feed_{pH}	Feed_{Alk} (mg CaCO₃/L)	AD1-AB1 System	AD1_{pH}	AD1_{Alk} (mg CaCO₃/L)	AB1_{pH}	AB1_{Alk} (mg CaCO₃/L)
Average:	-	4.8	11	Average:	7.5	534	7.7	739
STD:		(1.1)	(13)	STD: (Days 72-146)	(0.3)	(127)	(0.1)	(136)
RUNS 2 & 3 (Alk Added)	NaHCO₃ (g/L)	Feed_{pH}	Feed_{Alk} (mg CaCO₃/L)	AD2-AB2 System	AD2_{pH}	AD2_{Alk} (mg CaCO₃/L)	AB2_{pH}	AB2_{Alk} (mg CaCO₃/L)
Average:	0.9	7.5	478	Average:	7.2	484	7.4	598
STD:	(0.4)	(0.4)	(157)	STD: (Days 72-146)	(0.4)	(141)	(0.5)	(82)

The average pH and alkalinity conditions in the biological reactors when alkalinity was not supplemented are reported in Tables 4.5 a) and b) [Run 1]. However, the content of the treatment units were temporarily exposed to some NaHCO₃ addition from Days 9–25.

4.2.3.3 UASB Reactor Volatile Fatty Acids

The concentrations of VFAs produced in both digesters over the entire duration of the investigation are presented in Tables L.7 a) – d) of *Appendix L.7* as well as plotted in Figures 4.4 a) and b). Only the main precursors of methane were monitored by GC analysis (i.e., acetic, propionic and butyric acids), practically every other day, from samples obtained from the UASB reactor recycling lines. The corresponding total VFAs to TA ratios that could be calculated are also presented in these tables.

Throughout the study, the resulting ratios were always found to be much below 0.4 (*Appendix L.7*) and they were only observed to be in the 0.1–0.2 range, on a few occasions, during the acclimation or unsteady influent flow periods (Run 2). Although such values typically indicate stable reactor conditions (Section 2.1.2.3), both the total VFAs and the buffering capacity of the anaerobic systems were consistently low. Overall, the total VFAs were only observed to be at 20(±25) mg/L in AD1 and 9(±16) mg/L in AD2, on average, when considering the days of stable influent flows and excluding the start-up period.

The highest total VFA concentrations in each UASB were recorded during the latter part of the acclimation period, when reactor influents were nearly at their targeted strengths. The greatest values were approximately of 115 mg VFA_{Total}/L and 102 mg VFA_{Total}/L in AD1 and AD2, respectively (on Day 17). The reduction in their concentration at the start of Run 1 is possibly due to the acclimation of the culture to the test substrate. However, no acid predominated and negligible levels were observed in both anaerobic reactors, over the experimental time course

[Figures 4.7 a) and b)]. Nonetheless, the reactor with the greatest soluble OLRs (i.e., AD1) was found to have the highest VFAs, on average.

Acetic acid was unusually low at all times (< 40 mg AA/L) and not always detected, especially during Run 1, where all VFAs were particularly low in both ADs. Butyric acid was generally not detected, but when it was, it was always the highest VFA in both UASBs. A tiny increase in propionic acid was also noticed in Run 3. Although not apparent during this run, slight fluctuations in total VFAs were noted during the first few days of Runs 1 and 2, when flow transitions were being made. Yet, no correlation was observed between OLRs (or SOLRs) and VFA levels and in spite of the low biogas and CH_4 productions (Section 4.2.3.4), no significant VFA increases arose. Thus, the methanogenic bacteria were either always very efficient in converting VFAs (i.e., acetate) to CH_4 and CO_2 or as a result of some form of inhibition (Sections 2.1.2.3 and 4.2.3.5), VFA's were virtually not being produced (Grady and Lim, 1980).

4.2.3.4 UASB Reactor Biogas Production

The small quantities of biogas produced daily in each UASB during the various stages of the study are presented in Tables L.8 a) – d) of *Appendix L.8*. The total biogas produced, the average biogas and CH_4 production rates as well as the average CH_4 yield factors in each reactor over each run (during the stable influent flow periods) are summarized in Table 4.10. The average methane-associated results were calculated based on the days where biogas composition (Section 4.2.3.5) was evaluated (*Appendix L.9*). Figures 4.6 a) and b) also present the daily (mL/d) and cumulative biogas productions (mL) observed throughout the entire experimental period. The latter were only found to be of approximately 12.3 L and 8.2 L in AD1 and AD2, respectively.

Although average and overall average COD conversions (Sections 4.2.2.1 and 4.2.3) were found to be reasonable in both ADs ($> 90\%$), their ability to produce biogas was very poor. Gas productions were found to be highly irregular and extremely low during all run conditions. On many days, no biogas generation was detected at all in either anaerobic reactor. Following the acclimation period, AD biogas production rates were estimated to range from nothing to about only 0.2 L/d, on average, while overall UASB CH_4 yields resulted in values estimated at approximately 0.03 L $\text{CH}_4/\text{g COD}_{\text{t rem}}$ in AD1 and 0.04 L $\text{CH}_4/\text{g COD}_{\text{rem}}$ in AD2. Since these were very far from the theoretical methane yield of 0.38 L $\text{CH}_4/\text{g COD}_{\text{rem}}$ (at 25°C), very little of the incoming COD that was removed was converted to methane. Thus, it was possibly transformed into new cells or biogas might have been escaping from the anaerobic systems owing to leaks and/or dissolved CH_4 .

losses in the (liquid) effluent. In addition, the low production of CH_4 could have been due to SRB activity (i.e., $\text{SO}_4^{2-} \rightarrow \text{H}_2\text{S}$) or denitrification [i.e., $\text{NO}_3^- \rightarrow \text{N}_{2(\text{gas})}$].

Table 4.10: Average Biogas and Methane Production Estimates in Both UASBs (Under Maintained Influent Flow Conditions)

AD1	Total Biogas (mL)	(L Biogas/d)	(L CH_4/d)	(L CH_4_{ave}/g $\text{COD}_{\text{t ave. rem}}$)
Start-Up	456 (Days 1-17)	0.018 (Days 5-17)	0.006	("M" from Eq. 2.2):
Run 1	1 368 (Days 18-61)	0.017 (Days 30-61)	0.011	0.008
Run 2	9 272 (Days 62-113)	0.17 (Days 72-113)	0.05	0.05
Run 3	1 216 (Days 114-146)	0 (Days 137-146)	0	0
Overall	12 312 mL (Days 1-146)	0.09 (84 Days)	0.03 ^s (or 0.04)	0.03
AD2	Total Biogas (mL)	(L Biogas/d)	(L CH_4/d)	(L CH_4_{ave}/g $\text{COD}_{\text{t ave. rem}}$)
Start-Up	640 (Days 1-17)	0.043 (Days 6-17)	0.013	("M" from Eq. 2.2):
Run 1	6 120 (Days 18-61)	0.194 (Days 31-61)	0.148	0.09
Run 2	80 (Days 62-113)	0.0011 (Days 72-113)	*0.002	0.005
Run 3	1 372 (Days 114-146)	0.053 (Days 137-146)	0.029	0.003
Overall	8 212 mL (Days 1-146)	0.08 (83 Days)	0.05 ^s (or 0.04)	0.04

Except for the total biogas productions, overall results were obtained by weighing the individual run results over the different number of stable influent flow days per run. ^sEstimated by multiplying the overall average biogas productions (L/d) by the overall average CH_4 fractions reported in Section 4.2.3.5. * CH_4 production > biogas production since the former result was based on the days where biogas composition was estimated.

Oddly some CH_4 fractions were detected on some days where there was no biogas production. A possible explanation would be that the quantity of biogas produced by the UASBs on those days was less than the amount of gas required to make the 2 wet tip meters flip (i.e., 76 mL and 40 mL). Thus, biogas fractions could have been distinguished even though no flips were registered *per se*. Although AD1 produced more biogas overall (mL), its biogas and methane production rates were generally smaller than those of AD2's, except for the results of the second

run. Nonetheless, the overall average biogas as well as the CH_4 production rates of each AD were similar at approximately 0.08 L/d and 0.04 L CH_4 /d, respectively.

Even if the rise in AD2's methane content seemed to correspond with a apparent increase and greater consistency in its biogas production during the mid to latter part of Run 1, no correlation was noted between biogas production and composition, when examining the results of both ADs. AD1 generated the most of its biogas during Run 2 (i.e., 9.3 L), when its highest average fraction of CO_2 and CH_4 (i.e., 64%) were recorded, while AD2 encountered its greatest average CH_4 fraction (i.e., 66%) when practically no biogas was produced. Oddly, the latter produced nearly all of its biogas during Run 1 (i.e., 6.1 L) when its average SS inventory, SB activity (based on SRRs) and total VFAs were not at their peak. In addition, even if it produced relatively less biogas on the whole, AD2 generated a higher overall CH_4 content. However, its CO_2 fraction was only slightly greater, on average [Figures 4.7 a) and b)].

4.2.3.5 UASB Reactor Biogas Composition

The composition of the biogas produced in both UASBs throughout the study is presented in Tables L.9 a) – d) of *Appendix L.9*. These results are also depicted in Figures 4.7 a) and b), where average CH_4 and CO_2 fractions observed during the maintained influent flow rate period of each run are reported. Their values, which ranged from 23–64 % CH_4 , with 1–6 % CO_2 , in AD1 and 40–66 % CH_4 , with 3–6 % CO_2 , in AD2 were presented in Tables 4.5 a) and b), with their corresponding STDs.

The first signs of methanogenesis were observed in the two anaerobic reactors during the acclimation period, where the initial methane fractions evolved on Day 7. However, CO_2 fractions were only detected on Day 25 (Run 1). These actually never got much better over time and the highest values reached were only of 9% (Run 2) and 10% (Run 3), in AD1 and AD2, respectively (*Appendix L.9*). Conversely, as shown on Figures 4.7 a) and b), the average UASB methane quality improved over Runs 1 – 2, with increasing influent flow rates and OLRs. Reasonable values of > 60 % CH_4 were maintained in both reactors during the latter run and this corresponded to the time where their greatest average COD removals were achieved [Tables 4.5 a) and b)]. Both anaerobic reactors actually reached CH_4 fractions as high as 72% towards the beginning of this run.

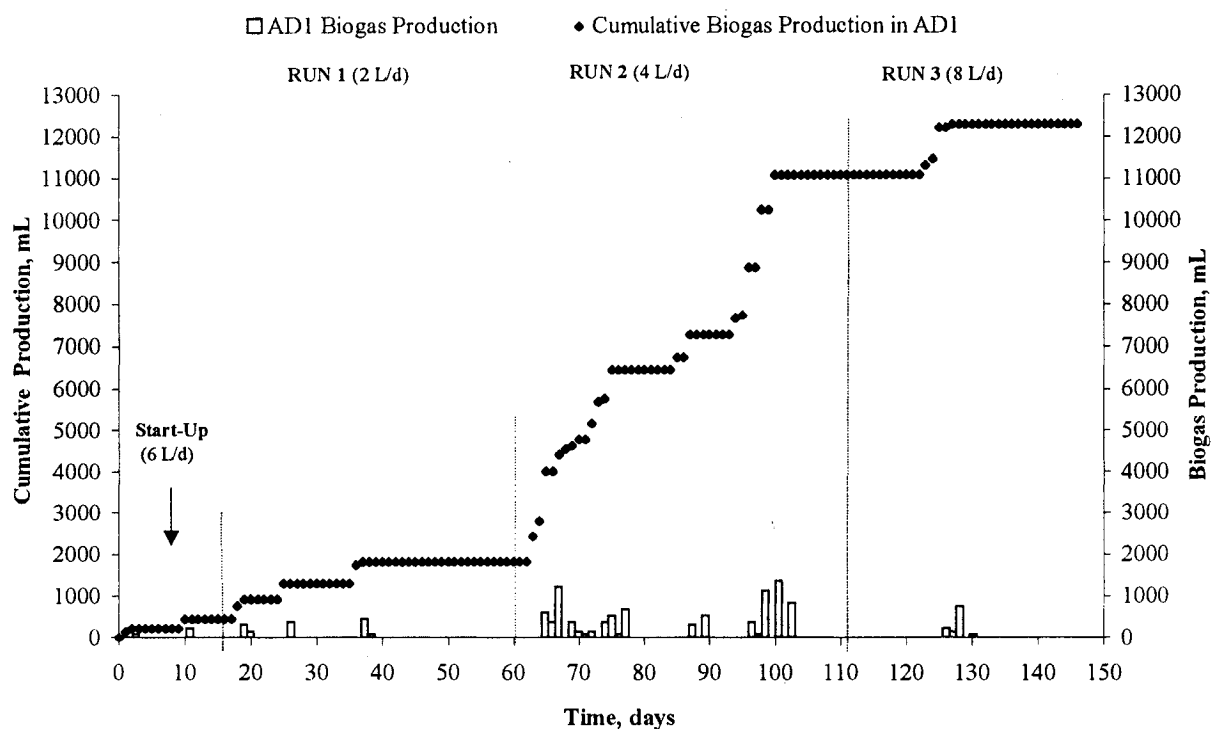


Figure 4.6 a): Biogas Production in AD1

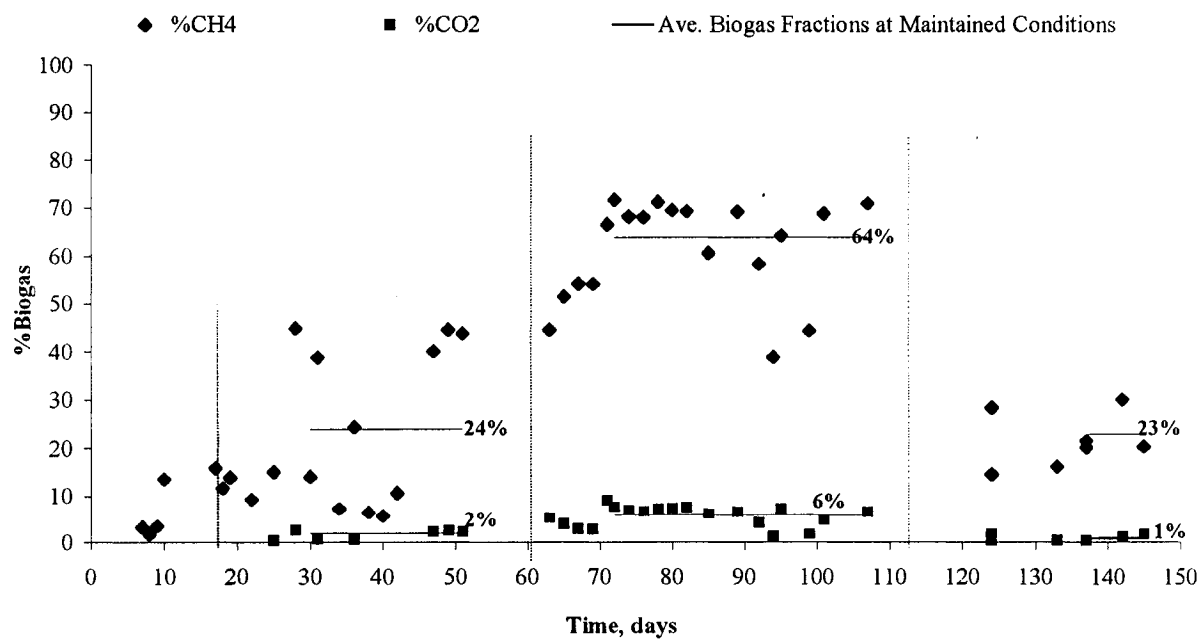


Figure 4.7 a): AD1 Biogas Quality

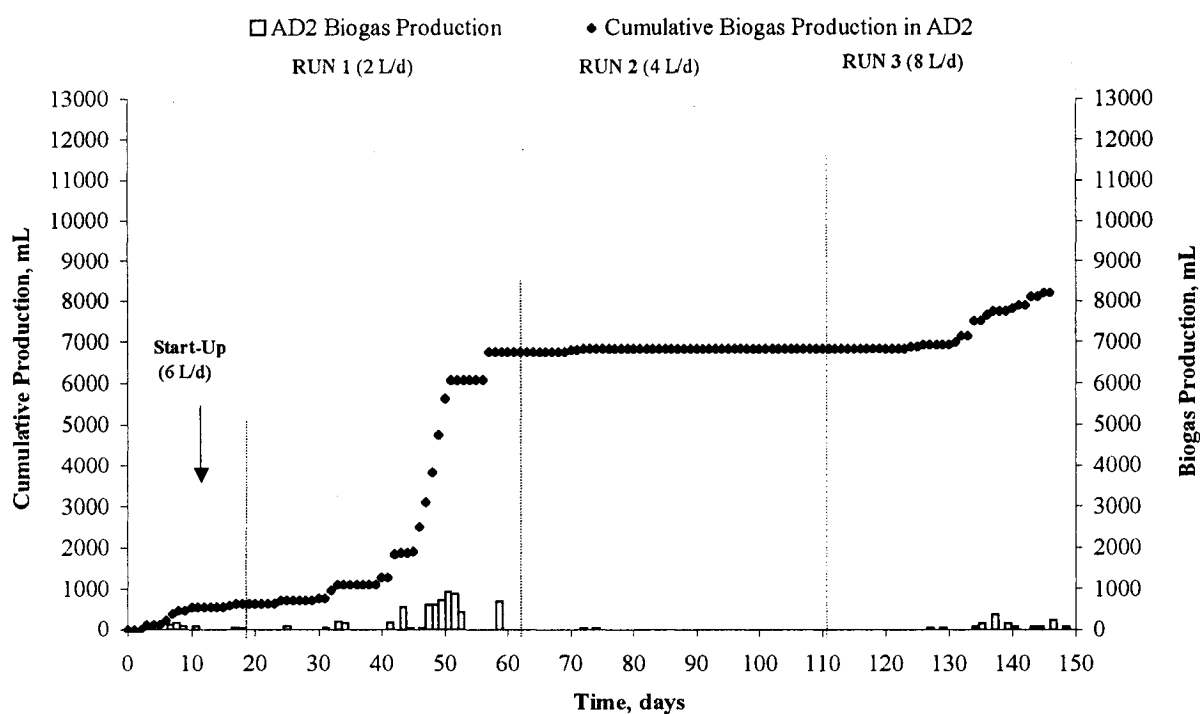


Figure 4.6 b): Biogas Production in AD2

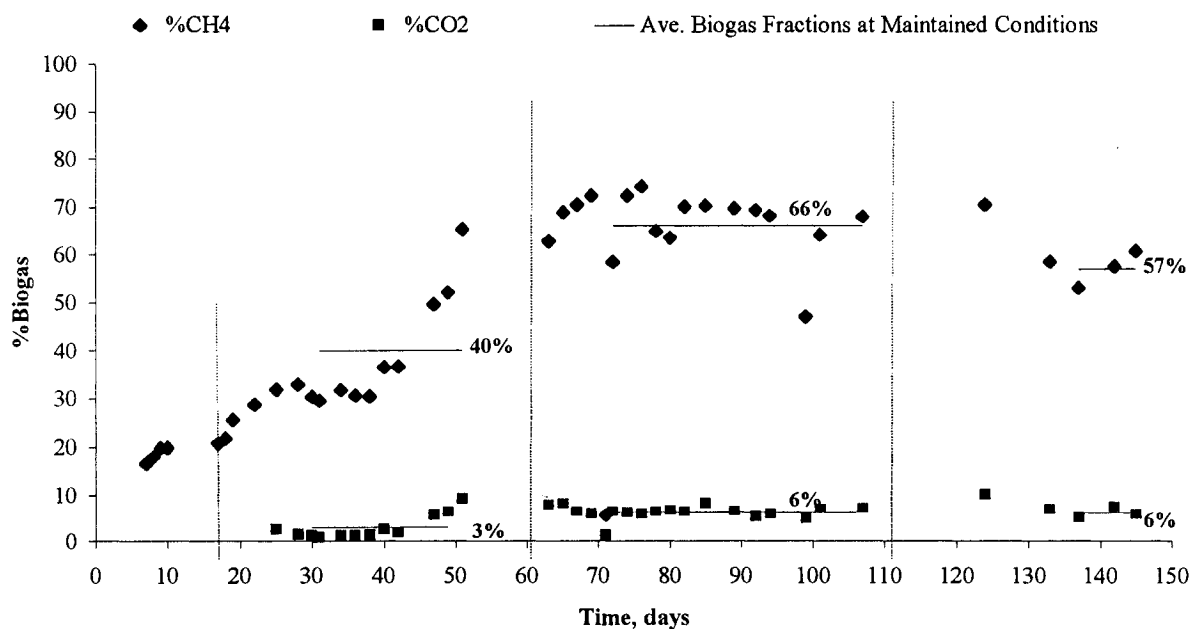


Figure 4.7 b): AD2 Biogas Quality

However, during the last run, a slight reduction was noted in AD2's methane quality and the average results in AD1 basically slipped back to some comparable to those observed during the first run. This could be due to the fact that the reactors were left on internal recycling for several days (Days 114–123) prior to Run 3's continuous feeding period. Yet, this was also the case between Runs 1 and 2 (Days 52–62) in order for UASB reactor assays to be conducted (Section 4.3). AD1 was also probably exposed to air, at least temporarily, during this problematic run. Its U-tube had to be removed on Day 131 since floating sludge was causing a continuous blockage in it, leading to spills. However, the notable drop in AD1's methane content during Days 34–42 of Run 1 remains unexplained.

The average CH_4 and CO_2 fractions over all runs (excluding the start-up phase) were found to be $44(\pm 24) \% \text{CH}_4$ and $4(\pm 3) \% \text{CO}_2$ in AD1 as well as $56(\pm 15) \% \text{CH}_4$ and $5(\pm 2) \% \text{CO}_2$ in AD2, for the time periods where the influent flow rates were considered to be steady. Although the overall average methane content in the UASBs was reasonable (compared to typical values for carbohydrate wastes), the average percentage of CH_4 found in AD1 during Run 1 and 3 was unsatisfactory. CO_2 fractions were also always a great deal lower than typical values for anaerobic processes (Section 2.1.2.2d), which resulted in particularly high N_2 fractions (*Appendix L.9*).

However, a somewhat similar outcome was reported in a study by Droste et al. (1987), where a dilute synthetic sugar wastewater of 300–1 000 mg COD/L was treated in an upflow sludge blanket filter, at HRTs < 12 h. These authors observed N_2 biogas fractions as high as 55% as well as CH_4 and CO_2 contents as small as 40 and 5%, respectively. Among other things, they suggested leaks and dissolved feed N_2 coming out of solution in the warmer reactor environment as possible reasons for such a high N_2 content. They also pointed out a few other biomass-retaining anaerobic system studies where high concentrations of N_2 were encountered in the off gas when dilute strength wastewaters were being treated.

When N_2 fractions are observed, it can certainly imply that the anaerobic environment is not well maintained and that air is probably seeping in. Thus, the UASBs were checked for leaks on many occasions during the present study since they are very common in lab-scale reactors (de Sousa and Foresti, 1996). Although no obvious ones were found, the airtight conditions of the reactors tried to be improved with the few means that were available (i.e., silicone and/or Teflon tape for joint sealing, clamps, etc.). However, the liquid level within their U-tubes was often even, but particularly in AD1 towards the latter portion of the experiment. The small pressure differentials observed within the anaerobic units were in agreement with the fact that little biogas was produced (Section 4.2.3.4).

It is also very plausible that the high N_2 recorded was due to the fact that biogas samples had to be brought to another floor, on which the GC was located, with a simple valved-syringe and rubber stopper. However, a gas-tight syringe was tried out for comparison, when one was available, but no difference in gas composition was noted. In addition to potential anaerobic system leaks through the numerous joints and connectors, the limited biogas generated (and less than desirable CH_4 fractions) could also have been affected by feed interruptions due mostly to pump failures, feed replenishment, reactor operational problems, set-up maintenance or repairs. Other possible causes include inaccurate wet tip meter calibration and their questionable reliability in recording actual biogas production. These were however serviced (i.e., water level, battery, ball bearing, silicone seal), prior to and on a few occasions during the study, to insure that they were functioning properly and their calibration was also checked (*Appendix L.8*).

Since the feed strength used was to remain constant in each reactor throughout the study and the treatment systems were fed in a relatively continuous mode, gas fractions and production should also have been relatively constant (Grady and Lim, 1980). However, the significant fluctuations observed owing to the degradation that occurred in the refrigeration unit (Section 4.2.1) could have had some impact on reactor biogas production and quality. A reduced amount of biogas and a lower CH_4 content was anticipated due to the dilute nature of the feed (Droste et al., 1987) as well as the ambient operating temperature of the UASBs, although not to the extent of the results obtained.

In any case, low biogas (or CH_4) production rates and CH_4 fractions often result from low sludge activity. In addition to the room temperature operation of the anaerobic reactors, it is reasonable to say that the SB channeling (i.e., poor substrate-biomass contact), which was noticed in both UASBs, probably played a part in the low granular biomass activity (i.e., low or nearly undetected VFA production) observed during the experiment (particularly when estimated in terms of SRRs). Substrate limiting conditions can also decrease the specific activity of sludge (Droste et al., 1987), and consequently their treatment ability, while reaction rates are slower at moderate temperatures compared to biogas-optimizing mesophilic temperatures. However, as a rule, it is not feasible to treat dilute wastewaters at high temperatures and especially not in large-scale systems (Speece, 1996). It would require significant amounts of additional energy to sustain such conditions.

Poor conversion of COD to CH_4 (low methane yields and fractions), limited biogas production as well as low anaerobe activity are all typical signs of potentially unhealthy (acetoclastic) methanogens and they are usually indicative of inhibition, toxicity or process upset.

In spite of reasonable UASB effluent pHs, owing to regular sodium bicarbonate additions (Section 4.2.3.2), such conditions resulted in the anaerobic units during the present study. Since little fermentation seemed to be taking place, maximum substrate degradation by the anaerobes was certainly not reached. When the methanogenic and acidogenic bacteria populations suffer from an imbalance, reductions in the production of biogas usually entail a fall in pH and a subsequent increase in VFAs as well as a rise in CO₂ fractions (Section 2.1.2.1). However, this did not occur (Sections 4.2.3.3 and 4.2.3.5).

While this was also not established, the methanogens or the acidogens could have been preferentially washed out. Yet, the granules did tend to develop a slightly lighter color over time, which is usually characteristic of the proliferation acid-forming bacteria (Section 2.1.3.3). Several of the toxicity and/or inhibition sources listed in Section 2.1.2.3 (i.e., heavy metals, inorganic cations, sulfides, ammonia (or ammonium), H₂ or H₂S accumulation, organic overloading) seem unlikely when considering the particularities of the experiment, but most of them were not monitored or quantified. Although the UASBs were not subjected to much organic stress (owing to the low applied OLRs and incoming COD concentrations), the continuous (Days 2–45) or batch (Days 70–146) recycling of settled aerobic sludge to the ADs could have induced O₂ toxicity or allowed aerobic microorganisms to negatively impact the anaerobic ones. In addition, the synthetic substrate's nutrient requirements could have been deficient. Other than constantly applying the chemical supplementation (*Appendix B*), they were not monitored after the preliminary characterization phase.

The small amount of anaerobic by-products generated (i.e., low VFAs, acetate and CH₄ gas) combined with the reasonable organic removals observed in both anaerobic units during the entire experiment [Tables 4.5 a) and b)] could simply indicate that they were under loaded or in a state of starvation. However, Grady and Lim (1980) report that a decline in CH₄ generation rate *without* a variation in pH or a VFA concentration build-up suggests that the system or the acidogens are somehow being inhibited. Thus, it is probable for the anaerobic process to have been disturbed prior to the acidification step. Hydrolysis and fat (or lipid) degradation have actually been reported to be rate-controlling steps under low operation temperatures and short SRTs (Section 2.1.2.1), in the case of the latter. Enfalac™ feed residue was sometimes visible in the granular beds of the ADs, but mostly at the bottom of AD1 over the final run.

4.3 UASB REACTOR GRANULAR BIOMASS CHARACTERISTICS AND PROFILES

In addition to the regular anaerobic parameters monitored during the study period, which were discussed in the previous section, complementary assays were conducted to quantify and follow the changes that occurred in the UASB biomass conditions. Acetoclastic activity (Section 4.3.1), SS (Section 4.3.2) and settling analyses (Section 4.3.3) were carried out on the initial inoculum (at least in duplicate), prior to start-up, as well as on settled sludge bed samples taken from sampling ports positioned throughout the depth of each bed (defined in Section 3.1.1.1), at the end of each experimental run (Runs 1 – 3). Both UASBs, seeded with anaerobic granular biomass, were assumed to initially have the same characteristics as the granular seeding material. Due to an increase in the anaerobic reactor SBHs compared to their initial volume (Table 4.14), the tops of the sludge beds were located just above the T1 and T2 ports when the first tests were performed and SS were sampled from the TAD1 and TAD2 ports by the end of the second experimental run, which resulted in three different bed sampling locations (i.e., top, middle and bottom).

The volumetric port elevations at which samples were extracted are reported in *Appendix L.11*. The same ones were used for all three assays within a same reactor, but the port heights differed slightly between the two anaerobic digesters. To minimize sludge removal from the beds, the assays were not carried out after the 17-day acclimation period and were only performed using single 10 mL grab samples (with the exception of the acetoclastic activity tests), where they were withdrawn with a straight connector fitted-syringe. Apart from performing these tests and for relatively few sampling accidents, where some granular biomass was lost, UASB reactor sludge was not deliberately wasted during the course of the study (Cocci et al., 1990a and 1990b; Kettunen et al., 1996). Therefore, the anaerobic units (as well as the aerobic basins) were essentially operated at their maximum sludge hold-up (Lettinga and Van Haandel, 1994). The latter authors report that periodic sludge discharges can actually be counteractive in some instances, particularly when biological post-treatment units are used.

4.3.1 VOLUMETRIC AND SPECIFIC ACETOCLASTIC ACTIVITY

The raw data from the batch AATs performed on the inoculum sludge and on all anaerobic granular biomass samples are reported in *Appendix L.10*. Based on these, Figures L.10 a) – d) present acetic acid concentration depletion (mg AA/L) per unit time (h) plots for all applicable assays. The volumetric acetoclastic activity rates (g AA/L·d) were derived from the slopes of the linearly regressed best-fit lines. The volumetric activities of the raw seed [Table L.10a)] and of the biomass, evaluated at the respective SB ports as well as for the average bed conditions, at the end

of the various runs, are reported at the bottom of Tables L.10 b) – d) [Appendix L.10], on a g AA/L·d and on a g COD_{rem}/L·d basis. All rates and deviations [as well as intercepts and coefficient of determination (R^2) values] were obtained with *Microsoft Excel*'s Linest function and all test values presented exclude the control vial results. Since the defined medium's quality seemed to be adequate during the first assays (i.e., low and constant control values), a control was omitted in the final test.

Table 4.11 summarizes the average acetoclastic activities of the granular sludge (and their deviations) at the various sampling locations as well as for the whole SB, at each particular run condition, and for the overall study period. These averages were computed from the corresponding sludge acetoclastic activities estimated at the beginning and the end of each run (Appendix L.10).

Table 4.11: Average Volumetric Activity Profiles of the Sludge Beds

June 30	Sludge Inoculum: (Appendix L.10)				0.63 ± 0.02 g AA _{ave} /L·d (0.68 ± 0.02 g COD _{rem} /L·d)			
Anaerobic Digester 1 (AD1)					Anaerobic Digester 2 (AD2)			
	Top (TAD1)	Middle (T1)	Bottom (B1)	SB Ave.	Top (TAD2)	Middle (T2)	Bottom (B2)	SB Ave.
After 2 L/d: (Run 1)	-	2.5±0.2 (2.7±0.2)	2.4±0.3 (2.6±0.3)	2.4±0.2 (2.6±0.2)	-	2.5±0.1 (2.7±0.1)	1.8±0.2 (1.9±0.2)	2.2±0.2 (2.3±0.2)
After 4 L/d: (Run 2)	0.57±0.02 (0.61±0.02)	2.6±0.2 (2.8±0.2)	2.5±0.3 (2.7±0.3)	1.9±0.2 (2.0±0.2)	0.95±0.03 (1.01±0.03)	2.7±0.1 (2.9±0.1)	2.0±0.2 (2.2±0.2)	1.9±0.1 (2.0±0.1)
After 8 L/d: (Run 3)	0.44±0.03 (0.48±0.03)	-	0.63±0.05 (0.67±0.06)	0.54±0.04 (0.58±0.04)	0.82±0.05 (0.88±0.06)	1.13±0.07 (1.21±0.07)	1.04±0.06 (1.11±0.07)	1.00±0.06 (1.36±0.07)
	Overall Ave.:			1.6 ± 0.1 (1.7 ± 0.1)		Overall Ave.:		1.7 ± 0.1 (1.9 ± 0.1)

[Values in parentheses, (), were converted from g AA_{rem}/L·d to g COD_{rem}/L·d; 1 g AA ≈ 1.07 g COD (Appendix M)].

Correspondingly, Table 4.12 reports the specific acetoclastic activities (g AA/g VSS·d) as well as the specific activities of the sludge (g COD/g VSS·d), and their respective deviations, evaluated per unit VSS at the end of the runs. The average specific activity profiles (g AA_{ave}/g VSS_{ave}·d or g COD_{ave}/g VSS_{ave}·d) and the results for the entire SBs during a run as well as over the whole experiment are presented in Table 4.13. The VSS concentrations (g VSS/L) derived from the results reported in Appendix L.11 and used to compute the specific activities (and deviations) are presented in Tables L.10 a) – d) [Appendix L.10]. The results given in Table 4.13 were essentially based on the average acetate utilization rates presented in Table 4.11 (g AA_{ave}/L·d) as well as on the average VSS concentrations assessed at the respective sampling locations

(g VSS_{ave}/L_{in} test bottles). The latter were calculated from the VSS content found in the 10 mL sludge samples at the beginning and end of each run (*Appendix L.10* or *L.11*).

Table 4.12: Sludge Bed Specific Activity Profiles at the End of the Various Runs

June 30		Sludge Inoculum:				0.101 ± 0.004 g AA _{ave} /g VSS _{ave} ·d (0.108 ± 0.004 g COD _{rem} /g VSS _{ave} ·d)			
Anaerobic Digester 1 (AD1)					Anaerobic Digester 2 (AD2)				
	Top (TAD1)	Middle (T1)	Bottom (B1)	SB Ave.		Top (TAD2)	Middle (T2)	Bottom (B2)	SB Ave.
After 2 L/d: (Run 1)	-	0.52±0.05 (0.56±0.05)	0.52±0.06 (0.56±0.07)	0.52±0.05 (0.56±0.06)		-	0.53±0.03 (0.56±0.03)	0.36±0.04 (0.38±0.04)	0.44±0.03 (0.47±0.03)
After 4 L/d: (Run 2)	0.087±0.003 (0.093±0.003)	0.115±0.005 (0.123±0.005)	0.096±0.007 (0.103±0.008)	0.100±0.004 (0.107±0.004)		0.233±0.007 (0.249±0.007)	0.200±0.007 (0.214±0.008)	0.170±0.005 (0.182±0.005)	0.196±0.004 (0.210±0.005)
After 8 L/d: (Run 3)	0.28±0.03 (0.30±0.04)	-	0.18±0.02 (0.19±0.02)	0.22±0.02 (0.23±0.02)		0.28±0.03 (0.30±0.03)	0.21±0.02 (0.23±0.02)	0.15±0.01 (0.16±0.02)	0.24±0.02 (0.26±0.02)

[Values in parentheses were converted from g AA_{rem}/g VSS·d to g COD_{rem}/g VSS·d (1 g AA ≈ 1.07 g COD)].

Table 4.13: Average Sludge Bed Specific Activity Profiles in g AA_{ave}/g VSS_{ave}·d (or g COD_{ave}/g VSS_{ave}·d)

Anaerobic Digester 1 (AD1)					Anaerobic Digester 2 (AD2)				
	Top (TAD1)	Middle (T1)	Bottom (B1)	SB Ave.		Top (TAD2)	Middle (T2)	Bottom (B2)	SB Ave.
After 2 L/d: (Run 1)	-	0.34±0.03 (0.36±0.03)	0.34±0.04 (0.36±0.04)	0.34±0.03 (0.36±0.03)		-	0.34±0.02 (0.37±0.02)	0.25±0.02 (0.26±0.02)	0.29±0.02 (0.32±0.02)
After 4 L/d: (Run 2)	0.087±0.003 (0.093±0.003)	0.33±0.03 (0.35±0.03)	0.30±0.03 (0.32±0.04)	0.32±0.03 (0.34±0.04)		0.233±0.007 (0.249±0.007)	0.40±0.02 (0.43±0.02)	0.28±0.02 (0.29±0.02)	0.35±0.02 (0.37±0.02)
After 8 L/d: (Run 3)	0.11±0.01 (0.12±0.01)	-	0.11±0.01 (0.12±0.01)	0.11±0.01 (0.12±0.01)		0.25±0.02 (0.27±0.02)	0.21±0.01 (0.22±0.01)	0.16±0.01 (0.17±0.01)	0.22±0.01 (0.24±0.01)
		Overall Ave.: 0.26 ± 0.02 (0.27 ± 0.03)					Overall Ave.: 0.29 ± 0.02 (0.31 ± 0.02)		

[Values in parentheses were converted from g AA_{rem}/g VSS·d to g COD_{rem}/g VSS·d (1 g AA ≈ 1.07 g COD)]

Based on the AAT test results, the raw inoculum was observed to have a low average specific acetoclastic activity rate of 0.101 ± 0.004 g AA/g VSS·d. It was possibly influenced by the room temperature incubation observed during the assay (and all others), as opposed to typical mesophilic test temperatures of 35°C. However, this condition was used with the intent to more accurately reflect the sludge activity level within the ambient temperature-operated UASBs. The low initial activity rate of the mesophilic seed originally used for the treatment of pulp mill wastewater might also be the result of prolonged (room temperature) storage, without feeding, prior to reactor start-up. As for the other types of batch tests (i.e., SS and settleability), another AAT was

also run on a duplicate seed sample shortly after its shipment (April 4), but its results were rejected because of operational problems encountered with the GC at that time.

However, the VRRs ($\text{g COD}_{\text{rem}}/\text{L}\cdot\text{d}$) and SRRs ($\text{g COD}_{\text{rem}}/\text{g VSS}\cdot\text{d}$) of the anaerobic granular sludge, based on incoming and outgoing UASB data, yielded much smaller values than those resulting from the AATs. The former results, which were presented and discussed in Section 4.2.3, were thought to better represent the actual microbial activity in the SBs. Therefore, the AAT results were essentially presented for comparison purposes.

Other than the significant difference in their magnitude, their decreasing trend with time also contrasted with the reactor data. However, they did show that the average specific activity significantly increased in both ADs, over the acclimation period and the first experimental run, compared to the initial state of the anaerobic seed (Table 4.13). As anaerobic reactor OLRs (and influent flow rates) were increased (or the HRTs reduced), their VRRs and SRRs were also observed to increase. Thus, these increases in activity rates suggest that the low-strength candidate feedstock exerted some stimulatory effects on the anaerobic organisms, even at ambient temperature.

Although the reactor data were favored, AAT results revealed that the activity levels in the UASB reactor sludge bed layers differed. A specific activity gradient was detected in the end-of-run conditions of AD2's SB, but it was less reflected in its average bed state. However, the average specific acetoclastic activity SB profiles in AD1 were found to be relatively uniform over a run. Though a gradation was also not observed for the volumetric activities of either reactor, generally, the specific and average specific activities were found to be highest in the top rather than in the bottom portion of the SBs. This could be the result of non-uniform mixing and substrate distribution as well as SB channeling. However, this outcome is typical in granular UASB reactors since methanogen-rich upper SB biomass is usually lighter and more active compared to heavier and less active bottom sludge particles, which tend to contain more acidogens (Speece, 1996).

4.3.2 *SUSPENDED SOLIDS INVENTORY*

Tables L.11 b) – d) of *Appendix L.11* present the SS profiles found along the height of the SBs and their mean VSS inventories as well as the average concentrations in both anaerobic reactors, at the end of the different run conditions. In addition, Figures 4.8 a) and b) respectively depict the observed variations in the distribution of the VSS concentrations within the SBs of AD1 and AD2, throughout the experiment.

Table 4.14 summarizes the average VSS concentrations and content in the AD sludge beds prior to reactor start-up, for each run condition as well as for the entire study. The average VSS characteristics reported [SS inventory in the SBs (g VSS) and concentrations in the entire reactors (g VSS/L)] were computed from the initial and final VSS concentrations found in the SBs (mg VSS/L) as well as from the average SBH estimates (*Appendix L.5*) and AD working volumes given in *Appendix L.11*.

Table 4.14: Average Suspended Solids Inventory in UASB Reactor Sludge Beds Over Time

UASB Reactors			VSS (mg/L)	$\frac{\text{g VSS}}{10 \text{ mL}}$	AD ^{ave} Volume (L)	*SBH (mL)	Total SB (g VSS)	VSS in ADs (g VSS/L)	%VSS
April 4/June 30	Inoculum Ave:		31 275	0.31	(3.80)	2 000	62.6	16.5	88.4
	STD ($\pm$):		3 446	0.03			6.9	2.0	0.7
*AD1	(Day 18-61)	Run 1 Ave.: (2 L/d)	36 259	0.3626	~3.85	2 221	80.5	20.9	89.4
	(Day 62-113)	Run 2 Ave.: (4 L/d)	39 755	0.3975	~3.85	2 469	98.1	25.5	89.8
	(Day 114-146)	Run 3 Ave.: (8 L/d)	^{\$} 25 092	^{\$} 0.2509	~3.85	*2 655	^{\$} 66.6	^{\$} 17.3	88.9
	Overall Average:		33 702	0.3370	(3.85)	2 448	81.7	21.2	89.4
AD2	(Day 18-61)	Run 1 Ave.: (2 L/d)	36 498	0.3650	~3.75	2 188	79.9	21.2	89.2
	(Day 62-113)	Run 2 Ave.: (4 L/d)	34 029	0.3403	~3.75	2 501	85.1	22.7	89.6
	(Day 114-146)	Run 3 Ave.: (8 L/d)	25 321	0.2532	~3.75	2 795	70.8	18.9	89.2
	Overall Average:		31 942	0.3194	(3.75)	2 495	78.6	21.0	89.3

*By the end of the study, AD1's SB split in two. A small part of it was floating by the TAD1 port, but the total bed had ~1 200 mL (*Appendix L.11*). ^{\$}For a more representative assessment of the average VSS content in AD1's SB, the VSS concentration estimate at the B1 port was used since the amount of sludge was much more significant at the bottom of the bed at that time (*Appendix L.11*).

The seed sludge had a high average VSS/TSS ratio of 0.88 and a mean VSS concentration of approximately 31.3 ± 3.4 g VSS/L. The average solids inventory in the original beds of both UASBs was estimated as 62.6 ± 6.9 g VSS and the average total VSS concentration initially present in each anaerobic reactor (including their blankets) was estimated as 16.5 ± 2.0 g VSS/L. The granular sludge in AD1 and AD2 contained relatively little inert substances and average FSS fractions respectively ranged from 10.2–11.1% and 10.4–10.8%. No inert fraction accumulation was observed since the FSS percentages were less than that of the highly organic seeding material.

The average VSS proportions of both ADs were slightly higher during the second run and their overall magnitudes were practically identical as well as not significantly greater than the seed's.

After the acclimation period and the first experimental run, similar average concentrations of VSS were found in the SBs of both AD's (~36 g VSS/L) and a significant increase was observed compared to the initial VSS concentration levels (*Appendix L.11*). After this point and as the SBHs rose, the SS concentrations measured in both UASBs generally declined (*Appendix L.11*), although the SB concentration of the higher organically loaded reactor (AD1) remained well above the conditions initially found in the inoculum. Even though a notable concentration decrease occurred in AD2 by the end of the second run, the average VSS concentration in its SB (mg VSS/L) over that run was still greater than its original condition, which was also the case for AD1. The considerable sludge loss encountered in the latter reactor by the end of the final run (*Appendix L.11*) corresponded with a significant SS concentration reduction and its SBH ultimately occupied only 1.200 L compared with 2.875 L in AD2 (*Appendix L.11*). In spite of this and even though the mean VSS concentration level in AD2's SB (mg VSS/L) remained relatively constant after the last run, the average VSS concentrations in the beds of both UASBs were also observed to be similar during Run 3 (~25 g VSS/L).

Despite the granular sludge flotation problems encountered in the UASBs and its settleability deterioration with time (Section 4.3.3), concentration gradients or SB stratification developed in both reactors (except for very few exceptions in AD1). Higher SS concentrations and %VSS fractions were found in the bottom portions than in the top portions of the beds and the latter were much greater compared to the FSS proportions (*Appendix L.11*, but average run profiles are not presented). The gradation in the solids estimates or the deviation between the SS concentration levels at the different SB port heights had a tendency to be more significant with operation time [*Appendix L.11*; Figures 4.8 a) and b)] as well as on average (not presented).

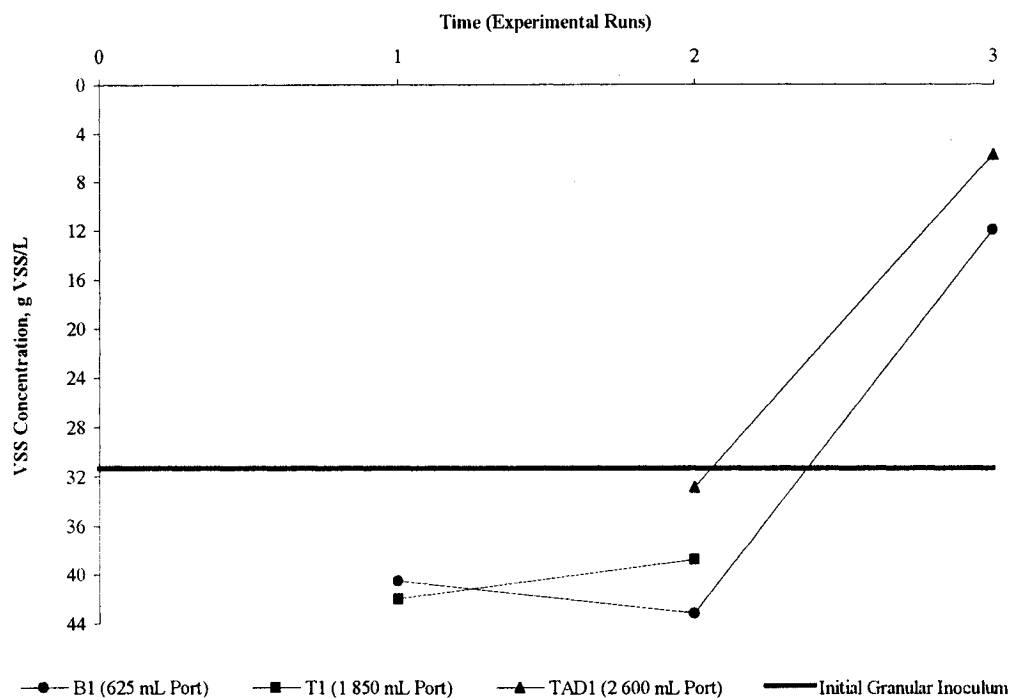


Figure 4.8 a): VSS Profile of AD1 at the End of Each Run Condition

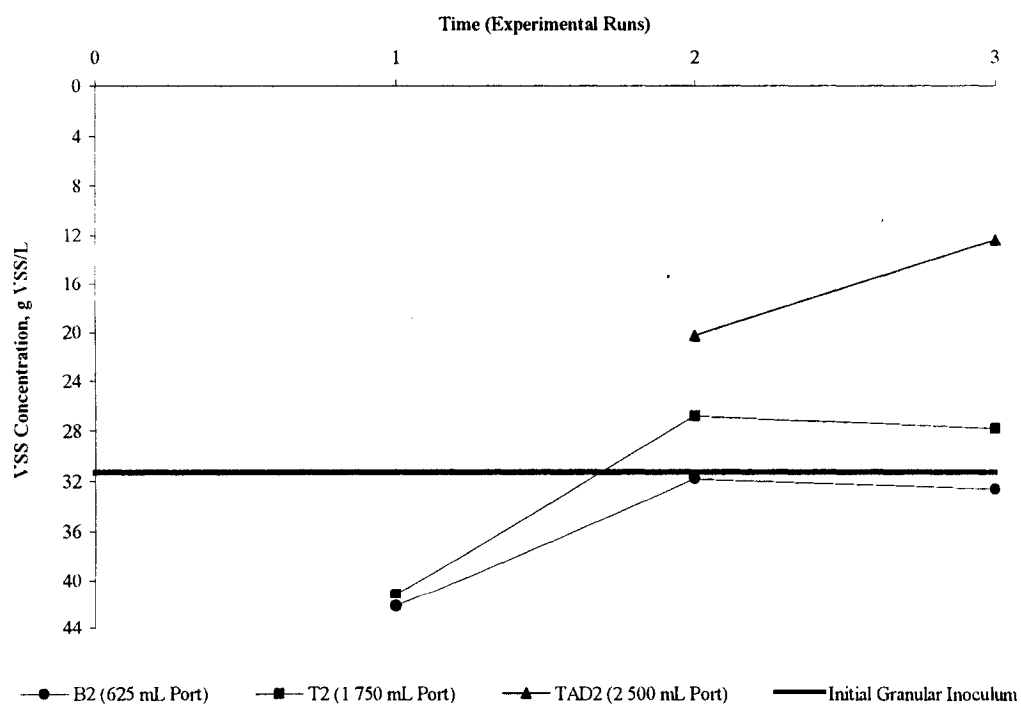


Figure 4.8 b): VSS Profile of AD2 at the End of Each Run Condition

From the results of Table 4.14, Table 4.15 presents the percent variations that occurred in the average SB solids inventory as well as the SS concentrations in the UASBs during the different runs. Comparisons were made with their original state (seed) as well as with the resulting average conditions at the end of the preceding run. All corresponding percent increases (or decreases) were analogous for the average bed solids (g VSS) and UASB SS concentrations (g VSS/L).

Table 4.15: Changes in Average UASB Solids Inventories and SS Concentrations

Feeding Days	Solids Inventory in Beds (g VSS)		AD SS Concentration (g VSS/L)	
	from: 62.6 g VSS seed	previous conditions	from: 16.5 g VSS/L seed	previous conditions
AD1	Run 1: (17+34 d)	+ 17.9 (28%)	+ 17.9 (28%)	+ 4.4 (27%)
	Run 2: (67 d)	+ 35.5 (57%)	+ 17.6 (22%)	+ 9.0 (54%)
	Run 3: (28 d)	+ 4.0 (6%)	- 31.5 (32%)	+ 0.8 (5%)
	Overall Ave:	+ 19.1 (31%)		+ 4.7 (28%)
AD2	Run 1: (17+34 d)	+ 17.3 (28%)	+ 17.3 (28%)	+ 4.8 (29%)
	Run 2: (67 d)	+ 22.5 (36%)	+ 5.2 (6%)	+ 6.2 (38%)
	Run 3: (28 d)	+ 8.2 (13%)	- 14.3 (17%)	+ 2.4 (14%)
	Overall Ave.:	+ 16.0 (26%)		+ 4.5 (27%)

(+) Increase; (-) Decrease; Values in parentheses represent the proportion of the increase or decrease (%) from either the average initial inoculum conditions or the average conditions prior to that particular run.

