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Treatment of Dilute Aircraft Deicing Fluid (ADF) using an Anaerobic Baffled Reactor (ABR)

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TREATMENT OF DILUTE AIRCRAFT DEICING FLUID (ADF) USING AN ANAEROBIC BAFFLED REACTOR (ABR)

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B.Sc. Industrial Biochemical Engineering

Thesis submitted to the
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Abstract

Successful treatment of low strength aircraft deicing fluid (ADF) was achieved using the Anaerobic Baffled Reactor (ABR), under different hydrological retention times (HRT) and COD influent concentrations. A total of 17 experimental runs were conducted in continuous mode, achieving COD removal efficiencies of over 75% for most of the conditions tested. During this experiment different ADF concentrations (0.04%, 0.07%, and 0.13% v/v) were continuously fed at different HRTs (24, 12, 6 and 3 hrs) at organic loading rates (OLR) varying between 0.3 and 6 kg COD/m^3/d. The performance of the reactor was determined by the COD removal efficiency at the different OLRs. The best COD removal was reached at high COD influent concentrations and HRTs, for the lowest HRTs essayed COD removal efficiency dropped to an average of 65% for the 3 COD influent concentrations tested. Methane production potential were close to the theoretical value of 0.39 L CH_4/g COD removed at 35°C.

The effect of biomass pre-acclimation was also evaluated and it was found to be effective in increasing specific acetoclastic activity for this type of wastewater in order to reduce the time needed for acclimation. It was found that the biomass specific acetoclastic activity changed with time all through the reactor. At the end of the experiment, a new distribution of different groups of microorganisms was established in each compartment of the reactor. Biomass specific acetoclatic activity improved two fold for the last three compartments and decreased almost the same magnitude for the first compartment. The different experimental conditions did not affect the settling characteristics of the granular biomass during this study.

The application of the empirical model proposed for Xing *et al.* (1991) did not described the performance of the ABR under the experimental conditions tested, instead experimental results were fitted to a linear model to describe the COD removal efficiency as a function of COD influent concentration and HRT. Model verification indicated that predicted response (COD removal efficiency) was in good agreement with experimental results.

Résumé

Le traitement de fluide de déglaçage d'avion (FDA) à basse concentration a été réussi avec succès en utilisant un réacteur à déflecteurs anaérobique (RDA), sous différentes conditions de temps de rétention hydrologique (TRH) et de concentration d'influent de demande chimique d'oxygène (DCO).

Un total de 17 essais experimentaux ont été effectué en mode continu, atteignant des efficacités d'élimination de DCO de plus de 75% pour la plupart des conditions expérimentées. Durant cette expérience, différentes concentrations de FDA (0.04%, 0.07%, et 0.13% v/v) ont été alimenté sous divers TRH (24, 12, 6 et 3 hrs) a des taux de chargement organique (TCO) variant entre 0.3 et 6 kg DCO/m^3•d. La performance du réacteur a été déterminé par l'efficacité d'élimination du DCO sous les divers TLO. La meilleure élimination de DCO a été atteinte avec des concentrations élevées d'influents de DCO et de grands TRH. Pour les plus petits TRH expérimentés, l'efficacité d'élimination est tombé a environ 65% en moyenne, pour les trois concentrations d'influents de DCO testées.

L'effet d'une pré-acclimatation a aussi été évaluée. Cet effet a été efficace pour ce type d'eaux contaminées, afin de réduire le temps nécessaire pour l'acclimatation.

Il a été trouvé que l'activité acétoclastique spécifique à cette biomasse a changé à travers tout le réacteur à la fin de l'expérience, ce qui indique une distribution de différents groupes de micro-organismes dans chacun des compartiments du réacteur. L'activité acétoclastique spécifique de la biomasse s'est améliorée du double pour les trois derniers compartiments, tout en diminuant de la même amplitude dans le premier.

La production potentielle de méthane a aussi été très proche de la valeur théorique de 0.39 L CH_4/g DCO éliminée à 35°C.

Les diverses conditions expérimentales n'ont pas affecté les caractéristiques de dépôt de la biomasse granulaire, lors de cette étude.

L'application du modèle empirique suggéré par Xing *et al* (1991) n'a pas prédit la performance du RDA sous les conditions expérimentales testées. Un modèle linéaire a plutôt été développé afin de décrire l'efficacité d'élimination du DCO comme une fonction de la concentration de l'influent DCO et du TRH. La validation du modèle

indique que la réponse prédite (l'efficacité d'élimination du DCO) s'accordait avec les résultats expérimentaux.

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Nomenclature

AAT	acetoclastic activity test
AAFEB	anaerobic attached film expanded bed reactor
ABR	anaerobic baffled reactor
Ac	acetate
ADF	aircraft deicing fluid
AEA	Association of European Airlines
AF	anaerobic filter
ATA	anaerobic toxicity test
AFBR	anaerobic fluidized bed reactor
BOD	biochemical oxygen demand
BOD_5	biochemical oxygen demand (after five days incubation)
CEPA	Canadian Environmental Protection Act
COD	chemical oxygen demand
COD:N:P	cod:nitrogen:phosphorus ratio
CSTR	completely stirred tank reactor
d	diameter
EC_{50}	concentration for 50% effect
EG	ethylene glycol
FAA	Federal Aviation Administration
FDP	freezing point depressing fluid
F/M	food-to-microorganism ratio
GC	gas chromatography
HABR	hybrid anaerobic baffled reactor
HPLC	high pressure liquid chromatography
HRT	hydraulic retention time
ISO	International Standards Organization
L	liter
Li^+	Lithium
MeBT	4(5)-methylbenzotriazole
MW	molecular weight

OLR	organic loading rate
PG	propylene glycol
R	recycling ratio
rpm	revolutions per minute
RT	retention time
SAE	Society of Automotive Engineers
SD	standard deviation
SFABR	split fed anaerobic baffled reactor
SMPs	soluble microbial products
SOLR	specific organic loading rate
SS	steady state
SRT	solids retention time
SWW	synthetic wastewater
TSS	total suspended solids
UASB	upflow anaerobic sludege blanket
VFA	volatile fatty acid
VSS	volatile suspended solids

k	first order reaction rate, d^{-1}
k_1	first order reaction rate, L/g VSS/d
k_{max}	maximum specific rate of substrate utilization, g COD/g VSS/d
k_d	endogenous decay coefficient, d^{-1}
K_s	affinity constant, mg/L
Q	flow rate, L/d
S	substrate, g/L
U	specific substrate utilization rate, mg COD/ mg VSS/d
v_{50}	upflow velocity resulting in 50% biomass washout, m/h
V_1	compartment 1 volume, L
Vr	rector volume, L
W_1	mass of granular sludge in compartment 1, g VSS
X	biomass concentration, g VSS/L

X_1	biomass in compartment 1, g VSS/L
X_N	biomass in compartment N, g VSS/L
Y	cell yield coefficient, mg VSS/ mg COD
B_0	parameter estimate
$\bar{y}$	average value
y	parameter response
$\hat{y}$	predicted value of y
R^2	coefficient of determination, a regression statistic

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CHAPTER 1

INTRODUCTION

In our modern society, flying as a means of transportation has become a necessity. As the air traffic increases, the need for safety in all its aspects is more demanding. Northern countries, face the additional challenge to provide safe traveling under severe winter conditions. To accomplish this, airports, as a part of their winter maintenance operations use deicing chemicals on a regular basis to make sure that critical aircraft surfaces are free of ice, snow or frost formation before take-off.

Aircraft Deicing Fluids (ADF) are applied to aircraft surfaces to remove snow, ice and frost and to prevent their subsequent accumulation or formation. These elements can alter the shape of the wings airfoil section and its surface flow characteristics, causing a loss of lift that can prevent the plane from taking off or cause it to crash if the ice develops in flight (Transport Canada, 1994). One of those occurrences happened at LaGuardia Airport in New York during a winter storm in March, 1992, where 27 passengers and crew lost their lives. In response, the U.S Federal Aviation Administration (FAA) imposed more stringent requirements for deicing activities, and, as a consequence, the amounts of ADF used in U.S. airports has increased.

The fluids used to deice/anti-ice aircraft in North America are usually composed of ethylene glycol (EG) or propylene glycol (PG) combined with water and other ingredients. The formulation is proprietary, but depending on the final use of the product some, may contain wetting agents, corrosion inhibitors, colorants and thickeners.

In Canada, air transportation is responsible for the greatest volume of release of EG to the environment. According to a survey carried out under authority of the Canadian Environmental Protection Act (CEPA), an estimated 7.7 kilo-tonnes of EG were used in 1996 for aircraft deicing/anti-icing operations (Environmental Canada, 2000). Test results have indicated that 16% of the glycol used to deice planes remains on the aircraft, 35% is blown behind the aircraft and about 50% falls to the ground in the vicinity of the aircraft following applications. (Simpson, 1997). Runoff from aircraft deicing activities has resulted in the release of large quantities of dilute glycol-based fluids to the aquatic environment. The very high biochemical oxygen demand (BOD_5) of deicers is their main impact on environment. (Miller, 1979; and Sabeh, 1991).

There is also evidence that the toxicity of ADF is increased by the presence of additives present in the formulation (e.g. triazoles, organic amine bases, etc.). Pillard (1995) found that ADF formulations were significantly more toxic to the water flea *Cercerodaphnia dubia,* and the fat head minnow, *Pimephales promelas,* than pure EG and PG. This indicates the need to develop systems able to remove the glycol and the additive contaminants from airport's runoffs, which can cause serious effects on the environment.

To date, there are reports that show the efficiency of anaerobic treatment of ADF (Darlington and Kennedy; 1988, Schoenberg *et al.*, 2001; Pham, 2002; and Zitomer and Tonuk, 2003) using different reactor configurations and treatment operation variables, however the literature available for dilute EG-based ADF wastewaters using the ABR is limited.

1.1 RESEARCH OBJECTIVES

The general objective of this study is to evaluate the performance of the Anaerobic Baffled Reactor (ABR) for the treatment of low-strength synthetic EG-based ADF wastewater, under different ADF concentrations, HRT and, mesophilic temperature conditions (35°C).

The specific objectives of this study are:

- Determine the optimum operation conditions for the treatment of dilute ADF in ABRs.
- Determine the efficiency of the ABR in terms of influent substrate concentration and HRT under mesophilic conditions.
- Evaluate changes in the characteristics of the granular biomass, such as acetoclastic activity and settling characteristics.
- Determine the effect of OLR and HRT on the suspended solids in the effluent.
- Use the empirical model proposed by Xing *et al.*, (1991) to describe the effects of influent ADF concentration and HRT on COD removal efficiency.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

2.1 AIRCRAFT DEICING/ANTI-ICING FLUIDS

2.1.1. Overview

The basic philosophy of using deicing/anti-icing fluids for aircraft is to remove frozen contaminants such as ice, snow and frost and to prevent their accumulation. According to Transport Canada (1998a), in winter, most aircraft related accidents have occurred when the aircraft was not deiced properly prior to take-off. The deicing process is intended to restore the aircraft to the clean configuration in which it was designed, so its aerodynamic characteristics will not be affected, and to avoid mechanical failures due to the presence of these contaminants. Canadian air regulations prohibit takeoff when frost, ice or snow is adhering to any critical surface of the aircraft. This is referred to as "The Clean Aircraft Concept" and it is essential to the maintenance of flight safety.

The methods used to deice aircraft may vary depending on the type of accumulation on the surface of the aircraft, the type of aircraft, the climate conditions and the resources at the airport. They can be divided into manual methods (e.g., use of brooms or ropes), chemical methods (e.g., solutions that have lower freezing point) and use of heat (e.g., use of designated warm areas to defrost aircraft). Each one has advantages and disadvantages, and depending on the situation, one can be more suitable or all of them used together. One of the more common deicing procedures involves

using hot water, freezing point depressing fluids (FDP) or solutions made of both. Each FDP has its own characteristics, uses and handling requirements.

2.1.2 Deicing/anti-icing Fluids, Types and uses

The FPDs used to deice aircraft in North America are usually composed of ethylene glycol or propylene glycol combined with water and other ingredients (Transport Canada, 1997b). They are classified in Types. Type I: in concentrated form these fluids contain 80% glycol; they have low viscosity and are normally used for deicing purposes. Type II ADFs have thickening agents added and contain no less than 50% glycol. They provide a greater protection than Type I against frost, ice or snow formation. Types II ADFs are affective anti-icers because of their high viscosity and pseudo-plastic behavior. Type IV ADFs, are relatively new entrants in the aircraft deicing market place. They are also composed of EG and PG, along with thickeners that allow the fluid to cling and adhere to the aircraft until its air speed is approximately 85 knots (157.4 km/h). This provides prolonged holdover times and makes it suitable for larger aircraft that reach a rotational speed of more than 85 knots during takeoff (Switzenbaum *et al.*, 2001).

These glycol-based deicing/anti-icing fluids are formulated to meet specific fluid specifications established by such organizations as the Society of Automotive Engineers (SAE), the Association of European Airlines (AEA) and the International Standards Organization (ISO). The appropriate fluid specification identifier should precede the word "type", e.g., SAE Type I and ISO Type II/IV, and they provide the minimum details to assess holdover time (Mudd, 1993). The ADFs most often used in

North America are comprised of EG or PG. Often Type I fluids are EG based and Type II/IV are PG-based. In Europe diethylene glycol-based fluids are more commonly used (Nitschke *et al.*, 1996) while in Canada EG-based fluids are predominantly used (Simpson, 1997).

2.1.3 Environmental Concerns

2.1.3.1 Oxygen Depletion

One of the environmental concerns regarding deicing and anti-icing operations is the glycol-component of the ADF fluids: both, EG and PG exert a high BOD_5. Pure PG was found to have a BOD_5 of approximately 1,000,000 mg/L and chemical oxygen demand (COD) of 1,848,000 mg/L, while pure EG had a BOD_5 value between 400,000 and 800,000 mg/L and a COD value of 1,408,000 mg/L. These values may vary depending on the type of fluid and the manufacturer. The ADF used in this project, Union Carbide UCAR XL 54 ADF (Type I) prior to dilution exerts a BOD_5 of 523,000 mg/L (Mulligan *et al.*, 1997) and COD values in the range of 700,000 to 800,000 mg/L (Pham, 2002).

The high BOD_5/COD of ADF ethylene glycol-based fluids is of particular concern to surface waters. Ethylene glycol partitions mainly into surface water or groundwater with little transfer to soil or air (vapor pressure is <0.2 mmHg); due to biodegradation, its half-life in water is estimated to be between 2 – 12 days. This high BOD_5 and aerobic biodegradability can deplete the dissolved oxygen from receiving waters.

2.1.3.2 Toxicity

ADF fluids also contain additives such as corrosion inhibitors, wetting agents, pH modifiers, and thickeners among others. Since the formulations are proprietary, it is difficult to determine the amounts or types of additives. As well, they vary within each Type, e.g., Type II fluids contain additional thickeners, which are not in Type I fluids. These additives have potential toxic effects, especially benzotriazoles, which are normally used as corrosion inhibitors. It has been shown that, additives increased the toxicity of ADF compositions, compared to that of pure EG and PG. (Jank *et al.*, 1973; Pilliard, 1995; Cancilla *et al.*, 1997). For example, Cornell *et al.*, (2000) investigated the effect of selected ADF-additives on the biodegradability of PG. The component chemicals studied were PG, the corrosion inhibitor 4(5)-methylbenzotriazole (MeBT) and proprietary mixes of corrosion inhibitors, buffers, and surfactants, referred to as the additive package or AdPack. Using enrichments of soil microorganisms acclimated to ADF, they found that MeBT decreased cell growth rates and yields more severely than the AdPack. Also the Microtox® test indicated that MeBT is the ADF component most toxic to microorganisms. On the other hand, results of the acute toxicity test indicated that the AdPack components were more toxic than MeBT to *Ceriodaphnia dubia* and *Pimephales promelas*, and both components were more toxic than PG alone.

In a more comprehensive study Pham (2002) found that concentrations above 2% volume of EG-based ADF (UCAR XL 54 ADF) had toxic effects on the granular anaerobic biomass used for her study. This is significant because this is the type of ADF that will be used for this study.

2.1.4. Aircraft Deicing Fluids Treatment Options

As mentioned before, the high BOD_5 associated with ADF has detrimental effects on the dissolved oxygen of receiving waters, which has become a serious environmental problem. In Canada, airports are major contributors to the total glycol released in to the environment, concentrations as high as 16,400 mg/L have been detected in storm water during the 1989-1990 winter season at Halifax International Airport (MacDonald *et al.*, 1993). For that reason the collection and treatment of deicing runoff has become mandatory. To ensure that contaminated runoffs are not discharged untreated into the environment, the CEPA has set a limit of 100 mg/L total glycol that can be discharged into receiving waters (Simpson, 1997). Currently Canadian airports have means of capturing and/or treating spent aircraft deicing fluids (e.g., through controlled drainage, vacuum collection, diversion to wastewater treatment plants, etc.) (Transport Canada, 1994).

Treatment alternatives for spent ADF can be grouped in three general options

- Recovery
- Off-site treatment
- On-site treatment.

2.1.4.1 Recovery

This treatment alternative is accomplished in a three-stage process, typically consisting of primary filtration, contaminant removal via ion exchange or nanofiltration and distillation (USEPA, 1994). It is applicable when the ADF collected has a minimum of 15% glycol. Lower percentages need expensive pre-concentration steps.

Recovery has several disadvantages: it requires high energy consumption; the feasibility of recovery is dependent on the collection system and on its ability to recover concentrated waste stream relatively free of other contaminants that may be present; mixtures are not recovered effectively; and the process is only effective for one type of glycol, either EG or PG-based ADF. For these reasons, amongst others, recovery is not widely practiced in North America. In 1999, there were only two pilot scale operations, one at the Denver International Airport and another tasted at L.B. Pearson Airport in Toronto. Recovery has been implemented mainly in Europe: Munich, Germany, Oslo, Norway and Lulea, Sweden (USEPA, 1999).

2.1.4.2 Off-site Treatment

In this case, the airport discharges the stream into a collection system or pays for trucking to a treatment/disposal facility. Some airports use the local wastewater treatment plant for this purpose, however to accomplish this, the plants need to have the capacity to accommodate the increase in organic loading. It has to be considered however that there is variability in strength of these wastes. Depending on the climatic conditions, the strength of the runoff can increase and the construction of equalization basins may be needed (Switzenbaum *et al.*, 2001). Another option is the use of engineered wetlands, which represent a low cost and a low maintenance option (Lorion, 2001). There are however several inconveniences regarding this option. To begin with, there are space constraints; wetlands require a considerable amount of land, which is not always available at airports close to big cities. The option to construct the wetland far from the airport represents additional challenges and expenses. Also, it is known that

glycol does not accumulate on water and that it is biodegradable, however the fate of the ADF additives is of toxicological concern and is not well understood or studied.

2.1.4.3 On-site Treatment

The on-site treatment option relies on the construction of aerobic or anaerobic biological treatment facilities at the airport, where the runoffs of deicing operations can be biologically treated or pretreated before disposal.

2.1.4.3.a Aerobic Biodegradation

Glycols have been used for long periods of time for many purposes other than deicing; hence their aerobic biodegradation is well understood (Pasternak, 1993). Also, there are many studies that show that both EG and PG are biodegradable under aerobic conditions (Staples, 1996; Kapalan *et al.*, 1982; Evans and David, 1974). Aerobic microorganisms use both EG and PG as sources of carbon and energy, some studies have shown that they are generally Gram-negative rods (Gonzalez *et al.*, 1972; Haines and Alexander, 1975).

Jank *et al.*, (1973) investigated the biodegradation of ADF (EG-based fluid) by the activated sludge process, they found that the biodegradation rates were faster at higher temperatures. In the same study they encountered operational problems such as filamentous bulking and scum formation when ADF was co-treated with raw sewage at 10°C and organic loadings greater than 0.15 kg BOD/kg mixed liquor suspended solids/day.

Sabeh and Narasiah (1992) studied the biological degradation of EG-based UCAR ADF-D, in a sequential aerobic biological reactor under different organic loads

and temperatures; they reported that the rate of removal of the ADF followed a first-order reaction rate, *k*. It was also found that, at 22°C with a food-to-microorganism ratio (F/M) of less than or equal to 2.8, a four-fold increase in F/M reduced *k* values by a factor of eight. This study demonstrated that UCAR ADF-D could be treated biologically and that the rate of removal was significantly affected by organic load and temperature

Using a batch-loaded aerobic fluidized bed reactor to treat EG-ADF, Safferman *et al.* (1998) reported good BOD_5 removals in lab-scale testing, however the waste stream was dilute (240 mg BOD/L). Aerobic treatment of ADF can be affective at low concentrations (Williams, 1998), which is not very convenient for this type of waste stream, because depending on weather conditions, concentrations can vary greatly. Additionally, treatment would require large amounts of energy (oxygen). Aerobic systems also have high growth yields and the production of large quantities of sludge represents an additional problem.

2.1.4.3.b Anaerobic Biodegradation

The development of high rate processes such as the anaerobic filter (AF), anaerobic expanded or anaerobic fluidized bed reactor (AFBR) and the UASB enabled greater opportunities for using anaerobic processes for the treatment of industrial wastewaters (Switzenbaum *et al.*, 2001). Anaerobic systems compared to aerobic systems require less energy, the nutritional requirements and the growth yields are lower, so the production of sludge is considerably less. In addition, anaerobic systems produce methane, which is a valuable byproduct. Anaerobic biomass has the ability to remain dormant for long periods of time and still maintain high activity (Jewell, 1987).

These advantages are relevant for deicing wastewater treatment, because such wastewaters have a large oxygen demand, they are usually nutrient-deficient and are seasonally generated (Switzenbaum *et al.*, 2001).

2.2 Anaerobic Digestion

Anaerobic digestion is the bacterial fermentation of organic materials into carbon dioxide and methane gas in an oxygen-free environment. This is a complex process, as shown in figure 2-1, but in general it involves three stages, with a metabolic group of bacteria associated with each one. In the first stage, fermentative bacteria produce mainly short chain fatty acids (propionate, acetate, butyrate, etc.), carbon dioxide and hydrogen. In the second stage, acetogenic bacteria use the volatile acids plus ethanol or lactate and produce acetate, carbon dioxide and hydrogen. Finally in the third stage, methanogenic bacteria use them to produce methane gas. Two classes of methanogenic bacteria are involved in this third stage: acetoclastic methanogens and hydrogen utilizing methanogens, this last group uses hydrogen and carbon dioxide to produce methane. According to McCarty and Smith (1986), acetic acid is the precursor of 72% of the methane formed from a complex waste. Methanogens are a very sensitive group of microorganisms. They are obligate anaerobes with a range of temperature for growing between 30°C to 35°C for the mesophiles . They can grow in a range of pH between 6.6 and 7.6, the optimal range being 7.0 to 7.2, hence anaerobic systems have to be well buffered.

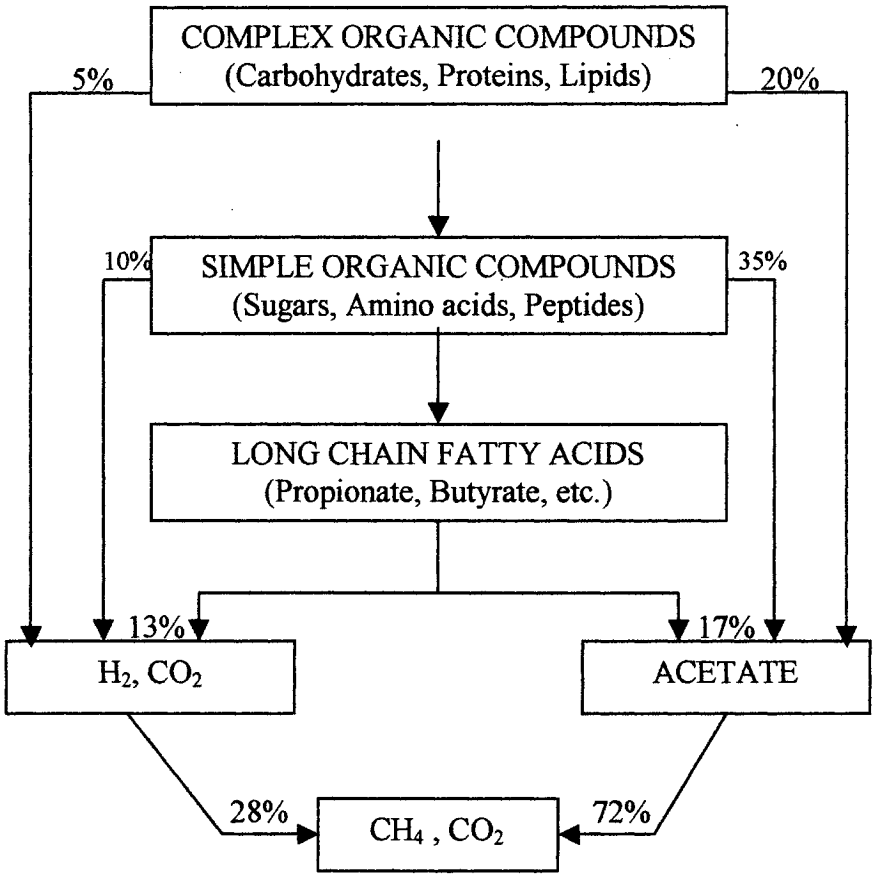

Figure 2- 1 Anaerobic Biotrasformation of ORganic Substances (Speece, 1996).

