EXCESS SLUDGE REDUCTION DURING ACTIVATED SLUDGE MUNICIPAL WASTEWATER TREATMENT BY INTEGRATING AN ANOXIC HOLDING TANK AND POST-ULTRASOUND TREATMENT TO ENHANCED BIOMASS MAINTENANCE METABOLISM

by

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Thesis Submitted to the
Faculty of Graduate and Postdoctoral Studies

Under the supervision of

Prof. Kevin J. Kennedy

In partial fulfillment of the requirements
For the PhD degree in Environmental Engineering

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Abstract

The exchange to the aeration basin of activated sludge biomass previously exposed to a controlled anoxic environment (HT) followed by low intensity ultrasound post-treatment (USPT) enhanced excess sludge minimization during aerobic biological treatment of municipal wastewater. HT biomass sonicated at low ES inputs (< 56KJ/gTS) decreased floc size by 41% and enhanced its metabolic activity by 50-250% compared to control. ES inputs >118 KJ/gTS caused HT biomass solubilization and irreversible loss of its metabolic activity and reflocculation ability. Surface response methodology and USPT optimization indicated that HT biomass activity was only enhanced at ES inputs <17 KJ/gTS. During continuous activated sludge processing (ASP) of real primary effluent the observed yield (Y_{obs}) decreased by 20% compared to control ASP at SF (stress factor) of 1 (biomass exchanged without USPT). At SF of 0.5, 1 and 1.5 (biomass exchanged with USPT) the Y_{obs} further decreased by 33, 25 and 44% respectively as compared to control. This indicated that combining biomass anoxic exposure with USPT enhanced sludge reduction by increasing microbial maintenance metabolism likely in combination with microbial flora shift in the ASP depending on SF. ASP effluent quality remained constant for SF of 0.5 and 1 (88 and 91% total chemical oxygen demand (tCOD) and total suspended solids (TSS) respectively) but decreased by 12 and 41% respectively at SF of 1.5. The ASP biomass activity and microbial stress (specific oxygen uptake rate (SOUR) and dehydrogenase activity DHA)) increased in average by 23% and 39% respectively under all SF conditions tested, suggesting that the experimental conditions induced metabolic stress on the ASP biomass with concomitantly lower Y_{obs}

Acknowledgements

I want to express my gratitude to Dr Kevin J. Kennedy for giving me the big opportunity of pursuing my graduate studies under his supervision. From the bottom of my hearth thank you very much Dr. Kennedy, for all the valuable lessons. I want also to express my infinite gratitude to Mr Francisco Aposaga for being such a professional and committed person to his work and a great friend to everybody. Thank you very much as well to Lise Rousseau, Yolande Hogan and Manon Racine for their always prompt and professional help. I want to thank all my colleagues: Zaman, Mashim, Gabriel, Cigdem, Anh, Jill, Liz, Mukesh (too many to write them all) for making of this little journey such great experience. May all of you grow older and prosper.

I want to say thank you very much to Dr. Roberto Narbaitz, Dr. Martin Ternan, Dr. Banu Ormeci, Dr. Chris Lan, and Mr Frederic Tremblay for being such positive role models and for their enthusiasm and commitment to teach and help.

I want to say thank you very much to my beautiful kids, to my big Juan Carlos Marin and to my little Ivan Augusto Marin, I simply adore you my guys. Thank you very much for being my motor, my inspiration and for making me so happy and proud with so little. Mil gracias to my mother Maria Luisa Hernandez and my sisters and brothers for all their prayers and unconditional love and support. And last but not least, I want to say merci beaucoup to my great friend Sylvain Perron, thank you very much for the 58 round trips to ROPEC (I swear one day I will buy you new tires). Thank you so much for lending me a hand when I really needed, people like you make of this world a nicer place. I humbly dedicate this work to you and to your priceless friendship.

Abbreviations

ASP	activated sludge process
ATP	adenosine triphosphate
BOD	biochemical oxygen demand
bCOD	biodegradable chemical oxygen demand
BW	bound water
CCD	central composite design
COD	chemical oxygen demand
COD:N:P	COD:nitrogen:phosphorus ratio
CST	capillarity suction time
CSTR	complete stirred tank reactor
CTC	5-cyano-2,3-ditolyltetrazolium chloride
DHA	dehydrogenase activity
DO	dissolved oxygen
ECD	equivalent circle diameter
ADHA	extracellular dehydrogenase activity
ES	specific energy
EXS	excess sludge
F/M	food to microorganism ratio
FTR	fill time ratio
HT	holding tank
HRT	hydraulic retention time
HSD	honest significance difference test

MBR	membrane bioreactor
MFI	micro-flow imaging
MLSS	mixed liquor suspended solid
MLTSS	mixed liquor total suspended solids
MLTVSS	mixed liquor total volatile solids
MOE	Ministry of Environment
MWW	municipal wastewater
nbCOD	non-biodegradable chemical oxygen demand
nbsCOD	soluble non-biodegradable chemical oxygen demand
OLR	organic loading rate
OMAFRA	Ontario Ministry of Agriculture, Food and Rural affairs
OMOE	Ontario Ministry of Environment
ORP	oxygen reduction potential
OSA	oxic-settling-anaerobic activated sludge process
PAOs	polyphosphate accumulating microorganisms
PE	population equivalent
pnbCOD	particulate non-biodegradable chemical oxygen demand
*p*NP	paranitrophenol
R	recycling ratio
RAS	return activated sludge
rbCOD	readily biodegradable chemical oxygen demand
ROPEC	Robert O. Pickard Environmental Center
RSM	response surface methodology

sbCOD	slowly biodegradable chemical oxygen demand
SBR	sequencing batch reactor
SF	stress factor
SOLR	specific organic loading rate
SOUR	specific oxygen uptake rate
SRT	solids retention time
SVI	sludge volume index
tCOD	total chemical oxygen demand
TCP	2,4,5-trichlorophenol
TCS	3,3’,4’,5-tetrachlorosalicylanilide
TDHA	total dehydrogenase activity
TDS	total dissolved solids
TKN	kjeldahl nitrogen
TOC	total organic carbon
TP	total phosphorous
TSS	total suspended solids
TS	total solids
TVS	total volatile solids
US	ultrasound
VER	volumetric exchange ratio
VIF	variance inflation factor
VSS	volatile suspended solids
WAS	waste activated sludge

WWTP	wastewater treatment plant
XTT	3’-{1-(phenylamino)-carbonyl]-3,4-tetrazolium} -bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate

Nomenclature

f_d	fraction of biomass that remains as cell debris, g VSS/m^3/d
k	first order reaction rate, 1/d
k_1	first order reaction rate, L/gVSS/d
k_{max}	maximum specific rate of substrate utilization, gCOD/gVSS/d
k_d	endogenous decay coefficient, 1/d
Q	flow rate, L/d
r_g	net biomass production rate, g VSS/m^3/d
r_{su}	substrate utilization rate, (units depends on the application of kinetic model)
$r_{X_T,VSS}$	total VSS production rate, g/m^3 /d
S	substrate concentration, mg/L
t_i	time for the *ith* phase, h
t_f	time for fill, h
t_c	total time of one cycle [$t_c = \Sigma t_i$], h
V	volume, L
X	biomass concentration, gVSS/L
$X_{o,i}$	influent nonbiodegradable VSS, g/m^3
Y	true growth yield coefficient, gVSS/g COD
Y_{bio}	net biomass yield, gVSS/g COD
Y_{obs}	observed growth yield, gVSS/g COD

ΔV_f	volume of wastewater fed to reactor, L
ΔV_w	sludge wasting volume, L
μ	specific growth rate, g VSS (new cells)/ g VSS /d

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Chapter 1

Introduction

The activated sludge process (ASP) is one of the most commonly used technologies for the biological treatment of municipal and industrial wastewaters all around the world. This process is very effective in the removal of soluble, colloidal and particulate organic substances which are biologically oxidized into carbon dioxide, water and cellular mass (excess sludge). The aerobic mode of metabolism is the most efficient in terms of energy recovered by the microbial biomass per unit of substrate processed. However, this results in a relatively large quantity of excess sludge (EXS) production. Although the ASP has high performance in terms of total chemical oxygen demand (tCOD) and suspended solids (TSS) removals its main drawback is the production of large amounts of EXS, which contains high fractions of water (>95% by weight) and volatile solids. Concomitantly large volumes of residual solids have to be managed and finally disposed of. Treatment, handling, and disposal of EXS can account for 25 to 65% of the total operational costs of a conventional activated sludge treatment plant (Perez-Elvira et al., 2006). According to Perez-Elvira et al. (2006) global implementation of new wastewater treatment regulations will result in a 40 to 60% increase in the number of wastewater treatment plants to be built in the next 10 years; between 1998 and 2005 in Europe this has already lead to an increase in EXS production of nearly 40%, which is now estimated at approximately 9.4 million tons of EXS (dry weight) every year. By the year 2010 it was expected that sludge production in the United States will exceed 10 million tons. In Canada in 2000, excess sludge production was approximately 388,700 tonnes of dry sludge per year (CH2MHill, 2000). Depending on the jurisdiction, management and ultimate disposal of EXS has traditionally been done by anaerobic digestion followed by land application, landfilling or incineration. However, these conventional methods are currently facing new challenges and roadblocks due to more stringent environmental regulations regarding air emission (greenhouse gases), land application and public acceptance. Hence, new approaches are needed in order to find effective and acceptable methods for the ultimate disposal of EXS or steps that can be taken to minimize EXS production within the biological activated sludge treatment process, thereby minimizing stress on ultimate disposal options. Based on current and future environmental regulations, the second option sounds more attractive; since this would not only reduce the costs associated with sludge handling and disposal; it would also allow for less mass going to land application or landfills and its associated deleterious effects on the environment.

During the last decade various methods have been studied and implemented in order to reduce EXS production within the ASP. Some methods rely on modifying the metabolic activity of the microorganisms responsible for waste degradation by withdrawal and disintegration of a portion of the EXS by mechanical, chemical or physical means and then returning it to the ASP aeration basin. The premise is that the treatment applied would dissolve a portion of the particulates into secondary substrates that are easier to assimilate once they are returned to the aeration tank, making their digestion easier thereby enhancing sludge reduction within the process. Various degrees of sludge disintegration have been achieved by using ozone (Ahn et al., 2002; Yusai and Shibata, 1994; Yusai et al., 1996; Kamiya et al., 1998) chemical treatments (Yamaguchi et al., 2006; Rocher et al., 2001), and mechanical disintegration (Muller, 2000; Tiehm et al., 1997; Zhang et al., 2007). However, as such approaches have a direct impact in the ASP aeration basin they can also have potential negative effects on the process performance. Therefore EXS minimization has to be evaluated taking into consideration that the ASP has to retain or improve its overall performance in terms of effluent quality and residual sludge characteristics. Another approach to minimizing EXS production relies on the use of higher forms of organisms or protozoan such as free-swimming or stocked ciliates, rotifers and flagellates to predate on bacteria in the ASP (Lee and Welander, 1996; Rensink and Rulkens, 1997). Although, high EXS reduction has been reported via this method (60-80%), such an approach demands high numbers of predatory organisms under constant culture conditions, which in reality are hard to achieve and maintain in the ASP. Yet another approach to minimizing EXS production relies on the fact that certain chemical substances induce uncoupling of the catabolic and anabolic biological paths, favouring the loss of metabolic energy as heat instead of using that energy for reproductive functions (Mayhew and Stephenson, 1998). Unfortunately, the compounds used as metabolic uncouplers present certain disadvantages, amongst them, once the bacteria experience acclimation to the uncoupler, the EXS reduction effect is reduced. Either larger doses of the uncoupler are required or a different type of uncoupler compound will be needed after a certain period of time. Additionally, some of the chemical uncouplers used (i.e. chlorophenol) are themselves toxic compounds and their fate or the fate of by-products of their metabolism in the receiving waters is unknown at this time.

It has also been reported that EXS production can be minimized by changing operational conditions in the ASP, such as using a low food to micro-organism (F/M) ratio, operating at long solid retention times or by increasing the oxygen concentration in the aeration basin (Sakai et al., 1992; Abbasi et al., 1999). Negligible sludge production has been reported when the sludge growth rate was close to the sludge decay rate which can be achieved under long solids retention time (Sakai et al., 1992). While this approach which is the basis of extended aeration processes and membrane reactors may be effective for minimizing excess sludge production it requires large reactor volumes and high energy consumption.

An alternate approach to minimizing sludge production was developed by Chudoba et al. (1992) who inserted an anaerobic holding tank in the sludge return line of a conventional ASP, which was referred to as the oxic-settling-anaerobic (OSA) activated sludge system. In this system, thickened EXS from the secondary clarifier was returned to the aeration tank with no chemical or mechanical treatment via an anaerobic holding tank. They observed that the biomass yield of this system was in the range of 0.13-0.29 g VSS/g COD which was significantly lower compared to the conventional ASP, which had a biomass yield of 0.28-0.47 g VSS/g COD. Chen et al. (2001) reported that besides the reduction in EXS, the OSA system also resulted in improved chemical oxygen demand (COD) removal and increased sludge settleability. In the OSA system, the anaerobic holding tank plays an important role in the sludge reduction process and according to Chudoba et al. (2001) the effectiveness of the anaerobic holding tank can be influenced by the sludge anaerobic exposure time, the oxygen reduction potential (ORP), and sludge concentration. To date two proprietary processes referred to as Cannibal™ and Biolysis E™ make use of this anaerobic/anoxic holding step and claim to reduce excess sludge production by almost 50%. In this process, biomass maintenance metabolism is enhanced as the aerobic biomass withdrawn from the secondary clarifier is converted to a dominantly facultative microbial population. By carefully controlling the anaerobic holding tank environment, the death and lysis of aerobic bacteria is achieved, this is believed to allow the low yield facultative bacteria to metabolize the soluble and particulate remains of the aerobes as well as any by products (Sheridan and Curtis, 2007). However, the mechanisms of sludge reduction in this type of systems are speculative at this time as most of the information available is proprietary.

The purpose of the present study is to assess the potential for EXS minimization in a modified continuous ASP which integrates an anoxic holding tank with post-sonication treatment prior to biomass recycling into the aeration basin and the performance of such modified ASP on effluent quality and residual sludge. Among the different types of disruption technologies, ultrasound has been already implemented and optimized at full scale wastewater treatment plants. Therefore most likely laboratory-scale results can be easily scaled-up to pilot and/or full-scale application. In addition, US treatment alone has been found to be effective in promoting excess sludge reduction (Ginestet, 2007). It is a premise of the present study that this novel approach for the aerobic biological treatment of municipal wastewater will produce a synergistic effect on the biomass metabolic requirements, as the biomass has to cope with the stressful environmental conditions imposed to the system (anoxic exposure time followed by sonication) leading to a certain degree of excess sludge reduction without compromising ASP performance in terms of effluent quality and residual sludge characteristics.

1.1 Research objectives

The present research has the following major hypothesis:

1 – It is hypothesized that aerobic excess sludge biomass exposed to an anoxic controlled environment followed by low intensity ultrasound treatment can synergistically result in enhancement of biomass maintenance metabolism mechanisms, which in turn can lead to minimize excess sludge reduction when the treated biomass is recycled to the aeration basin without having detrimental effects on the activated sludge process performance.

This research intends to contribute to the fundamental research knowledge of the wastewater treatment industry with the following specific objectives:

- Determine (based on the current literature) the application of a Cannibal-type activated sludge processes for excess sludge reduction and highlight the main contributions and gaps in the field.
- Determine the performance and sludge production of a laboratory scale sequencing batch reactor (SBR) for the treatment of municipal wastewater (primary effluent) to set base line conditions for further comparisons with the modified ASP process proposed in the present study.
- Determine the effects of low intensity ultrasound treatment on the holding tank biomass bioactivity, biomass solubilization and residual sludge characteristics as it relates to excess sludge reduction.
- Determine the impact of low intensity ultrasound treatment on biomass morphology and floc stability previously exposed to a controlled anoxic environment (the holding tank) as it relates to excess sludge reduction.
- Determine the effects of the exchange of non-sonicated biomass from the controlled anoxic holding tank on SBR performance in terms of effluent quality

(total suspended solids, total COD) and residual sludge characteristics (settleability, dewaterability) during continuous biological aerobic treatment of real municipal wastewater (primary effluent) as it relates to excess sludge reduction.

- Determine the effect of the exchange of different fractions of post-sonicated biomass from the controlled anoxic holding tank on SBR performance in terms of effluent quality (total suspended solids, total COD) and residual sludge characteristics (settleability, dewaterability) during continuous biological aerobic treatment of real municipal wastewater (primary effluent) as it relates to excess sludge reduction.
- Determine the extent of the proposed modified activated sludge process for excess sludge minimization during aerobic treatment of real municipal wastewater with concomitant minimal detrimental effects on effluent quality and residual sludge characteristics.