At the lowest applied loadings, the VSS content of both ADs initially showed a significant and similar increase of approximately 28%, when weighed against the original bed conditions (62.6 g VSS). This corresponded to the time when the average sludge settling characteristics of the UASBs were at their best (Table 4.17) and when their average specific acetoclastic activities experienced the greatest increase (Table 4.13). Even though the average VSS concentration in AD2's SB decreased, average bed solids contents and reactor SS concentrations continued to increase in both anaerobic units, but they rose by approximately 3 times more in AD1. This was mainly due to the fact that the latter's SBH had attained 3.250 L by the end of the second run (compared to 2.775 L in AD2), which resulted in final conditions of 124.4 g VSS and 32.3 g VSS/L, respectively (*Appendix L.11*). The greatest average activity level in AD2's SB was also observed to correspond to its greatest average SS inventory, although this was not the case in AD1. The aforementioned improvements can be indicative of biomass growth or at least of some stimulatory effects of the feed on the anaerobes.

During the last experimental run, where the highest organic loads and flow rates were applied, both reactors experienced considerable reductions in their average VSS content (Tables 4.14 and 4.15) and this also correlated with an important decrease in their average activity levels, based on the AAT results (Table 4.13). However, the 32% VSS drop encountered in AD1, can be partly explained by a sampling mishap (where a significant but unknown amount of SS were lost) as well as the substantial sludge flotation. Since its sludge settling properties continued to worsen over time (Section 4.3.3), granules eventually started to discharge into the ABs (*Appendix L.4*). At 66.6 g VSS and 17.3 g VSS/L, on average, the resulting solids corresponded to levels approximately 5–6% above its starting conditions.

Yet, a greater overall VSS inventory and concentration was sustained in the average SB of the higher loaded reactor, at 82 g VSS and 34 g VSS/L, respectively, compared to 79 g VSS and 32 g VSS/L for the lower organic loaded reactor (Table 4.14). The percent variations in the resulting solids inventories compared to the mean initial SB conditions represented approximately +30% in AD1 and +26% in AD2, on the whole. However, similar overall reactor VSS concentrations of 21 g VSS/L and VSS/TSS ratios of 89% were observed, on average, in both UASBs, over the entire experimental time course, but a slightly greater average SBH was observed in AD2.

Although the average settled sludge bed volumes (SBH in mL) were found to increase (Table 4.14) over the course of the experiment, their values were only rough approximations since they were visually estimated from calibrated graduations placed along the height of the UASBs (Section 3.2.1.1g). To try to find the most accurate estimation of the true SBHs, the anaerobic reactors were vigorously shaken and left to settle for about 1.5 h prior to the measurements. However, the top of the beds were often not quite level and it was sometimes difficult to distinguish between the supernatant blanket and the SB.

Also, due to the SB flotation that was encountered by both ADs (Section 4.3.3), to various extents, SB compaction got progressively poorer over time. Furthermore, the effects of reactor feeding and internal recycling seemed to make matters worse since expanded SBHs (not presented) were observed to be significantly greater than settled SBHs. A few times, AD1's SB had to be poked with a long rod in order to try to get it to settle, particularly in cases when it had actually risen above the outlet port. Access to the bed was achieved via a port in the lid and the reactor was exposed to air, at least for a short time, which most probably added to reactor stress.

Therefore, the SB volume measurements taken after the end of the first experimental run are believed to be overestimated, at least to some extent, since sludge settleability showed apparent

signs of deterioration, particularly in AD1 (Section 4.3.3). Since the buoyant SBs were most likely not influenced by the extremely low biogas production rates (Section 4.2), this assumption might explain the suspiciously large increase of 0.875 L found in AD1's SBH, over only 48 days of additional operation time (end of Run 1 – 2).

4.3.3 **SETTLING VELOCITY (SETTLEABILITY)**

The tabulated settleability data acquired from tests performed on the inoculum granules and on UASB reactor SS are presented in *Appendix L.12* [Tables L.12 a) – g)]. The TSS fractions (%) that exited from the upflow velocity tube and the cumulative TSS wash out (%) plotted against the applied upflow velocities are also presented in this appendix [Figures L.12 a) – j)], for all applicable AD samples. These typical settling (or upflow velocity) profile curves allow the assessment of general sludge quality, changes in bed granularity during reactor operation as well as possible variations in the settling properties of the sludge along the height of the beds over time.

The average results of a duplicate test carried out on the inoculum [Table L.12a iii) and Figure L.12a)] provide an estimate of the original granular biomass properties prior to its exposure to the experimental Enfalac™ substrate. The average upflow velocity at which 50% of sludge samples were washed out (V_{50}) was evaluated to be 66.9 ± 0.6 m/h. The first two fractions of the percent TSS distribution curve only constituted approximately 4% of the total average sample. Also, its form was clearly unimodal (with one peak), which is typical of homogeneous sludge usually made up of granules of similar shapes and sizes. Since little granule loss occurred at the lower upflow velocities, the unusually large V_{50} (obtained by interpolation for a 50% loss from the mean cumulative solids plot) suggested that the granular seed was a high-quality sludge with good initial settling capabilities even for high upflow velocities. The STD of the inoculum's average V_{50} was obtained from the results of the duplicate test [Table L.12a i) and ii)].

The resulting 50% granule washout upflow velocities (V_{50}) for the various UASB samples (evaluated at the end of each run) are reported in Table 4.16 and they were obtained similarly to those of the seed, from the cumulative upflow velocity profiles [Figures L.12 b) – d) in *Appendix L.12*]. Thus, the values summarized in Table 4.17 are assumed to represent the average V_{50} estimates in the SBs during a particular run and over the entire experiment. However, the (end-of-run) upflow velocity profiles presented in Figures L.12 e) – j) of *Appendix L.12* are mostly qualitative in nature and they mainly depict the changes in SB quality over the operation time of the reactors.

Although the two UASB reactors were assumed to have the same settleability characteristics as the inoculum, Enfalac™-acclimated sludge developed poorer settling properties in both ADs since the average V_{50} values reported for all run conditions were smaller than that of the seed (Table 4.17). In terms of the average sludge beds, a significant declining trend was observed as the study progressed. The V_{50} range in AD1 and AD2's SBs was respectively of 17–59 and 41–57 m/h, on average. Since higher V_{50} values represent superior settling sludge (Andras et al., 1989), AD2 had better average settleability, with an overall V_{50} of 47(±9) m/h compared to 35(±22) m/h for AD1. From beginning to end, the average sludge settling velocity reductions corresponded to approximately 48 and 29%, for AD1 and AD2 respectively.

Table 4.16: Settling Profiles of the UASB Reactor Sludge Beds at the End of the Runs

April 5 and June 20		Average V_{50} of Inoculum:				66.9 ± 0.6 m/h			
Settling Velocity Tests Performed After Runs 1 – 3:		V_{50} of Anaerobic Digester 1 (AD1)				V_{50} of Anaerobic Digester 2 (AD2)			
		Top (TAD1)	Middle (T1)	Bottom (B1)	SB Average	Top (TAD2)	Middle (T2)	Bottom (B2)	SB Average
Sept. 12	(After 2 L/d):	-	54	48	51 (±3)	-	47	50	48 (±2)
Nov. 18/19	(After 4 L/d):	9	25	29	21 (±10)	38	56	35	43 (±12)
Dec. 16	(After 8 L/d):	20	-	10	15 (±5)	25	45	46	39 (±12)

[Values in parentheses represent the variations within the bed heights or layers (± V_{50}).]

Table 4.17: Average 50% Washout Velocities (V_{50}) in the UASB Reactor Sludge Beds

Ave. V_{50} (m/h)	Anaerobic Digester 1 (AD1)				Anaerobic Digester 2 (AD2)			
	Top (TAD1)	Middle (T1)	Bottom (B1)	SB Average	Top (TAD2)	Middle (T2)	Bottom (B2)	SB Average
Run 1:	-	60	58	59 (±1)	-	57	59	57 (±1)
Run 2:	9	39	39	29 (±17)	38	51	42	44 (±7)
Run 3:	14	-	19	17 (±5)	31	50	41	41 (±9)
	Overall Ave.			35 ^s (±22)	Overall Ave.			47 ^s (±9)

[Values in parentheses represent the variations within the bed heights or ^swithin the bed averages (± V_{50}).]

Over time, some changes in granularity and differences in settling characteristics were also observed within the layers of the sludge beds. The most noticeable deterioration in the settling conditions was observed in AD1 when SB properties were re-evaluated after the second experimental run. This was clearly reflected in the average V_{50} values, which fell from 59(±1) m/h to 29(±17) m/h. Also, AD1's end-of-run upflow velocity profile plots show that a large portion of

granular sludge exited at the lowest upflow velocity. This was particularly apparent in the top section sample for which a V_{50} of only 9 m/h was estimated. Thus, there was an apparent shift to the left in the TSS distribution, when comparing the conditions at the conclusion of first and second runs. Although the upper portion showed some improvement by the end of the last run, in terms of its average V_{50} (14 m/h), the deterioration was significantly worse in the lower part of the bed (B1), where the V_{50} estimate decreased to 19 m/h, on average. After the final run, no test could be performed on the middle section of AD1 since the sludge bed had completely split in two. Only SB remnants with small and fairly fluffy granules remained by the top port while a relatively larger amount was present at the bottom.

However, these results were not surprising since this particular reactor was plagued by granule and SB flotation problems that were visible even before settling tests were performed. SB channeling was observed in both UASB reactors and it started practically as soon as reactor operation got under way. As the experiment progressed, the granules seemed to develop a relatively slimy texture as well as a lighter color and became finer, especially in the top sections of the SBs. These changes were more apparent in the more problematic AD1. Some white feed residue was also observed within the beds, on occasion, but particularly in the bottom portion of AD1. This degenerating reactor, which did not show signs of recovery and probably required re-inoculation to improve its poor level of performance (Section 4.2), was shut down after 146 days of operation mainly because granules were continuously plugging the overflow port and causing important operational problems.

In general, AD2 was less problematic than AD1 and seemed to sustain larger as well as more granular-natured sludge particles. However, the V_{50} values evaluated in the various SB layers at the end of the runs presented more variability beyond the second run (Table 4.19), as did the average SB estimate for the last run. Even though a decreasing trend was observed in all average V_{50} estimates within its SB, the velocities assessed in its middle and bottom sections after the second and final runs, respectively, showed much improvement. The upflow velocity distributions [Fig. L.12 h) – j)] demonstrated rather multimodal profiles (with two peaks), which usually suggest a more heterogeneous sludge, at least compared to the inoculum and AD1. However, AD2 was shut down at the same time as AD1 since it also did not exhibit particularly good performance and also experienced considerable effluent solids wash out (Section 4.2), especially during the final experimental run.

The average specific activities, SBHs and SS content of the AD sludge beds that correspond to the mean settling results presented are summarized above in Tables 4.13 and 4.14,

respectively. Granules in the lower part of the SBs were not always found to have better average settleability and they generally had the lowest inert solids fractions (*Appendix L.11*). This outcome is rather uncharacteristic for granular sludge-UASBs (Section 2.1.3). However, FSS are recognized as the heaviest constituents of biomass and when they prevail, good settling sludge usually results. Settleability reductions or losses in bed granularity were not necessarily accompanied by a decline in the activity of the biomass (Section 4.3.1), but this was also found in an anaerobic SBR study, where a synthetic soluble sucrose/acetate wastewater was treated at low OLRs and SOLRs (Kennedy et al., 1991). Furthermore, despite the fact that AD1's average sludge settling properties clearly got worse during the second run, its mean SS inventory was largest at this time (although it dropped significantly over the final run).

When comparing the sum of the TSS fractions exited during the settling tests, in terms of g TSS [Tables L.12 a) – g) in *Appendix L.12*], to the TSS content found in all the 10 mL SS samples analyzed [Tables L.11 a) – d) in *Appendix L.11*], all the percent SS recoveries (Table 4.18) were found to be very good, particularly when considering the average TSS values in the entire SBs. However, the STD of the seed's average TSS recovery was quite large since the sums of the TSS exited fractions for the two inoculum samples tested (on a g TSS basis) resulted in fairly different values. This is probably because their sampling techniques differed (wide-bore pipet versus syringe fitted with straight connector). A large discrepancy between their upflow velocity estimates for the 6th pump setting [Table L.12a i) and ii) in *Appendix L.12*] was also noted.

Table 4.18: TSS Recoveries (%), on a g TSS basis, for the 10 mL UASB samples

April 4/5 & June 20/30		Average for Inoculum:				~100 ± 29%			
Comparison of Settling Velocity Tests and SS Tests		Anaerobic Digester 1 (AD1)				Anaerobic Digester 2 (AD2)			
		Top (TAD1)	Middle (T1)	Bottom (B1)	*SB Average	Top (TAD2)	Middle (T2)	Bottom (B2)	*SB Average
Sept. 12	(After 2 L/d):	-	86	»100	>100	-	>100	»100	>100
Nov.18/19	(After 4 L/d):	83	>100	90	92	>100	>100	93	>100
Dec. 16	(After 8 L/d):	»100	-	»100	»100	»100	96	96	>100

*The mean TSS fraction sums of the SB layers after each particular run are not presented in *Appendix L.12*.

The reactor upflow velocities (m/h) reported in Table 4.19 consider the total volumetric flow rates (V_{up} in L/d) that were assumed to be applied at the inlets of the two UASBs during the various operating conditions. Their values include the different targeted influent flow rate conditions [6 L/d (Start-up), 2 L/d (Run 1), 4 L/d (Run 2) and 8 L/d (Run 3)] as well as the

corresponding internal recycle rates [Figures 3.5 b) – e) in Section 3.1.1.1]. The upward velocities differed slightly for AD1 and AD2 since the diameters of both anaerobic units were not exactly the same. The surface areas of AD1 and AD2 were estimated as 81.71 and 76.98 cm², respectively. Thus, the upflow velocities reported in Tables 4.16 and 4.19 were calculated by dividing the total incoming volumetric flows either by the cross-sectional area of the settling test tube or by that of the UASB reactors, respectively.

Table 4.19: UASB Reactor Liquid Upflow Velocity Estimates

Total Applied Upflow Velocities:		V _{up} (L/d)	V _{up} in AD1 (m/h)	V _{up} in AD2 (m/h)
Max. upflow velocity Including recycle rates:	Start-up (Days 1-17):	~42	0.21	0.23
(Q _{infl.} ~2 L/d)	Run 1 (Days 18-44):	~14	0.07	0.08
	(Days 45-61):	~10	0.05	0.05
(Q _{infl.} ~4 L/d)	Run 2 (Days 62-113):	~12	0.06	0.06
(Q _{infl.} ~8 L/d)	Run 3 (Days 114-146):	~16	0.08	0.09

The total upward velocities (V_{up}) applied to the UASBs (Table 4.19) were observed to be much less than 1 m/h (i.e., typical for UASBs) and when compared to the various average V₅₀ results obtained with the settling (or upflow) test device (Table 4.17), all the values were much less than the latter ones (V_{up} ≪ V₅₀). Thus, solids washout certainly should not have been a concern with the incoming flow rates used and it was certainly not attributable to biogas uplift since its production was extremely low in both ADs (*Appendix L.8*). However, as mentioned above, both anaerobic units experienced important SB flotation problems as well as relatively poor effluent quality in terms of SS concentrations (*Appendix L.4*). This suggests that fairly significant washout occurred even under very low applied upflow velocities (V_{up}). Although microscopic studies were not performed, it is not unreasonable to say that *Methanosaeta* acetoclasts could have prevailed in the UASBs since acetate levels were very low (Section 4.2.3.3). These are however less affected by the presence of O₂ as well as usually less prone to washout (Section 2.1.3.3).

The initial influent flow of 6 L/d (Days 2–17) and internal recycle rates were significantly reduced (by a factor of 3) after the start-up period [Figure 3.5 c) in Section 3.1.1.1] since the effect of the incoming liquid flow rates on the SB was questioned. By doing so, it was hoped that the experiment could be carried on with less operational difficulties and that the UASBs would be able to cope with the SB channeling, expansion and flotation that had already been observed. However, it was not the intent to apply greater influent flow rates or to operate at higher HRTs during the

acclimation period than during the experimental runs since this is not the preferred approach for reactor start-up. As the AB baffles were installed towards the end of the first experimental run (Day 45), the recycling streams from the activated sludge clarifiers to the UASBs were also eliminated to further reduce the incoming flow rates.

However, as explained in Section 3.1.3.1, the main cause of granule flotation was eventually not believed to be entirely due to the total incoming flow rates since it still occurred in the settled SBs, in some instances during the 146-day study, when all pumps were off for an extended period. Influent and recycling flow rates could have been underestimated during pump calibration. However, it is very unlikely that the desired flow settings (or combined flows) were off to the point to cause the applied V_{up} values to lie above the estimated V_{50} values or even above the lowest applied upflow tube velocity. Although the design of the T-shaped inlet is intended to reduce dead spaces in the SB, its constrictions might have somewhat increased the influent V_{up} values. The practical 5 min TSS fraction collection times typically used in settling tests usually introduce a positive bias in the cumulative plots (Andras et al., 1989). However, since they only slightly overestimate the V_{50} results, true V_{50} values would not be very much closer to those reported in Table 4.19.

As far as sludge flotation problems are concerned, perhaps the reactor acclimation time was insufficient. The change in substrate source from the more concentrated pulp and paper mill effluent to the dilute synthetic milk-based wastewater might have negatively affected the settling characteristics of the original granular sludge. The granules might have been starving and they could have developed hollow regions that could have caused them to become buoyant due to endogenous decay. The growth of filamentous organisms (i.e., sludge bulking) may possibly be to blame for the settling problems. However, microscopic studies were not performed and bacterial composition was not examined. The sludge particles might have been affected by the fat-content of the experimental Enfalac™ feed, where hydrolysis could have been somewhat inhibited by the potential coating of their external surfaces (Brown and Pico, 1979). Guiot (1992) reports that granules covered with fat can cause massive and sudden flotation to occur (Section 2.2.3). Since the circumferences of the UASBs were relatively small (~4 in.), reactor-bridging effects could also have been a potential cause of SB flotation.

As the settled SBHs of both reactors increased over time, their expanded form eventually exceeded the height of the internal recycling ports (Section 3.1.1.1) and even reached the underside of the biorings, even though the anaerobic units were far from being full of sludge (*Appendix L.11*). Consequently, the recirculation pumps could have crushed the granules and affected the granularity

of the beds, at least to some extent. This could also have been responsible for the observed reduction in particle size and the development of more voluminous sludge. However, it is postulated that the plastic biorings (acting as the GLSS) were not an efficient means of sludge retention since relatively high effluent TSS resulted in both UASBs [Tables 4.5 a) and b), Table 4.6 or *Appendix L.4*]. However, their average SRTs remained quite reasonable throughout the experimental time course [Tables 4.5 a) and b) in Section 4.2] since anaerobic sludge was not intentionally discharged other than for sampling purposes. Although AD2's average SRT decreased significantly from Run 2 to 3, during all runs, the mean SRTs in both UASBs remained greater than the recommended design SRT of 20 d at 24°C (Section 2.1.2.2c).

Table 4.20 summarizes the average overall characteristics of the original anaerobic granular inoculum as well as of the SB conditions observed in each UASB unit during their entire operational time. Even though some notable variations were observed from run to run, over the height of each particular SB, as well as when comparing the corresponding responses in both reactors, overall, the average AD biomass conditions did not vary immensely from those of the initial beds nor between the greater (AD1) and less organically loaded (AD2) beds (Table 4.20). Although specific acetoclastic activities (Section 4.3.1) and SS contents improved to some extent, sludge settling properties demonstrated evident deterioration compared to the conditions of the original seeding material. This had been detected from visual observations as well as from high effluent SS and reactor operational problems. Oddly, the greater average VSS content and concentration in the SB of the higher loaded unit (AD1) did not correspond to greater reactor settling properties or average acetoclastic activities, overall. However, based on the VRRs and SRRs results (Table 4.7), the volumetric and specific activities were always found to be much greater in AD1 than in AD2.

Table 4.20: Average Anaerobic Sludge Characteristics Over the Entire Study

Granular Biomass Properties	Inoculum	AD1	AD2
Specific Acetoclastic Activity ($\text{g AA}_{\text{rem}}/\text{g VSS}\cdot\text{d}$)	0.10	0.26	0.29
Volumetric Acetoclastic Activity ($\text{g AA}_{\text{rem}}/\text{L}\cdot\text{d}$)	0.6	1.6	1.7
SS: VSS concentration ($\text{g VSS}/\text{L}_{10 \text{ mL samples}}$)	31	34	32
SB inventory ($\text{g VSS}_{\text{Bed}}$)	63	82	79
SS in entire UASB ($\text{g VSS}/\text{L}_{\text{Reactor}}$)	16	21	21
VSS/TSS ratio	0.88	0.89	0.89
Settleability or V_{50} (m/h)	67	35	47

4.4 KINETIC CONSTANTS

Bench-scale treatability studies usually allow the estimating of process kinetic coefficients ultimately used in modeling and design as well as in predicting full-scale system performance. However, due to insufficient data (i.e., only 3 quasi-stable run conditions), Lineweaver-Burke plots (Section 2.1.2.4) were not performed. Thus, the only overall reactor kinetic constants that could be estimated were the maximum specific substrate utilization rates (k) and the overall observed yield coefficients ($Y_{\text{obs}} = \Delta \text{VSS}_{\text{prod}} / \Delta \text{COD}_{\text{rem}}$). The k values for each reactor during each run were previously presented in Table 4.7 as the average SRRs as well as the specific activities from the AAT results ($\text{g COD}_{\text{rem}}/\text{g VSS}\cdot\text{d}$). The results based on continuous reactor data, presented in Table 4.21, were weighed over the various stable influent flow periods while the anaerobic batch assay results were weighed over the time between reactor SS samplings.

Table 4.21: Average Overall Reactor Kinetic Constants

	AD1	AD2
$k_{\text{AAT tests (g COD}_{\text{rem}}/\text{g VSS}\cdot\text{d)}}$	0.27	0.31
$k_{\text{Reactor data (g COD}_{\text{s rem}}/\text{g VSS}_{\text{ave}}\cdot\text{d)}}$	0.023	0.008
$Y_{\text{AD (g VSS/g COD}_i\text{)}}$	0.104	0.058
	AB1	AB2
$k_{\text{Reactor data (g COD}_{\text{s rem}}/\text{g VSS}\cdot\text{d)}}$	0.44	0.51
$Y_{\text{AB (g VSS/g COD}_i\text{)}}$	0.601 or $*(-0.159)$	-0.155

*Excludes the results of Run 3 since a large amount of granules went into AB1 (i.e., $\Delta \text{g VSS}_{\text{prod}} = +2.231$)

CHAPTER 5

CONCLUSIONS

In spite of evident signs of UASB reactor instability, the low-strength synthetic dairy substrate investigated was found to be amenable to anaerobic treatment, even under low applied organic loading rates and ambient temperature conditions.

Based on the results of the bench-scale study, the following specific conclusions can be drawn:

- The organic removal efficiencies of both UASB reactors were not affected by the comparable reduction in the HRTs and increase in total OLRs investigated, which ranged from approximately 2 to 0.5 days and 0.5 to 2 g COD_i/L·d, respectively. This was also the case for the aerobic basins.
- At $23.5 \pm 0.8^\circ\text{C}$ and for the range of operation conditions examined, the anaerobic units were quite proficient at reducing influent organic matter since they achieved COD removals of approximately 90%. However, the combined anaerobic-aerobic treatment systems attained levels of 94 and 96%, respectively. Thus, the activated sludge polishing processes only contributed marginally to organic strength reduction.
- Owing to the low organic loads applied, the maximum specific activities achievable in the UASB reactors only ranged from 0.006-0.08 and 0.004-0.03 g COD_s/g VSS·d in AD1 and AD2, respectively. Since their COD removals were reasonable, the systems should be able to treat much greater organic loadings at lower HRTs.
- Contrary to the COD removals, no solids reduction took place in the anaerobic units. Since biomass flotation problems developed and the biorings were inefficient at retaining solids, high particulate COD fractions and SS concentrations resulted in their effluents. The overall TSS reductions in both systems were also found to decrease considerably, over the runs, with the increases in hydraulic and organic loading rates. Thus, the aerobic basins were responsible for the TSS removals observed.
- The settling properties of the SB granules of both UASBs worsened with operation time. They were lower than those of the inoculum and showed no sign of recovery. The plaguing sludge

flotation problems were so severe in AD1 that the floating bed split in two distinct parts during the final run. Although the biomass inventories decreased noticeably in both reactors, their SRTs remained greater than 20 d.

- Although some biogas was generated, CH_4 as well as CO_2 gas fractions were detected and influent organic matter was removed, which implies that anaerobic degradation was occurring, there were obvious indications of process upset and/or inhibition in the UASBs. Even though their pH were maintained in a sensible operating range, the biogas production, methane yields and VFA levels in both anaerobic units were practically negligible throughout the course of experiment while N_2 gas fractions were high.
- The relatively high fat content of the mainly soluble and inoffensive simulated dairy wastewater was suspected to exert a marked influence on the anaerobic microorganisms.
- No scum or foam was observed in the anaerobic units while slight foaming was only noticed in AB2 on one occasion. Throughout the study, both treatment systems were relatively odor free and, in general, the performance of both treatment systems was similar in many respects.

CHAPTER 6

RECOMMENDATIONS

Some suggestions for the improvement of the current research project include:

- Implementing a continuous stirring apparatus in the feed refrigeration tank.
- Applying a better influent dilution approach or assessing the effects of feed strength on system performance by using one combined treatment system and varying its influent concentration over different runs.
- Applying a longer sludge acclimation period as well as an initial anaerobic unit HRT that would be longer than the ones to be considered during subsequent steady state conditions.
- Optimizing influent substrate distribution and increasing the internal mixing in the sludge bed.
- Using larger diameter anaerobic reactors to avoid possible wall bridging effects.
- Using a more efficient G/L/S separator than the simple plastic flexirings and/or incorporating a solids capture device (or settling vessel) such as the one used by van den Berg and Kennedy (1983).
- Performing intentional anaerobic granular biomass wastage at regular intervals.
- Reducing UASB reactor headspace volumes and/or constantly (or semi-continuously) purging them with nitrogen to enhance anaerobic process conditions.
- Performing a Biochemical Methane Potential (BMP) test on the synthetic wastewater used to assess its anaerobic biodegradability and methane production potential.
- Using a more sophisticated biogas measuring device and pumps with digital flow readouts would allow for greater operational control, particularly of the recirculation rates.
- Using narrow and deep aeration basins, as opposed to wide and shallow ones, as well as the insertion of some form of cloth, mesh or membrane might help to contain the MLSS.
- Using mixed liquor seed of greater initial concentration (i.e., $\geq 3\,500$ mg VSS/L) is also advised.
- Operating and monitoring of the treatment systems until actual steady state conditions are reached.

Based on the current study, further research work could include the following:

- Evaluating the treatment capabilities of the proposed anaerobic-aerobic system under potentially stressed conditions such as lower operating temperatures and/or greater influent concentrations, over a broader range of HRTs and applied OLRs.
- Assessing bioreactor and combined treatment performance in treating an actual dairy or domestic wastewater.
- Assessing process kinetics for reactor design and system modeling by conducting a greater number of continuous operation runs under steady state conditions.
- Investigating the effects of Enfalac[™]-acclimated biomass on UASB and dual anaerobic-aerobic system performance.
- Monitoring the fate of the substrate's lipid and LCFA content as well as assessing its impact on the anaerobic granules.
- Investigating the probable detrimental effects of oxygen and aerobic sludge recycling on the anaerobic bacteria.
- Elucidating the mechanism(s) involved in the biogas production inhibition of the anaerobic units, which was not accompanied by acid accumulation.
- Conducting microscopic investigations to determine if granule hollowing was the cause of the sludge flotation problems observed.
- Conduct tracer studies to determine the level of short-circuiting/dead space in the UASBs as well as to estimate their mixing regime and the extent of substrate-biomass contact.
- Comparing the results of the current study with the extent of treatment that would be achieved by a sole activated sludge process, under comparable conditions.
- Conducting a similar experiment with an anaerobic contact-activated sludge polishing process configuration or investigating the use of phase separation, which is reported to be advantageous when treating high carbohydrate wastewaters.
- Investigating the nutrient removal potential of the anaerobic-aerobic configuration.

REFERENCES

- Abeling, U. and C. F. Seyfried (1992), "Anaerobic and Aerobic Treatment of High-Strength Ammonium Wastewater-Nitrogen Removal Via Nitrite," *Water Science and Technology*, 26, 5-6, pp. 1007-1015.
- Ackman, R. G. (1972), "Porous Polymer Bead Packings and Formic Acid Vapor in the GLC of Volatile Free Fatty Acids", *Journal of Chromatographic Science*, 10, pp. 560-565.
- Andras, E. (1987), *Initial Biofilm Formation of Acetoclastic Methanogenic Bacteria*, M. A. Sc. Thesis, University of Ottawa, Canada.
- Andras, E., Kennedy, K. J. and D. A. Richardson (1989), "Test for Characterizing Settleability of Anaerobic Sludge", *Environmental Technology Letters*, 10, pp. 463-470.
- Aulenbach, D. B. and R. V. Hallock (1972), "Treatment of Milk Truck Washing Wastes by Anaerobic Digestion," *Proceedings of the 27th Industrial Waste Conference*, Purdue University, pp. 126-136.
- Backman, R. C., Blanc, F. C. and J. C. O'Shaughnessy (1985), "The Treatment of Dairy Wastewater by The Anaerobic Up-Flow Packed Bed Reactor," *Proceedings of the 40th Industrial Waste Conference*, Purdue University, pp. 361-372.
- Barber, W. P. and D. C. Stuckey (1999), "The Use of the Anaerobic Baffled Reactor (ABR) for Wastewater Treatment: a Review," *Water Research*, 33, 7, pp. 1559-1578.
- Barbosa, R. A. and G. L. Sant'Anna, Jr. (1989), "Treatment of Raw Domestic Sewage in an UASB Reactor," *Water Research*, 23, 12, pp. 1483-1490.
- Bargman, R. D. (1968), *Anaerobic Sludge Digestion*, Water Pollution Control Federation, Manual of Practice no. 16, Lancaster Press, Lancaster, PA.
- Bernet, N., Delgenes, N., Akunna, J. C., Delgenes, J. P. and R. Moletta (2000), "Combined Anaerobic-Aerobic SBR for the Treatment of Piggery Wastewater," *Water Research*, 34, 2, pp. 611-619.
- Bitton, G. (1994), *Wastewater Microbiology*, Wiley-Liss, Inc., New York.
- Blanc, F. C. and R. Navia (1991), "Treatment of Dairy Wastewater by Chemical Coagulation," *Proceedings of the 45th Industrial Waste Conference*, Purdue University, pp. 681-689.
- Bonkoski, W. A., Sointio, J. E. and G. R. Gillespie (1983), "Anaerobic-Aerobic Treatment of a Wheat Starch Plant Effluent A Case History," *Proceedings of the 38th Industrial Waste Conference*, Purdue University, pp. 781-787.
- Brown, H. B. and R. F. Pico (1979), "Characterization and Treatment of Dairy Wastes in the Municipal Treatment System," *Proceedings of the 34th Industrial Waste Conference*, Purdue University, pp. 326-333.

- Buswell, A. M., Boruff, C. S. and C. K. Wiesman (1932), "Anaerobic Stabilization of Milk Waste," *Industrial and Engineering Chemistry*, 24, 12, pp. 1423-1425.
- Carucci A., Lindrea, K., Majone, M. and R. Ramadoori (1995), "Dynamics of the Anaerobic Utilization of Organic Substrates in an Anaerobic/Aerobic Sequencing Batch Reactor," *Water Science and Technology*, 31, 2, pp. 35-43.
- Castillo, A., Cecchi, F. and J. Mata-Alvarez (1997), "Combined Anaerobic-Aerobic System to Treat Domestic Sewage in Coastal Areas," *Water Research*, 31, 12, pp. 3057-3063.
- Chapra, S. C. and R. P. Canale (1998), *Numerical Methods for Engineers: With Programming and Software Applications*, 3rd ed., WCB/McGraw-Hill, Boston.
- Chevalier, Pierre et collaborateurs. (1996), "*Technologies d'assainissement et prévention de la pollution*", collection sciences de l'environnement, Télé-université, Presses de l'Université du Québec, Sainte-Foy, Québec.
- Choi, E. and C. E. Burkhead, (1984), "Anaerobic Treatment of Dairy Wastes using Fixed-Film and Without-Media Reactors," *Proceedings of the 39th Industrial Waste Conference*, Purdue University, pp. 223-233.
- Chynoweth, D. P. and R. Isaacson. (1987), *Anaerobic Digestion of Biomass*, Elsevier Applied Science. New York.
- Çiftçi, T and I. Öztürk (1995), "Nine Years of Full-Scale Anaerobic-Aerobic Treatment Experience with Fermentation Industry Effluents," *Water Science and Technology*, 32, 12, pp. 131-139.
- Cocci, A. A., Landine, R. C., Loedolter, M. and S. Beuttel (1990a), "Case History: Anaerobic-Aerobic Pretreatment of Confectionary Waste," *Dairy, Food and Environmental Sanitation*, 10, 3, pp. 146-150.
- Cocci, A. A., Burke, B. F., Landine, R. C. and D. L. Blickenstaff (1990b), "Anaerobic-Aerobic Pretreatment of a Dairy Waste: A Case History," *Proceedings of the Industrial Wastes Symposia: 63rd Annual Conference of the Water Pollution Control Federation*, Washington, D.C., October 7-11th, pp. 242-253.
- Collivignarelli, C., Urbini, G., Farneti, A., Bassetti, A. and U. Barbaresi (1990), "Anaerobic-Aerobic Treatment of Municipal Wastewaters with Full-Scale Upflow Anaerobic Sludge Blanket and Attached Biofilm Reactors," *Water Science and Technology*, 22, 1-2, pp. 457-482.
- Curi, K. and W. Wesly Eckenfelder, Jr. (1976), *Theory and Practice of Biological Wastewater Treatment*, *Proceedings of the NATO Advanced Study Institute on Theory and Practice of Biological Wastewater Treatment*, Sijthoff & Noordhoff International Publishers B. V., Alphen aan den Rijn, The Netherlands.
- de Sousa, J. T. and E. Foresti (1996), "Domestic Sewage Treatment in an Upflow Anaerobic Sludge Blanket – Sequencing Batch Reactor System", *Water Science and Technology*, 33, 3, pp. 73-84.

- de Zeeuw, W. and G. Lettinga (1980), "Acclimation of Digested Sewage Sludge During Start-Up of an Upflow Anaerobic Sludge Blanket (UASB) Reactor," *Proceedings of the 35th Industrial Waste Conference*, Purdue University, pp. 39-47.
- Droste, R. L. (December, 1997a), *Laboratory Exercises in Environmental Engineering*, Introduction to Environmental Engineering Undergraduate Course (CVG-2131) Lab Manual, Department of Civil Engineering, University of Ottawa, Canada.
- Droste, R. L. (1997b), *Theory and Practice of Wastewater Treatment*, John Wiley & Sons, Inc., New York.
- Droste, R. L., Guiot, S. R., Gorur, S. S. and K. J. Kennedy (1987), "Treatment of Domestic Strength Wastewater with Anaerobic Hybrid Reactors," *Water Pollution Research Journal of Canada*, 22, 3, pp. 474-490.
- Eckenfelder Jr., W. W. (1989), *Industrial Water Pollution Control*, 2nd Edition, McGraw-Hill Series in Water Resources and Environmental Engineering, New York.
- Elmitwalli, T. A., Zandvoort, M. H., Zeeman, G., Bruning, H. and G. Lettinga (1999), "Low Temperature Treatment of Domestic Sewage in Upflow Anaerobic Sludge Blanket and Anaerobic Hybrid Reactors," *Water Science and Technology*, 39, 5, pp. 177-185.
- Ferguson, J. F. (1994), "Anaerobic and Aerobic Treatment for AOX Removal," *Water Science and Technology*, 29, 5-6, pp. 149-162.
- Field, J. A., Stams, A. J. M., Katom, M. and G. Schraa (1995), "Enhanced Biodegradation of Aromatic Pollutants in Cocultures of Anaerobic and Aerobic Bacterial Consortia," *Antonie van Leeuwenhoek*, 67, pp. 44-77.
- Filho, B. C., de Figueiredo, R. F., and E. A. A. Nour (1996), "Anaerobic Pretreatment of Dairy Liquid Effluents," *Proceedings of the 51st Industrial Waste Conference*, Purdue University pp. 323-331.
- Fox, P. and V. Venkatasubbiah (1996), "Coupled Anaerobic/Aerobic Treatment of High-Sulfate Wastewater with Sulfate Reduction and Biological Sulfide Oxidation," *Water Science and Technology*, 34, 5-6, pp. 359-366.
- Garuti, G., Dohanyos, M. and A. Tilche (1992), "Anaerobic-Aerobic Combined Process for the Treatment of Sewage with Nutrient Removal: the ANANOX Process," *Water Science and Technology*, 25, 7, pp. 383-394.
- Goronszy, M. C. (1990), "Batch Reactor Treatment of Dairy Wastewaters: A Case History," *Proceedings of the 44th Industrial Waste Conference*, Purdue University, pp. 795-805.
- Grady, C. P. L., JR. and H. C. Lim (1980), *Biological Wastewater Treatment: Theory and Applications*, Marcel Dekker, New York.
- Grady, C. P. L., JR., Daigger, G. T. and H. C. Lim (1999), *Biological Wastewater Treatment*, Marcel Dekker, New York.

- Guiot, S. R. (1992), "Bioimmobilization in Anaerobic Reactors", *Proc. of the Bioprocessos Anaerobios para el Tratamiento de Efluentes Industriales*, by the Universidad Autonoma Metropolitana (Iztapalapa) and the Instituto Mexicano del Petroleo in Mexico City, pp. 1-14.
- Guiot, S. R., Arcand, Y. and C. Chavarie (1992a), "Advantages of Fluidization on Granule Size and Activity Development in Upflow Anaerobic Sludge Bed Reactors," *Water Science and Technology*, 26, 3-4, pp. 897-906.
- Guiot, S. R., Pauss, A. and J. W. Costerton (1992b), "A Structured Model of the Anaerobic Granule Consortium," *Water Science and Technology*, 25, 7, pp. 1-10.
- Gunnerson, C. G. and D. C. Stuckey (1986), *Anaerobic Digestion Principles and Practices for Biogas Systems*, Integrated Resource Recovery Series, UNDP Project Management Report no. 5, World Bank Technical Paper no. 49, Washington, D.C.
- Haberl, R., Atanasoff, K. and R. Braun (1991), "Anaerobic-Aerobic Treatment of Organic High-Strength Industrial Waste Water," *Water Science and Technology*, 23, 10-12, pp. 1909-1918.
- Hamza, A. (1982), "Treatability of Dairy Wastewater," *Proceedings of the 37th Industrial Waste Conference*, Purdue University, pp. 311-319.
- Hanaki, K., Matsuo, T. and M. Nagase (1981), "Mechanism of Inhibition Caused by Long-Chain Fatty Acids in Anaerobic Digestion Process," *Biotechnology and Bioengineering*, 23, pp. 1591-1610.
- Harper, W. J. and C. W. Hall (1976), *Dairy Technology and Engineering*, Avi Publishing Company Inc., Connecticut.
- Heertjes, P. M. and R. R. van der Meer (1979), "Comparison of Different Methods for Anaerobic Treatment of Dilute Wastewaters," *Proceedings of the 34th Industrial Waste Conference*, Purdue University, pp. 790-803.
- Hoover, S. R., Jasewicz, L., Pepinsky, J. B. and N. Porges (1951), "Assimilation of Dairy Wastes by Activated Sludge", *Sewage and Industrial Wastes*, 23, 2, pp. 167-173.
- Hoover, S. R. and N. Porges (1952), "Assimilation of Dairy Wastes by Activated Sludge". *Sewage and Industrial Wastes*, 24, 3, pp. 306-312.
- Hovious, J. C. (1972), *Anaerobic Treatment of Synthetic Organic Wastes*, Office of Research and Monitoring Environmental Protection Agency, Union Carbide Corporation Chemicals and Plastics Division Research and Development Department, South Charleston, West Virginia.
- Huang, J. and V. T. Bates (1979), "Treatment of Milk Waste by Rotating Biological Contactors Using Pure Oxygen," *Proceedings of the 34th Industrial Waste Conference*, Purdue University, pp. 483-498.
- Huster, R. and C. H. Möbius (1990), "Performance Increase of Aerobic-Biological Waste Water Treatment Plants in Papermills by Anaerobic Pretreatment of High-Strength Partial Flows," *Water Science and Technology*, 22, 7-8, pp. 287-288.

- International Association on Water Quality, IAWQ Specialist Group on Anaerobic Digestion, (January 1999), *Newsletter*, no. 5.
- Jorden, W. L., Pohland, F. G. and B. H. Kornegay (1971), "Evaluating Treatability of Selected Industrial Wastes," *Proceedings of the 26th Industrial Waste Conference*, Purdue University, pp. 514-529.
- Kasapgil, B., Anderson, G. K. and O. Ince (1994), "An Investigation Into the Pre-Treatment of Dairy Wastewater Prior to Aerobic Biological Treatment," *Water Science and Technology*, 29, 9, pp. 205-212.
- Kennedy, K. J. (1985), *Startup and Steady State Kinetics of Anaerobic Downflow Stationary Fixed Film Reactors*, Ph.D Thesis, University of Ottawa, Canada.
- Kennedy, K. J. (1999), *Anaerobic Digestion* (CVG-5179), compiled course notes and scientific papers, University of Ottawa.
- Kennedy, K. J., Sanchez, W. A., Hamoda, M. F. and R. L. Droste (1991), "Performance of Anaerobic Sludge Blanket Sequencing Batch Reactors," *Research Journal Water Pollution Control Federation*, 63, 1, pp. 75-83.
- Kettunen, R. H., Pulkkinen, E. M. and J. A. Rintala (1996), "Biological Treatment at Low Temperatures of Sulfur-Rich Phenols Containing Oil Shale Ash Leachate," *Water Research*, 30, 6, pp. 1395-1402.
- Kobayashi, H. A., Stenstrom, M. K. and R. A. Mah (1983), "Treatment of Low Strength Domestic Wastewater Using the Anaerobic Filter," *Water Research*, 17, 8, pp. 903-909.
- Koster, I. W. and A. Cramer (1987), "Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids," *Applied and Environmental Microbiology*, pp. 403-409.
- Lalman, J. A. and D. M. Bagley (2000), "Anaerobic Degradation and Inhibitory Effects of Linoleic Acids," *Water Research*, 34, 17, pp. 4220-4228.
- Lalman, J. A. and D. M. Bagley (2001), "Anaerobic Degradation and Methanogenic Inhibitory Effects of Oleic and Stearic Acids," *Water Research*, 35, 12, pp. 2975-2983.
- Landine, R. C., Brown, G. J., Steeves, A. L., Bough, W. A., Bybee, E. C. and E. D. Burke (1987). "Anaerobic Treatment of Dairy Wastewater," *Proceedings of the 42nd Industrial Waste Conference*, Purdue University, pp. 701-705.
- Langenhoff, A. A. M. and D. C. Stuckey (2000), "Treatment of Dilute Wastewater Using an Anaerobic Baffled Reactor: Effect of Low Temperature," *Water Research*, 34, 15, pp. 3867-3875.
- Lentz, E. M. (1996), *Treatment of Landfill Leachate Using Upflow Anaerobic Sludge Sequencing Batch Reactor Technology*, M. A. Sc. Thesis, University of Ottawa, Canada.

- Lettinga, G. (1996), "Sustainable Integrated Biological Wastewater Treatment," *Water Science and Technology*, 33, 3, pp. 85-98.
- Lettinga, G. and J. N. Vinken (1980), "Feasibility of the Upflow Anaerobic Sludge Blanket (UASB) Process for the Treatment of Low-Strength Wastes," *Proceedings of the 35th Industrial Waste Conference*, Purdue University, pp. 625-634.
- Lettinga, G. and L. W. Hulshoff Pol (1991), "UASB-Process Design for Various Types of Wastewaters," *Water Science and Technology*, 24, 8, pp. 87-107.
- Lettinga, G., de Man, A., van der Last, A. R. M., Wiegant, W., van Knippenberg, K., Frijns, J. and J. C. L. van Buuren (1993), "Anaerobic Treatment of Domestic Sewage and Wastewater," *Water Science and Technology*, 27, 9, pp. 67-73.
- Lettinga, G., de Zeeuw, W., Wiegant, W. and L. Hulshoff Pol (1987), *Bioenvironmental Systems* (CH. 5: "High-Rate Anaerobic Granular Sludge UASB-Reactors for Wastewater Treatment"), Volume I, pp.131-155.
- Lettinga, G., Hulshoff Pol, L. W., Koster, I. W., Wiegant, W. M., de Zeeuw, W. J., Rinzema, A., Grin, P. C., Roersma, R. E. and S. W. Hobma (1984), "High Rate Anaerobic Waste-Water Treatment Using the UASB Reactor under a Wide Range of Temperature Conditions," *Biotechnology and Genetic Engineering Reviews*, 2, pp.253-284.
- Lettinga, G., Roersma, R. and P. Grin (1983), "Anaerobic Treatment of Raw Domestic Sewage at Ambient Temperature Using a Granular Bed UASB Reactor," *Biotechnology and Bioengineering*, 25, pp. 1701-1723.
- Lettinga, G., van Velsen, A. F. M., Hobma, S., de Zeeuw, W. and A. Klapwijk (1980), "Use of the Upflow Sludge Blanket (USB) Reactor Concept for Biological Wastewater Treatment, Especially for Anaerobic Treatment," *Biotechnology and Bioengineering*, 22, pp. 699-734.
- Liu, H. W., Lo, S. N. and H. C. Lavalley (1996a), "Theoretical Study on Two-Stage Anaerobic-Aerobic Biological Treatment of a CTMP Effluent Part 1: Effects of Operating Conditions on System Behavior," *Water Quality Research Journal of Canada*, 31,1, pp. 1-19.
- Liu, H. W., Lo, S. N. and H. C. Lavalley (1996b), "Theoretical Study on Two-Stage Anaerobic-Aerobic Biological Treatment of a CTMP Effluent Part 2: Optimization of Treatment System," *Water Quality Research Journal of Canada*, 31,1, pp. 21-35.
- Maat, D. Z. and S. S. Gorur (1990), "Start-Up and Performance Testing of a Full Scale UASB Anaerobic Wastewater Treatment Facility," *Proceedings of the 44th Industrial Waste Conference*, Purdue University, pp. 209-214.
- Malaspina, F., Stante, L., Cellamare, C. M. and A. Tilche (1995), "Cheese Whey and Cheese Factory Wastewater Treatment with a Biological Anaerobic-Aerobic Process," *Water Science and Technology*, 32, 12, pp. 59-72.
- Manjunath, D. L., Mehrotra, I. and R. P. Mathur (1990), "Treatment of Cane Sugar Mill Wastewater in Upflow Anaerobic Sludge Blanket (UASB) Reactors", *Proceedings of the 44th Industrial Waste Conference*, Purdue University, pp. 215-223.

- Maragno, A. L. F. C. and J. R. Campos (1992), "Treatment of Wastewater with a Low Concentration of Organics using an Anaerobic Fluidized Bed Reactor," *Water Science and Technology*, 25, 7, pp. 179-181.
- Masters, G. M. (1998), *Introduction to Environmental Engineering and Science*, 2nd ed., Prentice Hall, Inc., New Jersey.
- McCarty, P. L. (1964), "Anaerobic Waste Treatment Fundamentals", *Public Works*, 95, 9-12, pp. 91-94; 95-99; 107-112; 123-126.
- McCarty, P. L. and D. P. Smith (1986), "Anaerobic Wastewater Treatment", *Environmental Science and Technology*, 20, 12, pp. 1200-1206.
- McCarty, P. L., Hahn, D. J., McDermott, G. N. and P. J. Weaver (1972), "Treatability of Oily Wastewaters from Food Processing and Soap Manufacture," *Proceedings of the 27th Industrial Waste Conference*, Purdue University, pp. 867-878.
- Mennerich, A. (1987), "Investigations on the Two-Stage Anaerobic-Aerobic Treatment of High Concentrated Landfill Leachates", *10th Annual Madison Waste Conference*, University of Wisconsin, Madison, WI., pp. 261-275.
- Metcalf and Eddy, Inc. (1991), *Wastewater Engineering: Treatment, Disposal, and Reuse*, 3rd Edition, revised by G. Tchobanoglous, McGraw-Hill Publishing Company, New York.
- Mikula, W. J., Reynolds, J. H., Middlebrooks, E. J. and D. George (1979), "Performance Characteristics of a Rotating Biological Contactor (RBC) Treating a Cheese Processing Wastewater," *Proceedings of the 34th Industrial Waste Conference*, Purdue University, pp. 335-353.
- Monroy-H., O., Vázquez-M., F., Derramadero, J. C. and J. P. Guyot (1995), "Anaerobic-Aerobic Treatment of Cheese Wastewater with National Technology in Mexico: The Case of "El Sauz", " *Water Science and Technology*, 32, 12, pp. 149-156.
- Monroy, O., Famá G., Meraz, M., Montoya, L. and H. Macarie (2000), "Anaerobic Digestion for Wastewater Treatment in Mexico: State of the Technology," *Water Research*, 34, 6, pp. 1803-1816.
- Obayashi, A. W. and J. M. Gorgan (1985), *Management of Industrial Pollutants by Anaerobic Processes*, Industrial Waste Management Series, Lewis Publishers, Inc., Michigan.
- Oleszkiewicz, J. A. and J. E. Hutchison (1990), "Anaerobic and Aerobic Options in Treating Food Industry Wastewater," *Proceedings of the 44th Industrial Waste Conference*, Purdue University, pp. 761-768.
- Ozturk, I., San, H.A. and V. Eroglu (1990), "Pilot and Full Scale Treatability Studies on Wastewaters from an Edible Oil Refining Industry," *Proceedings of the 44th Industrial Waste Conference*, Purdue University, pp. 769-776.