Two classes of acetate-metabolizing methanoges have been identified: *Methanosaeta*, which grows as short rods and *Methanosarcina*, which grows as clumps (Speece, 1996). These two classes differ in their affinity for acetate (K_s) and their maximum specific utilization rates (U). *Methanoseta* has a high substrate affinity (K_s = 20 mg/L), but a low U (2-4 g COD/g VSS/d), and *Methanosaeta* has a low affinity for acetate (K_s = 400 mg/L), but the maximum specific utilization rate is much higher (U = 6-10 g COD/g VSS/d) (Speece, 1996).

In most situations, the rate-limiting step in methane production from organic materials is the step involved in the breakdown of the complex organic materials into fermentation products such as acetate and hydrogen. However, in the case of dilute or soluble effluents, the rate-limiting step is controlled by the slowest growing group, which in this situation is represented by the methanogens, specifically the propionic and acetic acid utilizing methanogens. In the absence of the former groups the systems have the tendency to accumulate propionic and acetic acids, with the concomitant decrease in the pH, which in many cases can lead to the failure of the system. For that reason in these types of system, it is fundamental to provide the optimum conditions for the activity of the acetic and propionic acid utilizers. According to Bryant (1978) hydrogen is a very important regulator in the methane formation. If the partial pressure of hydrogen builds up, the degradation of many organic compounds stops. This can lead to the formation of reduced compounds, which still keep much of their energy, rendering a deficient treatment of the original organic compounds.

In general, anaerobic systems have been used for the treatment of organic sludges, manures and high strength industrial wastes. The process was not considered to be practical to treat low strength wastes (<1000 mg COD/L). As mentioned before, advances in reactor design and in the understanding of the microbial processes that occur in anaerobic digestion have enabled reactors to maintain a high solids retention time (SRT 20-100d), while keeping the hydraulic retention time (HRT) to a minimum (as low as 1.3 hrs). Shorter HRTs have resulted in smaller reactor size. These designs have made possible the treatment of a variety of dilute soluble wastes more economically (Specee, 1996).

Anaerobic digestion of low strength wastewater results in the production of significantly smaller amounts of biological sludge, compared to aerobic systems (Langenhoff *et al.*, 2000). The production of biogas is limited given the nature of the low strength wastes, but in this case this is not that important compared with the savings in energy (oxygen supply), nutrient requirements, as well as sludge treatment and disposal, that anaerobic digestion has over aerobic treatment technology.

2.3 Anaerobic Digestion of Deicing Fluids

The anaerobic degradation of EG follows various synchronized steps. In the first stage, EG is dismutated to produce acetate and ethanol. The alcohols produced are then oxidized to their corresponding volatile fatty acids with the reduction of protons to form hydrogen. Ethanol and propionate oxidation to acetate is favorable only under low hydrogen partial pressure. Finally the acetate is metabolized via acetoclastic methanogenesis to carbon dioxide and methane. As shown below.

Reaction	Chemical Equations	ΔG°' Kcal
1) EG ⟶ Acetate + Ethanol		
$HOCH_2CH_2OH$ ⟶	$0.5CH_3COO^- + 0.5H^+ + 0.5CH_3CH_2OH + 0.5H_2O$	-21.7
2) Ethanol ⟶ Acetate		
$CH_3CH_2OH + H_2O$ ⟶	$CH_3COO^- + H^+ + 2H_2$	+2.3
3) Acetate ⟶ Methane		
$CH_3COO^- + H_2O$ ⟶	$HCO_3^- + CH_4$	-7.4
4) Hydrogen ⟶ Methane		

$$4H_2 + H^+ + HCO_3^- \longrightarrow CH_4 + 3\,H_2O \qquad -32.4$$

According to Veltman *et al.*, (1998) the overall reaction would proceed in accordance with the following balance:

$$HOCH_2CH_2OH + 0.25H_2O \longrightarrow 1.25CH_4 + 0.75\,H^+ + 0.75\,HCO_3^-$$

While it is possible to write balanced chemical equations for the complete anaerobic mineralization of EG and the reaction is thermodynamically possible under the conditions present in anaerobic systems, this alone does not prove that the reactions will proceed. It is also important to consider the consortia of microorganisms that, working together, will mineralize the glycols. And that is much more complicated, because some steps may lead to the formation of intermediate products and not all steps may be equivalent energetically (Veltman *et al.*, 1998).

Dwyer and Tiedje (1983) studied the biodegradation of EG and PG, using methanogenic enrichments from a municipal anaerobic digester sludge and reported that the rates of degradation for ethylene, diethylene and polyethylene glycol were inversely related to the number of ethylene oxide monomers per molecule and ranged from 0.84 to 0.13 mM ethylene oxide units degraded per h. They also proposed two main metabolic stages in the degradation of EG, the first one being the generation of ethanol and acetate, once the EG was depleted, the ethanol could be consumed to produce methane and more acetate

Darlington and Kennedy (1988) treated EG-based ADF wastewater (5-20 g COD/L) in a UASB reactor at 35°C and reported removal efficiencies of 70-98% at OLR as high as 38.7 Kg COD/$m^3_{reactor}$/day.

Mulligan *et al.* (1997) treated EG-based Union Carbide UCAR XL 54 deicing fluid using multi-plate reactors at 39°C. They reported COD removal of 90% at an OLR of 16.5 Kg COD/m^3 $_{reactor}$ /day, with an influent COD of 8,500 mg/L.

There are also full-scale examples of ADF-treatment facilities using anaerobic technology. The Albany, New York, International Airport has used successfully, a 700 L anaerobic fluidized bed reactor (AFBR) for treatment of spent PG-based ADF. At 30°C and an influent COD concentration of 500 mg/L (OLR = 15 Kg COD/m^3 /d) 95% COD removal has been reported (Switzenbaum *et al.*, 2001).

Schoenberg *et al.* (2001) studied the kinetics of anaerobic degradation of both EG and PG based Type I ADF using suspended-growth fill-and-draw continuous reactors. At 35°C and substrate feed concentration of 9000 mg COD/L. They reported near-complete anaerobic degradability and first order degradation rate constants: 3.5 d^{-1} for PG-based ADF and 5.2 d^{-1} for EG-based ADF. Also, it was noted that a decrease of 10°C had little effect on biodegradability, but at temperatures lower than 10°C, the reactor showed signs of stress (reduction of COD removal and increased concentrations of intermediate metabolites such as ethanol, acetate and propionate).

Pham (2002) investigated the treatment of EG-based UCAR XL 54 ADF Type I using UASB reactors, granular biomass and mesophilic conditions (35°C). Pham noticed that granular biomass exposed to concentrations of 2% by volume ADF or higher, were inhibited. The results from anaerobic toxicity tests (ATA) showed that granules exposed to concentrations of 4 and 8% ADF by volume were discolored and after nine days biogas production stopped. Accordingly the reactor feed concentrations used were 0.8, 1.2 and 1.6% ADF by volume, which corresponded to values of 6.4, 9.1

and 11.8 g COD/L, respectively. For 1.2% ADF the reactor performed very well, with removals up to 90%. The maximum volumetric rate of substrate utilization, and the half-velocity constant K_s were 28.4 g COD/L d and 648 mg COD/L, respectively. It was only at higher loading rates (specific organic loading rate 0.5 g COD/g VSS/d) that treatment performance was negatively affected. Pham (2002) also studied the fate of ADF additives. Ethylene glycol degradation experiments showed that at the additive concentrations used in ADF, they did not have significant effects on methanogenesis, but concentrations of nonylphenol above 100 mg/L had inhibitory effects on the acetoclastic activity.

Zitomer and Tonuk (2003) made a comparative study of the removal of acidified and non-acidified PG-based ADF, in anaerobic complete-mix stirred tank reactor (CMSTR), anaerobic filter (AF), and anaerobic fluidized bed reactor (AFBR) at temperatures between 35°C and 11°C. They reported maximum specific removal rates of 0.93, 0.30 and 0.45 g COD/g VSS/d. They also report a Y_{obs} of 0.039 mg VSS/mg COD at SRT of 30 days and. The most significant increase in COD removal was a result of biomass immobilization and increased biomass concentration in AF and AFBR.

Even if the literature on the anaerobic treatment of ADF has increased in recent years, to date there are not many reports on the use of the Anaerobic Baffled Reactor (ABR) for the removal of such wastewaters. One of the inconveniences with UASB is the loss of biomass, especially at high organic loading rates and low HRTs. Reactor configurations that can separate the SRT from the HRT (UASB, Fixed Film) are among the most successful for the anaerobic treatment of wastewater. According to

Switzenbaum and Jewell (1980) the anaerobic attached film expanded bed reactor (AAFEB) can achieve high loading rates (120 kg COD/m^3 /d) but their operation is expensive and difficult.

In our lab the ABR was used for the treatment of a synthetic EG-based ADF wastewater. At 35°C, and 1% ADF (corresponding to 7000 mg COD/L) by volume the reactors achieved a 95% COD removal, at HRTs of 1.5 d and OLR of 14 kg COD (Barriault, 2003).

No studies have looked at the application of ABR for treatment of dilute ADF runoffs (COD <700 mg/L), which is a significant ADF waste at airports.

2.4 Anaerobic Baffled Reactor

As mentioned earlier, the development of high-rate reactors has increased the applications of anaerobic technology for the treatment of medium strength wastewaters. These designs achieve high loading rates, while keeping a small size, thanks to their ability to separate the SRT from the HRT. The biomass within the system remains almost constant and active. The ABR is categorized as these type of reactors. It is a compartmentalized reactor, the compartmentalization is the result from a series of vertical baffles, which force the wastewater to flow over and under them as it travels from the inlet to the outlet of the reactor. Each compartment consist of an up-flow and a down-flow section (Figure 2-2).

The ABR presents several advantages over other well-established technologies.

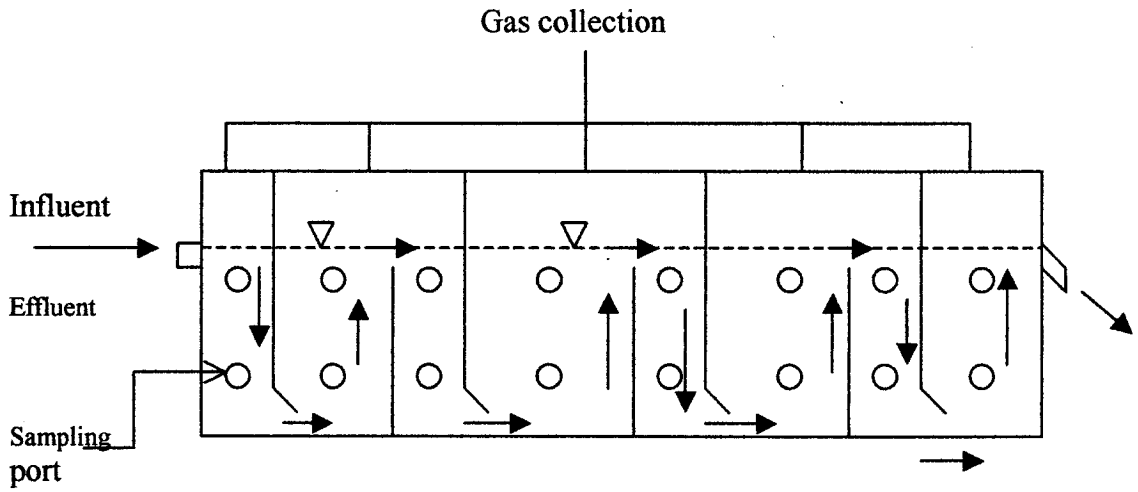

Figure 2- 2 Representation of the ABR.

- Simple Design: It does not need any moving parts or mechanical mixing, also it does not require special gas or sludge separation.
- Low capital and operational cost: Inexpensive to construct and require low maintenance.
- Low sludge generation and high solids retention time.
- High stability to hydraulic shock loadings and organic shocks.

According to Weiland and Rozzi (1991) another significant advantage of the ABR is its ability to separate acidogenesis and methanogenesis longitudinally down the reactor. This allows the reactor to behave as a two-phase system, in which acidogenic bacteria accumulate in the first compartments (Cohen *et al.*, 1982). For these reasons the ABR could be considered as an attractive option for wastewater treatment. There are however some disadvantages associated with it. For instance:

- The requirement to build shallow reactors to maintain acceptable liquid and gas up-flow velocities.
- No removal of inert solids.

Advantages out-weight the disadvantages, and the ABR represents an attractive option for the treatment of low-strength wastewaters.

2.4.1 Evolution of the Anaerobic Baffled Reactor

In 1981, McCarthy and co-workers developed the ABR by removing the rotating disks of an anaerobic rotating biological contactor. Granular biomass within the reactor raised and settled due to flow characteristics and gas production, but moved down the reactor at a slow rate.

From the original design of McCarty and co-workers, the ABR has had several modifications. Most of them were introduced with the purpose to increase the SRT or for the treatment of wastes with high solids content. (Barber and Stuckey, 1999).

Fannin *et al.*, (1981) added vertical baffles to a plug-flow reactor treating high solids sea kelp slurry, in order to avoid the slow growing methanogens being replaced by the influent solids. At a constant loading rate of 1.6 kg COD/m^3/d, they observed that the production of methane increased by 85% with a yield of 0.34 m^3/kg VSS, after the baffles were added. Bachmann *et al.*, (1983) studied the effects of narrowing the down-flow chambers and slanting the baffle edges. This increased the treatment performance of the reactors.

Tilche and Yang (1987) for the treatment of high strength wastes made further modifications to the ABR. The reactor they used incorporated a solids settling chamber

after the final compartment. This allowed the solids washed out from the baffled section of the reactor to be collected and recycled again to the first compartment. They also added packing (Pall rings) to the liquid phase of each compartment of the baffled section to entrap the buoyant bioflocs produced during high gas production. By doing this they achieved higher loading rates and treatment efficiency.

Boopathy and Sieves (1991) which worked with swine wastewater reduced the up-flow liquid velocities in the first compartment of a two chambers unit. Performance was measured against another ABR having the same dimensions and an additional third chamber. They reported that the addition of the third chamber provided an increased SRT and superior performance.

Uyanik (2003) modified the feeding strategy for the ABR for treatment of high strength brewery wastewater. According to Uyanik (2003), by splitting the feed along the length of the ABR, a number of desirable characteristics can be encouraged, such as low OLR, longer HRTs, longer SRT in the initial compartments and greater availability of food for the microorganisms in the final compartment of the reactor. This split fed anaerobic baffled reactor (SFABR) could offer more stability during start-up and shock loading regimes, by eliminating the high specific loading conditions in the first compartments of the reactor. In this experiment the organic loadings were increased stepwise from 0.9 to 10.5 kg COD/L and the HRT was kept at 2 days and a COD removal of 90% was reported. Uyanik also found that the SFABR produced a more homogeneous microbial ecology in each compartment compared to the normal fed ABR.

2.4.2 Low -Strength Wastewater Treatment Using the ABR

Barber and Stuckey (1999) reported that the ability of ABRs for the treatment of low-strength wastewaters had been successfully accomplished. However, the number of reports on dilute wastewater was very limited. Dilute wastes, provide a low mass transfer driving force between biomass and substrate, which would decrease the biomass activity according to Monod kinetics. On the other hand, one of the advantages of low-strength wastewater treatment is the potential increase in the SRT due to lower biogas production, which is beneficial, because these wastes can be treated at lower HRTs.

Orozco (1998) noted decreasing biogas production when increasing HRTs, while treating dilute municipal wastewaters. It was suggested that biomass in the last compartment could suffer starvation at high HRTs. According to Kato *et al.* (1997), treating such a wastes at low retention times can increase the hydraulic turbulence, which can lower apparent K_s (saturation constant) values, enhancing treatment efficiency.

Witthauer and Stuckey (1982) used the ABR to treat dilute synthetic grey-water at low volumetric loading rates and high HRTs and observed irregular COD removal rates. They associated this effect with the low initial biomass contained in the ABR (less than 3 g VSS/L). They recommended that for the treatment of this waste, higher initial biomass concentrations would be much better.

Langenhoff *et al.* (2000) studied the influence of HRT on start-up conditions and performance of ABRs. They used four reactors (10 L and eight compartments each) for the treatment of a complex dilute soluble-colloidal wastewater (milk, colloidal

rice and dog food). The initial feed concentration was 500 mg COD/L, HRT of 80 hrs, and 35°C. The HRT was gradually reduced to 6 hrs. For all HRTs tested the COD removal was greater than 80%. However, when the HRT of one of the reactors was further reduced to 1.3 hrs, the removal efficiency decreased to 40%, in a four day period. They also noticed that, as the HRTs were reduced, the production of soluble microbial products (SMPs) increased (especially in reactors fed with colloidal material). They found that, the removal efficiency for the colloidal waste was slightly lower compared with that of the soluble waste but in general, it was good for both. They concluded that a two-phase dispersion model could describe the mixing characteristics of the reactors and the eight compartments behaved like eight separate CSTRs in series. In the same study they investigated the effect of temperature on the performance of the ABR while maintaining constant OLR and HRT. At 35°C removal rates were up to 95%. Which decreased to 70% at 20°C, and 60% at 10°C. It was also noted that SMPs increased with the decrease in temperature.

Langenhoff *et al.*, (2000) concluded that even at low temperatures the treatment of low-strength wastewater was effective, however the addition of a an aerobic polishing step would be useful to treat the amount of SMPs produced at 10°C.

Manariotis and Griogoropoulus (2002) used a 14.7-L, three-chamber ABR for the treatment of a complex low-strength soluble synthetic wastewater containing raw cane sugar, nonfat dried milk and corn starch (300 to 400 mg COD/L), at 26°C, and HRTs of 24 and 12 hrs, which corresponded to an OLR of 0.30 and 0.66 kg COD/m^3/d, respectively. The reactors were seeded and feeding started after 8 days. They reported COD removals of 87% and 91%, respectively, after 42 days of operation. After two

years without feeding the ABRs were reactivated and had an average COD removal of 85.3%, after only 10 days of reactivation. They also noticed that lowering the temperature to 16°C had no effect on the removal efficiency. They concluded, that the ABR offered a promising system for the direct anaerobic treatment of intermittently produced low-strength wastewaters, but more research needed to be done in order to establish appropriate design parameters.

However, it is important to mention that the synthetic wastewater used in the previous experiment was an easily biodegradable substrate, and had a low TSS content, which is not always the case for municipal wastewaters or seasonally generated wastes.

Barker *et al.* (2000) investigated the chemical composition, molecular weight (MW) distribution and biodegradability (both aerobic and anaerobic) of soluble microbial products (SMPs) in ABR treating low-strength wastewater. They used two ABRs with a working volume of 10 L, and eight compartments of equal volume. The ABRs were kept at 10°C, 20°C and 35°C and the feed consisted of a pasteurized semi-skimmed milk diluted with tap water to a final COD of 500 mg/L. ABR1 and ABR2 were seeded with 13 and 6 g VSS/L, respectively, and were operated under the following conditions:

- ABR1: at different temperatures (10°C, 20°C and 35°C) and HRTs (6, 10, 20, 40 and 80 hrs)
- ABR2: at constant temperature of 35°C and different HRTs (1.3, 2.8,4, 5, 6, 10, 20, 40, and 80 hrs)

They reported that high MW materials were concentrated in the middle compartments of the ABR, and formed 22% of the effluent COD. This fraction was found to be 86% degradable under aerobic conditions and only 4% under anaerobic conditions. These materials could be the products of cell lysis, which are known to have a high MW. On the other hand, low MW compounds were mainly found in the first compartment and represented the highest portion of the effluent COD (36%), being more degradable under anaerobic conditions (33%) than in aerobic conditions (17%). These materials were believed to be products associated with substrate metabolism and biomass growth or the products of degradation of high MW compounds.

They also noted that the SMP production decreased with decreasing HRT and increased with decrease in temperature. It was concluded that this type of waste could be treated successfully by anaerobic systems, however the addition of an aerobic process may be necessary to achieve discharge standards.

There are also several full-scale, and pilot-scale experiences reported using the ABR for the treatment of low-strength wastewaters.

Orozco (1997), reported the performance of a full-scale plant, treating domestic waste from a small town in Colombia (<2500 inhabitants) where two reactors of 197 L capacity and eight compartments have been operated at 15°C, OLR of 0.85 kg COD/m^3/d with a 70% removal efficiency. The reactor experienced some problems during early operation, primarily hydraulic shocks increased biomass washout. Once this problem was solved the ABRs were stable and the increase of OLR from 0.4 to 2 kg COD/m^3/d had no effect on performance. However the installation of a polishing lagoon was necessary to achieve discharge quality effluents.

Damas et al. (2001) reported the construction of a 3200 L, ABR for the treatment of domestic wastewater in KwaZulu Natal, South Africa. The ABR has eight compartments. The height of the baffle drops across the reactor to facilitate the ease of flow through the reactor. The feed consisted of approximately 50% mixture of domestic wastewater, and textile manufacturing effluent. They reported that the ABR was not seeded, due to difficulties to transport the sludge, and that , the feed concentration to the reactor could not be controlled, since they got feed from the municipal wastewater works. The influent COD to the ABR ranged between 50 and 400 mg/L, and COD reductions ranged from 60 – 90% at HRTs below 20 – 60 hrs. They concluded that, a better COD reduction is expected in the future when the reactor is located at the proper place in the wastewater treatment plant and is fed with 100% domestic effluent. Also, the removal of pathogens could be possible due to the long solid retention times achieved in the reactor (not reported).

2.4.3 Hydrodynamics of the ABR

Several studies on the ABR have focused on the hydrodynamics and on the degree of mixing within the reactor. It is believed that these have a direct influence on the mass transfer and on the performance of the reactor (Barber and Stuckey, 1999).

Grobicki and Stuckey (1992), conducted a series of residence time distribution studies. In their study, they tracked the fate of the inert tracer: Li^{-} in the effluent of a number of baffled reactors (four to eight) chambers both with biomass and without biomass at various HRTs. They incorporated the data into the "Dispersion" and "Tanks in series" models (Levenspiel, 1999). They reported that the ABR could be

characterized as series of well mixed CSTRs, with low dead space (<8% hydraulic dead space in empty reactors, and 18% with the addition of 8 g VSS/L). They attributed the low mixing between adjacent compartments to the baffle arrangement, and correlated that the ABR can be modeled as a series of ideal stirred tanks, corresponding to the number of actual compartments present. They also noted that this characterization is more accurate at low HRTs (below 20 hrs) than at higher HRTs, especially at high OLR were gas production increases the mixing in each compartment.

According to Barber and Stuckey (1999) there is a need to investigate other factors that affect mixing such as biogas mixing effects, viscosity changes due to extracellular polymer production and biomass particle size, among others.

In our laboratory, Barriault (2003) performed ARB mixing studies (same configuration as present study), with dilute Rhodamine W.T. as a tracer, and HRTs of 36 h, 6 h and 1 hr. The outlet tracer concentrations were monitored with time and measured using a flourometer and a calibration curve in order to determine the number of ideal CSTR-in-series. The residence time distribution studies in the reactor without granular biomass revealed that the reactor behaved as 4 theoretical CSTR-in-series

2.4.4 Modeling of the ABR

Attempts have been made to model the treatment performance in the ABR. The most common model uses first order kinetics to represent the change of substrate concentration with time, as follows:

$$\frac{dS}{dt} = -kS \qquad (2.1)$$

S= substrate concentration (g COD/L)

k=first order reaction constant (d^{-1})

In order to determine the decrease in substrate concentration with the increase in biomass concentration the following equation is introduced, which is also first order with respect to substrate and biomass concentration.

$$\frac{dS}{dt} = -k_1 SX \qquad (2.2)$$

X = active biomass (g VSS/L)
k_1= first order reaction rate constant (L/g VSS/d)

Equation (2.2) has been used for modeling aerobic systems due to its simplicity and it also can be used in anaerobic digestion (Chynoweth and Isaacson, 1987). Equation (2.2) does account for the effect of substrate limitation and biomass concentration on the reaction rate, which is important in the case of dilute wastewaters. To account for the whole range of substrate concentration that affects bacterial substrate removal the next equation is often used:

$$\left(\frac{dS}{dt}\right) = - \frac{k_{\max} X_b S}{K_s + S} \qquad (2.3)$$

This equation, called the Monod equation, has been shown to describe the utilization of substrate in most continuous-growth systems in substrate-limited conditions (van Haandel and Lettinga, 1994), where:

k_{max}= maximum specific rate of substrate utilization (g COD/g VSS/d)
X_b= active biomass (g VSS/L)
S= substrate concentration (g/L)
K_s= half-velocity rate constant (g/L)

The substrate utilization rate (k_{max}) and the half-velocity rate constant (K_s) can be determined using the linear form of Lineweaver-Burke equation:

$$\left(\frac{X}{dS/dt}\right) = \frac{1}{U} = \frac{K_s}{k_{max}}\frac{1}{S} + \frac{1}{k_{max}} \qquad (2.4)$$

Where U is the specific substrate utilization rate (g COD/g VSS/d), and represents the rate of substrate consumption of substrate per unit biomass. Rearranging (2.2) and (2.4):

$$\left(\frac{dS/dt}{X}\right) = U = -k_{max}S \qquad (2.5)$$

From (2.5) k_{max} and K_s can be determined graphically. It is important to mention that the use of the previous equations has some inconveniences due to its linear graphical fit and caution is advised in the use of it without experimentation in the particular system.

Based on basic kinetics several models have been proposed for the ABR as shown in table 2-1 .