1.2 Thesis structure

The thesis is developed and presented in journal manuscript format. Figure 1.1 indicates how the different chapters in the thesis interrelate. Chapter 2 presents the main findings from a critical literature review on the current approaches for excess sludge reduction at the source during municipal wastewater treatment and on the effect of ultrasound treatment on excess sludge reduction during aerobic biological treatment of municipal wastewater. In addition, some important concepts on aerobic bacterial metabolism and its implications for municipal wastewater treatment and excess sludge production are also reviewed.

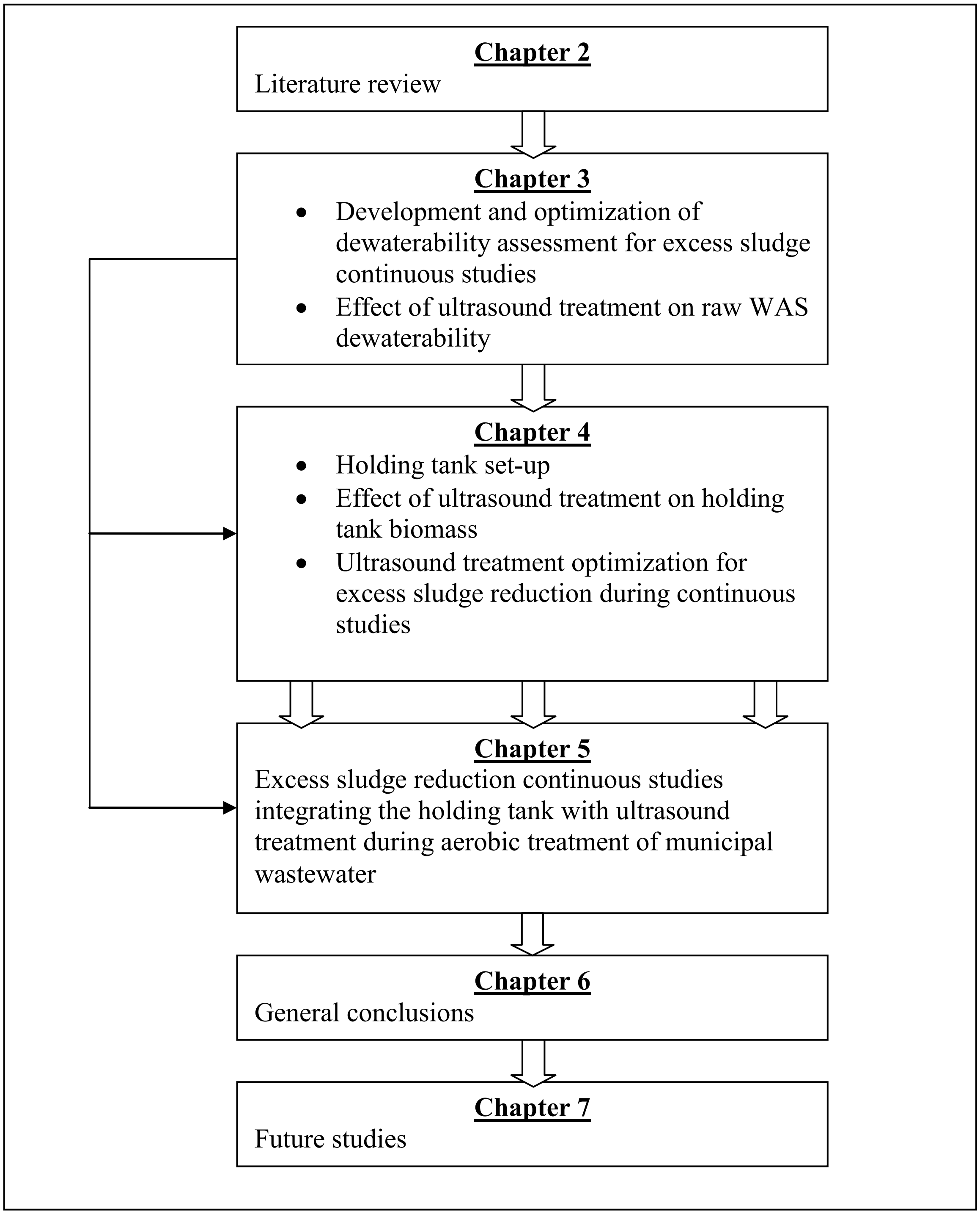

Figure 1.1: Thesis structure and development. The arrows indicate the sequence and interrelation between the different chapters.

Since the aerobic sequencing batch reactor (SBR) was the chosen technology to study excess sludge reduction in the present research, chapter 2 includes also an introduction to SBR technology as well as the main concepts used during SBR operation for aerobic municipal wastewater treatment. From chapter 2, it was clear that the role of ultrasound for excess sludge minimization during municipal wastewater treatment although very positive it is still very contradictory. It was also observed that ultrasound effects depend not only on the sample physical-chemical characteristics but also on the type of equipment used. Furthermore, in the current literature most of the reported studies have used various synthetic wastewaters as a surrogate for typical municipal wastewater. This lack of consistency as well as the use of ideal but different surrogate substrates makes it difficult to compare studies and to determine the real extent of standalone ultrasound treatment for excess sludge minimization at the source. This requires more research in order to understand the fundamental mechanisms of ultrasound treatment that could trigger excess sludge reduction at the source as well as its long term effects on the ASP performance with real municipal wastewater. In the present study the development of a sound methodology for ultrasound treatment was initially undertaken with excess sludge from the aeration basin at ROPEC (raw sludge). During this initial stage the methodologies used in further batch and continuous studies were developed and fine-tuned such as the optimization of the ultrasound equipment, micro flow imaging (MFI), and dewaterability assessment by a centrifugal method. Chapter 3 presents results on the effect of US treatment on dewaterability of raw waste activated sludge. Excess sludge dewaterability of is one of the most important unit operations during excess sludge management, handling and final disposal. Chapter 4 presents results on the effect of ultrasound specific energy input (ES) on holding tank's biomass bioactivity (assayed by the specific oxygen uptake rate and enzymatic assay XTT) as well as floc and cellular solubilization. The wide range of ES input assayed during this portion of the present study indicated that through US pretreatment it is possible to trigger two different mechanisms for sludge minimization once the biomass is recycled into the activated sludge process via the controlled anoxic holding tank step: sludge minimization via cryptic-growth and sludge minimization via enhanced maintenance metabolism: cryptic-growth is an energy intensive approach and it proved not to be a feasible option to pursue under the available laboratory resources. Therefore, the mechanism of enhanced maintenance metabolism for sludge minimization was further investigated. In addition, chapter 4 also presents results on the optimization of the controlled anoxic holding tank operation coupled with ultrasound treatment and the best operational conditions to enhance biomass bioactivity. In chapter 5; optimized operational conditions for the anoxic holding tank coupled with low intensity ultrasound treatment were integrated with an aerobic SBR activated sludge process treating real municipal wastewater operating at 5d solids retention time (SRT). Chapter 5 presents results on the effect of different proportions of post-sonicated biomass recycling to the aeration basin on activated sludge process excess sludge production and effluent quality during treatment of primary effluent based on the mechanism of enhanced maintenance metabolism. Several important activated sludge parameters including excess sludge production, effluent quality, biomass concentration, biomass activity in the aeration basin as well as residual excess sludge characteristics are reported. In Chapter 6 the overall conclusions found during the present study in relation to the initial objectives are summarized. Chapter 7 outlines some recommendations for future studies.

Chapter 2

Background and Literature Review

2.1 Activated sludge process background

Due to its economical and reliable operation the activated sludge process (ASP) is still the preferred technology for the treatment of municipal and many industrial wastewaters. In this biological process, the removal of organic pollutants and suspended solids is achieved by the action of a heterogeneous group of aerobic microorganism, which oxidizes them into CO_2, H_20 and a flocculent microbial suspension (excess sludge) that once settled can be separated from the treated water. The aerobic mode of metabolism is the most efficient in terms of energy recovered by the microbial biomass per unit of substrate processed. However this results in a relatively large quantity of excess sludge production. In 1914, Arden and Lockett presented the results of a new process for the treatment of municipal sewage. In their pioneer work, they realized the importance of air supply to this biological process and tried different ways to force air into bottles filled with sewage. After a certain amount of time a sludge formation occurred. Once the liquid on top was decanted, fresh sewage was added to the bottles, and the process was repeated again. They called this accumulated material “activated sludge”; after raw sewage was mixed with this activated sludge and air, the sewage became easy to clarify; leaving a clear liquid that would not putrefy (Schneider, 2006). A number of ASPs and design configurations have evolved since that conception, mainly to correct shortcomings, to improve its performance and extend the process to treat not only carbonaceous materials but also nutrients such as ammonia-N and phosphorous which are found in municipal wastewater. Current technological advances in equipment, electronics and process control as well as a better understanding of microbiological metabolism, have lead to sophisticated designs that incorporate more than one biological process (i.e. nitrification, denitrification, phosphorus removal) and improved effluent quality (Metcalfe and Eddy, 2003; Bitton, 2005). Overall, during the last two decades ASP process modifications have been developed to:

1) Improve oxygen supply efficiency (conventional aeration, step aeration, pure oxygen aeration processes, tapered aeration, etc.)
2) Increase treatment capacity (high rate process, completely mixed aerated systems, plug flow processes, sequencing batch reactors, etc.)
3) Reduce excess sludge production (contact stabilization, extended aeration process, membrane bioreactor)

2.1.1 Activated sludge process description

A conventional activated sludge process which is used mainly for carbonaceous substrate removal is illustrated in Figure 2.1. The conventional ASP consists of three components:

1) An aeration tank, where aerobic oxidation of carbonaceous organic matter takes place. The micro-organisms responsible for biological oxidation are maintained in suspension and oxygen is supplied through either mechanical or diffused aeration. Primary effluent entering the aeration basin is mixed with return activated sludge (RAS) from the final clarifier to form the mixed liquor in the aeration basin.

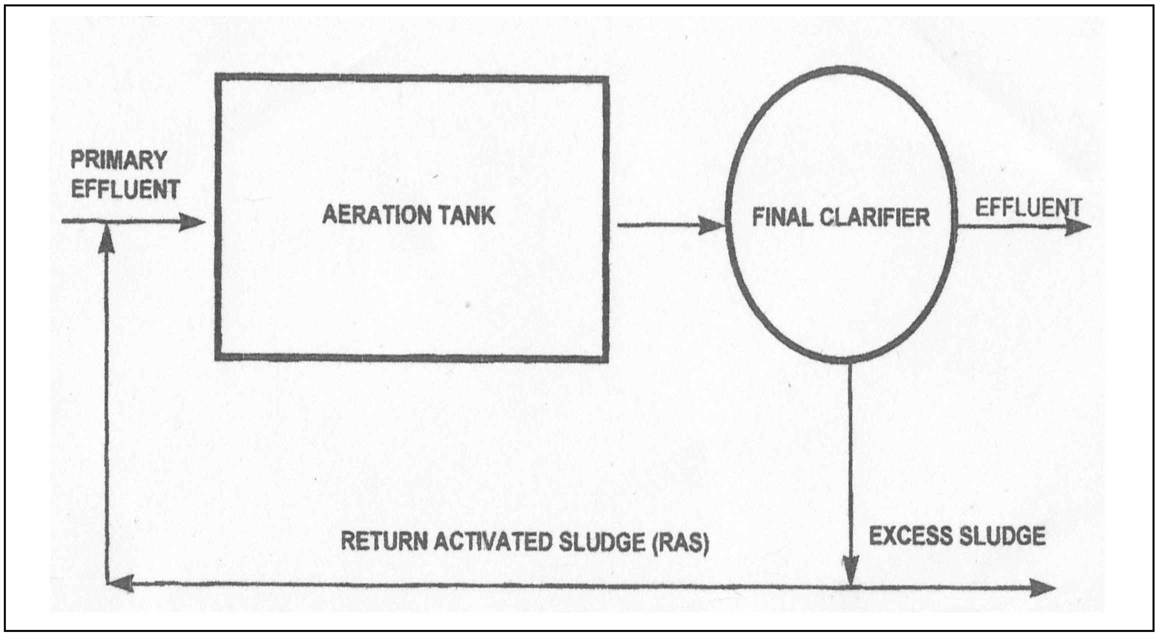

Figure 2.1: Conventional activated sludge process (adapted from Bitton, 2005)

2) A final clarifier (sedimentation/settling tank) in which, the microbial flocs (excess sludge) produced in the aeration basin are separated from the treated effluent.

3) A return activated sludge (RAS) recycle system, which returns a portion of the excess sludge from the settling basin to the aeration tank in order to maintain a specified biomass concentration in the aeration tank. The recycling of this portion of excess biomass makes the mean cell residence time (sludge retention time) greater than the hydraulic retention time (HRT), which allows the process to maintain a large inventory of microorganisms that effectively oxidize organic compounds in a relatively short time. Excess sludge is also wasted from the RAS stream.

Overall, the treatment performance of an ASP (carbonaceous organic matter removal rate and efficiency) depends on the microbial biomass concentration and specific biological activity in the aeration tank; the microbial biomass concentration is controlled by the amount of returned sludge to the aeration tank, while specific biomass activity is maintained by supplying adequate dissolved oxygen. There are some standard parameters commonly used for controlling the ASP, amongst them: the solids retention time (SRT), which is the most critical parameters and the food to microorganism ratio (F/M) ratio, which gives an indication of the amount of substrate available to the microorganisms in the aeration tank. F/M ratios of 0.2 to 0.6 kg COD/kg VSS and a SRT of 3 to 15 days are normally suggested for achieving satisfactory performance and reliable operation with the conventional ASP (Metcalfe and Eddy, 2003). It is important to note that both SRT and F/M ratio depend on the mixed liquor total suspended solids (MLTSS) concentration in the aeration tank. For the conventional ASP, the MLTSS concentration is usually maintained around 3,000 mg/L in order to avoid oxygen supply limitations and to facilitate good solids separation in the final clarifier (settling basin). Aeration tank MLTSS concentration, F/M ratio and SRT are controlled by regulating the amount of return activated sludge and excess sludge wastage. Table 2.1 shows some typical values of F/M ratio, SRT and MLTSS for various modifications of the activated sludge process, note that the sludge production in all processes is high, except for the extended aeration process, which has a low treatment capacity but requires higher oxygen input and large reactor volume.

Table 2.1: Typical operational parameters in various activated sludge processes (adapted from WEF, 1998)

Parameter	Activated sludge process				
	Conventional	**Step aeration**	**Pure oxygen**	**High rate treatment**	**Extended aeration**
F/M ratio (kg BOD/kg VSS-day)	0.2-0.4	0.2-0.4	0.2-0.1	0.4-1.5	0.05-0.15
SRT (day)	3-15	5-15	8-20	4-15	20-30
BOD removal (%)	45-90	45-90	85-95	75-90	75-90
MLSS (g/L)	1.5-3.0	1.5-3.0	6.0-8.0	4.0-10.0	4.0-7.0
Air supply rate (m^3/kg BOD)	45-90	45-90	-	24-45	90-125
Sludge growth yield (kg SS/kg COD)	0.4-0.7	0.4-0.5	0.3-0.5	0.7-0.8	0.2-0.3

2.2 Excess sludge production in the activated sludge process

Municipal wastewater is generated from domestic and industrial sources; the volume produced depends on population density and economical development. However, independently of the amount of wastewater produced, the standards to discharge treated effluent to the environment are set. In Ontario, Canada effluent discharge regulations for BOD_5 and TSS from a conventional ASP treating municipal wastewater are 25 mg/L and 25 mg/L respectively. Hence, the system used for its treatment has to be robust enough to handle the high variability in mass load and volumetric load associated with municipal wastewaters. The major components in municipal wastewater are suspended solids, biodegradable carbonaceous organics, nutrients and pathogens, from which toxic organic compounds and pathogens represent serious health concerns to humans and the environment. In order to protect the aquatic environment from contamination, wastewater must undergo treatment prior to discharge. Wastewater treatment is accomplished by using a combination of treatment unit operations and processes and depending on the degree of treatment desired can be classified as: primary, secondary, tertiary or advanced treatment. Secondary treatment is commonly used to treat municipal and some industrial wastewaters. A typical scheme of a conventional municipal wastewater treatment process is shown in Figure 2.2. Note the different combination of unit operations depending on effluent quality desired (excess sludge treatment included).