- Page, I. C., Ott, C. R., Pottle, D. S., Cocci, A. A. and R. C. Landine (1999), "Anaerobic-Aerobic Treatment of Dairy Wastewater: A Pilot Study," *Hazardous and Industrial Wastes*, Proceedings of the 31st Mid-Atlantic Industrial and Hazardous Wastes Conference, pp.68-79.
- Parkin, G. F. and W. F. Owen (1986), "Fundamentals of Anaerobic Digestion of Wastewater Sludges," *Journal of Environmental Engineering, American Society of Civil Engineers (ASCE)*, 112, 5, pp. 867-920.
- Parsons, W. A. (1977), "Activated Sludge Plant Effluent Polishing," *Proceedings of the 32nd Industrial Waste Conference*, Purdue University, pp. 24-28.
- Pavlostathis, S. G. and E. Giraldo-Gomez (1991), "Kinetics of Anaerobic Treatment: A Critical Review", *Critical Reviews in Environmental Control*, by CRC Press Inc., 21(5-6), pp. 411-490.
- Perle, M., Shlomo, K. and G. Shelef (1995), "Some Biochemical Aspects of the Anaerobic Degradation of Dairy Wastewater," *Water Research*, 29, 6, pp. 1549-1554.
- Pham, Thi Tham (2002), *Anaerobic Degradation of Aircraft Deicing Fluid (ADF) in Upflow Anaerobic Sludge Blanket (UASB) Reactors and the Fate of ADF Additives*, Ph.D Thesis, University of Ottawa, Canada.
- Pipes, W. O. (1977), "Microbiology of Dairy Waste Activated Sludge Separation Problems," *Proceedings of the 32nd Industrial Waste Conference*, Purdue University, pp. 303-309.
- Pretorius, W. A. (1971), "Anaerobic Digestion of Raw Sewage," *Water Research*, 5, pp. 681-687.
- Rebac, S., vanLier, J. B., Lens, P., Stams, A. J. M., Dekkers, F., Swinkels, K. T. M. and G. Lettinga (1999), "Psychrophilic Anaerobic Treatment of Low Strength Wastewaters," *Water Science and Technology*, 39, 5, pp. 203-210.
- Reemtsma, T. and M. Jekel (1997), "Dissolved Organics in Tannery Wastewaters and their Alteration by a Combined Anaerobic and Aerobic Treatment," *Water Research*, 31, 5, pp. 1035-1046.
- Rinzema, A., Boone, M., van Knippenberg, K. and G. Lettinga (1994), "Bacterial Effect of Long Chain Fatty Acids in Anaerobic Digestion," *Water Environment Research*, 66, 1, pp. 40-49.
- Rittmann, B. E. and P. L. McCarty (2001), *Environmental Biotechnology: Principles and Applications*, McGraw-Hill Companies Inc., Boston.
- Robinson, R. K. (1990), *Dairy Microbiology: The Microbiology of Milk*, Vol. I, 2nd ed., Elsevier Applied Science Publishing Co., New York.
- Samson, R. et al. (1984), "Dairy Waste Treatment Using Industrial-Scale Fixed-Film and Upflow Sludge Bed Anaerobic Digesters: Design and Start-Up Experience," *Proceedings of the 39th Industrial Waste Conference*, Purdue University, pp. 235-241.
- Sanz, I. and F. Fdz-Polanco (1990), "Low Temperature Treatment of Municipal Sewage in Anaerobic Fluidized Bed Reactors," *Water Research*, 24, 4, pp. 463-469.

- Savard, Catherine (2001), *Anaerobic Treatment of Landfill Leachate Using a Peat Moss Filter for Pre-Treatment*, M. A.Sc. Thesis, University of Ottawa, Canada.
- Saw, C. B. (1986), "Comparison of the Anaerobic Contact and Packed Bed Processes for the Treatment of Edible Oil Wastewaters," *Proceedings of the 41st Industrial Waste Conference*, Purdue University, pp. 178-187.
- Sayed, S. K. I. and A. A. Fergala (1995), "Two-Stage UASB Concept for Treatment of Domestic Sewage Including Sludge Stabilization Process," *Water Science and Technology*, 32, 11, pp. 55-63.
- Shen, C. F., Rousselle, D and S. R. Guiot (1995), "Feasibility of Synchronous Aerobic/Anaerobic Processes with a Single Upflow Sludge Bed System," *Proceedings of the 50th Industrial Waste Conference*, Purdue University, pp. 385-393.
- Simpson, D. E. (1971), "Investigations on a Pilot-Plant Contact Digester for the Treatment of a Dilute Urban Waste," *Water Research*, 5, pp. 523-532.
- Spaulding, R. A. (1948), "The Anaerobic Digestion Rates of Milk Wastes," *Proceedings of the 4th Industrial Waste Conference*, Purdue University, pp. 40-44.
- Speece, R. E. (1983), "Anaerobic Biotechnology for Industrial Wastewater Treatment," *Environmental Science and Technology*, 17, 9, pp.416a-427a.
- Speece, R. E. (1996), *Anaerobic Biotechnology for Industrial Wastewaters*, Archae Press, Nashville, TN.
- Stall, T. R. and J. H. Sherrard (1978), "Evaluation of Control Parameters for the Activated Sludge Process," *J. Water Pollution Control Federation*, 50, 3, pp. 450-457.
- Standard Methods for the Examination of Water and Wastewater* (1995), 19th ed., American Public Health Association, Washington, DC.
- Stover, E. L. and R. Gonzalez (1987), "Anaerobic Pilot Plant Studies for Dairy Plant Wastewaters," *Proceedings of the 42nd Industrial Waste Conference*, Purdue University, pp. 693-700.
- Tanaka, T., Kitagawa, M. and E. Ohkubo (1991), "Energy Saving, High Biological Phosphorus Removal and Improvement of Sludge Settleability on Full-Scale Anaerobic-Aerobic Activated Sludge Process," *Water Science and Technology*, 23, 4-6, pp. 801-810.
- Tang, N. H., Torres, C. L. and R. E. Speece (1995), "Treatment of Low Strength Domestic Wastewater by Using Upflow Anaerobic Sludge Blanket Process," *Proceedings of the 50th Industrial Waste Conference*, Purdue University, pp. 437-448.
- Tchobanoglous, G. and E. D. Schroeder (1985), *Water Quality: Characteristics, Modeling, Modification*, Adisson-Wesley, Reading, Mass.

- Thirumurthi, D., Austin, T. P., Ramalingaiah and S. Khakhria, (1986), "Anaerobic/Aerobic Treatment of Municipal Landfill Leachate," *Water Pollution Research Journal of Canada*, 21, 1, pp. 8-20.
- Torpy, M. F. (1988), *Anaerobic Treatment of Industrial Wastewaters*, Pollution Technology Review no. 154, Noyes Data Corporation, Park Ridge, New Jersey.
- Tracy, K. D. and S. N. Hong (1990), "Upgrading Wastewater Treatment Plants with Anaerobic Selectors," *Water Science and Technology*, 22, 7-8, pp. 35-43.
- Ulrix, R. P. (1994), "Anaerobic/Aerobic Combination Treats High-Strength Wastewater," *Water-Engineering & Management*, 141, 2, pp. 22-23 and 33.
- van den Berg, L. and K. J. Kennedy (1983), "Dairy Waste Treatment with Anaerobic Stationary Fixed Film Reactors," *Water Science and Technology*, 15, 8/9, pp. 359-368.
- van der Last, A. R. M. and G. Lettinga (1992), "Anaerobic Treatment of Domestic Sewage Under Moderate Climatic (Dutch) Conditions Using Upflow Reactors at Increased Superficial Velocities," *Water Science and Technology*, 25, 7, pp. 167-177.
- Van der Steen, P., Brenner, A., Van Buuren, J. and G. Oron (1999), "Post-Treatment of UASB Reactor Effluent in an Integrated Duckweed and Stabilization Pond System," *Water Research*, 33, 3, pp. 615-620.
- van Haandel, A. C. and G. Lettinga (1994), *Anaerobic Sewage Treatment A Practical Guide for Regions with a Hot Climate*, John Wiley & Sons, New York.
- van Huyssteen, J. J. (1967), "Gas Chromatographic Separation of Anaerobic Digester Gases Using Porous Polymers", *Water Research*, 1, pp. 237-242.
- Viraraghavan, T., and S. R. Kikkeri (1990), "Anaerobic Filter Treatment of Dairy Wastewater at Low Temperatures," *Proceedings of the 44th Industrial Waste Conference*, Purdue University pp. 199- 208.
- Vieira, S. M. M. and A. D. Garcia, Jr. (1992), "Sewage Treatment by UASB-Reactor Operation Results and Recommendations for Design and Utilization," *Water Science and Technology*, 25, 7, pp. 143-157.
- Vuoriranta, P., Rintala, J. and H. Morange (1987), "Anaerobic-Aerobic Versus Aerobic Treatment of Concentrated Pulp and Paper Mill Effluent," *Water Science and Technology*, 19, 7, pp 1219-1221.
- Wang, X., Mize, T.H., Saunders, F. M. and S. A. Baker (1997), "Biotreatability Test of Bleach Wastewaters from Pulp and Paper Mills," *Water Science and Technology*, 35, 2-3, pp. 101-108.
- Water Environment Federation (WEF), Task Force on Wastewater Residuals Stabilization, (1995), *Wastewater Residuals Stabilization*, Manual of Practice FD-9, Alexandria, VA.

- WEF Manual of Practice no. 8, ASCE Manual and Report on Engineering Practice, no. 76, (1991), *Design of Municipal Wastewater Treatment Plants*, vol. II, CH. 18: Sludge Stabilization, pp. 1255-1382.
- Wheatley, A. (1990), *Anaerobic Digestion: A Waste Treatment Technology*, Elsevier Applied Science, London, England.
- Wu, Wei-Min, Hickey, R. F., Bhatnagar, L., Jain, M. K. and J. G. Zeikus (1990), "Fatty Acid Degradation as a Tool to Monitor Anaerobic Sludge Activity and Toxicity," *Proceedings of the 44th Industrial Waste Conference*, Purdue University, pp. 225-232.
- Young, L. Y. (unknown), "Anaerobic Biodegradability Assay", *Biotransformation and Biodegradation*, pp. 802-805.
- Young, J. C. and T. J. Irwin (1999), "Treatability Test Assessment", *Industrial Wastewater*, Jan./Feb. issue, pp. 37-42.
- Zitomer, D. H. and R. E. Speece (1993), "Sequential Environments for Enhanced Biotransformation of Aqueous Contaminants", *Environmental Science and Technology*, 27, 2, pp.227-244.

APPENDIX A: PRELIMINARY CALCULATIONS FOR REACTOR VOLUME ESTIMATES

Based on the following treatment configuration (Figure A.1) and assumptions (i.e., CM activated sludge process with recycle, steady state conditions, etc.), the subsequent *Excel* spreadsheet results present the rough preliminary analysis that was performed to obtain reactor volume estimates (and scale-ups) for the experimental configuration (Figure A.2).

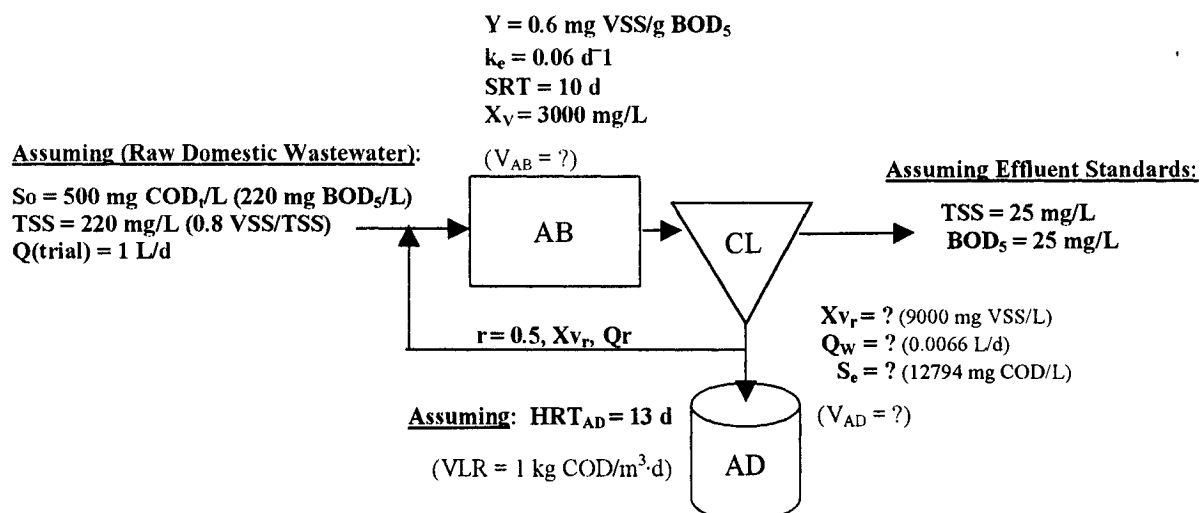


Figure A.1: Conventional Activated Sludge Process – Anaerobic Digester

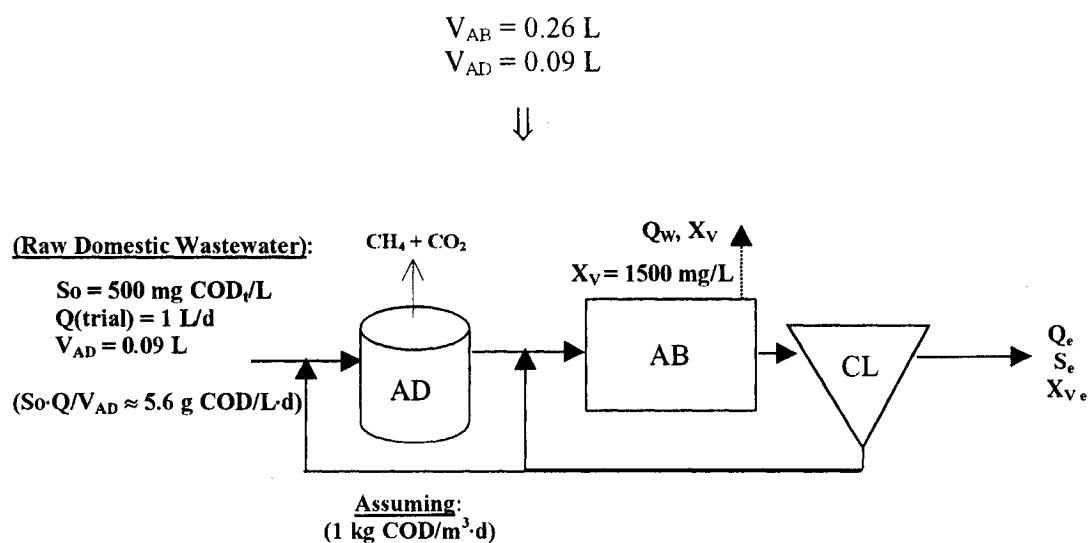


Figure A.2: Coupled Anaerobic Digester – Activated Sludge Process

The Conventional Activated Sludge – Anaerobic Digester configuration analysis yielded :

Anaerobic Digester Influent	
$X_{v,r} = 9000 \text{ mg VSS/L}$	
$Q_{WAS} = 0.0066 \text{ L/d}$	
$S_e (\text{COD}_{WAS}) = 12794 \text{ mg/L}$	
$\text{VLR} = 1 \text{ g COD/L}\cdot\text{d}$ (assuming HRT = 13 d)	

Thus,

Reactor Volumes (L)		Flow Rate (L/d)
Anaerobic	Aerobic	
0.09	0.26	1 L/d
0.05	0.15 (≈ 0.2)	
5	20	100 L/d
2.5	10	50 L/d
1	4	20 L/d

Unfortunately, the 1.5 L ADs that were first used (with ~ 0.5 L SBs) were not able to handle the planned incoming flow rate of approximately 20 L/d and reactor start-ups failed (Section 3.1.3.1). Therefore, larger available anaerobic reactors (~ 3.7 L working volumes with 2 L SBHs) and a lower influent feed flow rate of approximately 6 L/d were employed in the actual study (Sections 3.1.1.1 and 3.1.3.2).

Conventional Activated Sludge - Anaerobic Digester (Figure A.1)

Domestic Wastewater

COD (mg/L) =	500
So (mg BOD ₅ /L) =	220
TSS (mg/L) =	220
Q design (L/d) =	1
ρ (kg/m ³) =	1000
VSS (mg/L) =	176

Activated Sludge Process

Y (mg VSS/mg COD) =	0.4
Y (mg VSS/mg BOD ₅) =	0.6
k _e (d ⁻¹) =	0.06
SRT (d) =	10
X _{VSS} =	3000
ρ _{WAS} =	1020
HRT (d) =	0.26
HRT (h) =	6.32

V (L) =	0.26
V (m ³) =	0.0003

r =	0.5
-----	-----

Q _r (L/d) =	0.5
X _r (mg VSS/L) =	9000
Q _{WAS} (L/d) =	0.0066
Q _{WAS} (m ³ /d) =	0.0000066
M _{WAS} (wet kg/d) =	0.007

Overall plant BOD removal efficiency

$$E (\%) = 89$$

Efficiency based on soluble BOD

$$E (\%) = 96$$

Waste Activated Sludge Stream:

COD (total) =	12794
BOD ₅ =	8743

Clarifier Effluent

Se (BOD ₅) =	25	(soluble)
Se (BOD ₅) =	9.3	(soluble)
X _{e TSS} =	25	
X _{e VSS} =	20	
COD =	13.6	(soluble)

Secondary Sludge Production

r _s =	-1200	mg COD/L d
Y _{obs} =	0.25	mg VSS/mg COD
P _{xb} =	0.00008	kg VSS/d
P _{xb} =	7.90E-05	kg VSS/L
P _{xb} =	0.0001	(kg TSS/d)
P _{xt} =	0.00007	dry kg TSS/d

Specific Substrate Utilization Rate

U =	0.40	mg COD _{rem} /mg VSS d
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F:M ratio

F:M =	0.42	mg COD _{fed} /mg VSS d
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Volumetric Loading Rate (Activated Sludge)

VLR =	1.25	kg COD/m ³ d
VLR =	0.67	kg VSS/m ³ d

For Anaerobic Digester Volume:

HRT (d) =	13
V(L) =	0.09

Volumetric Loading Rate (Anaerobic Digester)

VLR =	1	kg COD/m ³ d
VLR =	1	kg VSS/m ³ d

APPENDIX B: NITROGEN AND PHOSPHORUS FEED SUPPLEMENTATION

The chemical nutrient additions for N and P were calculated as follows to supplement approximately 3.5 mg N/L and 0.8 mg P/L to the feed during its preparation, for the entire length of the experiment, based on the theoretical *aerobic* COD: N: P + 10% (=110: 5.5: 1.1) versus an experimental COD: N: P of 106: ~2: 0.3 (CH. 4, Section 4.1). Nitrogen was added with Fisher Scientific 99% reagent grade L-(+)-Glutamic Acid [$\text{C}_3\text{H}_5\text{NH}_2(\text{COOH})_2$ = 147.13 g/mol] while phosphorus was added with BDH Inc. 97.5-101% anhydrous Di-Potassium Hydrogen Orthophosphate (K_2HPO_4 = 174.18 g/mol) (dibasic) laboratory reagent.

Nitrogen supplement:

$$\frac{3.5 \text{ mg N/L} \times 147.13 \text{ g C}_3\text{H}_5\text{NH}_2(\text{COOH})_2/\text{mol}}{14.007 \text{ g N/mol}} = 36.764 \text{ mg glutamic acid/L}$$

For 20 L of feed $\Rightarrow 36.764 \text{ mg glutamic acid/L} \times 20 \text{ L} = 735.28 \text{ mg} = 0.735 \text{ g glutamic acid}$

For 26 L of feed $\Rightarrow 36.764 \text{ mg glutamic acid/L} \times 26 \text{ L} = 955.86 \text{ mg} = 0.956 \text{ g glutamic acid}$

Phosphorus supplement:

$$\frac{0.8 \text{ mg P/L} \times 174.18 \text{ g K}_2\text{HPO}_4/\text{mol}}{30.974 \text{ g P/mol}} = 4.499 \text{ mg di-potassium hydrogen orthophosphate/L}$$

For 20 L of feed $\Rightarrow 4.499 \text{ mg K}_2\text{HPO}_4/\text{L} \times 20 \text{ L} = 89.98 \text{ mg} = 0.090 \text{ g K}_2\text{HPO}_4$

For 26 L of feed $\Rightarrow 4.499 \text{ mg K}_2\text{HPO}_4/\text{L} \times 26 \text{ L} = 116.97 \text{ mg} = 0.117 \text{ g K}_2\text{HPO}_4$

APPENDIX C: CHARACTERIZATION ANALYSES ONLY PERFORMED ON THE IRON FORTIFIED ENFALAC™ FEED

C.1 TOTAL (TDS), VOLATILE (VDS) AND FIXED DISSOLVED SOLIDS (FDS)

Method 2540 C. was used to determine the TDS in 1 000 mg COD_l/L Iron fortified Enfalac™ liquid feed mixture samples. Several 50 mL samples of filtrate and washings, collected in dry porcelain evaporation dishes, from TSS analyses (Section 3.2.1.1c) performed on these samples, were used for the TDS determinations. They were evaporated to dryness in the Blue M Electric Company Stabil-Therm Gravity drying oven, set to $180 \pm 2^{\circ}\text{C}$, and were then treated according to Method 2540 E. for VDS analyses (APHA, 1995). The FDS were also determined mathematically from the difference between TDS and VDS results.

C.2 TOTAL, VOLATILE AND FIXED SOLIDS

Method 2540 B. (APHA, 1995) was used to determine the TS on several feed samples and the FS were determined similarly to the FDS. The TS procedure was performed on 100 mL well mixed samples yielding less than 200 mg residue. The method suggests the use of an analytical balance of 0.1 mg sensitivity, but as for the TSS/VSS analysis and the TDS/VDS tests, a Sartorius analytical balance, capable of weighing to 0.01 mg, was used for all weightings.

C.3 PERCENT TOTAL, VOLATILE AND FIXED SOLIDS IN SOLID AND SEMISOLID SAMPLES

As described in APHA (1995), Method 2540 G. was used to determine, in %, the TS, VS and FS fractions of the raw Enfalac™ powder used to make the feed mixture. The FS were determined mathematically from the difference between TS and VS results or by calculation. Powdered samples were weighed with a Mettler P1210 analytical balance, with a sensitivity of 10 mg, and the sample size used was 25 g. Since TDS, VS and %VS were determined, all evaporating dishes used were pre-conditioned at 550°C for 1 hour.

C.4 TOTAL PHOSPHORUS AND TOTAL DISSOLVED PHOSPHORUS ANALYSES

The total phosphorus (TP) and total dissolved phosphorus (TDP) fractions analyzed were both subjected to the 4500-P B. 5. Persulfate Digestion Method followed by the 4500-P D. Stannous Chloride Method (APHA, 1995), except that these procedures were applied to filtrate samples in the case of the TDP determination. Samples were analyzed shortly after the feed mixture was prepared. All glassware used in this analysis was initially acid-washed with hot dilute HCl and thoroughly rinsed with distilled water. Commercial detergents, containing phosphates,

were never used to clean them and they were only water pressure-washed in a dishwasher between analyses.

Because soluble or TDP was to be performed, samples were filtered through 0.45 μm Pall Gelman non-sterile GN-6 Metrical[®] 47 mm membrane filters with a filtration apparatus composed of a 300 mL Micro Filtration System (MFS) glass membrane filter funnel, clamped to a PYREX[®] fritted glass support and stopper, and a large Erlenmeyer flask connected to a vacuum pump. The filtrate was successively subjected to the two techniques stated above to analyze TDP and a similar but separate analysis was performed on unfiltered aliquots to determine TP. Filtration through membrane filters of this pore size is simply a replicable analytical technique designed to distinguish dissolved from suspended forms of phosphorus.

All filters were pre-washed by soaking them in distilled water. Fifty of them were soaked in 2 L of distilled water for 1 h, the water was changed and they were re-soaked for an additional 3 hours. Because prolonged filtration times were encountered, the hard-to filter TDP samples were pre-filtered through the same 1.2 μm GF/C grade glass-micro fiber filters used for the SS analysis, to increase the filtration rate.

C.4a Persulfate Digestion Method

The method proceeded by adding 1 drop of phenolphthalein indicator solution followed by 1 mL of H_2SO_4 to each 50 mL of well-mixed sample in 125 mL Erlenmeyer flasks. Instead of $\text{K}_2\text{S}_2\text{O}_8$, 0.4 g of individually weighed solid ammonium persulfate $[(\text{NH}_4)_2\text{S}_2\text{O}_8]$ was used. Before sterilization, sample Erlenmeyer flask tops and necks were covered with Kraft paper. They were then heated at 121°C, for 30 minutes, at slow exhaust mode, in a Market Forge Sterilmatic Autoclave capable of developing 98 to 137 kPa, and neutralized with 1N NaOH. Once cooled, the autoclaved samples that underwent a volume reduction were made up to 100 mL with distilled water, in 100 mL volumetric flasks. Most samples were diluted to be able to read feed sample concentration values from a calibration curve (*Appendix F*), which was prepared with a series of standards made from an anhydrous KH_2PO_4 stock solution.

After this preliminary digestion step converting phosphorus to orthophosphate (orthophosphate phosphorus), all samples were treated to form molybdenum blue, an unstable colored compound proportional to the amount of phosphate present, and analyzed with a spectrophotometer after performing the colorimetric Stannous Chloride Method.

C.4b *Stannous Chloride Method*

Again, after adding 1 drop of indicator to 100 mL diluted and digested samples, no pink color ever developed. Both color-producing reagents were then added to all samples per APHA (1995). Their light absorbance was determined at a 690 nm wavelength with a Bausch & Lomb Spectronic 70 Spectrophotometer, equipped with Bausch & Lomb 20 mm test tubes (320-1 000 nm usable range). The instrument was calibrated and operated according to the procedures in the *Laboratory Exercises in Environmental Engineering* lab manual (Droste, 1997a).

Color absorbencies were measured between 10 and 12 minutes after reagent addition and equal timing conditions were maintained for all determinations (samples, blanks and standards) because color develops progressively at first and later fades. Feed sample phosphorus concentrations ($\text{PO}_4^{-3}\text{-P}$) were determined from an average calibration curve of absorbance as a function of concentration that yields a near linear relation (*Appendix F*). Although unfiltered (i.e., TP) milky feed samples were slightly turbid, color development did not seem to be hindered.

The simple digestion technique described was directly selected and used, since the equipment to perform it was available. During the series of analyses on the feed, the phenolphthalein indicator used changed from an alcoholic solution to the appropriate aqueous disodium salt solution, when it was made available (as of June 21). However, no changes in the results were noticed following replication.

C.5 **5-DAY BIOCHEMICAL OXYGEN DEMAND TEST**

Fresh oxygen saturated dilution water was prepared by adding 1 mL/L each of magnesium sulfate, calcium chloride and ferric chloride stock solutions as well as VWR ready to use pH 7.2 phosphate buffer solution for BOD to 20 L of distilled water, to give it optimal conditions and quality. Because nitrification inhibition was desired, 10 mg/L of 2-chloro-6-(trichloro methyl) pyridine (TCMP) was also added to it and left to dissolve. It was aerated overnight with a Drummond Scientific pipet-aid[®] and diffuser stones, always stored in a 20°C incubator but not tested with the glucose-glutamic acid check.

The Enfalac[™] feed sample to be tested required the addition of a seed source since it did not contain a population of microorganisms that could oxidize the biodegradable organic matter in the sample. The seeding material used was supernatant taken from un-disinfected, biologically treated effluent of an acclimated and active UASB reactor (University of Ottawa). Although the reactor was fed with aircraft deicing fluid, its anaerobic granular sludge came from the same source as the one used in this experiment. The seed was adapted to the feed waste investigated by adding small

daily increments of it to the seed and continuously aerating the mixture in the lab for a few days. It was assumed to contain a satisfactory microbial population and was ready for direct addition to each BOD bottle at the beginning of the analysis.

Because the BOD_5 content of the feed was unknown, the general guidelines from the *Process Control Laboratory Course manual for Wastewater Treatment Plant Operators* (1982) served as a guide in selecting appropriate dilutions (*Appendix M*). The 5210 B. 5-day BOD test procedure used (APHA, 1995), consisted of adding 5 drops of acclimated seed to 300 mL extra clean standard BOD sample bottles and duplicate seed control bottles prior to dilutions. This method was combined with the 4500-O G. Membrane Electrode Method (APHA, 1995), for the initial and final DO measurements of all bottles, before and after incubation. The same calibrated ORION Model 860 DO Meter and DO electrode used for the aerobic basin DO monitoring were used in this analysis. The probe was however equipped with an overflow funnel, fixing ring and rotor stirring accessory to adapt it to the BOD bottles, which were placed on a Fisher Scientific Flexa-mix magnetic stirrer.

The average BOD_5 in the sample could be computed from the oxygen concentration reductions in the wastewater dilutions and the seed controls, after the incubation period (Example Calculation in *Appendix M*). Although samples were analyzed promptly after making the feed, they were brought to room temperature prior to preparing the dilutions. The unseeded dilution water blanks were run to verify dilution water quality and incubation bottle cleanliness. Four blanks were analyzed with each sample batch. For the test to be meaningful, the results are restricted by several criteria presented in *Appendix M*.

C.6 TOTAL KJELDAHL NITROGEN AND ORGANIC NITROGEN ANALYSES

The 4500-N_{org} C. Semi-Micro-Kjeldahl Method (APHA, 1995) was used to determine the TKN and org. N content of fresh feed samples. TKN concentrations were obtained by direct sample digestion followed by distillation and subsequent on-line titration. The org. N concentrations were determined similarly although ammonia removal was first performed since TKN is defined as the sum of org. N and NH_3 -N. Samples were also analyzed shortly after feed preparation and sampling. A de-chlorinating reagent was not used to remove residual chlorine in feed samples prepared with tap water.

For analysis of both TKN and org. N, undiluted 50 mL samples were digested, under a fume hood, in a BÜCHI 430 Digester. This digestion apparatus was equipped with a glass suction tube and outlet, to vent copious SO_3 fumes produced, and 8 heating elements, which accommodated the

300 mL BÜCHI digestion/distillation vessels used. 10 mL of Kjeldahl digestion reagent solution was added to each sample. For org. N determinations, ammonia was removed prior to distillation by adding 3 mL of borate buffer to 50 mL samples, adjusting their pH to 9.5 with 6 N NaOH and a combination electrode pH meter (consisting of the same Fisher Accumet® Model 320 used for pH determinations) and by finally boiling off 30 mL.

According to the digestion steps in the unit's instruction manual, to minimize sample digestion time, each of the two heating unit power controls was first set to its maximum power (setting 10) until the samples just about started to boil. They were then reduced to a medium setting of 4-5, but making sure they continuously boiled until the solutions became pale green and the copious white fumes diminished. Because the digestion time depends on many factors (i.e., type of samples, sample volumes, type of reagents), it is determined by trial and error. However, it is suggested to digest samples for an additional 15-30 minutes after the solutions become clear. The sample digestion causes their volumes to decrease significantly and prior to distillation, no sample exceeded 30 mL. Samples were usually covered and left to cool overnight.

The next day, sample distillation with automatic metered addition of distilled water and 32% NaOH solution, was carried out, during approximately 5 minutes, in a pre-heated BÜCHI 322 Distillation unit, equipped with a BÜCHI 342 Control unit. After the addition of 10 mL of sodium hydroxide-thiosulfate reagent to each sample and their distillation, 30-40 mL of distillate was collected in the receiving vessel. Then, 10 mL of indicating boric acid solution (prepared with isopropyl alcohol), acting as the absorbent receiving solution, was used for the titrimetric finish.

Manual titrations were performed with a METROHM 614 Impulsomat used in conjunction with a METROHM 632 pH meter, calibrated to pH 4.0 with a standard, and a METROHM 655 Dosimat (a motor-driven piston buret). A 0.05 N HCl solution was used to titrate the distillate to a preset pH 4.65 end-point. Because this was difficult to achieve exactly, the resulting pH and volume of titrant added were linearized and interpolation to this desired pH was performed (*Appendix M*). Standards and reagent distilled water blanks, treated identically to the samples, were also carried through all the steps of the procedure with each batch of samples.

Two different standards were prepared (*Appendix G*). The one made with NH_4Cl yielded a 20 mg/L standard for $\text{NH}_3\text{-N}$, while the other prepared with Glutamic acid $[\text{C}_3\text{H}_5\text{NH}_2(\text{COOH})_2]$, yielding a 20 mg/L standard for org. N. Combined together, they served as a 40 mg/L standard for the TKN determination. All blanks, standards and reagents were prepared with distilled water, not with ammonia-free water, since the ammonia levels in the distilled water used, determined with the ammonia-selective electrode, had very low levels (i.e., $< 10^{-8}$ M; Table 4.1 and *Appendix M*). The

tap water used to prepare the feed samples was also found to have low ammonia levels (i.e., $\ll 10^{-8}$ M; Table 4.1 and *Appendix M*)

C.7 AMMONIA DETERMINATION

Dissolved NH_3 concentrations were determined on un-distilled and un-digested aqueous feed samples by Direct Calibration using a meter with direct millivolt readout. The analytical technique followed, based on the 4500- NH_3 D. Ammonia-Selective Electrode Method (APHA, 1995), was that of the instruction manual of the ammonia electrode used.

Potentiometric measurements were achieved with an ORION Model 95-12 Ammonia Gas-Sensing membrane electrode and a Fisher Accumet Model 750 digital pH/Ion Meter, compatible with standard specific ion electrodes. The electrometer operated in the relative millivolt measurement mode (readability to 0.1 mV). The electrode was prepared and used according to the manufacturer's *Instruction Manual*, with its outer body containing ORION Ammonia Electrode filling solution A slope check was also performed prior to every analysis to verify proper electrode operation. The change in electrode potential was observed for a tenfold concentration change of a 0.1 M NH_4Cl standard (a -54 to -60 mV/decade millivoltage range difference should result for $25 \pm 5^\circ\text{C}$ solutions).

Before all measurements, ORION Ammonia pH-Adjusting ISA (Ionic Strength Adjustor) was added to all solutions during magnetic stirring, with a 1 to 50 ratio, to ensure they had similar ionic strengths, to reduce potential interfering ion effects and to bring them to the proper operating range (pH 11 to 14 and level of dissolved species below 1 M). Above this molarity, ammonia is rapidly lost to the air. Sample turbidity and color was said not to affect the measurements and a blue color was observed in all cases, after ISA addition.

Semi-logarithmic calibration curves (*Appendices I.1* and *I.2*) were always determined by serial dilutions from a fresh customer prepared 1 M ammonium chloride (NH_4Cl) standard solution (*Appendix H*). Electrode potential measurements (mV) were plotted against corresponding NH_3 concentrations (log axis), in molar (M) units, from the most dilute to the most concentrated. Sample concentrations, corresponding to stable potential meter readings, were determined by comparison to the standards and by applying the appropriate curve equation obtained by regression (i.e., Typical Direct Measurement Ammonia Electrode Calibration Curves, *Appendix I.1*).

Samples were analyzed right away after a feed batch was made. Distilled water was also used instead of ammonia-free water in checking the slope and preparing the standards since NH_3 levels were found to be very low in distilled water (Table 4.1 and *Appendix M*). All solutions were

allowed to come to room temperature before measurements and were kept covered between them. The electrode was also always thoroughly rinsed with distilled water between measurements. During solution analyses, to minimize possible temperature increases due to mixing, some insulating material was placed between the sample beaker and the American Dade Tek-Pro® Tek-Stir® Hotplate/Magnetic Stirrer used.

Since duplicate feed sample ammonia concentrations (Table 4.1), found by the *Direct Calibration Method* (Appendices I.1 and M), fell below 4×10^{-6} M (0.07 ppm NH₃ or 0.06 ppm as N), *Low-Level Measurements by Direct Calibration* (Appendices I.2 and M) were performed. For this method, the feed sample concentration was also determined with a calibration curve and by applying non-linear regression curve fittings (Typical Low-Level Measurement Ammonia Electrode Calibration Curve, Appendix I.2). The technique required longer electrode stabilization and response times (5-10 minutes), due to possible NH₃ absorption from the air. The electrode tip was kept immersed in a 10^{-3} M standard with ISA, between measurements, and in a 0.1 M standard without ISA, overnight, but was placed in a pH 4.0 buffer for several minutes, between measurements, to speed them up.

C.8 NITRATE DETERMINATION

Nitrate concentrations were determined on aqueous feed samples, based on the 4500-NO₃⁻ D. Nitrate Electrode Method (APHA, 1995), but according to the *Direct Measurement Procedure using a mV meter* found in the instruction manual of the nitrate electrode used.

The measurements were achieved with an ORION Model 90-02 Double Junction Sure-Flow® Reference Electrode, used with a ORION Model 93-07 Nitrate Half-Cell membrane Electrode, and the same Fisher Accumet Model 750 digital pH/Ion Meter as for the NH₃ measurements (Appendix C.7). The nitrate and reference electrodes were prepared and used according to the manufacturer's *Instruction Manuals*. The reference electrode's outer chamber was filled before each daily use with ORION ionplus® Optimum Results™ F Reference Electrode filling solution and its level was always kept at least 2.5 cm higher than the sample level. Its inner chamber was however filled and replaced weekly with fresh green Double Junction Reference Electrode inner filling solution and kept at least 2.5 cm above the level of the outer solution.

Most of the NO₃⁻ procedure measures were followed for NO₃⁻ determinations. During analysis, the nitrate electrode was always immersed to about half the length of its sensing module but never past the rubber washer while the reference electrode was submerged to the same depth in the same beaker. The fill hole was left uncovered during measurements, as called for. The Model

90-02 Double Junction Reference Electrode (that can be stored in air between sample measurements) was stored in distilled water, which is acceptable for short periods, while the Model 93-07 Nitrate Electrode was stored in a 10^{-2} M nitrate standard.

The electrode's slope value, sample potential measurements and the semi-logarithmic calibration curves (*Appendices J.1 and J.2*) were determined in the same manner as for the NH_3 electrode (*Appendix C.7*). The curves were prepared from a fresh 1 M calcium nitrate calibration standard (*Appendix H*). ORION ionplus[®] Nitrate Ionic Strength Adjustor (ISA) was added in all solutions, diluting them in the same 50:1 ratio with ISA. Nitrate Interference Suppressor Solution was never used, but 1 M boric acid preservation solution (*Appendix J.1*) was added, in a 1:100 ratio, to all standards and samples, to inhibit biological growth and prevent biological degradation of the solutions.

Since feed sample NO_3^- concentrations (Table 4.1) found by the *Direct Measurement* technique (*Appendices J.1 and M*) were less than 1.4 ppm as N (6.2 ppm as NO_3^-) or 10^{-4} M NO_3^- (in the non-linear range of the nitrate electrode), *Low-Level Measurements by Direct Measurement* were performed (*Appendices J.2 and M*) with a 100:1 ratio (50:0.5 and 25:0.25 also used) of solution to Low-Level ISA solution (*Appendix J.2*). Contrary to the NH_3 electrode, the response time of this electrode is faster at low-level ranges.

APPENDIX D: SOLUTIONS USED IN THE VFA ANALYSIS

The following two solution preparation procedures were taken from C. M. Elliott's (Department of Civil Engineering, University of Ottawa Water Pollution Laboratory, Canada) Gas Chromatography of Volatile Fatty Acids procedure, in an unpublished document (May, 1989).

20 g/L Internal Standard (ISTD)

This solution was made by first preparing a 20 g/L stock solution of isobutyric acid. The working solution was then prepared in a 1 L volumetric flask by combining 100 mL of the isobutyric acid stock solution (20 g/L), 40 mL of distilled formic acid and 12 mL of 10 N sulfuric acid. The rest of the flask is topped to 1 L with distilled water.

20 g/L VFA Standard Mixture (2% acetic acid, 2% propionic acid and 2% butyric acid)

Stock solutions containing 20 g/L of acetic acid, 20 g/L of propionic acid and 20 g/L of butyric acid were prepared using reagent grade acids. Standardization of the solutions can be achieved by titrating with NaOH for the solution contents to be within 0.5% of 20 g/L. The working VFA standard mixture can then be prepared in a 1 L volumetric flask by combining 100 mL of the acetic acid stock solution (20 g/L), 100 mL of the propionic acid stock solution (20 g/L) and 100 mL of the butyric acid stock solution (20 g/L). The rest of the flask is topped to 1 L with distilled water.

Once prepared, the ISTD and standard mixture solutions were refrigerated at 4°C in glass-stoppered flasks, sealed with Parafilm[®].

APPENDIX E: FLOW METER CALIBRATION CURVE

For 2 to 8 L/d total influent flow rates to the anaerobic reactors, the desired water inputs from AD2's CHT were to vary from approximately 1 to 4 L/d, throughout the experiment, to dilute the incoming feed. As stated in Section 3.2.1.3b, a Fisher Scientific Lab-Crest Mark III flow meter with a 0.32 cm ($\frac{1}{8}$ in.) sapphire float could regulate liquid input rates from 0.72 (0.5 mL/min) to 33.13 L/d (23.01 mL/min), which corresponds to scale readings 2 to 12 (Figure E.1).

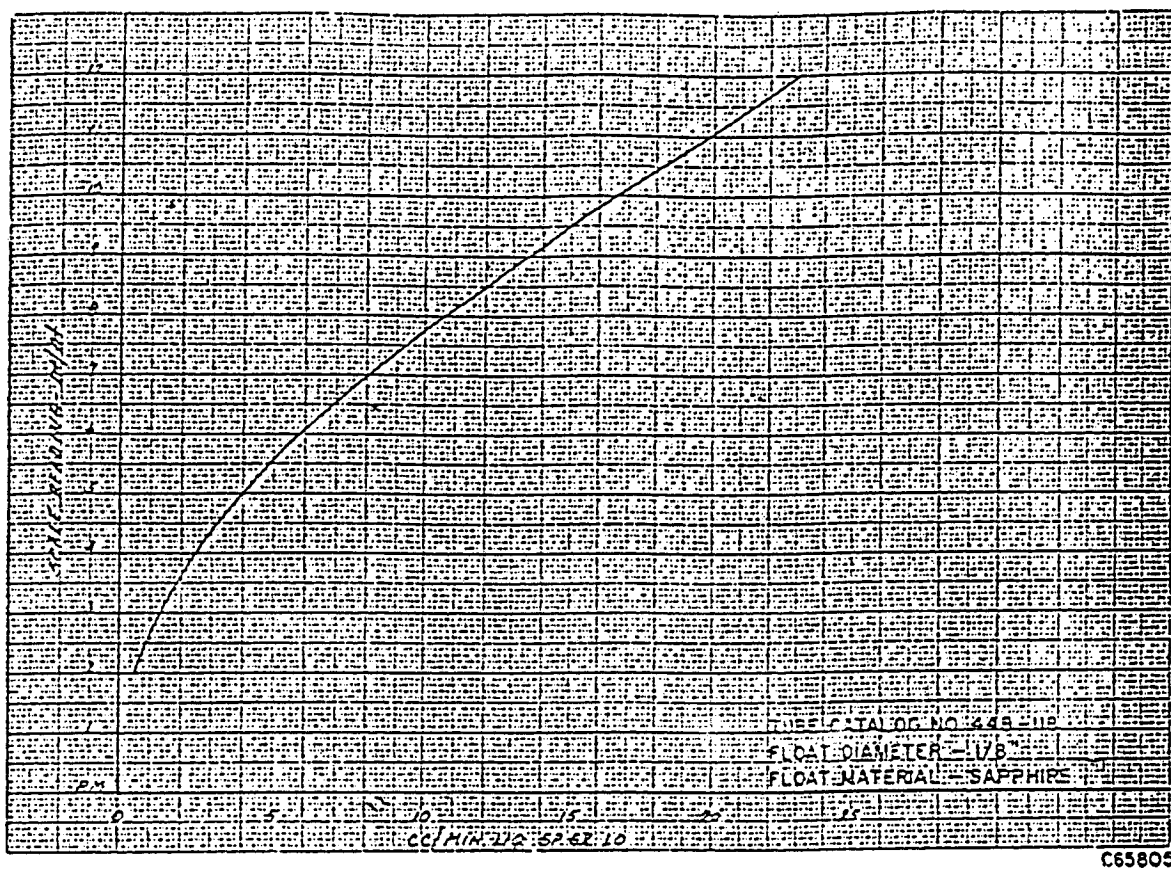


Figure E.1: Fisher Scientific Lab-Crest Mark III Flow Meter Calibration Curve For Liquid Flow Rates With a 0.32 cm ($\frac{1}{8}$ in.) Sapphire Float (from Instruction Manual)

Example for Run 2 (4 L/d):

According to the calibration curve (Figure E.1), if a 2 L/d (1.39 mL liquid/min) water input was desired, the meter was set and ideally maintained to scale reading 3.

However, for the wet tip meters (Section 3.2.1.1e), a reasonable flow meter scale setting was chosen and their calibration airflow rate was determined using the appropriate Fisher Scientific Lab-Crest Mark III flow meter calibration curve, for a 0.32 cm ($\frac{1}{8}$ in.) stainless steel float (not presented).

APPENDIX F: AVERAGE PHOSPHORUS CALIBRATION CURVE

The total and dissolved phosphorus concentrations contained in the 1 000 mg COD_t/L Iron Fortified Enfalac™ feed mixture (725 mg Enfalac™/L), prior to chemical nutrient addition [glutamic acid (N) and di-potassium hydrogen orthophosphate (P)], were determined from Figure F.1. In 3 instances [March 19, April 3 and June 21 (aqueous phenolphthalein indicator)], spectrophotometric absorbance measurements (results not presented) were repeated on the same series of fresh standard dilutions. The results were averaged (Table F.1) and used to create the spectrophotometer's average phosphate calibration curve.

Table F.1: Average Spectronic 70 Phosphate Calibration Curve Absorbance Measurements

Standard Dilutions (mg PO ₄ ³⁻ -P/L)	Average Absorbance	Transmittance (%)
0.0	0.000	100
0.1	0.043	91
0.2	0.131	74
0.4	0.237	58
0.6	0.375	42
0.8	0.481	33
1.0	0.590	26

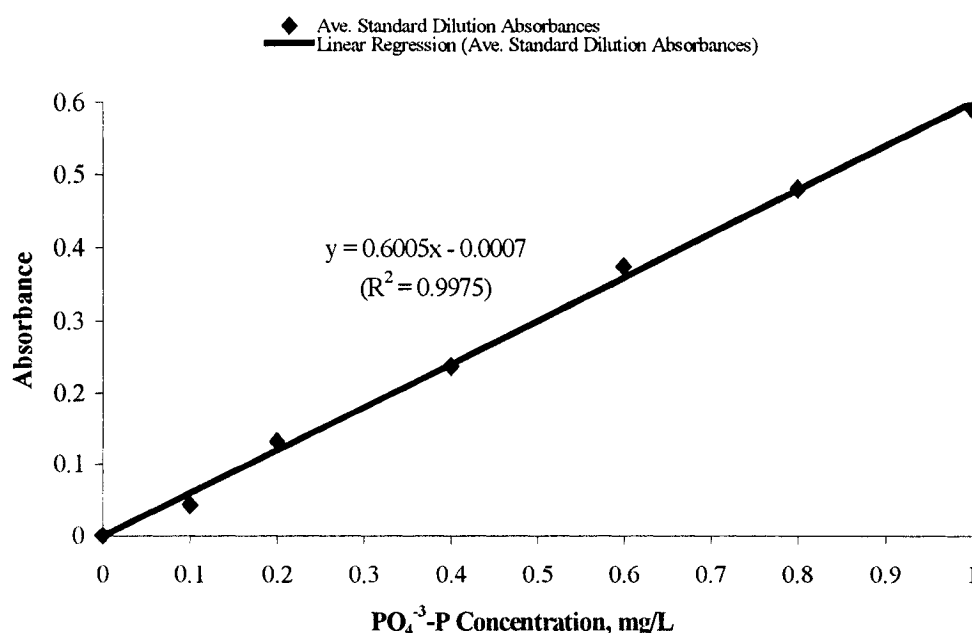


Figure F.1: Average Spectronic 70 Phosphate Calibration Curve (March-June 2000)

APPENDIX G: ORGANIC NITROGEN AND AMMONIA NITROGEN STANDARD SOLUTIONS FOR TKN ANALYSIS

Organic nitrogen standard solution with Glutamic acid:

$$\frac{20 \text{ mg N/L} \times 147.13 \text{ g C}_3\text{H}_5\text{NH}_2(\text{COOH})_2/\text{mol}}{14.007 \text{ g N/mol}} = 210.08 \text{ mg glutamic acid/L}$$

NH₃ standard solution with Ammonium Chloride:

$$\frac{20 \text{ mg NH}_4^+\text{-N/L} \times 53.49 \text{ g NH}_4\text{Cl/mol}}{14.007 \text{ g NH}_4^+\text{-N/mol}} = 76.38 \text{ mg ammonium chloride/L}$$

TKN Standard: TKN-N = Org. N + NH₄-N = 20 mg N/L + 20 mg NH₄-N/L = 40 mg TKN-N/L

Before using the digestion procedure in *Appendix C.6*, the one described in APHA (1995) was followed, but caused several digestions vials to crack. It consisted in first setting each heating unit to its medium heating power (setting 5) to boil samples and then to their maximum setting of 10, to digest the samples for 30 additional minutes. This proved to be a very lengthy process calling for several hours of digestion. To prevent bumping and cracking of vials, both 2 to 3 (8-9 mm) and 5 (3-4 mm) glass beads were tried out. In addition to the cracking vials, uneven heating from the elements caused other digestion difficulties. On the attempts where the vials broke, the digested samples were transferred to another digestion vessel by adding several distilled water rinsings.

APPENDIX H: NH₃ AND NO₃⁻ STANDARD SOLUTIONS FOR CALIBRATION CURVES

The NH₃ standards were prepared with BDH Inc. AnalaR[®] 99.8% ammonium chloride analytical reagent (NH₄Cl = 53.49 g/mol) while the NO₃⁻ standards were prepared with BDH Inc. Assurance 99.0-103.0% calcium nitrate tetrahydrate [Ca(NO₃)₂ · 4H₂O = 236.15 g/mol]. Both NH₃ and NO₃⁻ calibration curves were made from serial dilutions of 1 M standard solutions. Distilled water was added to the amount of reagents calculated below, in 100 mL volumetric flasks. These initial 1 M standard solutions were successively diluted to construct calibration curves for the 2 different ions. 10 mL samples were pipeted from the previous 100 mL distilled water-filled volumetric flasks to make second standard solutions, and so on, until the desired range of standards had been prepared. This was always repeated with fresh standards for each batch of feed samples analyzed.