Bachmann *et al.* (1983), represented the biomass particles in the mass bed as fluidized spheres with surface area through which the solute must diffuse for bacterial consumption. In their first development, they considered the concept of liquid-layer mass transfer. The model was good for describing performance at low OLR, however at higher OLRs it failed. One of the reasons could be their assumption of a constant diffusion-layer depth, which is not likely at high loading rates. In their second approach they considered a series of of completely mixed dispersed growth reactors using Monod kinetics, their results were not very realistic, since diffusional limitations were not considered. (Equation 1, Table 2-1).

In a further study, Bachmann *et al.* (1985), applied a fixed film model to an ABR treating high strength wastewater, the model predicted a decrease in efficiency with decreasing influent substrate concentration at constant loading rates, however the experimental results, demonstrated the opposite. (Equation 2, Table 2-1).

Table 2- 1 Model Equations for ABR Systems (Barber and Stuckey, 1999).

No.	Substrate Model Equation	Reference
1	$\frac{dS}{dt} = -aCS^{q} + QSo - QS,\ S = So - \left(\frac{a}{Q}\right)CS^{q}$	Bachmann *et al.*1983
2	$D_f\left(\frac{\partial^2 Sf}{\partial z^2}\right) = \left(\frac{kSfXf}{K_s + Sf}\right)$	Bachmann *et al.*, 1985
3	$Df\left\{\frac{\partial^2 Sf}{\partial r^2} + 2r\left(\frac{\partial Sf}{\partial r}\right)\right\} = \left(\frac{kXfSf}{K_s + Sf}\right)$	Nachaiyasit and Stuckey, 1995

Where a = surface area per unit reactor volume (L^{-1}); C = variable-order reaction coefficient; D_f = molecular diffusivity in biofilm (L^2t^{-1}); k = maximum specific rate of substrate utilization (t^{-1}); K_s = half-velocity constant (ML^{-3}); Q = specific flow rate (Vt^{-1}); q = variable order reaction order; r = radious of a three-dimensional spherical particle (L); R = recycle ratio; S= substrate concentration (ML^{-3}); S_n = influent substrate concentration (ML^{-3}); S_f = substrate concentration in biofilm (ML^{-3}); S_n = effluent substrate concentration (ML^{-3}); W = mass of biomass (M); z = distance normal to biofilm surface (L); Numerical subscripts refer to compartment number.

Nachaiyasit and Stuckey (1995) derived a spherical model (Equation 3, Table 2-1) using Monod kinetics combined with molecular diffusion, based on the following assumptions:

a) Substrate concentration could be described by a single parameter: COD.

b) Biomass concentration could be described by a single parameter VSS.

c) Biomass growth is constant during balanced growth.

d) The biological reactions of importance occurred at constant temperature and pH.

The model developed performed better for high OLRs (8 to 15 g COD/L) and long HRTs, than for short HRTs and low OLRs. One of the inconveniences of this model was that it relied on kinetic constant calculations that had to be determined using bioassays for each compartment once the reactor was at steady state, (Bachmann *et al.*, 1985). However, bottle bioassays may not be representative of reactor performance. It was concluded that while the predictive capacity of the spherical model was not always good, it was useful for understanding interactions between the system parameters.

According to Barber and Stuckey (1999) a combination of theoretical considerations and experimental findings can be used together in order to generate models with a more realistic fit. So far most of the modeling has used acetoclastic methane production as the rate-limiting step. Due to the structure of the ABR it is expected a shift in the population dynamics of the two species (*Methanosarcina* and *Methanosaeta*) responsible for acetate consumption. Both groups differ in kinetic abilities and both will have a different effect on each compartment. For low-strength wastes, acetate loading will be low and this will encourage growth of *Methanosaeta*, while for high strength wastes, where high VFA concentrations are expected in the acidification zone (Barber and Stuckey, 1999) *Methanosarcina* are expected to predominate in the reactor.

2.5 Empirical Model Proposed

Xing *et al.* (1991), used a modification of the ABR called the Hybrid Anaerobic Baffled Reactor (HABR), which was described previously in section 2.4.2, and developed a mathematical model to describe reactor performance. They used the concept of completely mixed reactors in series and first order kinetics. However, kinetic constants were un-consistent and the model did not work.

For the treatment of dilute ADF in this study the following first order reaction rate model, will be used under the following assumptions:

a) Acetoclastic methane production is the rate-limiting step.

b) All substrate utilization occurs in the granular sludge bed.

c) The sludge bed is completely mixed due to gas production.

According to this, a mass balance for the first chamber can be established as follows:

$$\frac{dS_1}{dt} V_1 = QSo + RQS_N - (1 + R)QS_1 - k_1 S_1 X_1 V_1 \qquad (2.6)$$

Where:

So = influent substrate concentration (g/L)
S_1 = substrate concentration in chamber 1 (g/L)
S_N = substrate concentration in the chamber n (the last chamber of the ABR) (g/L)
V_1 = working volume of chamber 1 (L)
Q = influent substrate flow rate (L/d)
R = recycling ratio.
k_1 = first order reaction rate constant (L/g VSS/d)
X_1 = theoretical average granular sludge concentration in chamber 1 (g/L)
X_1 is defined as follows

$$X_1 = \frac{W_1}{V_1} \qquad (2.7)$$

Where W_1 = mass of granular sludge in chamber 1. (g).

At steady state $dS_1/dt = 0$, and eqn.(2.8) reduces to

$$0 = QSo + RQS_n - (1+R)QS_1 - k_1S_1X_1V_1$$

Resolving for S_1

$$S_1 = \frac{So + RS_N}{1 + R + k_1X_1V_1/Q} \tag{2.8}$$

And eqn. (2.7) implies

$$W_1 = X_1V_1 \tag{2.9}$$

Substituing eqn. (2.9) into eqn. (2.8), it becomes:

$$S_1 = \frac{So + RS_N}{1 + R + k_1W_1/Q} \tag{2.10}$$

Using the same derivation method, from the substrate balance equations established for each subsequent chamber of the ABR yields the following effluent substrate concentrations expressions:

$$S_2 = \frac{S_1(1+R)}{1 + R + k_2W_2/Q} \tag{2.11}$$

$$S_3 = \frac{S_2(1+R)}{1 + R + K_3W_3/Q} \tag{2.12}$$

For S_n

$$S_n = \frac{S_{n-1}(1+R)}{1+R+K_nW_n/Q} \qquad (2.13)$$

Where:

$S_2, S_3, \ldots, S_n$ = substrate concentrations in camber 2, chamber 3,..., chamber n (g/L)
$k_2, k_3, \ldots, k_n$ = first order reaction rate constant in chamber 2, chamber 3,..., chamber n (L/g VSS/d).

Introducing expressions for S_{n-1}; $S_{n-2}, \ldots, S_1$ consequently into the expression of S_n and arranging it, yields:

$$S_n = \frac{So(1+R)^{n-1}(So+RS_n)}{(1+R+k_1W_1/Q)(1+R+k_2W_2/Q)\ldots(1+R+k_nW_n/Q)} \qquad (2.14)$$

Resolving for S_n from eqn. (2.14) and arranging it, results in

$$S_n = \frac{So(1+R)^{n-1}}{(1+R+k_1W_1/Q)(1+R+k_2W_2/Q)\ldots(1+R+k_nW_n/Q)-(1+R)^{n-1}R} \qquad (2.15)$$

For an n stage ABR, the effluent of the n^{th} chamber is the effluent of the whole reactor, so eqn. (2.15) becomes the generalized formula for simulating the kinetic performance of the ABR.

For this study, the ABR has four compartments and since the recycling effect will not be examined eqn. (2.15) is further reduced to:

$$S_4 = \frac{So}{(1+k_1W_1/Q)(1+k_2W_2/Q)(1+k_3W_3/Q)(1+k_4W_4/Q)} \qquad (2.16)$$

Equation (2.16) will be applied to the results of this experiment in order to get the performance of the ABR for the treatment of low-strength ADF wastewater.

CHAPTER 3

MATERIALS AND METHODS

In the present study, the anaerobic digestion of dilute EG-based ADF was investigated by using both batch and continuous experiments. In addition, the characterization of the biomass was determined by means of settling characteristics and acetoclastic activity during the different experimental conditions.

3.1 Batch Experiments

Batch experiments were carried out at 35°C in 160-ml glass serum bottles using different concentrations of ADF predetermined for this study (see Table 3-2). The biomass used was ADF-acclimatized. Batch experiments were used to determine and better understand ADF biodegradability.

Anaerobic granular biomass was diluted with different concentrations of ADF (0.04%, 0.07% and 0.13% v/v); to give biomass concentrations of approximately 10 g VSS/L. Bottles were shaken at 100 rpm. Biogas production, soluble COD removed and VFA consumption were measured after 24 hrs. Subsequent measurements were carried every other day. Two types of controls were used: one control had biomass diluted with tap water and the other control had mineral salts media (without ADF), the bottles were sacrificed at the end of the study for VSS determination.

3.2 Continuous Experiments

3.2.1 Apparatus

The continuous experiments were performed with two identical ABRs. The reactors were made of Plexiglas and had the following dimensions: 56 cm long, 30.5 cm high, and 25.5 cm wide. Both reactors, had four compartments, and each compartment was comprised of a down-flow section (4.5 cm long), and an up-flow section (9.5 cm long). The height of the liquid within the reactor was 21 cm, giving a total liquid working volume of 32 L for the reactor, and 8 L for each compartment. The down-flow section ended in a 45° angled portion, which was used to direct the fluid and improve the mixing of the up-flow section. Each section of the compartments had two sampling ports: the upper-port for the soluble fraction and lower port for the sludge. Each compartment had an individual gas-collection port. However, for this study, only total gas production was considered. These ports were connected by means of plastic tubing (380 PVC, Nalgene®) and directed to one single gas measurement device. As shown in Figure 3-1.

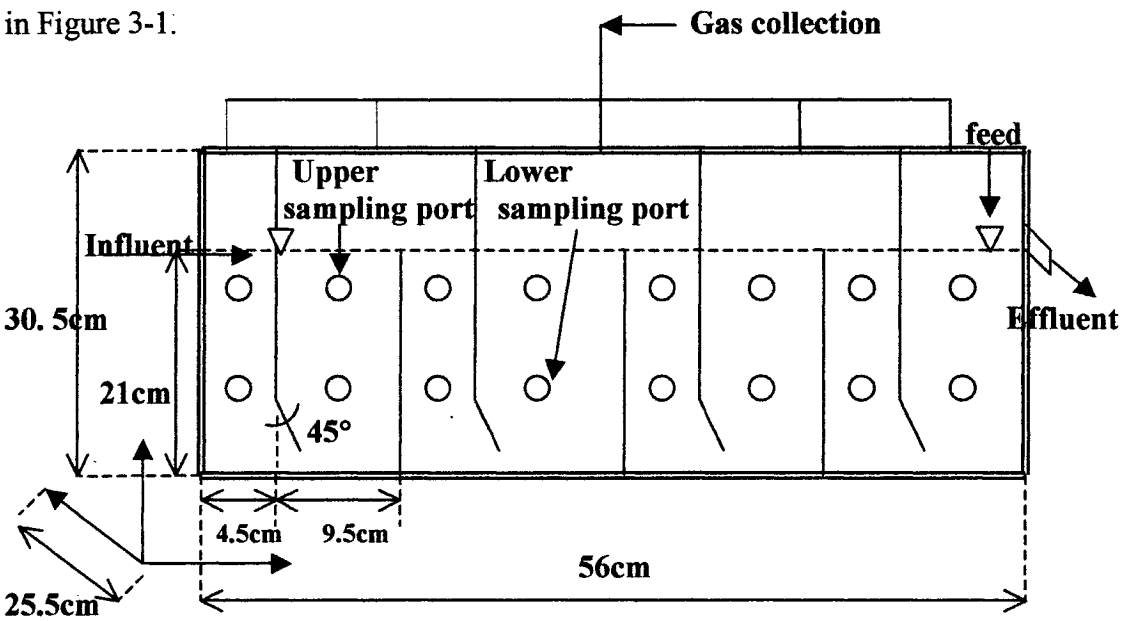

Figure 3- 1 Dimensions of the 4-Compartment ABR Used for ADF Study.

SWW flowed from the top of the down-flow section of the first compartment and through the portion of granular biomass retained in that section and then up through the bed section in the up-flow section. The process was repeated for each compartment. Finally the treated effluent exited from the upper-port at the top of the up-flow section of the fourth compartment.

3.2.2 Process Description

The two bench-scale ABRs were inoculated with 10 L of anaerobic granular biomass to give an overall concentration of 10.5 g VSS/L, and they were set in a temperature-controlled room (35°C ± 2°C). The inoculum was obtained from Lake Utopia Paper (New Brunswick, Canada) a chemical thermal mechanical pulp treatment plant. Although the effect of recycling was not part of this study, the reactors were equipped with internal recycling lines, to be used only in case of excessive VFA production. Under such conditions, feeding could be stopped and the reactors put in full recycling mode until the concentration of VFA were reduced to a safe operational level within the reactor (< 500 mg acetic acid/L). When needed, recycle would be drawn from the upper sample port in the up-flow section of the fourth compartment. As shown in figure 3-2 the SWW fed to the reactors was taken from an insulated main feed circulation line in order to avoid shocks to the biomass within the reactors due to temperature differences. The treated effluent exiting through the outlet port in the fourth compartment flowed to a U-tube, which helped to maintain anaerobic conditions within the reactors, and it was then, accumulated in tanks for its further disposal.

Total biogas production was measured by wet-tip meters, which were connected to the top portion of the reactors. The gas, liquid and sludge sampling-process was

accomplished by using the different ports located in the reactors. Thus, a sampling port was used in order to collect compartment samples and, to monitor the reactor more efficiently.

Clear, 380 PVC tubing (Nalgene®) was used to connect feed tanks, pumps and reactors in the system. The tubing used for the pump head was a food-grade Norprene® (LABCOR). This tubing was replaced every two weeks to avoid spills or feed variations due to worn tubing. The tubing connections from the feed tank to the pumps and the reactors were cleaned each week or, as needed in order to avoid feed deterioration due to microbial growth.

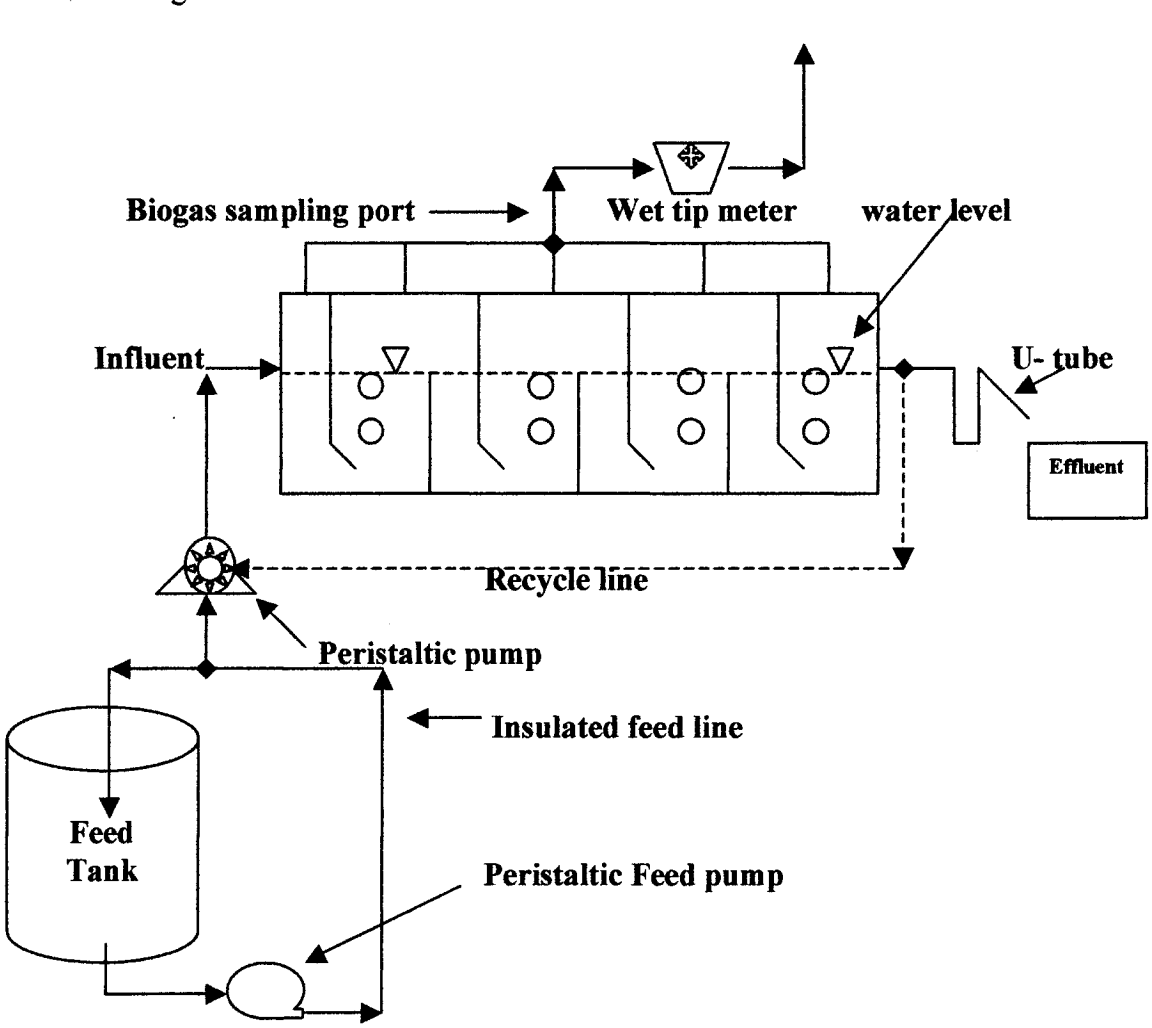

Figure 3- 2 Experimental Set-up.

3.2.3 Experimental Program

After inoculation the reactors were set in recycle mode for a four-day-period. After that, continuous feeding started at long retention time (80 hrs) and high strength SWW (4500 mg COD/L). This was done to expose the biomass within the reactors to the EG component of the ADF and for acclimation. This acclimation process was done for a period of two months, during which the production of VFAs as well as the pH were closely monitored. If the VFAs reached levels higher than 500 mg acetate/L or the pH decreased to less than 7, the reactors were set in full recycling mode until the normal conditions of operation were restored (VFAs concentrations < 500 mg acetate/L and pH>7). Under normal acclimation start-up conditions the feed concentration was reduced gradually and the HRT was also gradually decreased until the base line conditions of 40 hrs HRT and initial feed concentrations of 750 mg COD/L were achieved. From this base line the feeding program of each run with dilute ADF was achieved by increasing the flow rate 5 – 10% each day at constant ADF concentration, or the ADF concentration was increased or decreased by an increment of 0.01% ADF per day at a constant HRT. The experimental program shown in Table 3-1 shows the order of the runs and the program of experimentation. During run to run transitions the levels of VFAs, pH and effluent soluble COD were closely monitored. Total experimentation time was approximately 7 months.

Reactor performance was assessed based on measurements made during steady state conditions. For the reactors to be considered in steady state, the levels of VFAs had to be constant (± 50 mg/L) as well as the removal efficiency (± 10%) for a period of 5 HRTs. Once this state was reached, reactors were kept at that condition for another

five HRTs. For each HRT VFA concentrations, pH, effluent soluble COD concentrations, compartment COD profiles, effluent TSS and VSS concentrations, biogas production and biogas composition were measured.

Table 3- 1 Experimental Program.

Reactor	Influent COD (mg/L)	HRT (H)	OLR (kg COD/m^3/d)
R1	300	24	0.3
R1	300	12	0.6
R1	300	6	1.2
R1	300	3	2.4
R2	500	24	0.5
R1	500	12	1
R1	500	6	2
R2	500	3	4
R2	750	24	0.75
R2	750	12	1.5
R2	750	6	3
R2	750	3	6

3.2.4 Feed Composition

For this study, the carbon source in the synthetic ADF wastewater was EG based UCAR XL-54 deicing fluid from Union Carbide. Experiments were conducted at ADF concentrations of 0.04%, 0.07% and 0.130% (v/v). This concentration range corresponded to dilute feed COD levels of 300, 500 and 750 mg/L, respectively. The feed solution was also composed of various other components to provide the system with adequate source of nitrogen, phosphorus, miscellaneous nutrients, and alkalinity, which was used for pH control. Yeast extract was used as a source of miscellaneous nutrients and, alkalinity was provided in 10% excess. Table 3-2 shows the components of the synthetic feed solution. The average COD:N:P ratio of the feed solution was 200:5:1.

To get the reactors to the steady state baseline, ADF SWW was prepared every other day with tap water and kept at 4°C. For the specific runs, the feed was prepared as needed based on a HRT basis. For longer HRTs (24 hrs and 12hrs) it was prepared every day with warm tap water (approximately 30°C) and kept in the temperature-controlled room. The strength was monitored regularly to ensure no deterioration. For the shorter HRTs (6 hrs and 3 hrs) the feed was prepared in the same way as before, but 2 or 3 times per day.

Table 3- 2 Composition of Synthetic ADF-based Feed Solution.

Component (g/L)	0.04%ADF	0.07%ADF	0.130%ADF
COD	0.3	0.5	0.75
NH_4HCO_3	0.06	0.1	0.15
$NaHCO_3$	2.1	2.37	2.42
$KHCO_3$	2.4	2.63	2.93
KH_2PO_4	0.006	0.01	0.015
K_2HPO_4	0.008	0.013	0.02
$(NH_4)_2SO_4$	0.015	0.025	0.038
Yeast Extract	0.003	0.006	0.008

3.3 Biomass Characterization

3.3.1 Specific Acetoclastic Activity Tests

Biomass acetoclastic activity was determined for the inoculum and for each compartment of both reactors at the end of the experimental runs, based on known procedures (Speece, 1996). Anaerobic granular biomass (10 ml) from each compartment of the ABR were anaerobically transferred to 160-ml glass serum bottles and diluted with 30 ml of defined medium (Table 3-3) to give a biomass concentration of approximately 10 g VSS/L. The sealed and capped serum bottles were shaken

overnight in the dark at 100 rpm and 35°C, for an initial acclimation. Acetic acid stock solution (0.2 mL, see table 3-4) was injected into the bottles to give an initial acetate concentration in the range of 1200-1500 mg/L. The acetate consumption rate was measured by monitoring the change in acetate concentration over time using gas chromatography (GC).

Table 3- 3 Composition of Defined Medium for Specific Acetoclastic Activity Test.

Component	Concentration (mg/L
NaCl	500
$CaCl_2 \cdot 2H_2O$	100
NH_4Cl	1894
$MgCl_2 \cdot 6H_2O$	100
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	10
$ZnSO_4 \cdot 7H_2O$	0.1
H_3BO_3	0.3
$FeCl_2 \cdot 4H_2O$	1.5
$CoCl_2 \cdot 6H_2O$	10
$MnCl_2 \cdot 4H_2O$	0.03
$NiCl_2 \cdot 6H_2O$	0.03
$AlK(SO_4)_2 \cdot 12H_2O$	0.1
Nicotinic acid	0.1
Cyanocobalamine	0.1
Thiamine	0.05
p-aminobenzoic acid	0.05
Pyridoxine	0.25
Pantothenic acid	0.025
KH_2PO_4	500
Resazurin	1.5
2-Methyl-*n*-butyric acid	102
$NaHCO_3$	3400

Table 3- 4 Acetic Acid Stock Solutions for Specific Acetoclastic Activity Test.

Component	Concentration (g/L)
Ammonium acetate	31.3
Potassium acetate	41.4
Sodium acetate	46.2
Glacial acetic acid	16.8

3.3.2 Settling Test

Biomass settling tests were determined based on the procedure described by Andras *et al.* (1989) A 10 mL granular biomass sample taken from the lower sampling port of the up-flow section of each compartment and was placed in a glass up-flow velocity test tube (L = 20 cm, ID = 1.9 cm); water at 35°C was pumped through the tube for five minutes at successively increasing flow-rates. The fractions of biomass exiting from the tube were collected on pre-weight Whatman® 185 mm diameter grade 1, filter paper and dried overnight at 105°C. The difference in weight gave the TSS fractions exited at each flow-rate. Settling curves were determined by plotting the cumulative TSS exiting fractions against up-flow velocity. The v_{50} was considered to be the velocity at which 50% of the granules were washed out.

Samples were analyzed at the end of each steady state. Settling velocity determinations were made in duplicate and the results were compared with the settling characteristics of the initial sludge used as inoculum.

3.4 Analytical Methods

3.4.1 Biogas Composition

Methane and carbon dioxide content of the gas produced, was determined by the GC method developed by Van Huyssteen (1967), using a Hewlett-Packard 5710a gas chromatograph, equipped with thermal conductivity detector and a model 3380A integrator. A Porepak T column (6.35 mm X 304.3 cm) was held at 70°C with a helium gas carrier flow rate of 40 ml/min. Biogas samples were taken from a port at the top of the reactor, with an airtight syringe and 0.5 ml of gas were injected for the determination.