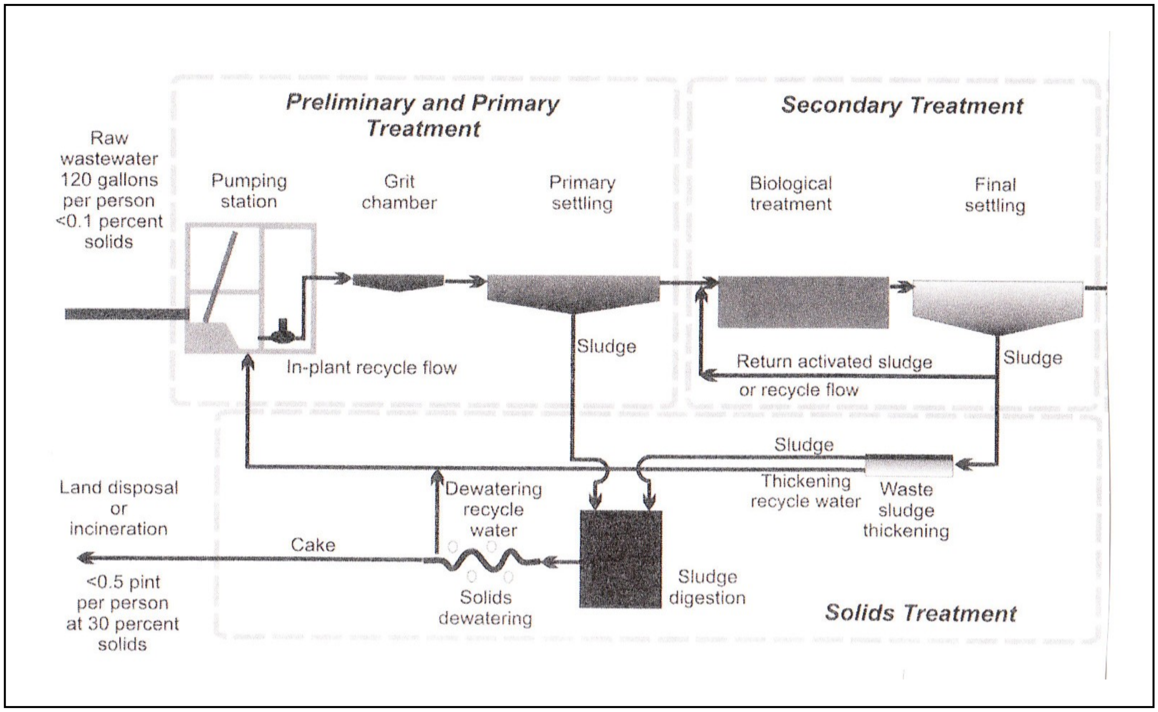

Figure 2.2: Schematic of a typical municipal wastewater treatment plant. Liquid treatment consists of preliminary treatment, primary sedimentation, biological treatment, final settling and disinfection prior to discharge. Solids treatment consists of digestion of primary and thickened secondary solids, mechanical dewatering and land disposal. (Adapted from Hammer, 2004)

From the total biochemical oxygen demand (BOD) that represents the raw influent into the wastewater system, primary treatment removes approximately 35% of the influent BOD, leaving the primary effluent wastewater with 40-50% suspended solids and 65% BOD content. The suspended solids and a large portion of the dissolved organic and inorganic compounds are removed during secondary treatment, through the biological activity of various aerobic microorganisms, mainly bacteria. A portion of the organic matter is oxidized into CO_2 and H_2O, while another portion is used for the synthesis of new cells, producing excess biomass, which is called waste activated sludge (WAS) (Metcalf and Eddy, 2003). The biochemical reaction for organics removal in the biological aerobic basin can be represented by the following expression:

$$\text{Organic matter} + O_2 + \text{nutrients} \xrightarrow{\text{microorganisms}} CO_2 + H_2O + \text{biomass} \qquad (2.1)$$

The most important purpose of the secondary biological treatment is to convert non-settleable (soluble and colloidal) solids to settleable biomass, known as flocs. The floc particle in wastewater treatment is very heterogeneous in composition and it contains living and dead bacterial cells as well as organic and inorganic materials, held together in a matrix by complex extracellular polymers and proteins, as shown in Figure 2.3.

The biomass produced during aerobic treatment in the ASP has to be wasted regularly in order to avoid sludge accumulation and to maintain an optimum biomass concentration in the aeration tank necessary to achieve satisfactory process performance.

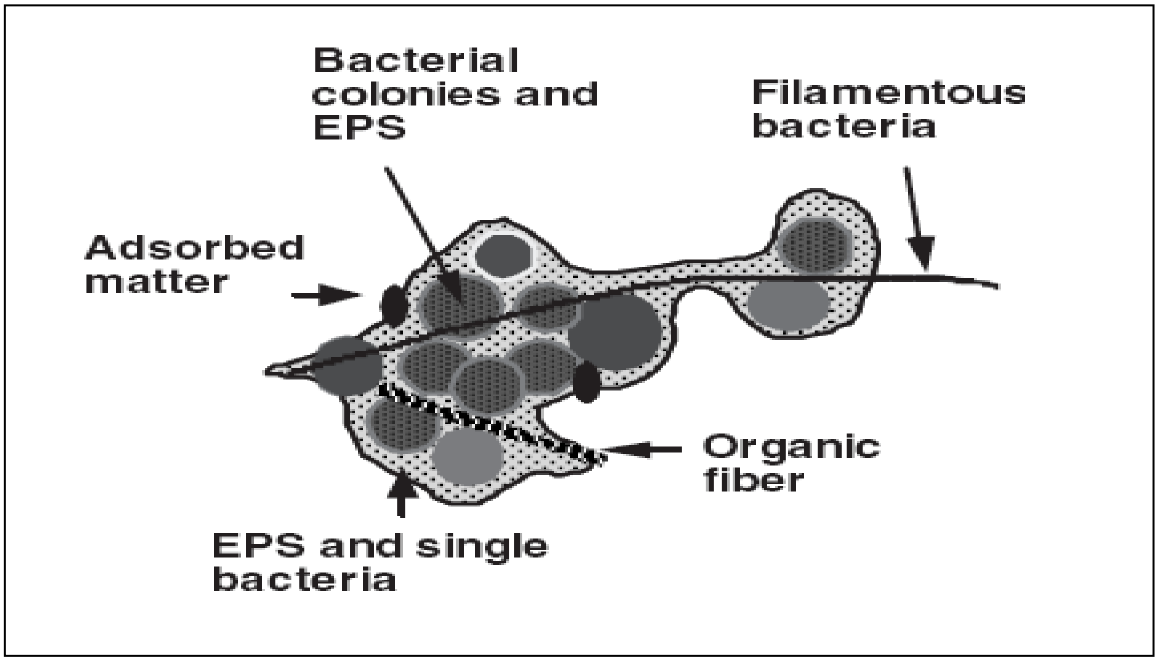

Figure 2.3: Main components in activated sludge floc (adapted from Nielsen et al., 2004)

As shown in Figure 2.2, excess sludge wasted from the secondary treatment is mixed with the primary sludge for further processing and/or final disposal. Table 2.2 presents the quantities of sludge produced from different wastewater treatment processes. It can be observed from Table 2.2 that even if the amount of sludge produced does vary with the type of operation a considerable amount of excess sludge is always produced. On average for a conventional ASP 80 kg dry excess sludge is generated for every 1000 m^3 of wastewater treated. For the City of Ottawa that treats an average of 545,000 m^3/d of municipal wastewater this translates into 43.6 and 15, 914 dry tonnes of excess sludge per day and per year respectively. Table 2.3 shows some physical characteristics of secondary sludge compared to primary sludge. It can be observed from Table 2.3 that both have different solids, water content and specific gravities.

Table 2.2: Quantity of excess sludge generated in different AS treatment processes (adapted from Metcalf and Eddy, 2003)

Treatment type	Dry solids kg/10^3 m^3 of wastewater treated	
	Range	Typical
Primary sedimentation	110-170	150
Activated sludge	70-100	80
Trickling filter	60-100	70
Extended aeration	80-120	100*
Aerated lagoon	80-120	120*
Filtration	12-24	20

*** Assuming no primary treatment**

Table 2.3: Physical characteristics of primary and secondary sludge (adapted from Metcalf and Eddy, 2003)

Sludge type	Water content %	Specific gravity	Dry solids %	
			Range	Typical
Primary	95	1.02	4-10	5
Secondary	99	1.005	0.5-1.5	0.8

Considering processing and disposal, not only is the mass of excess solids produced an important factor but also the volume of excess sludge generated (i.e. City of Ottawa 43.6 dry tonnes/d with 0.8% solids). Water content and specific gravity affect the volume of sludge produced in a particular treatment process. The excess sludge generated from a conventional ASP has to be dewatered for volume reduction and further processed for final disposal, which makes the dewatering process not only expensive to manage but sometimes a critical factor due to local regulations regarding its final disposal.

2.2.1 Excess sludge production and organics removal in the activated sludge process

According to Metcalf and Eddy (2003), the kinetics of microbial growth determines substrate utilization and biomass production in the ASP. A detailed description of microbial kinetics is out of the scope of the present study and only the most important terms related to biomass production will be further developed.

During biological treatment the removal of organic compounds and cell growth take place at the same time, the ratio of biomass produced relative to the amount of substrate consumed is defined as the biomass growth yield (Y). For the treatment of municipal wastewater containing a large number of organic compounds, Y is based on a measurable parameter reflecting the overall organic compound consumption, such as BOD, total organic carbon (TOC) or chemical oxygen demand (COD). When O_2 and N_2 are used as electron acceptors during organic compounds oxidation, the microorganisms produce energy in the form of adenosine triphosphate (ATP), which in turn is used to build new cellular constituents and increase cell mass to a certain level, above which, the cell will divide. Due to environmental constraints and other factors the cellular biomass present in the system may decay. This process called lysis leads to the release of organic matter into the bulk liquid, which can be reutilized by the bacteria to generate more cells (cryptic growth). Part of the energy produced from the biological oxidation of organics is not used for growth but to maintain the cell's integrity, cell repair and activity, this fraction is called maintenance metabolism and it groups and accounts for all non-growth activities (i.e. osmosis equilibration, transport of nutrients, repair, and motility amongst others). The goal for sludge reduction in the ASP then is to somehow decrease the overall biomass yield.

Equation 2.2 shows the relationship between the rate of growth and the rate of substrate utilization.

$$r_g = -Yr_{su} - K_d X \qquad (2.2)$$

Where, r_g is net biomass production rate (g VSS/m^3/d), r_{su} is the substrate utilization rate (units depend on the application of kinetic model), Y is the yield coefficient, (gVSS/g

COD, or BOD, or TOC), k_d is the endogenous decay coefficient, (g VSS/ g VSS/d) and X is the biomass concentration (g VSS)

Dividing eq. 2.2 by X, equation 2.3 is obtained

$$\mu = \frac{r_g}{X} = Yr_{su} - k_d \qquad (2.3)$$

Where, μ is the specific growth rate, g VSS (new cells)/ g VSS /d

The specific growth rate (μ) is a function of the substrate concentration and the endogenous decay coefficient. The kinetic coefficients used to determine the rate of substrate utilization and growth rate in an ASP depend on certain variables such as the wastewater type, microbial population and temperature. Table 2.4 shows typical kinetic coefficients for the conventional ASP for the removal of organics from domestic wastewater. The coefficients presented in Table 2.4, represent the net effect on the simultaneous degradation of a variety of different wastewater constituents by a heterogeneous mix of aerobic microorganisms. In the ASP the kinetic coefficients that describe biological growth are related to the active biomass (X) or volatile suspended solids (VSS). However, VSS accounts for more than the active biomass; it also includes the extracellular polysaccharides in the floc matrix as well as cell debris (from cells endogenous decay) and non biodegradable VSS (from influent wastewater). Although both later fractions are relatively small, their determination is important in order to know the real excess sludge production in the ASP, especially for this work where the chosen treatment (ultrasound) depending on the intensity of treatment will most likely increase the amount of cell debris.

The VSS production in an aeration tank can then be determined as:

$$r_{X_T,VSS} = -Yr_{su} - k_d X + f_d(k_d)X + \frac{QX_{o,i}}{V} \qquad (2.3)$$

Where, $r_{X_T,VSS}$ is the total VSS production rate (g/m^3 /d), Q is the influent flow rate (m^3/d) $X_{o,i}$ is influent non-biodegradable VSS (g/m$^{3)}$ and f_d is the fraction of biomass that remains as cell debris (g VSS/m^3/d)

Table 2.4: Typical kinetic coefficients for the activated sludge process (adapted from Metcalf and Eddy, 2003)

Coefficient		**Units**	**Value (at 20ºC)**	
			range	**typical**
K	Maximum specific substrate utilization rate	gCOD/g VSS/d	2-10	5
K_s	Half velocity constant	Mg/L BOD Mg/L COD	25-100	60
Y	Yield coefficient	mgVSS/mg BOD mgVSS/mg COD	0.4-0.8 0.3-0.6	0.6 0.4
K_d	Endogenous decay	g VSS/g VSS /d	0.06-0.15	0.10

In the case of municipal wastewater, the true biomass yield is difficult to determine due to the variety of organic compounds that make up the influent, and the great variety of microorganisms that form the activated sludge floc. Consequently, two terms are commonly used in the design and analysis of biological treatment processes (Metcalf and Eddy, 2003):

1) The net biomass yield (Y_{bio}), which is used to estimate the amount of active microorganisms in the system

$$Y_{bio} = -\frac{r_g}{r_{su}} \qquad (2.4)$$

2) The observed yield (Y_{obs}), which accounts for the total volatile solids produced in the system and is determined as follows

$$Y_{obs} = \frac{r_{X_T,VSS}}{r_{su}} \qquad (2.5)$$

Another way to estimate Y in the ASP is by stoichiometry. Rewriting equation 2.1 in the form

$$v_1(organics) + v_2O_2 + v_3NH_3 + v_4PO_4^{3-} \xrightarrow{microorganisms} v_5(newcells) + v_6CO_2 + v_7H_2O \quad (2.6)$$

Where v_i is a stoichiometric coefficient; if the organic strength of the wastewater entering the aeration tank is determined by its COD (dissolved and particulate fractions) then, during biological treatment a portion of influent COD is oxidized into CO_2 and water and the remaining portion is converted into biomass. COD oxidation requires oxygen and biomass growth produces excess sludge; thus oxygen supply and sludge production are two very important issues related to the operation of an ASP. According to Metcalf and Eddy (2003), oxygen supply accounts for 30-40% of the total operational cost of an ASP treatment plant, while according to Perez-Elvira et al. (2006) sludge treatment and disposal accounts for 25-60%. If it is assumed that glucose is the major organic carbonaceous source in the wastewater and biomass composition is described by the formula $C_5H_7O_2N$, then neglecting nutrients other than N_2 equation (2.6) can be written as:

$$3C_6H_{12}O_6 + 8O_2 + 2NH_3 \xrightarrow{microorganisms} 2C_5H_7O_2N + 8CO_2 + 14H_2O \qquad (2.7)$$

Biomass production and oxygen requirements can be estimated from (2.7) as:

$$Y = \frac{\Delta(C_5H_7NO_2)}{\Delta(C_6H_{12}0_6)} = \frac{2(113g/mol)}{3(180g/mol)} = 0.42\frac{g_{cells}}{g_{glucose}} \tag{2.8}$$

In addition to growth, microorganisms undergo endogenous respiration, Henze and Mladenovski (1991) categorized the endogenous respiration in three sequential steps: death, hydrolysis and growth, hydrolysis being the limiting step. In practice COD and VSS are used to represent the organic matter and the new biomass (Metcalf and Eddy, 2003), therefore, in order to represent Y in terms of COD, the COD of glucose has to be determined. Writing a balanced stoichiometric reaction for the oxidation of glucose to CO_2 the COD of glucose can be determined

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \tag{2.9}$$

From equation 2.9

$$COD_{glucose} = \frac{\Delta O_2}{\Delta(C_6H_{12}O_6)} = \frac{6(32g/mol)}{180g/mol} = 1.07\frac{g_{O_2}}{g_{glucose}} \tag{2.10}$$

Hence, from equations 2.8 and 2.10, the theoretical Y expressed in terms of COD is 0.39 g cells/g COD used. However, due to cell maintenance functions the actual Y would be lower than the theoretical Y determined. Proceeding in the same way, the biomass COD equivalent is 1.42 g O_2/g cells. Then the total oxygen needed for substrate oxidation and endogenous respiration can be determined from the difference between the COD removed and the COD equivalent of biomass produced. This implies that the oxygen requirement for a conventional ASP is inversely related to the sludge production;

lower sludge production has higher oxygen requirements. Oxygen required for each unit of COD removed can be determined by equation 2.11.

Oxygen consumed = COD removed – cells produced as COD equivalent (2.11)

Oxygen consumed = 577.8 g O_2 – 320.9 g O_2 = 256.9 g O_2

From equation 2.11 the oxygen consumed per unit of COD removed is 0.44 g O_2/g COD. If 0.44 g of oxygen is required for each gram of COD removed, the remaining portion (1-0.44 = 0.56 g) of the removed COD is assumed to be converted into biomass. For each gram of COD removed, 0.39 g (0.56/1.42) of biomass is produced, which represents the true biomass growth Y. When biomass undergoes death, decay and cell maintenance, a portion of their cell mass is converted into soluble secondary substrates that are further oxidized, reducing the amount of cells produced. The observed yield (Y_{obs}), which includes both cell growth and cell decay, is less than the true yield (Y) (Metcalf and Eddy, 2003). The relationship between observed yield and true yield can be described by equation 2.12.

$$Y_{obs} = \frac{Y}{1 + k_d SRT} \qquad (2.12)$$

Where, Y_{obs} is the observed growth yield (g VSS/g COD), Y is the true growth yield (g VSS/g COD), k_d is the endogenous decay (1/day) and SRT is the solids retention time, (day).