1 M NH₃ standard with ammonium chloride, in 100 mL volumetric flasks:

$$53.49 \text{ g/mol NH}_4\text{Cl} \times \frac{1 \text{ mol NH}_4\text{Cl}}{1 \text{ mol NH}_4} \times \frac{1 \text{ mol NH}_4}{\text{L}} = 53.49 \text{ g NH}_4\text{Cl}/1000 \text{ mL} = 5.349 \text{ g NH}_4\text{Cl}/100 \text{ mL}$$

1 M NO₃⁻ standard with calcium nitrate, in 100 mL volumetric flasks:

$$236.15 \text{ g/mol Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O} \times \frac{1 \text{ mol Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}}{2 \text{ moles NO}_3} \times \frac{1 \text{ mol NO}_3}{\text{L}} = 118.075 \text{ g Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}/1000 \text{ mL} = 1.808 \text{ g Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}/100 \text{ mL}$$

APPENDIX I: AMMONIA ELECTRODE CALIBRATION CURVES

Appendix I.1: *Direct Calibration Method*

Although more points were measured, only 2 standards, differing in concentration by 10-fold, are needed to bracket unknown sample concentrations in the linear response range of the electrode. For the *Low-Level* measurement procedure ($< 4 \times 10^{-6}$ M, 0.07 ppm NH_3 or 0.06 ppm as N), a few points were also measured in the non-linear regions. For both *Low-Level* and *Direct Calibration* procedures, new calibration curves were prepared with fresh standards, for repeat feed sample analysis, from 1 000 mg COD_L feed batches, without nutrient supplements. An example of a typical NH_3 *Direct Measurement* calibration curve is given below.

Table I.1: NH_3 Electrode Potential Measurements of Standards by *Direct Calibration* (June 8)

Molarity (M)	Electrode Potential Reading (mV)
10^{-2}	-22.1
10^{-3}	40.6
10^{-4}	98.8
10^{-5}	133.4
10^{-6}	139.2
10^{-7}	140.2
10^{-8}	140.6

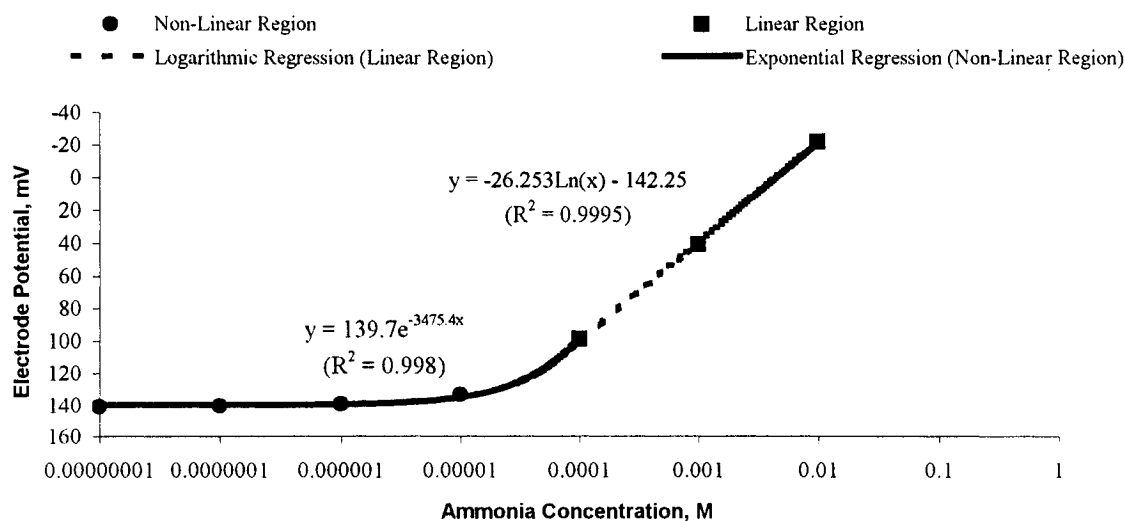


Figure I.1: Typical *Direct Calibration* Ammonia Electrode Calibration Curve (June 8)

Appendix I.2: Low-Level Measurement Method

Increments of a 10^{-3} M calibration solution were added as follow to a 125 mL distilled water-filled Erlenmeyer flask with (2 mL) ISA in order to construct a *Low-Level* calibration curve (Figure I.2). The solution, similar in composition to low concentration samples, was prepared by serial dilutions from a 0.1 M NH_4Cl standard (*Appendix H*). The electrode potentials (mV) were measured after each solution increment addition and the feed sample concentration determined from the resulting curve, similarly to the *Direct Calibration Technique*.

Table I.2: Additions of 10^{-3} M Standard for *Low-Level* Ammonia Measurement Calibration Curve (July 9)

Step	Graduated Pipet Size (mL)	Volume of 10^{-3} M Solution Added (mL)	Molarity (M)	Electrode Potential Reading (mV)
1.	1	0.10	9.8×10^{-7}	133.6
2.	1	0.10	2.0×10^{-6}	131.5
3.	1	0.20	3.9×10^{-6}	127.7
4.	1	0.20	5.8×10^{-6}	125.2
5.	1	0.40	9.7×10^{-6}	121.0
6.	2	2.00	2.9×10^{-5}	111.8
7.	2	2.00	4.7×10^{-5}	105.1

Table 2 from the *Ammonia Electrode Instruction Manual* (p. 14)

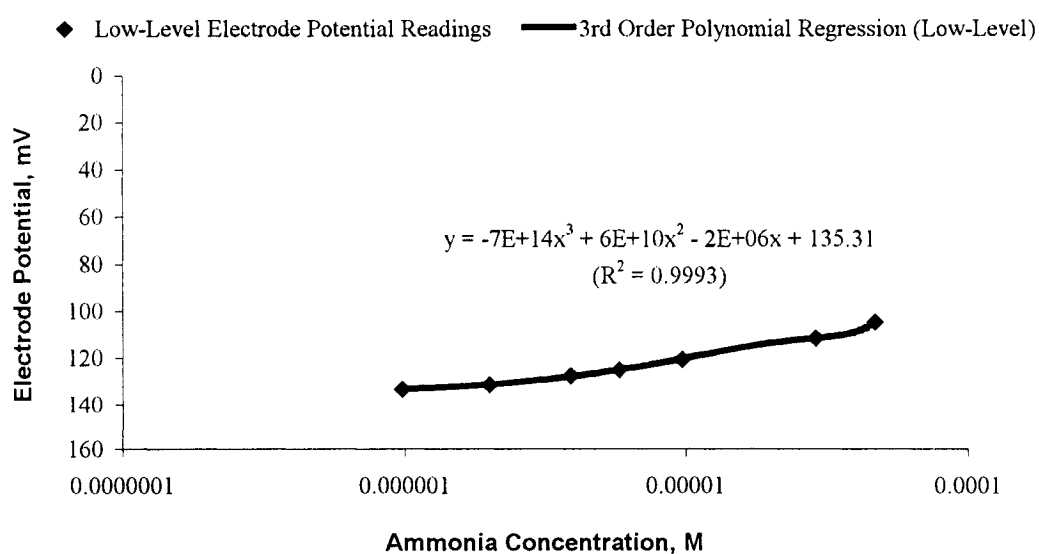


Figure I.2: Typical *Low-Level* Ammonia Electrode Calibration Curve (July 9)

APPENDIX J: NITRATE ELECTRODE CALIBRATION CURVES

Appendix J.1: Direct Measurement Procedure

Only 2 standards, differing in concentration by 10-fold, are also sufficient to bracket unknown sample concentrations in the NO_3^- electrode's linear response range (greater than 10^{-4} M NO_3^- , 6.2 ppm NO_3^- or 1.4 ppm as N). More points were also measured for more accuracy. At low concentrations (*Low-Level* measurements), multi-point calibration was also used to compensate for the electrode's non-linearity. As for the NH_3 electrode, with each sample analyzed from Enfalac™ feed batch mixtures (without nutrient supplements), new calibration curves were prepared with fresh standards, for both *Low-Level* and *Direct Measurement* procedures. An example of a typical NO_3^- *Direct Measurement* calibration curve is given below.

Table J.1: NO_3^- Electrode Potential Measurements of Standards by *Direct Calibration* (July 7)

Molarity (M)	Electrode Potential Reading (mV)
10^{-1} M	28.7
10^{-2} M	82.9
10^{-3} M	132.4
10^{-4} M	184.4
10^{-5} M	219.5
10^{-6} M	225.8

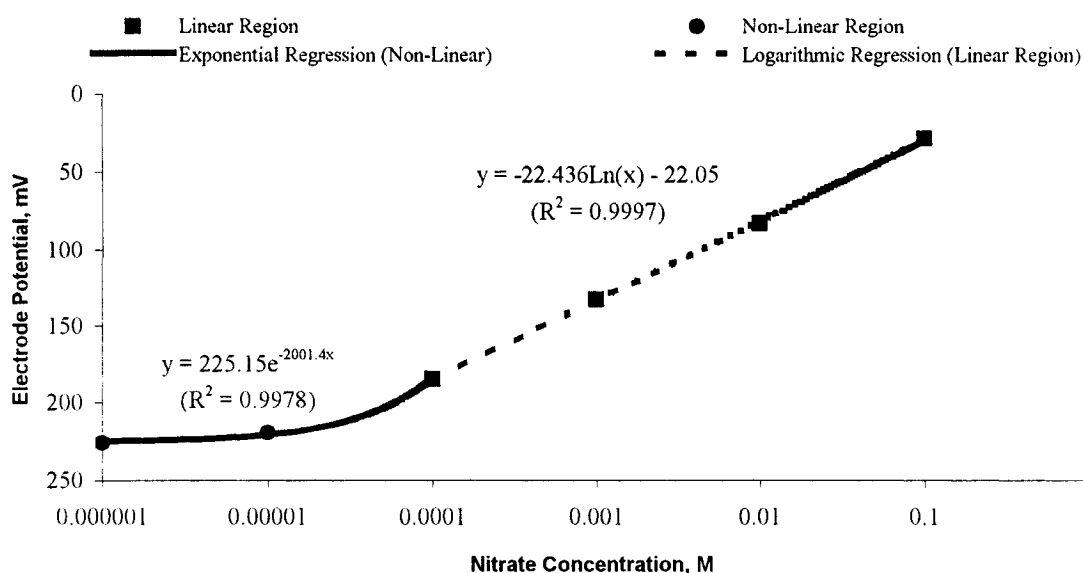


Figure J.1: Typical *Direct Measurement* Nitrate Electrode Calibration Curve (July 7)

1 M Boric Acid Preservative Solution (Appendix C.8):

This solution was prepared by dissolving 6.2 g of boric acid in 100 mL of boiling water and letting the solution cool down.

Appendix J.2: Low-Level Measurements

Increments of a 10^{-3} M standard solution, made by serial dilutions from a 0.1 M nitrate standard (*Appendix H*), were added as follow to a 150 mL distilled water-filled beaker, with Low-Level ISA, in order to construct a *Low-Level* calibration curve (Figure J.2). The electrode potentials (mV) were measured after each increment addition and the sample concentrations determined from the resulting curve, similarly to the *Direct Measurement Technique*, but via this calibration solution, similar in composition to low concentration samples.

Table J.2: Additions of 10^{-3} M Standard for *Low-Level* Nitrate Measurement Calibration Curve (July 7)

Step	Graduated Pipet Size (mL)	Volume of 10^{-3} M Solution Added (mL)	Molarity (M)	Electrode Potential Reading (mV)
1.	1	0.10	10^{-6}	242.9
2.	1	0.10	2.0×10^{-6}	241.6
3.	1	0.20	3.9×10^{-6}	238.8
4.	1	0.20	5.9×10^{-6}	235.9
5.	1	0.40	9.8×10^{-6}	230.5
6.	2	2.00	2.9×10^{-5}	218.9
7.	2	2.00	4.7×10^{-5}	210.9

Table 2 from the Nitrate Electrode Instruction Manual (p. 33)

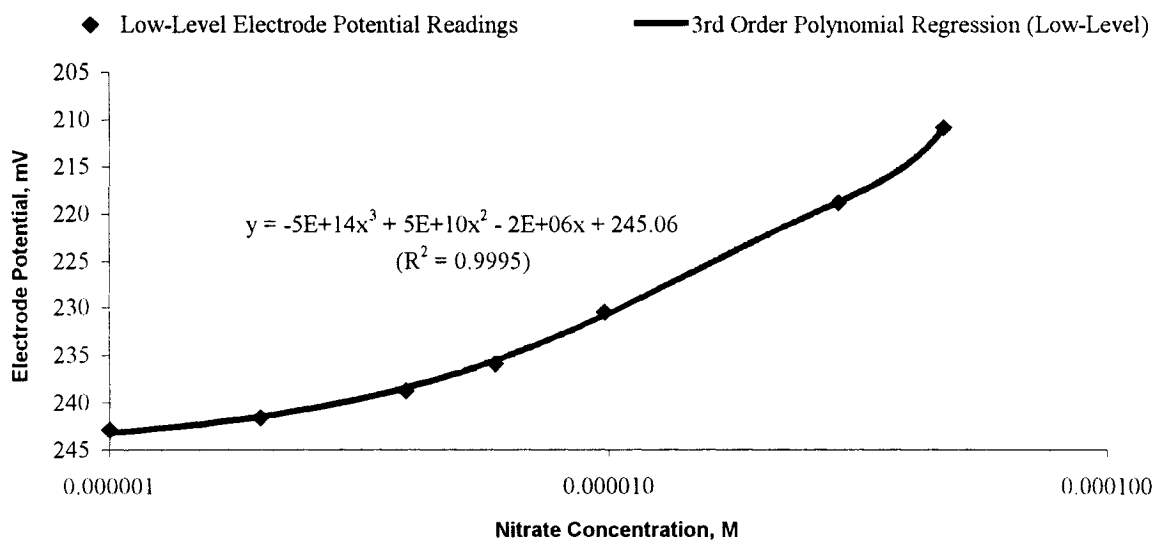


Figure J.2: Typical *Low-Level* Nitrate Electrode Calibration Curve (July 7)

Low-Level ISA Solution (Appendix C.8):

A Low-Level ISA solution, to be used for Low-Level nitrate measurements, was prepared by diluting 20 mL of Nitrate ISA in 100 mL of distilled water.

APPENDIX K: SOLUTIONS FOR ATA AND AAT TESTS

The following solution preparation procedures are not found in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition (APHA, 1995). The ones for the Defined Growth Medium, the Stock Solutions used in the Defined Growth Medium, the Acetate Stock Solutions, the Phosphate Buffer and the Resazurin as well as the Cysteine Na_2S solutions were taken from the *Anaerobic Methods manual* (Kennedy, 1999). Distilled water was substituted for glass-distilled water in all these solutions.

Defined Growth Medium

Stock solutions were combined in the proportions listed below. The resulting solution was boiled for 5 minutes in a large Erlenmeyer flask; 3.4 g of NaHCO_3 was added and it was cooled to room temperature. It was transferred to a clean Wheaton bottle, topped to 1 L with distilled water and flushed with N_2 . The lid was sealed with Parafilm[®] and the bottle stored in a refrigerator.

Table K.1: Defined Growth Medium Solutions

Stock Solution	Volume (mL)
Distilled water	900
Mineral I	10
Mineral II	1
Vitamin B	1
Phosphate	10
Resazurin	15
2-methyl-n-butyric acid	1

(Table K.2, gives the breakdown of components contained in these stock solutions. The refrigerated stock solutions used to prepare the medium were those left behind by Mrs. Catherine Savard, another graduate student.)

Table K.2: Stock Solutions Used in the Growth Medium

Stock Solution	Components	Concentration in distilled water (g/L)
Mineral I ⁽¹⁾	NaCl	50
	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	10
	NH_4Cl	189.4
	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	10
Mineral II	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	10
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.1
	H_3BO_3	0.3
	$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	1.5
	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	10
	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.03
	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.03
	$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	0.1
Vitamin B	Nicotinic acid	0.1
	Cyanocobalamine	0.1
	Thiamine	0.05
	p-aminobenzoic acid	0.05
	Pyridoxine	0.25
	Pantothenic acid	0.025
Phosphate	KH_2PO_4	50
Resazurin	(Following page)	0.1
2-methyl-n-butyric acid ⁽²⁾		102

⁽¹⁾ Dissolved in 0.01M HCl rather than distilled water.

⁽²⁾ Adjusted to pH 6.5.

Acetate Stock Solutions:***Solution A:***

12.8 g of ammonium acetate and 16.4 g of potassium acetate put in 100 mL of distilled water.

Solution B:

18.3 g of sodium acetate and 6.7 g of glacial acetic acid put in 100 mL of distilled water.

Phosphate Buffer (pH = 7.5)

(Not made by the student, but borrowed from Mrs. Tham Pham, graduate student, for AAT test controls.)

13.9 g of monobasic sodium phosphate and 26.825 g of dibasic sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) were dissolved in 500 mL Milli-Q water. 0.1 mL of Resazurin was added to the solution, transferred in a round bottom flask with boiling beads. It was brought to a boil, while flushing with N_2 . Flushing was continued for 15 min, once heat was turned off, and 5 mL of Cysteine Na_2S was added at this time. Flushing was continued until the solution was clear and colorless. Another 1 mL of Cysteine Na_2S was added. It was then anaerobically dispensed in a pre-flushed serum bottle. The bottle containing the buffer was finally capped, sealed and refrigerated.

Resazurin and Cysteine Na_2S solutions:***1 g/L Resazurin (for the Phosphate Buffer and diluted 1:10 for the Defined Growth Medium)***

0.1 g of Resazurin was dissolved in 100 mL of distilled water. The solution was capped, sealed and refrigerated in foil-wrapped serum bottles.

Cysteine Na_2S

2.5 g of cysteine HCl were dissolved in 50 mL of distilled water and flushed with N_2 for 15 min. The pH is to be adjusted to 10 with freshly prepared 3 N NaOH. 2.5 g of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ were then added, but these crystals must be white and clear. The solution was diluted up to 200 mL and transferred to a 500 mL round bottomed flask with boiling beads. It was brought to a boil, while flushing with N_2 . Once boiling and the heat turned off, flushing was continued for another 30 sec. While cooling, flushing of the headspace continued. The solution was then anaerobically transferred in pre-flushed 160 mL serum bottles. The bottles containing the final colorless Cysteine solution were finally capped, sealed and refrigerated.

APPENDIX L.1: ATA DATA

TABLE L.1 a): ATA Biogas Volume Readings (June 4-July 6, 2000)

Date	Gas Reading	Time (h)	Time (d)	Test Bottles	Initial (mL)	Final (mL)	Biogas Volume (mL)	Average (mL)	Cumulative Gas Production (mL)
June 4 th	11:00 PM	t = 0	0	Control - 1	30.80	34.30	3.50	4.30	4.30
				Control - 2	31.00	36.10	5.10		
				250 mg/L - 1	31.60	35.00	3.40	4.00	4.00
				250 mg/L - 2	31.60	36.20	4.60		
				500 mg/L - 1	31.80	35.40	3.60	3.35	3.35
				500 mg/L - 2	31.80	34.90	3.10		
				1 000 mg/L - 1	31.80	35.70	3.90	3.25	3.25
				1 000 mg/L - 2	31.80	34.40	2.60		
	30 secs	0.00003	0	Control - 1	30.80	80.60	48.40	48.90	53.20
				Control - 2	31.00	81.60	49.40		
				250 mg/L - 1	31.60	100.00	67.80	67.80	71.80
				250 mg/L - 2	31.60	100.00	67.80		
				500 mg/L - 1	31.80	98.40	66.10	60.90	64.25
				500 mg/L - 2	31.80	88.00	55.70		
				1 000 mg/L - 1	31.80	79.20	46.90	40.60	43.85
				1 000 mg/L - 2	31.80	66.60	34.30		
	1 min	0.0007	0	Control - 1	31.90	74.40	42.50	42.45	95.65
				Control - 2	31.90	74.30	42.40		
				250 mg/L - 1	31.90	76.80	44.90	45.90	117.70
				250 mg/L - 2	32.00	78.90	46.90		
				500 mg/L - 1	32.00	77.90	45.90	45.90	110.15
				500 mg/L - 2	32.00	NA			
				1 000 mg/L - 1	32.00	79.60	47.60	46.50	90.35
				1 000 mg/L - 2	32.00	77.40	45.40		
June 5 th	11:00 PM	24	1.00	Control - 1	32.00	36.40	4.40	4.15	98.80
				Control - 2	32.00	35.90	3.90		
				250 mg/L - 1	32.00	39.20	7.20	7.30	125.00
				250 mg/L - 2	32.00	39.40	7.40		
				500 mg/L - 1	32.00	39.00	7.00	6.00	116.15
				500 mg/L - 2	32.00	37.00	5.00		
				1 000 mg/L - 1	32.00	44.00	12.00	12.00	102.35
				1 000 mg/L - 2	32.00	44.00	12.00		
	6:30 PM	43.5	1.81	Control - 1	32.00	34.20	2.20	2.40	102.20
				Control - 2	32.00	34.60	2.60		
				250 mg/L - 1	32.00	NA		2.00	127.00
				250 mg/L - 2	32.00	34.00	2.00		
				500 mg/L - 1	32.00	36.20	4.20	4.18	120.33
				500 mg/L - 2	32.05	36.20	4.15		
				1 000 mg/L - 1	32.00	38.30	6.30	5.73	108.08
				1 000 mg/L - 2	32.05	37.20	5.15		
	6:30 PM	87.5	2.81	Control - 1	32.05	33.70	1.65	1.80	104.00
				Control - 2	32.05	34.00	1.95		
				250 mg/L - 1	32.05	34.70	2.65	2.20	129.20
				250 mg/L - 2	32.05	33.80	1.75		
				500 mg/L - 1	32.05	33.60	1.55	2.10	122.43
				500 mg/L - 2	32.05	34.70	2.65		
				1 000 mg/L - 1	32.05	36.20	4.15	4.65	112.73
				1 000 mg/L - 2	32.05	37.20	5.15		
	6:30 PM	91.5	3.81	Control - 1	32.10	33.00	0.90	0.80	104.80
				Control - 2	32.10	32.80	0.70		
				250 mg/L - 1	32.10	32.85	0.75	0.93	130.13
				250 mg/L - 2	32.10	33.20	1.10		
				500 mg/L - 1	32.10	34.60	2.50	2.15	124.58
				500 mg/L - 2	32.10	33.90	1.80		
				1 000 mg/L - 1	32.10	36.00	3.90	3.60	116.33
				1 000 mg/L - 2	32.10	35.40	3.30		
June 6 th	6:30 PM	115.5	4.81	Control - 1	32.10	33.00	0.90	0.80	104.80
				Control - 2	32.10	32.80	0.70		
				250 mg/L - 1	32.10	32.85	0.75	0.93	130.13
				250 mg/L - 2	32.10	33.20	1.10		
				500 mg/L - 1	32.10	34.60	2.50	2.15	124.58
				500 mg/L - 2	32.10	33.90	1.80		
				1 000 mg/L - 1	32.10	36.00	3.90	3.60	116.33
				1 000 mg/L - 2	32.10	35.40	3.30		

APPENDIX L.1: ATA DATA

(Continued) TABLE L.1 a): ATA Biogas Volume Readings (June 4-July 6, 2000)

Date	Gas Reading	Time (h)	Time (d)	Test Bottles	Initial (mL)	Final (mL)	Biogas Volume (mL)	Average (mL)	Cumulative Gas Production (mL)
June 12 th	8:00 PM	189	7.88	Control - 1	32.20	35.40	3.20	3.70	108.60
				Control - 2	32.20	36.40	4.20		
				250 mg/L - 1	32.20	36.50	4.30	3.55	133.88
				250 mg/L - 2	32.20	35.00	2.80		
				500 mg/L - 1	32.20	41.00	8.80	7.30	131.88
				500 mg/L - 2	32.20	38.00	5.80		
				1 000 mg/L - 1	32.20	33.90	1.70	1.50	117.83
				1 000 mg/L - 2	32.20	33.50	1.30		
June 14 th	9:30 PM	238.5	9.84	Control - 1	32.20	35.40	3.20	3.00	111.50
				Control - 2	32.20	35.00	2.80		
				250 mg/L - 1	32.20	36.20	4.00	3.90	137.58
				250 mg/L - 2	32.20	36.00	3.80		
				500 mg/L - 1	32.20	36.40	4.20	3.15	135.03
				500 mg/L - 2	32.20	34.30	2.10		
				1 000 mg/L - 1	32.20	38.10	5.90	5.50	123.33
				1 000 mg/L - 2	32.20	37.30	5.10		
June 17 th	7:30 PM	308.5	12.85	Control - 1	32.30	34.20	1.90	3.60	116.10
				Control - 2	32.30	37.60	5.30		
				250 mg/L - 1	32.30	40.00	7.70	7.20	144.78
				250 mg/L - 2	32.30	39.00	6.70		
				500 mg/L - 1	32.30	39.20	6.90	7.40	142.43
				500 mg/L - 2	32.30	40.20	7.90		
				1 000 mg/L - 1	32.30	39.70	7.40	6.95	130.28
				1 000 mg/L - 2	32.30	38.80	6.50		
June 19 th	6:30 PM	355.5	14.81	Control - 1	32.30	35.30	3.00	4.10	118.20
				Control - 2	32.30	37.50	5.20		
				250 mg/L - 1	32.30	36.60	4.30	4.75	148.53
				250 mg/L - 2	32.40	37.60	5.20		
				500 mg/L - 1	32.40	37.00	4.60	4.60	147.03
				500 mg/L - 2	32.40	37.00	4.60		
				1 000 mg/L - 1	32.30	36.90	4.60	5.55	135.83
				1 000 mg/L - 2	32.30	38.80	6.50		
June 22 th	6:30 p.m.	428.5	17.77	Control - 1	32.40	38.70	6.30	6.60	125.80
				Control - 2	32.40	39.30	6.90		
				250 mg/L - 1	32.40	40.50	8.10	7.40	156.93
				250 mg/L - 2	32.40	39.10	6.70		
				500 mg/L - 1	32.40	39.20	6.80	6.90	153.93
				500 mg/L - 2	32.40	39.40	7.00		
				1 000 mg/L - 1	32.40	38.90	6.50	7.15	142.98
				1 000 mg/L - 2	32.40	40.20	7.80		
June 29 th	6:40 PM	597.67	24.90	Control - 1	32.60	41.90	9.30	8.85	134.65
				Control - 2	32.60	41.00	8.40		
				250 mg/L - 1	32.60	43.80	11.20	11.20	168.13
				250 mg/L - 2	32.60	43.80	11.20		
				500 mg/L - 1	32.60	42.80	10.20	10.40	164.33
				500 mg/L - 2	32.60	43.20	10.60		
				1 000 mg/L - 1	32.60	42.30	9.70	10.65	153.63
				1 000 mg/L - 2	32.60	44.20	11.60		
July 6 th	4:40 PM	763.67	31.82	Control - 1	33.60	44.00	10.40	10.30	144.95
				Control - 2	33.60	43.80	10.20		
				250 mg/L - 1	33.60	42.60	9.00	8.70	176.83
				250 mg/L - 2	33.60	42.00	8.40		
				500 mg/L - 1	33.60	41.90	8.30	7.80	172.13
				500 mg/L - 2	33.60	40.90	7.30		
				1 000 mg/L - 1	33.60	41.00	7.40	8.70	162.33
				1 000 mg/L - 2	33.60	43.60	10.00		

APPENDIX L.1: ATA DATA

TABLE L.1 b): ATA Biogas Production

Elapsed Time (h) (d)		Cumulative Biogas Production (mL)			
		Ave. Control (0% Feed)	Ave. 250 mg COD _L /L (8.3% Feed)	Ave. 500 mg COD _L /L (16.7% Feed)	Ave. 1 000 mg COD _L /L (33.3% Feed)
0.0	0.00	0	0	0	0
24.0	1.00	95.65	117.70	110.15	90.35
43.5	1.81	99.80	125.00	116.15	102.35
67.5	2.81	102.20	127.00	120.33	108.08
91.5	3.81	104.00	129.20	122.43	112.73
115.5	4.81	104.80	130.13	124.58	116.33
189.0	7.88	108.50	133.68	131.88	117.83
238.5	9.94	111.50	137.58	135.03	123.33
308.5	12.85	115.10	144.78	142.43	130.28
355.5	14.81	119.20	149.53	147.03	135.83
426.5	17.77	125.80	156.93	153.93	142.98
597.67	24.90	134.65	168.13	164.33	153.63
763.67	31.82	144.95	176.83	172.13	162.33
Δ Days		Daily Biogas Production (mL/d)			
0.00	0	0.00	0.00	0.00	0.00
1.00	1	95.65	117.70	110.15	90.35
0.81	1	5.12	9.01	7.41	14.81
1.00	1	2.40	2.00	4.18	5.73
1.00	1	1.80	2.20	2.10	4.65
1.00	1	0.80	0.93	2.15	3.60
3.07	3	1.21	1.16	2.38	0.49
2.06	2	1.46	1.89	1.53	2.67
2.91	3	1.24	2.47	2.54	2.39
1.96	2	2.09	2.42	2.35	2.83
2.96	3	2.23	2.50	2.33	2.42
7.13	7	1.24	1.57	1.46	1.49
6.92	7	1.49	1.26	1.13	1.26

APPENDIX L.2: pH, ALKALINITY, AB TEMPERATURE AND DO MEASUREMENTS

TABLE L.2 a i): Feed-AD1-AB1-B1 pH, Alk, AB Temperature and DO Results During Start-Up (Days 1-17)

Date	DAY	Feed and Alk. Added	NaHCO ₃ (g/L)	Feed _{PH}	Feed _{ALK} (mg/L as CaCO ₃)	AD1 Effl. PH	AD1 Effl. ALK (mg/L as CaCO ₃)	AB1 PH	AB1 ALK (mg/L as CaCO ₃)	AB1 DO (mg O ₂ /L)	AB1 Temp. (°C)	B1 PH	B1 ALK (mg/L as CaCO ₃)
24-Jul		1000 mg COD/L FEED, supplemented with P & N		6.7	30								
24-Jul	1	24L-100 mg COD/L FEED, suppl. with P & N		7.2	28	8.0		8.0		NA	18.8		
25-Jul	2	18L-100 mg COD/L FEED				7.7		8.8		8.5	18.8		
26-Jul	3	20L-200 mg COD/L FEED				8.2							
27-Jul	4	17L-200 mg COD/L FEED				7.7				8.0	20.1		
28-Jul	5	OFF (~200 mg COD/L)		6.7		7.7							
29-Jul	6	ON (~20L/400 mg COD/L)				7.5							
30-Jul	7	19L-400 mg COD/L FEED				7.5	287						
31-Jul	8	19.5L-600 mg COD/L FEED				7.2				7.8	21.1		
1-Aug	9	NO ALK/-600 mg COD/L		6.2	28	7.1	230						
	9	-800 mg COD/L (6g NaHCO ₃ /15L)		7.2	233								
	9	-600 mg/L (16g NaHCO ₃ /15L)		7.7	599								
	9	-600 mg COD/L (Total of 20g NaHCO ₃ /15L)	1.3	7.8	753								
2-Aug	10	-800 mg COD/L (10g ALK/5L)	2.0	7.7	765	7.3	417			8.2	21.0		
3-Aug	11	-800 mg COD/L		7.8	1120	7.4	497						
7-Aug	15	-800 mg COD/L (10g ALK/5L)	2.0										
8-Aug	16	-1000 mg COD/L (35g ALK/16L)	2.2	7.8	1087	7.8	687						
9-Aug	17	-1000 mg COD/L		7.8	1275	7.8	1088						

FEED DAYS : ~9 Days
START-UP : 17 Days

AVERAGE: 1.9
STD: -

8.1 20.0
0.3 1.1

TABLE L.2 b i): Feed-AD1-AB1-CL1-B1 pH, Alk, DO and AB Temperature Results During Run 1 (Days 18-61)

Date	DAY	Feed and Alk. Added	NaHCO ₃ (g/L)	Feed _{PH}	Feed _{ALK} (mg/L as CaCO ₃)	AD1 Effl. PH	AD1 Effl. ALK (mg/L as CaCO ₃)	AB1 PH	AB1 ALK (mg/L as CaCO ₃)	AB1 DO (mg O ₂ /L)	AB1 Temp. (°C)	B1 PH	B1 ALK (mg/L as CaCO ₃)
10-Aug	18	40g ALK/20L (feed = 24L)	2.0	7.4	1158					5.4	20.9		
16-Aug	24	16L old feed/40g ALK		8.1	543	7.9	1268	9.0		7.9	19.4		
17-Aug	25			8.0		7.9							
18-Aug	26			6.0	30	8.1	1155	8.9	1235	9.0	18.8		
20-Aug	28	NO ALK/15L Fresh Feed											
21-Aug	29	NO ALK/15L Fresh Feed (feed ~17L)											
23-Aug	31			4.0	0	8.1	720	8.8		NA	18.5	8.8	870
24-Aug	32					7.7		8.6	620	8.5	18.8	8.6	671
25-Aug	33	NO ALK/15L Fresh Feed		5.8	13	7.8	585			8.0	21.0		
26-Aug	34	NO ALK/15L Fresh Feed (BLENDER)				7.7		8.5	500	7.8	21.3	8.4	475
28-Aug	36	Day old feed (precipitates)		4.2	0	7.4	440						
29-Aug	37	NO ALK/20 L Fresh Feed (MIXER)											
31-Aug	39												
1-Sep	40	(NO ALK/5 Day old feed)		5.5	20	7.8	227	8.1	215	8.2	21.8	8.1	240
3-Sep	42	NO ALK/20 L Fresh Feed				7.7				7.6	22.4		
5-Sep	44												
6-Sep	45												
8-Sep	47												
9-Sep	48												
10-Sep	49	NO ALK/20 L Fresh Feed				7.4				7.7	20.9		
11-Sep	50												
12-Sep	51	(NO ALK/2 Day old feed)		3.5	0	7.8	133	7.9	65	7.0	21.9	7.7	82
22-Sep	61									7.1	21.1		

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

FEED DAYS : ~24 Days
RUN 1 : 44 Days

AVERAGE
STD

NA 4.8 11 11 13 121 7.7 421 8.5 445 8.0 20.6 8.5 564
NA 1.1 13 22 121 0.2 244 0.3 208 0.3 1.3 0.3 270
STD of Ave 1.7 3 3 3 58 7 4 4 48

APPENDIX L.2: pH, ALKALINITY, AB TEMPERATURE AND DO MEASUREMENTS

TABLE L.2 c i): Feed-AD1-AB1-B1 pH, Alk, AB Temperature and DO Results During Run 2 (Days 62-113)

Date	DAY	Feed and Alk. Added	NaHCO ₃ (g/L)	Feed _{pH} (mg/L as CaCO ₃)	Feed _{ALK} (mg/L as CaCO ₃)	AD1 EM _{pH} (mg/L as CaCO ₃)	AD1 EM _{ALK} (mg/L as CaCO ₃)	AB1 _{pH} (mg/L as CaCO ₃)	AB1 _{ALK} (mg/L as CaCO ₃)	AB1 _{DO} (mg O ₂ /L)	AB1 Temp. (°C)	B1 _{pH}	B1 _{ALK} (mg/L as CaCO ₃)
23-Sep	62	20g Alk./20L Fresh Feed	1.0	7.5	563	6.7	440	7.5	98	NA	20.1	6.9	63
24-Sep	63			7.6	520	7.6	585			8.1	20.0		
26-Sep	65	NO Alk./20L Fresh Feed								8.5	20.8		
27-Sep	66			4.0	0	6.9	250						
29-Sep	68			6.4	27								
30-Sep	69	(26L Fresh Feed, before ALK. Added) After 30g Alk. added/26L Fresh Feed	1.2	7.4	670	6.6	480			7.7	21.3		
1-Oct	70			6.8	515	7.5	671			7.6	21.5		
3-Oct	72	(3 Day old feed/ 30g Alk.)		7.9	628								
72	72	10g Alk./26L Fresh Feed	0.4	7.0	NA	6.9	464			8.0	19.9		
4-Oct	73					7.3	300						
6-Oct	75	(3 Day old feed/10g Alk.)		7.1	210	7.4	520			8.4	19.8		
75	75	20g Alk./26L Fresh Feed	0.8	7.6	463					8.1	20.5		
9-Oct	78					7.6	357						
10-Oct	79	10g Alk./26L Fresh Feed	0.4	6.4	167								
11-Oct	80					7.1	445						
13-Oct	82	20g Alk./26L Fresh Feed	0.8	7.4	417								
15-Oct	84					7.4	570			8.6	19.7		
16-Oct	85	20g Alk./26L Fresh Feed	0.8	6.8	390	7.4	570						
17-Oct	86			7.8	425	7.5	540			8.3	19.8		
19-Oct	88	10g Alk./26L Fresh Feed	0.4	7.5	217	7.6	365						
21-Oct	90												
22-Oct	91	30 g Alk./26 L Fresh Feed	1.2										
23-Oct	92			7.7	653	7.6	653			8.6	19.9		
24-Oct	93	15g Alk./26 L Fresh Feed	0.6										
25-Oct	94	(Day old feed, 15g Alk./26L)		7.1	260	7.4	523			8.0	20.7		
26-Oct	95	30g Alk./26L Fresh Feed	1.2										
27-Oct	96			7.6	650	7.6	721	7.8	614	7.9	20.3	7.7	552
29-Oct	98	10g Alk./26L Fresh Feed	0.4										
30-Oct	99			6.9	177	7.1	350						
1-Nov	101	20g Alk./26L fresh feed	0.8										
102	102	(3 Day old feed/20g Alk.)		7.9	444	7.2	526			8.2	20.0		
5-Nov	105	20g Alk./26L Fresh feed	0.8	7.7	460								
106	106												
7-Nov	107	(3 Day old feed/20g Alk.)		7.7	427	7.3	564						
8-Nov	108	10g Alk./26L Fresh Feed	0.4	7.1									
9-Nov	109												
13-Nov	113	(5 Day old)											

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

FEED DAYS : ~36 Days
RUN 2 : 52 Days

AVERAGE: 0.7
STD: -
STD of Ave. (%): 5

7.7
NA
20.3
0.6
3

8.2
0.3
4

614
NA
7.8
NA

505
125
25

7.4
0.2
3

436
165
38

7.4
0.4
5

7.7
NA
20.3
0.6
3

552
NA

APPENDIX L.2: pH, ALKALINITY, AB TEMPERATURE AND DO MEASUREMENTS

TABLE L.2 a (i): Feed-AD2-AB2-B2 pH, Alk, AB Temperature and DO During Start-Up (Days 1-17)

Date	DAY	ALK./Feed added	NaHCO ₃ (g/L)	Feed _{pH}	Feed _{ALK} (mg/L as CaCO ₃)	AD2 Effl _{pH}	AD2 Effl _{ALK} (mg/L as CaCO ₃)	AB2 _{pH}	AB2 _{ALK} (mg/L as CaCO ₃)	AB2 _{DO} (mg O ₂ /L)	AB2 Temp. (°C)	B2 _{pH}	B2 _{ALK} (mg/L as CaCO ₃)
24-Jul	1	1000 mg COD/L FEED, supplemented with P & N 24L/-100 mg COD/L FEED, suppl. with P & N		6.7	30								
25-Jul	2	18L/-100 mg COD/L FEED		7.2	28	8.0		8.3		NA	18.5		
26-Jul	3	20L/-200 mg COD/L FEED				7.8		8.8		8.5	18.9		
27-Jul	4	17L/-200 mg COD/L FEED				7.8							
28-Jul	5	OFF (-200 mg COD/L)		6.7		7.3				8.2	18.9		
29-Jul	6	ON (-20L/400 mg COD/L)				7.0							
30-Jul	7	19L/-400 mg COD/L FEED				7.1							
31-Jul	8	19.5L/-600 mg COD/L FEED				7.0	183						
1-Aug	9	NO ALK./-600 mg COD/L		6.2	28	6.9				7.9	20.9		
	9	NO ALK./-600 mg COD/L		7.2	233	7.0	178						
	9	-600 mg COD/L (6g NaHCO ₃ /15L)		7.7	588								
	9	-600 mg COD/L (Total of 20g NaHCO ₃ /15L)	1.3	7.8	753								
2-Aug	10	-800 mg COD/L (10g Alk./5L)	2.0	7.7	765	7.1	292			8.0	21.0		
3-Aug	11	-800 mg COD/L		7.8	1120	7.4	385						
7-Aug	15	-800 mg COD/L (10g Alk./5L)	2.0										
8-Aug	16	-1000 mg COD/L (35g Alk./16L)	2.2	7.8	1087	7.4	332						
9-Aug	17	-1000 mg COD/L		7.8	1275	7.3	500						

FEED DAYS: ~9 Days
START-UP: 17 Days

AVERAGE: 1.9
STD: -

8.2 19.6
0.3 1.2

TABLE L.2 b (i): Feed-AD2-AB2-B2 pH, Alk, AB Temperature and DO Results During Run 1 (Days 18-61)

Date	DAY	ALK./Feed added	NaHCO ₃ (g/L)	Feed _{pH}	Feed _{ALK} (mg/L as CaCO ₃)	AD2 Effl _{pH}	AD2 Effl _{ALK} (mg/L as CaCO ₃)	AB2 _{pH}	AB2 _{ALK} (mg/L as CaCO ₃)	AB2 _{DO} (mg O ₂ /L)	AB2 Temp. (°C)	B2 _{pH}	B2 _{ALK} (mg/L as CaCO ₃)
10-Aug	18	40g Alk./20L (feed = 24L)	2.0	7.4	1158					8.1	19.8		
16-Aug	24	16L old feed/40g Alk.		8.1	543	7.6	1225			8.0	18.8		
17-Aug	25			8.0		7.5		8.7					
18-Aug	26			6.0									
20-Aug	28	NO ALK./15L Fresh Feed			30								
21-Aug	29					7.7	615	8.6	649	9.2	18.2		
23-Aug	31	NO ALK./15L Fresh Feed (feed=17L)								NA	17.8		
24-Aug	32			4.0	0	7.6	325					8.4	410
25-Aug	33			5.8	13	7.8	245	8.8		8.5	18.9		
26-Aug	34	NO ALK./15L Fresh Feed				7.0		8.3	274	8.3	20.3	8.1	340
28-Aug	36	NO ALK./15L Fresh Feed (BLENDER)				6.8							
29-Aug	37	Day old feed (precipitates)		4.2	0	6.8	165	8.0	185	8.1	20.8	8.0	175
31-Aug	39	NO ALK./20 L Fresh Feed (MIXER)				7.6							
1-Sep	40									8.3	20.8		
3-Sep	42	(NO ALK./5 Day old feed)		5.5	20	7.3	100			7.9	21.9	7.8	111
5-Sep	44	NO ALK./20 L Fresh Feed				7.8		7.7	100				
6-Sep	45			3.5	0								
8-Sep	47					7.0				8.0	20.7		
9-Sep	48												
10-Sep	49	NO ALK./20 L Fresh Feed				7.7	116	7.5	35	7.2	21.3	7.4	40
11-Sep	50												
12-Sep	51	(NO ALK./2 Day old feed)				7.7				7.2	20.9		
22-Sep	61												

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

FEED DAYS: ~24 Days
RUN 1: 44 Days

AVERAGE: NA
STD: NA
STD of Ave. (%): 22

7.4 190 8.2 186 8.2 20.0 8.0 259
0.4 94 0.5 87 0.2 1.3 0.3 139
5 49 6 47 3 7 4 54

APPENDIX L.2: pH, ALKALINITY, AB TEMPERATURE AND DO MEASUREMENTS

TABLE L.2 c ii): Feed-AD2-AB2-B2 pH, Alk, AB Temperature and DO Results During Run 2 (Days 62-113)

[illegible]

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

FEED DAYS: ~36 Days
RUN 2: 52 Days

AVERAGE:
STD:
STD of Ave. (%):

7.4	436
0.4	165
5	38

7.1	447
0.3	124
5	28

7.7	540
NA	NA

20.2
0.6
3

7.7	464
NA	NA

APPENDIX L.2: pH, ALKALINITY, AB TEMPERATURE AND DO MEASUREMENTS

TABLE L.2 d i): Feed-AD1-AB1-B1 pH, Alk, AB Temperature and DO Results During Run 3 (Days 114-146)

Date	DAY	Feed and Alk. Added	NaHCO ₃ (g/L)	Feed _{PH} (mg/L as CaCO ₃)	AD1 Effl. _{PH} (mg/L as CaCO ₃)	AD1 Effl. _{ALK} (mg/L as CaCO ₃)	AB1 _{PH} (mg/L as CaCO ₃)	AB1 _{ALK} (mg/L as CaCO ₃)	AB1 _{DO} (mg O ₂ /L)	AB1 Temp. (°C)	B1 _{PH}	B1 _{ALK} (mg/L as CaCO ₃)
14-Nov	114	30g Alk./26L Fresh Feed	1.2	7.7	7.5	844						
20-Nov	120	20g Alk./26L Fresh Feed	0.8	7.7								
24-Nov	124	30g Alk./26L Fresh Feed	1.2	7.6	7.1	748			8.9	18.6		
28-Nov	128	30g Alk./26L Fresh Feed	1.2	7.6	7.5	720						
1-Dec	131	30g Alk./26L Fresh Feed	1.2	7.6	7.5	720						
3-Dec	133	30g Alk./26L Fresh Feed	1.2	7.6	7.5	720						
4-Dec	134	(Day old feed/30g Alk.)	0.8	7.6	7.6	490						
5-Dec	135	20g Alk./26L Fresh Feed	0.8	7.6	7.6	423						
7-Dec	137	(2 Day old/20g Alk.)	0.8	7.6	7.7	693						
8-Dec	138	30g Alk./26L Fresh Feed	1.2	7.6	7.7	693	7.7	884	8.2	17.9	7.7	640
10-Dec	140	30g Alk./26L Fresh Feed	1.2	7.7	7.7	693						
11-Dec	141	(2 Day old/30g Alk.)	0.8	7.4	7.7	617			8.2	18.6		
12-Dec	142	30g Alk./26L Fresh Feed	1.2	7.7	7.7	617						
14-Dec	144	20g Alk./26L Fresh Feed	0.8	7.6	7.7	580	7.6	720	NA	19.7	7.9	536
15-Dec	145	(Day old, 20g Alk.)	1.2	7.7	7.7	556						
16-Dec	146	30g Alk./26L Fresh Feed	1.2	7.6	8.2	683						

Shaded areas were not considered in the maintained run condition parameter averages and standard deviations. Calculations were based on data obtained once stable flows were reached during the run.

FEED DAYS: ~12 Days
RUN 3: 33 Days

AVERAGE: 1.0 7.6 553 7.8 643 7.7 802 8.2 18.7 7.8 588
STD: - 0.1 113 0.3 54 0.05 82 0 0.7 0.1 52
STD of Ave. (%): 1 20 8 1 10 0 4 1 9

TABLE L.2 d ii): Feed-AD2-AB2-B2 pH, Alk, AB Temperature and DO Results During Run 3 (Days 114-146)

Date	DAY	Feed and Alk. Added	NaHCO ₃ (g/L)	Feed _{PH} (mg/L as CaCO ₃)	AD2 Effl. _{PH} (mg/L as CaCO ₃)	AD2 Effl. _{ALK} (mg/L as CaCO ₃)	AB2 _{PH} (mg/L as CaCO ₃)	AB2 _{ALK} (mg/L as CaCO ₃)	AB2 _{DO} (mg O ₂ /L)	AB2 Temp. (°C)	B2 _{PH}	B2 _{ALK} (mg/L as CaCO ₃)
14-Nov	114	30g Alk./26L Fresh Feed	1.2	7.7	7.6	729						
20-Nov	120	20g Alk./26L Fresh Feed	0.8	7.7								
24-Nov	124	30g Alk./26L Fresh Feed	1.2	7.6	6.9	712			8.1	20.2		
28-Nov	131	30g Alk./26L Fresh Feed	1.2	7.6	7.7	745						
3-Dec	133	30g Alk./26L Fresh Feed	1.2	7.6	7.7	745						
4-Dec	134	(Day old feed/30 g Alk.)	0.8	7.6	7.6	490						
5-Dec	135	20g Alk./26L Fresh Feed	0.8	7.6	7.6	423						
7-Dec	137	(2 Day old/20 g Alk.)	0.8	7.6	7.7	654						
8-Dec	138	30g Alk./26L Fresh Feed	1.2	7.6	7.7	654	7.7	656	7.6	19.1	7.7	805
10-Dec	140	30g Alk./26L Fresh Feed	1.2	7.7	7.7	656						
11-Dec	141	20g Alk./26L Fresh Feed	0.8	7.4	7.7	483			8.0	19.8		
12-Dec	142	(2 Day old/20g Alk.)	1.2	7.7	7.5	460						
14-Dec	144	30g Alk./26L Fresh Feed	1.2	7.7	7.7	460						
15-Dec	145	20g Alk./26L Fresh Feed	0.8	7.6	7.7	754	6.9	NA	NA	20.0	8.3	636
16-Dec	146	(Day old, 20g Alk.)	1.2	7.7	7.7	754						
18-Dec	148	30g Alk./26L Fresh Feed	1.2	7.6	8.1	632						

Shaded areas were not considered in the maintained run condition parameter averages and standard deviations. Calculations were based on data obtained once stable flows were reached during the run.

FEED DAYS: ~12 Days
RUN 3: 33 Days

AVERAGE: 1.0 7.6 553 7.8 625 7.3 656 7.8 19.8 8.0 721
STD: - 0.1 113 0.3 122 0.4 NA 0.2 0.5 0.3 85
STD of Ave. (%): 1 20 3 20 5 3 2 4 12

APPENDIX L.3: COD DATA

TABLE L.3 a i): COD Results for AD1-AB1 System During Start-up (Day 1-17)

DATE	DAY	Feed Concentration	FEED TOTAL (mg/L)	SOLUBLE (mg/L)	AD1 Em. TOTAL (mg/L)	SOLUBLE (mg/L)	B1 (or AB1 Em.) TOTAL (mg/L)	SOLUBLE (mg/L)	%COD Rem. AD1 Feed-AD1 Em. ($S_{10}-S_{99}$)/ S_{10}	%COD Rem. AB1 AD1 Em.-B1 ($S_{10}-S_{99}$)/ S_{10}	%COD Rem. Syst. 1 Feed-B1 ($S_{10}-S_{99}$)/ S_{10}
24-Jul	1	~100 mg COD/L feed	80	74	80	80					
25-Jul	2				320	NA					
26-Jul	3	~200 mg COD/L feed	114	32	240	234					
27-Jul	4		169	146	469	80			53		
28-Jul	5				720	160	($Q_{AD1}=6$ L/d)				
29-Jul	6	~400 mg COD/L feed	295		NA						
30-Jul	7		236		NA						
31-Jul	8	~800 mg COD/L feed	469		217						
1-Aug	9	(Day old 600 mg COD/L feed/20g NaHCO ₃)	466								
2-Aug	10	(~800 mg COD/L, 10g Alk.)	275		193						
3-Aug	11		693		142						
4-Aug	12		234		227						
5-Aug	13	~1000 mg COD/L	946								

*The feed concentration was progressively increased to 1 000 mg COD/L during the acclimation period.