3.4.2 Volatile Fatty Acids Quantification

VFA quantification was accomplished by a gas chromatographic method with an internal standard (Ackman, 1972), using a Hewlett-Packard 5840A gas chromatograph equipped with a flame ionization detector, an auto sampler, a model 5840 integrator and a Chromosorb 101 packed column (304.8 cm X 2 mm ID, 80/100 mesh size). The oven temperature was 180°C, and the injection temperature was 250°C. The detector temperature was maintained at 350°C. The flow rate of the formic acid saturated helium carrier gas was 15 ml/min. Samples analyzed for VFA concentrations were centrifuged at 5000 rpm for 5 min in a micro centrifuge, and the supernatant was diluted with an equal volume of internal standard containing 1000 mg/L isobutyric acid prior to GC injection.

3.4.3 pH Measurement

The pH was measured using a Fisher Accumet pH meter model 925 (Fisher Scientific, Pittsburgh, Penn.) employing a glass electrode. Sensitivity of the unit was 0.05 pH units.

3.4.4 VSS and TSS Determination

Total and volatile suspended solids determinations were based on procedures in Standard Methods (APHA, 1998). Well-mixed biomass samples were filtered through a pre-weighed GF/C Fiberglas filter (VWR Canlab, Canada) and the residue retained on the filter was dried overnight to a constant weight in a 103 to 105°C oven. The increase in weight of the filter represented the TSS portion. The dried filter was ignited at 550°C in a muffle furnace for 20 min. The difference between ash and dry weights represented the fixed fraction of the sample, and the difference between the TSS and the fixed fraction was considered as the VSS portion.

3.4.5 COD Analysis

Two different techniques were used for the soluble COD analysis: colorimetric and titrimetric techniques were determined based on procedures in Standards Methods (APHA, 1998). Samples were taken from the upper sample port at the up-flow section of each compartment. These samples were centrifuged (Sorvall® SS-3 Automatic centrifuge, Newtown, Conn., USA) for 15 minutes at 10,000 rpm and the supernatant was used for the determination. COD analysis was performed in duplicate.

For the Colorimetric technique a Perkin-Elmer spectrophotometer (Coleman 295, USA) was used to measure the light absorbance at 600 nm of the prepared COD samples. Known concentrations of potassium hydrogen phthalate were used to calibrate the spectrophotometer.

For the titrimetric technique (values of COD lower than 400 mg COD/L), prepared samples were titrated with ferrous ammonium sulphate (FAS) 0.1M. Ferroin indicator solution was used to indicate the end-point of the titration.

CHAPTER 4

RESULTS AND DISCUSSION

Two ABRs were operated over a period of 7 months. Acclimation took place during the first two months, while the last 5 months were dedicated to the operation of the reactors at predetermined steady-state conditions, to obtain experimental data to determine performance with dilute ADF. The major parameters examined during the period were the influence of influent substrate concentration, hydraulic retention time (HRT) and organic loading rate (OLR) on ABR performance, including COD removal, settling characteristics and acetoclastic activity of the granular biomass.

The HRT and OLR were based on a reactor working volume of 32 L, the volume occupied by the granular biomass was not considered. Since HRT is equal to the volume of the reactor divided by the flow rate, the reported HRT and OLR were conservative for this study.

4.1 Start-up and Acclimation

Both lab-scale reactors were seeded with Lake Utopia anaerobic granular biomass (10.5 g VSS/L) and allowed four days of stabilization by recycling only. After that period, acclimation was started under the following conditions: 80h HRT and 4500 mg COD/L feed (OLR 1.35 kgCOD/m^3/d). Measurement of VFAs in both reactors revealed the prompt appearance and accumulation of acetic and propionic acids, which fluctuated in all compartments; butyric acid was observed in the last 2 compartments of each reactor. For reactor 1, the concentrations of acetic acid ranged from 186 mg/L (day 12) to 1806 mg/L (day 19), with a low value of 37 mg/L at day 22

for the first compartment. For reactor 2, acetic acid concentrations were 54 mg/L on day 12, 1176 mg/L on day 19 and, 21 mg acetic acid/L on day 22, also for the first compartment. These irregular profiles were observed in both reactors and, in all compartments during the first 25 days of operation. The principal trend observed was the accumulation of VFAs in the last two compartments. Full recycling only operation was needed to decrease the amount of VFAs present. After what seemed to be stable operation (day 27 to 29) the HRT was decreased to 60h. As a consequence, VFA concentrations again increased dramatically in all compartments, reaching a concentration of 1750 mg of acetic acid/L, 300 mg of propionic acid/L and 600 mg of butyric acid/L, in all compartments of both reactors in just 36 hrs; and the value of the pH went down to 6.8. At that point, the reactors were again set in full recycling mode, and pH and VFAs levels were closely monitored. This type of behavior is quite normal in anaerobic digestion, and according to Speece (1996), to fully stabilize and acclimatize these types of systems an average of 60 days is needed. Acclimation to a specific substrate is very important, as well as the start-up strategy. Barber and Stuckey (1999) treated sucrose wastewater with two ABRs at 35°C, they reported that after a few weeks of operation at 4 g COD/L with HRT decreasing from 80, 60 and 40 hours, the performance of the reactors deteriorated significantly, the reactors "soured" and subsequently failed. Reasons for this type of behavior were attributed to quality of the sludge as well as its handling and storage. In the case of the current ADF experiment the granular biomass had been in the laboratory for a period of over 24 months, at room temperature in sealed containers, this could have had an effect on the distribution of the microbial consortia or its ability to metabolize certain intermediates, such as propionic

acid. It is also important to consider the effect of the toxicity associated with ADF, since the innoculum was originally from a pulp and paper mill.

The evolution of VFAs during startup for each compartment of both reactors is shown in figure 4-1 and 4-2. From figures 4-1 and 4-2 the general trend observed in all compartments was the increase of VFAs, especially in the last two compartments, where the concentrations of VFAs were highest.

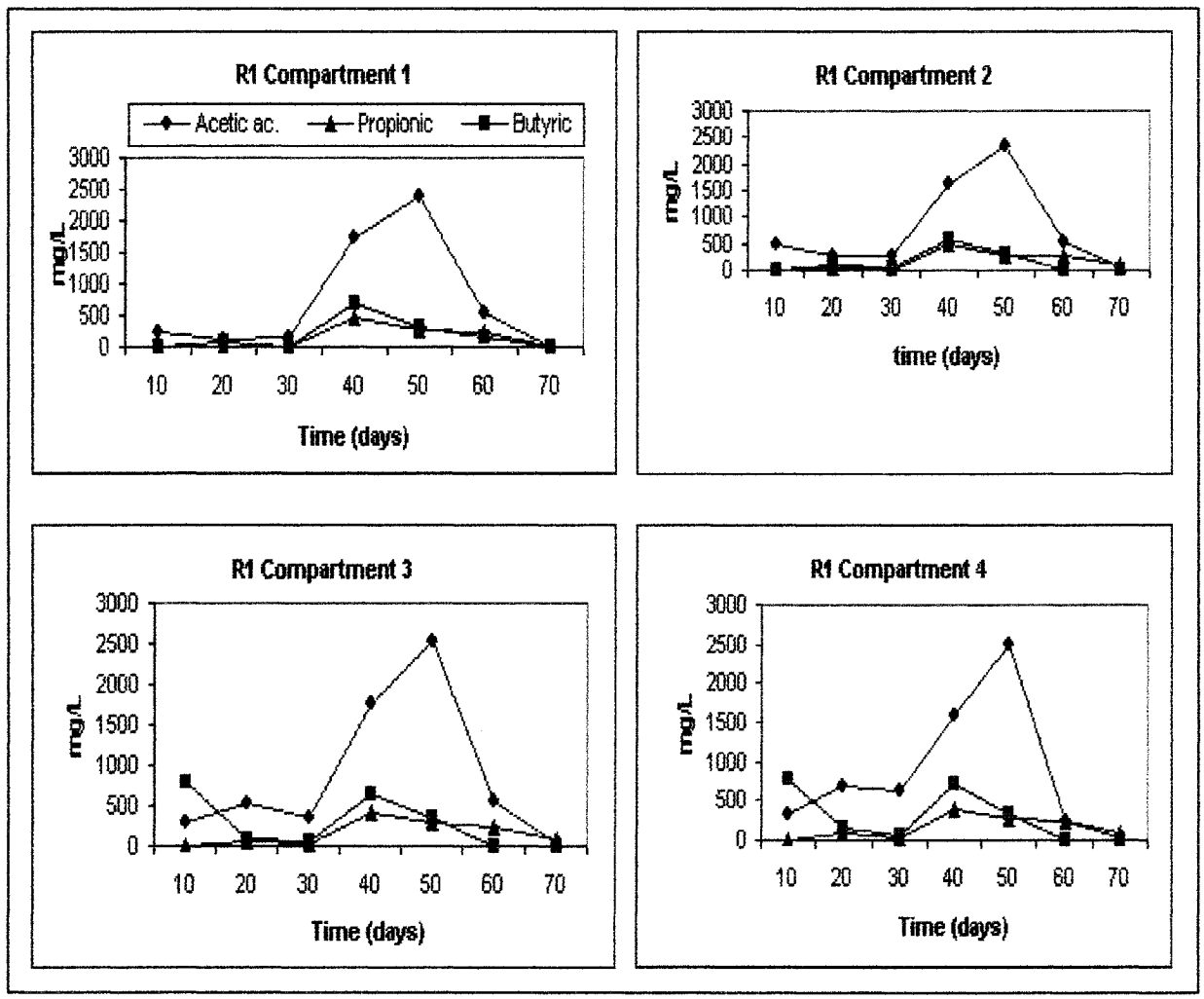

Figure 4- 1 VFAs Profile for Reactor 1.

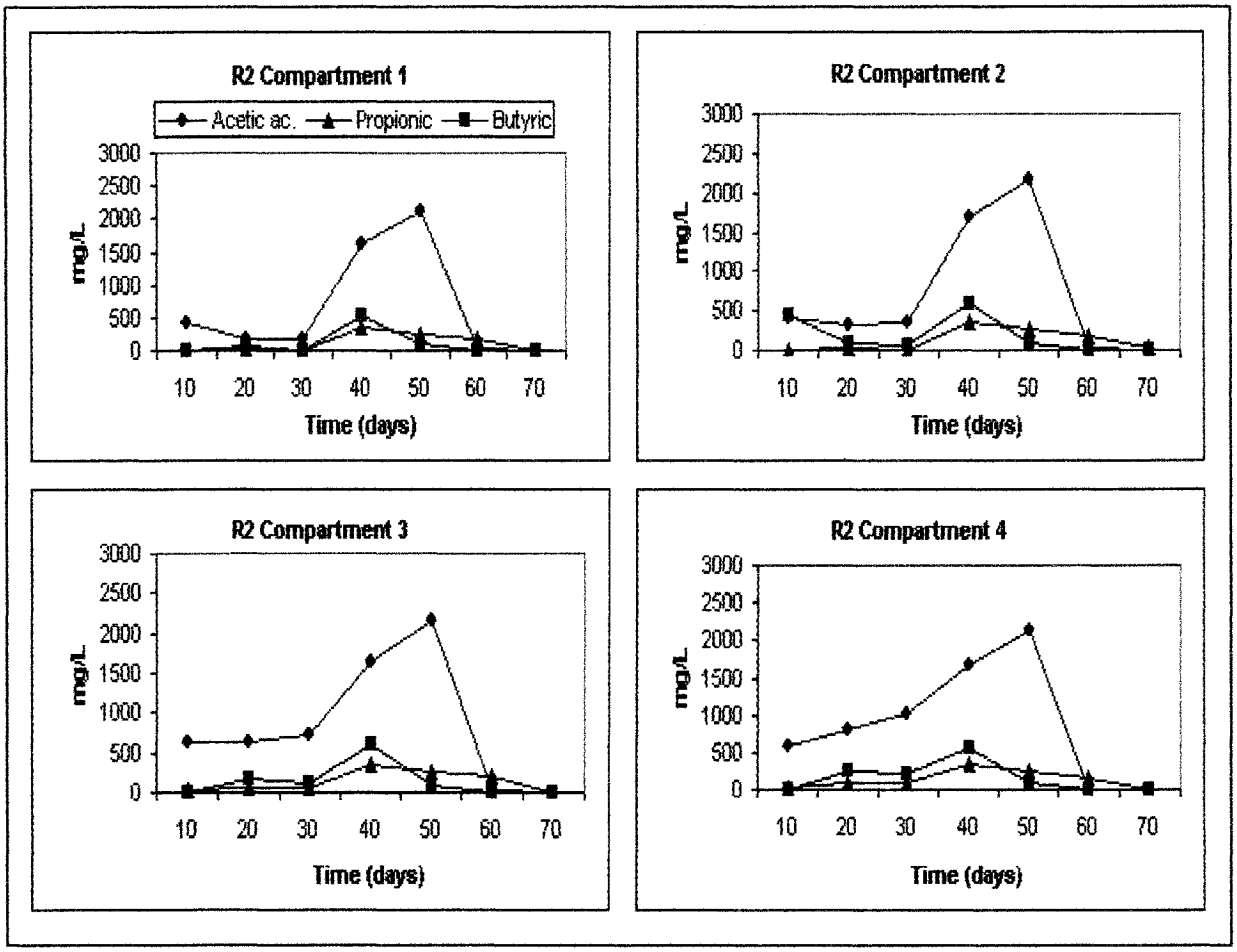

Figure 4- 2 VFAs Profile for Reactor 2.

Once the reactors were set in full recycling mode they behaved as complete mixed systems, maintaining equal concentrations of acids in all compartments, which can be observed from day 30 to day 40. The recycling flow rate was increased in order to improve the mixing within the reactors, and as a result the concentration of propionic and butyric acid started to decline. In order to avoid disruption in the structure of the granules, the up flow velocity in the reactors, never went up to the v_{50} determined for the granules (27.6 m/h). Also from figures 4-1 and 4-2, it can be seen that the acetic acid concentration started to increase even more after day 40. This increase was due to the consumption of propionic and butyric acids, respectively, the same behavior was

observed in each compartment of both reactors. By day 60, butyric acid had completely disappeared from the four compartments of reactor 2 and was present at low concentration in reactor 1; after day 70, VFA concentrations were lower than 50 mg/L, with propionic acid being the most persistent. Also after day 50, rapid consumption of acetic acid and the concomitant mesure in total biogas production were observed (Figure 4-3). The total biogas production measurement during this period suffered from mechanical problems and was not reliable. The low biogas production of reactor 1 compared to reactor 2 was the consequence of operational and mechanical problems associated with it, and the figure is shown just to illustrate the effect that consumption of acetic acid had on total biogas production. The composition of the biogas by day 60 was 65% methane for reactor 1 and 68% for reactor 2.

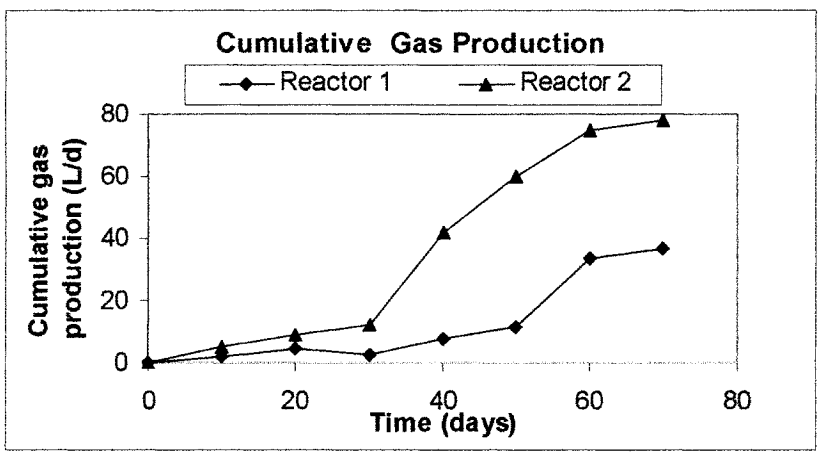

Figure 4- 3 Cumulative Total Biogas Produced During Acclimation.

The pH was constantly changing in each compartment, independently of the excess buffering capacity added to the feed. pH, stayed in the range of 6.6 to 6.8. The general trend was a decrease in pH in the last compartments of both reactors. After

day 50 the pH did not change, and was neutral in all compartments, with a constant value of 7.1. Changes in pH in individual compartments was believed to be part of the acclimation process as biomass adapted to the flow and load conditions.

The height of the sludge bed did not increase during the startup period. Due to hydraulic effects the biomass in the down flow section of each compartment re-allocated itself in the upper section, however the net increase was low. The method used for sludge determination was visual, sludge bed height combined with sludge bed SS concentration, consequently the measure was prone to error in the bed height reading. Also the increase or decrease of the bed does not account for dead or alive cells, however, the reading gives an adequate idea of the amount of biomass.

It is important to describe the operational problems that reactor 1 had at the beginning of its operation: the feed-pump malfunctioned and the HRT was higher than desired; also a tubing connection broke, which resulted in a mayor liquid leak and air entering the system. Reactor 2 never sustained any mechanical problems or disturbance during its operation, but the gas meter device had to be replaced. In general both reactors showed the same behavior and both recovered their stability almost at the same rate. Reactor 1 acclimation took one week more to achieve complete stabilization, than reactor 2. Startup showed that the biomass was able to handle stressful situations successfully, even the presence of air in the system, with good recovery, and that acclimation was a unique process for this type of wastewater and required about 2 months. In the case of all wastewater treatment experiments it is important to spend the time to acclimate the biomass to ensure that results of SS tests are based on biomass performance that is acclimated and able to metabolize the SWW. Once the propionic

acid disappeared from the reactors, continuous feeding was re-started and steady-state transitions were smooth without further problems or accumulation of any VFAs.

4.1.1 Pre-acclimation with Lactose

According to Gavala and Lyberatos (2001), the adaptation of an anaerobic culture to a specific substrate brings significant changes to its microbial population. Among those is the biomass ability to successfully consume different substrates and the evolution of intermediate products such as acetic and propionic acids. At the end of the acclimation this can yield a microbial population very different from the original culture. Aguilar *et al.* (1995) investigated the degradation of VFAs by differently enriched methanogenic cultures. Those cultures were pre-acclimated with glucose and acetate, in order to determine if pre-treatment enhanced the ability to treat synthetic media of protein. They found that the glucose-pretreated culture performed better in terms of the consumption of VFAs and low accumulation of propionic acid.

In the present study a side experiment was also conducted to see if the pre-acclimation enhanced acclimation to ADF. In order to determine the effect of pre-acclimation on consumption and accumulation of VFAs, the biomass was pre-acclimatized with another substrate prior to exposure to ADF. A five-liter-reactor was filled with 3 liters of biomass (10.5 g VSS/L) and was fed with 5 L of a 10 g/L lactose solution for 1 month. Control reactor was treated in the same way but without lactose. After 1 month of pre-acclimation both reactors were fed with dilute ADF (300 mg COD/L) SWW for a period of 3 weeks, the HRT was 7 days. Once acclimation ended, biomass samples were anaerobically transferred to serum bottles with different

concentrations of ADF, and were assayed for VFA, COD consumption, as well as acetoclastic activity and settling characteristics.

From figure 4-4, it can be observed that the biomass that was pre-acclimatized with lactose, then acclimated with ADF performed better in terms of COD removal and in the VFAs consumption than biomass without pretreatment (figure 4-5). Also from figures 4-4c it can be observed that the test bottles that contained biomass pre-acclimated with lactose consumed the substrate faster (determined by the slope of the COD curve) than the bottles containing biomass which had not been pre-acclimated with lactose (4-5c). For example, for ADF concentrations of 750 and 500 mg COD/L the COD removal rates were 2.9, and 2.7 mg COD/L/h respectively, while for the control the values were 2.4 and 1.3 mg COD/L/h for the same concentrations. The consumption of acetic (Figure 4-4a), and propionic acids was also faster for the bottles pretreated with lactose and the accumulation of propionic acid was near zero (Figure 4-4b). For the bottles with no treatment the profiles of acetic acid were more irregular (Figure 4-5b) showing more fluctuations in time, and the concentration of propionic acid was also higher (Figure 4-5b). In figures 4-4a and 4-4b; and 4-5a and 4-5b only the ADF concentrations of 500 and 750 mg COD/L are shown because at 300 mg COD/L there was no accumulation of any acid and the consumption of both acids was complete.

Even though in both cases the maximum consumption of COD was in the first 15 days, the biomass pre-acclimated with lactose consumed COD faster. The gas production was also measured (Figures 4-4d and 4-5d), the absence of a lag phase for 500 and 750 mg COD/L indicated that the consortia was better acclimated to the VFAs

produced, also for the same biomass the gas production of the lactose-acclimatized batch at 500 mg COD/L was higher than at 750 mg COD/L, which was unexpected (Figure 4-4d).

Also, for pre-acclimated biomass after day 10 the maximum production of biogas occurred, which is in accordance with the COD and VFAs consumption data.

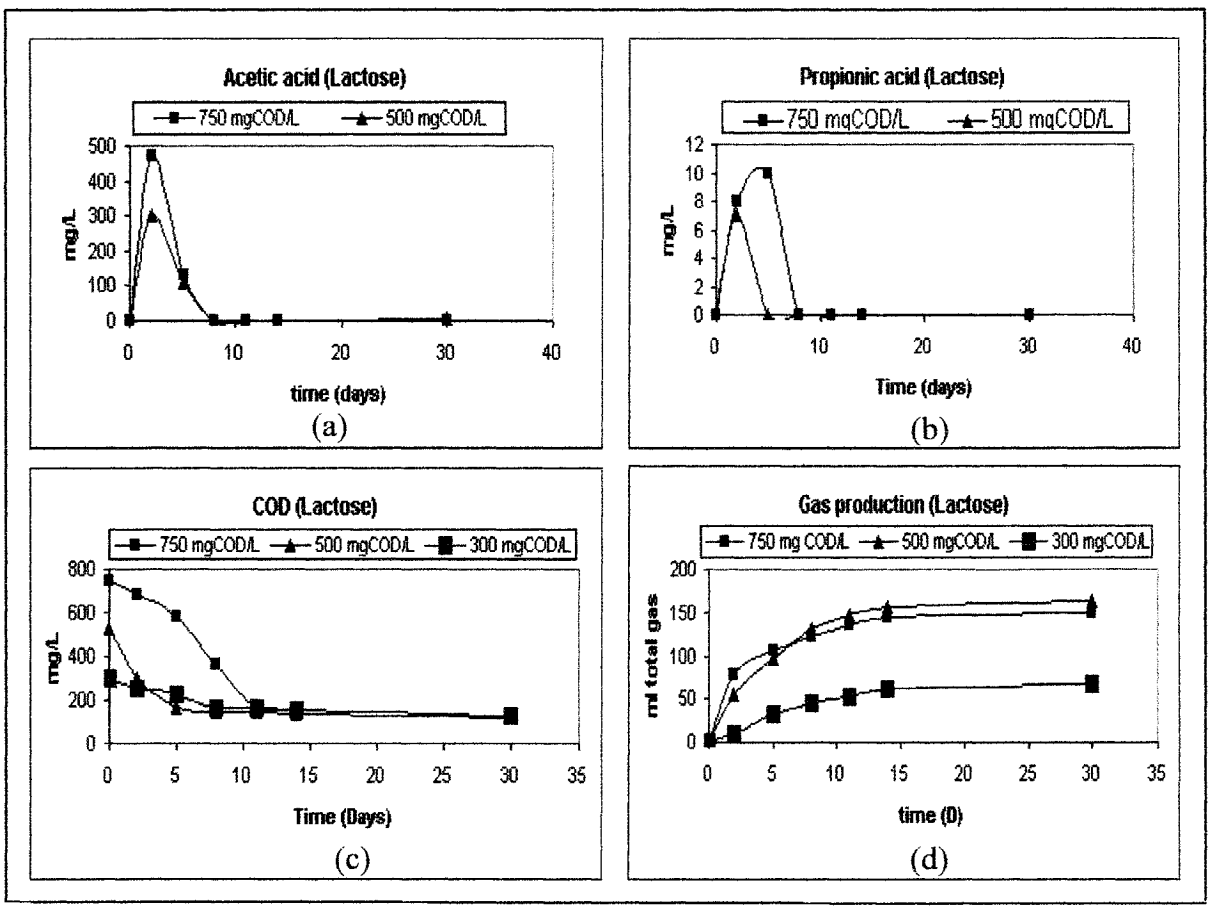

Figure 4- 4 Lactose Pre-acclimation Results.

Pretreatment with lactose also modified the settling properties of the granules, this was in accordance with other reports (Tay J.H. *et al*, 2001; and Gavala and Lyberatos, 2001) that showed that granules grown in the presence of sugars presented better settling characteristics than granules grown under other conditions (protein or

acetate-fed systems). From figure 4-6, it can be seen that there was a favorable increase in v_{50} for the lactose pre-acclimated biomass, with v_{50} of 33.4 m/h compared to 27.6 m/h for the control no pre-acclimated with lactose. The development of granular biomass with good settling characteristics is desirable in this type of reactors, because that ensures a good biomass inventory. Also, from figure 4-6, it is important not only to notice the increase of the v_{50}, of the granules pre-acclimatized with lactose, but also the shape of the graph, which in this case indicates that at higher flow velocities it is still possible to maintain a good biomass inventory, since at higher upflow velocities the amount of granular biomass washed out will be less.