In equation 2.12, k_d accounts for loss of cell mass due to oxidation of internal storage products to obtain energy for cell maintenance, cell death, and predation by higher forms of organisms (Van Loosdrecht and Henze, 1999). SRT and k_d are generally lumped together. Since a decrease in cell mass due to endogenous metabolism or endogenous decay is assumed to be proportional to the biomass concentration present, the effects of endogenous decay on sludge production will be more pronounced at a longer SRT.

Figure 2.4 shows a correlation between sludge production, SRT and temperature. From Figure 2.4 it can be observed that sludge production normally decreases with an increase in SRT. Hence, low sludge production can be achieved in extended aeration activated sludge processes and membrane reactor (MBR) due to their longer SRT (> 20 days). Since the F/M ratio is inversely related to SRT, low sludge production can also be achieved by operating an ASP at low F/M.

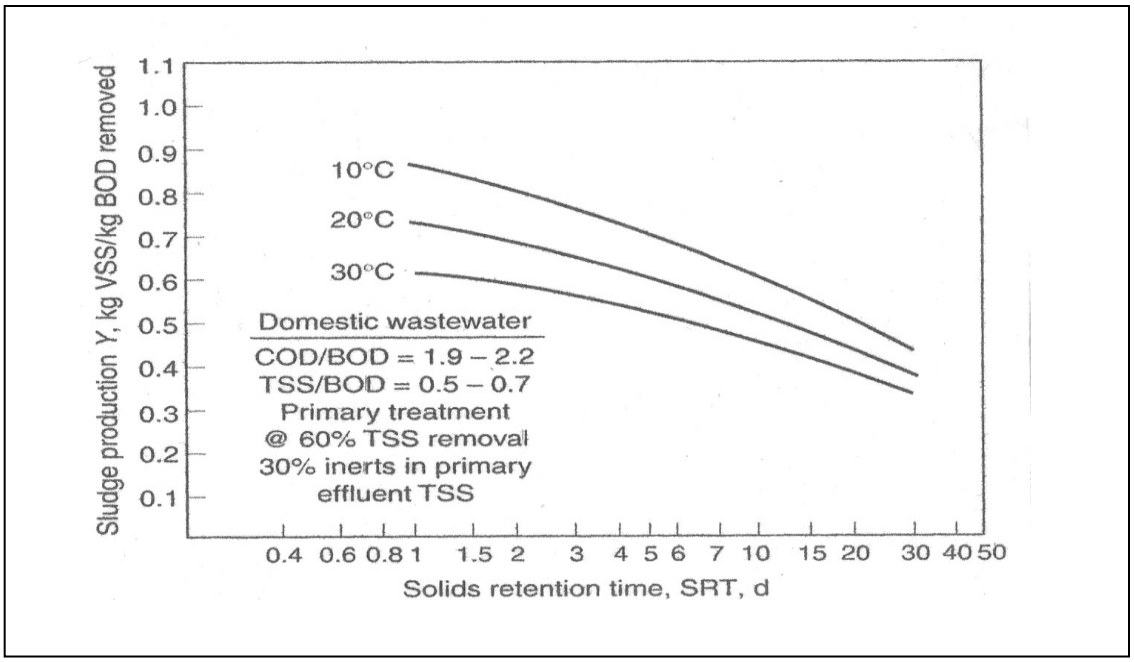

Figure 2.4: Net sludge production (from primary effluent) versus SRT and temperature (adapted from Metcalf and Eddy, 2003)

2.3 Sludge production and regulation in Canada

In Canada, the proportion of the population served by municipal wastewater treatment, increased from 64% to 78% between 1990 and 1997 (Woods and Lyzell, 2003). Due to the country’s current economical growth, this proportion is expected to increase in the near future which will cause an increase in excess sludge production. Table 2.5 shows the sludge generation in Canada at 387,166 dry tons for the year 2001. According to the City of Ottawa, Canada's sludge production in 2005 reached 679,590 dry ton /year of which 50% is from ASP, this represents a 2 fold increase in sludge production in 5 years. In the particular case of Ottawa, Ontario sludge production presents monthly and yearly variations, however, between 40,000 and 45,000 metric tonnes are generated yearly of which 50% is from ASP as shown in Figure 2.5 (City of Ottawa, 2007).

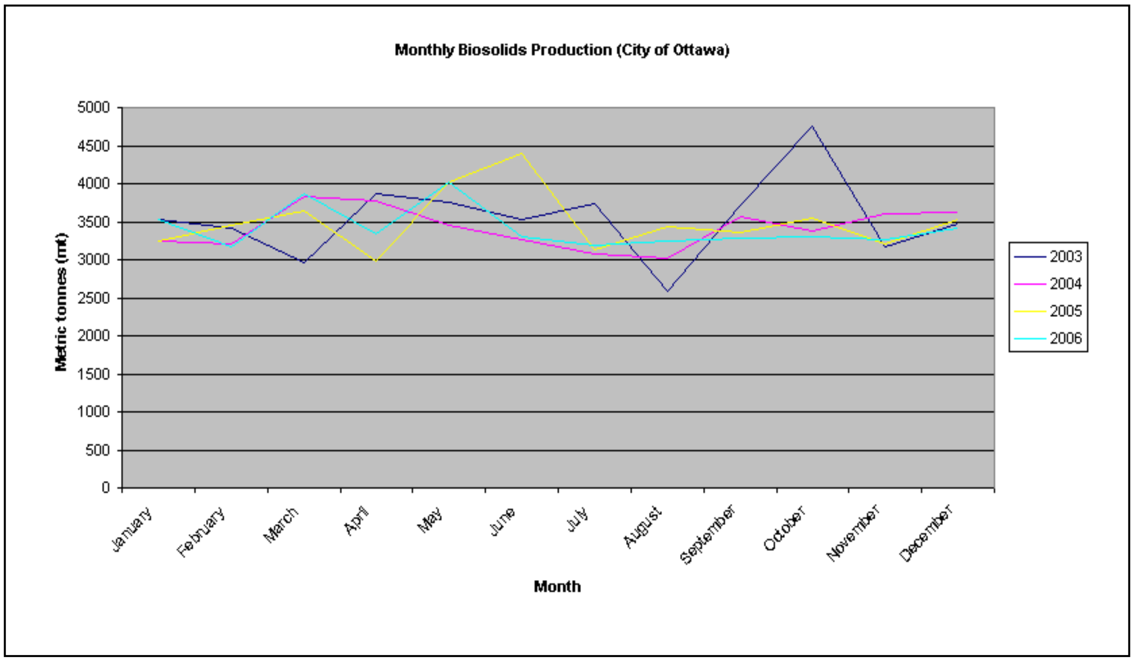

Figure 2.5: Excess primary and secondary sludge production for the city of Ottawa, ON, Canada (adapted from City of Ottawa, 2007)

Table 2.5: Overall municipal sludge generation in Canada (adapted from Wood and Layzell, 2003)

	Population[1]	Population served by water treatment[2]	Sludge production[3]	
			kg/day	t/year
Canada	21,585,857	16,836,968	1,060,729	387,166

1 Statistics Canada, CANSIM II 051-00001
2 Assumes 78% of wastewater undergoes primary, secondary or tertiary treatment.
3 Assumes sludge production of 0.063 kg/person/day

In order to protect the aquatic environment, public health and safety, the Federal and Provincial governments have regulations that limit the quantity of substances of concern discharged into the environment via the municipal sewer system (Ratnaparkhe and Sertic, 2006). However, Canada's governing structure can lead to confusion regarding the role of the various levels of government when dealing with sludge. The federal and the provincial government have independent legislation aimed at protecting air and water quality and in the case of municipal sludge (biosolids is the term used in Canadian legislation) some jurisdictions have regulations that target specific contaminants that may be present in sludge, while others have regulatory means to address how sludge could be re-used, hence the role of each level of government is not always clearly understood (LeBlank, 2007). In Ontario, biosolids can be composted, landfilled, incinerated, land applied and pelletized. However, in all cases they must be used or disposed of at a site that has an appropriate Certificate of Approval issued by the Ontario Ministry of Environment (MOE). Ontario legislation has no provision for excluding biosolids from being defined as a waste, even when sludge meets the standards set out in the Guidelines for the Utilization of Biosolids and Other Wastes on Agricultural Land (LeBlank, 2007). Therefore, biosolids are always defined as a waste even if they are treated and stabilized via anaerobic digestion or composting (Lewis, 2006)

Sludge disposal is expensive and problematic. In Canada, the sale of sludge as fertilizer is not currently permitted. Therefore, in most regions, the favoured approach is to spread the sludge on agricultural lands, where it acts as a soil amendment. Sites are selected according to stringent criteria set out by provincial government environmental agencies. Those criteria are intended to minimize contamination of surface or groundwater supplies, avoid nuisance and odour complaints. Usually lands where crop growth is intended for animal consumption are selected. However, areas where all the criteria may be adequately met are in short supply, leaving as an alternative sludge disposal in landfill sites. But as in the case of land application the shortage of landfill sites is generating an increase in the costs of sludge handling, transportation and tipping fees.

Therefore, developing technologies to reduce the excess sludge production within the activated sludge system are of growing interest.

2.4 Costs associated with excess sludge handling and disposal

Operation and maintenance costs of a secondary wastewater treatment plant, including the cost of sludge treatment and disposal can be divided into four major categories: personnel, energy, chemicals and maintenance. Personnel and energy costs account for about 85% of the total cost, 36% of the total plant operating cost is the energy consumption in aeration and pumping systems (wastewater and sludge included). Sludge treatment alone, accounts for more than 32% of the total plant energy consumption (Tsagarakis et al., 2003). If sludge handling, transportation and final disposal are added then the total cost for sludge treatment and disposal may be up to 40-65% of the total operating cost.

The ideal solution to alleviate both cost and space availability problems would be the direct reduction of sludge within the activated sludge process, even if the amount of primary sludge due to its nature can not be reduced substantially the reduction of ASP sludge (secondary sludge) is possible, and which accounts for 40-60% of the total sludge produced at secondary biological wastewater treatment plants (Odegaard, 2004).

2.5 Excess sludge reduction

During the last decade efforts have been made to reduce the amount of sludge generated during biological activated sludge treatment of wastewater. Due to the presence of significant amounts of inorganic and non-biodegradable solids in wastewater that are difficult to destroy even with additional treatments, the total elimination of solids discharge is unrealistic (Andreottola and Foladori, 2006). The performance of the ASP relies to a large extent on the amount of the return activated sludge (RAS) recycled to the aeration tank; hence, an excessive reduction in excess sludge production can compromise the process removal efficiency and quality of the final effluent. In this study, the term "excess sludge reduction" is defined according to the one proposed by Odegaard (2004) and refers to the reduction of dry mass of sludge within the biological treatment process such as the ASP, and not with the mass of sludge reduction as a result of further chemical/physical treatment such as thickening, dewatering, digestion or incineration.

As mentioned in section 2.2.1, the main target for excess sludge reduction is to decrease the overall Y during biological treatment of municipal wastewater by direct methods or to enhance biomass non-growth activities (maintenance activities) to decrease Y indirectly. Figure 2.6 shows the cycle of sludge production and some ways to decrease it. From Figure 2.6 it can be observed that sludge reduction can be accomplished in several ways: 1) by replacing high energy producing electron acceptors such as O_2 and nitrate or by decreasing their efficiency (anaerobic treatment of municipal wastewater or metabolism uncoupling), 2) by enhancing maintenance requirements, allowing less energy for growth, 3) by enhancing lysis and decay of biomass, which released products could be reutilized by other bacteria in the system and 4) by enhancing the biodegradability of accumulated inert organic materials (Ginestet, 2007).

Recent literature reviews (Odegaard, 2004; Perez-Elvira et al., 2006; Andreotolla and Foladori, 2007) corroborate that excess sludge reduction can be achieved by using the above mentioned approaches.

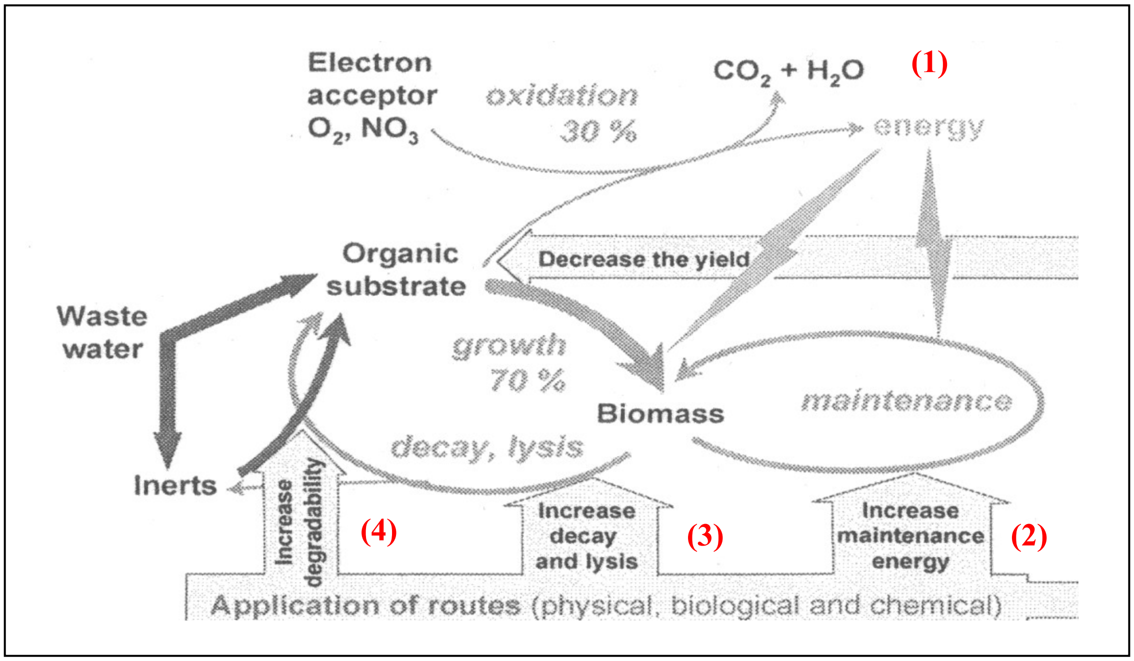

Figure 2.6: Possible ways to decrease excess sludge production (adapted from Ginestet, 2007)

Perez-Elvira et al. (2006) categorized the different processes according to the location within the WWTP train were the sludge reduction step takes place; they identified three main locations: in the wastewater line (i.e. around the ASP), in the sludge line (i.e. anaerobic digestion of the sludge) and in the final waste (i.e. incineration). Figure 2.7 shows a simplified wastewater treatment line, the red star indicates the locations in the treatment train where excess sludge reduction can be achieved. For the purposes of the present study, only the processes in the water line are considered. Table 2.6 shows the main approaches used to achieve sludge reduction in the water line.

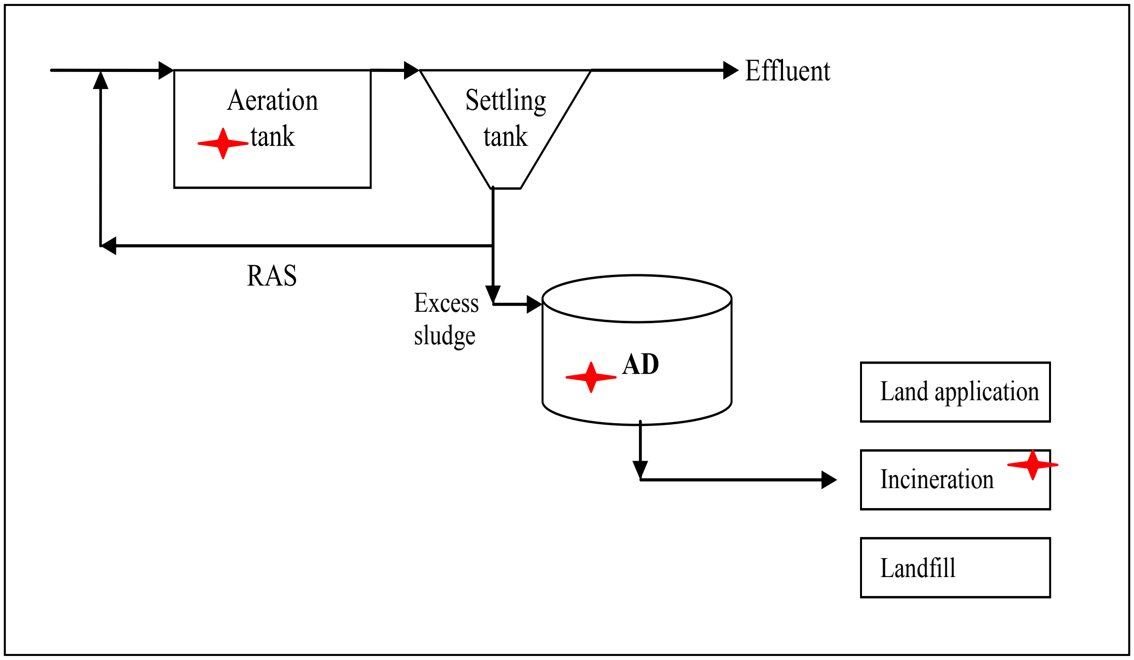

Figure 2.7: Main locations in the wastewater treatment process where excess sludge reduction can be achieved (indicated with the red star).