START-UP : ~6 L/d

TABLE L.3 b i): COD Results for the AD1-AB1 System During Run 1 (Days 18-61)

DATE	DAY	Feed Concentration	FEED TOTAL (mg/L)	SOLUBLE (mg/L)	AD1 Em. TOTAL (mg/L)	SOLUBLE (mg/L)	B1 (or AB1 Em.) TOTAL (mg/L)	SOLUBLE (mg/L)	%COD Rem. AD1 Feed-AD1 Em. ($S_{10}-S_{99}$)/ S_{10}	%COD Rem. AB1 AD1 Em.-B1 ($S_{10}-S_{99}$)/ S_{10}	%COD Rem. Syst. 1 Feed-B1 ($S_{10}-S_{99}$)/ S_{10}
10-Aug	18	Fresh 1000 mg COD/L feed (40g Alk./20L)	497		136		68				
17-Aug	25		418		113						
18-Aug	26		940		95		88				
20-Aug	28	Fresh 1000 mg COD/L feed (No Alk.)	NA		125						
21-Aug	29										
22-Aug	30		339		122		73				
23-Aug	31	Fresh 1000 mg COD/L feed (No Alk.)	993		154		76				
24-Aug	32		421		126		72				
25-Aug	33	Fresh 1000 mg COD/L feed (No Alk.)	1258	NA	257	35	63	20	97	92	98
26-Aug	34		387	253			58	52	48	NA	87
27-Aug	35		203	123	175	106					
28-Aug	36		928	721							
29-Aug	37	Fresh 1000 mg COD/L feed (No Alk.), Blender	498	184			62	54	86		89
30-Aug	38		268	192	132	38	45				
31-Aug	39		1181	813							
1-Sep	40	New 1000 mg COD/L (20L, No Alk.), Mixer	694	1411		55	48	42	92		94
3-Sep	42		466	210	443	45	40	40	90	91	91
4-Sep	43			183							
5-Sep	44			148			51	29			
6-Sep	45	Fresh 1000 mg COD/L feed (No Alk.)	1002	676	MODIFICATIONS			TO	ABS		
7-Sep	46		966	411	NA	NA	162	41	NA	NA	96
8-Sep	47						457	121			
9-Sep	48		777	314			180	82			89
10-Sep	49		347	199			43	34			90
11-Sep	50	Fresh 1000 mg COD/L feed (No Alk.)	1333	624							
12-Sep	51		837	411	351	99	73	53	88	85	94
22-Sep	61		411	252			33	33			92

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

(Left NEW TWAS to aerate in 3.5 L ABS on Sept. 13)

RUN 1 : ~2 L/d

AVERAGE: 777 412 161 60 37 89 92 90

STD: 380 238 51 11 11 3 1 3

STD of Ave. (%): 49 58 32 16 30 4 1 3

APPENDIX L.3: COD DATA

TABLE L.3 c i): COD Results for the AD1-AB1 System During Run 2 (Days 62-113)

DATE	DAY	Feed Concentration	FEED		AD1gm.		B1 (or AB1gm.)		%COD Rem. AD1	%COD Rem. AB1	%COD Rem. Syst. 1
			TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	Feed-AD1gm. (S ₁₀ -S ₅₀)/S ₁₀	AD1gm.-B1 (S ₁₀ -S ₅₀)/S ₁₀	Feed-B1 (S ₁₀ -S ₅₀)/S ₁₀
23-Sep	62	Fresh 1000 mg COD/L feed (20g Alk./26L)	873	809							
24-Sep	63		745	555			575	102			86
26-Sep	65		877	413	165	89	105	20	87	88	97
27-Sep	66		609	305							
	66	Fresh 1000 mg COD/L feed (No Alk.)	1016	745			349	68			93
28-Sep	67		877	339	274	81	372	98	88	75	90
29-Sep	68		556	305			135	34			94
30-Sep	69		420	406							
	69	Fresh 1000 mg COD/L feed (before Alk. add.)	1354	1219							
	69	Fresh 1000 mg COD/L feed (30g Alk./26L)	948	526	379	141	508	102	85	73	89
2-Oct	71		800	474	NA	83	307	85	88	NA	89
3-Oct	72	Fresh 1000 mg/L feed (10g Alk./26L)	874	800							
4-Oct	73		800	304	797	72	207	40	88	95	93
5-Oct	75		533	237	NA	41	85	24	92	NA	95
	75	Fresh 1000 mg COD/L feed (20g Alk./26L)	748	600	NA						
9-Oct	78		728	304	NA	59	41	34	92	NA	95
10-Oct	79	Fresh 1000 mg COD/L feed (10g Alk./26L)	1287	815	NA	48	115	44	96	NA	97
11-Oct	80		874	452							
13-Oct	82		512	156	NA	72	92	28	85	NA	95
	82	Fresh 1000 mg COD/L feed (20g Alk./26L)	748	1044							
16-Oct	85	Fresh 1000 mg COD/L feed (20g Alk./26L)	1007	600							
17-Oct	86		852	556	NA	72	81	18	92	NA	98
19-Oct	88		800	528	568	62	300	NA	90	NA	NA
	88	Fresh 1000 mg COD/L feed (10g Alk./26L)	1673	376							
21-Oct	90		880	315	445	38	113	38	94	91	94
22-Oct	91	Fresh 1000 mg COD/L feed (30g Alk./26L)	1049	508							
23-Oct	92		838	409	NA	41	232	25	95	NA	97
25-Oct	94		496	179	1246	86	248	28	83	98	94
	94	Fresh 1000 mg COD/L feed (15g Alk./26L)	1213	807							
27-Oct	96		832	442	1542	81	113	59	90	98	93
	96	Fresh 1000 mg COD/L feed (30g Alk./26L)	1037	830							
30-Oct	99		687	317							
	99	Fresh 1000 mg COD/L feed (10g Alk./26L)	819	574	1723	98	212	40	88	98	95
1-Nov	101		687	344	556	38	113	61	94	89	91
2-Nov	102		556	278							
	102	Fresh 1000 mg COD/L feed (20g Alk./26L)	884	541							
4-Nov	104		621	312							
5-Nov	105	Fresh 1000 mg COD/L feed (20g Alk./26L)	1147	705	458	50	78	21	96	95	98
8-Nov	108		805	607							
	108	Fresh 1000 mg COD/L feed (10g Alk./26L)	884	672							
9-Nov	109		687	442	NA	206	219	47	70	NA	93
13-Nov	113		536	212	NA	208	358	121	81	NA	77

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

RUN 2: ~4 L/d

AVERAGE:	794	472	564	61	167	40	92	93	95
STD:	195	159	141	19	62	12	4	3	2
STD of Ave. (%):	25	34	25	31	37	29	4	3	2

TABLE L.3 d i): COD Results for the AD1-AB1 System During Run 3 (Days 114-146)

DATE	DAY	Feed Concentration	FEED		AD1gm.		B1 (or AB1gm.)		%COD Rem. AD1	%COD Rem. AB1	%COD Rem. Syst. 1
			TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	Feed-AD1gm. (S ₁₀ -S ₅₀)/S ₁₀	AD1gm.-B1 (S ₁₀ -S ₅₀)/S ₁₀	Feed-B1 (S ₁₀ -S ₅₀)/S ₁₀
14-Nov	114	Fresh 1000 mg COD/L Feed (30g Alk./26L)	440	295							
20-Nov	120										
24-Nov	124										
	124	Fresh Feed (20g Alk./26L)	1013	624		115	835	67	89		93
1-Dec	131	Fresh Feed (30g Alk./26L)	868	439							
2-Dec	132		476	NA	1375	89	1857	67	81	95	86
4-Dec	134	Fresh Feed (30g Alk./26L)	893	859							
	134		630	603	217	38		38	94	82	94
5-Dec	135	Fresh Feed (20g Alk./26L)									
7-Dec	137		796	469	875	127	289	58	84	91	93
	137	Fresh Feed (20g Alk./26L)	1568	868							
8-Dec	138	Fresh Feed (30g Alk./26L)									
10-Dec	140		892	573	579	86	1857	58	90	90	93
	140	Fresh Feed (20g Alk./26L)	1043	850	3118	129	1435	53	88	98	95
12-Dec	142	Fresh Feed (30g Alk./26L)	756	504							
13-Dec	143		953	772							
14-Dec	144	Fresh Feed (20g Alk./26L)	592	384	290	77	1776	70	87	76	88
15-Dec	145		894	567							
16-Dec	146	Fresh Feed (30g Alk./26L)									

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

RUN 3: ~8 L/d

AVERAGE:	829	576	434	97	1690	60	87	86	92
STD:	180	154	205	27	224	7	3	9	3
STD of Ave. (%):	22	27	47	27	13	12	3	10	3

DATE	DAY	Feed Concentration	lwL AD2 TOTAL (mg/L)	SOLUBLE (mg/L)	AD2 BWL TOTAL (mg/L)	SOLUBLE (mg/L)	%COD g _{max} AD2 lwL AD2-AD2BWL (S _{AD2} -S _{AD2BWL})/%S _{AD2}	%COD g _{max} AB2 AD2g _{max} -B2 (S _{AD2} -S _{B2})/%S _{AD2}	%COD g _{max} Syst. 2 lwL AD2-B2 (S _{AD2} -S _{B2})/%S _{AD2}
24-Jul	1	-100 mg COD/L feed							
25-Jul	2								
26-Jul	3								
28-Jul	3	-200 mg COD/L feed							
27-Jul	4		352		160				
28-Jul	5		211	32	208	160	95		
29-Jul	6				160		24		
31-Jul	8				176	160			
1-Aug	9	-400 mg COD/L feed							
		-800 mg COD/L feed	572						
		(Day old 600 mg COD/L feed/20g NaHCO ₃)							
2-Aug	10		579		154				
8-Aug	16	(-800 mg COD/L, 10g Alk.)			142				
9-Aug	17	-1600 mg COD/L	162		109				
					178				

The feed concentration was progressively increased to 1 000 mg COD/L during the acclimation period.

TABLE L.3 b II): COD Results for the AD2-AB2 System During Run 1 (Days 18-31)

DATE	DAY	Feed Concentration	Wt. AD2 TOTAL (mg/L)	SOLUBLE (mg/L)	AD2 em. TOTAL (mg/L)	SOLUBLE (mg/L)	B2 (or AB2)em. TOTAL (mg/L)	%COD em. AD2 Wt. AD2-AD2em. (S ₁₀ -S ₁₀)/S ₁₀	%COD em. AB2 Wt. AD2-AB2 (S ₁₀ -S ₁₀)/S ₁₀	%COD em. Sync. 2
16-Aug	18	Fresh 1000 mg COD/L feed (40g Alk./20L)	538		74		155			
17-Aug	25				85		81			
18-Aug	26				111		53			
20-Aug	28	Fresh 1000 mg COD/L feed (NO Alk.)	876		73		54			
21-Aug	29		824		63					
22-Aug	30		741							
23-Aug	31	Fresh 1000 mg COD/L feed (No Alk.)			125		49			
24-Aug	32				182		41			
25-Aug	33									
26-Aug	34	Fresh 1000 mg COD/L feed (No Alk.)	782	107		46	44	94		96
27-Aug	35						52			
28-Aug	36		1695	388		35	39	96		98
29-Aug	37	Fresh 1000 mg COD/L feed (No Alk.) Blender					33			
30-Aug	38		1849	184	170	46	33			
31-Aug	39	New 1000 mg COD/L (20L No Alk.) Mixer					28			
1-Sep	40		740	184	225	44	81	94		95
3-Sep	42		924		105	76	57	92		96
4-Sep	43						29			
5-Sep	44						24			
7-Sep	45	Fresh 1000 mg COD/L feed (No Alk.)	681		428	148	117		75	
8-Sep	46						105			
9-Sep	47		2229	269	301	155	177	93	60	95
10-Sep	48						56			
11-Sep	49	Fresh 1000 mg COD/L feed (No Alk.)	1049	82	347	64	103	94	83	98
12-Sep	50						40			
13-Sep	51						23			

that the paper areas were not considered in the estimates from here and of course the empty flows were considered stable during the run.

RUM 1

[illegible]

APPENDIX L.3: COD DATA

TABLE L.3 c ii): COD Results for the AD2-AB2 System During Run 2 (Days 62-113)

DATE	DAY	Feed Concentration	Infl. AD2		AD2 em.		B2 (or AB2em.)		%COD Rem. AD2	%COD Rem. AB2	%COD Rem. Syst. 2
			TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	Infl. AD2-AD2em. (S ₁₀ -S ₂₀)/S ₁₀	AD2em.-B2 (S ₁₀ -S ₂₀)/S ₁₀	Infl. AD2-B2 (S ₁₀ -S ₂₀)/S ₁₀
23-Sep	62	Fresh 1000 mg COD/L feed (20g Alk./26L)									
24-Sep	63										
25-Sep	65		1780	230	533	81	782	74	95	87	96
26-Sep	65										
27-Sep	66										
	66	Fresh 1000 mg COD/L feed (No Alk.)									
28-Sep	67		4333	891	463	102	142	81	98	83	98
29-Sep	68										
30-Sep	69										
	69	Fresh 1000 mg COD/L feed (before Alk. add.)									
	69	Fresh 1000 mg COD/L feed (30g Alk./26L)	2437	542	376	54	248	34	98	91	99
2-Oct	71		800	148		70	122	39	88		94
3-Oct	72	Fresh 1000 mg/L feed (10g Alk./26L)									
4-Oct	73		978	89	299	81	115	55	92	82	94
5-Oct	74										
6-Oct	75										
	75	Fresh 1000 mg COD/L feed (20g Alk./26L)			921	80	41	39		98	
9-Oct	78		904	96	855	55	89	41	94	94	95
10-Oct	79	Fresh 1000 mg COD/L feed (10g Alk./26L)									
11-Oct	80		830	156		47	115	40	94		95
12-Oct	81										
13-Oct	82										
	82	Fresh 1000 mg COD/L feed (20g Alk./26L)				54	121	39			
15-Oct	84										
16-Oct	85	Fresh 1000 mg COD/L feed (20g Alk./26L)									
17-Oct	86		1800	183	862	74	115	28	96	97	98
19-Oct	88										
	88	Fresh 1000 mg COD/L feed (10g Alk./26L)	896	193	178	47	121		95		
21-Oct	90		1410	146	299	46	113	28	97	91	98
22-Oct	91	Fresh 1000 mg COD/L feed (30g Alk./26L)									
23-Oct	92			146	424	50	150	28		93	
25-Oct	94										
	94	Fresh 1000 mg COD/L feed (15g Alk./26L)		179	286	48	212	30		90	
27-Oct	96										
	96	Fresh 1000 mg COD/L feed (30g Alk./26L)	950	136	1075	90		30	91	97	97
29-Oct	98										
30-Oct	99										
	99	Fresh 1000 mg COD/L feed (10g Alk./26L)	1478	179	227	54	115	34	98	85	98
1-Nov	101		1161	115	299	62	113	38	95	87	97
2-Nov	102										
	102	Fresh 1000 mg COD/L feed (20g Alk./26L)									
4-Nov	104										
5-Nov	105	Fresh 1000 mg COD/L feed (20g Alk./26L)		459	424	30	115	24		94	
8-Nov	108										
	108	Fresh 1000 mg COD/L feed (10g Alk./26L)									
9-Nov	109			146	796	62	252	28		96	
13-Nov	113			344	1351	110	230	68		95	

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

RUN 2: ~4 L/d		AVERAGE:	1076	146	305	59	123	34	94	89	96
		STD:	247	32	85	16	33	8	2	5	1
		STD of Ave. (%):	23	22	28	27	27	24	2	5	1

TABLE L.3 d ii): COD Results for the AD2-AB2 System During Run 3 (Days 114-146)

DATE	DAY	Feed Concentration	Infl. AD2		AD2 em.		B2		%COD Rem. AD2	%COD Rem. AB2	%COD Rem. Syst. 2
			TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	TOTAL (mg/L)	SOLUBLE (mg/L)	Infl. AD2-AD2em. (S ₁₀ -S ₂₀)/S ₁₀	AD2em.-B2 (S ₁₀ -S ₂₀)/S ₁₀	Infl. AD2-B2 (S ₁₀ -S ₂₀)/S ₁₀
14-Nov	114										
20-Nov	120	Fresh 1000 mg COD/L Feed (30g Alk./26L)									
24-Nov	124										
	124	Fresh Feed (20g Alk./26L)	1495	178	302	38	283	31	97	90	98
1-Dec	131	Fresh Feed (30g Alk./26L)	905	145	2388	57	217	48	94	98	95
2-Dec	132										
4-Dec	134	Fresh Feed (30g Alk./26L)	3335	54	832	48	109	38	99	95	99
5-Dec	135	Fresh Feed (20g Alk./26L)									
7-Dec	137										
	137	Fresh Feed (20g Alk./26L)		175	796	173		28		97	
8-Dec	138	Fresh Feed (30g Alk./26L)									
10-Dec	140										
	140	Fresh Feed (20g Alk./26L)	838	181	1906	49	410	38	94	98	95
12-Dec	142	Fresh Feed (30g Alk./26L)	1098	38			109	23		98	
13-Dec	143		971	205							
14-Dec	144	Fresh Feed (20g Alk./26L)									
15-Dec	145			287	1537	43		18		99	
16-Dec	146	Fresh Feed (30g Alk./26L)									

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

RUN 3: ~8 L/d		AVERAGE:	1052	195	1513	43	109	27	94	98	95
		STD:	300	49	405	6	9			1	
		STD of Ave. (%):	29	25	27	13		32		1	

APPENDIX L.4: SS DATA

TABLE L.4 a i): TSS & VSS Results for AD1-AB1 System During Start-up (Days 1-17)

DATE	DAY	Feed Concentration	FEED		AD1 Effl.		In AB1		*After SS add. from (B1 on previous day)		B1	%TSS Removal			
			TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	X _{AD1} Feed-AD1	X _{AB1} AD1-B1	System 1 Feed-B1	
21-Jul	INITIALLY						1449	974							
24-Jul	1	~100 mg COD/L feed													
26-Jul	3	~ 200 mg COD/L feed					213	100							
29-Jul	6	~ 400 mg COD/L feed													
31-Jul	8														
1-Aug	9	~ 600 mg COD/L feed (Day old 600 mg COD/L feed/20g NaHCO ₃)			149	115	124	121			59			61	
2-Aug	10	(~800 mg COD/L, 10g Alk.)													
8-Aug	16		196	185	179	160									
9-Aug	17	~1000 mg COD/L	243	219	297	256									
NR- No Removal															

START-UP

TABLE L.4 b i): TSS & VSS Results for the AD1-AB1 System During Run 1 (Days 18-61)

DATE	DAY	Feed Concentration	FEED		AD1 Effl.		In AB1		*After SS add. from (B1 on previous day)		B1	%TSS Removal			
			TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	X _{AD1} Feed-AD1	X _{AB1} AD1-B1	System 1 Feed-B1	
10-Aug	18	Fresh 1000 mg COD/L feed (40g Alk./20L)	64	47	26	41	455	335			8	60			
11-Aug	19		48	44	108	65		180			15				
18-Aug	26	Fresh 1000 mg COD/L feed (No Alk.)	127	126	900	779					30	NR			
21-Aug	29	Fresh 1000 mg COD/L feed (No Alk.)	133	131	261	221			198	105	9	NR			
23-Aug	31	Fresh 1000 mg COD/L feed (No Alk.), Blender			293	216			249	148	8	NR			
26-Aug	34		120	119	482	395			403	242	71				
28-Aug	36	New 1000 mg COD/L (20L, No Alk.), Mixer			373	335			272	167	34				
30-Aug	38										NA				
31-Aug	39										15				
1-Sep	40										30				
3-Sep	42										8				
5-Sep	44										7				
6-Sep	45	Fresh 1000 mg COD/L feed (No Alk.)	173	172							17				
7-Sep	46				646	539	180	119			11				
8-Sep	47								423	214	38				
9-Sep	48		12	8	366	288	63	41	258	161	5				
10-Sep	49	Fresh 1000 mg COD/L feed (No Alk.)					12	2			7				
11-Sep	50				115	102					NA				
12-Sep	51	(On Sept. 11, did not add settled solids back in AB's and threw out their contents on Sept. 12)									3				
22-Sep	61														
NR- No Removal															

*Started batch recycling SS back to AB1 on Day 36.

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

NR- No Removal

AVERAGE: 111 106 352 292 280 165 9 7 98 93
STD: 46 51 99 88 87 57 1 1 1 1
STD of Ave. (%): 42 47 28 30 31 35 11 13 1 1

RUN 1

APPENDIX L.4: SS DATA

TABLE L.4 c i): TSS & VSS Results for the AD1-AB1 System During Run 2 (Days 62-113)

DATE	DAY	Feed Concentration	FEED		AD1 Eff.		In AB1		*After SS add. from		B1		%TSS Removal		
			TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	Before solid add.		(B1 on previous day)		TSS (mg/L)	VSS (mg/L)	X _{AD1} Feed-AD1	X _{AB1} AD1-B1	System 1 Feed-B1
23-Sep	62	Fresh 1000 mg COD/L feed (20g Alk./26L)	46	43			1143	750							
24-Sep	63		37	32							18	9			53
25-Sep	64						529	510							
26-Sep	65	Fresh 1000 mg COD/L feed (No Alk.)	NA	NA	106	81	83	34	148	114	37	21		85	NR
27-Sep	66		40	41							108	56			
28-Sep	67		37	35	170	117			163	131	48	24	NR	72	NR
29-Sep	68								349	201	83	31			
30-Sep	69		40	30											
	69	Fresh 1000 mg COD/L feed (30g Alk./26L)	55	41	94	73	48	34	198	146	301	202	NR	NR	NR
1-Oct	70	(Started weekly batch recycling to AD's ...)							180	148	21	10			
2-Oct	71				188	118	73	37	144	94	43	22		74	
3-Oct	72	Fresh 1000 mg/L feed (10g Alk./26L)	43	45							35	14			18
4-Oct	73				406	353			142	119	49	21		88	
5-Oct	74	(8 hrs after solid add. from Oct. 3 on Oct. 4)							156	116	41	23			
6-Oct	75	Fresh 1000 mg COD/L feed (20g Alk./26L)	42	30	312	252			212	125	36	21	NR	89	14
7-Oct	76	(4 hrs after solid add. from Oct. 5 on Oct. 6)							161	138					
8-Oct	77								140	98	36	19			
9-Oct	78	(AB1 'Before' from Oct. 7 add., 'After' Oct. 9)			2174	1927	(152)	(87)	179	148	30	14		99	
10-Oct	79	Fresh 1000 mg COD/L feed (10g Alk./26L)	42	38					214	157	74	41			NR
11-Oct	80				550	481			172	127	38	19		93	
12-Oct	81								130	108	31	26			
13-Oct	82	Fresh 1000 mg COD/L feed (20g Alk./26L)			1140	823									
14-Oct	83								175	120					
15-Oct	84										51	31			
16-Oct	85	Fresh 1000 mg COD/L feed (20g Alk./26L)	48	42					200	123					
17-Oct	86				4156	3716					50	27		99	
18-Oct	87								366	269					
19-Oct	88	Fresh 1000 mg COD/L feed (10g Alk./26L)	52	47	378	200					48	23	NR	87	7
20-Oct	89		53	45	556	451			188	115	80	27	NR	89	NR
21-Oct	90	Fresh 1000 mg COD/L feed (30g Alk./26L)	143	141											
22-Oct	91		134	121	871	680			298	215	53	NA	NR	94	61
23-Oct	92		158	137											
24-Oct	93	Fresh 1000 mg COD/L feed (15g Alk./26L)	37	30	129	91			292	203	86	35	NR	49	33
25-Oct	94		NA	NA					311	208					
27-Oct	96	Fresh 1000 mg COD/L feed (30g Alk./26L)	188	172	578	425					72	32	NR	87	61
28-Oct	97		137	124											
30-Oct	99	Fresh 1000 mg COD/L feed (10g Alk./26L)	61	56	638	488			285	201	148	79	NR	77	NR
	99	(After add. extra solids from Bs during SBH (5L))							595	435					
1-Nov	101		59	53	301	216	(305)	(211)	422	320	60	32	NR	80	NR
2-Nov	102		165	153											
3-Nov	103	Fresh 1000 mg COD/L feed (20g Alk./26L)	129	120											
4-Nov	104		57	47											
5-Nov	105	Fresh 1000 mg COD/L feed (20g Alk./26L)	125	117	139	71			692	292	51	29	NR	83	59
6-Nov	106		184	170											
7-Nov	107	Fresh 1000 mg COD/L feed (10g Alk./26L)	122	119											
8-Nov	108		120	111	6139	5836			431	328	170	138	NR	90	NR
9-Nov	109		87	79	9075	8015			1271	1085	813	401	NR	88	NR
13-Nov	113														

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

NR- No Removal

RUN 2	AVERAGE:	87	79	510	394	273	188	50	26	NR	89	41
	STD:	51	48	183	156	153	92	14	7		4	22
	STD of Ave. (%):	59	61	36	40	56	49	27	29		5	54

TABLE L.4 d i): TSS & VSS Results for the AD1-AB1 System During Run 3 (Days 114-146)

DATE	DAY	Feed Concentration	FEED		AD1 Eff.		In AB1		*After SS add. from		B1		%TSS Removal		
			TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	Before solid add.		(B1 on previous day)		TSS (mg/L)	VSS (mg/L)	X _{AD1} Feed-AD1	X _{AB1} AD1-B1	System 1 Feed-B1
14-Nov	114	Fresh 1000 mg COD/L Feed (30g Alk./26L)	129	108					1215	978					
20-Nov	120	Fresh Feed (20g Alk./26L)	49	40	8671	7818			148	128	208	163	NR	88	NR
24-Nov	124	Fresh Feed (30g Alk./26L)	61	43											
1-Dec	131				319	198			778	678	102	68		88	
2-Dec	132	Fresh Feed (30g Alk./26L)	52	41	227	78			2900	2511	547	480	NR	NR	NR
4-Dec	134	Fresh Feed (20g Alk./26L)	45	38											
5-Dec	135	Fresh Feed (20g Alk./26L)	63	44	193	101			1963	1699	148	114	NR	23	NR
7-Dec	137	Fresh Feed (30g Alk./26L)	134	114											
8-Dec	138	Fresh Feed (20g Alk./26L)	95	76	131	60			3596	3145	235	194	NR	NR	NR
10-Dec	140	Fresh Feed (30g Alk./26L)	27	NA											
12-Dec	142	Fresh Feed (30g Alk./26L)			684	578			3710	3284	1319	1108		NR	
13-Dec	143		47	39											
14-Dec	144	Fresh Feed (20g Alk./26L)	58	40	179	105			1784	1569	182	128	NR	9	NR
16-Dec	146	Fresh Feed (30g Alk./26L)													

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

NR- No Removal

RUN 3	AVERAGE:	71	58	168	89	2763	2419	182	145	NR	16	NR
	STD:	35	30	33	25	1031	910	47	43		7	
	STD of Ave. (%):	49	51	20	28	37	38	26	29		43	

APPENDIX L.4: SS DATA

TABLE L.4 a ii): TSS & VSS Results for AD2-AB2 System During Start-up (Days 1-17)

DATE	DAY	Feed Concentration	FEED TSS (mg/L) VSS (mg/L)	AD2 Effl. TSS (mg/L) VSS (mg/L)	In AB2 Before solid add. TSS (mg/L) VSS (mg/L)	*After SS add. from (B2 on previous day) TSS (mg/L) VSS (mg/L)	B2 TSS (mg/L) VSS (mg/L)	%TSS Removal X _{AD2} Feed-AD2 X _{AB2} AD2-B2 System 2 Feed-B2
21-Jul	INITIALLY				1449	974		
24-Jul	1	~100 mg COD/L feed						
26-Jul	3	~100 mg COD/L feed			180	82		
27-Jul	3	new 200 mg/L feed						
29-Jul	4	Day old 200 mg/L feed					29	14
31-Jul	6	~ 400 mg COD/L feed						
31-Jul	8				170	110	30	25
1-Aug	8	~ 600 mg COD/L feed						10
2-Aug	9	(Day old 600 mg COD/L feed/20g NaHCO ₃)						
8-Aug	16	(~800 mg COD/L, 10g Alk.)	196 185	63 48				
9-Aug	17	~1000 mg COD/L	243 219	262 233				NA

NR- No Removal

START-UP

TABLE L.4 b ii): TSS & VSS Results for AD2-AB2 System During Run 1 (Days 18-61)

DATE	DAY	Feed Concentration	FEED TSS (mg/L) VSS (mg/L)	AD2 Effl. TSS (mg/L) VSS (mg/L)	In AB2 Before solid add. TSS (mg/L) VSS (mg/L)	*After SS add. from (B2 on previous day) TSS (mg/L) VSS (mg/L)	B2 TSS (mg/L) VSS (mg/L)	%TSS Removal X _{AD2} Feed-AD2 X _{AB2} AD2-B2 System 2 Feed-B2
10-Aug	18	Fresh 1000 mg COD/L feed (40g Alk./20L)						
11-Aug	19		64 47	16 13	254 159		37 27	
18-Aug	26			55 45	169 104		9 3	
21-Aug	29						25 23	
23-Aug	31	Fresh 1000 mg COD/L feed (No Alk.)	48 44	109 88			10 9	
26-Aug	34	Fresh 1000 mg COD/L feed (No Alk.)	127 126	683 537			6 5	
28-Aug	36	Fresh 1000 mg COD/L feed, Blender	133 131	286 244			3 1	
30-Aug	38			430 370			2 1	
31-Aug	39	New 1000 mg COD/L (20L, No Alk.) Mixer	120 119	474 413			5 2	
1-Sep	40			100 75			14 11	
3-Sep	42						3 1	
5-Sep	44							
6-Sep	45	Fresh 1000 mg COD/L feed (No Alk.)	173 172					
7-Sep	46			140 113				
8-Sep	47			126 91	180 110		11 9	
9-Sep	48							
10-Sep	49	Fresh 1000 mg COD/L feed (No Alk.)	12 8	28 24	257 186		40 27	
11-Sep	50				272 189		73 50	
12-Sep	51	(On Sept. 11, did not add settled solids back in AB's and threw out their contents on Sept. 12)			67 37		7 5	
22-Sep	61	(Left NEW TWAS to aerate in 3.5 L AB's on Sept. 13)			23 21		4 2	

*Started batch recycling SS back to AB1 on Day 36.

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

NR- No Removal

RUN 1

AVERAGE:	111	106	397	342	154	84	4	2	NR	99.2	96
STD:	46	51	98	88	30	9	2	2		0.3	1
STD of Ave (%)	42	47	25	26	20	11	44	81		0.3	1

APPENDIX L.4: SS DATA

TABLE L.4 c ii): TSS & VSS Results for AD2-AB2 System During Run 2 (Days 62-113)

DATE	DAY	Feed Concentration	FEED		AD2 EM.		In AB2		*After SS add. from (B2 on previous day)		B2		%TSS Removal		
			TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	Before solid add. TSS VSS	Before solid add. TSS VSS	TSS VSS	TSS VSS	TSS (mg/L)	VSS (mg/L)	X _{AD2} Feed-AD2	X _{AB2} AD2-B2	System 2 Feed-B2
23-Sep	62	Fresh 1000 mg COD/L feed (20g Alk./26L)	46	43			1853	1235							
24-Sep	63		37	32							189	115			NR
25-Sep	64						1073	649							
26-Sep	65	Fresh 1000 mg COD/L feed (No Alk.)	NA	NA	157	114	57	30	181	154	57	32		84	NR
27-Sep	66		40	41							85	40			NR
28-Sep	67		37	35	182	142			148	129	12	4	NR	93	67
29-Sep	68								132	117	78	43			
30-Sep	69		40	30											
	69	Fresh 1000 mg COD/L feed (30g Alk./26L)	55	41	143	104	213	129	227	168	260	237	NR		NR
1-Oct	70	(Started 'weekly' batch recycling to AD's ...)							122	87	58	51			
2-Oct	71				343	127	33	19	148	98	58	51		84	
3-Oct	72	Fresh 1000 mg/L feed (10g Alk./26L)	43	45							NA	10			
4-Oct	73				274	208			185	114	35	21		87	
5-Oct	74	(8 hrs after solid add. from Oct. 3 on Oct. 4)							161	109					
6-Oct	75	Fresh 1000 mg COD/L feed (20g Alk./26L)	42	30	345	283			177	118	48	28	NR	89	6
	75	(4 hrs after solid add. from Oct. 5 on Oct. 6)							100	78	39	21			
7-Oct	76								140	102					
8-Oct	77								178	114	35	28			
9-Oct	78	(AB1 'Before' from Oct. 7 add., 'After' Oct. 9)			779	639			171	120	34	26		98	
10-Oct	79	Fresh 1000 mg COD/L feed (10g Alk./26L)	42	38					131	108	39	30			6
11-Oct	80				1725	1427			102	82	80	49		95	
12-Oct	81								125	100	137	102			
13-Oct	82	Fresh 1000 mg COD/L feed (20g Alk./26L)			138	83									
14-Oct	83								128	89					
15-Oct	84										67	55			
16-Oct	85	Fresh 1000 mg COD/L feed (20g Alk./26L)	48	42					110	92	49	30		88	
17-Oct	86				410	332			307	214					
18-Oct	87										108	87	NR	NR	NR
19-Oct	88	Fresh 1000 mg COD/L feed (10g Alk./26L)	52	47	97	57			293	222	47	33	NR	44	12
20-Oct	89		53	45	84	53									
21-Oct	90	Fresh 1000 mg COD/L feed (30g Alk./26L)	143	141					348	224	150	100	NR	21	NR
22-Oct	91		134	121	190	167									
23-Oct	92		158	137					347	216	65	49	NR	61	33
24-Oct	93	Fresh 1000 mg COD/L feed (15g Alk./26L)	37	30	186	95			197	154	134	85	NR	55	29
25-Oct	94		NA	NA											
26-Oct	95	Fresh 1000 mg COD/L feed (30g Alk./26L)	188	172	388	234									
27-Oct	96		137	124					415	323	67	43	NR	98	32
28-Oct	97	Fresh 1000 mg COD/L feed (10g Alk./26L)	61	56	2994	2573			508	376					
29-Oct	98	(After add. extra solids from Bs during SBH (5L))					(305)	(211)	376	299	157	108	NR	46	NR
1-Nov	101		59	53	290	245									
2-Nov	102		185	153											
3-Nov	103	Fresh 1000 mg COD/L feed (20g Alk./26L)	129	120											
4-Nov	104		57	47											
5-Nov	105	Fresh 1000 mg COD/L feed (20g Alk./26L)	125	117	131	73			563	432	105	89	NR	20	16
6-Nov	106		184	170											
7-Nov	107	Fresh 1000 mg COD/L feed (10g Alk./26L)	122	119											
8-Nov	108		120	111	699	730									
9-Nov	109		122	111					617	463	115	100	NR	87	4
10-Nov	110		87	79	1258	1005			371	286	60	48	NR	95	3

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

NR- No Removal

RUN 2	AVERAGE:	87	79	316	245	246	181	51	36	NR	88	25
	STD:	51	48	81	57	138	105	15	12		1	9
	STD of Ave. (%):	59	61	26	23	56	58	30	33		1	36

TABLE L.4 d ii): TSS & VSS Results for AD2-AB2 System During Run 3 (Days 114-146)

DATE	DAY	Feed Concentration	FEED		AD2 EM.		In AB2		*After SS add. from (B2 on previous day)		B2		%TSS Removal		
			TSS (mg/L)	VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	Before solid add. TSS VSS	Before solid add. TSS VSS	TSS VSS	TSS VSS	TSS (mg/L)	VSS (mg/L)	X _{AD2} Feed-AD2	X _{AB2} AD2-B2	System 2 Feed-B2
14-Nov	114	Fresh 1000 mg COD/L Feed (30g Alk./26L)	129	108					282	202					
20-Nov	120	Fresh Feed (20g Alk./26L)	49	40	152	59			274	196	203	153	NR	NR	NR
24-Nov	124	Fresh Feed (30g Alk./26L)	61	43											
1-Dec	131				1185	972			378	301	78	48		94	
2-Dec	132				904	684			405	325	50	24	NR	94	4
4-Dec	134	Fresh Feed (30g Alk./26L)	52	41											
5-Dec	135	Fresh Feed (20g Alk./26L)	45	38											
7-Dec	137	Fresh Feed (20g Alk./26L)	63	44	224	131			703	586	59	32	NR	74	7
8-Dec	138	Fresh Feed (30g Alk./26L)	134	114											
10-Dec	140	Fresh Feed (20g Alk./26L)	95	78	509	361			808	688	21	14	NR	96	78
12-Dec	142	Fresh Feed (30g Alk./26L)	27	NA											
13-Dec	143				328	254			436	355	31	12		90	
14-Dec	144	Fresh Feed (20g Alk./26L)	47	39											
16-Dec	146	Fresh Feed (30g Alk./26L)	58	40	788	683			1085	973	174	125	NR	77	NR

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

NR- No Removal

RUN 3	AVERAGE:	71	58	418	308	758	651	26	13	NR	93	7
	STD:	35	30	90	54	268	256	5	1		3	
	STD of Ave. (%):	49	51	22	17	35	39	20	9		3	

APPENDIX L.5: UASB REACTOR OPERATION CONDITIONS

TABLE L.5 a i): AD1 Conditions and Performance During Start-up (Day 1-17)

DAY	DATE	AD1				$(Q_{\text{eff}} \times X_{\text{eff}})$				TOTAL				SOLUBLE				Batch Fed Sludge Recycled to AD1 (mL)
		Q_{AD1}	(L/d)	V_{AD1}	(L)	HRT_{AD1}	(d)	Washout $_{\text{AD1}}$	$(g \text{ VSS/d})$	SRT_{AD1}	(d)	$(g \text{ COD}_{\text{rem}}/L \cdot d)$	OLR_{AD1}	$(g \text{ COD}_{\text{rem}}/L \cdot d)$	OLR_{AD1}	$(g \text{ COD}_{\text{rem}}/L \cdot d)$	VR_{AD1}	SBH_1
1	24-Jul	Feed on 8:30PM	4.57	4.50	0.98													2.000
2	25-Jul	16	3.85	3.85	0.25													
3	26-Jul	8	3.85	3.85	0.50													
4	27-Jul	3	3.85	3.85	1.20													
5	28-Jul	(pump died)	3.85	3.85	1.28													
6	29-Jul	6	3.90	3.90	0.65													
7	30-Jul	3	3.85	3.85	1.17													
8	31-Jul	3	3.85	3.85	0.71													
9	1-Aug	6	4.25	4.25	0.86													
10	2-Aug	4	3.85	3.85	0.96													
11	3-Aug	7	3.85	3.85	0.56													
12	4-Aug																	
13	5-Aug																	
14	6-Aug																	
15	7-Aug																	
16	8-Aug																	
17	9-Aug																	

Different peristaltic pump used on July 30. General Power Shutdown in building on Aug. 3 (11 PM-4 AM).
Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE: 3.9 0.9
STD: 2 0.2 0.2
STD of Ave. (%): 4 27

TABLE L.5 b i): AD1 Conditions and Performance During Run 1 (Days 18-61)

AD1		(Q _{eff} × X _{eff})				TOTAL				SOLUBLE				SOLUBLE		Batch Fed Sludge Recycled to AD1 (mL)
DAY	DATE	(L/d)	(L)	(d)	(g VSS/d)	(g COD _{rem} /L·d)	(d)	(g COD _{rem} /L·d)	(g COD _{rem} /L·d)	(g COD _{rem} /L·d)	(g COD _{rem} /L·d)	(g COD _{rem} /L·d)	(L)	(L)		
18	10-Aug	4.57	4.50	0.98												
25	17-Aug	2.86	4.00	1.40												
28	18-Aug	1.50	3.85	2.42												
28	20-Aug	1.21	3.925	3.24												
29	21-Aug	0.86	4.250	4.78	0.2	403										
30	22-Aug	1.83	4.375	2.39												
31	23-Aug	2.09	4.500	2.15	0.4	205										
32	24-Aug	1.63	4.375	2.68												
33	25-Aug	2.12	4.250	2.00												
34	26-Aug	2.10	3.950	1.86												
35	27-Aug	2.15	3.85	1.79	4.8	17										
36	28-Aug	1.66	4.250	2.56	1.3	64										
37	29-Aug	1.74	4.350	2.50												
38	30-Aug	2.07	4.200	2.03	1.3	61										
38	31-Aug	1.95	4.125	2.12												
40	1-Sep	1.83	4.00	2.19	2.3	35										
42	3-Sep	2.87	3.85	1.34	2.3	35										
43	4-Sep	ND	3.85													
44	5-Sep	1.71	3.85	2.25												
45	6-Sep	OFF/3.1	3.85		MODIFICATIONS				10				2.000			
46	7-Sep	1.58	3.85	2.44	0.8	94										
47	8-Sep	2.26	3.85	1.68												
48	9-Sep	1.78	3.85	2.16	0.5	157										
49	10-Sep															
49	11-Sep	2.38	3.85	1.62												
50	12-Sep	2.28	3.85	1.69	0.2	348										
51	13-Sep	2.22	3.85	1.73												
51	14-Sep	OFF	3.85													

APPENDIX L.5: UASB REACTOR OPERATION CONDITIONS

TABLE L.5 c i): AD1 Conditions and Performance During Run 2 (Days 62-113)

AD1		$(Q_{AD1} \cdot X_{AD1})$						TOTAL	TOTAL	SOLUBLE	SOLUBLE	Batch Fed Sludge Recycled to AD1 (mL)
DAY	DATE	(L/d) Q_{AD1}	(L) V_{AD1}	(d) HRT_{AD1}	(g VSS/d) $Washout_{AD1}$	(d) SRT_{AD1}	(g COD _{reg} /L·d) OLR_{AD1}	(g COD _{rem} /L·d) VRR_{AD1}	(g COD _{reg} /L·d) OLR_{AD1}	(g COD _{rem} /L·d) VRR_{AD1}	(L) SBH_1	
62	23-Sep	OFF	3.825								2.275	
63	24-Sep	2.4	3.875	1.64			0.48		0.34	0.34		
65	26-Sep	2.3	3.85	1.71			0.40	0.34	0.24	0.21		
66	27-Sep	2.2	3.85	1.73	0.1	720	0.47		0.30			
67	28-Sep	2.7	3.85	1.45	0.3	314	0.47	0.41	0.23	0.21		
68	29-Sep	2.6	3.85	1.46			0.38		0.21			
69	30-Sep	2.8	3.85	1.36	0.2	475	0.65	0.56	0.80	0.51	2.350	
70	1-Oct	2.3	3.85	1.67								60
71	2-Oct	3.2	3.85	1.22	0.4	268	0.49	0.42	0.39	0.33		
72	3-Oct	4.2	3.85	0.93			0.73		0.65			80
73	4-Oct	4.1	3.85	0.95	1.4	66	0.63	0.56	0.32	0.28		
74	5-Oct	4.2	3.85	0.92								350
75	6-Oct	4.3	3.85	0.91	1.5	66	0.71	0.65	0.46	0.43		
76												
77												
78	9-Oct	3.9	3.85	0.98	7.8	13	0.74	0.68	0.31	0.29	2.375	350
79	10-Oct	4.7	3.85	0.82			1.55	1.49	0.75	0.73		
80	11-Oct	4.5	3.85	0.85	2.2	45	0.79		0.53			
81	12-Oct	4.1	3.85	0.94								360
82	13-Oct	4.8	3.85	0.80	3.9	25	0.79	0.68	0.75	0.64		
83	14-Oct	4.3	3.85	0.90								
84	15-Oct	4.6	3.85	0.84							2.375	360
85	16-Oct	4.8	3.85	0.85			1.19		0.71			
86	17-Oct	4.3	3.85	0.90	15.9	8	0.94	0.87	0.62	0.56		360
87	18-Oct	4.2	3.85	0.93	1.2	79	1.23	1.10	0.49	0.44		
88												
89												
90	21-Oct	4.2	3.85	0.91	1.9	51	0.73	0.68	0.35	0.33	2.400	360
91	22-Oct	4.1	3.85	0.93			1.13		0.55			
92	23-Oct	3.9	3.85	1.00	2.6	37	0.84	0.80	0.41	0.39		360
93												
94	25-Oct	3.9	3.85	0.98	0.4	276	0.87	0.72	0.40			360
95												
96	27-Oct	4.4	3.85	0.88	1.9	53	1.07	0.96	0.73	0.66	2.400	360
97												
98	29-Oct	3.9	3.85	0.99								360
99	30-Oct											
100												
101	1-Nov	4.0	3.85	0.97	1.9	51	0.78	0.69	0.46	0.41		360
102	2-Nov	4.5	3.85	0.86	1.4	69	0.80	0.76	0.40	0.38		
103		4.5	3.85	0.85			0.84		0.48			
104	4-Nov	4.4	3.85	0.87			0.71		0.36			
105	5-Nov	4.2	3.85	0.92	0.3	330	1.25	1.19	0.77	0.73	2.500	360
106												
107	8-Nov											
108		4.5	3.85	0.86			0.98		0.74			
109	9-Nov	4.4	3.85	0.88	25.4	4	0.78	0.54	0.50	0.35		360
110												
111	13-Nov	3.7	3.85	1.05	29.3	3	0.51	0.31	0.20	0.12	3.250	360
112												
113												

*Started batch recycling SS back to AD1 on Day 70.

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.	4.3	3.85	0.91	1.8	58	0.9	0.8	0.5	0.5	2.469	Average SBH (L)
STD:	0.3		0.06	0.4	13	0.2	0.3	0.2	0.2		
STD of Ave. (%):	6		7	25	23	27	36	32	34		

TABLE L.5 d i): AD1 Conditions and Performance During Run 3 (Days 114-146)

AD1		$(Q_{AD1} \cdot X_{AD1})$						TOTAL	TOTAL	SOLUBLE	SOLUBLE	Batch Fed Sludge Recycled to AD1 (mL)
DAY	DATE	(L/d) Q_{AD1}	(L) V_{AD1}	(d) HRT_{AD1}	(g VSS/d) $Washout_{AD1}$	(d) SRT_{AD1}	(g COD _{reg} /L·d) OLR_{AD1}	(g COD _{rem} /L·d) VRR_{AD1}	(g COD _{reg} /L·d) OLR_{AD1}	(g COD _{rem} /L·d) VRR_{AD1}	(L) SBH_1	
114	14-Nov		3.85								3.100	
120	20-Nov		3.85									
124	24-Nov											
124		4.5	4.00	0.88	35	2	1.15	1.02	0.71	0.63	3.000	
131	1-Dec		3.85									
132	2-Dec	4.1	3.85	0.93	0.6	62	0.93	0.75				
134	4-Dec											
134		6.1	4.00	0.65	0.5	139	1.06	1.00	1.01	0.85	3.000	
135	5-Dec	7.2	3.85	0.54			1.17		1.23			
137	7-Dec											
137		8.4	3.85	0.46	0.9	78	1.74	1.47	1.03	0.88		350
138	8-Dec	8.9	3.85	0.43			3.83		2.01			
140	10-Dec											
140		9.6	3.85	0.40	0.6	116	2.23	2.01	1.43	1.29	2.500	350
142	12-Dec	8.8	3.85	0.44			2.39	2.09	1.95	1.71		
143	13-Dec	8.8	3.85	0.44	5	13	1.73		1.15			350
144	14-Dec	8.3	3.85	0.47			2.05		1.66			
145	15-Dec	8.8	3.85	0.44			1.36	1.18	0.88	0.77		350
146	16-Dec	8.9	3.85	0.43	0.9	72	2.06		1.31		1.200	(60 mL for sampling)

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.	8.8	3.85	0.44	0.8	89	1.9	1.7	1.3	1.2	2.655	Average SBH (L)
STD:	0.4		0.02	0.2	24	0.4	0.4	0.4	0.4		
STD of Ave. (%):	4		4	24	27	18	26	28	37		

APPENDIX L.5: UASB REACTOR OPERATION CONDITIONS

TABLE L.5 c ii): AD2 Conditions and Performance During Run 2 (Days 62-113)

AD2		(Q FEED + Q H ₂ O)	(Q _{eff} X _{eff})		(VSS)	TOTAL	Total	SOLUBLE	SOLUBLE	2.275 (80 mL for sampling)	
		(L/d)	(L)	(d)	(g VSS/d)	(d)	(g COD _{reg} /L·d)	(g COD _{rem} /L·d)	(g COD _{reg} /L·d)	(g COD _{rem} /L·d)	(L)
DAY	DATE	Q _{AD2}	V _{AD2}	HRT _{AD2}	Washout _{AD2}	SRT _{AD2}	OLR _{AD2}	VRR _{AD2}	OLR _{AD2}	VRR _{AD2}	SBH ₂
62	23-Sep	OFF	3.50								2.185
63	24-Sep	2.7	3.75	1.40							
65	26-Sep	2.2	3.75	1.89	0.3	338	1.04	0.99	0.14	0.13	
66	27-Sep	1.6	3.75	2.09							
67	28-Sep	5.2	3.75	0.72	0.7	117	6.01	5.87	0.96	0.94	
68	29-Sep	2.6	3.75	1.44							
69	30-Sep	2.4	3.75	1.59	0.2	350	1.53	1.50			2.250
70	1-Oct	3.8	3.75	1.59							60
71	2-Oct	4.0	3.75	0.93	0.9	93	0.85	0.57	0.16	0.14	
72	3-Oct	4.7	3.75	0.80							
73	4-Oct	4.8	3.75	0.78	1.0	86	1.26	1.15	0.11	0.10	2.350
74	5-Oct	4.3									350
75	6-Oct	5.1	3.75	0.74	1.4	60					
76											
77											
78	9-Oct	3.0	3.75	1.23	1.9	44	0.73	0.69	0.08	0.07	350
79	10-Oct	4.4	3.75	0.85							
80	11-Oct	3.9	3.75	0.97	5.5	16	0.85	0.80	0.16	0.15	
81	12-Oct	4.2	3.75	0.90							350
82	13-Oct	4.3	3.75	0.87	0.4	239					
83	14-Oct	2.4									
84	15-Oct	2.1	3.75	1.76							2.425
85	16-Oct	3.1	3.75	1.21							350
86	17-Oct	5.1	3.75	0.73	1.7	51	2.46	2.36	0.22	0.21	350
87	18-Oct										
88	19-Oct	2.9	3.75	1.30	0.2	520	0.69	0.65	0.15		350
89											
90	21-Oct	2.3	3.75	1.62	0.1	700	0.87	0.84	0.09	0.09	350
91	22-Oct	5.5	3.75	0.68							
92	23-Oct	5.6	3.75	0.67	0.9	91			0.22		350
93											
94	25-Oct										350
95		3.8	3.75	0.99	0.4	239			0.18		
96	27-Oct										350
97		6.0	3.75	0.62	1.4	61	1.52	1.36	0.22	0.20	
98	29-Oct	4.0	4.875	1.22							2.750
99	30-Oct										
100		4.8	3.75	0.78	12.4	7	1.89	1.82	0.23	0.22	350
101	1-Nov	3.4	3.75	1.11	0.8	103	1.04	0.99	0.10	0.10	350
102	2-Nov	3.0	3.75	1.27							
103											
104	4-Nov	2.3	3.75	1.62							
105	5-Nov	4.6	3.75	0.82	0.3	294			0.56		2.875
106	6-Nov										
107	8-Nov										
108		2.9	3.75	1.28							
109	9-Nov	2.3	3.75	1.63	1.7	51			0.09		350
110											
111	13-Nov	5.2	3.75	0.73	5.2	17			0.47		

*Started batch recycling SS back to AD1 on Day 70.