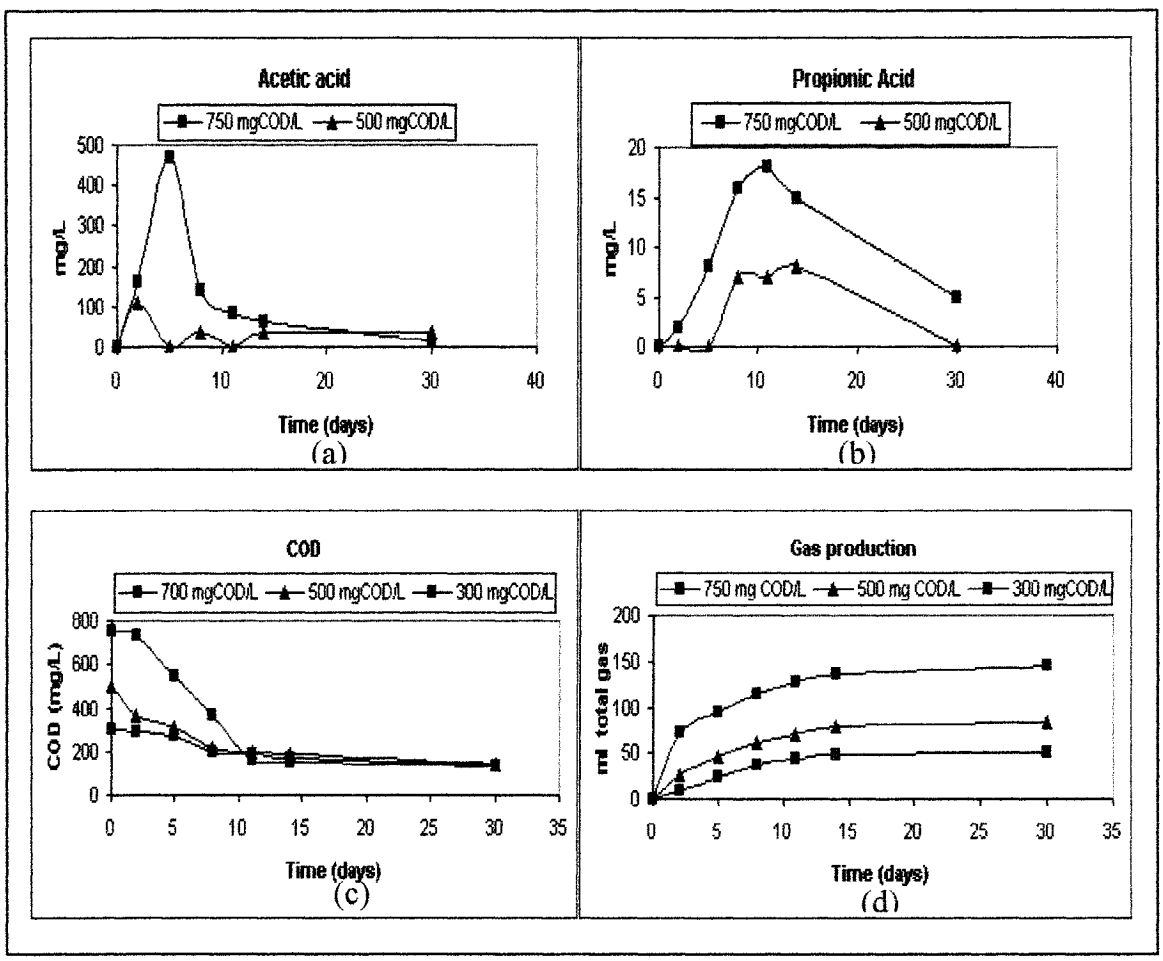

Figure 4- 5 No Pre-acclimation With Lactose Results.

The acetoclastic activity of the granules pre-acclimatized with lactose also increased substantially. Biomass that was lactose pre-acclimated had a specific acetoclastic activity of 0.36 g/g VSS/d versus 0.21 g/g VSS/d for the control without lactose pre-acclimation, as can be seen in table 4-1. The previous results showed that pre-acclimation to a supplementary carbon source can be very effective for enhancing the acclimation to ADF wastewater.

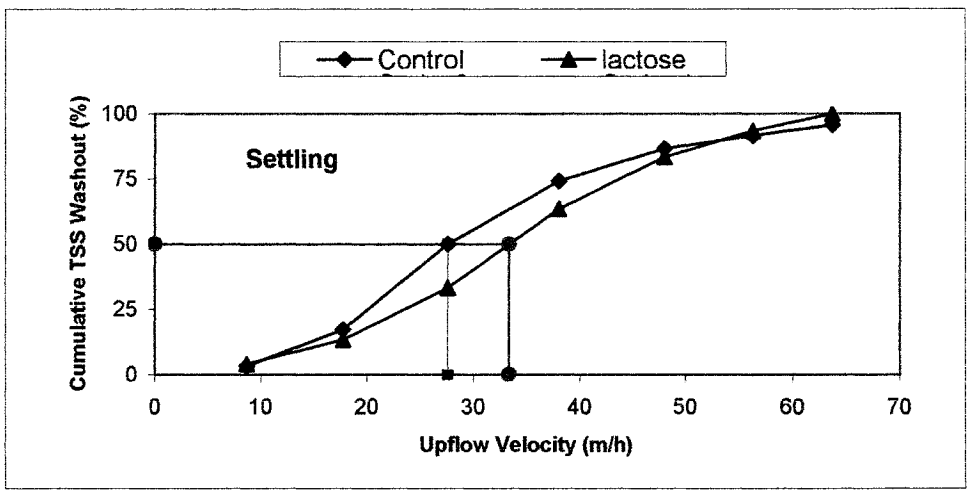

Figure 4- 6 Biomass Settling Characteristics After Lactose Pre-acclimation.

Table 4- 1 Specific Acetoclastic Activity for Lactose Pre-acclimation Study.

	Acetoclastic activity (g acetate/g VSS •d)
Inoculum	0.20
Acclimation with Lactose	0.36
Acclimation without Lactose	0.21

It should be noted that biomass used in continuous study did not undergo pre-acclimation with lactose. Biomass used in continuous study was subjected to stand ADF only acclimation. Further work is required on the benefits of pre-acclimation.

4.2 Continuous Experiments

Table 4-2 summarizes the average steady-state data for the 12 experimental conditions evaluated and figure 4-7 shows the typical $COD_{influent}$, $COD_{effluent}$ during the period of time of this study for reactor number two. More detailed results and standard deviation can be found in appendix C.

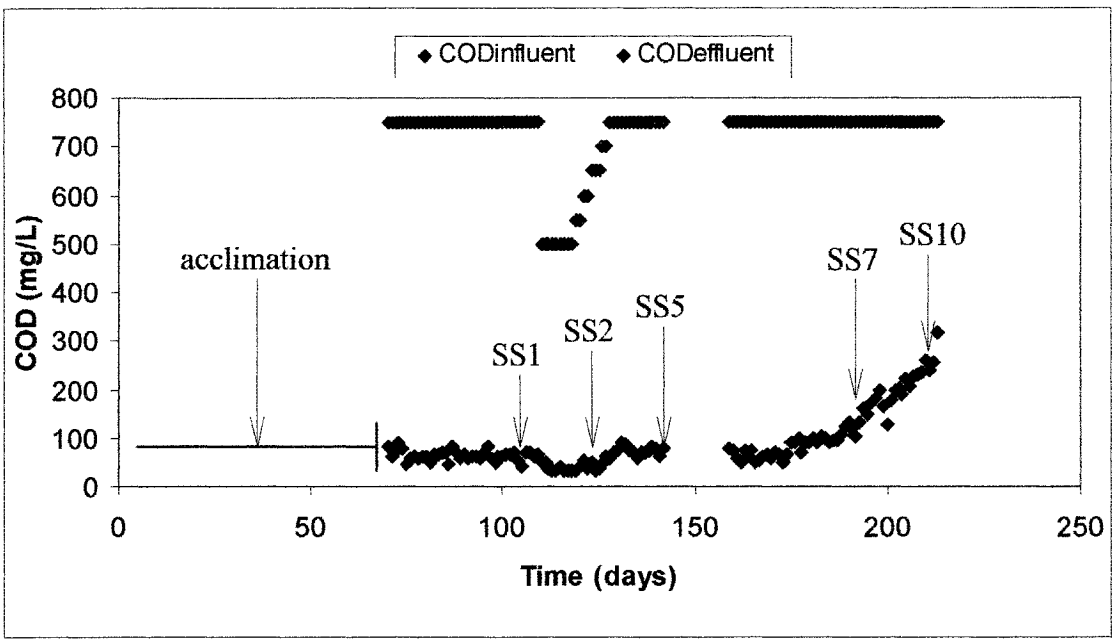

Figure 4- 7 Typical Performance Data for Reactor 2. $COD_{influent}$ effluent COD During the Continuous Study of this Experiment. Arrows Indicate the Steady-State conditions.

Results are presented for COD removal, effluent VSS, gas production and composition. During the various run conditions a range of different OLRs were covered and the COD removal efficiency ranged from 61% to 93%. It is important to note the long SRTs achieved. Even at HRT of 3 hrs the SRT was over 28 d, indicating that biomass washout was not a problem.

Table 4- 2 Steady-State Data of Overall ABR Performance

RUN	HRT	OLR	Influent COD	Effluent COD	COD Removal	Total Gas Production	Gas Composition	Effluent VSS	SRT
	(h)	(kg COD/m^3 d)	(mg/L)	(mg/L)	(%)	(L/d)	(% CH4)	(mg/L)	(d)
1	24	0.75	750	59.65	92	7.3	63	13.7	3627
2	24	0.5	500	34	93.2	6.71	70	20.6	2367
3	24	0.3	300	42	87.8	2.37	68	15	2397
4	12	1.5	750	71.7	90.4	14.2	69	51	334.4
5	12	1	500	36	92.7	11.21	74	41.2	566.6
6	12	0.6	300	33	89	4.2	71	26.5	583.8
7	6	3	750	172	77	14	69	64	150.2
8	6	2	500	128	74.4	26.8	70	65.5	161.3
9	6	1.2	300	74	75.4	7.3	71	36	269.4
10	3	6	750	239	68	36.09	74	170.5	28
11	3	4	500	166	66.8	26.29	73	152	32
12	3	2.4	300	116.4	61.2	10.37	70	114	36

4.2.1 ABR Performance versus Hydraulic and Organic Loading Rates

The HRT versus the COD removal efficiency for the ABR (measured as a percentage of influent COD removed) is shown in figure 4-8. From the figure it can be observed that at hydraulic retention times greater than 12 hrs the removal efficiency was constant ; between 12 and 6 hrs HRT, COD removal started to decrease, but it was still over 74% and for HRTs lower than 6 hours the performance declined even more. At 3 hrs HRT the removal efficiency decreased to an average of 65%, for the 3 waste concentrations tested, which indicated that the use of the ABR under these conditions was still feasible as a pretreatment process but most likely an additional aerobic polishing stage may be needed for further stabilization. Figure 4-9 shows the OLR versus COD removal efficiency. It can be observed that the best conditions were reached at low OLRs, and that as the OLR increased, the removal efficiency decreased. However it is important to mention that the OLRs were reached under different conditions of initial COD concentration and flow rate. For 750 mg COD/L under the

different HRTs tested, it was observed that at a high OLR (6 kg COD/m^3/d) the removal efficiency was still acceptable at 68% but for 300 mg COD/L at a OLR of 2.4 kg COD/m^3/d the removal efficiency was 62%.

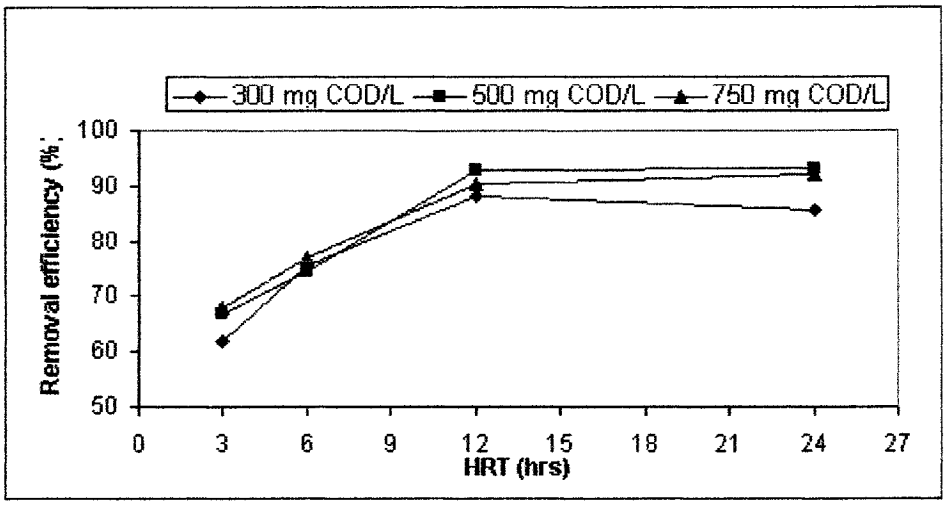

Figure 4- 8 HRT versus COD Removal Efficiency.

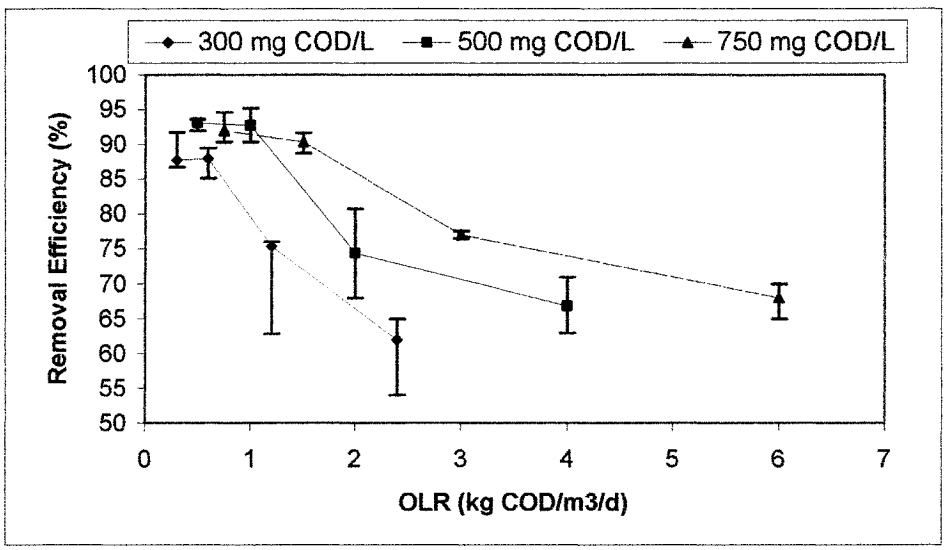

Figure 4- 9 OLR versus COD Removal Efficiency.

Also, for OLRs between 1-2 kg COD/m^3/d, 2-4 kg COD/ m^3/d and 4-6 kg COD/ m^3/d corresponding to HRTs less than 12 hrs for waste concentrations of 300, 500 and 750 mg COD/L, respectively, the COD removal efficiency decreased to 75.4%, 74.4% and 77%, respectively, indicating that ABR performance with dilute waste was strongly influence by substrate-microbe contact time (i.e., HRT). From figure 4-9 it can also be observed that the lowest COD removal efficiency was for the lowest influent substrate concentration. While this was likely due to a combination of ABR operating conditions and dilute influent concentration, a part of the poor performance could be attributed to the fraction of COD in the effluent that is not biodegradable that may be attributed to products of microbial origin that are generated within the reactor, under conditions of low substrate concentration. In figure 4-10 the effect of influent concentration on removal efficiency is shown. For the same influent concentration, the removal efficiency was almost constant, independently of HRT. Thus it can be said that for dilute ADF the ABR performance is more dependent on the HRT rather than initial feed concentration.

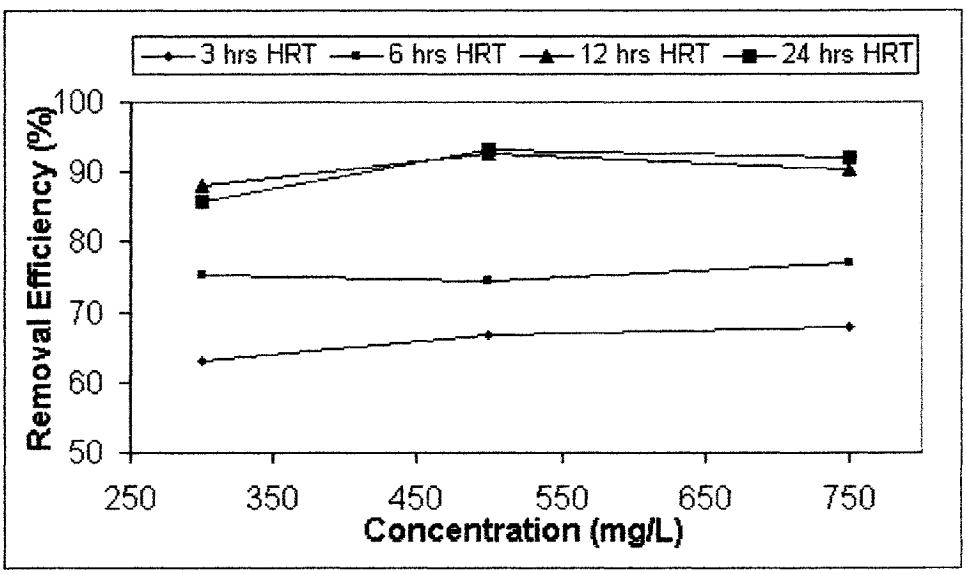

Figure 4- 10 Influent COD Concentration versus COD Removal Efficiency.

4.2.2. Biogas Production and Composition

From figure 4-11, it can be seen that total biogas production increased as the OLR increased. More variability was seen at high OLR values which was to be expected as HRT decreased and COD removal decreased. The average methane yield was 0.29 ± 0.05 L/g $COD_{removed}$ for the 12 experimental run conditions and is near the theoretical maximum of 0.395 L/g $COD_{removed}$ at 35°C. Interestingly; methane yields for experimental runs at shorter HRT were lower than runs at longer HRT. In general, result values between 0.29-0.37 L/g $COD_{removed}$ are the most common; due to experimental error as well as biomass generation, also. It is important to consider that a portion of methane was dissolved in the liquid effluent, and it could account for this lower methane yields since 0.032 L CH_4/L effluent is the methane solubility at 35°C (Switzenbaum, 1978).

Table 4-3 shows the mass balances during the 12 steady-state ABR runs. Based on the first law of thermodynamics, and assuming no COD accumulation, the total COD entering the ABR is equal to the COD leaving the reactor, thus the ratio COD_{in}/COD_{out}

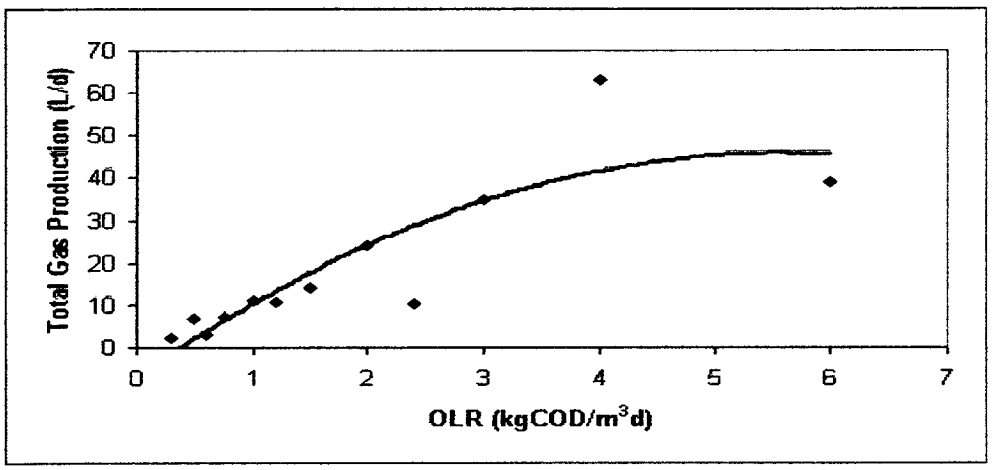

Figure 4- 11 OLR versus Total Biogas Production (Curve shows only the trend).

Table 4- 3 Mass Balance for the Steady-state Experimental Conditions

RUN	Flow rate	Soluble COD influent	Soluble COD effluent	CH_4 Produced	COD IN	COD OUT	Mass of COD removed	CH_4 prod/g COD removed
	(L/d)	(g/L)	(g/L)	(L/d)	(gCOD/d)	(gCOD/d)	(g/day)	(L/g removed)
1	33	0.75	0.059	7.87	24.8	24.6	22.80	0.35
2	33	0.5	0.034	6.10	16.5	19.3	15.38	0.40
3	33	0.3	0.042	2.39	9.9	10.1	8.51	0.28
4	66	0.75	0.071	14.80	49.5	47.6	44.81	0.33
5	66	0.5	0.036	11.20	33.0	36.2	30.62	0.37
6	66	0.3	0.035	4.20	19.8	18.3	17.49	0.24
7	132	0.5	0.152	14.00	66.0	66.3	45.94	0.30
8	132	0.75	0.172	26.80	99.0	101.4	76.30	0.35
9	132	0.3	0.074	7.30	39.6	39.0	29.83	0.24
10	264	0.75	0.239	39.06	198.0	183.7	134.90	0.29
11	264	0.5	0.166	26.29	132.0	132.0	88.18	0.30
12	264	0.3	0.139	10.37	79.2	84.5	42.50	0.24

Assuming no accumulation : $COD_{in} = COD_{effluent} + COD_{methane}$

$CODin = COD_{influent} \times$ Flow Rate

$CODout = (COD_{effluent} \times \text{Flow Rate}) + (CH_{4produced}/0.395) + (0.032 \times \text{Flow Rate}/0.395)$

0.395 l/g COD is the methane potential at 35 °C.

0.032 L CH_4/Leffluent is the methane solubility at 35°C. (Switzenbaum, 1978).

is equal to one. However, some discrepancies can be observed as the COD_{in}/COD_{out} ratio varied between 0.86 and 1.08. The closeness of the mass balance to a value of 1 give us confidence in our overall performance results and methane yields. Small differences are most likely attributed to the measurement error rather than to the experimental conditions. For example, wet tipgas meters some times simply stopped working (low battery), resulting in data variability.

Also from table 4-2 it can be observed that the methane content of the gas changed between runs. However the average composition was 70%±3% methane and 30%±3% carbon dioxide, on a volume basis, and agreed with other values reported for the anaerobic treatment of ADF (Pham, 2002).

4.2.3. Effluent Volatile Suspended Solids

Effluent suspended solids concentration is an important parameter for effluent quality. The ABR, which is characterized as a high rate reactor, separates the SRT from the HRT. As mentioned previously and shown in table 4-2 SRT ranged from 28 to 3627 d. This important characteristic makes this type of system attractive for the treatment of dilute wastewaters because acceptable effluent quality can be obtained at low HRTs, i.e., the reactors can have a smaller footprint. Fig 4-12 shows the relationship between HRT and the effluent VSS for all runs. From figure 4-12a, it can be observed that as the HRT decreased the VSS concentration in the effluent increased (shorter SRT). On the other hand, if OLR versus effluent VSS (figure 4-12b) are compared, it can be observed that at high OLR (short HRT), there was also an increase in VSS concentration in the effluent. This behavior was expected, since at high OLRs and concomitant short HRTs there was both more production of new biomass as well as biogas. As the anaerobic granules are acted upon by the hydraulic and buoyant forces in the ABR, they can be carried though the ABR in the SWW flow and exit the reactor. The largest VSS loss of about 170 mg/L occurred at 3 hrs HRT and 750 mg COD/L. It should be noted that at the HRT of 24 hrs VSS in the effluent (without clarification step) were close to the effluent standards values set for secondary wastewater treatment (25 mg/L).

For a determined HRT, the SRT was almost constant and independent of the initial feed concentration (Table 4-2). For instance, with an HRT of 24 hrs, the SRT was very high above 2300 d and for all purposes could be considered almost constant for the three COD feed concentrations tested. The same can be observed for the HRT

of 3 hours where the SRT were lower but is also in the same range for the three SWW concnetrations. In this latter case, the SRT is between 28-36 days, which is greater than the duplication time of methanogenic bacteria. Even under these short HRT, and high OLR conditions the ABR was still able to maintain a high biomass inventory even though the COD removal performance was the lowest at about 56%.

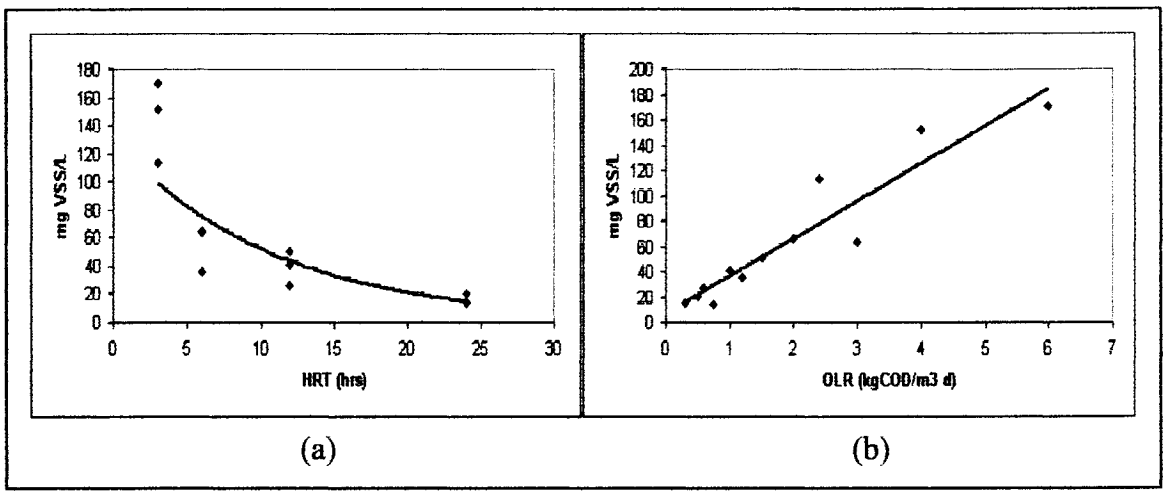

Figure 4- 12 HRT and OLR versus Effluent VSS (Curves Show the General Trends Only).