Table 2.6: Sludge minimization processes (adapted from Perez_Elvira et al., 2006)

Processes that reduce the yield coefficient	Lysis-cryptic growth	Chemical oxidation, ozonation	FS[1]
		Chlorination	FS
		Integration of chemicals and heat treatment	FS
		High purity oxygen process	FS
		Enzymatic reactions	FS
	Maintenance metabolism	Membrane bioreactor	EM, IN
	Uncoupling metabolism	Chemical uncoupler	EM[1]
	Predation on bacteria	Two-stage system	EM
		Oligochaetes (worms)	EM
Processes with low yield coefficient		Anaerobic/aerobic systems	EM, IN

FS:full scale application, EM: embrionaire, laboratory scale;IN: innovatibe

From Table 2.6 it can be observed that processes that reduce the yield coefficient via lysis and cryptic growth are achieved through high intensity thermal, mechanical, enzymatic or ozone treatments, while the processes with a low yield coefficient depend mainly on restricting/limiting sludge growth in an anaerobic/anoxic environment or enhancing non-growth biomass metabolic activity. Mason and Hamer (1987) studied cell growth on released cellular products and described it as cryptic growth; when a external force is applied to a portion of the sludge, microbial cells undergo death and lysis during which substrates and nutrients are released to the bulk liquid and taken up by living bacteria as substrate, the extent of lysis greatly depends on the type of physical or chemical force applied. Solubilization of cell products is greater than growth on the lysis products (released substrate), which results in an overall net decrease in cell mass produced. There are two stages in cryptic growth: lysis and biodegradation; lysis is considered to be the limiting step and can be accomplished by specific treatments (mechanical, chemical or biological). When the treated sludge is returned to the aeration tank, degradation of the secondary substrate takes place, which results in a reduction of excess sludge production. However, the specific mechanisms involved in such phenomena are not yet well understood.

2.6 Treatment technologies to reduce excess sludge production in the waterline

Figure 2.8A represents the conventional ASP, where RAS is recycled to maintain a determined concentration of MLTSS in the aeration tank without any treatment that would influence biomass yield. Figure 2.8B shows potential to reduce the biomass yield by inducing cryptic growth, severe disruptive treatment of all or a portion of the excess RAS is necessary to facilitate the release of cellular components and other secondary organics into the medium. Once the excess sludge has been treated all or a portion of the RAS is then recycled to the aeration tank for secondary oxidation to reduce the overall biomass yield.

Figure 2.8D represents a typical oxic-settling-anaerobic activated sludge process system that alters the microbial consortia within the aeration tank thereby reducing biomass yield via increased metabolic metabolism, there is no RAS treatment prior to its recycling to the aeration tank. Figure 2.8C is the novel RAS recycle proposed in the present study: an approach that combines processes D and C with option of partial treatment bypass. The process depicted in Figure 2.8C has the potential to combine the advantages of cryptic growth and/or increased maintenance metabolism depending on the intensity of the chosen pretreatment or the anoxic conditions used in the holding tank.

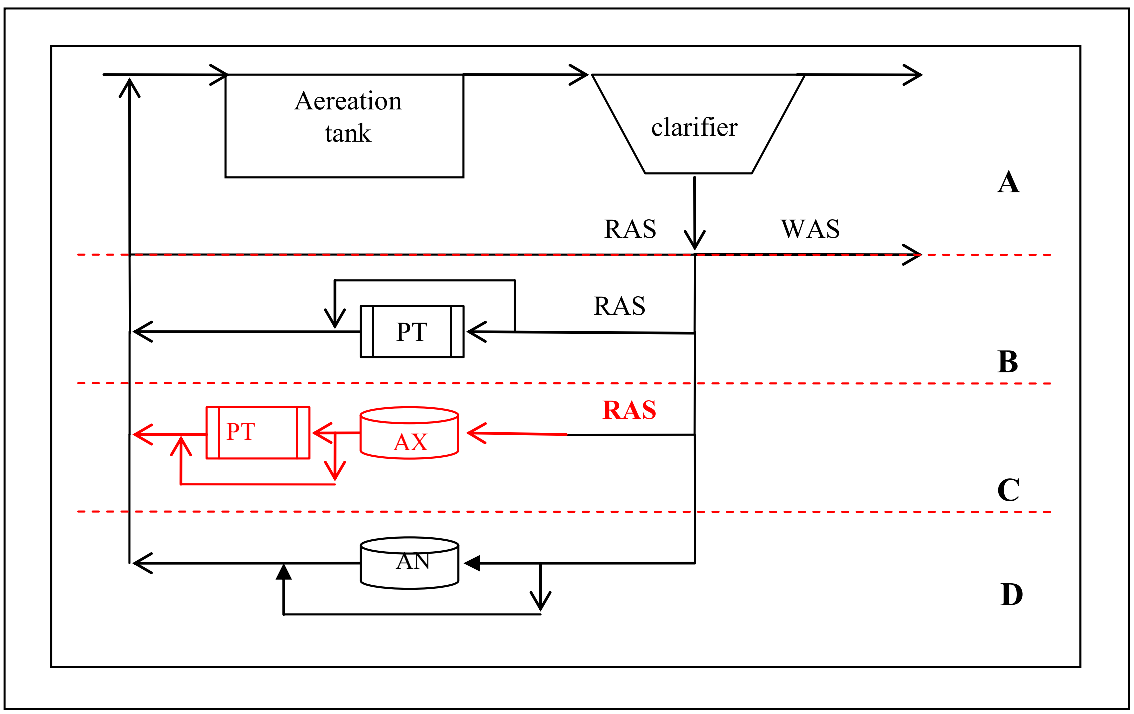

Figure 2.8: Different strategies for recycling excess sludge in the ASP. PT (treatment), AN (anaerobic), and AX (anoxic).

Excess sludge treatment as shown in Fig 2.8B or proposed in 2.8C is achieved by chemical, thermal or mechanical means. Several studies have reported on the effectiveness of disruptive treatment technologies shown in Table 2.7 for excess sludge reduction via enhanced cryptic growth

Table 2.7: Main treatment technologies used in excess sludge reduction at the source (adapted from Perez-Elvira et al., 2007; Andreottola and Foladori, 2007)

Treatment technology	Agent used
Chemical	Ozone, alkaline hydrolysis, chlorine
Thermal	Thermal hydrolysis at temperatures >150 ºC.
Mechanical	Stirred ball mills, ultrasound, high pressure homogenizers

The use of such technologies, besides helping to achieve significant excess sludge reduction might also help reduce operational costs in subsequent sludge treatment processes, such as anaerobic digestion, conditioning, dewatering and final disposal, even if the inclusion of any of them in a WWTP represents an initial investment and energy consumption.

2.6.1 Use of ozone as pretreatment for excess sludge reduction

Ozone is a strong chemical oxidant; it has already been applied in wastewater treatment for the removal of refractory COD from secondary effluent and also to help control sludge bulking and foaming (Metcalf and Eddy, 2003). Recently, the use of ozone has been attempted to reduce excess sludge production in the ASP by enhancing cryptic growth (Yasui and Shibata, 1994; Yasui et al., 1996; Sakay et al., 1992; Kamiya and Hirotsuki, 1998; Egemen et al., 2001, Ahn et al., 2002; Deleris et al., 2000). Excess sludge is exposed to ozone in order to induce cell lysis and solubilization; treated sludge is then returned to the aeration tank for oxidation of the soluble organics generated. It is believed that after ozone exposure, part of the excess sludge is mineralized to CO_2 and water, while another fraction is solubilized to biodegradable organics. Microbial growth in an ASP under such conditions results in a net lower microbial growth yield compared to a conventional ASP. According to Deleris et al. (2000) more than 50% of the carbon obtained after ozonation of the RAS is biodegradable.

Yasui and Shibata (1994) applied doses of 0.05 mg to 0.20 O_3/g SS and dilution rates of 0.1 to 0.3/day to a portion of excess sludge that was then returned to the ASP aeration tank. The aeration tank had a concentration of 4.2 g MLTSS/L and BOD influent concentration of 1000 mg/L. They reported that after six weeks of operation under such conditions no sludge wasting had been necessary, and that the reactor's biomass concentration remained constant. In a full scale application, Yusai et al. (1994) reported that sludge wastage was not necessary after ten months of operation; although, an increase in refractory organic carbon was observed in the final effluent. There was no report of changes in the microbial consortia within the ASP or any other explanation of why the Y decreased.

Sakai et al. (1997) developed a combined system in which sludge from a continuous ASP with synthetic wastewater was withdrawn, subjected to ozonation and returned to the aeration tank. They reported up to 50% sludge reduction with ozone doses of 10 mg/g MLTSS/ d. In another study, when ozone dose was increased to 20 mg/ g MLTSS /d, no excess sludge was produced (Kamiya and Hirotsuji, 1998). Unfortunately, no results were provided on the effect of the pretreatment on the degree of cell lysis or solubilisation of organic and recalcitrant inorganic components as well as the quality of the final effluent or final sludge characteristics (i.e settling, dewaterability). Additionally, taking into account they were working with soluble synthetic wastewater without inert materials the sludge reduction is likely overestimated.

Egemen et al. (1999) following the same approach as Sakai et al. (1997), reported 40 to 60% excess sludge reduction after ozonation using a synthetic wastewater, excess sludge was ozonated for 3 hrs every day and then returned to the aeration tank. In a pilot study Ahn et al. (2002), studied the feasibility of ozonation (0.1 to 2 mg O_3/g MLSS) in order to reduce excess sludge production in an ASP. They reported that ozonation resulted in mass reduction by mineralization, achieving an average of 55% excess sludge reduction under the experimental conditions tested as well as volume reduction by improvement of dewaterability characteristics. They concluded that mass reduction in microbial biomass in the ASP was enhanced as ozone dose increased.

2.6.2 Use of Chlorination as treatment for excess sludge reduction

Chorine has been used extensively in water/wastewater disinfection; recently, it has also been used to enhance excess sludge reduction via cryptic growth. Saby et al. (2002) subjected excess sludge to chlorine dose of 133 mg/mg MLTSS/d, the chlorinated sludge was then returned to the aeration tank resulting in sludge reduction of 65% compared to the untreated control. However, compared to the control, the settleability of the sludge was reduced as well as a reported increase in soluble COD concentration in the effluent. While it was not addressed in the manuscript, the use of chlorine for pretreatment of RAS can result in concerns related to the formation of trihalomethanes in the effluent that might originate in this process due to the high organic content of ASP sludge.

2.6.3 Use of alkaline and heat treatment for excess sludge reduction

Thermal and mechanical treatments mainly results in the breaking of the cell wall and membrane of microorganisms due to its sensitivity to shear and to changes in temperature. Thermal treatment for excess sludge reduction use temperatures from 40 to 120 ºC, with different heating rates and holding times. Canales et al. (1994) used a membrane reactor (MBR) with long SRT to treat synthetic wastewater when returned activated sludge passed through a thermal loop (50, 70 and 90 °C) and then recycled to the MBR. 60% excess sludge reduction was achieved versus the control. They found that the best condition for cell death and solubilization was a fast heating rate (12°C/min, no holding time reported and 90 °C). However, the characteristics of the final effluent or residual sludge were not mentioned. In addition, the role of the long SRT in the MBR as it may pertain to minimizing sludge production was not addressed. In order to further enhance cell solubilization, some studies have also incorporated alkaline or acid treatments in addition to heat. Rocher et al. (2001) combined an alkaline treatment with high temperatures when treating a synthetic wastewater; NaOH was added to excess activated sludge to a final pH of 10 and then subjected to 60 °C for 20 min and returned to the aeration tank. They reported an increased release of dissolved carbon and solubilisation after pretreatment and 37% excess sludge reduction was achieved under these experimental conditions versus the control without reducing ASP performance in terms of COD removal. However secondary effects pertaining to sludge dewaterability or settleability were not discussed.

2.6.4 Use of high purity oxygen and high oxygen concentration for excess sludge reduction

Low sludge production has also been reported by increasing oxygen transfer rates and concentration of dissolved oxygen (DO) in the ASP. However, the mechanisms of excess sludge reduction with high DO concentrations are not clearly known.

Wunderlich et al. (1985), reported a sludge reduction from 0.38 to 0.28 mg VSS/mg COD (about 25% excess sludge reduction) when high purity O_2 was used in the ASP treating synthetic wastewater.

Abassi et al. (1999) investigated the influence of different sludge loadings and oxygen concentrations on excess sludge production. They found that excess sludge production was enhanced with an increase in loading rate, while, a decrease in excess sludge production was observed by increasing the oxygen concentration. Glucose-based synthetic media was fed to a 28 L reactor at different F/M ratios (0.53, 0.71 and 1.70 mg BOD_5 mg /MLTSS /d and dissolved oxygen concentrations (2-6 mg O_2/L). They reported that a rising of dissolved O_2 concentration in the reactor lead to a reduction of excess sludge at all F/M ratios tested. It was postulated that an increase of O_2 concentration in the bulk liquid leads to a deep diffusion of O_2 in to the AS floc which increases the aerobic volume inside the floc, allowing the hydrolysed microorganisms in the matrix to be degraded. They concluded that as O_2 diffuses deeper into layers of the sludge flocs compared to substrate, concomitantly endogenous decay and cell lyses is enhanced and as result there is a lower sludge production. However, the impact on final sludge characteristics in terms of settling, activity and dewaterability were not addressed.

2.6.5 Use of enzymatic process as treatment for excess sludge reduction

The use of biological agents for cell disruption is a technology to induce excess sludge production in the ASP. Some proprietary designs have already been applied successfully at plant scale such as the S-TE and Biolysis E®. Both processes make use of thermophilic enzymes to induce microbial cell disruption before being returned to the ASP. In the Biolysis E® process a portion of RAS is withdrawn, thickened and sent to a thermophilic anaerobic digester operating at 50-60 ºC. It is believed that such conditions stimulate the development of a certain type of microorganism that attacks the outer membrane of the bacteria in the sludge, reducing their ability to reproduce. Once the treated sludge is sent back to the aeration tank 30 to 80% excess reduction has been observed (Lebrum et al., 2003). Unfortunately, most of the information is proprietary and few details were available to confirm the claims.

2.6.6 Use of mechanical treatment for excess sludge reduction

Mechanical treatment has high potential for excess sludge reduction; mechanical shear enhances the breaking of microbial cells and flocs through cavitation facilitating the release of soluble fractions to the media. However, when low energy is applied to the sludge, it is believed that the flocs disintegrate without actual microbial cellular breakage (Muller, 2000). Energy is provided by pressure, translational and rotational energy. The following methods of mechanical treatment have been already applied in order to decrease ASP excess sludge production: stirred balls mills (SBM), high pressure homogenizers (HPH), ultrasonic homogenizers (UH), high performance pulse technique (HPP) and lysat-centrifugal-technique.

Lehne et al. (2001) studied the effect of mechanical treatment on municipal sludge disintegration. Different ultrasonic homogenizers, a high pressure homogenizer and a stirred ball mills were used under different conditions of energy input. In this experiment the degree of disintegration was determined by measuring the particle size after disruption; as the energy input increased the particle size decreased. They concluded that for the stirred ball mills, the optimum was reached at low ball diameter values. Figure 2.9 shows the disintegration results from mechanical disruption. From figure 2.9 it can be observed that as the energy input increases higher degrees of disintegration are reached and also at higher TSS concentrations better performance of the different devices.

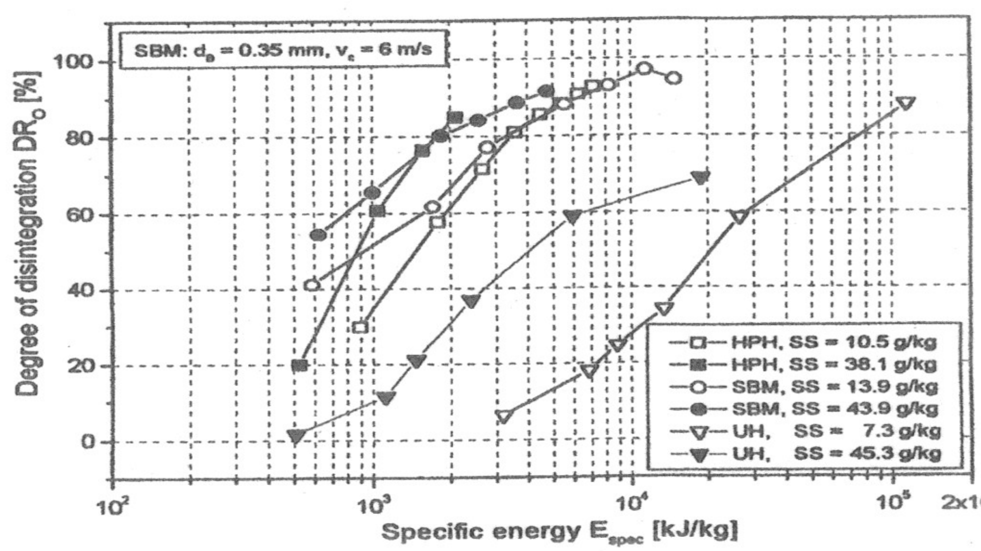

Figure 2.9: Comparison of different disintegration methods on excess sludge: HPH (high pressure homogenizer), SBM (stirred ball mill) and UH (ultrasound homogenizer). (Adapted from Lehne et al., 2001)

Nolasco et al. (2002) used a mechanical mill (Kady^{TM} mill) to disrupt secondary sludge produced from a pulp and paper plant. After disintegration the sludge was recycled to the aeration tank. They found that the recycling of the sludge did not have an adverse effect on reactor performance (COD removal) and that the use of this technology was found effective in reducing excess sludge production by 60%. However, due to the use of synthetic wastewater the extent of excess sludge reduction achieved in this process might be lower if real wastewater had been used.