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.:	4	3.75	1.0	1.2	78	1.1	1.0	0.16	0.14	2.501 Average SBH (L)
STD:	1	0.22	0.3	0.4	22	0.4	0.4	0.06	0.06	
STD of Ave. (%):	27	6	30	31	28	38	38	38	42	

TABLE L.5 d ii): AD2 Conditions and Performance During Run 3 (Days 114-146)

AD2		(Q FEED + Q H ₂ O)	(Q _{eff} X _{eff})		(VSS)	TOTAL	Total	SOLUBLE	TOTAL	2.655 (120 mL for sampling)	
		(L/d)	(L)	(d)	(g VSS/d)	(d)	(g COD _{reg} /L·d)	(g COD _{rem} /L·d)	(g COD _{reg} /L·d)	(g COD _{rem} /L·d)	(L)
DAY	DATE	Q _{AD2}	V _{AD2}	HRT _{AD2}	Washout _{AD2}	SRT _{AD2}	OLR _{AD2}	VRR _{AD2}	OLR _{AD2}	VRR _{AD2}	SBH ₂
114	14-Nov		3.75								2.990
120	20-Nov		3.75								
124	24-Nov										
125		4.2	3.75	0.90	0.2	286	1.67	1.62	0.20	0.19	2.750
131	1-Dec		3.75								
132	2-Dec	5.7	3.75	0.66	5.5	13	1.37	1.28	0.22	0.21	
134	4-Dec										
134		7.3	3.75	0.51	4.9	15	6.51	6.42	0.11	0.10	350
135	5-Dec	7.0	3.75	0.54							2.750
137	7-Dec	8.6	3.75	0.44	1.1	63			0.40		350
138											
138	8-Dec	8.5	3.75	0.44							
140	10-Dec	9.2	3.75	0.41	3.3	21	2.065	1.94	0.45	0.42	350
141											
142	12-Dec	8.0	3.75	0.47							2.750
143	13-Dec	8.0	3.75	0.47	2.0	35	2.069		0.44		350
144	14-Dec	7.8	3.75	0.48							
145	15-Dec	8.4	3.75	0.45					0.64		350
146	16-Dec	8.4	3.75	0.44	5.8	12					2.875 (90 mL for sampling)

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.:	8.4	3.75	0.45	3	28	2.067	1.9	0.48	0.4	2.795 Average SBH (L)
STD:	0.5		0.02	0.7	7	0.002		0.09		
STD of Ave. (%):	5		5	24	24	0.08		20		

APPENDIX L.6: AERATION BASIN OPERATION CONDITIONS

TABLE L.6 a i): AB1 Conditions and Performance During Start-up (Day 1-17)

AB1		(Q _{AB1} -Q _{AD1 EM})	(V _{CL1} -11L)	(Q _{eff} -X _{eff})			TOTAL	TOTAL	SOLUBLE	SOLUBLE
DAY	DATE	(L/d) Q _{AB1}	(L) V _{AB1}	(d) HRT _{AB1}	(g VSS/d) Washout _{AB1}	(d) SRT _{AB1}	(g COD _{fed} /L·d) OLR _{AB1}	(g COD _{rem} /L·d) VRR _{AB1}	(g COD _{fed} /L·d) OLR _{AB1}	(g COD _{rem} /L·d) VRR _{AB1}
1	24-Jul	Feed on 6:30PM	2.0							
2	25-Jul	28	2.0	0.07			1.11		1.11	
3	26-Jul	12	2.0	0.17			1.92			
4	27-Jul	15	2.0	0.13			3.56		0.61	
5	28-Jul	(AD1 pump died)	2.0							
6	29-Jul	15	2.0	0.13						
8	31-Jul	18	2.0	0.11	0.4	1				
9	1-Aug	15	2.0	0.13			1.66			
10	2-Aug	18	2.0	0.11			1.74			
16	8-Aug	16	2.0	0.13			1.14			
17	9-Aug	19	2.0	0.11			2.12			

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE. 17 2.0 0.12
 STD: 2 0.01
 STD of Ave. (%): 9 9

TABLE L.6 b i): AB1 Conditions and Performance During Run 1 (Days 18-61)

AB1		(Q _{AB1} -Q _{AD1 EM})	(V _{CL1} -11L & 0.7L)	(Q _{eff} -X _{eff})			TOTAL	TOTAL	SOLUBLE	SOLUBLE
DAY	DATE	(L/d) Q _{AB1}	(L) V _{AB1}	(d) HRT _{AB1}	(g VSS/d) Washout _{AB1}	(d) SRT _{AB1}	(g COD _{fed} /L·d) OLR _{AB1}	(g COD _{rem} /L·d) VRR _{AB1}	(g COD _{fed} /L·d) OLR _{AB1}	(g COD _{rem} /L·d) VRR _{AB1}
18	10-Aug	8.57	2.0	0.23	0.020					
25	17-Aug	2.86	2.0	0.70			0.19			
26	18-Aug	1.59	2.0	1.26			0.09			
28	20-Aug	1.21	2.0	1.65			0.06			
29	21-Aug	4.89	2.0	0.41	0.013		0.31			
30	22-Aug	5.83	2.0	0.34			0.36			
31	23-Aug	6.09	2.0	0.33	0.051		0.47			
32	24-Aug	5.63	2.0	0.36			0.35			
33	25-Aug	6.12	2.0	0.33						
34	26-Aug	6.10	2.0	0.33	0.013		0.78	0.72	0.11	0.10
35	27-Aug	6.15	2.0	0.33						
*36	28-Aug	5.66	2.0	0.35	0.012	18	0.50		0.30	
36										
37	29-Aug	5.74	2.0	0.35						
38	30-Aug	6.07	2.0	0.33	0.017	18	0.40		0.12	
39	31-Aug	5.95	2.0	0.34						
40	1-Sep	5.83	2.0	0.34	0.104	5			0.16	
42	3-Sep	6.87	2.0	0.29	0.086	4	1.52	1.38	0.15	0.14
43	4-Sep		2.0							
44	5-Sep	5.71	2.0	0.35	0.014					
45	6-Sep	OFF/3.5 L ABs	2.8		MODIFICATIONS			TO	ABs	
46	7-Sep	1.58	2.8	1.77	0.017					
47	8-Sep	2.29	2.8	1.22						
48	9-Sep	1.78	2.8	1.57	0.053	8				
49	10-Sep	2.38	2.8	1.18	0.005					
49										
50	11-Sep	2.28	2.8	1.23	0.009		0.29	0.24	0.08	
51	12-Sep	2.22	2.8	1.26	0.007					
61	22-Sep	OFF	2.8							

*Started batch recycling SS back to AB1 on Day 36.

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE. 6.0 2.0 0.34 0.014 18 0.5 0.7 0.13 0.12
 STD: 0.3 0.02 0.002 0.06 0.2 0.03 0.02
 STD of Ave. (%): 6 5 15 0.33 34 23 18

APPENDIX L.6: AERATION BASIN OPERATION CONDITIONS

TABLE L.6 c i): AB1 Conditions and Performance During Run 2 (Days 62-113)

AB1		($Q_{AB1}=Q_{AD1}$)	($V_{CL1} \sim 0.7L$)	($Q_{em} \cdot X_{em}$)			TOTAL	TOTAL	SOLUBLE	SOLUBLE
DAY	DATE	(L/d) Q_{AB1}	(L) V_{AB1}	(d) HRT_{AB1}	(g VSS/d) $Washout_{AB1}$	(d) SRT_{AB1}	(g COD _{fed} /L-d) OLR_{AB1}	(g COD _{rem} /L-d) VRR_{AB1}	(g COD _{fed} /L-d) OLR_{AB1}	(g COD _{rem} /L-d) VRR_{AB1}
62	23-Sep	OFF	2.8							
63	24-Sep	2.4	2.8	1.18	0.02					
65	26-Sep	2.3	2.8	1.24	0.05	7	0.13	0.12	0.07	0.063
66	27-Sep	2.2	2.8	1.26	0.12					
67	28-Sep	2.7	2.8	1.05	0.06	6	0.26	0.20	0.08	0.058
68	29-Sep	2.6	2.8	1.08	0.08	7				
69	30-Sep	2.8	2.8	0.99	0.57	0.7				
70	1-Oct	2.3	2.8		0.02	18				
71	2-Oct	3.2	2.8	0.89	0.07	4			0.09	
72	3-Oct	4.2	2.8	0.67	0.06		1.16	1.10	0.10	0.10
73	4-Oct	4.1	2.8	0.69	0.09	4				
74	5-Oct	4.2	2.8	0.67	0.10	3				
75	6-Oct	4.3	2.8	0.66	0.09	4			0.06	
76										
77										
78	9-Oct	3.9	2.8	0.71	0.06	7			0.08	
79	10-Oct	4.7	2.8	0.59	0.19	2			0.08	
80	11-Oct	4.5	2.8	0.62	0.09	4				
81	12-Oct	4.1	2.8	0.68	0.11	3				
82	13-Oct	4.8	2.8	0.58					0.12	
83	14-Oct	4.3	2.8	0.65						
84	15-Oct	4.6	2.8	0.61	0.14					
85	16-Oct	4.6	2.8	0.62						
86	17-Oct	4.3	2.8	0.66	0.11				0.11	
87	18-Oct	4.2	2.8	0.67	0.09	8	0.84		0.09	
88										
89										
90	21-Oct	4.2	2.8	0.66	0.11	3	0.67	0.62	0.06	0.05
91	22-Oct	4.1	2.8	0.68						
92	23-Oct	3.9	2.8	0.73					0.06	
93	24-Oct									
94	25-Oct									
95		3.9	2.8	0.72	0.14	4	1.74	1.70	0.12	0.12
96	27-Oct									
97		4.4	2.8	0.64	0.14	4	2.42	2.33	0.13	0.12
98	29-Oct	3.9	2.8	0.72						
99	30-Oct									
100		4.0	2.8	0.70	0.32	3	2.45	2.39	0.14	0.13
101	1-Nov	4.5	2.8	0.62	0.14	6	0.89	0.79	0.06	0.05
102	2-Nov	4.5	2.8	0.62						
103										
104	4-Nov	4.4	2.8	0.63						
105	5-Nov	4.2	2.8	0.67	0.12	5	0.68	0.65	0.08	0.07
106										
107										
108	8-Nov									
109	9-Nov	4.4	2.8	0.64	0.60	1			0.32	
110										
111	13-Nov	3.7	2.8	0.77	1.47	1			0.27	

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.	4.3	2.8	0.66	0.11	4	0.9	0.8	0.09	0.07
STD:	0.3		0.04	0.04	2	0.2	0.2	0.03	0.02
STD of Ave. (%)	6		7	36	39	24	28	30	29

TABLE L.6 d i): AB1 Conditions and Performance During Run 3 (Days 114-146)

AB1		($Q_{AB1}=Q_{AD1}$)	($V_{CL1} \sim 0.7L$)	($Q_{em} \cdot X_{em}$)			TOTAL	TOTAL	SOLUBLE	SOLUBLE
DAY	DATE	(L/d) Q_{AB1}	(L) V_{AB1}	(d) HRT_{AB1}	(g VSS/d) $Washout_{AB1}$	(d) SRT_{AB1}	(g COD _{fed} /L-d) OLR_{AB1}	(g COD _{rem} /L-d) VRR_{AB1}	(g COD _{fed} /L-d) OLR_{AB1}	(g COD _{rem} /L-d) VRR_{AB1}
114	14-Nov		2.8							
120	20-Nov		2.8							
124	24-Nov									
124		4.5	2.8	0.62	0.7	0.5			0.19	
131	1-Dec									
132	2-Dec	4.1	2.8	0.68	0.3	7	2.02	1.92	0.13	0.13
134	4-Dec									
134		6.1	2.8	0.46	2.8	2	0.47	0.39	0.08	0.07
135	5-Dec	7.2	2.8	0.39						
137	7-Dec									
137		8.4	2.8	0.33	1.0	5	2.04	1.86	0.38	0.35
138	8-Dec	8.9	2.8	0.31						
140	10-Dec									
140		9.6	2.8	0.29	1.9	5	1.99	1.79	0.30	0.27
142	12-Dec	8.8	2.8	0.32			9.80	9.64	0.40	0.40
143	13-Dec	8.8	2.8	0.32	9.8	1				
144	14-Dec	8.3	8.0	0.34						
145	15-Dec	8.8	2.8	0.32			0.91	0.69	0.24	0.18
146	16-Dec	8.9	2.8	0.32	1.1	3				

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.	8.8	2.8	0.32	1.3	4	1.7	1.5	0.33	0.27
STD:	0.4		0.01	0.5	1	0.6	0.7	0.08	0.08
STD of Ave. (%)	4		4	36	29	38	45	23	30

APPENDIX L.6: AERATION BASIN OPERATION CONDITIONS

TABLE L.6 a ii): AB2 Conditions and Performance During Start-up (Day 1-17)

AB2		($Q_{AB2}=Q_{AD2}$ Eff.)	($V_{CL2}=13L$)	$(Q_{eff} \cdot X_{eff})$		(VSS)	TOTAL	TOTAL	SOLUBLE	SOLUBLE
		(L/d)	(L)	(d)	(g VSS/d)	(d)	(g COD _{fed} /L-d)	(g COD _{rem} /L-d)	(g COD _{fed} /L-d)	(g COD _{rem} /L-d)
DAY	DATE	Q_{AB2}	V_{AB2}	HRT _{AB2}	Washout _{AB2}	SRT _{AB2}	OLR _{AB2}	VRR _{AB2}	OLR _{AB2}	VRR _{AB2}
1	24-Jul	Feed on 8:30PM	2.0							
2	25-Jul	off (AD2 pump died)	2.0							
3	26-Jul	28.1	2.0	0.07			2.25		2.25	
4	27-Jul	22.0	2.0	0.09	0.14		2.29		1.76	
5	28-Jul	15.0	2.0	0.13			1.32		2.58	
6	29-Jul	16.0	2.0	0.13						
7	30-Jul	16.0	2.0	0.13	0.10	2				
8	31-Jul	16.0	2.0	0.13						
9	1-Aug	16.0	2.0	0.13			1.23			
10	2-Aug	18.0	2.0	0.11			1.28			
16	8-Aug	24.0	2.0	0.08			1.31			
17	9-Aug	18.5	2.0	0.11			1.65			

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.: 18 2.0 0.12
 STD: 3 0.02
 STD of Ave. (%): 17 15

TABLE L.6 b ii): AB2 Conditions and Performance During Run 1 (Days 18-61)

AB2		(Q _{AB2} =Q _{AD2} Eff.)	V _{CL2} =13 L (and 0.7 L)	(on Sept. 8)	(Q _{eff} ·X _{eff})	(VSS)	TOTAL	TOTAL	SOLUBLE	SOLUBLE
		(L/d)	(L)	(d)	(g VSS/d)	(d)	(g COD _{fed} /L·d)	(g COD _{rem} /L·d)	(g COD _{fed} /L·d)	(g COD _{rem} /L·d)
DAY	DATE	Q _{AB2}	V _{AB2}	HRT _{AB2}	Washout _{AB2}	SRT _{AB2}	OLR _{AB2}	VRR _{AB2}	OLR _{AB2}	VRR _{AB2}
18	10-Aug	8.1	2.0	0.25	0.11					
25	17-Aug	2.9	2.0	0.70			0.11			
26	18-Aug	3.1	2.0	0.64	0.01	22	0.10			
28	20-Aug	0.5	2.0	4.08			0.02			
29	21-Aug	5.0	2.0	0.40	0.02		0.16			
30	22-Aug	8.0	2.0	0.25			0.45			
31	23-Aug	5.9	2.0	0.34	0.02		0.37			
32	24-Aug	6.4	2.0	0.31			0.58			
33	25-Aug	6.2	2.0	0.32						
34	26-Aug	7.3	2.0	0.27	0.02				0.17	
35	27-Aug	6.2	2.0	0.32						
*36	28-Aug	8.2	2.0	0.24	0.004	46			0.14	
36										
37	29-Aug	6.8	2.0	0.30						
38	30-Aug	5.6	2.0	0.36	0.002	82	0.48	0.40	0.13	0.11
39	31-Aug	5.2	2.0	0.39						
40	1-Sep	5.3	2.0	0.38	0.002	65	0.60	0.50	0.12	0.10
42	3-Sep	6.7	2.0	0.30	0.03	5	0.35	0.22	0.26	0.16
43	4-Sep	5.8	2.0	0.34						
44	5-Sep	5.6	2.0	0.36	0.002					
45	6-Sep	OFF/3.5 L ABs	2.8		MODIFICATIONS			TO	ABs	
46	7-Sep	1.8	2.8	1.52	0.02		0.28	0.21	0.10	0.07
47	8-Sep	2.6	2.8	1.08						
48	9-Sep	2.9	2.8	0.97	0.08	7	0.31	0.19	0.16	0.10
49	10-Sep	3.0	2.8	0.92	0.15					
49										
50	11-Sep	2.7	2.8	1.03	0.01		0.34	0.31	0.06	0.06
51	12-Sep	2.1	2.8	1.34	0.004					
61	22-Sep	OFF (Sept. 13-22)	2.8							

*Started batch recycling SS back to AB1 on Day 36.

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.: 6.2 2.0 0.33 0.003 65 0.5 0.45 0.16 0.103
 STD: 0.8 0.04 0.001 18 0.1 0.05 0.06 0.005
 STD of Ave. (%): 13 13 37 28 24 11 34 5

APPENDIX L.6: AERATION BASIN OPERATION CONDITIONS

TABLE L.6 c ii): AB2 Conditions and Performance During Run 2 (Days 62-113)

AB2		($Q_{AB2}=Q_{AD2}$)			$(Q_{eff} \cdot X_{eff})$ (VSS)		TOTAL	TOTAL	SOLUBLE	SOLUBLE
DAY	DATE	(L/d) Q_{AB2}	(L) V_{AB2}	(d) HRT_{AB2}	(g VSS/d) Washout $_{AB2}$	(d) SRT_{AB2}	(g COD $_{fed}$ /L-d) OLR_{AB2}	(g COD $_{rem}$ /L-d) VRR_{AB2}	(g COD $_{fed}$ /L-d) OLR_{AB2}	(g COD $_{rem}$ /L-d) VRR_{AB2}
62	23-Sep	OFF	2.8							
63	24-Sep	2.7	2.8	1.04	0.31					
65	26-Sep	2.2	2.8	1.26	0.07	8	0.42	0.37	0.064	0.06
66	27-Sep	1.8	2.8	1.56	0.07					
67	28-Sep	5.2	2.8	0.54	0.02	17	0.86	0.71	0.19	0.16
68	29-Sep	2.6	2.8	1.07	0.11	3				
69	30-Sep	2.4	2.8	1.19	0.56	1	0.32	0.29	0.05	0.04
70	1-Oct	3.8	2.8	0.60	0.19	1				
71	2-Oct	4.0	2.8	0.69	0.21	1			0.10	
72	3-Oct	4.7	2.8	0.60	0.05					
73	4-Oct	4.8	2.8	0.58	0.10	3	0.51	0.42	0.14	0.11
74	5-Oct	4.3	2.8	0.65	0.12	3				
75	6-Oct	5.1	2.8	0.55	0.11	2	1.66	1.59	0.14	0.14
76										
78	9-Oct	3.0	2.8	0.92	0.08	4	0.71	0.67	0.06	0.06
79	10-Oct	4.4	2.8	0.63	0.13	2				
80	11-Oct	3.9	2.8	0.73	0.19	1				
81	12-Oct	4.2	2.8	0.67	0.42	1				
82	13-Oct	4.3	2.8	0.65						
83										
84	14-Oct	2.4	2.8	1.17						
85	15-Oct	2.1	2.8	1.31	0.12					
86	16-Oct	3.1	2.8	0.91						
87	17-Oct	5.1	2.8	0.55	0.15		1.58	1.53	0.14	0.13
88	18-Oct	2.9	2.8	0.97	0.25		0.18		0.05	
89										
90	21-Oct	2.3	2.8	1.21	0.08	8	0.25	0.22		
91	22-Oct	5.5	2.8	0.51						
92	23-Oct	5.6	2.8	0.50	0.56	1	0.85	0.80	0.10	0.09
93										
94	25-Oct	3.8	2.8	0.74	0.18	3	0.39	0.35	0.06	0.06
95										
96	27-Oct	6.0	2.8	0.47	0.51	1	2.31	2.24	0.19	0.19
97										
98	29-Oct	4.0	2.8	0.70						
99	30-Oct									
100										
101	1-Nov	4.8	2.8	0.58	0.21	5	0.39	0.33	0.09	0.08
102	2-Nov	3.4	2.8	0.83	0.36	2	0.36	0.31	0.08	0.07
103		3.0	2.8	0.95						
104	4-Nov	2.3	2.8	1.21						
105	5-Nov	4.6	2.8	0.61	0.41	3	0.70	0.66	0.05	0.05
106										
108	8-Nov									
109	9-Nov	2.9	2.8	0.96						
110										
111	9-Nov	2.3	2.8	1.22	0.23	6	0.63	0.61	0.05	0.05
112										
113	13-Nov	5.2	2.8	0.54	0.25	3	2.49	2.37	0.20	0.19

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.:	4	2.8	0.8	0.14	4	0.5	0.4	0.08	0.08
STD:	1		0.2	0.05	2	0.2	0.2	0.03	0.03
STD of Ave. (%):	27		30	36	46	38	47	39	33

TABLE L.6 d ii): AB2 Conditions and Performance During Run 3 (Days 114-146)

AB2		($Q_{AB2}=Q_{AD2}$ EM.)	$V_{CL2}=0.7L$		$(Q_{eff} \cdot X_{eff})$ (VSS)		TOTAL	TOTAL	SOLUBLE	SOLUBLE
DAY	DATE	(L/d) Q_{AB2}	(L) V_{AB2}	(d) HRT_{AB2}	(g VSS/d) Washout $_{AB2}$	(d) SRT_{AB2}	(g COD $_{fed}$ /L-d) OLR_{AB2}	(g COD $_{rem}$ /L-d) VRR_{AB2}	(g COD $_{fed}$ /L-d) OLR_{AB2}	(g COD $_{rem}$ /L-d) VRR_{AB2}
114	14-Nov		2.8							
120	20-Nov		2.8							
121										
124	24-Nov									
125										
131	1-Dec	4.2	2.8	0.67	0.64	1	0.45	0.40	0.06	0.051
132	2-Dec	5.7	2.8	0.49	0.26	3	4.84	4.74	0.12	0.113
134	4-Dec	7.3	2.8	0.38	0.18	5	2.18	2.08	0.13	0.120
135	5-Dec	7.0	2.8	0.40						
137	7-Dec	8.6	2.8	0.33	0.27	6	2.44	2.36	0.53	0.51
138	8-Dec	8.5	2.8	0.33						
140	10-Dec	9.2	2.8	0.30	0.13	15	6.29	6.16	0.16	0.158
141										
142	12-Dec	8.0	2.8	0.35			3.13	3.07	0.11	0.106
143	13-Dec	8.0	2.8	0.35	0.09	11				
144	14-Dec	7.8	2.8	0.36						
145	15-Dec	8.4	2.8	0.33			4.62	4.56	0.13	0.128
146	16-Dec	8.4	2.8	0.33	1.05	3				

Shaded areas were not considered in the parameter averages and standard deviations. Results were based on data where influent flows were considered stable during the run.

AVE.:	8.4	2.8	0.34	0.11	13	5	5	0.13	0.13
STD:	0.5		0.02	0.02	2	2	2	0.03	0.03
STD of Ave. (%):	5		5	16	16	34	34	20	20

APPENDIX L.7: UASB REACTOR VFA CONCENTRATION DATA

TABLE L.7 a): VFA Concentration Results During Start-Up (Days 1-17)

Date	DAY	AD1- Anaerobic Digester 1			TOTAL VFA _{AD1} mg/L	AD1 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD1} ALK _{AD1}	AD2- Anaerobic Digester 2			TOTAL VFA _{AD2} mg/L	AD2 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD2} ALK _{AD2}
21-Jul	Anaerobic Sludge & TWAS in INITIAL Reading	Acetic	Propionic	Butyric				Acetic	Propionic	Butyric			
22-Jul		1.851	1.385	ND	3.236			0.680	1.161	ND	1.841		
23-Jul		ND	ND	ND	NA			ND	ND	ND	NA		
24-Jul	1	ND	ND	ND	ND			ND	1.492	ND	1.492		
25-Jul	2	3.112	3.705	ND	6.817			ND	1.754	ND	1.754		
26-Jul	3	1.719	0.577	ND	2.296			ND	1.837	ND	1.837		
27-Jul	4	2.358	3.304	ND	5.662			ND	1.593	ND	1.593		
28-Jul	5	2.475	2.370	ND	4.845			ND	1.344	ND	1.344		
29-Jul	6	1.371	2.284	ND	3.655			ND	1.044	ND	1.044		
30-Jul	7	3.691	4.740	ND	8.431	287	0.03	ND	2.621	ND	2.621	183	0.01
31-Jul	8	ND	1.800	ND	1.800			ND	1.559	ND	1.559		
1-Aug	9	2.398	3.775	ND	6.173	230	0.03	ND	1.745	ND	1.745	178	0.01
2-Aug	10	3.355	4.487	ND	7.842	417	0.02	ND	1.962	ND	1.962	292	0.01
3-Aug	11	10.881	8.556	47.036	66.473	497	0.13	0.635				385	
4-Aug	12	(VFA machine down)											
7-Aug	15	11.602	16.602	61.888	90.092	687	0.14	12.407	15.771	66.099	94.277	332	0.26
8-Aug	16	18.413	18.441	61.800	98.654	1088	0.11	13.878	12.723	59.039	85.640	500	0.20
9-Aug	17	24.052	23.733	67.303	115.088			18.244	19.903	64.126	102.273		

ND-Not Detected

START-UP

TABLE L.7 b): VFA Concentration Results During Run 1 (Days 18-61)

Date	DAY	AD1- Anaerobic Digester 1			TOTAL VFA _{AD1} mg/L	AD1 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD1} ALK _{AD1}	AD2- Anaerobic Digester 2			TOTAL VFA _{AD2} mg/L	AD2 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD2} ALK _{AD2}
10-Aug	18	Acetic	Propionic	Butyric				Acetic	Propionic	Butyric			
11-Aug	19	3.112	4.217	ND	7.329			2.117	3.459	ND	5.576		
14-Aug	22	2.648	4.155	ND	6.803			2.319	4.545	ND	6.864		
17-Aug	25	11.086	9.109	44.506	64.701	1268	0.04	4.162	5.870	37.141	47.123	1225	0.004
18-Aug	26	7.494	6.940	40.539	54.973			2.529	2.191	ND	4.720		
20-Aug	28	5.533	5.526	ND	11.059			ND	2.392	ND	2.392		
22-Aug	30	3.503	4.496	ND	7.999			ND	2.433	ND	2.433		
24-Aug	32	ND	1.168	ND	1.168			ND	1.015	ND	1.015		
26-Aug	34	ND	ND	ND	NA	720	NA	ND	1.091	ND	1.091	325	0.003
28-Aug	36	ND	1.974	ND	1.974	585	0.003	ND	ND	ND	NA	245	NA
30-Aug	38	ND	2.054	ND	2.054			ND	1.145	ND	1.145		
1-Sep	40	ND	1.112	ND	1.112			ND	1.733	ND	1.733		
3-Sep	42	ND	ND	ND	NA			ND	ND	ND	NA		
5-Sep	44	ND	0.921	ND	0.921	227	0.007	ND	ND	ND	NA	100	NA
6-Sep	45	1.658	ND	ND	1.658			ND	ND	ND	NA		
7-Sep	46	5.107	3.467	ND	8.574			ND	1.163	ND	1.163		
9-Sep	48	ND	ND	ND	NA			ND	ND	ND	NA		
11-Sep	50	ND	ND	ND	NA			ND	ND	ND	NA		
12-Sep	51	ND	ND	ND	NA	133		ND	ND	ND	NA	116	
22-Sep	61												

Shaded areas were not considered in the maintained run condition parameter averages and standard deviations. They are based only on VFA concentrations that were detected once stable flows were reached during the run.

ND-Not Detected; NA- Not Applicable

RUN 1

AVERAGE

STD

3

2

ND

2

NA

0.005

0.002

ND

1.2

0.3

ND

1.2

0.3

NA

0.003

NA

APPENDIX L.7: UASB REACTOR VFA CONCENTRATION DATA

TABLE L.7 c): VFA Concentration Results During Run 2 (Days 62-113)

Date	DAY	AD1- Anaerobic Digester 1			TOTAL VFA _{AD1} mg/L	AD1 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD1} ALK _{AD1}	AD2- Anaerobic Digester 2			TOTAL VFA _{AD2} mg/L	AD2 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD2} ALK _{AD2}
		Acetic	Propionic	Butyric				Acetic	Propionic	Butyric			
23-Sep	62	11,286	9,330	42,653	63,269	440	0.14	7,455	4,801	31,040	43,296	410	0.11
24-Sep	63	6,698	3,567	ND	10,265	585	0.02	4,203	2,756	ND	6,959	470	0.01
26-Sep	65	21,964	15,701	26,898	64,563			14,360	11,394	38,883	64,637		
28-Sep	67	10,178	8,935	33,617	52,730			6,847	6,378	29,870	43,095		
30-Sep	69	4,105	2,798	ND	6,903			4,382	2,095	ND	6,477		
2-Oct	71	10,666	8,306	46,007	64,979			16,384	11,389	47,491	75,264		
5-Oct	74	8,758	5,070	ND	13,828			5,658	3,586	ND	9,244		
7-Oct	76	4,596	3,725	ND	8,321	520	0.02	3,978	3,218	ND	7,196	390	0.02
9-Oct	78	8,003	2,474	ND	10,477	357	0.03	6,111	2,323	ND	8,434	312	0.03
11-Oct	80	8,711	5,505	ND	14,216			8,325	5,124	ND	13,449		
13-Oct	82	39,920	11,879	41,155	92,954			13,724	4,417	ND	18,141		
15-Oct	85	7,771	8,401	43,090	59,262			4,965	4,018	ND	8,983		
18-Oct	87	3,670	2,684	ND	6,354			1,426	1,362	ND	2,788		
20-Oct	89	8,058	3,667	ND	11,725			2,379	ND	ND	2,379		
23-Oct	92	3,841	3,917	ND	7,758			1,060	1,614	ND	2,674		
25-Oct	94	5,241	4,693	ND	9,934			3,784	3,359	ND	7,143		
30-Oct	99	8,977	6,644	41,052	56,673			4,548	4,086	ND	8,634		
7-Nov	107	4,652	3,081	ND	7,733			0,860	1,741	ND	2,601		
13-Nov	113												

Shaded areas were not considered in the maintained run condition parameter averages and standard deviations. They are based only on VFA concentrations that were detected once stable flows were reached during the run.
 ND-Not Detected; NA- Not Applicable

RUN 2
 AVERAGE: 9 5 43 28 0.02 6 4 47 13 0.023
 STD: 9 3 2 29 0.01 5 3 NA 19 0.004

TABLE L.7 d): VFA Concentration Results During Run 3 (Days 114-146)

Date	DAY	AD1- Anaerobic Digester 1			TOTAL VFA _{AD1} mg/L	AD1 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD1} ALK _{AD1}	AD2- Anaerobic Digester 2			TOTAL VFA _{AD2} mg/L	AD2 Em. ALK. mg/L as CaCO ₃	TOT. VFA _{AD2} ALK _{AD2}
		Acetic	Propionic	Butyric				Acetic	Propionic	Butyric			
14-Nov	114	0,767	1,374	ND	2,141	748	0.03	1,525	1,064	ND	2,589		
18-Nov	118	13,319	10,267	ND	23,586			5,929	4,972	ND	10,901		
3-Dec	133	17,677	19,306	ND	36,983	693	0.05	8,316	3,318	ND	11,634	712	0.02
7-Dec	137	8,371	10,511	ND	18,882	617	0.01	0,205	ND	ND	0,205	654	0.02
11-Dec	141	1,238	6,483	ND	7,721	580	0.08	1,354	2,103	ND	3,457	460	0.01
12-Dec	142	7,289	39,599	ND	46,888	683		1,316	1,076	ND	2,392	754	0.003
15-Dec	145												
16-Dec	146												

Shaded areas were not considered in the maintained run condition parameter averages and standard deviations. They are based only on VFA concentrations that were detected once stable flows were reached during the run.
 ND-Not Detected; NA- Not Applicable

RUN 3
 AVERAGE: 9 19 ND 28 0.05 3 2 ND 4 0.01
 STD: 7 15 18 0.03 4 1 5 0.01

APPENDIX L.8: UASB REACTOR BIOGAS PRODUCTION DATA

TABLE L.8 a): Anaerobic Biogas Production During Start-Up (Days 1-17)

DAY	DATE	AD1 (Battery counter) Wet Tip Meter Readings	AD2 (Digital counter) Wet Tip Meter Readings	AD1 Cumulative Gas Prod. (mL) 76 mL/dp	AD2 Cumulative Gas Prod. (mL) 40 mL/dp	AD1 Gas Production (mL) 76 mL/dp	AD2 Gas Production (mL) 40 mL/dp	(-Morning Room Temperature (°C)	Readings) Refrigerator Temperature (°C)
		17686	2794	0	0	0	0	23.8	4.0
1	24-Jul	17688	2794	152	0	152	0	23.8	6.0
2	25-Jul	17689	2794	228	0	76	0	23.9	5.5
3	26-Jul	17689	2797	228	120	0	120	23.8	6.0
4	27-Jul	17689	2797	228	120	0	0	24.1	5.0
5	28-Jul	17689	2797	228	120	0	0	24.0	4.5
6	29-Jul	17689	2800	228	240	0	120	24.2	7.0
7	30-Jul	17689	2804	228	400	0	160	24.5	
8	31-Jul	17689	2806	228	480	0	80	24.4	4.0
9	1-Aug	17689	2806	228	480	0	0	24.4	3.5
10	2-Aug	17692	2808	456	560	228	80	24.2	4.0
11	3-Aug	17692	2808	456	560	0	0		
12	4-Aug	17692	2808	456	560	0	0		
13	5-Aug	17692	2808	456	560	0	0		
14	6-Aug	17692	2808	456	560	0	0		
15	7-Aug	17692	2808	456	560	0	0		
16	8-Aug	17692	2808	456	800	0	40	24.2	2.5
17	9-Aug	17692	2810	456	840	0	40	24.1	4.0

Shaded areas were not considered for maintained influent flow condition values reported in Tables 4.5 a) and b).

START-UP	Total Biogas Production (mL):	456	640	Ave. Temperature:	24.1	4.7
	Ave. Biogas Production (L/d):	0.027	0.038		STD:	0.2
	Ave. Biogas Production at Stable Influent Flows (L/d):	0.018	0.043			1.3

TABLE L.8 b): Anaerobic Biogas Production During Run 1 (Days 18-61)

DAY	DATE	AD1 (Battery counter) Wet Tip Meter Readings	AD2 (Digital counter) Wet Tip Meter Readings	AD1 Cumulative Gas Prod. (mL) 76 mL/dp	AD2 Cumulative Gas Prod. (mL) 40 mL/dp	AD1 Gas Production (mL) 76 mL/dp	AD2 Gas Production (mL) 40 mL/dp	(-Morning Room Temperature (°C)	Readings) Refrigerator Temperature (°C)
		17686	2810	760	840	304	0	23.8	5.0
18	10-Aug	17686	2810	812	840	152	0	23.7	
19	11-Aug	17688	2810	812	840	0	0		
20	12-Aug	17688	2810	812	840	0	0		
21	13-Aug	17688	2810	812	840	0	0	23.9	
22	14-Aug	17688	2810	812	840	0	0		
23	15-Aug	17688	2810	812	840	0	0	23.8	3.0
24	16-Aug	17688	2812	812	720	0	80	24.0	3.5
25	17-Aug	17703	2812	1292	720	380	0	23.6	3.0
26	18-Aug	17703	2812	1292	720	0	0		
27	19-Aug	17703	2812	1292	720	0	0		
28	20-Aug	17703	2812	1292	720	0	0	23.4	2.5
29	21-Aug	17703	2812	1292	720	0	0	21.8	3.5
30	22-Aug	17703	2813	1292	760	0	40	20.9	3.0
31	23-Aug	17703	2813	1292	760	0	0	21.2	2.5
32	24-Aug	17703	2818	1292	960	0	200	20.9	3.0
33	25-Aug	17703	2822	1292	1120	0	160	23.0	4.0
34	26-Aug	17703	2822	1292	1120	0	0	23.7	5.1
35	27-Aug	17703	2822	1292	1120	0	0	23.6	5.0
36	28-Aug	17709	2822	1748	1120	456	0	23.9	5.0
37	29-Aug	17710	2822	1824	1120	76	0	24.1	5.0
38	30-Aug	17710	2822	1824	1120	0	0	24.2	5.0
39	31-Aug	17710	2822	1824	1120	0	0	24.2	5.0
40	1-Sep	17710	2826	1824	1280	0	160	23.9	10.0
41	2-Sep	17710	2826	1824	1280	0	0		
42	3-Sep	17710	2840	1824	1940	0	560	24.2	8.5
43	4-Sep	17710	2841	1824	1880	0	40	23.8	8.0
44	5-Sep	17710	2841	1824	1880	0	0	23.5	7.5
45	6-Sep	17710	2842	1824	1920	0	40		
46	7-Sep	17710	2857	1824	2520	0	600	23.7	9.5
47	8-Sep	17710	2872	1824	3120	0	600	23.6	8.0
48	9-Sep	17710	2890	1824	3840	0	720	23.7	7.0
49	10-Sep	17710	2913	1824	4760	0	920	23.7	7.0
50	11-Sep	17711	2935	1824	5640	0	880	24.1	7.0
51	12-Sep	17711	2946	1824	6080	0	440	24.3	7.5
52	13-Sep	17711	2946	1824	6080	0	0		
53	14-Sep	17711	2946	1824	6080	0	0		
54	15-Sep	17711	2946	1824	6080	0	0		
55	16-Sep	17711	2946	1824	6080	0	0		
56	17-Sep	17711	2946	1824	6080	0	0		
57	18-Sep	17711	2963	1824	6760	0	680		
58	19-Sep	17711	2963	1824	6760	0	0		
59	20-Sep	17711	2963	1824	6760	0	0		
60	21-Sep	17711	2963	1824	6760	0	0		
61	22-Sep	17717	2963	1824	6760	0	0	23.5	4.0

*Checked functioning of battery counter wet tip meter. Shaded areas were not considered for maintained influent flow condition values reported in Tables 4.5 a) and b).

RUN 1	Total Biogas Production (mL):	1368	6120	Ave. Temperature:	23.4	5.4
	Ave. Biogas Production for Run 1 (L/d):	0.031	0.14		STD:	1.0
	Ave. Biogas Production at Stable Influent Flows (L/d):	0.017	0.19			2.2

APPENDIX L.8: UASB REACTOR BIOGAS PRODUCTION DATA

TABLE L.8 c): Anaerobic Biogas Production During Run 2 (Days 62-113)

DAY	DATE	AD1	AD2	AD1	AD2	AD1	AD2	(-Morning Readings)	
		(Battery counter)	(Digital counter)	Cumulative Gas Prod.	Cumulative Gas Prod.	Gas Production	Gas Production	Room	Refrigerator
		Wet Tip Meter	Wet Tip Meter	(mL)	(mL)	(mL)	(mL)	Temperature	Temperature
		Readings	Readings	76 mL/tip	40 mL/tip	76 mL/tip	40 mL/tip	(°C)	(°C)
62	23-Sep	17717	2963	1824	6760	0	0	23.5	3.0
63	24-Sep	17725	2963	2432	6760	608	0	23.4	7.0
64	25-Sep	17730	2963	2812	6760	380	0	23.1	7.5
65	26-Sep	17746	2963	4028	6760	1216	0	23.1	6.5
*66	27-Sep	17788	2988	4028	6760	0	0	23.3	6.0
67	28-Sep	17785	2988	4408	6760	380	0	23.0	7.0
68	29-Sep	17787	2988	4560	6760	162	0	22.8	7.0
69	30-Sep	17788	2988	4636	6760	76	0	24.1	7.0
70	1-Oct	17790	2989	4788	6800	152	40	24.7	6.5
71	2-Oct	17790	2989	4788	6800	0	0	24.5	6.0
72	3-Oct	17795	2990	5168	6840	380	40	24.5	7.0
73	4-Oct	17802	2990	5700	6840	532	0	24.4	7.0
74	5-Oct	17803	2990	5776	6840	76	0	23.8	6.5
*75	6-Oct	17812	3000	6460	6840	684	0	23.4	6.6
76	7-Oct	17812	3000	6460	6840	0	0	24.1	7.0
77	8-Oct	17812	3000	6460	6840	0	0		
78	9-Oct	17812	3000	6460	6840	0	0	24.1	6.5
79	10-Oct	17812	3000	6460	6840	0	0	23.3	7.0
80	11-Oct	17812	3000	6460	6840	0	0	23.6	6.0
81	12-Oct	17812	3000	6460	6840	0	0		6.5
82	13-Oct	17812	3000	6460	6840	0	0	24.3	6.0
83	14-Oct	17812	3000	6460	6840	0	0	24.5	7.0
84	15-Oct	17812	3000	6460	6840	0	0	24.5	7.0
85	16-Oct	17816	3000	6764	6840	304	0	23.9	7.0
86	17-Oct	17816	3000	6764	6840	0	0	23.5	7.5
87	18-Oct	17823	3000	7296	6840	532	0	23.8	6.5
88	19-Oct	17823	3000	7296	6840	0	0	24.2	7.0
89	20-Oct	17823	3000	7296	6840	0	0	23.9	6.5
90	21-Oct	17823	3000	7296	6840	0	0	24.6	6.5
91	22-Oct	17823	3000	7296	6840	0	0	24.0	7.0
92	23-Oct	17823	3000	7296	6840	0	0	23.4	7.0
93	24-Oct	17823	3000	7296	6840	0	0	23.7	6.0
94	25-Oct	17828	3000	7676	6840	380	0	23.9	6.0
95	26-Oct	17829	3000	7752	6840	76	0	24.0	6.0
96	27-Oct	17844	3000	8892	6840	1140	0	24.2	6.0
97	28-Oct	17844	3000	8892	6840	0	0	24.0	7.0
98	29-Oct	17862	3000	10280	6840	1368	0	23.8	
99	30-Oct	17862	3000	10280	6840	0	0	23.9	6.0
100	31-Oct	17873	3000	11096	6840	836	0	23.9	7.0
101	1-Nov	17873	3000	11096	6840	0	0	23.8	7.0
102	2-Nov	17873	3000	11096	6840	0	0	23.5	6.5
103	3-Nov	17873	3000	11096	6840	0	0	23.6	6.5
104	4-Nov	17873	3000	11096	6840	0	0	23.5	7.5
105	5-Nov	17873	3000	11096	6840	0	0	23.3	8.0
106	6-Nov	17873	3000	11096	6840	0	0	23.1	7.0
107	7-Nov	17873	3000	11096	6840	0	0	23.9	7.0
108	8-Nov	17873	3000	11096	6840	0	0	23.5	6.5
109	9-Nov	17873	3000	11096	6840	0	0		
113	13-Nov	17873	3000	11096	6840	0	0		

*Serviced both wet tips (Sept 27) and the digital one again, on Oct. 6, to make sure it was working properly. Shaded areas were not considered for maintained influent flow condition values reported in Tables 4.5 a) and b).

RUN 2		Total Biogas Production (mL):	9272	80	Ave. Temperature:		23.8	6.7
		Ave. Biogas Production for Run 2 (L/d):	0.18	0.0015	STD:		0.5	0.8
		Ave. Biogas Production at Stable Influent Flows (L/d):	0.17	0.0011				

TABLE L.8 d): Anaerobic Biogas Production During Run 3 (Days 114-146)

DAY	DATE	AD1	AD2	AD1	AD2	AD1	AD2	(-Morning Readings)	
		(Battery counter)	(Digital counter)	Cumulative Gas Prod.	Cumulative Gas Prod.	Gas Production	Gas Production	Room	Refrigerator
		Wet Tip Meter	Wet Tip Meter	(mL)	(mL)	(mL)	(mL)	Temperature	Temperature
		Readings	Readings	76 mL/tip	40 mL/tip	76 mL/tip	40 mL/tip	(°C)	(°C)
122	22-Nov	18167	3522	11096	6840	0	0		
123	23-Nov	18170	3522	11324	6840	228	0		
124	24-Nov	18172	3523	11476	6880	152	40	22.7	7.5
125	25-Nov	18182	3523	12236	6880	760	0	23.3	7.5
126	26-Nov	18182	3524	12236	6920	0	40		
127	27-Nov	18183	3524	12312	6920	76	0	23.5	7.0
128	28-Nov	18183	3524	12312	6920	0	0		
129	29-Nov	18183	3524	12312	6920	0	0		
130	30-Nov	18183	3524	12312	6920	0	0		
*131	1-Dec	3524	18184	12312	6996	0	76	23.2	6.5
132	2-Dec	3524	18186	12312	7148	0	152	22.7	8.0
133	3-Dec	3524	18186	12312	7148	0	0	22.7	8.0
134	4-Dec	3524	18181	12312	7528	0	380	22.6	7.5
135	5-Dec	3524	18181	12312	7528	0	0	23.1	7.0
136	6-Dec	3524	18183	12312	7680	0	152	23.0	8.0
137	7-Dec	3524	18184	12312	7756	0	76	22.0	8.0
138	8-Dec	3524	18184	12312	7756	0	0	22.5	8.0
139	9-Dec	3524	18184	12312	7756	0	0	22.1	4.5
140	10-Dec	3524	18185	12312	7832	0	76	22.2	4.0
141	11-Dec	3524	18186	12312	7908	0	76	22.5	7.0
142	12-Dec	3524	18186	12312	7908	0	0		
143	13-Dec	3524	18189	12312	8136	0	228	22.7	8.0
144	14-Dec	3524	18189	12312	8136	0	0	22.3	7.5
145	15-Dec	3524	18200	12312	8212	0	76	22.4	8.0
146	16-Dec	3524	18200	12312	8212	0	0	22.1	7.0

*Exchanged battery counter wet tip with digital counter wet tip and U-tube was removed in AD1. Effluent flowed directly to AB1. Shaded areas were not considered for maintained influent flow condition values reported in Tables 4.5 a) and b).

RUN 3		Total Biogas Production (mL):	1216	1372	Ave. Temperature:		22.6	7.3
		Ave. Biogas Production for Run 3 (L/d):	0.037	0.042	STD:		0.4	1.2
		Ave. Biogas Production at Stable Influent Flows (L/d):	0.00	0.053				
		Total Biogas Production:	12312 mL	8212 mL	Overall Average Temperature:		23.5	6.2
		Estimated Overall Ave. Biogas Production Rates:	0.08 L/d	0.06 L/d	STD:		0.8	1.6
			0.02 L/L _{avg} ·d	0.02 L/L _{avg} ·d				

APPENDIX L.9: UASB REACTOR BIOGAS COMPOSITION DATA

TABLE L.9 a): Biogas Composition Results, in %, During Start-Up (Days 1-17)

DAY	Date	AD1			AD2			(%) TOTAL	(%) TOTAL
		N ₂ (%)	CH ₄ (%)	CO ₂ (%)	N ₂ (%)	CH ₄ (%)	CO ₂ (%)		
1	24-Jul	Was at 5% retention window (RTW). Machine not printing well on Days 3-5.							
2	25-Jul								
3	26-Jul								
4	27-Jul	N2	slight	Poor	Bad injection				
5	28-Jul	N2	slight	GC Integrator Printout	N2	some	GC Integrator		
6	29-Jul	Changed to 10% RTW.							
7	30-Jul	96.7	3.3	ND	83.3	16.7	ND	100	100
8	31-Jul	98.3	1.7	ND	81.9	18.1	ND	100	100
9	1-Aug	96.5	3.5	ND	80.1	19.9	ND	100	100
10	2-Aug	86.3	13.7	ND	80.0	20.0	ND	100	100
17	9-Aug	84.0	16.0	ND	79.1	20.9	ND	100	100

ND-Not Detected

START-UP

TABLE L.9 b): Biogas Composition Results, in %, During Run 1 (Days 18-61)

DAY	Date	AD1			AD2			(%) TOTAL	(%) TOTAL
		N ₂ (%)	CH ₄ (%)	CO ₂ (%)	N ₂ (%)	CH ₄ (%)	CO ₂ (%)		
18	10-Aug	88.2	11.8	ND	78.1	21.9	ND	100	100
19	11-Aug	86.1	14.0	ND	74.2	25.8	ND	100	100
22	14-Aug	90.6	9.4	ND	71.2	28.9	ND	100	100
25	17-Aug	84.4	15.2	0.4	65.3	32.1	2.6	100	100
28	20-Aug	52.4	44.9	2.7	65.5	33.1	1.4	100	100
30	22-Aug	85.8	14.20	ND	68.5	30.4	1.1	100	100
31	23-Aug	60.5	38.9	0.6	69.5	29.6	0.8	100	100
34	26-Aug	92.6	7.4	ND	66.8	32.0	1.2	100	100
36	28-Aug	75.0	24.4	0.6	68.0	30.8	1.2	100	100
38	30-Aug	93.6	6.5	ND	68.2	30.5	1.3	100	100
40	1-Sep	94.2	5.8	ND	60.7	36.6	2.6	100	100
42	3-Sep	89.2	10.8	ND	61.3	36.8	1.9	100	100
47	8-Sep	57.5	40.1	2.3	44.6	49.7	5.7	100	100
49	10-Sep	52.8	44.7	2.5	41.4	52.2	6.4	100	100
51	12-Sep	53.8	43.9	2.3	25.4	65.3	9.2	100	100
61	22-Sep								

Shaded areas were not considered in the maintained run condition parameter averages and standard deviations. Calculations were based on data obtained once stable flows were reached during the run.

RUN 1	AVERAGE:	75	24	2	56	40	3
	STD:	18	17	1	15	13	3

APPENDIX L.10: AAT DATA AND RESULTS

TABLE L.10 a): Volumetric AAT Data for Lake Utopia Granular Sludge Inoculum (June 30)

	S1	S2	Ave. Sample	Control	Blank
Time (h)	Acetic Acid (mg AA/L)	Acetic Acid (mg AA/L)	Acetic Acid (mg AA/L)	Acetic Acid (mg AA/L)	Acetic Acid (mg AA/L)
0	1527.47	1563.13	1545.30	2.70	1455.71
1.5	1534.22	1508.45	1521.34	1.08	1450.98
3.0	1891.17	1930.55	1910.86	3.04	1864.48
4.5	1893.27	1856.40	1874.84	5.54	1814.85
Lag Time: 6.0	1375.72	1431.04	1403.38	1.24	1357.66
12.0	1288.54	1231.50	1260.02	5.04	1394.28
24.0	1051.42	875.06	963.24	1.39	1294.62
29.0	889.80	671.47	780.63	2.68	1327.10
32.0	858.18	600.70	729.44	20.54	1371.29

AAT Test and Control Bottle Content: 40 mL Defined Medium + 10 mL Sludge = 50 mL		
Average g VSS in 10 mL Seed Sludge Sample =	0.3128	g VSS (Appendix L.11)
Average VSS Concentration in Test Bottle (50 mL) =	6.2560	g VSS/L
Volumetric Acetoclastic Activity = AAT Slope (g AA/L·d) =	0.63	g AA/L·d
	(0.68	g COD _{rem} /L·d)
Slope Deviation =	0.02	g AA/L·d
	(0.02	g COD _{rem} /L·d)

Average Volumetric Acetoclastic Activity of the Inoculum:	0.63 ± 0.02	g AA/L·d
	(0.68 ± 0.02	g COD _{rem} /L·d)

Shaded values were not used in the volumetric acetoclastic activity computations (6 h-lag time was encountered).