4.2.4 COD Profiles

COD profiles were created in order to observe the consumption of influent COD through the four compartments of the ABRs. The COD profiles were obtained at each steady-state for the different run conditions. From a previous mixing study in this laboratory, using an identical ABR with the same number of compartments, it was shown that the reactor behaved as four CSTRs-in-series (Barriault, 2003). Since each compartment behaved as a completely mixed reactor, the effluent of the first compartment was considered as the influent for the second compartment and so on.

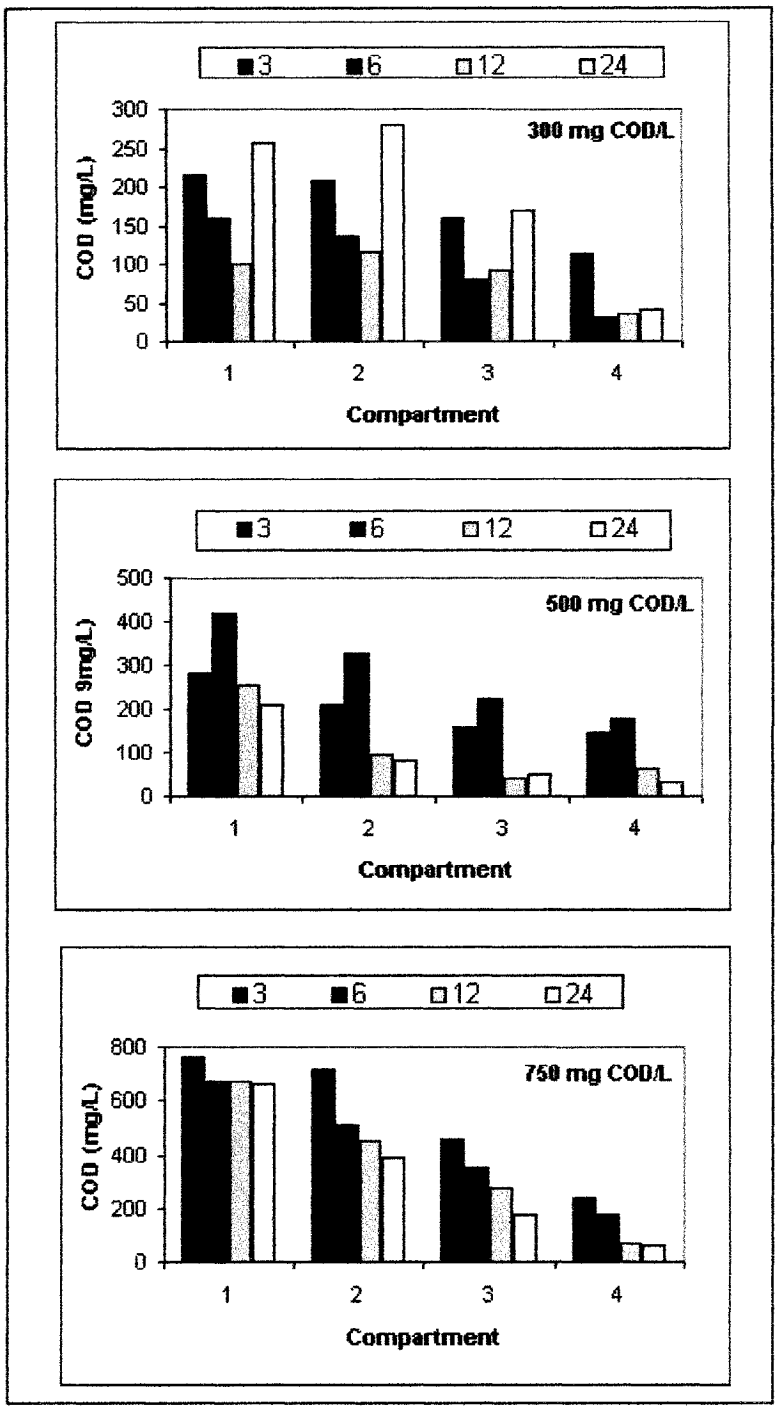

Figure 4- 13 Different COD profiles for ADF Treatment at 3,6, 12 and 24h HRT.

Figure 4-13 shows the typical COD profiles at various HRTs for the three ADF concentrations tested. It can be observed that at lower COD influent concentrations,

the COD concentration from compartment to compartment was more irregular with lower concentrations in the first two compartments than, the profile-behavior when treating higher COD influent concentrations. At low influent concentrations, the COD concentration did not always decrease from one compartment to the next as expected. Instead, there was an increase in some experimental conditions: for 300 mg COD /L and 24 hrs HRT, the COD increased from compartment 1 to compartment 2, and the same effect was observed for the same compartments at 12 hrs HRT. At higher influent concentrations the COD concentration was maintained high in each compartment. It would suggest that a lower concentrations and hence lower OLR only the first two compartments were being stressed while at higher OLR and concentrations the biomass in more compartments was being stressed. The increase in COD for some compartments was observed at the lowest and at the highest OLRs, and even if the measurement was slightly and within the normal variability of the sample, it could be explained because low or high substrate concentrations can create stresses on the system. As a response to the stress the microorganisms produce soluble microbial products (SMP). According to Barker and Stuckey (1999) there are many reasons for the production of SMP, but normally under stressful conditions SMP production is favored. This effect was expected to be observed in the last compartments, which were supposed to be in starvation, but was normally seen in the first two compartments. Hence, it can be said that these products were somehow produced in the first two compartments, but that they were consumed to a certain extent in the last compartments, which explains why the effect was not observed. However, more research needs to be done to confirm this. For the rest of the runs and the rest of the compartments the general trend observed was

a COD reduction as the SWW moved through the reactor from the first compartment to the last one.

The same trend was observed for the VFAs. For the above mentioned compartments, there was no relationship between the higher influent COD and the VFA concentration, as shown in figure 4-14. From figure 4-14, it can be seen that even if an increase in COD occurred in certain compartments, a decrease in VFA was sometimes observed at the same time. In some cases for compartment one, the COD measured was high and the levels of acetic acid were rather low (more evidence of SMP production). It is also difficult to address the percentage removal of each compartment, because as mentioned before, in some cases the amount of COD in the subsequent compartment was higher or at least the same. However, the four compartments functioned together for the total stabilization of the SWW in the system. At certain OLR the first and second compartments showed signs of stress (low COD removal, high VFA) and this effect disappeared in the last compartments. However, if the last compartments started to be stressed the total performance of the reactor decreased, and the accumulation of VFA started to increase gradually in each compartment.

This effect was observed at the end of the experiment. It was intended to continue the experiment and take the reactor to 1.5 hrs HRT with 750 mg COD/L. However, once the HRT was decreased to two hrs the ABR performance in terms of COD removal dropped even more with the final COD removal being 35% and the apparition of butyric acid in the last compartment (350 mg/L).

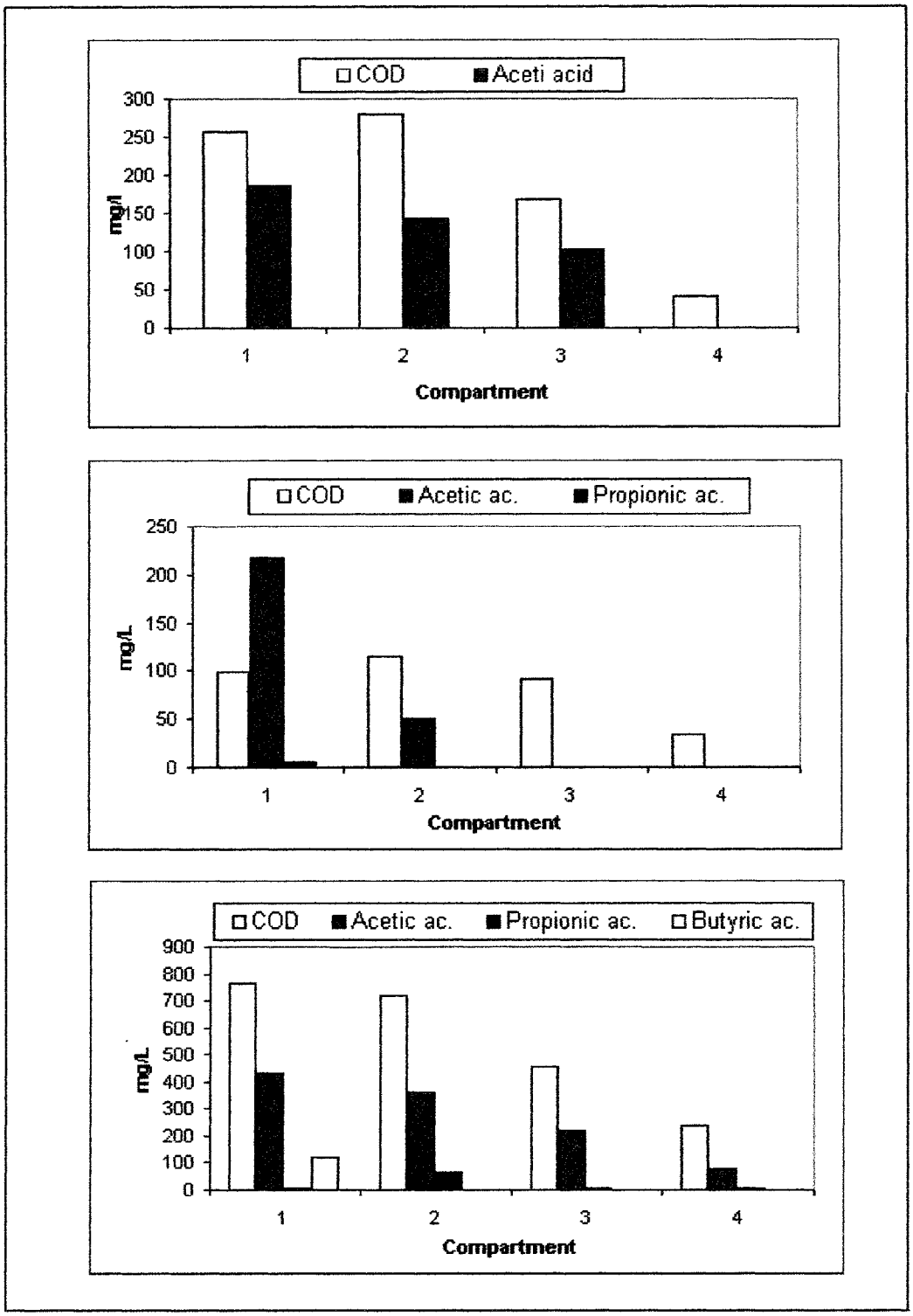

Figure 4- 14 VFAs Levels for Experimental Runs 3, 6 and 10.

It is known that the ABR and its compartmentalization favors the separation of acidogenesis and methanogenesis. Thus, the measurement of the methane production for each compartment could have given more clues, about the extent of both processes, but the gas measurement was done for the total system. Since the first compartment showed the highest COD and VFA concentrations, it can be stated that this compartment was more the acidogenic portion of the reactor. In the last three compartments, the total consumption of VFA took place, such in these last compartments methanogenesis took place, the exact extent is unknown and was dependent on the experimental conditions. However, the four compartments had an active participation in the total stabilization of the reactor and treatment of the dilute ADF.

4.3 Biomass Characterization

4.3.1 Biomass Settling Characteristics

The success of high rate anaerobic reactors such as the ABR is related to its ability to maintain a good settling biomass, which allows a high SRT and good process stability independent of the HRT. As shown in table 4-2 the ABR operated with very long SRT ranging from 28-3256 d. At the lowest SRT it is important to see if the biomass has been loosing its ability to settle since this could eventually result in biomass washout. In this experiment the settling characteristics of the biomass were measured at the end of each steady-state condition for the four different compartments of the reactors and the results were compared with those of the seed granular biomass. This type of test only provides qualitative comparisons, and the v_{50} is considered to be

the velocity at which 50% of the granular biomass would be washed out. Table 4-4 shows the v_{50} values obtained for the different experimental conditions.

Table 4- 4 Settling Velocity (v_{50}) for Granular Biomass After Steady-States.

			v_{50} (m/h)			
Seed Granular Biomass			27.6			
Run	$COD_{influent}$ Mg/L	HRT hrs	Compartment 1	Compartment 2	Compartment 3	Compartment 4
1	750	24	26.6	25.8	27.1	26.9
2	500	24	26.3	25.2	27.3	26
3	300	24	23.2	25.7	25.1	26.7
4	750	12	25.8	30.2	27.4	26.4
5	500	12	26	27.7	26.8	28.4
6	300	12	30.4	31.4	25.9	27.5
7	750	6	24.3	24	25.8	28.1
8	500	6	24.2	24.1	23.2	26.3
9	300	6	23	27.6	26.7	29.8
10	750	3	23.8	26	25.2	26.4
11	500	3	21	23.6	25.4	26.3
12	300	3	19.2	24.1	25.8	28.1
Average			24.5 ± 2.8	26.3 ± 2.5	26 ± 1.2	27.2 ± 1.2

From table 4-4 it can be observed that no substantial change occurred in the settling characteristics of the biomass during the experimental conditions. The average v_{50} of 25.99 m/h and SD of 2.25 for all test conditions suggested that the acclimation process was complete and that granular biomass remained stable under a wide range of operational conditions, independently of the feed concentration or HRT. These results agreed with other studies on settling characteristics of anaerobic granular biomass. Pham (2002) reported an initial v_{50} of 32 m/h and at the end of her experiment, the average v_{50} was 23 ±1.4 m/h, and Barriault (2003) also reported no change in the granular biomass treating ADF in ABRs, the initial v_{50} in this case was 49.7±7 m/h and

at the end of her experiment the average v_{50} of the anaerobic granular biomass was 47±1 m/h.

For the last two experimental conditions tested, the v_{50} for the first compartments was slightly lower (19.2 and 21 m/h) compared to the rest of the values obtained, that was believed to be due to a crust-type formation around the granules that was observed in the first compartment of each reactor. The first compartment of the reactors was exposed to the highest loading rates and after prolonged exposure to the ADF feed, the granules started losing their original coloration. The same effect was observed in both reactors and the v_{50} under the conditions tested did not change significantly from that of the seed as can be seen in the average values in table 4-4, specially in the last two compartments, which is also important in terms of stability, because in the last compartments the washout could have an adverse effect on the total performance of the reactor if it is considered that methanogenic microorganisms are distributed more favorably in this portion of the reactor. The typical S shape of the curves for the determination of v_{50} was kept during all the experimental conditions. Figure 4-15 shows a typical curve, in this case is for the same granular biomass for the run number 1 (750 mg COD/L influent concentration and 24 hrs HRT) and for the run number 10 (750 mg COD/L influent concentration and 3 hrs HRT). Appendix B shows a typical granule size distribution obtained during the different conditions of up-flow velocity.

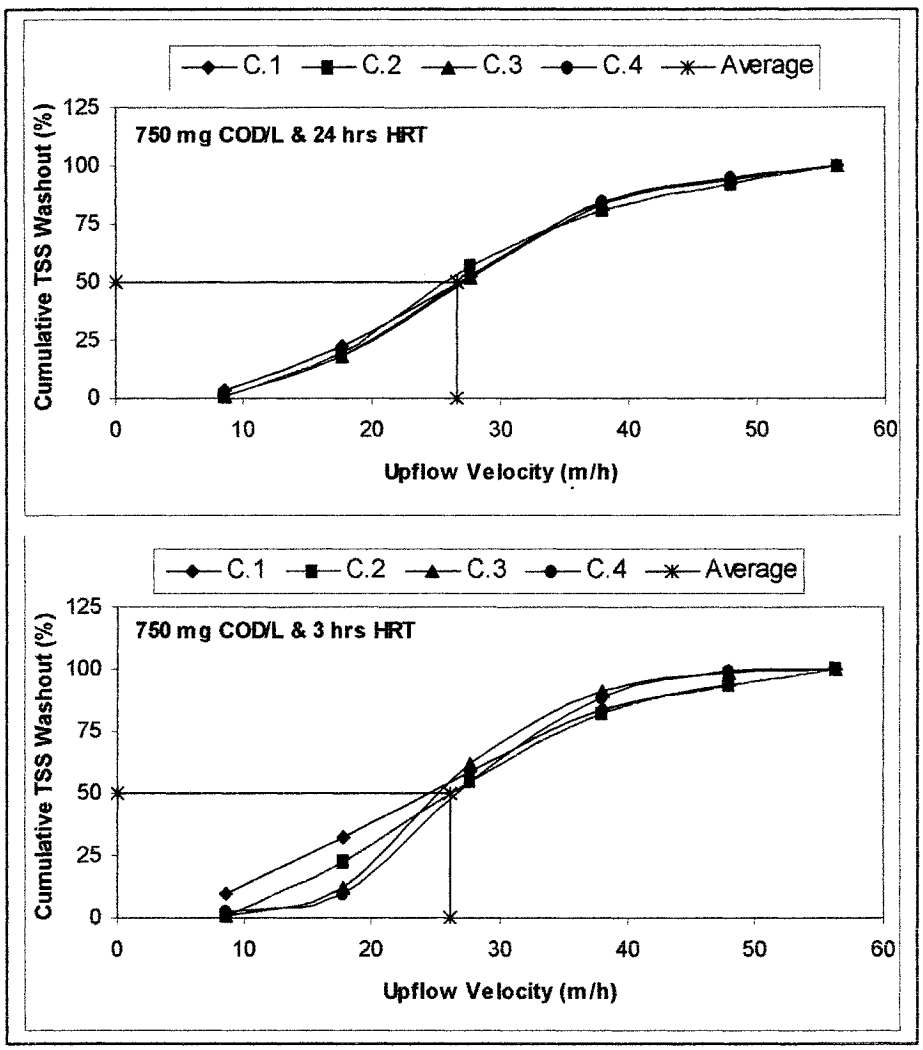

Figure 4- 15 Typical Settling Curve for Granular Biomass During ADF Study.

4.3.2 Yield and Endogenous Decay

To obtain the yield and endogenous decay for the experimental conditions, a mass balance was performed on the reactor biomass. From the general equation

$$\frac{dX}{dt}V_R = QX_0 - QX_e + V_R(-Yr_{SU} - k_d X) \qquad (4.17)$$

Where V_R = reactor volume, L
Q = flow rate, L/d
X = active biomass concentration in the reactor, mg VSS/L
X_0 = biomass concentration in the feed, mg VSS/L
X_e = biomass concentration in the effluent, mg VSS/L
Y = cell yield coefficient, mg VSS/ mg COD
r_{su} = rate of substrate utilization, mg COD/ L/d
k_d = endogenous decay coefficient, d^{-1}

Since the biomass in the feed is zero, and at steady state, dX/dt is also equal to zero, rearranging equation (4.17)

$$\frac{QX_e}{V_R X} = \frac{1}{\theta_c} = -Y\frac{r_{SU}}{X} - k_d = YU - k_d \qquad (4.18)$$

where θ_c = solids retention time (SRT), d
$1/\theta_c$ = net specific growth rate, d^{-1}
U = specific substrate utilization rate, mg COD/mg VSS/d

By plotting the net specific growth rate against the specific substrate utilization rate, the biomass yield coefficient and the endogenous decay rate can be determined.

For this experiment the values obtained are shown in table 4-5. From table 4-5, it can be observed that the values of Y for the highest concentrations are relatively constant. However for the lowest ADF concentration, there is an increase of Y, which was not expected due to even lower substrate limitations. However, it could be argued that Y tended to increase with low feed concentration.

Table 4- 5 Yield and Endogenous Decay Coefficients for ADF Treatment.

Influent COD mg/L	Y mg VSS/mg COD	k_d d^{-1}
750	0.011	0.0092
500	0.017	0.0095
300	0.027	0.0116

It is important to mention that the reactors were operated under alternating influent substrate concentrations at pre-determined HRT values. Hence, previous operational conditions could have had an effect on latter results. Also the yield and endogenous decay coefficients are general results obtained from a balance on the whole reactor, from some of the previous results it was observed that the reactor had the advantage of separating acidogenesis and methanogenesis in various compartments, so different values of Y could be expected for different sections of the reactor since methanogenic bacteria have higher duplication times than acidogenic bacteria. Since the extent of both acidogenic and methanogenic phases in individual compartments is unknown, the general approach shown above is appropriate and is in the range reported for anaerobic digestion of a similar type of wastewater (Pham, 2002). The net increase in biomass was very low for both reactors as suggested by the low Y values, is favorable in one sense because at the low values of yield, sludge wasting would not be necessary over long periods of operation. Additionally, the low endogenous decay values (0.009-0.001 d^{-1}) suggest that active granular biomass can be maintained for long periods of operational down time (i.e., summer seasons). This was demonstrated once the steady-state experiment was over, the reactors were shutdown for a period of 4 months. To simulate summer shutdown. Both reactors were re-started with dilute ADF and they reached a stable baseline of 40 hrs HRT with 750 mg COD/L with a 93% COD removal in only one week. This restart showed that once the biomass was acclimatized, and the different groups of microorganisms developed within each compartment, the start-up was fast and stable and, the activity of the biomass remained constant during storage at 35°C. On the other hand, the low yields are bad should a

sudden loss of granular biomass occur. A prolonged period of time would be required to replenish the biomass inventory.

4.3.3 Specific Acetoclastic Activity

Table 4-6 shows the results of the specific acetoclastic methanogenic activity tests determined for the seed and at the end of the experiment for each compartment. From table 4-6, it can be observed that there was a change in the specific acetoclastic activity through the different compartments of the reactor compared to the seed. Even if the batch serum bottle activity test does not reflect the exact conditions in the reactor, it can give an idea about the biomass activity, since its activity is constant and only the environment change.

Table 4- 6 Specific Acetoclastic Activity for Granular Biomass at the End of the Experiment.

	Acetoclastic Activity g Ac/g VSS/d
Seed	0.20±0.01
Compartment 1	0.04±0.01
Compartment 2	0.33±0.02
Compartment 3	0.36±0.04
Compartment 4	0.30±0.01

For the first compartment, there was a decrease in specific acetoclastic activity compared with that of the granular biomass used as seed. This is reasonable since this part is considered to be the acidogenic portion of the reactor where acetic acid is generated from the COD in the influent. In general, the specific acetoclastic methanogenic activity within the reactor increased in each compartment, which also indicated that different groups of bacteria were selectively developed in each compartment. The higher the specific acetoclastic activity the greater the proportion of

acetoclastic methanogens compared to other microbes that make up the consortia. Also, from table 4-6 it can be observed that the last compartment had also a high specific acetoclastic activity (0.3 g/g VSS/d), which was not expected, due to the lack of acetic acid in this compartment for most of the experimental conditions. This may suggest that the biomass in this compartment is the least unchanged from the inoculum and that under conditions of shock this compartment would serve to enhance the total stability of the ABR reactor.

4.4 ABR Modeling

4.4.1 First Order Reaction Rate Constant

Based on equation 2.2, which was the model applied to the reactor, the k_1 or first order reaction rate can be calculated for the ABR treatment of dilute ADF.

For this experiment it was considered that both reactors had the same performance as a single reactor. This assumption was considered reasonable since both were identical in dimensions and both were seeded with the same initial amount of anaerobic granular biomass and at the same time. Furthermore, as discussed in the section on preliminary acclimation both reactors were observed to behave in a similar manner. Additionally, both reactors were taken to a base line steady state condition for 5 HRTs with 750 mg COD/L prior to sampling. Kinetic results are shown in table 4-7. From this table, it can be observed that variability between the two reactors was small, thus both reactors were treated as equal, and it was assumed that changes in the process response would be due to the experimental run condition, rather than in the reactor used.

Based on the previous statement the overall first order reaction rate k_1 obtained for the different initial feed concentrations are shown in table 4-8.

Table 4- 7 Steady-State Results for Base-line Conditions.

Reactor	Influent COD	COD Removal	Total Gas Production	CH4 Percentage	CH4 Potential	Effluent VSS
	mg/L	%	L/d	%	$L/gCOD_{rem}$	mg/L
R1	750	94.6	8.3	63	0.37	15.1
R2	750	93	7	68	0.34	12.8

Table 4- 8 First Order Reaction Rate (k_1) for the Different Feed Concentrations.

Feed concentration mg COD/L	300	500	750
k_1 (L/g VSS/d)	0.24	0.24	0.48

From table 4-8, it can be observed that k_1 is constant for low concentrations (300 and 500 mg COD/L) but doubles its value for the highest concentration tested. Since different combinations of concentration and flow rate generate different OLR, it can be said that k_1, which is an indication of the consumption rate of COD is constant under certain ranges of OLR and the performance is expected to be the same, which makes the system stable under varying conditions of influent concentration and HRT. Additionally, a significant change in the reaction rate occurred when moving to 750 mg COD/L from the more dilute concentrations tested, indicating that concentration impacted the rate of substrate removal. It is also important to mention that the k_1 values in table 4-8 were calculated using the ABR as a whole, and assuming that the effluent substrate concentration is the same concentration within the reactor, the COD

driving force and the compartmentalization of the reactors were ignored, thus the results have to be used with some caution.