2.6.7 Use of metabolic un-couplers for excess sludge reduction

As mentioned in section 2.5, there is a direct relationship between the rate of substrate removal and excess sludge production; the higher the rate of substrate removal, the higher the excess sludge production. Several studies have shown that significant reduction in sludge production can be achieved by restricting/limiting sludge growth under the presence of metabolic inhibitors (Low and Chase, 1998; Chen et al., 1999; Strand et al., 1999; Low et al., 2000). Excess sludge reduction could be explained by the theory of energy uncoupling/spilling; which is a discrepancy in the energy balance between catabolism and anabolism. In bacterial cells, growth, replication and maintenance processes are achieved through complex metabolic pathways. Catabolism is a reaction series that reduces the organic compounds and produces free energy in the form of ATP; while anabolism involves the use of a portion of the energy produced during catabolism to synthesize new cell materials. Under normal conditions, catabolic and anabolic reactions are tightly coupled. However, it has been observed that energy requirements for non-growth activities (in particular for maintenance functions) increases in the presence of certain compounds or culture conditions, the energy wastage can be increased by the uncoupling of oxidative phosphorylation, before the ATP is formed, or by energy spilling after the ATP is produced (Liu, 2001). This is believed to reduce the amount of energy available for the synthesis of new cells resulting in less excess sludge production.

Metabolic un-couplers include a diverse group of compounds, most of them lipophillic weak acids, amongst them: paranitrophenol (*p*NP), nitrophenol, chlorophenol, 3,3',4',5-tetrachlorosalicylanilide (TCS), 2,4,5-trichlorophenol (TCP), and cresol. In the case of *p*NP, excess sludge reduction of 49% have been reported (Low et al., 2000), and no sludge production at *p*NP concentrations of 120 mg/L was reported (Low and Chase, 1998). However, the use of such compounds represents many technical and environmental problems such as higher O_2 consumption, the direct introduction of toxic materials to the ASP and concomitantly to the treated effluent, which can reach the aquatic environment with unknown effects. Furthermore, once the microbial population acclimates to the specific un-coupler the sludge reduction effect most likely decreases, which demands a constant exchange of either un-coupler concentration or type.

2.6.8 Use of protozoa and metazoan for excess sludge reduction

Protozoa and metazoans make up approximately 5% of the total dry weight of wastewater biomass and their presence indicates a healthy bacterial population in the ASP. In addition, the low sludge production in trickling filters and rotating biological contactors has been attributed to a great extent to the contribution of predatory organisms (Metcalf and Eddy, 2003). It has been reported that protozoa and metazoan can achieve lower sludge yields by predatory grazing. Lee and Welander (1996), employed protozoa and metazoan to achieve 60-80% decrease in the overall biomass production in an ASP treating pulp and mill effluent. Mixed liquor from the aeration tank was treated in a separate reactor to increase the growth of dispersed bacteria and then fed to another reactor where the protozoa and metazoans metabolized bacterial cells. They reported Y in the range of 0.1 and 0.23 kg TSS/kg COD removed.

Cech (1994) observed a decrease in phosphorous removal efficiency in an ASP laboratory-scale reactor as the protozoa and metazoan numbers increase. It was concluded that the use of predatory activity to reduce the sludge production is not suitable for nutrient removal plants. In addition, implementation of such an approach requires the ASP to be controlled at constant operation conditions to sustain a high population of protozoa, which is difficult to obtain at full-scale operations.

2.6.9 Use of the Oxic-Settling-Anaerobic System (OSA) for excess sludge reduction

The OSA system is a modification of the conventional ASP, which inserts an anaerobic sludge zone in the sludge return line. The insertion of an anaerobic sludge zone in the ASP has already been assayed for controlling filamentous growth and enhance nutrient removal (Metcalf and Eddy, 2003) and more recently for excess sludge reduction (Chudoba et al., 2001; Chen et al., 2001; Chen et al., 2003). In this process, the repeated passage of activated sludge microorganisms through an anaerobic sludge zone or reactor may create conditions of physical stress; hence, a considerable part of energy produced under anaerobic conditions (normally available for biomass growth) would be used for maintenance functions or wasted, which is believed to result in the reduction of excess sludge. Up to 50% reduction in excess sludge production has been achieved without affecting effluent quality and sludge settleability (Chen et al., 2003). Various proprietary technologies currently available in the market make use of this holding step such as the Cannibal Process™, Biolysis E™, which have reported up to 60% excess sludge reduction (Sheridan and Curtis, 2007). In the anoxic/anaerobic holding tank, no additional influent substrate is added and sludge concentration is kept high, as well as the retention time of the recirculated sludge.

2.7 Use of ultrasound technology to improve excess sludge reduction

Ultrasonic waves have been extensively used in industry, signalling, medicine and many other fields including sludge treatment (Thiem et al., 1997; Wang et al., 1999; Thiem et al., 2001) and more recently for excess sludge reduction (Andreottola and Foladori, 2007; Zhang et al., 2007). Ultrasound is a mechanical disruption process; when used at high intensities for sludge disintegration of microbial cells some other compounds in the floc matrix are also broken down into simpler forms due to the generation of oxidizing compounds such as hydroperoxyl ($HO_2^{\bullet}$) and hydrogen peroxide (Adewuyi, 2001). Some of the advantages and disadvantages of ultrasound technology are given in Table 2.8. According to Khanal et al. (2008), the high capital and operation costs might decrease as the technology matures. Hence, ultrasound seems a promising technology as can be observed in the advantages part in Table 2.8.

Table 2.8: Advantages of ultrasound used as a treatment technology for excess sludge reduction at the source (adapted from Khanal et al., 2008)

Advantages	• Compact design • Easy to retrofit within existing systems • Efficient operation • Complete process automation • Potential control of filamentous bulking • Improved VS destruction and dewaterability • Improved biosolids quality
Disadvantages	• High capital cost • High operating cost • Limited long term performance data of full-scale systems

2.7.1 Ultrasound fundamentals

Ultrasound is a sound wave at a frequency above the normal hearing range of an average person (>20 KHz). Figure 2.10 shows the relative frequencies of sound waves, and the range of ultrasound waves most commonly used in environmental studies.

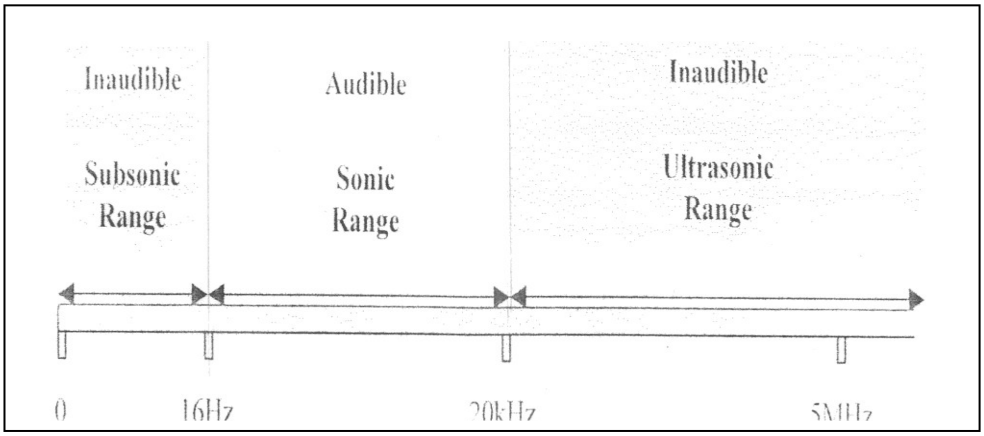

Figure 2.10: Sound waves at different frequencies (adapted form Khanal et al., 2008)

When the ultrasound wave propagates in a liquid medium such as sludge it generates a repeating pattern of compressions and rarefactions called cavitation. Cavitation is basically the formation, growth, and sudden collapse of bubbles in liquids (Adewuyi et al., 2001). In the rarefaction part of the ultrasonic wave; when the liquid is stretched microbubbles form because of reduced pressure. These microbubbles contain vaporized liquid or gas that was previously dissolved in the liquid, and can be either stable about their average size for many cycles or transient when they grow to certain size and violently collapse during the compression part of the wave (Adewuyi et al., 2001; Khanal et al., 2008). According to Adewuyi et al., (2001) once the formed bubbles collapse, they can produce high local temperatures and pressures sometimes above 5000 K and 1000 atm, respectively (Figure 2.11).

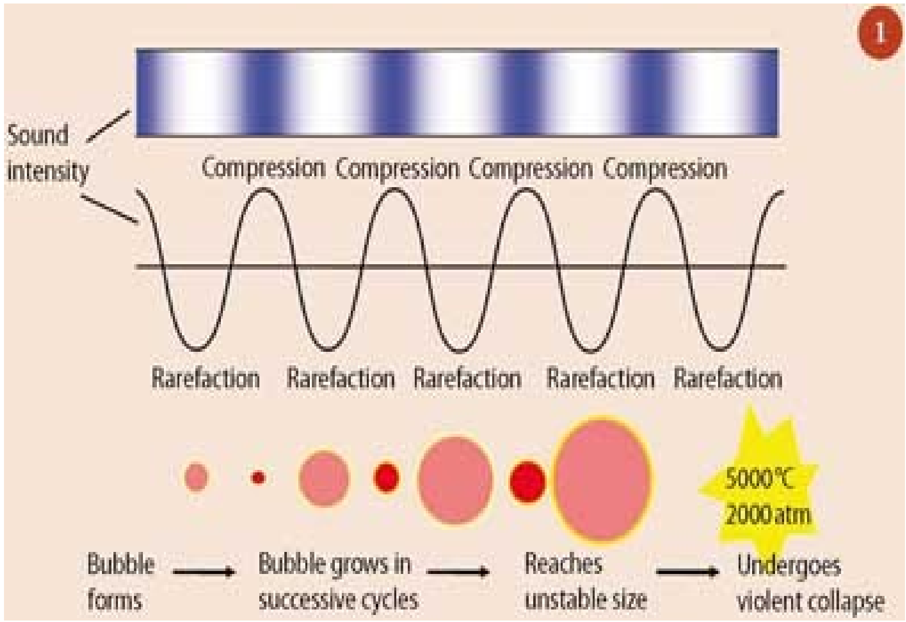

Figure 2.11: Microbubbles collapsing due to cavitation (adapted from http://www.deafwhale.com/strandedwhale/barotrauma.htm)

These conditions even though very short-lived generate powerful hydro mechanical shear forces in the bulk liquid surrounding the bubbles, which can disrupt adjacent bacterial cells or the floc matrix by extreme shear forces, rupturing the cell wall and membranes (Khanal et al., 2008). Also, at high temperatures the lipids in the cytoplasmic membrane are decomposed resulting in holes within the membrane, facilitating the release of intracellular material to the aqueous phase which can be used for cryptic re-growth (Wang et al., 2005).

2.7.2 Delivery and quantification of ultrasound energy

The three major components of an ultrasound (US) system are the converter (the transducer), booster and horn (or sonotrode) (Khanal et al., 2008). Figure 2.12 shows a diagram of a typical 20 kHz lab scale US system with the functions of every part mentioned before. Horn design is considered to be one of the most important factors affecting sludge disintegration, however its design (horn, probe, etc) is often proprietary (Khanal et al., 2008)

In order to determine the efficiency of US technology it is necessary to measure the power (KW) or energy (KJ) input needed to achieve effective sludge disintegration. The most important parameters used in US technology applied to environmental samples are given in Table 2.9.

Table 2.9: Main parameters used in ultrasound technology applied to sludge treatment (adapted from Khanal et al., 2008)

Parameter	Units	Definition
Specific energy input	kWs/kg TS	Energy input per unit of sludge to achieve certain degree of disintegration
Ultrasonic dose	kWs/L	Energy supplied per unit volume of sludge
Ultrasonic density	W/L	Power supplied per unit volume of sludge
Ultrasonic intensity	W/cm^2	Power supplied per unit of converter

2.8 Ultrasound technology applied to excess sludge reduction

US technology has been commonly used in sludge line applications (anaerobic treatment of WAS) in order to improve parameters such as: solids destruction, biogas production, sludge volume reduction and dewaterability, and sludge quality amongst others (Hogan et al., 2004). However, for the specific purpose of excess sludge reduction in the water line of the ASP the literature is still limited.

Zhang et al. (2007) studied the effect of ultrasound on excess sludge reduction in an ASP fed with synthetic wastewater. 3 SBR's with total capacity of 7 L were operated in cycles of 6 hrs (4 h aeration, 1 h for settling and 30 min each for feed and withdraw).

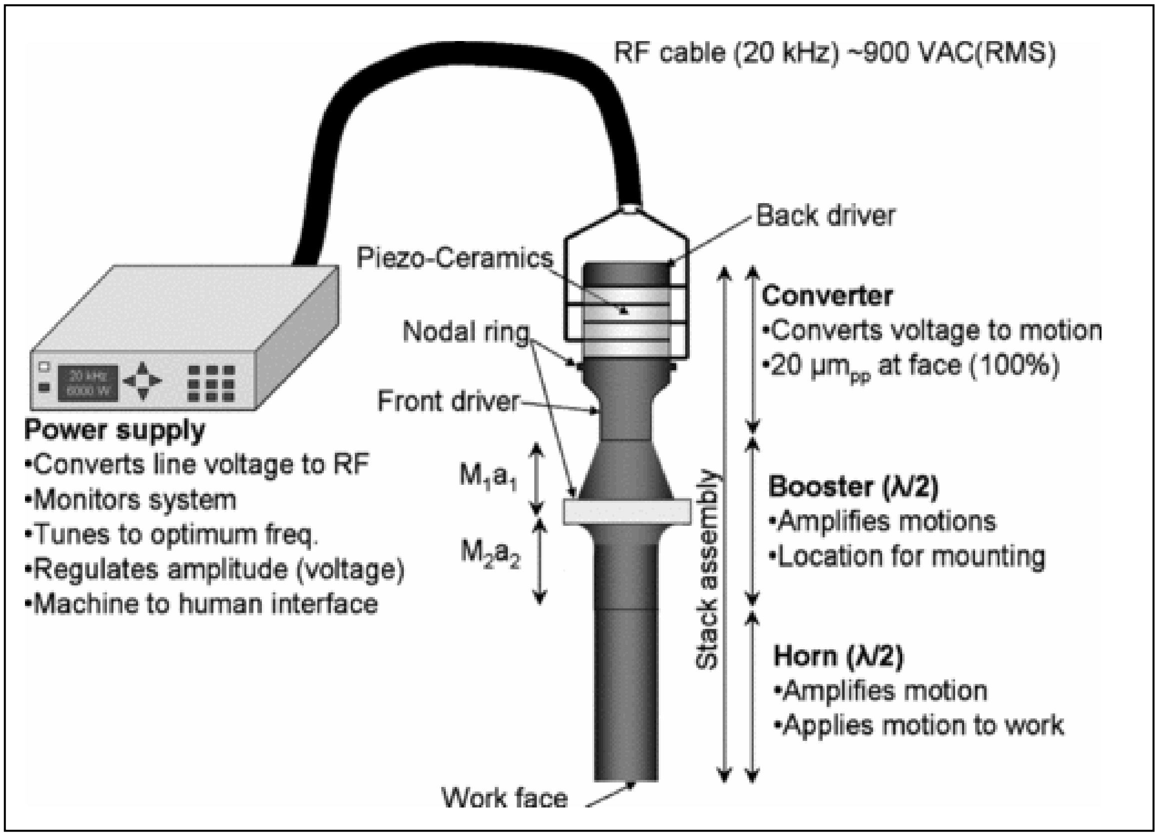

Figure 2.12: Typical laboratory-scale ultrasound system (adapted form Kahnal et al., 2008)

It was reported that ultrasound (25 kHz) effectively liquefied the sludge. The most favourable conditions for excess sludge reduction were sludge sonication ratio of ¾, ultrasound intensity of 120 kW/kgDS and sonication time of 15 min to achieve 91% sludge reduction without affecting the process performance. However, high concentrations of phosphorus were observed in the effluent.