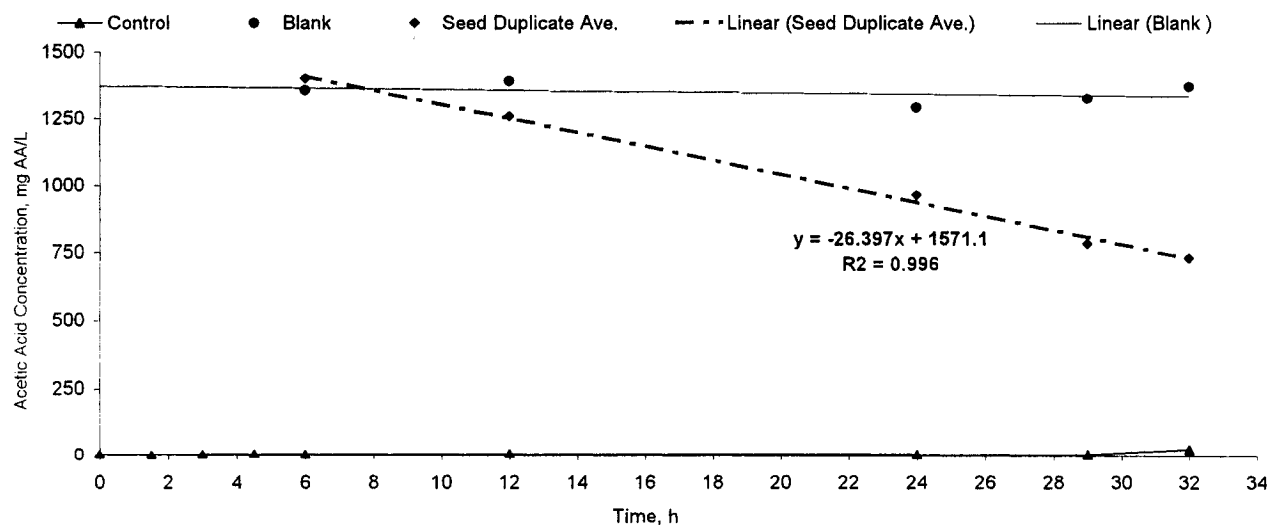


Figure L.10 a): Average AAT on Lake Utopia Granular Sludge Inoculum (June 30)

APPENDIX L.10: AAT DATA RESULTS

TABLE L.10b i): Volumetric AAT Results on AD1 Granular Sludge, at Top (T1) and Bottom (B1), on Sept. 13, after Run 1 (2 L/d)

Time (h)	T1 Acetic Acid (mg AAL)	B1 Acetic Acid (mg AAL)	AD1 Average Acetic Acid (mg AAL)	CT1 Acetic Acid (mg AAL)	CB1 Acetic Acid (mg AAL)	Ave. Control 1 Acetic Acid (mg AAL)	Blank Acetic Acid (mg AAL)
0	1308.73	1639.78	1474.255	3.68	3.94	3.61	1238.21
1.5	1565.78	1699.26	1612.52	0.00	0.00	0.00	1332.43
3.0	1447.98	1508.22	1478.10	7.14	0.00	3.57	1425.85
4.5	1390.62	1368.62	1379.62	0.00	0.00	0.00	1394.39
6.0	777.64	796.86	787.25	2.44	0.91	1.68	1501.64
7.5	539.22	690.71	614.97	6.49	3.81	5.15	1406.60
123.0	4.26	3.374	3.81	8.47	4.47	6.47	1331.38

AAT Test Bottle Volume: 40 mL Defined Medium + 10 mL Sludge = 50 mL

Estimated g VSS in 10 mL Sludge Added to Bottle T1 = 0.4197 g VSSIL
 VSS Concentration in Bottle T1 = 8.3946 g VSSIL
 T1 Volumetric Acetoclastic Activity = AAT Slope (g AAL-d) = 4.36
 Slope Deviation = 0.42 g AAL-d
 Estimated g VSS in 10 mL Sludge Added to Bottle B1 = 0.4051 g VSSIL
 VSS Concentration in Bottle B1 = 8.1024 g VSSIL
 B1 Volumetric Acetoclastic Activity = AAT Slope (g AAL-d) = 4.24
 Slope Deviation = 0.52 g AAL-d

Volumetric Acetoclastic Activity at Top (T1) after Run 1:	4.36 ± 0.42 g AAL-d (4.66 ± 0.44 g COD _{acet} -d)
Volumetric Acetoclastic Activity at Bottom (B1) after Run 1:	4.24 ± 0.52 g AAL-d (4.53 ± 0.56 g COD _{acet} -d)
Ave. Volumetric Acetoclastic Activity in AD1's SB after Run 1:	4.30 ± 0.44 g AAL-d (4.60 ± 0.47 g COD _{acet} -d)

Shaded values were not considered in the best fit linear regressions used to compute the volumetric acetoclastic activities

Lag Time:

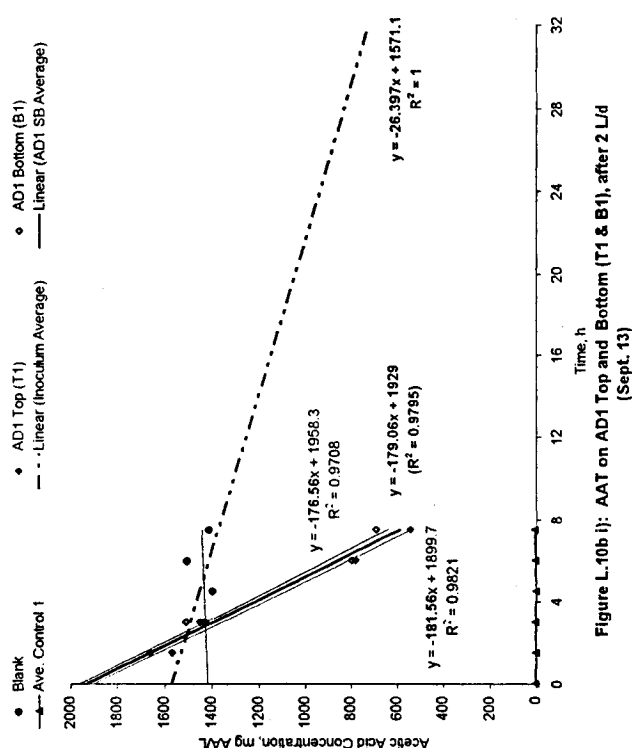


Figure L.10b i): AAT on AD1 Top and Bottom (T1 & B1), after 2 L/d (Sept. 13)

TABLE L.10b ii): Volumetric AAT Results on AD2 Granular Sludge, at Top (T2) and Bottom (B2), on Sept. 13, after Run 1 (2 L/d)

Time (h)	T2 Acetic Acid (mg AAL)	B2 Acetic Acid (mg AAL)	AD2 Average Acetic Acid (mg AAL)	CT2 Acetic Acid (mg AAL)	CB2 Acetic Acid (mg AAL)	Ave. Control 2 Acetic Acid (mg AAL)	Blank Acetic Acid (mg AAL)
0	1419.16	1759.69	1589.43	17.67	17.81	17.74	1238.21
1.5	1734.91	1743.09	1739.00	12.65	12.71	12.68	1332.43
3.0	1551.81	1657.09	1604.45	11.15	9.40	10.28	1425.85
4.5	1476.35	1569.75	1523.05	4.71	2.06	3.38	1394.39
6.0	956.20	1176.96	1066.58	3.61	16.59	10.10	1501.64
7.5	675.77	1044.82	860.29	5.21	3.62	4.42	1406.60
123.0	0.00	0.00	0.00	2.84	1.52	2.18	1331.38

AAT Test Bottle Volume: 40 mL Defined Medium + 10 mL Sludge = 50 mL

Estimated g VSS in 10 mL Sludge Added to Bottle T2 = 0.4118 g VSSIL
 VSS Concentration in Bottle T2 = 8.2368 g VSSIL
 T2 Volumetric Acetoclastic Activity = AAT Slope (g AAL-d) = 4.34
 Slope Deviation = 0.23 g AAL-d
 Estimated g VSS in 10 mL Sludge Added to Bottle B2 = 0.4226 g VSSIL
 VSS Concentration in Bottle B2 = 8.4518 g VSSIL
 B2 Volumetric Acetoclastic Activity = AAT Slope (g AAL-d) = 3.00
 Slope Deviation = 0.31 g AAL-d

Volumetric Acetoclastic Activity at Top (T2) after Run 1:	4.34 ± 0.23 g AAL-d (4.65 ± 0.24 g COD _{acet} -d)
Volumetric Acetoclastic Activity at Bottom (B2) after Run 1:	3.00 ± 0.31 g AAL-d (3.21 ± 0.33 g COD _{acet} -d)
Ave. Volumetric Acetoclastic Activity in AD2's SB after Run 1:	3.67 ± 0.28 g AAL-d (3.93 ± 0.30 g COD _{acet} -d)

Shaded values were not considered in the best fit linear regressions used to compute the volumetric acetoclastic activities

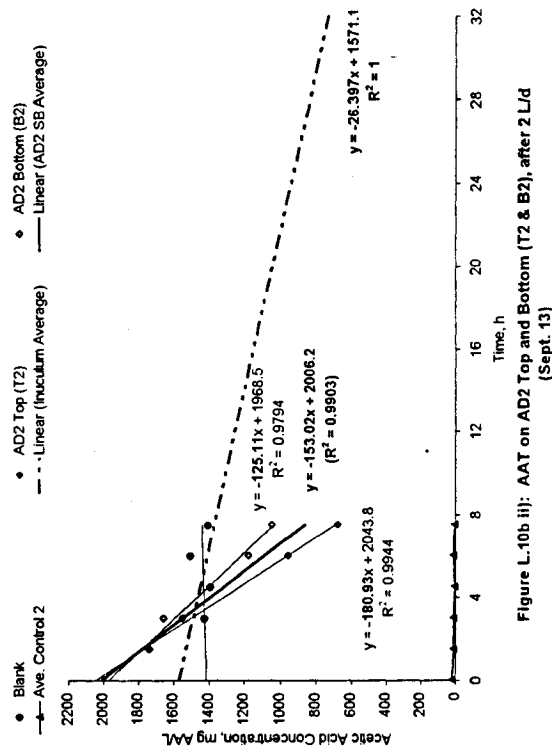


Figure L.10b ii): AAT on AD2 Top and Bottom (T2 & B2), after 2 L/d (Sept. 13)

APPENDIX L.10: AAT DATA RESULTS

TABLE L.10c ii: Volumetric AAT Results on AD1 Granular Sludge, at Top (TAD1), Middle (T1) and Bottom (B1), on Nov. 14, after Run 2 (4 L/d)

Time (h)	TAD1 Acetic Acid (mg AAL)	T1 Acetic Acid (mg AAL)	B1 Acetic Acid (mg AAL)	AD1 Average Acetic Acid (mg AAL)	CTAD1 Acetic Acid (mg AAL)	CT1 Acetic Acid (mg AAL)	CB1 Acetic Acid (mg AAL)	Ave. Control 1 Acetic Acid (mg AAL)	BLANK Acetic Acid (mg AAL)
0	1172.81	1200.21	1112.18	1161.73	16.01	5.10	19.22	13.44	1317.03
1.5	1230.10	1181.03	1180.97	1197.37	27.12	139.54	33.49	66.72	1304.68
3.0	1282.25	1202.51	1113.58	1199.45	21.53	130.96	26.08	59.52	1435.93
4.5	1240.02	1169.10	1135.90	1181.67	25.79	4.23	12.39	14.14	1380.43
6.0	1184.54	1041.47	1022.23	1082.75	13.83	3.06	9.71	8.86	1512.90
9.0	1111.32	844.11	936.18	963.87	10.76	1.56	7.95	6.75	1453.79
15.0	1002.82	749.43	647.66	799.99	4.81	2.42	2.95	3.39	1440.59
25.0	741.62	381.43	407.13	510.06	0.00	0.00	1.92	0.64	1434.57

AAT Test Bottle Volume: 40 mL Defined Medium + 10 mL Sludge = 50 mL
 Estimated g VSS in 10 mL Sludge Added to Bottle TAD1 = 6.3289 g VSS
 VSS Concentration in Bottle TAD1 = 6.3780 g VSS/L
 TAD1 Volumetric Acetoclastic Activity = AAT Slope (g AA/L-d) = 0.57 g VSS/L
 Slope Deviation = 0.57 g AAL-d
 Estimated g VSS in 10 mL Sludge Added to Bottle T1 = 0.02 g VSS/L
 T1 Volumetric Acetoclastic Activity = AAT Slope (g AA/L-d) = 0.3875 g VSS/L
 Slope Deviation = 0.89 g AAL-d
 Estimated g VSS in 10 mL Sludge Added to Bottle B1 = 0.04 g VSS/L
 B1 Volumetric Acetoclastic Activity = AAT Slope (g AA/L-d) = 0.83 g VSS/L
 Slope Deviation = 0.06 g AAL-d

Volumetric Acetoclastic Activity at Top (TAD1) after Run 2:	0.57 ± 0.02	g AAL-d	(0.61 ± 0.02	g COD _{max} /L-d)
Volumetric Acetoclastic Activity at Middle (T1) after Run 2:	0.89 ± 0.04	g AAL-d	(0.95 ± 0.04	g COD _{max} /L-d)
Volumetric Acetoclastic Activity at Bottom (B1) after Run 2:	0.83 ± 0.06	g AAL-d	(0.89 ± 0.07	g COD _{max} /L-d)
Ave. Volumetric Acetoclastic Activity in AD1's SB after Run 2:	0.77 ± 0.03	g AAL-d	(0.82 ± 0.03	g COD _{max} /L-d)

Shaded values were not considered in the best fit linear regressions used to compute the volumetric acetoclastic activities.

TABLE L.10c iii: Volumetric AAT Results on AD2 Granular Sludge, at Top (TAD2), Middle (T2) and Bottom (B2), on Nov. 14, after Run 2 (4 L/d)

Time (h)	TAD2 Acetic Acid (mg AAL)	T2 Acetic Acid (mg AAL)	B2 Acetic Acid (mg AAL)	AD2 Average Acetic Acid (mg AAL)	CTAD2 Acetic Acid (mg AAL)	CT2 Acetic Acid (mg AAL)	CB2 Acetic Acid (mg AAL)	Ave. Control 2 Acetic Acid (mg AAL)	BLANK Acetic Acid (mg AAL)
0	1083.64	966.85	1011.68	1020.72	4.82	4.85	17.33	9.00	1317.03
1.5	1217.16	1236.01	1089.53	1180.90	143.24	148.96	33.23	109.48	1304.68
3.0	1201.75	1244.37	1036.86	1160.99	146.14	134.44	24.63	101.74	1435.93
4.5	1141.00	1114.35	977.32	1077.56	17.98	21.37	25.02	21.46	1380.43
6.0	1049.59	1032.01	881.72	987.77	6.69	4.47	8.79	6.65	1512.90
9.0	937.82	881.23	808.87	875.97	3.28	2.47	6.81	4.19	1453.79
15.0	675.70	647.61	460.38	594.56	3.70	2.58	1.81	2.70	1440.59
25.0	326.43	210.03	51.133	195.87	2.43	4.28	0.00	2.24	1434.57

AAT Test Bottle Volume: 40 mL Defined Medium + 10 mL Sludge = 50 mL
 Estimated g VSS in 10 mL Sludge Added to Bottle TAD2 = 4.2030 g VSS
 VSS Concentration in Bottle TAD2 = 4.0600 g VSS/L
 TAD2 Volumetric Acetoclastic Activity = AAT Slope (g AA/L-d) = 0.95 g VSS/L
 Slope Deviation = 0.95 g AAL-d
 Estimated g VSS in 10 mL Sludge Added to Bottle T2 = 0.03 g VSS/L
 T2 Volumetric Acetoclastic Activity = AAT Slope (g AA/L-d) = 0.2686 g VSS/L
 Slope Deviation = 5.3720 g AAL-d
 Estimated g VSS in 10 mL Sludge Added to Bottle B2 = 0.04 g VSS/L
 B2 Volumetric Acetoclastic Activity = AAT Slope (g AA/L-d) = 0.3184 g VSS/L
 Slope Deviation = 6.3680 g AAL-d

Volumetric Acetoclastic Activity at Top (TAD2) after Run 2:	0.95 ± 0.03	g AAL-d	(1.01 ± 0.03	g COD _{max} /L-d)
Volumetric Acetoclastic Activity at Middle (T2) after Run 2:	1.07 ± 0.04	g AAL-d	(1.15 ± 0.04	g COD _{max} /L-d)
Volumetric Acetoclastic Activity at Bottom (B2) after Run 2:	1.08 ± 0.03	g AAL-d	(1.16 ± 0.04	g COD _{max} /L-d)
Ave. Volumetric Acetoclastic Activity in AD2's SB after Run 2:	1.03 ± 0.02	g AAL-d	(1.11 ± 0.02	g COD _{max} /L-d)

Shaded values were not considered in the best fit linear regressions used to compute the volumetric acetoclastic activities.

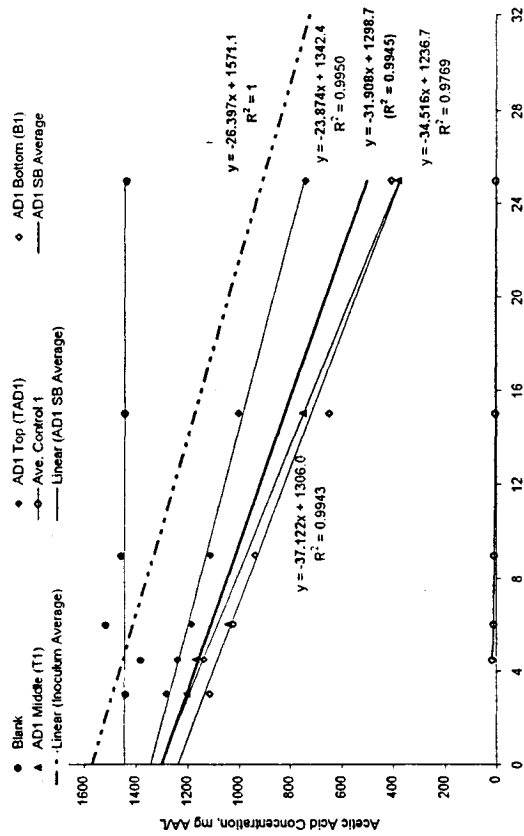


Figure L.10c ii: AAT on AD1 Top, Middle & Bottom (TAD1, T1 & B1), after 4 L/d (Nov. 14)

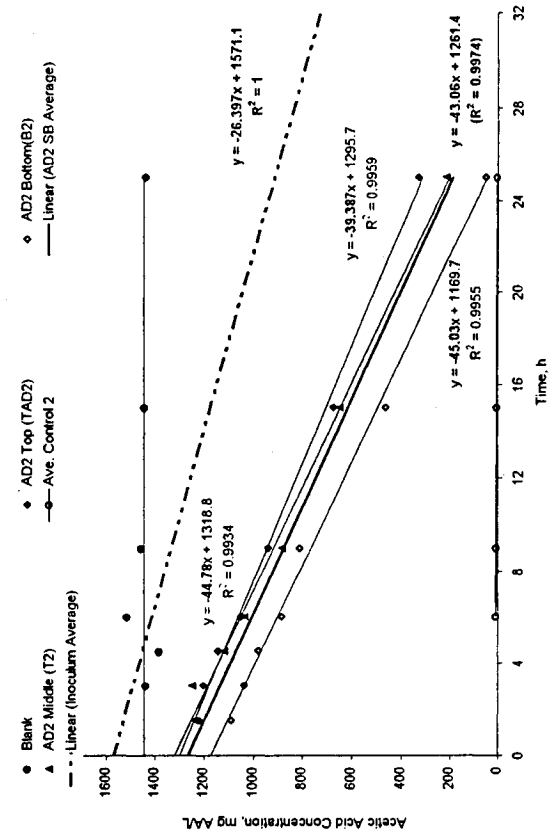


Figure L.10c iii: AAT on AD2 Top, Middle & Bottom (TAD2, T2 & B2), after 4 L/d (Nov. 14)

APPENDIX L.10: AAT DATA RESULTS

TABLE L.10d i): Volumetric AAT on AD1 Granular Sludge, at Top (TAD1) and Bottom (B1), on Dec. 16, after Run 3 (8 L/d)

Time (h)	TAD1 Acetic Acid (mg AAL)	B1 Acetic Acid (mg AAL)	AD1 Average Acetic Acid (mg AAL)	Blank Acetic Acid (mg AAL)
0	1366.90	1607.08	1486.99	1634.87
1.5	1326.58	1289.07	1307.83	1419.35
3.0	1575.41	1191.90	1383.66	1562.19
4.5	1261.02	1496.84	1378.93	1479.02
6.0	1184.94	1078.84	1131.89	1326.11
7.5	1420.46	1197.55	1309.02	1351.15
12.5	1148.66	1059.33	1104.00	1430.78
23.25	1058.47	868.78	963.63	1270.28

AAT Test Bottle Volume: 40 mL Defined Medium + 10 mL Sludge = 50 mL

Estimated g VSS in 10 mL Sludge Added to Bottle TAD1 = 0.0578 g VSS

Biomass Concentration in Bottle TAD1 = 1.1560 g VSS/L

TAD1 Volumetric Acetoclastic Activity = AAT Slope (g AA/L/d) = 0.32 g AA/L/d

Slope Deviation = 0.04 g AA/L/d

Estimated g VSS in 10 mL Sludge Added to Bottle B1 = 0.1192 g VSS

Biomass Concentration in Bottle B1 = 2.3840 g VSS/L

B1 Volumetric Acetoclastic Activity = AAT Slope (g AA/L/d) = 0.43 g AA/L/d

Slope Deviation = 0.05 g AA/L/d

Volumetric Acetoclastic Activity at Top (TAD1) after the last run: 0.32 ± 0.04 g AA/L/d

Volumetric Acetoclastic Activity at Bottom (B1) after the last run: 0.43 ± 0.05 g AA/L/d

Ave. Volumetric Acetoclastic Activity in AD1's SB after the last run: 0.38 ± 0.04 g AA/L/d

(0.41 ± 0.04 g AA/L/d)

Shaded values were not considered in the best fit linear regressions used to compute the volumetric acetoclastic activities.

No controls were run during the assay and g VSS values from the SS analysis are presented in Appendix L.11.

The g VSS estimated from the Bottom (B1) sample was used in the computation of the average specific acetoclastic activity of the bed at the end of Run 3.

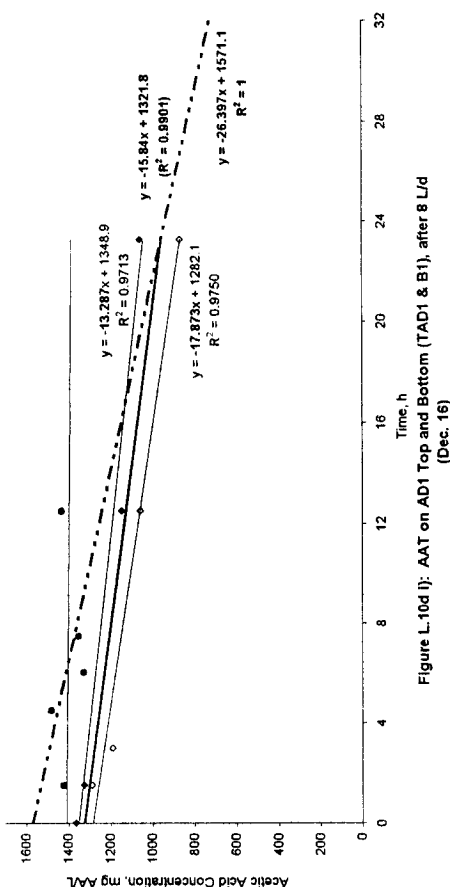


Figure L.10d i): AAT on AD1 Top and Bottom (TAD1 & B1), after 8 L/d (Dec. 16)

TABLE L.10d ii): Volumetric AAT on AD2 Granular Sludge, at Top (TAD2), Middle (T2) and Bottom (B2), on Dec. 16, after Run 3 (8 L/d)

Time (h)	TAD2 Acetic Acid (mg AAL)	T2 Acetic Acid (mg AAL)	B2 Acetic Acid (mg AAL)	AD2 Average Acetic Acid (mg AAL)	Blank Acetic Acid (mg AAL)
0	1589.45	1422.78	1257.37	1589.45	1634.87
1.5	1294.51	1139.03	1078.68	1170.74	1419.35
3.0	1473.03	1280.25	1188.68	1376.64	1562.19
4.5	1267.92	1080.42	966.68	1105.07	1479.02
6.0	1428.20	1009.20	996.05	1144.48	1326.11
7.5	1364.63	1042.11	902.91	1103.22	1351.15
12.5	1097.03	583.67	555.32	778.67	1430.78
23.25	928.17	232.89	224.10	461.72	1270.28

AAT Test Bottle Volume: 40 mL Defined Medium + 10 mL Sludge = 50 mL

Estimated g VSS in 10 mL Sludge Added to Bottle TAD2 = 0.1245 g VSS

VSS Concentration in Bottle TAD2 = 2.4900 g VSS/L

TAD2 Volumetric Acetoclastic Activity = AAT Slope (g AA/L/d) = 0.71 g AA/L/d

Slope Deviation = 0.08 g AA/L/d

Estimated g VSS in 10 mL Sludge Added to Bottle T2 = 0.2784 g VSS

VSS Concentration in Bottle T2 = 5.5682 g VSS/L

T2 Volumetric Acetoclastic Activity = AAT Slope (g AA/L/d) = 1.18 g AA/L/d

Slope Deviation = 0.09 g AA/L/d

Estimated g VSS in 10 mL Sludge Added to Bottle B2 = 0.3263 g VSS

VSS Concentration in Bottle B2 = 6.5252 g VSS/L

B2 Volumetric Acetoclastic Activity = AAT Slope (g AA/L/d) = 1.00 g AA/L/d

Slope Deviation = 0.09 g AA/L/d

Volumetric Acetoclastic Activity at Top (TAD2) after the last run: 0.71 ± 0.08 g AA/L/d

(0.76 ± 0.08 g AA/L/d)

Volumetric Acetoclastic Activity at Middle (T2) after the last run: 1.18 ± 0.09 g AA/L/d

Volumetric Acetoclastic Activity at Bottom (B2) after the last run: 1.00 ± 0.09 g AA/L/d

(1.1 ± 0.1 g AA/L/d)

Ave. Volumetric Acetoclastic Activity in AD2's SB after the last run: 1.2 ± 0.1 g AA/L/d

(1.3 ± 0.1 g AA/L/d)

Shaded values were not considered in the best fit linear regressions used to compute the volumetric acetoclastic activities.

No controls were run during the assay and g VSS values from the SS analysis are presented in Appendix L.11.

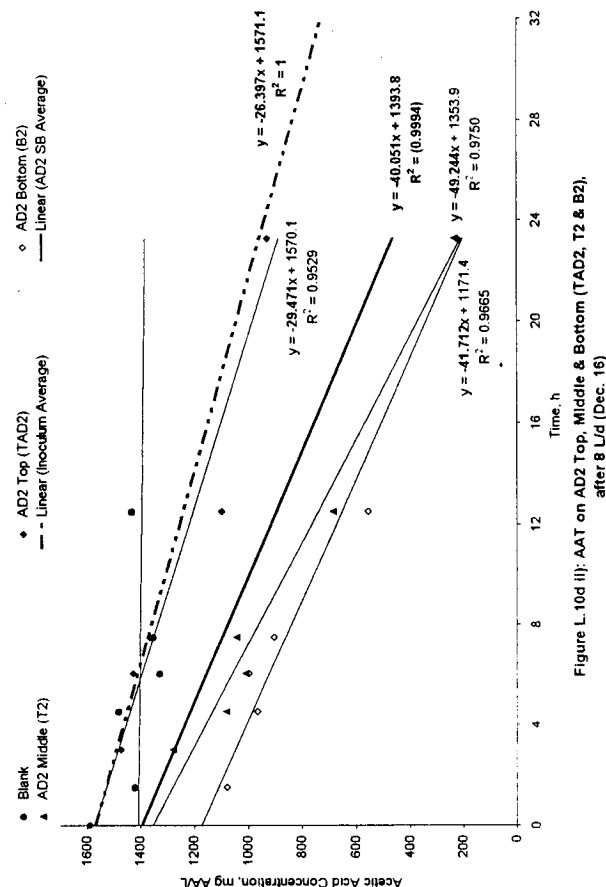


Figure L.10d ii): AAT on AD2 Top, Middle & Bottom (TAD2, T2 & B2), after 8 L/d (Dec. 16)

APPENDIX L.11: UASB REACTOR SUSPENDED SOLIDS INVENTORY AND PROFILES

TABLE L.11 a): Average Suspended Solid Test on Lake Utopia (NB) Granular Sludge Inoculum Duplicates (April 4 and June 30)

Date	Dish & filter (g)	After 105°C (g)	After 550°C (g)	V (mL)	TSS (mg/L)	VSS (mg/L)	FSS (mg/L)	g VSS 10 mL	Initial SBH (mL)	Total SB (g VSS)	AD Volume (L)	UASB VSS Conc. (g VSS/L)	%VSS (for port)	%FSS (for port)
4-Apr	2.44842	2.77341	2.48839	10	32499	28502	3997	0.2850	2000	57.0	3.85	14.8	87.7	12.3
	2.48081	2.80898	2.52034	10	32817	28864	3953	0.2886		57.7	(AD1)	15.0	88.0	12.0
30-Jun	2.42843	2.83311	2.47361	10	40468	35950	4518	0.3595	2000	71.9	3.75	19.2	88.8	11.2
	2.41786	2.77443	2.45659	10	36557	31784	3873	0.3178		63.6	(AD2)	17.0	89.1	10.9
Bed Ave.: 35360 31275 0.4128														
STD: 3689 3446 293 0.0345														
										62.6		16.5	88.4	11.6
										6.9		2.0	0.7	0.7

TABLE L.11 b): Suspended Solid Profile of Granular Sludge (Sept. 12), after Run 1 (2 L/d)

Anaerobic Reactor	Sampling Port	Port Height (mL)	Dish & filter (g)	After 105°C (g)	After 550°C (g)	V (mL)	TSS (mg/L)	VSS (mg/L)	FSS (mg/L)	g VSS 10 mL	SBH (mL)	Total SB (g VSS)	AD Volume (L)	UASB VSS Conc. (g VSS/L)	%VSS (for port)	%FSS (for port)
AD1	T1	1850	2.45067	2.91673	2.49700	10	46906	41973	4633	0.4197	2375	98.0	3.85	25.4	90.1	9.9
	B1	625	2.43545	2.88191	2.47679	10	44646	40512	4134	0.4051					90.7	9.3
Bed Ave.: 45626 41243 4383 0.4124																
STD (% of Ave.): 2 2 6 2																
AD2	T2	1750	2.46093	2.91919	2.50735	10	45826	41184	4642	0.4118	2350	98.0	3.75	26.1	89.9	10.1
	B2	625	2.47766	2.94736	2.52477	10	46970	42259	4711	0.4226					90.0	10.0
Bed Ave.: 46398 41722 4677 0.4172																
STD (% of Ave.): 1 1 1 1																
															89.9	10.1
															0.1	0.5
															90.4	9.6
															0.4	4

TABLE L.11 c): Suspended Solid Profile of Granular Sludge (Nov. 18), after Run 2 (4 L/d)

Anaerobic Reactor	Sampling Port	Port Height (mL)	Dish & filter (g)	After 105°C (g)	After 550°C (g)	V (mL)	TSS (mg/L)	VSS (mg/L)	FSS (mg/L)	g VSS 10 mL	SBH (mL)	Total SB (g VSS)	AD Volume (L)	UASB VSS Conc. (g VSS/L)	%VSS (for port)	%FSS (for port)
AD1	TAD1	2600	2.43758	2.80591	2.47700	10	36833	32891	3942	0.3289	3250	124.4	3.85	32.3	89.3	10.7
	T1	1850	2.45693	2.8850	2.50101	10	43157	38749	4408	0.3875					89.8	10.2
B1		625	2.39934	2.88185	2.45023	10	48251	43162	5089	0.4316					89.5	10.5
															89.5	10.5
Bed Ave.: 42747 38267 4480 0.3827																
STD (% of Ave.): 13 13 13 13																
AD2	TAD2	2500	2.44397	2.67234	2.46933	10	22837	20301	2536	0.2030	2775	73.1	3.75	19.5	88.9	11.1
	T2	1750	2.44750	2.74680	2.47816	10	29930	26864	3066	0.2686					89.8	10.2
B2		625	2.45374	2.80666	2.48824	10	35292	31842	3450	0.3184					90.2	9.8
															89.6	10.4
Bed Ave.: 29353 26336 3017 0.2634																
STD (% of Ave.): 21 22 15 22																
															1	6

TABLE L.11 d): Suspended Solid Profile of Granular Sludge (Dec. 16), after Run 3 (8 L/d)

Anaerobic Reactor	Sampling Port	Port Height (mL)	Dish & filter (g)	After 105°C (g)	After 550°C (g)	V (mL)	TSS (mg/L)	VSS (mg/L)	FSS (mg/L)	g VSS 10 mL	*SBH (mL)	Total SB (g VSS)	AD Volume (L)	UASB VSS Conc. (g VSS/L)	%VSS (for port)	%FSS (for port)
*AD1	TAD1	2600	2.45852	2.52393	2.46614	10	6541	5779	762	0.0578	1200	14.3	3.85	3.7	88.4	11.6
	B1	625	2.39441	2.52898	2.40981	10	13457	11917	1540	0.1192					88.6	11.4
Bed Ave.: 9999 5848 1151 0.0885																
STD (% of Ave.): 35 35 34 35																
AD2	TAD2	2500	2.41477	2.55690	2.43240	10	14213	12450	1763	0.1245	2875	69.9	3.75	18.6	87.6	12.4
	T2	1750	2.42693	2.73904	2.46063	10	31211	27841	3370	0.2784					89.2	10.8
B2		625	2.41706	2.78195	2.45569	10	36489	32626	3863	0.3263					89.4	10.6
															88.7	11.3
Bed Ave.: 27304 24306 2999 0.2431																
STD (% of Ave.): 43 43 37 43																
															1	9

*On Dec. 16, AD1's sludge bed was cracked and split in two. A small part of it was floating at the TAD1 port level. At that time, the total bed had a volume of approximately 1200 mL.

For a more representative estimate of the average SS content in the SBs and of its total concentration in AD1, the average VSS was taken as the VSS value at the B1 port (~11.9 g VSS/L) since the amount of sludge was much more significant at the bottom of the reactor.

APPENDIX L.12: UASB REACTOR SETTLING VELOCITY PROFILE DATA

TABLE L.12a i): Initial Settling Test on Lake Utopia (NB) Granular Sludge Inoculum (April 5)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Upflow Velocity (m/h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)
1	2	5	0.083	420	0.0004	0.0050	17.78	2.28796	2.29953	0.01157	5.14	0.01157	5.14
2	3	5	0.083	800	0.0008	0.0096	33.86	2.23945	2.23971	0.00026	0.12	0.01183	5.25
3	4	5	0.083	1240	0.0012	0.0149	52.48	2.23918	2.25675	0.01757	7.80	0.02940	13.06
4	6	5	0.083	1740	0.0017	0.0209	73.64	2.26418	2.39479	0.13061	58.01	0.16001	71.06
5	8	5	0.083	2920	0.0029	0.0350	123.58	2.18668	2.25184	0.06516	28.94	0.22517	100.00
Water Temperature = 21°C				Tube diam. (m) =	0.0190								
				Tube area (m ²) =	0.0003								
				V (mL) =	10								
Sum:										0.22517	100.00		

TABLE L.12a ii): Initial Settling Test on Lake Utopia (NB) Granular Sludge Inoculum (June 20)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Upflow Velocity (m/h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)
1	2	5	0.083	362	0.00036	0.0043	15.32	2.15320	2.16548	0.01228	2.55	0.01228	2.55
2	3	5	0.083	789	0.00079	0.0096	33.39	2.20291	2.20825	0.00534	1.11	0.01762	3.65
3	4	5	0.083	1220	0.00122	0.0146	51.63	2.25174	2.37767	0.12593	26.11	0.14355	29.77
4	6	5	0.083	2250	0.00225	0.0270	95.23	2.30081	2.62368	0.32287	66.95	0.46642	96.72
5	8	5	0.083	2918	0.00292	0.0350	123.50	2.17148	2.18728	0.01580	3.28	0.48222	100.00
Water Temperature = 23°C				Tube diam. (m) =	0.0190								
				Tube area (m ²) =	0.0003								
				V (mL) =	10								
Sum:										0.48222	100.00		

TABLE L.12a iii): Duplicate Average from April 5 & June 20

Upflow Velocity (m/h)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)
16.55	0.01193	3.84	0.01193	3.84
33.63	0.00280	0.61	0.01472	4.45
52.08	0.07175	16.96	0.08647	21.41
84.44	0.22674	62.48	0.31322	83.89
123.54	0.04048	16.11	0.35370	100.00
Sum:				

UASB Circumference:
UASB Diameter (~4 inches):
UASB Surface Area:

AD1	AD2
32 cm	31 cm
10.2 cm	9.9 cm
81.71 cm ²	76.98 cm ²

For the Max. applied upflow velocity (V_{up}) of .42 L/d
in the UASBs, which included all set internal recycling :

Upflow Velocity = -42 L/d = 42000 cm³/d
(8 L/d + 12 L/d + 24 L/d)
(Refer to Fig. 3.6a)
AD1 = 42000
AD2 = 545.60
0.21
0.23

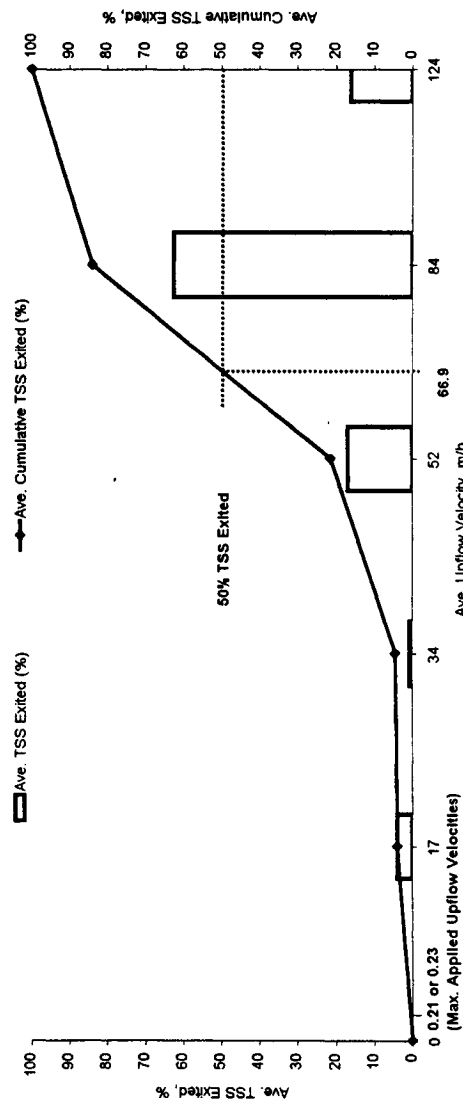


Figure L.12a: Average Settling Velocity Test on Lake Utopia Granular Sludge Duplicate (April 5 and June 20)

APPENDIX L.12: UASB REACTOR SETTLING VELOCITY PROFILE DATA

TABLE L.12b i): Settling Test Results on AD1 Granular Sludge, at Top (T1), on Sept. 12, after Run 1 (2 L/d)

Filter- No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m³)	Flow (m³/h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)	Upflow Velocity (m/h)
1	2	5	0.083	370	0.0004	0.0044	2.37589	2.36853	0.04264	10.62	0.04264	10.62	15.66
2	3	5	0.083	875	0.0009	0.0105	2.31685	2.33757	0.02072	5.16	0.06336	15.78	37.03
3	4	5	0.083	1220	0.0012	0.0146	2.25868	2.38566	0.12698	31.62	0.19034	47.40	51.63
4	6	5	0.083	0.0262	0.0022	0.0262	2.17354	2.37271	0.18917	49.60	0.38951	97.01	92.27
5	8	5	0.083	3150	0.0032	0.0378	2.21978	2.23180	0.01202	2.99	0.40153	100.00	133.32
Water Temperature = 23°C		Sum:											
						Tube diam. (m) = 0.0190				100.00		0.40153	
						Tube area (m²) = 0.0003							
						V (mL) 10							

TABLE L.12b ii): Settling Test Results on AD1 Granular Sludge, at Bottom (B1), on Sept. 12, after Run 1 (2 L/d)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³ /h)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)	Upflow Velocity (mh)
1	2	5	0.083	395	0.083	0.0047	2.24675	2.25128	0.00453	0.80	0.00453	0.60	16.72
2	3	5	0.083	790	0.008	0.0095	2.22134	2.43487	0.01353	28.44	0.21806	29.05	33.44
3	4	5	0.083	1290	0.013	0.0155	2.09383	2.28170	0.18787	25.02	0.40593	54.07	54.60
4	6	5	0.083	2185	0.022	0.0262	2.17061	2.50792	0.33731	44.93	0.74324	99.00	92.48
5	8	5	0.083	2900	0.029	0.0348	2.32287	2.33047	0.00750	1.00	0.75074	100.00	122.74
Water Temperature = 23°C				V (mL)	10			Sum.	0.75074	100.00			

TABLE L.12c i): Settling Test Results on AD2 Granular Sludge, at Top (T2), on Sept. 12, after Run 1 (2 L/d)

Filter: No.	Pump Setting	Time		Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited		Cumulative TSS (g)	Cumulative TSS Exited (%)	Upflow Velocity (m/h)
		(min)	(h)						(g)	(%)			
1	2	5	0.083	375	0.0004	0.0045	2.28580	2.44618	0.16238	33.47	0.16238	33.47	15.87
2	3	5	0.083	855	0.0009	0.0103	2.29780	2.29887	0.00107	0.22	0.16345	33.69	36.19
3	4	5	0.083	1250	0.0013	0.0150	2.31483	2.24080	0.01597	25.96	0.28942	59.65	52.90
4	6	5	0.083	2210	0.0022	0.0265	2.15318	2.32482	0.17164	35.37	0.46106	95.02	93.54
5	8	5	0.083	2890	0.0029	0.0347	2.24345	2.26759	0.02414	4.98	0.48520	100.00	122.32
Water Temperature				V (mL)		10		Sum:		0.48520		100.00	

TABLE L.12c II): Settling Test Results on AD2 Granular Sludge, at Bottom (B2), on Sept. 12, after Run 1 (2 L/d)

Filter: No.	Pump Setting	Time		Water Volume Collected		Water Volume Collected (m ³)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited		Cumulative TSS (g)	Cumulative TSS Exited (%)	Upflow Velocity (m/h)
		(min)	(h)	(mL)	(mL)					(g)	(%)			
1	2	5	0.083	415	0.00042	0.00498	2.17349	2.30870	0.13521	23.04	0.1352	23.04	17.56	
2	3	5	0.083	780	0.00078	0.00936	2.22328	2.24070	0.01742	2.97	0.1526	26.01	33.01	
3	4	5	0.083	1240	0.00124	0.01488	2.37456	2.48522	0.16065	27.38	0.3133	53.39	52.48	
4	6	5.5	0.082	2890	0.00244	0.02862	2.27105	2.51562	0.24457	41.68	0.5579	93.07	93.88	
5	8	5	0.083	2690	0.00289	0.03468	2.20720	2.17825	0.02895	4.93	0.5868	100.00	122.32	
Water Temperature = 23°C				V (mL)		10			Sum:		0.58681	100.00		

APPENDIX L.12: UASB REACTOR SETTLING VELOCITY PROFILE DATA

TABLE L.12f i): Last Settling Test on AD1 Granular Sludge, at Top (TAD1), on Dec. 16, after Run 3 (8 L/d)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)	Uplow Velocity (m/h)
1	2	5	0.083	405	0.000405	0.0049	2.15251	2.22328	0.07075	41.60	0.07075	41.60	17.14
2	3	5	0.083	805	0.000805	0.0097	2.13054	2.22709	0.09655	56.77	0.1673	98.37	34.07
3	4	5	0.083	1220	0.001220	0.0146	2.30433	2.30433	0.00278	1.63	0.17008	100.00	51.63
Water Temperature = 23°C				Tube diam. (m) =	0.0190								
				Tube area (m ²) =	0.0003								
				V (mL) =	10 (not all sludge)								
								Sum:	0.17008	100.00			

TABLE L.12f ii): Last Settling Test on AD1 Granular Sludge, at Bottom (B1), on Dec. 16, after Run 3 (8 L/d)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)	Uplow Velocity (m/h)
1	2	5	0.083	380	0.00038	0.0046	2.16289	2.3254	0.18985	79.88	0.18985	79.88	16.08
2	3	5	0.083	830	0.00083	0.0100	2.21447	2.24093	0.02648	12.44	0.19631	92.32	35.13
3	4	5	0.083	1220	0.00122	0.0146	2.28173	2.28718	0.00545	2.56	0.20178	94.89	51.63
4	6	5	0.083	2160	0.00216	0.0259	2.11840	2.12727	0.01087	5.11	0.21263	100.00	91.42
				V (mL) =	10			Sum:	0.21263	100.00			

TABLE L.12g i): Last Settling Test on AD2 Granular Sludge, at Top (TAD2), on Dec. 16, after Run 3 (8 L/d)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)	Uplow Velocity (m/h)
1	2	5	0.083	435	0.00044	0.0052	2.18392	2.28979	0.10587	45.79	0.10587	45.79	18.41
2	3	5	0.083	830	0.00083	0.0100	2.21969	2.23446	0.02377	10.28	0.12964	56.07	35.13
3	4	5	0.083	1220	0.00122	0.0146	2.27036	2.33731	0.06695	28.96	0.19659	85.03	51.63
4	6	5	0.083	2170	0.00217	0.0280	2.13763	2.17225	0.03462	14.97	0.23121	100.00	91.84
Water Temperature = 24°C				V (mL) =	10			Sum:	0.23121	100.00			

TABLE L.12g ii): Last Settling Test on AD2 Granular Sludge, at Middle (T2), on Dec. 16, after Run 3 (8 L/d)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)	Uplow Velocity (m/h)
1	2	6.5	0.108	565	0.00057	0.0052	2.12466	2.17567	0.05101	16.95	0.05101	16.95	18.39
2	3	5	0.083	810	0.00081	0.0097	2.15109	2.16708	0.01599	5.31	0.06700	22.27	34.28
3	4	5	0.083	1280	0.00128	0.0154	2.22797	2.38679	0.15882	52.78	0.22582	75.05	54.17
4	6	5	0.083	2150	0.00215	0.0258	2.31288	2.38797	0.07509	24.95	0.30091	100.00	91.00
				V (mL) =	10 (not all sludge)			Sum:	0.30091	100.00			

TABLE L.12g iii): Last Settling Test on AD2 Granular Sludge, at Bottom (B2), on Dec. 16, after Run 3 (8 L/d)

Filter. No.	Pump Setting	Time (min)	Time (h)	Water Volume Collected (mL)	Water Volume Collected (m ³)	Flow (m ³ /h)	Filter (g)	After 105°C (g)	TSS Exited (g)	TSS Exited (%)	Cumulative TSS (g)	Cumulative TSS Exited (%)	Uplow Velocity (m/h)
1	2	5	0.083	440	0.00044	0.0052	2.20079	2.27915	0.06936	19.86	0.06936	19.86	17.35
2	3	5	0.083	840	0.00084	0.0097	2.14744	2.17793	0.02529	7.24	0.09465	27.11	35.76
3	4	5	0.083	1240	0.00124	0.0146	2.29011	2.32318	0.12207	34.96	0.21672	62.07	51.85
4	6	5	0.083	2160	0.00216	0.0259	2.14160	2.34160	0.13246	37.93	0.34918	100.00	91.42
				V (mL) =	10			Sum:	0.34918	100.00			

APPENDIX L.12: UASB REACTOR SETTLING VELOCITY PROFILE DATA

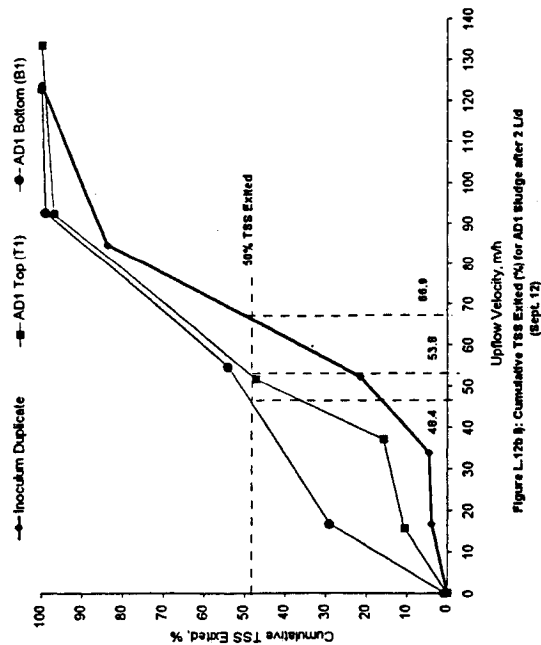


Figure L.12b i: Cumulative TSS Exited (%) for AD1 Sludge after 2 L/d (Sept. 12)

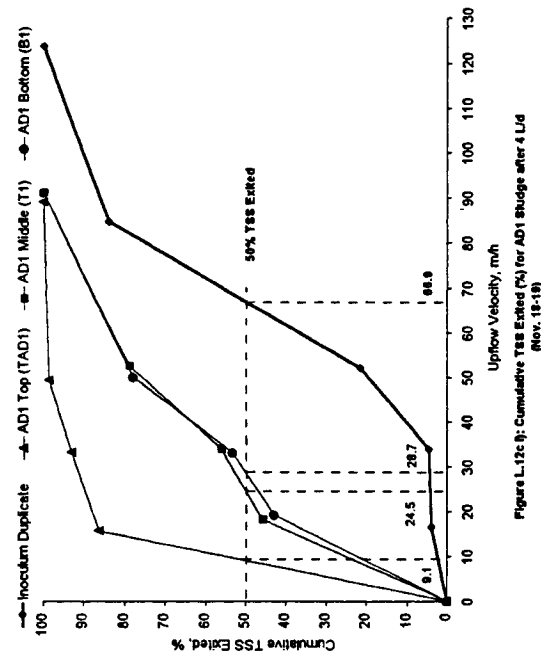


Figure L.12c i: Cumulative TSS Exited (%) for AD1 Sludge after 4 L/d (Nov. 18-19)