This concept of a first order reaction model was also applied to each compartment of the ABR and the values of k_1 obtained are shown in table 4-9.

Table 4- 9 First Order Reaction Rate (k_1) for Each Different Compartment.

Compartment	1	2	3	4
Influent COD (mg/L)	k_1 (L/gVSS/d)	k_1 (L/gVSS/d)	k_1 (L/gVSS/d)	k_1 (L/gVSS/d)
300	0.08	0.007	0.05	0.1
500	-0.007	0.024	0.08	0.05
750	0.5	0.1	0.24	0.24

As mentioned before, if k_1 is considered a measure of the COD consumption rate, the negative value obtained make no sense. However, a physical interpretation means that in that compartment acidogenesis predominated and under those conditions there was no net consumption of COD. This is obvious for the first compartment where the production of VFAs occurred. For the lowest concentration (300 mg COD/L) the low value in the second compartment is difficult to explain, this could be attributed to the production of soluble microbial products (SMP) given the stress associated with low substrate concentrations (Barker *et al.*, 2000), also at the highest concentration the two first compartments function as acidogenic units, meanwhile the last two compartments have the same activity, in general the highest value is always in the last compartment, which was not expected due to substrate limitation conditions. Somehow the biomass contained in those compartments kept a high activity, independently of the COD or VFAs present, which indicates that they were using another source of energy.

4.4.2 ABR Proposed Modeling

To overcome the restrictions of considering the reactor as a black box, the empirical model proposed by Xing *et al.*, (1991) was modified, and instead of using the k_l with the units of d^{-1}, the previous values of k_l shown in table 4-9 were used in empirical model equations 2.6 to 2.16 and the assumptions formulated were applied to the different results obtained during the different run conditions.

These results are shown in figure 4-16 and summarized in table 4-10. From the figure it can be seen that the model proposed did not fit the values well, it overestimated the performance of the ABR at low HRT and underestimated it at higher HRTs. However, experimental results showed that the performance of the reactor depended strongly on HRT and that as the HRT decreased the COD removal did the same. Also, from figure 4-16, it can be observed that residuals versus the HRT were not random and indicated certain tendency or model innacuracy. In their report Xing *et al.*, (1991) found good correlation between the model and the predictions, however the conditions were different, possibly at higher influent concentrations the model would give more realistic predictions of the system performance than at lower influent concentrations. Additionally, it should be noted that there was some mathematical inconsistency with the k_l values calculated by Xing *et al.*, (1991).

Table 4- 10 Empirical Model Results (1st Order Reaction Model).

Run	HRT	Influent Concentration	COD removal	COD removal predicted	Residual
	(hrs)	(mg/L)	(%)	(%	
1	24	750	92	84	-7.96
2	24	500	93.2	80.5	-12.63
3	24	300	87.5	85.4	-2.22
4	12	750	90.4	80.7	-9.66
5	12	500	92.7	78	-14.64
6	12	300	88	80.3	-7.65
7	6	750	77	76.5	-0.47
8	6	500	74.4	76.4	2.04
9	6	300	75.5	78.5	3.14
10	3	750	68	74.7	6.70
11	3	500	66.8	75.7	8.94
12	3	300	61.2	76.74	15.74

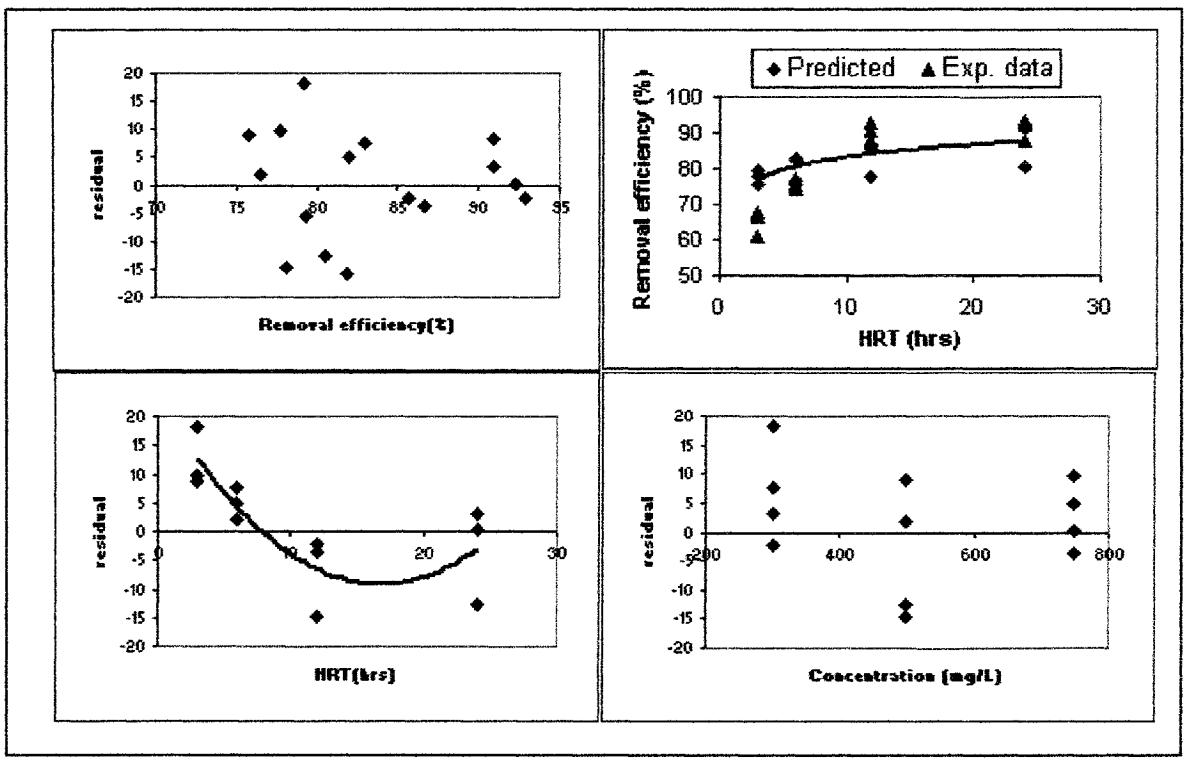

Figure 4- 16 Residual Plots for Emperical Model (1st Order Reaction Model).

4.4.3 Linear Model

To overcome the inaccuracy of the 1st order reaction rate model, the data were fitted to an empirical linear model in order to know if the performance of the reactor could be described in more precise way, and in terms of HRT and influent COD concentration. A series of five fitting exercises were run with the data by the method of least squares, using SAS® System for Linear Models. Equation (4.19) shows the best fitted model obtained to describe the performance of the reactor.

$$Y = 39.09 + 0.048\,X_1 + 4.68\,X_2 - 3.85E^{-5}\,X_1^2 - 0.128\,X_2^2 \qquad (4.19)$$

Where:

X_1 = initial feed concentration, mg/L
X_2 = HRT, hrs
Y = performance (as % COD removed)

From table 4-11, it can be observed that equation 4.19 gave a good estimate of the real performance of the reactor with a 95% confidence interval, 98% of the performance could be explained by the parameters. It is important to note that the Pr values that were significant corresponded to B2 and B4, which corresponded to HRT. The low value of the Durbin-Watson analysis (2.28), indicated that the was no correlation between runs and that each of them could be considered as a random event. Also for the analysis of residual plots figure 4-17 it can be observed that no general trend is observed and that the dispersion of the data, indicates an adequate linear model.

Table 4- 11 Linear Model Parameters.

Influent concentration (mg/L)	HRT (hrs)	Performance (%)	Predicted Performance (%)	Lower 95%	Upper 95%	Residual
750	24	92	92.22	89.47	94.97	-0.22
500	24	93.2	92.16	89.41	94.91	1.04
300	24	87.75	88.65	85.90	91.40	-0.90
750	12	90.4	91.42	88.82	94.02	-1.02
500	12	92.77	91.36	88.76	93.97	1.41
300	12	88	87.86	85.27	90.46	0.14
750	6	77	77.17	75.08	79.27	-0.17
500	6	74.4	77.12	75.02	79.21	-2.72
300	6	75.44	73.61	71.52	75.70	1.83
750	3	68	66.59	64.03	69.14	1.41
500	3	66.8	66.53	63.98	69.09	0.27
300	3	61.95	63.02	60.47	65.58	-1.07

	Parameter	t value	Pr > |t|
B0	39.09986	7.28	0.0002
B1	0.04831	2.24	0.0598
B2	4.68255	15.42	<,0001
B3	-0.00003846	-1.9	0.0986
B4	-0.12823	-11.96	<,0001

Sum of Squared Residuals	18.96436
Predicted Residual SS (Press)	48.94405

R-square	0.9866
Adj R-Square	0.9789

Durbin-Watson D	2.288
1st Order Autocorrelation	-0.175
Number of observations	12

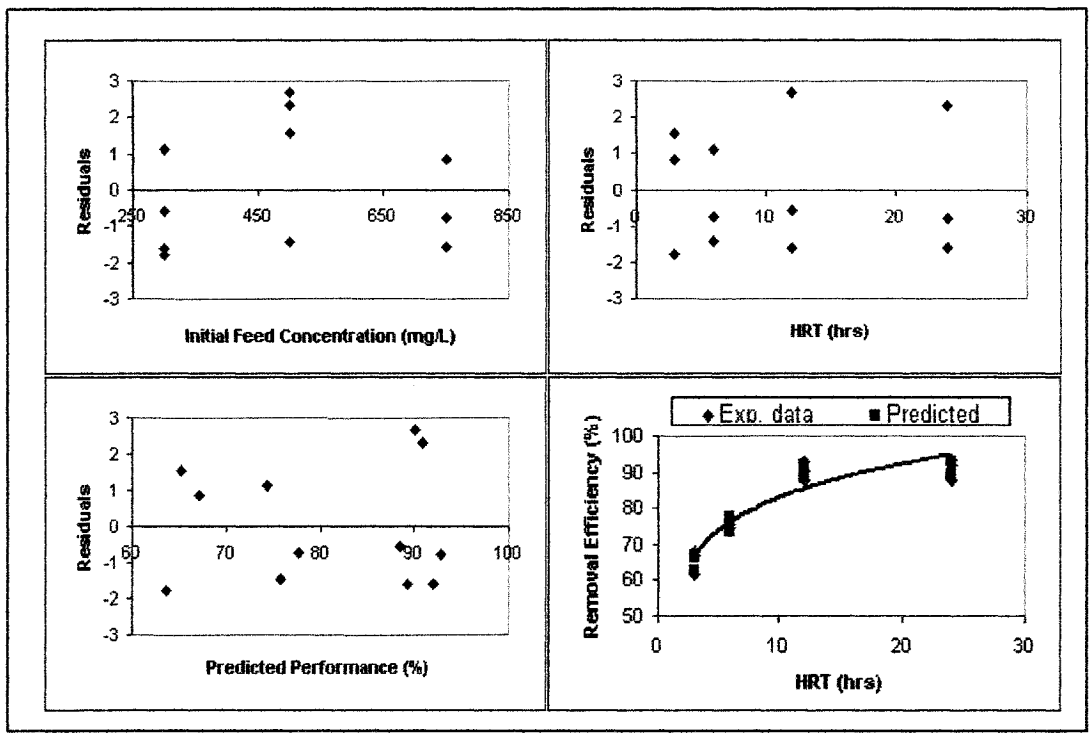

Figure 4- 17 Residual Plots for Linear Model.

4.4.4 Model Verification

After completion of the experimental conditions, three additional runs were carried out for model verification purposes. The results are presented in table 4-12. From the table it can be observed that the removal efficiency predicted using equation 19 slightly changed with the predicted values at the same experimental conditions. This change is small, and overestimated COD removal with decreasing concentration. However the error was less than 5%.

Table 4- 12 Comparison of COD Removal Efficiency to Predicted Values.

Run	Influent concentration	HRT	Performance	Predicted performance
	(mg/L)	(hrs)	(%)	(%)
1	750	24	93.3	92.3
2	500	12	91.8	96.3
3	300	24	82.6	88.6

CHAPTER 5

CONCLUSIONS

The ABR proved to be an effective technology for the treatment of low strength ADF-based wastewater, reaching COD removal efficiencies over 75% for most of the experimental conditions assayed. Better COD removal efficiencies were achieved at the highest concentration and HRTs over 6 hrs. For the 3 hrs HRT the removal efficiency was still acceptable, but an additional polishing treatment would be necessary to achieve effluent discharge standards, also for the lowest concentration the non-degradable COD masked the true removal efficiency of the reactor. The ABR reactor would not likely be affective for the treatment of ultra-low ADF concentrations (range lower than 200 mg COD/L)

Initial pre-acclimation of biomass is effective for the treatment of this type of wastewater in order to reduce the time needed for total biomass acclimation and should be further investigated.

The different experimental conditions evaluated did not affect the settling characteristic of the biomass, which remained constant through the experiment.

The specific acetoclastic activity of the biomass changed throughout the reactor, which indicated a distribution of different microorganisms in each compartment of the reactor. This finding supports the idea that the configuration of this reactor can effectively separate acidogenesis and methanogenesis in a single reactor. This could have ramifications in terms of special treatment applications such as sulfate reduction or maybe denitrification. Biomass specific acetoclastic activity for the last three compartments improved almost two-fold. Compartments two and three had

the highest specific acetoclastic activity. For compartment one the decrease in specific acetoclastic activity suggested the location of a more acidogenic culture.

Methane production was close to the theoretical value of 0.39 L CH_4/g COD removed at 35°C for the base-line conditions but decreased during the experimental runs. This may be attributed to a change in biomass yield over time, as compartmentalization of activity changed or the biomass became fully acclimated to the waste.

The low biomass yield was also in agreement with those reported for similar types of wastewater. Additionally, the low endogenous decay coefficient obtained is favorable for this type of seasonal wastewater.

The linear experimental model described the performance of the reactor more accurately compared to the 1st order model proposed by Xing *et al* (1991). It could be observed that the performance of the ABR on dilute ADF depended on HRT under the experimental conditions tested. Results also indicated that for the concentrations tested there was no interaction between the HRT and the initial feed concentration (see Appendix A).

CHAPTER 6

FUTURE STUDIES

- Investigate the formation of SMP produced for this type of wastewater on the ABR.
- Investigate biomass pre-acclimation with other substrate prior to ADF treatment in a continuous lab-scale reactor.
- Investigate the affect of adding a polishing step to the reactor in order to decrease the COD concentration in the effluent.
- Investigate a method to retain the biomass at short HRT in the last compartment
- Investigate if the effect of recycling at lower HRT could improve performance of the reactor.
- Investigate the performance of the reactor at lower temperatures for the treatment of ADF.
- Investigate the effect of adding or reducing the number of compartments on the ABR performance.
- Investigate the co-digestion of ADF and another type of wastewater (municipal) using the ABR.

REFERENCES

Ackman RG. (1972). Porous polymer bead packing and formic acid vapour in GLC of volatile fatty acids. *J. Chromatogr. Sci.* **10**:560–565.

Aguilar, A., Casas, C., and Lema, J.M. (1995). Degradation of volatile fatty acids by different enriched methanogenic cultures: kinetics and inhibition. *Wat. Res.* **29**(2), 505-509.

Ahn, Y-H., and Speece R.E. (2003). Settleability assessment protocol for anaerobic granular sludge and its application. *Water SA*, **29**(4), 419-426.

Andras, E., Kennedy, K.J., and Richardson, D.A. (1989). Test for characterization of anaerobic sludge. *Environ. Tech. Let.* **10,** 463-470.

Bachmann, A., Beard, V.L., and McCarty P.L. (1985). Performance characteristics of the anaerobic baffled reactor. *Wat. Res.* **19**(1), 99-106.

Barber, W.P. and Stuckey, D.C. (1999). The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. *Wat. Res.* **33**(7), 1559-1578.

Barker, D.J., Salvi, S.M.L., Langenhoff, A.A.M. and Stuckey, D.C. (2000). Soluble microbial products in ABR treating low-strength wastewater. *J. Environ. Eng.* **126**(3), 239-249.

Barker, D.J. and Stuckey, D.C. (1999). A review of soluble microbial products (SMP) in wastewater treatment systems. *Wat. Res.* **33**(14), 3063-3082.

Barriault, Michelle (2003). *Personal communication*, University of Ottawa, Canada

Battersby, N.S., and Wilson, V. (1989). Survey of anaerobic biodegradation potential of organic chemicals in digesting sludge. *Appl. Environ. Microbiol.* **55**(2), 433-439.

Boopathy, R. and Sieves, D.M. (1991). Performance of a modified anaerobic baffled reactor to treat swine waste. *Trans. ASAE.* **34**(6), 2573-2578.

Boopathy, R. and Tilche, A. (1991). Performance of a modified anaerobic baffled reactor to treat swine waste. *Trans. ASAE.* **34**(6), 2573-1578.

Bryant, M.P. (1979). Microbial methane production-theoretical aspects. *J. Anim. Sci.*, **48**, 193-201.

Cancilla, D.A.,Holtkamp, L.M. and Fang, X. (1997). Isolation and characterization of microtox-active components from aircraft deicing/antiacing fluid additives in a perched water monitoring well at an international airport. *Environ. Sci. Tech.* **32**, 3834-3835.

Chynoweth, D.P. and Isaacson R. (1987). Anaerobic Digestion of Biomass, Elsevier Applied Science, New York, NY.

Cohen, A., Breure, A.M., van Andel, J.G. and van Deursen, A. (1982). Influence of phase separation on the anaerobic digestion of glucose. II. Stability and kinetic responses to shock loadings. *Wat. Res.* **16**, 449-445.

Cornell, J.S., Pilliard, D. A., and Hernandez, M.T. (2000). Comparative measures of the toxicity of components chemicals in aircraft deicing fluid. *Environ. Toxicol. Chem.* **19**(6). 1465-1472.

Cox, D.P. (1978). The biodegradation of polyethylene glycols. *Adv. Appl. Microbiol.* **23,** 173-194.

Cox, D.P. (1996). Masculinity at risk, pesticides and male fertility. *J. Pestic. Reform* **16**, 2-7.

Damas, P., Bell, J., Foxon, K.M., Broukeart, C.J. (2001). Pilot-scale study of an anaerobic baffled reactor for the treatment of domestic wastewater. Proceedings of the IWA Conference on Water and Wastewater Management for Developing Countries, Putra World Trade Centre, Kuala Lumpur.

Darlington, C. and Kennedy, K.J. (1998). Biodegradation of aircraft deicing fluid in an upflow anaerobic sludge blanket (UASB) reactor. *J. Environ. Sci. Health* **A33**(3), 339-351.

Dwyer, D.F. and. Tiedje, J.M (1983). Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia. *Appl. Environ. Microbiol.* **46**, 185-190.

Environmental Canada (1994). Glycol guidelines, p. 840-842. *In* Canada Gazette Part I. Environmental Canada, Evaluation and Interpretation Branch, Ottawa.

Environmental Canada (2000). Canadian Environmental Protection Act –Priority Substances List- Supporting document for the environmental assessment of ethylene glycol. Commercial Chemicals Evaluation Branch, Environmental Canada, Hull, Quebec.

Evans, W.H. and David, E.J. (1974). Biodegradation of mono-, di-, and triethylene glycols in river waters under controlled laboratory conditions. *Wat. Res.* **8**, 97-100.

Fannin, K.F. Srivastra, V.J., Conrad, J.R., and Chynoweth, D.P. (1981). Marine biomass program: anaerobic digester system development. Annual Report for General Electric Company, IGT Project 20502. Institute of Gas Technology, IIT Centre, 3424 S. State Street, Chicago, IL 60616.

Gavala, H.N., and Lyberatos, G. (2001). Influence of anaerobic culture acclimation on the degradation kinetics of various substrates. *Biotechnol. Bioeng* **74**(3), 181-195.
Gonzalez, C.F.,Taber, W.A., and Zeitoun, M.A. (1972). Biodegradation of ethylene glycolby a salt-requiring bacterium. *Appl. Microbiol.* **24**, 911-919.

Grobicki, A. and Stuckey, D.C. (1991). Performance of the anaerobic baffled reactor under steady-state and shock loading conditions. *Biotechnol. Bioeng* **37**, 344-355.

Grobicki, A. and Stuckey, D.C. (1992). Hydrodynamic characteristics of the anaerobic baffled reactor. *Wat. Res.* **26**(3), 371-378.

Gruden, C.L., Sarahann, M.D., and Hernandez, M.T. (2001). Fate and toxicity of aircraft deicing fluid additives through anaerobic digestion. *Water Environ. Res.* **73**, 72-79.

Haines, J.R. and Alexander, M. (1975). Microbial degradation of polyethylene glycols. *Appl. Microbiol.* **29**, 621-625.

Jank, B.E., Guo, H.M., and Cairns, V.W. (1973). Biological treatment of airport wastewater containing aircraft deicing fluids. *Wastewater Technology Center, Environmental Protection Service.* Report No. EPS 4-WP-73-5.

Jank, B.E., Guo H.M. and Cairns V.W. (1974). Activated sludge treatment of airport wastewater containing aircraft deicing fluids. *Water Res.* **8**, 875-880.

Jewell, W.T. (1987). Anaerobic sewage treatment. *Environ. Sci. Technol.* **21**(1), 14-21.

Kaplan, D.L., Walsh, J.T. and Kaplan, A.M. (1982) Gas chromatographic analysis of glycols to determine biodegradability. *Environ. Sci. Technol.* **16**, 723-725.

Kato, M.T., Field, J.A., and Lettinga, G. (1997). The anaerobic treatment of low strength wastewaters in UASB and EGSB reactors. *Wat. Sci. Tech.* **36**(6-7), 373-382.

Kilroy, A.C. and Grey, N.F. (1992). Pilot plant investigation of the treatability of ethylene glycol by activated sludge. *Environ. Tech.* **13**, 293-300.

Langenhoff, A.A.M., and Stuckey, D.C. (2000). Treatment of dilute wastewater using an anaerobic baffled reactor: effect of low temperature. *Wat. Res.* **34**(15), 3867-3875.

Langenhoff, A.A.M., Intrachandra, N., and Stuckey, D.C. (2000). Treatment of dilute soluble and colloidal wastewater using an anaerobic baffled reactor: influence of hydraulic retention time. *Wat. Res.* **34**(4), 1307-1317.

Levenspiel O. (1999). *Chemical Reaction Engineering*, 3rd Edition, John Wiley & Sons, New York.

Lorion, R. (2001). Constructed wetlands: passive systems for wastewater treatment. Available from: http//clu-in.org/download/constructed_wetlands.pdf

Macarie, H. (2000). Overview of the application of anaerobic treatment to chemical and petrochemical wastewaters. *Wat. Sci. Tech.* **42**, 201-214.

McCarty, P.L., and Smith, D.P. (1986). Anaerobic wastewater treatment. *Environ. Sci. Tech.*, **20**, 1200-1206.

MacDonald, D.D., Cuthbert, I.D., Outridge, P.M., Ruthman, R.T., and Walker, S.L. (1993). Canadian water quality guidelines for the protection of aquatic life for ethylene glycol, diethylene glycol, and propylene glycol. *Scientific Series Report*. Environmental Canada.

Manariotis, D.I. and Grigoropoulos, S. G. (2002). Low-strength wastewater treatment using an anaerobic baffled reactor. *Wat. Env. Res.* **74**(2), 170-176.

Miller, L.M. (1979). Investigation of selected potential environmental contaminants: ethylene glycols, propylene glycols, and butylenes glycols. USEPA Report #560/79-006, Office of Toxic Substances, Washington, DC.

Mudd, L.E. (1993). Design of aircraft deicing facilities. Advisory Circular No. 150/5300-14. Available from: http://www.faa.gov/arp/pdf/5300-14.pdf

Mulligan, C., Chebib, J., and Safi, B. (1997). Anaerobic treatment of aircraft deicing fluid agent using the SNC-Lavalin multiple reactor. SNC Research Corp.'s presentation at the AWMA's 90th Annual Meeting & Exhibition, June 8-13, Toronto, Ontario, Canada.

Nachaiyasit, S. and Stuckey, D.C. (1995). Microbial response to environmental changes in an anerobic baffled reactor (ABR). *Antonie van Leeewenhoek* **67** 111-123.

Nachaiyasit S. and Stuckey D.C (1997a). The effect of shock loads on the performance of an anaerobic baffled reactor (ABR). 1. Step changes in feed concentration at constant retention time. *Wat. Res.* **31**(11), 2737-2746.

Nachaiyasit, S. and Stuckey, D.C (1997b). The effect of shock loads on the performance of an anaerobic baffled reactor (ABR). 2.Step and transient hydraulic shocks at constant feed strength. *Wat. Res.* **31**(11), 2747-2754.

Nitschke, L., Wagner, H., Metzner, G., Wilk, A., and Huber, L. (1996). Biological treatment of wastewaters containing glycols from deicing agents. *Wat. Res.* **30**, 644-648.

Novak, L.J., Holtze, K., Kent, R.A., Jefferson, C., and Anderson, D. (2000). Acute toxicity of storm water associated with de-icing/anti-icing activities at Canadian airports. *Environ. Toxicol. Chem.* **19**, 1846-1855.

Montgomery, D.C. (2001). *Design and Analysis of Experiments.* 5th Edition, John Willey &Sons, Arizona.