In a comparative study of sludge reduction routes, Ginetest (2007) identified ultrasound as one of the best technologies to achieve sludge reduction. Results from a study conducted at TU Braunschweig (Germany) reported 80% excess sludge reduction. However, the details of the study were not presented.

2.9 Sequencing batch reactor

In the present study it was decided to use the sequencing batch reactor (SBR) to study excess sludge reduction. SBR for wastewater treatment is an ASP that operates in a sequence of fill, react, settle and draw cycles (Mace and Mata-Alvarez, 2002). The unit operations involved in a SBR are equivalent to those in a conventional ASP (aeration, sedimentation), the main difference being that in the conventional ASP those processes take place in different tanks, while in a SBR they occur sequentially in the same reactor. Hence, SBR operates in time rather than space.

Research on SBRs started as early as 1970. The basic scientific assumption that governed the development of SBR technology was that periodic exposure of the microorganisms to defined process conditions is effectively achieved in a batch-feeding system in which exposure time and frequency of exposure can be set independently of any inflow conditions (Wilderer et al., 2001). SBR technology offers a great versatility in mode of operation, since aerobic and anaerobic phases can be implemented depending on the final effluent parameters desired; effluent concentrations as low as 10 mg TSS/L, 5 mg BOD/L and 5 mg total nitrogen/L have been achieved using SBR technology for municipal wastewater treatment (Wilderer et al., 2001). Nowadays SBR technology is gaining a lot of acceptance all over the world due to the combination of the facts mentioned before and relatively less expensive operational costs, compared to continuous processes. Some of the most important advantages of the SBR technology are: simple automation, easiness to modify operational conditions, ability to select robust microbial communities, the capacity to retain treated effluent before release and the ability to adjust aeration, and the selection of the number of tanks in operation to meet loading conditions (Wilderer et al., 2001). However, as with any other technology, inappropriate design and operation will cause the system to fail.

SBR technology has reached an advanced state of development; however compared with the continuous flow systems the knowledge base for SBR during practical situations is still limited.

2.9.1 SBR process description

The SBR process is characterized by a series of phases per cycle, the common phases are: fill, react settle, draw and idle; phases such as fill and react can be modified to attain certain process goals (i.e. they can be anoxic, aerated, mixed, non-mixed). Table 2.10 explains the main characteristics of each period of time within a SBR cycle and Figure 2.13 illustrates the main cycles that take place during a typical SBR application for wastewater treatment. Not all the cycles need to be carried out; the specific goals of the treated effluent dictate the type and number of phases.

The volume of wastewater into the reactor is ΔV_f and is added to the initial fixed volume in the SBR and the volume of solids (MLTSS) V_0; then at the end of the fill phase the reactor contains:

$$V_{\max} = V_0 + \Delta V_f \qquad (2.13)$$

Table 2.10: Main characteristics of time phases in one SBR cycle (Wilderer et al., 2001)

Time period	Main characteristics
Fill	Effluent can be raw or primary settled wastewater, it also can be slow or dump fill, depending on the process goals. Some organics degradation takes place in this period of time
React	Biological reactions which started during fill take place in this portion of the cycle. It can also be modified depending on process goals; usually sludge wasting takes place in this stage. Time dedicated to react can be as high as 50% or more of the total cycle time.
Settle	Solids separation take place in this stage and under quiescent conditions
Decant	Effluent withdrawn takes place in this period of time, the mechanism and time allocated for decant has to be design so that no solids exit with the effluent
Idle	This is the period between draw and fill, this stage can also be used for sludge wasting.

Sludge wasting (ΔV_w) can take place during react or after the clarified effluent (ΔV_d) has been discharged. This completes a cycle and the SBR is then available to start another one.

According to Wilderer et al. (2001), the SBR process is characterized for the typical process parameters as in a conventional continuous ASP such as sludge age, loading rate, and by the following set of parameters:

t_i	time for the *ith* phase
t_f	time for fill
t_c	total time of one cycle [$t_c = \Sigma t_i$]
FTR	fill time ratio, t_f / t_c
VER	Volumetric exchange ratio [$\Delta V_f / V_{\max}$]
HRT	hydraulic retention time [$nV_{\max}Q^{-1}$], where n is the number of tanks

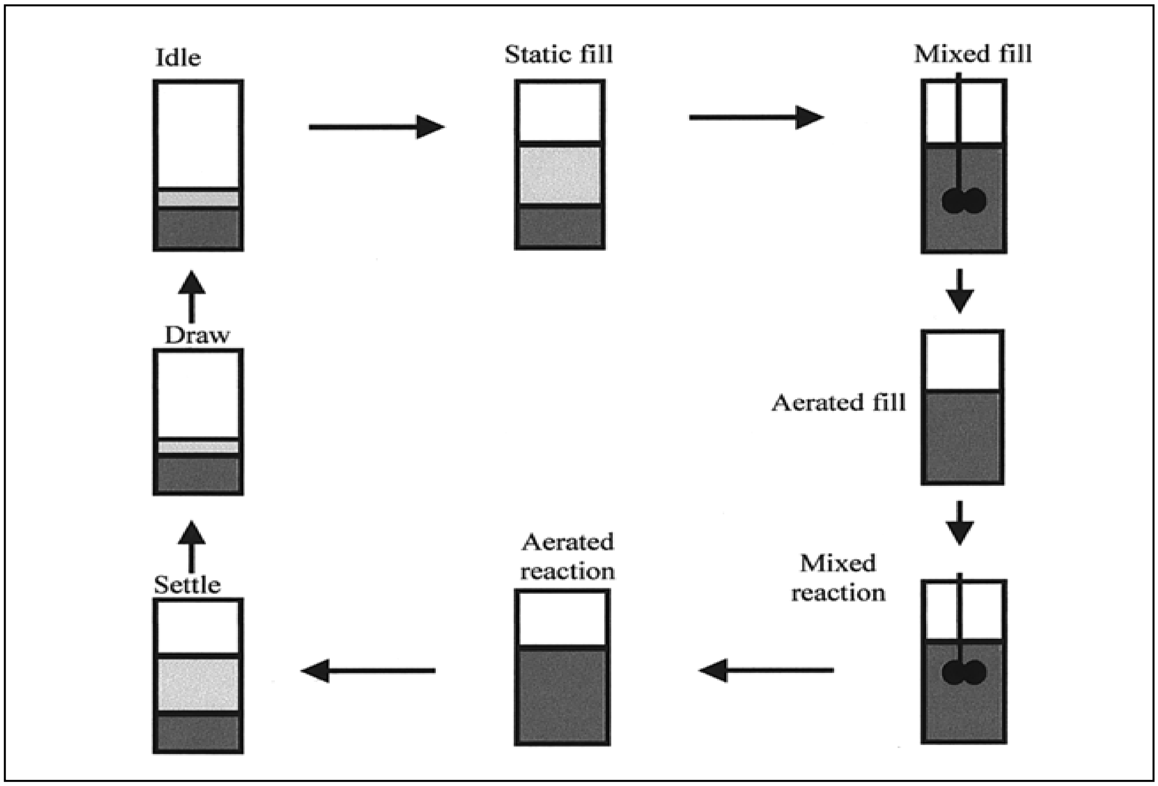

Figure 2.13: Schematic of a SBR operation during one cycle (adapted from Mace and Mata-Alvarez, 2002)

2.9.2 SBR process kinetics

During the react phase of a typical SBR operation for wastewater treatment batch kinetics applies, hence the changes in substrate concentration can be determined by the following mass balance (Metcalf and Eddy, 2003):

$$\frac{ds}{dt}V = QS_0 - QS + r_{su}V \qquad (2.14)$$

Where

$$r_{su} = -\frac{\mu_m XS}{Y(K_s + S)} \qquad (2.15)$$

Since Q = 0 for a batch reaction, the substrate concentration is determined by

$$\frac{dS}{dt} = -\frac{\mu_m XS}{Y(K_s + S)} \qquad (2.16)$$

If equation 2.16 is integrated with respect to time

$$K_s \ln\frac{S_0}{S_t} + (S_0 - S_t) = X\left(\frac{\mu_m}{Y}\right)t \qquad (2.17)$$

In this way by performing a mass balance on the solids in the reactor:

Mass of solids on the full reactor = mass of settled solids

$$V_T X = V_s X_s \qquad (2.18).$$

2.9.3 SBR applications in excess sludge reduction

SBR technology application for the treatment of municipal wastewater has been increasing in the last decades, particularly in Europe and Asia. Mace and Mata-Alvarez (2002) presented an extensive literature review on the use of SBR technology for different effluents, but the major application of SBR is still for the treatment of municipal wastewater, especially in small communities or in places with space limitations. In the particular case of the use of SBR for excess sludge reduction the literature is still limited.

Zheng et al. (2007) studied the effect of the metabolic un-coupler TCP in two SBRs (20 L). They reported that after 90 days of operation and at TCP doses of 2 mg/L the reactors achieved an excess sludge reduction of 47%, compared to control, however the settling characteristics of the sludge decreased with operating time. The SBRs were incubated with aerobic activated sludge from a municipal wastewater treatment plant (Shanghai, China) and fed with municipal wastewater (600–700 mg COD/L). The operating cycle at room temperature was 8 hr (10 min fill, 6.0 hr aeration, 1.5 hr settling, and 20 min for drawing phases). DO and MLTSS concentrations in the aeration tanks were kept at about 4 mg/L and 2,000 mg/L, respectively.

Goel and Noguera (2006) studied simultaneously, excess sludge reduction and enhanced phosphorous removal in a lab scale SBR. They reported 16-33% less excess sludge production after 120 days of operation. The two SBRs (2 L) were operated with 4 cycles/day, consisting of three stages. Stage I was a 2-h anaerobic period that included the input of synthetic wastewater 5 min after the initiation of the stage. Anaerobic conditions were maintained by constantly bubbling the mixed liquor with nitrogen gas at a rate of 1 L/min. Stage II was a 3-h aerobic react period during which the mixed liquor was constantly supplied with air (1 L/min) to maintain dissolved oxygen concentrations above 5 mg/L. Stage III corresponded to 55 min of settling followed by a 5 min decant period. The pH of the mixed liquor was maintained at 7.0±0.2 by adding acid or base using an automated pH controller. At the end of each cycle, 670 mL of supernatant were decanted from the reactor, and an equal volume of synthetic wastewater was provided at the beginning of the next cycle, which corresponded to 18 h HRT.

2.10 Summary from the critical literature review

From the reviewed literature it is clear that through US treatment, excess sludge minimization during municipal wastewater treatment can be achieved. However, the US mechanisms responsible for such an effect are not clearly understood. And although some studies have suggested that the main mechanism is the production of a soluble substrate released by lysis of the portion of sludge pretreated and recycled to the aeration basin for cryptic re-growth (Wei et al., 2003; Strunkmann et al., 2006); others have considered that the main mechanism triggered by US may be enhanced maintenance metabolism (Rai et al., 2004; Cao et al., 2006). Therefore, there is a need for research that can help elucidate the mechanisms that trigger excess sludge minimization when US treatment is applied. This would allow for an optimal design and application of US technology at pilot and full-scale applications. In a comparative evaluation of sludge reduction routes, Ginestest (2007) raised a series of questions for the many different approaches to minimize excess sludge:

- Biomass metabolic behaviour: how will biomass react under the specific pretreatment conditions?
- Biomass population changes and diversity: how will the biological communities shift upon treatment conditions?
- Process efficiency: how will the quality and quantity of residual sludge change? How will the effluent quality change? Can a modeling approach predict process performance?

If the very same questions were to be asked exclusively regarding ultrasound technology, for excess sludge minimization at the source, the current literature would still not able to answer them. Unfortunately, US technology for excess sludge minimization is still a topic of debate especially if integrated in an OSA process. In addition, there is still a lot of contradiction amongst the published studies regarding the potential of US technology for excess sludge production at the source or the proposed methodologies although reproducible at laboratory scale are not attainable at pilot or full scale applications. Finally, the current literature review also suggested that more research is needed with real municipal wastewater, as some of its constituents highly affect the amounts of total solids produced, which are usually lacking in synthetic wastewaters. The use of synthetic wastewater likely leads to an overestimation of the real potential of US technology for excess sludge minimization.

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Chapter 3

Effect of Ultrasound Specific Energy Input and Solids Concentration on Dewaterability of Raw Waste Activated Sludge

Juan Marin and Kevin J. Kennedy

3.1 Abstract

This manuscript presents results on the effect of low frequency ultrasound (US) treatment on raw waste activated sludge (WAS) dewaterability. WAS dewaterability was shown to be dependent on sample total solids (TS) concentration and US specific energy (ES) input. At US ES inputs lower than 56 KJ/gTS capillary suction time (CST) increased on average 85% for all WAS concentrations tested as compared to the control WAS, indicating potential deterioration in dewaterability. At higher ES inputs (>118 KJ/gTS) WAS solubilization increased and particle size decreased but the CST did not improve respect to the control but it was on average 11% lower than at 56 KJ/gTS suggesting that at 56 KJ/gTS was the worst condition for dewaterability. Particle size decreased with increased ES input, but the concentration of particles/mL in the size range of 1 to 227 μm increased on average 700% at ES inputs of 11 and 56 KJ/gTS, then decreased to 200% at ES inputs higher than 118 KJ/gTS compared to the non-sonicated WAS control as solubilisation dominated over particle disruption at the higher ES inputs. It is believed that the increased number of small and most likely charged particles produced at 11-56 KJ/gTS interacted at the filter pore interface causing clogging and decreasing the water flux which had a detrimental effect on the CST determination. On the other hand, assessment of WAS dewaterability by the direct centrifugation method showed increased solids capture and bound water content up to 25% on average for all TS concentrations tested compared to the control, indicating an overall improvement in the liquid-solid separation for all US treatment conditions assayed. CST determination combined with treatments that result in WAS solubilization and particle reduction is an unreliable stand alone technique to estimate dewaterability. Only direct methods such as centrifugation should be used alone or in combination with CST to assess the extent of emerging treatment technologies on WAS dewaterability.

Keywords CST, dewaterability, floc structure, ultrasound, WAS

3.2. Introduction

Excess waste activated sludge (WAS) management is one of the greatest challenges the wastewater treatment industry now faces. Stricter effluent quality discharge regulations combined with new sewer expansions required by economic development and population growth, will lead to increased WAS production despite tighten environmental regulations regarding its final disposal (Valexaire et al., 2008). WAS contains in excess of >98% water (by weight) combined with inorganic particles, microbial biomass, extracellular polymeric substances (EPS) and multivalent cations (Neyens et al., 2004). While the specific physicochemical interactions between these components are still not well understood, they do result in the cohesive properties needed for WAS flocculation and sedimentation. Concomitantly, these same properties result in inherently poor WAS dewaterability, even with the addition of polymers. Water content as high as 95% still remains in the thickened WAS (TWAS) after thickening and dewatering operations (Yin et al., 2006). Dewaterability depends on many specific WAS characteristics such as protein (PN), polysaccharide (PS) contents, PS/PN ratio in EPS, pH, viscosity, particle size, volatile to fixed solids (VS/FS) ratio, surface charge and type of wastewater treated (King and Foster, 1990; Higging and Novak, 1997; Novak et al., 2003; Guan et al., 2003; Erdincler and Vesilind, 2003; Pinjing et al., 2009). Hence, due to the high number of variables and combined interactions involved, a mechanistic model predicting WAS dewaterability is unreliable and consequently empirical approaches have been found to be of more practical value at full-scale applications (Feng et al., 2009).

WAS dewaterability is one of the least understood unit operations in wastewater treatment; paradoxically it is also the bottle-neck of handling and costs associated with its final disposal which can be as high as 50% of a wastewater treatment plant operation and maintenance (Andreottola and Foladori, 2006). Skidmore (2005) reported that a two fold increase in solids content in dewatered cake would reduce WAS landfill disposal costs by almost 50%. Furthermore, sludge volume reduction can also make a significant contribution towards lowering anaerobic digestion implementation costs by decreasing digester volume. Ultrasound (US) is a mechanical disruption technology that has been applied for WAS and TWAS disintegration at full-scale wastewater treatment plants. US uses cyclic sound pressure at frequencies >20 kHz, to produce microbubbles that grow to unstable sizes then collapse violently producing powerful shear forces, high localized temperatures and pressures able to disrupt microbial cell walls (Khanal et al., 2007). US is believed to enhance WAS dewaterability by disrupting the EPS-cell interface and breaking microbial cell walls and membranes releasing bound water from the WAS matrix (Fleming and Wingender, 2001; Wang et al., 2006). Among the various advanced sludge treatments (AST) proposed to enhance WAS dewaterability (thermal and thermo-chemical hydrolysis, advanced chemical oxidation, stirred ball mill and high pressure homogenizer disintegration) US has a number of specific advantages that may make it more suitable for full-scale applications: it can be introduced into the wastewater treatment process without major modifications to the plant infrastructure and no secondary by-products or toxic compounds are known to be produced (Feng et al., 2009). However, the role of US on WAS dewaterability is contradictory. While improved WAS dewaterability after US treatment has been reported (Erdincler and Vesilind 2003;

Skidmore 2005; Feng et al., 2009) its deleterious effect is also indicated in related literature (Dewil et al., 2006; Wang et al., 2006; Zhao et al., 2008). According to Dewil et al. (2006) EPS and cell wall disruption from US treatment would increase free water content which should improve dewaterability; however, simultaneously the US energy also produces a greater distribution of smaller particles as a result of floc size reduction. Fine particulates cause the clogging of capillary suction time (CST) filtering paper which would increase filtering time indicating a negative impact on WAS dewaterability. Since the CST method was not developed for WAS treatment dewaterability scenarios, as such it may not be well suited for such studies. Given that no other AST has provided such contradictory results more research is needed to help elucidate mechanisms that might have a major impact on raw WAS dewaterability after US treatment.