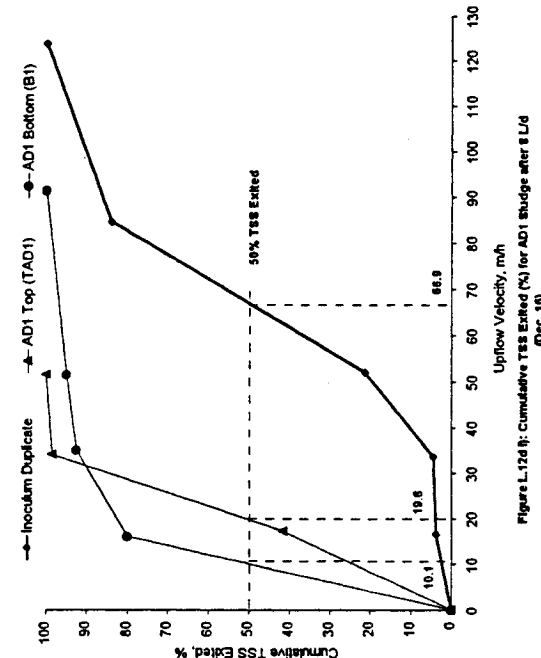


Figure L.12d i: Cumulative TSS Exited (%) for AD1 Sludge after 8 L/d (Dec. 16)

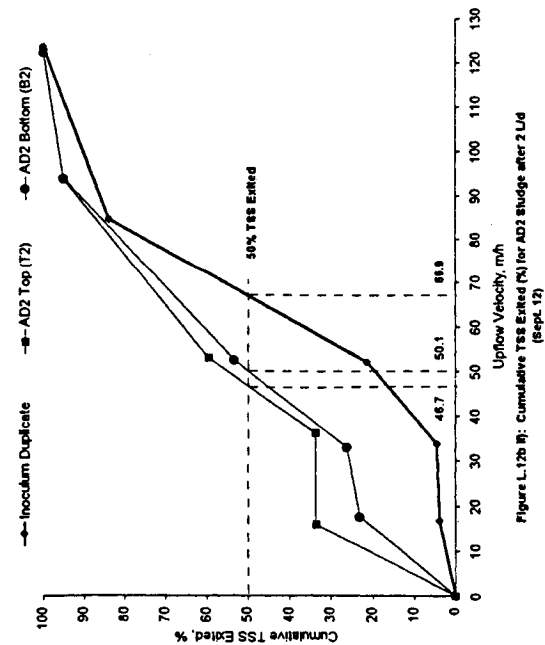


Figure L.12b ii: Cumulative TSS Exited (%) for AD2 Sludge after 2 L/d (Sept. 12)

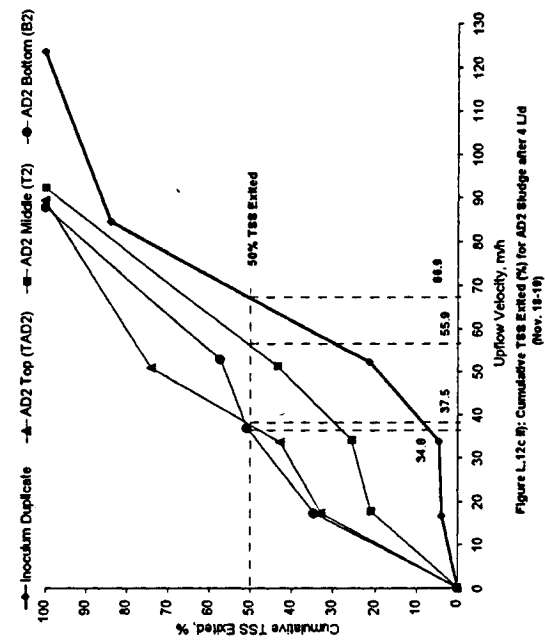


Figure L.12c ii: Cumulative TSS Exited (%) for AD2 Sludge after 4 L/d (Nov. 18-19)

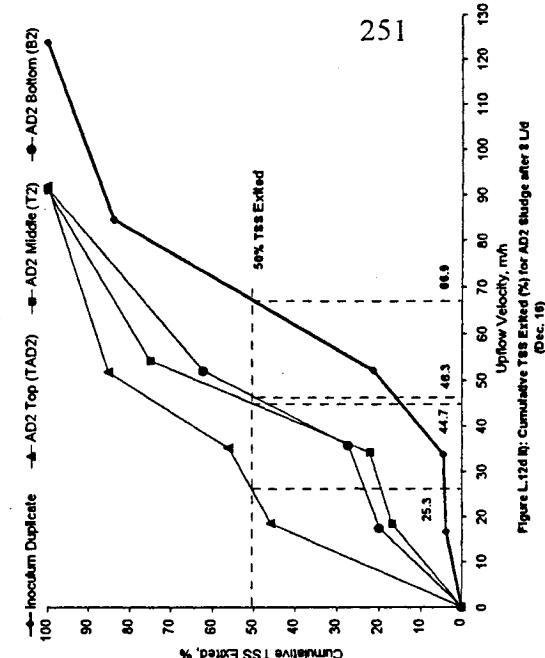
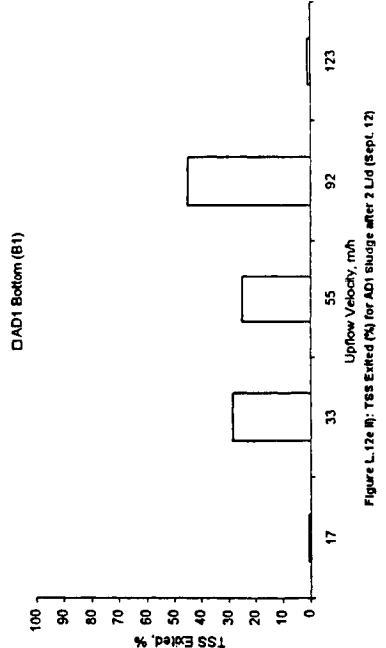
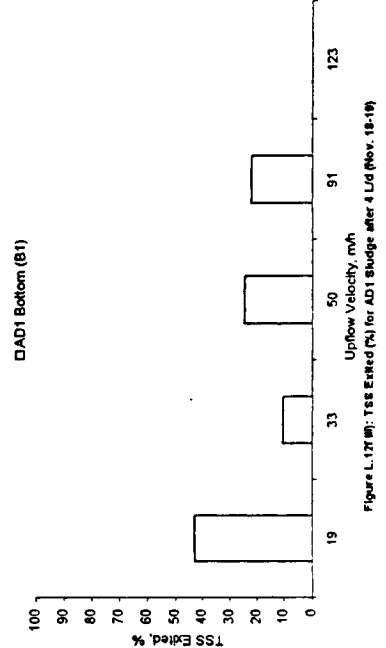
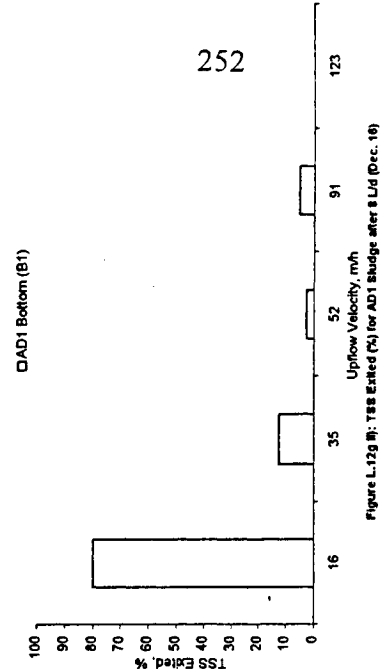
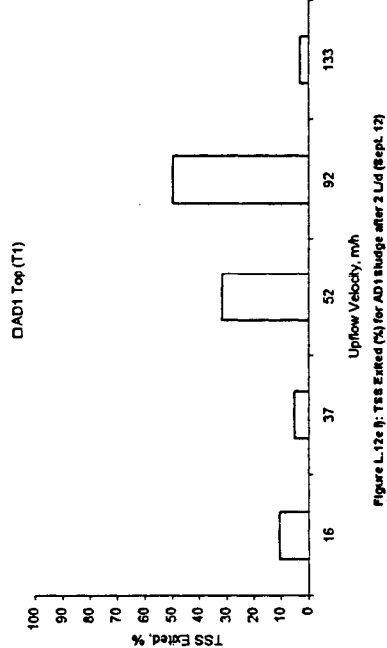
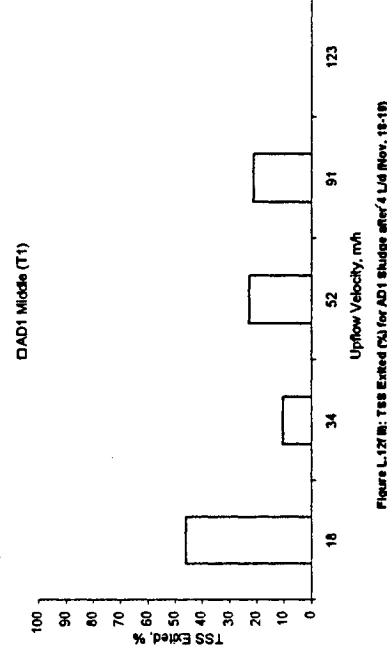
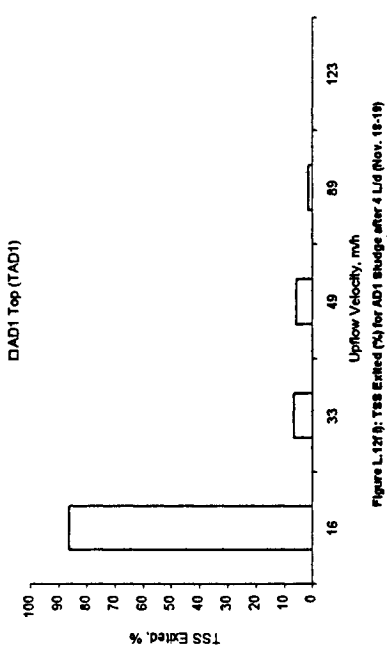
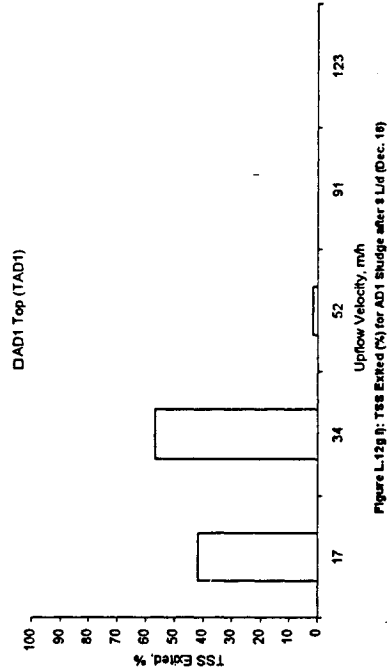
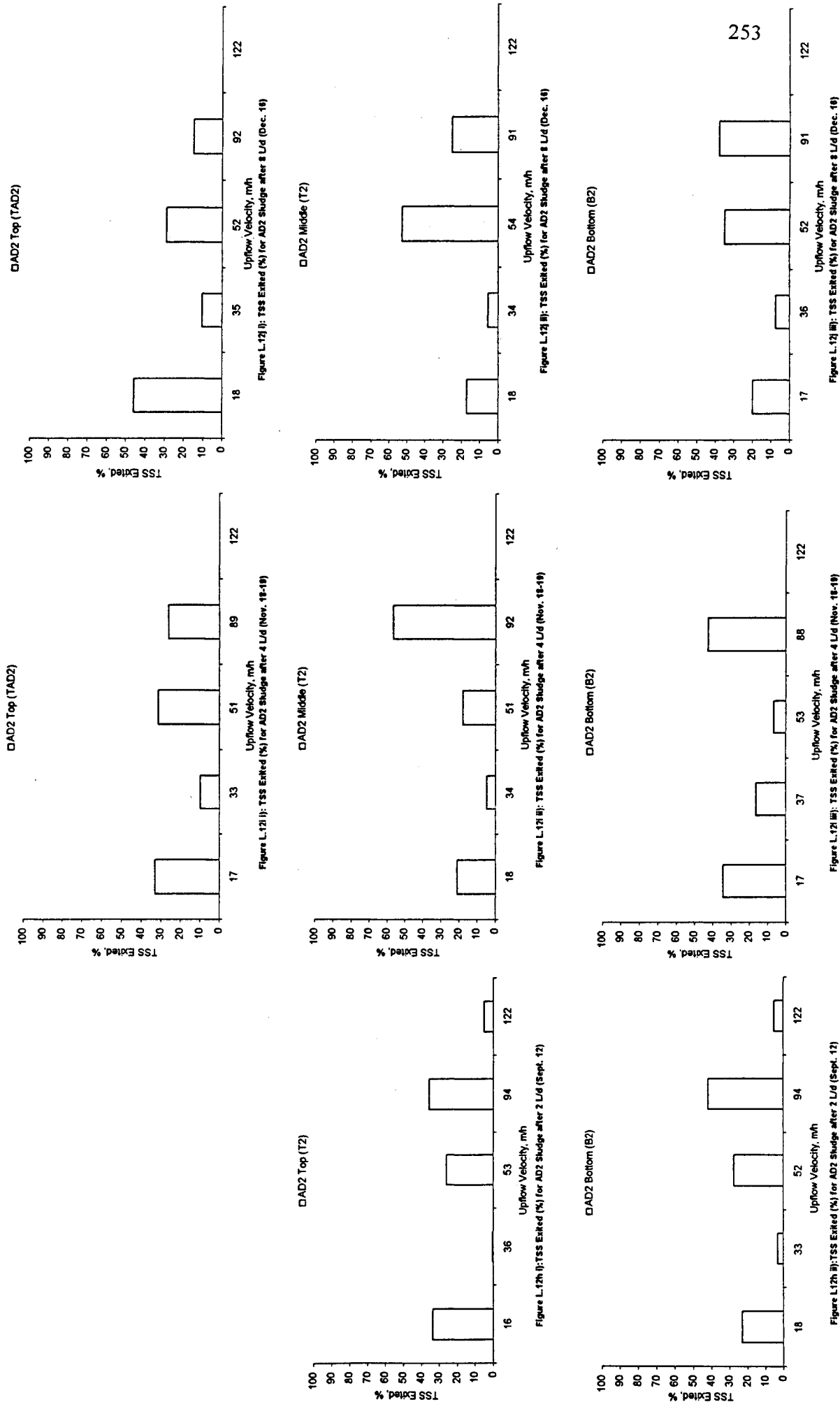


Figure L.12d ii: Cumulative TSS Exited (%) for AD2 Sludge after 8 L/d (Dec. 16)

APPENDIX L.12: UASB REACTOR SETTLING VELOCITY PROFILE DATA



APPENDIX L.12: UASB REACTOR SETTLING VELOCITY PROFILE DATA



APPENDIX M: PARAMETER EXAMPLE CALCULATIONS

[Most calculation equations used are from either *Standard Methods* (APHA, 1995) or *Laboratory Exercises in Environmental Engineering Lab Manual* (Droste, 1997a)]

Total alkalinity (TA) was calculated as follows to end-point pH 4.5 (potentiometric titration):

$$\text{Total Alkalinity, mg CaCO}_3/\text{L} = \frac{t \times N \times 50\,000}{V} \quad (\text{M.1})$$

where t = volume of total titrant to reach pH 4.5, mL
 N = normality of titrant (0.02N H_2SO_4)
 50 000 = unit conversion factor
 V = sample volume, mL

Example:

The raw anaerobic granular sludge supernatant sample data (Table 4.2) from April 3 ($\text{pH}_i = 7.9$, $t = 135.60$ mL, $V = 30$ mL) and July 21 ($\text{pH}_i = 7.5$, $t = 93.00$ mL, $V = 20$ mL) yielded:

$$\text{TA, mg CaCO}_3/\text{L} = \frac{135.60 \times 0.02 \times 50\,000}{30} = 4520 \text{ and } 4650$$

$$\text{Ave. TA} = 4585 \text{ mg/L as CaCO}_3$$

All total and soluble CODs (COD_t and COD_s) were calculated similarly with the following equation. The example presents the raw data from the feed sample characterization (Table 4.1) once the approximate amount of dry Enfalac™ powder mixture (725 mg Enfalac™/L) needed to prepare a 1 000 mg COD_t/L feed was determined by trial and error:

Table M.1: 1000 mg COD_t/L Enfalac™ Feed Sample Data

Sample	DF	s (mL)	Ave. s (mL)	COD_t (mg/L)	s (mL)	Ave. s (mL)	COD_s (mg/L)
KHP ₁ (40%)	2.5	1.76	1.74	192.60 × 2.5 = 482 ≈ 500	-	-	-
KHP ₂ (40%)		1.72					
M ₁	-	3.10	3.12	M = 0.0963	3.20	3.22	M = 0.0933
M ₂	-	3.13		(M=3 mL/3.12 mL × 0.1)	3.23		(M=3 mL/3.22 mL × 0.1)
b ₁	-	2.98	(b = 2.99)	-	NA	(b = 3.15)	-
b ₂	-	3.00			3.15		
5%	20	-	-	-	2.90	2.92	* 34.34 × 20 = 686
	20	-	-		2.93		
10%	10	2.31	2.26	112.49 × 10 = 1 125	2.65	2.64	76.88 × 10 = 769
	10	2.21			2.62		
20%	5	1.70	1.70	198.78 × 5 = 994	2.11	2.16	148.53 × 5 = 743
	5	1.70			2.20		

(*Discarded since < 50 mg/L before DF adjustment) **Average: 1 059**

STD: 65

Average: 756

STD: 13

$$\text{mg COD/L} = \frac{(b-s) \times M \times 8\,000}{V} \times \text{DF} \quad (\text{M.2})$$

where b = average volume of FAS used for the blank, mL
 s = average volume of FAS used for the sample, mL
 M = average molarity of the FAS titrant
 $8\,000$ = unit conversion factor
 V = sample volume, 5 mL
 DF = dilution factor

Example:

The total COD results for the 10% diluted sample duplicates (ave. $s = 2.26$ mL), tested on June 1, with the respective average blank and FAS molarity titrant volumes (ave. $b = 2.99$ mL and ave. $M = 3.12$ mL) gave,

$$\text{mg COD/L} = \frac{(2.99 - 2.26) \times 0.0963 \times 8\,000}{5} \times 10 = 1125$$

Ranges and STD determined from average FAS volumes of diluted sample duplicates (Table 4.1).

All total, volatile and fixed solids (TSS, VSS and FSS) were calculated with the following equations. The example shown is for diluted TWAS samples used to re-seed the ABs on Sept. 13 (Day 52), after the aeration basin modifications (3.5 L and baffle addition), to try to rectify important SS washout problems. The diluted mixture ($\text{DF} \approx 1.52/50$) was left to aerate from Sept. 13-23, until the start of Run 2, where the influent flow rate was progressively increased to 4 L/d. The two duplicate test results given below are summarized and presented in Table 4.3:

TABLE M.2: Diluted TWAS SS Raw Data Set

DATE	Dish & Filter (g)	After 103°C (g)	After 550°C (g)	V (mL)	TSS (mg/L)	VSS (mg/L)	FSS (mg/L)
Sept. 12	2.48624	2.50468	2.49239	10	1 844	1 229	615
	2.54041	2.55903	2.54663	10	1 862	1 240	622
Sept. 23	2.41755	2.42915	2.42160	10	1 160	755	405
	2.44759	2.45885	2.45139	10	1 126	746	380
<i>Average:</i>					1 498	992	506
<i>STD:</i>					410	279	131

$$\text{mg TSS/L} = \frac{(D - F) \times 1\,000}{V} \quad (\text{M.3 a})$$

where F = weight of filter and aluminum evaporation dish, mg
 D = weight of filter, dish and dried solid residue (103°C), mg
 $1\,000$ = unit conversion factor
 V = sample volume, mL

$$\text{mg TSS/L} = \frac{(2504.68 - 2486.24) \times 1000}{10} = 1844$$

Example: From Method 2540 D. (APHA, 1995) and for one of the Sept.12 duplicates,

$$\text{mg VSS/L} = \frac{(D - I) \times 1000}{V} \quad (\text{M.3 b})$$

where D = weight of filter, dish and dried solid residue, mg
I = weight of filter, dish and solid residue after ignition (550°C), mg

Example: From Method 2540 E. (APHA, 1995) and for one of the Sept.12 duplicates,

$$\text{mg VSS/L} = \frac{(2504.68 - 2492.39) \times 1000}{10} = 1229$$

$$\text{FSS} = \text{TSS} - \text{VSS} \quad (\text{M.3 c i})$$

$$\text{or} \quad \text{mg FSS/L} = \frac{(I - F) \times 1000}{V} \quad (\text{M.3 c ii})$$

where F = weight of filter and aluminum dish, mg of , mg
I = weight of filter, dish and solid residue after ignition, mg

Example: Method 2540 E. (APHA, 1995) and for one of the Sept.12 duplicates,

$$\text{FSS} = \text{TSS} - \text{VSS} = 1844 - 1229 = 615 \text{ mg FSS/L}$$

$$\text{mg FSS/L} = \frac{(2492.39 - 2486.24) \times 1000}{10} = 615$$

Percent COD removals for the anaerobic digesters, the activated sludge processes as well as the overall treatment systems were calculated as follow (Droste, 1997b):

$$\eta = \% \text{ COD Removal} = \left(\frac{S_o - S_e}{S_o} \right) \times 100 \quad (\text{M.4})$$

where COD_{infl.} = influent COD, mg COD_i/L
COD_{eml.} = effluent COD, mg COD_s/L

Like percent COD removals, percent TSS removals for the anaerobic digesters, the activated sludge processes as well as the overall treatment systems were calculated as follow (Parkin and Owen, 1986):

$$\% \text{ SS Removal} = \left(\frac{\text{SS}_{\text{Infl.}} - \text{SS}_{\text{Effl.}}}{\text{SS}_{\text{Effl.}}} \right) \times 100 \quad (\text{M.5})$$

The hydraulic retention times (HRT) for the anaerobic reactors and aerobic basins were calculated with the following equation (Droste, 1997b):

$$\text{HRT} = \theta_d = \frac{V_r}{Q} \quad (\text{M.6})$$

where θ_d = HRT = hydraulic retention time, d
 V_r = reactor volume, L
 Q = influent flow rate to the system, L/d

The equation (Droste, 1997b) used to calculate the solids retention times (SRT) or effective sludge ages for the anaerobic and aerobic reactors with and without recycle was:

$$\text{SRT} = \theta_x = \frac{\text{Mass of sludge in the reactor}}{\text{Mass removal rate of sludge from the reactor}} = \frac{V_r X_v}{Q_e X_e} \quad (\text{M.7 a})$$

where θ_x = SRT = Solids retention time or sludge age, d
 X_v = average concentration of VSS in the reactor, mg VSS/L
 Q_e = volumetric flow rate of the reactor effluent, L/d
 X_e = VSS concentration in Q_e , mg VSS/L

The volumetric organic loading rates (OLR_i and OLR_s), in g $\text{COD}_{\text{fed}}/\text{L}\cdot\text{d}$, were calculated with the following equation, for the anaerobic reactors and aerobic basins:

$$\text{OLR} = \frac{\text{COD} \times Q}{V_r} \quad (\text{M.8})$$

where COD = chemical oxygen demand of the influent, g COD/L
 Q = influent flow rate, L/d
 V_r = reactor volume, L

The volumetric (organic) removal rates (VRR_i and VRR_s), in g $\text{COD}_{\text{fed}}/\text{L}\cdot\text{d}$, were calculated with the following equation, for the anaerobic reactors and aerobic basins:

$$\text{VRR} = \text{OLR} \times \% \text{ COD Removal} \quad (\text{M.9})$$

The specific organic loading rates (SOLR_t and SOLR_s), in COD_{fed}/g VSS·d were calculated with the following equation, for the anaerobic reactors and aerobic basins:

$$\text{SOLR} = \frac{\text{COD} \times Q}{X_{\text{VSS}} \times V_r} \quad (\text{M.10})$$

where X_{VSS} = total VSS in reactor, g VSS (solids test x SBH)

The specific (Organic) removal rates (SRR_t and SRR_s) were calculated with the following equation, for the anaerobic reactors and aerobic basins:

$$\text{SRR} = \text{SOLR} \times \% \text{ COD Removal} \quad (\text{M.11})$$

The biomass concentration (X_{VSS} , gVSS) in the AD reactors (*Appendix L.11* and Table 4.15) was calculated as follows:

$$\begin{aligned} \text{AD Biomass Concentration (g VSS/L)} &= \frac{\text{g VSS}}{10 \text{ mL}} (\text{SS test}) \times \text{SBH (mL)} \\ &= \frac{\text{total g VSS in SB}}{\text{total } V_r \text{ (L)}} \end{aligned} \quad (\text{M.12 a})$$

The biomass concentration (X_{VSS} , gVSS) in the aeration basins was calculated as follows:

$$\text{AB Concentration (g VSS/L)} = \frac{\text{g VSS}}{10 \text{ mL}} (\text{VSS test}) = \frac{\text{g VSS}}{\text{L}} \quad (\text{M.12 b})$$

The deviations of the AAT slopes representing the deviations on the specific acetoclastic activity values (*Appendix L.10*) were also calculated with the above equation, where the AAT slope values were simply replaced with the slope deviation values found with Microsoft Excel's Linest function on the applicable data.

The specific acetoclastic activity, in g AA/g VSS·d, could then be converted in g COD_{rem}/g VSS·d via the oxidation reaction of acetic acid (CH₃COOH):



The relation between the acetic acid (60 g AA/mol) and oxygen (64 g COD/2 mol) formula weights yields:

$$\frac{1 \text{ g AA} \times 64 \text{ g O}_2}{60 \text{ g AA}} = X \text{ g O}_2 \text{ (as COD)}$$

$$1 \text{ g AA} = 1.07 \text{ g COD}$$

In *Appendix L.12*, the respective upflow settling velocities were calculated as follow. for the for the various flow (m³/h) settings, where the flows equaled the water volume collected (m³) divided by the time (h):

$$\text{Upflow velocity (m/h)} = \frac{\text{flow (m}^3/\text{h)}}{\text{Settling Test tube area (0.0003 m}^2\text{)}} \quad (\text{M.14 a})$$

The experimental upflow velocities for the UASB reactors (Table 4.16) were also calculated as follows:

$$\text{Upflow velocity (m/h)} = \frac{\text{flow (cm}^3/\text{h)}}{\text{AD reactor cross-sectional area (81.71 cm}^2\text{)} \times 100} \quad (\text{M.14 b})$$

Characterization Parameters Only Performed on Enfalac[™] Feed:

The total, volatile and fixed dissolved solids (TDS, VDS and FDS) were calculated similarly to TSS/VSS/FSS. The calculation example is from one of the June 22 duplicate test data (Raw data is not shown but averages are presented in Table 4.1.):

$$\text{mg TDS/L} = \frac{(D - F) \times 1000}{V} \quad (\text{M.15 a})$$

where F = weight of porcelain evaporation dish, mg
D = weight of dish and dried solid residue (180°C), mg

Example: From Method 2540 C. (APHA, 1995),

$$\text{mg TDS/L} = \frac{(66\,831.1 - 66\,811.2) \times 1000}{50} = 398$$

$$\text{mg VDS/L} = \frac{(D - I) \times 1000}{V} \quad (\text{M.15 b})$$

where D = weight of dish and dried solid residue, mg
I = weight of dish and solid residue after ignition (550°C), mg

Example: From Method 2540 E. (APHA, 1995),

$$\text{mg VDS/L} = \frac{(66\,831.1 - 66\,815.1) \times 1\,000}{50} = 320$$

$$\text{or mg FDS/L} = \frac{(I - F) \times 1\,000}{V} \quad (\text{M.15 c ii})$$

$$\text{FDS} = \text{TDS} - \text{VDS} = 398 - 320 = 78 \text{ mg FDS/L} \quad (\text{M.15 c i})$$

where F = weight of porcelain evaporation dish, mg
I = weight of dish and solid residue after ignition, mg

Example: From Method 2540 E. (APHA, 1995),

$$\text{mg FDS/L} = \frac{(66\,815.1 - 66\,811.2) \times 1\,000}{50} = 78$$

The total, volatile and fixed solids (TS, VS and FS) were calculated with the following equations. The example presented is also from one of the June 22 duplicate test data (Raw data is also not shown but averages are presented in Table 4.1.):

$$\text{mg TS/L} = \frac{(D - F) \times 1\,000}{V} \quad (\text{M.16 a})$$

where F = weight of porcelain evaporation dish, mg
D = weight of dish and dried solid residue (103°C), mg

Example: From Method 2540 B. (APHA, 1995),

$$\text{mg TS/L} = \frac{(71\,804.7 - 71\,729.0) \times 1\,000}{100} = 757$$

$$\text{mg VS/L} = \frac{(D - I) \times 1\,000}{V} \quad (\text{M.16 b})$$

where D = weight of dish and dried solid residue, mg
I = weight of dish and solid residue after ignition (550°C), mg

Example: From Method 2540 E. (APHA, 1995),

$$\text{mg VS/L} = \frac{(71\,804.7 - 71\,736.5) \times 1\,000}{100} = 682$$

$$FS = TS - VS = 757 - 682 = 75 \text{ mg FS/L} \quad (\text{M.16 c i})$$

$$\text{or} \quad \text{mg FS/L} = \frac{(I - F) \times 1000}{V} \quad (\text{M.16 c ii})$$

where F = weight of porcelain evaporation dish, mg
I = weight of dish and solid residue after ignition, mg

Example: From Method 2540 E. (APHA, 1995),

$$\text{mg FS/L} = \frac{(71\,736.5 - 71\,729.0) \times 1000}{100} = 75$$

The total, volatile and fixed solids (%TS, %VS and %FS), in solid Enfalac™ powder samples, were calculated with the following equations. Data from one of the June 22 test duplicates was also used in each of the example calculations (Raw data is not shown but averages are presented in Table 4.1.):

$$\% \text{ TS} = \frac{(D - F) \times 100}{(S)} \quad (\text{M.17 a})$$

where F = weight of porcelain evaporation dish, mg
D = weight of dish and dried solid residue (103°C), mg
S = weight of (wet) sample, mg

Example: From Method 2540 G. 3.b. (APHA, 1995),

$$\% \text{ TS} = \frac{(132\,278.2 - 107\,802.4) \times 100}{(25\,000)} = 97.90$$

$$\% \text{ VS} = \frac{(D - I) \times 100}{(D - F)} \quad (\text{M.17 b})$$

where I = weight of dish and solid residue after ignition (550°C), mg

Example: From Method 2540 G. (APHA, 1995),

$$\% \text{ VS} = \frac{(132\,278.2 - 108\,438.2) \times 100}{(132\,278.2 - 107\,802.4)} = 97.40$$

$$\%FS = \frac{(I - F) \times 100}{(D - F)} \quad (M.17c)$$

where F = weight of porcelain evaporation dish, mg
D = weight of dish and dried solid residue, mg
I = weight of dish and solid residue after ignition, mg

Example: From Method 2540 G. (APHA, 1995),

$$\%FS = \frac{(108\,438.2 - 107\,802.4) \times 100}{(132\,278.2 - 107\,802.4)} = 2.60$$

The phosphorus concentration in the feed (Table 4.1), prior to chemical supplement addition, was determined using the average phosphate calibration curve equation (*Appendix F*), with $R^2 = 0.9975$, found from repeat standard dilution spectrophotometric absorbance measurements:

$$y = 0.6005x - 0.0007 \quad (M.18)$$

Table M.3: 1 000 mg COD/L Feed Batch Sample (June 21)

Phosphorus Type	Dilution Factor (%)	Absorbance (y)
Total	10	0.207
Dissolved	10	0.056

Example: By rearranging the equation, the results yielded,

$$\text{Total Phosphorus} = [(0.207 + 0.0007)/0.6005] \times 10 = 3.46 \text{ mg PO}_4^{-3}\text{-P/L and}$$

$$\text{Dissolved Phosphorus} = [(0.056 + 0.0007)/0.6005] \times 10 = 0.94 \text{ mg PO}_4^{-3}\text{-P/L}$$

Results in Table 4.1 are the averages of replicate tests performed on a few feed samples.

Enfalac™ powder package content descriptions state that it contains 30 mg P/100 mL for a normal dilution. The theoretical phosphorous content of 17 g packages used to prepare 120 mL bottles ($17 \text{ g}/120 \text{ mL} = 141\,666.7 \text{ mg Enfalac}^{\text{TM}}/\text{L}$) would be 300 mg P/L. Therefore, a 725 mg Enfalac™/L sample (~1 000 mg COD_t/L) should contain:

$$141\,666.7 \text{ mg Enfalac}^{\text{TM}}/\text{L} \rightarrow 300 \text{ mg P/L (for a 120 mL bottle)}$$

$$725 \text{ mg Enfalac}^{\text{TM}}/\text{L} \rightarrow X \text{ mg P/L}$$

$$\text{Solving for X yields, } X = 1.54 \text{ mg P/L}$$

However, the average experimental result found for this parameter was 2.74 mg/L PO₄⁻³ as total P (Table 4.1).

The average BOD₅ contained in the feed was calculated with the following equation, where 5 mL of acclimated seed material was added directly to duplicate sample dilution and seed control bottles:

$$\text{mg BOD}_5/\text{L} = \frac{[(\text{DO}_i^s - \text{DO}_5^s) - (\text{DO}_i^c - \text{DO}_5^c) \times f] \times 300}{V} \quad (\text{M.18 a})$$

where DO_i = initial dissolved oxygen concentration, mg/L
DO₅ = dissolved oxygen concentration after 5 days, mg/L
s = refers to sample
c = refers to seed control
V = sample volume, mL
300 = BOD bottle volume, mL

and f is defined by:

$$f = \frac{\text{volume of seed added to the sample, mL}}{\text{volume of seed added to the control, mL}} = \frac{5 \text{ mL}}{5 \text{ mL}} = 1 \quad (\text{M.18 b})$$

For result reliability, the following BOD analysis criteria (APHA, 1995) are to be respected and the results rejected if not:

- Dilution water blanks DO uptake (DO_i^{BL}-DO₅^{BL}): ≤ 0.2-0.5 mg/L and preferably not more than 0.1 mg/L
- Seed Controls DO uptake (DO_i^C-DO₅^C): 0.6 mg/L ≤ (DO_i^C-DO₅^C) ≤ 1 mg/L
- Sample Dilutions: Residual DO (DO₅ ≥ 1 mg/L) and DO depletion (DO_i^S-DO₅^S) ≥ 2 mg/L

Table M.4: Average BOD₅ Content of 1 000 mg COD/L Feed Sample (June 28-July 3)

Sample	DO _i , (°C)	Duplicate	DO ₅ , (°C)	Duplicate	DO _i - DO ₅	Duplicate	Ave. DO _i - DO ₅	300/V	BOD ₅
Blank 1	9.0 (21.3)	9.0 (21.3)	8.0 (19.6)	8.1 (19.7)	<i>1.0</i>	<i>0.9</i>	-	-	-
Blank 2	8.8 (20.6)	8.7 (20.7)	8.1 (19.5)	8.2 (19.6)	<i>0.7</i>	0.5	0.5	-	-
Seed Control 1	8.7 (20.9)	8.7 (20.7)	7.4 (19.8)	8.0 (19.6)	<i>1.3</i>	0.7	0.7	-	-
0.5 mL	8.6 (20.8)	8.6 (20.7)	7.4 (19.9)	7.1 (19.8)	<i>1.2</i>	<i>1.5</i>	-	600	NA
1 mL	8.6 (20.9)	8.6 (20.8)	6.7 (19.9)	6.8 (20.1)	<i>1.9</i>	<i>1.8</i>	-	300	NA
2 mL	8.6 (20.8)	8.6 (20.8)	5.7 (19.9)	5.7 (19.8)	2.9	2.9	2.9	150	660
3 mL	8.6 (20.9)	8.6 (20.9)	4.5 (19.4)	4.6 (19.6)	4.1	4.0	4.05	100	670
6 mL	8.5 (20.9)	8.6 (21.0)	<i>0.9 (19.7)</i>	<i>0.6 (19.6)</i>	<i>7.6</i>	<i>8.0</i>	-	50	NA
10 mL	8.5 (21.0)	8.5 (21.1)	<i>0 (20.0)</i>	<i>0 (19.9)</i>	<i>8.5</i>	<i>8.5</i>	-	30	NA

Ave. BOD₅ = 665 mg/L

In Table M.4, the italicized data were rejected by not meeting the criteria stipulated above. Since 2 sample dilution results met the above sample dilution criteria, both for residual DO and DO depletion, as well as the other criteria, and there was no evidence of toxicity or anomaly, they were averaged (APHA, 1995) and presented in Table 4.1.

Example:

From Table M.4, for the 2 mL sample dilution duplicates, with $f = 1$ and $DF = 2$,

$$\text{Ave. mg BOD}_5/\text{L} = \{[(2.9) - (0.7)] \times 150\} \times 2 = 660$$

The feed's BOD_5 value was initially estimated to be roughly 667 $\text{mg BOD}_5/\text{L}$, from an approximate 1 000 $\text{mg COD}_t/\text{L}$ feed stock solution since BOD_5 can conservatively be converted to a COD value by multiplying it by 1.5, according to Droste (1997b). The *Process Control Laboratory Course Manual for Wastewater Treatment Plant Operators* (1982) general guidelines also states that BOD values in the range of 100 to 300 mg/L can be expected for typical raw domestic wastewater. Therefore, the feed mixture was diluted by half ($DF = 2$) and sample dilutions were prepared based on the ones suggested in the guidelines, in the volumes presented in Table M.4, to ensure some satisfactory results, in the acceptable range.

Average total kjeldahl nitrogen (TKN-N) and organic nitrogen (org. N) concentrations in duplicate feed samples were calculated with the following equations as well as the average recovery of nitrogen from the respective average standards (*Appendix G*). Average ammonia nitrogen ($\text{NH}_3\text{-N}$) contained in feed sample distillate was also estimated by direct distillation and titration [4500- NH_3 B. and C. (APHA, 1995)], from 25 mL sample volumes, and compared to the results found with the ammonia-selective electrode method (calculations described below).

Parameter result calculations could only be done after interpolating the volumes of HCl required to titrate the blanks, samples and standards to pH 4.65.

Example: Solving for X (mL) for one of the org. N blanks (July 18),

$$V_{\text{HCl}} = 0.43 \text{ mL; pH} = 4.82$$

$$V_{\text{HCl}} = ? \text{ mL; pH} = 4.65$$

$$V_{\text{HCl}} = 0.47 \text{ mL; pH} = 4.38$$

$$\frac{0.47 - 0.43}{4.82 - 4.38} = \frac{X - 0.43}{4.82 - 4.65}$$

$$X = V_{\text{HCl}} = 0.4454 \text{ mL}$$

The other org. N blank's HCl volume (July 18) gave 0.4276 mL and the average $\text{blk}_{\text{org N}} = 0.4365 \text{ mL}$ (Table M.5). All blank, sample and standard interpolations were determined similarly to the above example for TKN, org. N and $\text{NH}_3\text{-N}$ parameters but other raw data are not presented. However, the average titrant volumes needed to titrate the various samples to pH 4.65 appear in Table M.5 while the average parameter results are presented in Table 4.1 (CH.4, Section 4.1).

Table M.5: Average Interpolated HCl Volumes (July 18)

Parameter	Sample Volume (mL)	Ave. Std (mL)	Ave. sp (mL)	Ave. blk (mL)	^s Average % Recovery of N
TKN-N	50	2.553	1.391	0.398	75
org. N	50	1.426	1.173	0.437	~ 70
NH ₃ -N	*25	1.030	0.539	0.443	82

*Sample volume should have been much greater than 25 mL [~250 mL for 5-10 mg NH₃-N/L (APHA, 1995)] since results yielded approximately 3 mg NH₃-N/L. ^sSee example calculations below.

$$\text{mg TKN-N/L} = \frac{(\text{sp}_{\text{TKN-N}} - \text{blk}_{\text{TKN-N}}) \times 700}{V} \quad (\text{M.19 a})$$

where sp = volume of HCl required to titrate sample to pH 4.65, mL
 blk = average volume of HCl required to titrate blank to pH 4.65, mL
 V = sample volume, mL
 700 = conversion factor = (14 g/mol N × M_{HCl} × 1 000 mg/g)
 M_{HCl} = molarity of HCl titrant, 0.05 mol/L

Example:

$$\text{mg TKN-N/L} = \frac{(1.391 - 0.398) \times 700}{50} = 13.90$$

$$\begin{aligned} \% \text{ Recovery}_{\text{TKN-N}} &= \left(\frac{(\text{Std}_{\text{TKN-N}} - \text{blk}_{\text{TKN-N}}) \times 700}{V} \right) \times 100 \quad (\text{M.19 b}) \\ &= \frac{(2.553 - 0.398) \times 14}{40} = \frac{30.17}{40} = 75\% \end{aligned}$$

where Std_{TKN-N} = average volume of HCl required to titrate the 40 mg/L standard to pH 4.65, mL

Solving for X (mg TKN-N/L), with 100% recovery (Table 4.1):

$$13.90 \text{ mg TKN-N/L (at 75\%)} \rightarrow X \text{ mg TKN-N/L (at 100\%)} = 18.53 \text{ mg TKN-N/L}$$

$$\text{mg org. N/L} = \frac{(\text{sp}_{\text{org. N}} - \text{blk}_{\text{org. N}}) \times 700}{V} \quad (\text{M.20 a})$$

Example:

$$\text{mg org. N/L} = \frac{(1.173 - 0.437) \times 700}{50} = 10.30$$

Std_{org. N} = average volume of HCl required to titrate the 20 mg/L standard to pH 4.65, mL

$$\begin{aligned} \% \text{Recovery}_{\text{org. N}} &= \left(\frac{\frac{(\text{Std}_{\text{org. N}} - \text{blk}_{\text{org. N}}) \times 700}{V}}{20 \text{ mg/L}_{(\text{org. N})}} \right) \times 100 & (\text{M.20b}) \\ &= \frac{(1.426 - 0.437) \times 14}{20} = \frac{13.85}{20} \cong 70\% \end{aligned}$$

Solving for X (mg org. N/L), for 100% recovery (Table 4.1):

$$10.30 \text{ mg org. N/L (at } \sim 70\%) \rightarrow X \text{ mg org. N/L (at } 100\%) = 14.71 \text{ mg org. N/L}$$

$$\text{mg NH}_3\text{-N/L} = \frac{(\text{sp}_{\text{NH}_3\text{-N}} - \text{blk}_{\text{NH}_3\text{-N}}) \times 700}{V} \quad (\text{M.21a})$$

Example:

$$\text{mg NH}_3\text{-N/L} = \frac{(0.539 - 0.443) \times 700}{25} = 2.69$$

$$\begin{aligned} \% \text{Recovery}_{\text{NH}_3\text{-N}} &= \left(\frac{\frac{(\text{Std}_{\text{NH}_3\text{-N}} - \text{blk}_{\text{NH}_3\text{-N}}) \times 700}{V}}{20 \text{ mg/L}_{(\text{NH}_3\text{-N})}} \right) \times 100 & (\text{M.21b}) \\ &= \frac{(1.030 - 0.443) \times 28}{20} = \frac{16.44}{20} = 82\% \end{aligned}$$

where Std_{NH₃-N} = average volume of HCl required to titrate the 20 mg/L standard to pH 4.65, mL

Solving for X (mg NH₃-N/L), for 100% recovery (Table 4.1):

$$2.69 \text{ mg NH}_3\text{-N/L (at } 82\%) \rightarrow X \text{ mg NH}_3\text{-N/L (at } 100\%) = 3.28 \text{ mg NH}_3\text{-N/L}$$

The range and STD of the parameter sample duplicate results were established like that of the COD data, by using the average blank value (and average standard value for average % recoveries of N) in the calculation equation of the same form, with each of the sample titration volumes.

NH₃ and NH₃-N concentrations in the feed were determined by *Direct Calibration*, from duplicate test results (June 8 and July 9) using *Microsoft Excel's* Trendline command to fit the experimental data. Both sample potential readings just fell within the electrode's non-linear region. For the test performed on June 8 and presented in *Appendix I.1*, the best-fit exponential regression calibration curve equation, with $R^2 = 0.998$, yielded:

$$y = 139.7e^{-3475.4x} \quad (\text{M.22})$$

Table M.6: Raw 1 000 mg COD_L/L Feed Sample (June 8)

Electrode Slope (mV)	Sample Electrode Potential Reading (mV)
59 (O.K.)	137.8

Example: From *Microsoft Excel's* equation, solving for x (moles/L),

$$137.8 = 139.7e^{-3475.4x}$$

$$x = 3.94 \times 10^{-6} \text{ M}$$

From the appropriate NH₃ concentration unit conversion factors, where 10⁻¹ M (moles/L) = 1700 ppm as NH₃ = 1400 ppm as N, the results found were therefore:

$$x = 0.07 \text{ mg NH}_3/\text{L} \text{ and } 0.06 \text{ NH}_3\text{-N/L}$$

Excel polynomial trend models also fit the data quite well. Therefore, *MATLAB's* polyfit built-in function was used to calculate and compare the results found by *Excel's* Trendline command. For the test performed on June 8, given the non-linear experimental data (Table I.1 in *Appendix I.1*), the second-order polynomial fit from *MATLAB* gave:

$$x = 3.29 \times 10^{-6} \text{ M} = 0.06 \text{ mg NO}_3^-/\text{L} \text{ and } 0.05 \text{ NO}_3\text{-N/L (similar to Excel's result)}$$

Low-Level NH₃ and NH₃-N feed sample concentrations were also determined (with *Microsoft Excel's* Trendline command) since the *Direct Calibration* results hovered around the test limits of $4 \times 10^{-6} \text{ M NH}_3$ (0.07 ppm NH₃ or 0.06 ppm as N). The best fit for the Low-Level

ammonia calibration curve presented in *Appendix I.2* (Table I.2) was a third-order polynomial, with $R^2 = 0.9993$. However, the Low-Level measurement test (July 9), gave the following mV readings:

Table M.7: Samples Tested on July 9 (Low-Level)

Electrode Slope (mV)	Electrode Potential Readings (mV)
57.9 (O.K)	Feed = 136.8 Tap water = 149.5 Distilled water = 138.9

From Table I.2, it can be seen that the sample's electrode potential reading yielded less than 9.8×10^{-7} M (0.017 NH_3 or $0.014 \text{ NH}_3\text{-N}$). Similarly, from their resulting potential measurements, tap and distilled water samples were found to have even less than 10^{-8} M NH_3 (0.00017 NH_3 or $0.00014 \text{ NH}_3\text{-N}$) during Direct Calibration tests [June 8 (Table I.1) and June 25]. Since available distilled water had only trace amounts of NH_3 , it was therefore used to prepare standards instead of ammonia-free water.

Direct Measurement NO_3 and $\text{NO}_3\text{-N}$ concentrations in the feed were also determined from duplicate test results (June 9 and July 7), using *Microsoft Excel's* Trendline command. Sample potential readings for both trials clearly fell in the non-linear region of the calibration curves. For the test performed on July 7 and presented in *Appendix J.1*, the best-fit exponential regression calibration curve equation, with $R^2 = 0.9978$, yielded:

$$y = 225.15e^{-2001.4x} \quad (\text{M.23a})$$

Table M.8: Raw 1 000 mg COD_L/L Feed Sample (July 7)

Electrode Slope (mV)	Sample Electrode Potential Reading (mV)
57.9 (O.K)	193.6

Example: From *Microsoft Excel's* equation, solving for x (moles/L),

$$193.6 = 225.15e^{-2001.4x}$$

$$x = 7.54 \times 10^{-5} \text{ M}$$

From the appropriate NO_3 concentration unit conversion factors, where 10^{-2} M (moles/L) = 620 ppm as NO_3^- = 140 ppm as N, the results found were therefore:

$$x = 4.67 \text{ mg NO}_3^-/\text{L} \text{ and } 1.06 \text{ NO}_3\text{-N/L}$$

For the test performed on July 7, given the non-linear experimental data (Table J.1 in *Appendix J.1*), the second-order polynomial fit from *MATLAB*'s polyfit function gave:

$$x = 6.99 \times 10^{-5} \text{ M} = 4.33 \text{ mg NO}_3^-/\text{L} \text{ and } 0.99 \text{ NO}_3\text{-N/L (similar to Excel's result)}$$

Using *Microsoft Excel*'s Trendline command, *Low-Level* NO_3^- and $\text{NO}_3\text{-N}$ feed sample concentrations were also determined since the above results fell below 10^{-4} M NO_3^- (6.2 ppm NO_3^- or 1.4 ppm as N), the test limit for the *Direct Measurement* method. From the test performed on July 7 and presented in *Appendix J.2* (Table J.2), the best-fit third-order polynomial regression calibration curve equation, with $R^2 = 0.9995$, gave:

$$y = -5\text{E}+14x^3 + 5\text{E}+10x^2 - 2\text{E}+06x + 245.06 \quad (\text{M.23 b})$$

Table M.9: Raw *Low-Level* 1 000 mg COD/L Feed Sample (July 7)

Electrode Slope (mV)	Sample Electrode Potential Reading (mV)
58.7 (O.K)	214.1

Example: From *Microsoft Excel*'s equation, solving for x (moles/L),

$$214.1 = -5\text{E}+14x^3 + 5\text{E}+10x^2 - 2\text{E}+06x + 245.06$$

$$x = 3.72 \times 10^{-5} \text{ M} = 2.31 \text{ mg NO}_3^-/\text{L} \text{ and } 0.52 \text{ NO}_3\text{-N/L}$$

From the *Low-Level* measurement test (July 7), the second-order polynomial fit from *MATLAB* gave:

$$x = 3.92 \times 10^{-5} \text{ M} = 2.43 \text{ mg NO}_3^-/\text{L} \text{ and } 0.55 \text{ NO}_3\text{-N/L (similar to Excel's result)}$$

For both types of electrodes, *Direct* and *Low-Level* measurements were applied in a similar fashion to find tap and distilled water parameter concentration results (Table 4.1). All results presented in Table 4.1 are average results from the various trials, using both *Microsoft Excel* and *MATLAB* for simple curve regression analysis. However, *Excel*'s Trendline command which allows to fit common trend models to data, limits its statistical content to the coefficient of determination value (R^2). Thus, it does not allow statistical inferences to be drawn regarding the model fit (Chapra and Canale, 1998).