Orozco, A. (1988). Anaerobic wastewater treatment using an open plug flow baffled reactor at low temperature. 5th International Symposium on Anaerobic Digestion Bologna, Italy, pp. 759-762.

Pasternak, G. (1993). Ethylene/Propylene glycol toxicity. TDSR Case Studies in Environmental Medicine. US Department of Health and Human Resources.

Pham, T.T. (2002). Anaerobic degradation of aircraft deicing fluid (ADF) in upflow anaerobic sludge blanket (UASB) reactors and the fate of ADF additives. Ph.D. Dissertation, Department of Chemical Engineering, University of Ottawa, Ottawa, ON. Canada.

Pilliard, D.A. (1995). Comparative toxicity of formulated glycol deicers and pure ethylene and propylene glycol to *Ceriodaphnia dubia* and *Pimephales promelas. Environ. Toxicol. Chem.* **14**, 311-315.

Safferman, S.I. Siruvalure, G.S., and Foppe, L.E. (1998). Deicing fluid treatment in batch-loaded aerobic aerobic fluidized bed reactor. *J. Environ. Eng.* **124**, 11-15.

Sabeh, Y. and Narasiah, K.S. (1992). Degradation rate of aircraft deicing fluid in a sequential biological reactor. Wat. Sci. Tech. 26, 2061-2064.

Schoenberg, T., Veltman, S., and Switzenbaum, M.S. (2001). Kinetics of anaerobic degradation of glycol-based Type I aircraft deicing fluids. *Biodegradation* **12**, 59-68.

Simpson, A. (1997). Aircraft deicing best management plans. Transport Canada's presentation at the AWMA's 90th Annual Meeting & Exhibition, June 8-13, Toronto, Ontario, Canada.

Speece, R.E. (1996). *Anaerobic technology for industrial wastewaters.* Archea Press, Tennesse.

Standard Methods (1998). *Standard Methods for the Examination of Water and Waste Water*, 20th ed., APHA, AWWA and WEF, Washington, D.C.

Staples, C. (1996). Assessment technologies. Report to the ethylene glycol panel of the chemical manufacturers association. Chemical Manufacturers Association.

Switzenbaum, M.S. (1978). *The anaerobic attached film expanded bed reactor for the treatment of dilute organic wastes*. Ph. D. Thesis, Cornell University, USA.

Switzenbaum, M.S. and Jewell, W.J. (1980). Anaerobic attached-film expanded-bed reactor treatment. *J. WPCF.*, **52**(7), 1953-1965.

Switzenbaum, M.S., Veltman S., Mericas D., Wagoner B., and Schoenberg T. (2001). Best management practices for airport deicing stormwater. *Chemosphere* **43**, 1051-1062.

Tay, J-H., He, Y-X., and Yan, Y-G. (2001). Improved anaerobic degradation of phenol with supplemental glucose. *J. Environ. Eng.* **127**(1), 38-45.

Tilche, A. and Yang, X. (1987). Light and scanning electron microscope observations on the granular biomass of experimental SBAF and HABR reactors. Proceedings of Gasmat Workshop, Netherlands. 170-178.

Transport Canada (1994). The clean aircraft concept. Airworthiness Notice No. B017.

Transport Canada (1997b). When in doubt... small and large aircraft. Aircraft critical surface contamination training. 4th ed. (TP 10643E).

Transport Canada (1998a). Glycol monitoring program. Annual Report 1997/98. Environmental Affairs (TP 12576).

USEPA (1994). Addendum to municipal wastewater management fact sheets: storm water best management practices. EPA-832-F-93-013, United States Environmental Protection Agency Office of Water, Washington, DC.

USEPA (1999). Storm water technology fact sheet. Airplane deicing fluid recovery systems. EPA-832-F-99-043, United States Environmental Protection Agency Office of Water, Washington, DC.

Uyanik, S. (2003). A novel anaerobic reactor: split fed anaerobic baffled reactor (SFABR). *Turkish J. Eng. Env. Sci.*, **27**, 339-345.

Uyanik, S., Sallis, P.J. and Anderson, G.K. (2002). The effect of polymer addition on granulation in an anaerobic baffled reactor (ABR). Part I: process performance. *Wat. Res.* **36**, 933-943.

Uyanik S., Sallis, P.J. and Anderson, G.K. (2002). The effect of polymer addition on granulation in an anaerobic baffled reactor (ABR). Part II: compartmentalization of bacterial populations. *Wat. Res.* **36**, 944-955.

van Haandel, A.C. and Lettinga, G. (1994). Anaerobic sewage treatment: A practical guide for regions with a hot climate, John Wiley and Sons, New York. NY.

van Huyssteen, J.J.(1967). Gas chromatographic separation of anaerobic digester gases using porous polymer. *Wat. Res.* **1**, 237-242.

Veltman, S., Schoenberg, T. and Switzenbaum, M.S. (1998). Alcohol and acid formation during the anaerobic decomposition of propylene glycol under methanogenic conditions. *Biodegradation* **9**, 113-118.

Weiland, P. and Rozzi, A. (1991). The start-up, operation and monitoring of high-rate anaerobic treatment systems: discussers report. *Wat. Sci. Technol.* **24**(8), 257-277.

Williams, J.B. (1998). The disposal of ethylene glycol in wastewater treatment plants. Presented at the Water Environmental Federation of Industrial Wastes Technical Conferences. Nashville, TN, USA.

Witthauer, D. and Stuckey, D.C. (1982). Laboratory studies on anaerobic processes to treat dilute organic wastes in developing countries. Study by IRCWDEAWAG Dunbendorf, Switzerland.

Xing, J., Boopathy R., and Tilche A. (1991). Model evaluation of hybrid anaerobic baffled reactor treating molasses wastewater. *Biomass and Bioeng.* **1**(5), 267-274.

Zitomer, D.H. and Tonuk, G.U. (2003). Propylene glycol deicer biodegradation kinetics: anaerobic complete-mix stirred tank reactors, filter, and fluidized bed. *J. Environ. Eng.* **129**(2), 123-129.

Appendix A Tukey's test for Interaction

During experimental runs, data were collected at steady state, five different samples, however there were no replicates. In many experiments this is a general case, and the absence of replicates is justified under certain conditions. In the case of this experiment, the volume of the reactor was high, so to have true replicates both reactors had to be run at the same time under the same conditions this was however over the time-period for this experiment, due to the nature of anaerobic process randomization is not possible because the run conditions depend on the former conditions the reactor was exposed. On the other hand having four reactors would have been not permissible due to space constraints, work load and expenses associated with the cost of the salts used to prepare the wastewater.

The analysis applied in the case was the two factor with one observation per cell (Montgomery, 2001) then the effects model is described as

$$Y = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk} \quad \{i=1,2,\ldots a.\ j=1,2,\ldots,b.\ k=1,2,\ldots n\} \qquad (A.20)$$

Where

Y= response
μ= overall meann effect
τ_i= effect of the ith level of the row factor A
β_j= effect of the jth level of column factor B
$(\tau\beta)_{ij}$= effect of the interaction between τ_i and β_j
ε_{ijk}= random error component.

Since the error variance can not be estimated the interaction con not be separated, as a consequence there are not test on main effects unless the interaction effect is zero. If the interaction is zero a plausible model is

$$Y = \mu + \tau_i + \beta_j + \varepsilon_{ijk} \quad \{i=1,2,\ldots a.\ j=1,2,\ldots,b.\ k=1,2,\ldots n\} \qquad (A.21)$$

Using the test developed by Tukey the presence of interaction can be detected, and a more accurate model can be given. In case of no interaction the linear model can be obtained by equation (21) and in case of interaction there are other remedial solutions.

In order to determine the interaction for this experiment the following table was constructed based on the measured results.

Table A-1 ANOVA for No Interaction Determination.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Fo	Pr>F
Concentration	33.35	3	16.67	2.133	0.0448
HRT	1362.05	2	452.01	58.07	<0.0001
Non-additivity	0.5541	1	0.5541	0.07	
Error	39.09	5	7.81		
Total	1413.77	11			

The Tukey's test for interaction with 95% confidence interval is $F_{0.5,1,5}$ is 6.61, since Fo<F, it can be concluded that there is no evidence of interaction in these data and from the main effects only HRT is significant.

Appendix B Biomass Granule Size Distribution

During settling tests, biomass separation was based on size and density. At lower up-flow velocities small granules and fines were obtained, while larger granules were obtained at higher up-flow velocities. At the end of each experimental run or steady state granular biomass was taken from the lower-portion of each compartment and its settlability was tested. Figure B-1 shows a picture of the different fractions obtained during the test, in this case the pictures shown are from compartment 2, run number 7 ,(750 mg COD/L as influent concentration and 6 hrs HRT). The same trend was observed for all the compartments, the size distribution did not change considerably from the one presented in the picture or from the granular biomass, the granular biomass used as seed had a bigger fraction of small granules and fines ($D \leq 1mm$) than the samples taken after the runs, this was attributed to the storage conditions.

Table B-1 shows the biomass average size distribution during the different conditions of up-flow velocity. Since the settling characteristics did not change during the experimental conditions these values can be used as a generalization for the different compartments of the ABR, during ADF treatment.

Table B-1 Size Distribution of Granular Biomass Under the Different Up-flow Velocities.

Fraction	Upflow velocity (m/h)	Average Diameter (mm)
1	8.6	D≤1.0
2	17.7	0.5≤D≤1.5
3	27.2	1.0≤D≤2.5
4	37.9	2.0≤D≤3.5
5	47.7	2.5≤D≤4.5
6	56.2	D≥4.5

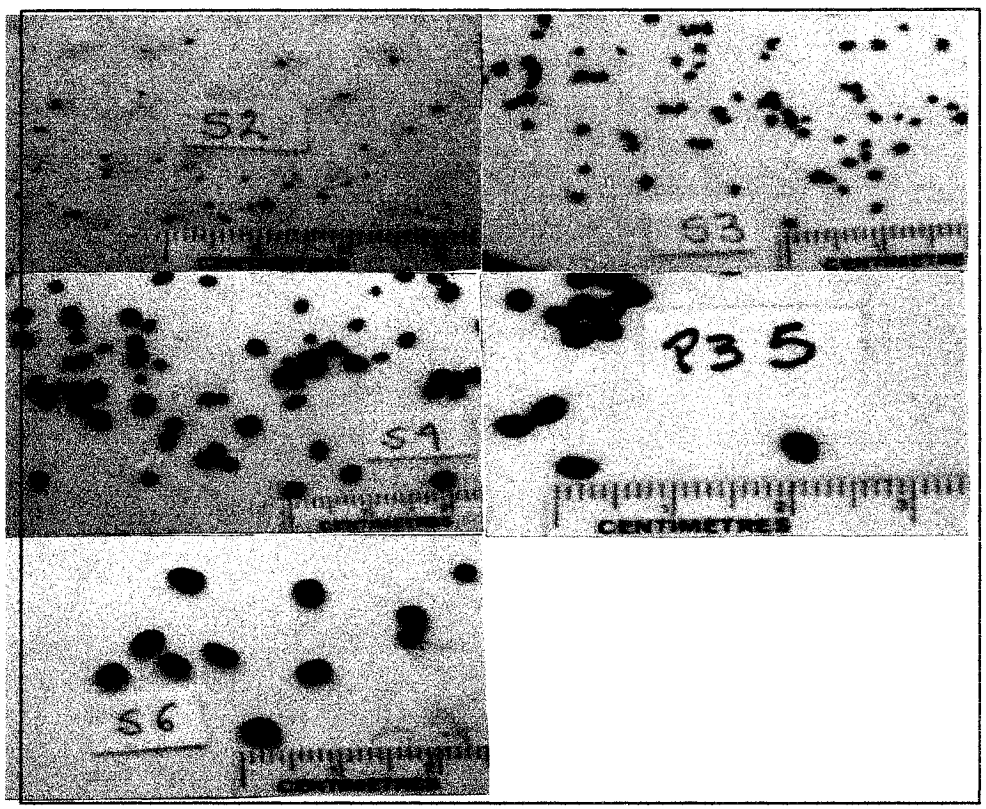

Figure B-1 Photograph of Different Fractions of Granular Biomass Sample Taken From Reactor 2, Compartment 3 After end of Run 10. S, Represents the Fraction Number.

Appendix C Statistic Analysis of Results

This Appendix contains the statistical analysis of the different steady-states, during which five differents measurements were taken for $COD_{effluent}$, biogas produced, effluent VSS.

Table C-1 Steady-State Data for Base-line Run. Reactor 1.

BASELINE CONDITIONS					
R1					
S-S	COD in	CODout	%removal	EffluentVSS	Gas Production
	mg/L	mg/L	%	mg/L	(L/d)
1	750	52.00	93.07	18.30	9.00
2	750	38.50	94.87	12.00	7.80
3	750	32.00	95.73	15.20	8.00
4	750	41.00	94.53	16.40	9.20
5	750	39.00	94.80	13.60	7.50
Mean	750	40.50	94.60	15.10	8.30
stddev	0.00	7.26	0.97	2.44	0.75
N	5.00	5.00	5.00	5.00	5.00
stderror	0.00	3.25	0.43	1.09	0.34
conf level	0.95	0.95	0.95	0.95	0.95
T	2.78	2.78	2.78	2.78	2.78
conf limit	0.00	9.02	1.20	3.03	0.94

Table C-2 Steady-State Data for Base-line Run. Reactor 2.

R2					
S-S	COD in	CODout	%removal	EffluentVSS	Gas Production
	mg/L	mg/L	%	mg/L	(L/d)
1	750	53.00	92.93	13.90	8.20
2	750	62.00	91.73	10.00	6.80
3	750	38.50	94.87	15.10	6.00
4	750	48.00	93.60	11.00	7.70
5	750	61.00	91.87	14.00	6.30
Mean	750	52.50	93.00	12.80	7.00
stddev	0	9.73	1.30	2.18	0.93
N	5	5.00	5.00	5.00	5.00
stderror	0	4.35	0.58	0.98	0.42
conf level	95%	0.95	0.95	0.95	0.95
T	2.78	2.78	2.78	2.78	2.78
conf limit	0	12.09	1.61	2.71	1.15

Table C-3 Settling Characteristics for Granular Biomass Used as Inoculum.

SETTLING VELOCITY V_{50}					
				mean	S.D.
			27.8	27.6	0.28
INOCULUM		V_{50}(m/h)	27.4		

Table C-4 v_{50} Determination at the End of Each Steady-State.

STEADY-STATE CONDITIONS						
Run	COD inf	HRT	Compartment1	Compartment 2	Compartment 3	Compartment 4
	(mgcod/l)	hrs	V50 (m/h)	V50 (m/h)	V50 (m/h)	V50 (m/h)
1	750	24	27.4	28	27.6	27.6
1	750	24	25.8	23.6	26.6	26.2
2	500	24	27	26.3	26.4	28
2	500	24	25.6	24.1	28.2	24
3	300	24	28.1	27	26.5	26.9
3	300	24	18.3	24.4	23.7	26.5
4	750	12	26.2	27.8	28	26.5
4	750	12	25.4	32.6	26.8	26.3
5	500	12	26	26.8	25	27.1
5	500	12	26	25.9	28.6	29.7
6	300	12	28.7	28	27	27
6	300	12	32.1	34.8	24.8	28
7	750	6	25.9	25.2	23	27.9
7	750	6	22.7	22.8	28.6	28.3
8	500	6	27.6	26	22	24
8	500	6	20.8	22.2	24.4	28.6
9	300	6	25	27.3	25.8	28.5
9	300	6	21	27.9	27.6	31.1
10	750	3	24	26	24.1	23.1
10	750	3	23.6	26	26.3	29.7
11	500	3	26.1	25.2	26.8	27.3
11	500	3	15.9	22	24	25.3
12	300	3	22	27.6	27	28
12	300	3	16.4	20.6	24.6	28.2

Table C-5 Average v_{50} Determined at the End of Each Steady-State for Each compartment.

	C1	C2	C3	C4
	V50 (m/h)	V50 (m/h)	V50 (m/h)	V50 (m/h)
Run	Mean	Mean	Mean	Mean
1	26.6	25.8	27.1	26.9
2	26.3	25.2	27.3	26
3	23.2	25.7	25.1	26.7
4	25.8	30.2	27.4	26.4
5	26	26.35	26.8	28.4
6	30.4	31.4	25.9	27.5
7	24.3	24	25.8	28.1
8	24.2	24.1	23.2	26.3
9	23	27.6	26.7	29.8
10	23.8	26	25.2	26.4
11	21	23.6	25.4	26.3
12	19.2	24.1	25.8	28.1

Table C-6 Steady-State Data for Run Number 1.

RUN	750 mg COD/L & 24 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	750	40.00	94.67	15.70	8.00
2	750	67.00	91.07	12.80	7.80
3	750	72.00	90.40	13.40	6.50
4	750	59.60	92.05	12.40	7.40
5	750	66.96	91.07	14.40	6.80
Mean	750	61.11	91.85	13.74	7.30
stddev	0	12.60	1.68	1.33	0.64
n	5	5.00	5.00	5.00	5.00
stderror	0	5.64	0.75	0.59	0.29
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	15.65	2.09	1.65	0.80

Table C-7 Steady-State Data for Run Number 2.

RUN	500 mg COD/L & 24 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	500	32.00	93.60	19.69	5.90
2	500	40.00	92.00	21.80	6.30
3	500	32.00	93.60	19.80	7.00
4	500	32.00	93.60	20.82	8.00
5	500	32.00	93.60	20.75	6.39
Mean	500	33.60	93.28	20.57	6.72
stddev	0	3.58	0.72	0.86	0.82
n	5	5.00	5.00	5.00	5.00
stderror	0	1.60	0.32	0.39	0.37
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	4.44	0.89	1.07	1.02

Table C-8 Steady-State Data for Run Number 3.

RUN	300 mg COD/L & 24 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	300	32.00	89.33	16.20	2.57
2	302	38.00	87.42	15.00	2.43
3	300	36.00	88.00	16.00	2.10
4	297	37.00	87.54	14.90	2.21
5	310	41.00	86.77	13.90	2.32
Mean	302	36.80	87.81	15.20	2.33
stddev	4.92	3.27	0.96	0.93	0.18
n	5.00	5.00	5.00	5.00	5.00
stderror	2.20	1.46	0.43	0.42	0.08
conf level	0.95	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	6.11	4.06	1.19	1.15	0.23

Table C-9 Steady-Satate Data for Run Number 4.

RUN	750 mg COD/L & 12 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	750	69.00	90.80	53.00	15.00
2	750	72.00	90.40	58.30	13.70
3	750	84.00	88.80	60.20	14.00
4	750	62.00	91.73	34.00	14.80
5	750	72.00	90.40	49.50	13.50
Mean	750	71.80	90.43	51.00	14.20
stddev	0	7.95	1.06	10.41	0.67
n	5	5.00	5.00	5.00	5.00
stderror	0	3.56	0.47	4.65	0.30
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	9.87	1.32	12.92	0.83

Table C-10 Steady-State Data for Run Number 5.

RUN	500 mg COD/L & 12 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	500	40.00	92.00	56.00	12.50
2	500	40.00	92.00	38.00	9.00
3	500	28.00	94.40	36.00	10.90
4	500	24.00	95.20	42.00	11.60
5	500	48.00	90.40	34.00	12.10
Mean	500	36.00	92.80	41.20	11.22
stddev	0	9.80	1.96	8.79	1.38
n	5	5.00	5.00	5.00	5.00
stderror	0	4.38	0.88	3.93	0.62
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	12.17	2.43	10.91	1.71

Table C-11 Steady-State Data for Run Number 6.

RUN	300 mg COD/L & 12 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	300	24.50	91.83	27.00	3.90
2	300	29.00	90.33	24.80	3.60
3	300	43.00	85.67	32.00	4.70
4	300	44.50	85.17	25.00	4.20
5	300	24.00	92.00	23.70	4.60
Mean	300	33.00	89.00	26.50	4.20
stddev	0	10.02	3.34	3.30	0.46
n	5	5.00	5.00	5.00	5.00
stderror	0	4.48	1.49	1.47	0.21
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	12.44	4.15	4.09	0.58

Table C-12 Steady-State Data for Run Number 7.

RUN	750 mg COD/L & 6 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	750	176.00	76.53	72.00	14.60
2	750	169.00	77.47	59.00	16.30
3	750	168.00	77.60	63.60	13.80
4	750	176.00	76.53	58.60	12.00
5	750	171.00	77.20	66.80	13.30
Mean	750	172.00	77.07	64.00	14.00
stddev	0	3.81	0.51	5.62	1.60
n	5	5.00	5.00	5.00	5.00
stderror	0	1.70	0.23	2.51	0.71
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	4.73	0.63	6.97	1.98

Table C-13 Steady-State Data for Run Number 8.

RUN	500 mg COD/L & 6 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	500	96.00	80.80	74.80	29.00
2	500	152.00	69.60	69.00	23.70
3	500	80.00	84.00	56.50	24.00
4	500	160.00	68.00	63.00	26.80
5	500	152.00	69.60	64.20	30.50
Mean	500	128.00	74.40	65.50	26.80
stddev	0	37.09	7.42	6.85	3.00
n	5	5.00	5.00	5.00	5.00
stderror	0	16.59	3.32	3.06	1.34
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	46.06	9.21	8.51	3.72

Table C-14 Steady-State Data for Run Number 9.

RUN	300 mg COD/L & 6 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	300	112.00	62.67	42.00	8.40
2	300	34.00	88.67	38.00	6.00
3	300	72.00	76.00	40.60	7.80
4	300	80.00	73.33	28.90	9.00
5	300	72.00	76.00	30.50	5.30
Mean	300	74.00	75.33	36.00	7.30
stddev	0	27.78	9.26	5.95	1.58
n	5	5.00	5.00	5.00	5.00
stderror	0	12.43	4.14	2.66	0.71
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	34.50	11.50	7.39	1.97

Table C-15 Steady-State Data for Run Number 10.

RUN	750 mg COD/L & 3 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	750	238.00	68.27	190.00	40.00
2	750	240.00	68.00	156.80	32.80
3	750	223.00	70.27	164.00	36.50
4	750	256.00	65.87	180.60	39.00
5	750	238.00	68.27	161.10	32.10
Mean	750	239.00	68.13	170.50	36.08
stddev	0	11.70	1.56	14.15	3.56
n	5	5.00	5.00	5.00	5.00
stderror	0	5.23	0.70	6.33	1.59
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	14.53	1.94	17.57	4.42

Tbale C-16 Steady-State Data for Run Number 11.

RUN	500 mg COD/L & 3 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	500	168.00	66.40	162.00	27.00
2	500	144.00	71.20	145.00	24.10
3	500	184.00	63.20	170.40	29.00
4	500	168.00	66.40	138.00	26.50
5	500	166.00	66.80	144.60	24.80
Mean	500	166.00	66.80	152.00	26.28
stddev	0	14.28	2.86	13.59	1.93
n	5	5.00	5.00	5.00	5.00
stderror	0	6.39	1.28	6.08	0.86
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	17.73	3.55	16.87	2.40

Table C-17 Steady-State Data for Run Number 12.

RUN	300 mg COD/L & 3 h HRT				
S_S	COD in	CODout	%removal	EffluentVSS	gas production
	mg/L	mg/L	%	mg/L	(L/d)
1	300	112.00	62.67	126.00	8.90
2	300	112.00	62.67	110.00	11.90
3	300	104.00	65.33	119.50	9.40
4	300	117.00	61.00	109.80	11.30
5	300	137.00	54.33	104.70	10.40
Mean	300	116.40	61.20	114.00	10.38
stddev	0	12.42	4.14	8.58	1.26
n	5	5.00	5.00	5.00	5.00
stderror	0	5.56	1.85	3.84	0.56
conf level	95%	0.95	0.95	0.95	0.95
t	2.78	2.78	2.78	2.78	2.78
conf limit	0	15.42	5.14	10.65	1.56

Table C-18 Verification Run Number 1

VERIFICATION RUNS			
RUN 1	750 mg CODL & 24 h HRT		
S-S	COD in	CODout	%removal
	mg/L	mg/L	%
1	750	64.60	91.39
2	750	48.00	93.60
3	750	39.10	94.79
4	750	54.00	92.80
5	750	45.50	93.93
Mean	750	50.24	93.30
stddev	0	9.64	1.29
n	5	5.00	5.00
stderror	0	4.31	0.57
conf level	95%	0.95	0.95
t	2.78	2.78	2.78
conf limit	0	11.97	1.60

Table C-19 Verification Run Number 2.

RUN 2	500 mg COD/L & 12 h HRT		
S-S	COD in	CODout	%removal
	mg/L	mg/L	%
1	500	49.00	90.20
2	500	38.70	92.26
3	500	36.50	92.70
4	500	42.00	91.60
5	500	38.80	92.24
Mean	500	41.00	91.80
stddev	0	4.88	0.98
n	5	5.00	5.00
stderror	0	2.18	0.44
conf level	95%	0.95	0.95
t	2.78	2.78	2.78
conf limit	0	6.06	1.21

Table C-20 Verification Run Number 3.

RUN 3	300 mg COD/L & 24 h HRT		
S-S	COD in	CODout	%removal
	mg/L	mg/L	%
1	300	56.00	81.33
2	300	49.80	83.40
3	300	61.00	79.67
4	300	52.40	82.53
5	300	46.30	84.57
Mean	300	53.10	82.30
stddev	0	5.67	1.89
n	5	5.00	5.00
stderror	0	2.53	0.84
conf level	95%	0.95	0.95
t	2.78	2.78	2.78
conf limit	0	7.04	2.35