The main objectives of the present study were to investigate the effect of US specific energy input (ES) on raw WAS dewaterability, in order to gain insight into the relationship between floc disruption, solid-liquid separation and filterability of raw WAS over a range of sample's total solids concentration (TS).

3.3 Materials and methods

3.3.1 Raw waste activated sludge

To avoid the influence of polymer-WAS interactions, raw WAS samples were obtained directly from the aeration basin at the Robert O. Pickard Environmental Center (ROPEC, Ottawa, ON) prior to any additive additions. ROPEC is one of the largest wastewater treatment plants (WWTP) in Canada, treating approximately 120 MGD of municipal wastewater (City of Ottawa, 2009). Primary influent is treated in a conventional plug flow aerobic waste activated sludge plant (ASP) operated at an average sludge retention time (SRT) of 5 days, indicative of a relatively young activated biomass sludge. WAS samples were collected in plastic containers on random days during the morning hours (7h00-8h30 a.m.) and transported immediately to the laboratory. Samples were then concentrated in a 20 L sedimentation tank at 4 ºC for 4 h and different WAS dilutions (from 0.15% to 2.5% total solids (TS) w/w) were prepared for US treatment. To avoid deterioration due to anoxic conditions and endogenous cellular activity WAS samples were kept no more than 2 days. Table 3.1 shows the main characteristics of WAS samples, as collected and after sedimentation.

Table 3.1: Raw WAS characterization

Parameter	raw WAS as collected	raw WAS sedimented
pH	6.7 ± 0.2[1]	6.5 ± 0.1
Total solids (% w/w)	0.16	2.9 ± 0.3
Volatile solids (%w/w)	0.11	1.8 ± 0.2
Total COD (mg/L)	1456 ± 137	35000 ± 253
Soluble COD (mg/L)[2]	198 ± 10	1200 ± 90
Alkalinity (mg $CaCO_3$/L)	207 ± 21	1230 ± 20
NH_3-N (mg/L)	31 ± 16	147 ± 28

[1] Data indicates the mean value ± standard deviation from 3 replicates
[2] Filtered sample (<0.45 µm)

3.3.2 Experimental approach

In related literature it has been suggested that one of the most critical parameters when applying US treatment (i.e. solubilization, enhanced anaerobic digestion or dewaterability) is raw WAS total solids (TS) concentration (Dewil et al., 2006; Gonze et al., 2003; Erdincler and Vesilind, 2003). Furthermore, most US treatment studies deal with thickened WAS (TWAS > 4% TS) in order to enhance anaerobic digestion. However, the effect of US treatment on raw WAS at dilute concentrations to enhance dewaterability has received little attention. In this study the effect of US specific energy (ES) input (10, 56, 118, 183 and 257 kJ/gTS) on raw WAS dewaterability was investigated under a wide range of dilute solids concentrations (0.15, 0.2, 0.25 and 0.3% TS) which are in the range commonly found in conventional ASP basins and two higher concentrations typically found after WAS sedimentation (1.2 and 2.5% TS). After samples sonication various parameters including particle size distribution, filterability, compactability and multivalent cations content were evaluated.

3.3.3 Apparatus and ultrasound treatment

Ultrasound treatment was performed with a bench scale ultrasound system Branson 102-CE (Branson Ultrasonics Corporation, USA, 20 kHz, tip surface area of 2.12 cm^2, peak power output of 276 ± 72.29 W). ES input was calculated according to equation 3.1 (Khanal et al, 2007).

$$ES = \frac{P * t}{V * TS} \tag{3.1}$$

Where P is the ultrasonic power (KW), t the ultrasound time (sec), V the sample volume (L) and TS the total solids concentration (g/L). It is important to mention that in the present study the ES input was reported as KJ/gTS instead of KJ/kgTS only to avoid the length in the reported numbers. ES input was maintained relatively constant by varying US intensity and sonication time at the different TS concentrations.

For US treatment, 500 ml raw WAS were placed in a 600 mL glass beaker; the ultrasonic horn was centered and immersed to a fixed depth of 3.5 cm below the air-water interface inside the beaker. During sonication the beaker was place in an ice bath to minimize temperature increases which were only observed at higher ES inputs. US treatment was performed in duplicate.

3.3.4 Dewaterability determination

After US treatment raw WAS dewaterability was determined using two methods: CST and a centrifugal method. CST determination was based on Standard Method 2710G (APHA, 1998), using a capillary suction timer (Model 440, Fann Instrument Co., TX, USA) with an 18mm sludge reservoir and chromatography grade paper number 17 (Venture Innovation, INC, Lafayette, LA, USA). In order to maintain the same humidity through CST measurements the paper was kept in desiccators at room temperature. For the CST test, a 5 mL sample was poured into the reservoir with a modified open mouth syringe, a digital timer indicated the time (sec) required for the water released from WAS to travel between two contact points on the chromatography paper. The CST for distilled water (3 ± 1 sec) was measured randomly during the present experiment and its value subtracted from the WAS CST. CST Measurements were performed in quadruplicate.

WAS moisture distribution and compactability were determined by a modified centrifugal method based on Skidmore (2005) using a Sorvall Legend^{TM} T_+/RT_+ Thermo Scientific tabletop centrifuge (Fisher, Canada). 50 mL samples were placed in pre-weighed centrifuge tubes and centrifuged at ~8000g for 1 hr. After centrifugation the supernatant was decanted and the tubes re-weighed, and by difference the volume of free water in grams was determined. The remaining WAS cake pellet was carefully removed and its water content determined by standard evaporation technique at 105 ºC. Water associated with the pellet was assumed to be the WAS bound water (percentage of water content per gram of WAS cake). Total water content was determined by the addition of free and bound water. Measurements were performed in duplicate.

3.3.5 Particle size distribution determination

Particle size distribution was measured using a Dynamic Particle Analyzer (DPA) 4100 series B (BrightWELL Technologies INC, Ottawa, Canada); equipped with an optical system, low magnification flow cell with particle detection in the range of 1 to 400 μm. DPA 4100 measured the size, shape, transparency, number count and concentration of liquid borne particles. A special 1 mL pipette tip (CPL model Neptune BT 1000, BrightWELL Technologies INC, Ottawa, Canada) was used for sample injection. The diluted sample volume was dispensed at a flow rate of 0.35 mL/min and analyzed for particle count (particles/mL) and mean particle size expressed as equivalent-circle-diameter- ECD (μm). ECD is the diameter of a circle that has the same area as the projected particle image, which allowed evaluating various irregularly shaped particles on the basis of a single consistent measurement. Due to the intrinsic non-circularity and constant breaking of the biomass floc, ECD is a more reliable unit to measure floc size reduction and fragmentation. Particle size analyses were performed immediately after sonication in duplicate.

3.3.6 Analytical methods

Sample analyses for control and US treated WAS were carried out at room temperature. pH was measured using a Fisher Accumet excel pH meter, XL 25 series (Fisher, Canada). TS, total suspended solids (TSS) and total dissolved solids (TDS) were measured in duplicate according to Standard Methods 2540B, 2540D and 2540C respectively (APHA, 1998). Total chemical oxygen demand (tCOD) measurements were performed on duplicate, based on the colorimetric Standard Method 5250D (APHA, 1998) using a Coleman Perkin–Elmer spectrophotometer model 295 at 600 nm light absorbance.

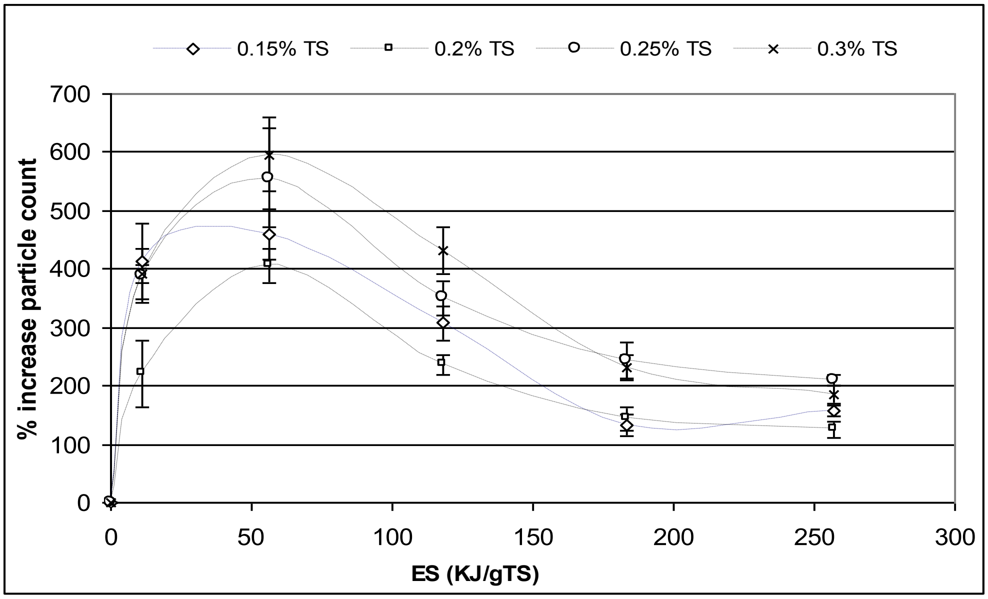
For soluble COD (sCOD) determination, US treated WAS and control samples were filtered through 0.45 μm nitrocellulose membrane filters (Fisher, Canada) prior to COD determination. Alkalinity was determined according to Standard Method 2320B (APHA 1998). Ammonia (NH_4^+) concentration was determined with an Orion ammonia electrode (model 95-12) connected to a Fisher Accumet® model 750 pH/ion meter (Fisher Sci., Ottawa, ON) according to Standard Method 4500D (APHA 1998). Concentration of multi-valent cations in samples was conducted in a nationally accredited third party laboratory (Exova-Accutest, www.exova.ca, Ottawa, Canada) using ICP-MS according to reference method AMMICPE0 M SM3120B-3500C. Cation determination was performed a total of 3 times during WAS US treatment, on both the supernatant and the WAS flocs.

Statistical analysis was performed using Minitab 15 (Minitab Inc., USA)

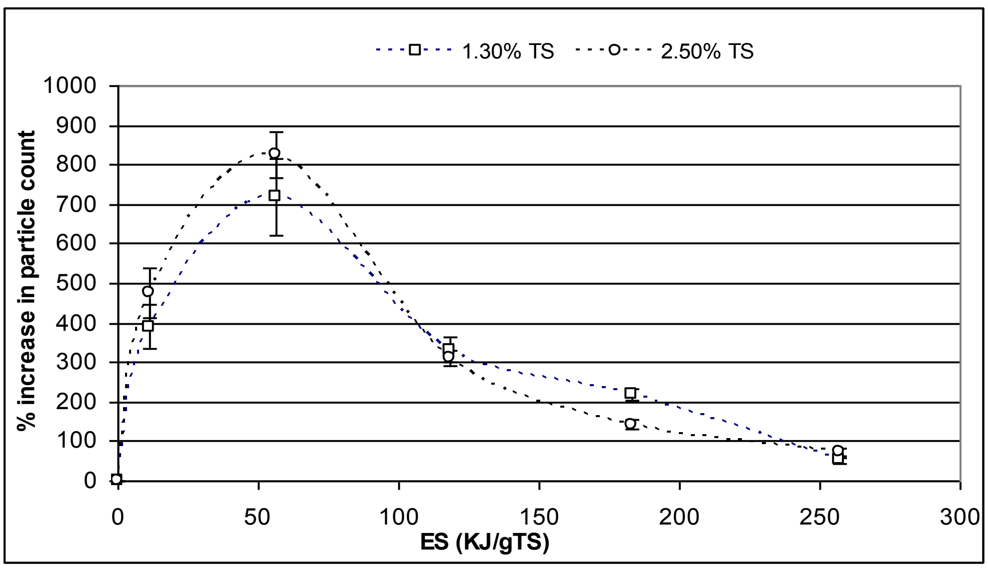
3.4 Results and discussion

3.4.1 Effect of ultrasound on floc size reduction

In the present study, and as reported in previous research (Show et al., 2007; Dewil et al., 2006; Gonze et al., 2003; Erdincler and Vesilind, 2003; King and Foster, 1990) US treatment resulted in a diminution of raw WAS particle size. This decrease in floc size concomitantly inferred an increase in particle numbers with increasing ES input. In general, as ES input increased in the range of 11 - 183 KJ/gTS, WAS ECD decreased from an average 9.5 μm to around 3.3 μm, independently of solids concentration. Additional ES input increase to 257 KJ/gTS resulted in only a marginal 1.3 ± 1% further reduction in floc ECD for both high and dilute WAS concentrations studied. These results suggested that for the WAS used in this study, a threshold ES input existed after which additional WAS disruption remained fairly constant and independent of TS concentration. Also, this result would have suggested that the maximum particle count exists once the threshold is achieved. However, based on particle count analysis a contradictory finding was determined. In Figure 3.1 the percent increase in particle count in the size range of 1 to 227 μm for this study is presented. It can be observed that for both dilute and concentrated WAS samples the number of particles in that particular size range started to increase considerably after ES input of 11 KJ/gTS to reach a maximum count at 56 KJ/gTS. Further ES increase to 118 and 183 KJ/gTS resulted in the number of particles in the same size range decreased and levelled off at the highest ES. The %particles increase at a given ES was greatest for the WAS with higher concentrations. A one-way ANOVA (Table 3.2) followed by Tukey's honestly significance difference (HSD) test were used to identify if there was a significant difference between ES inputs and particle numbers increase. From Table 3.2 it can be observed that the $F>F_{crit}$, which indicated that the results were significantly different at $p< 0.05$. In addition, the Tukey's 95% simultaneous confidence intervals indicated that the results for 56 KJ/gTS are significantly higher than those of control and the rest of the experimental conditions tested, suggesting that the optimum condition to produce greatest increase of particles in the size range of 1 to 227 μm (Figure 3.1b) are achieved at this ES input.



a)



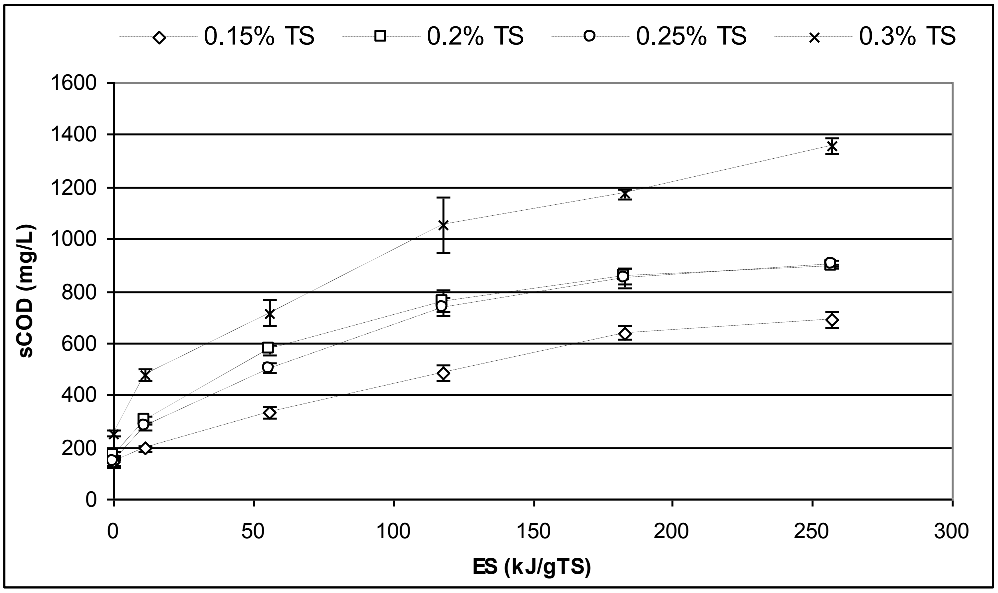
b)

Figure 3.1: Percent (%) increase in particle numbers compared to control in the size range from 1 to 227 µm. (a) Data is presented for dilute WAS (b) for sedimented WAS, in both cases data represent the mean value and error bars the standard deviation from six replicates. Data points are joined to indicate the trend

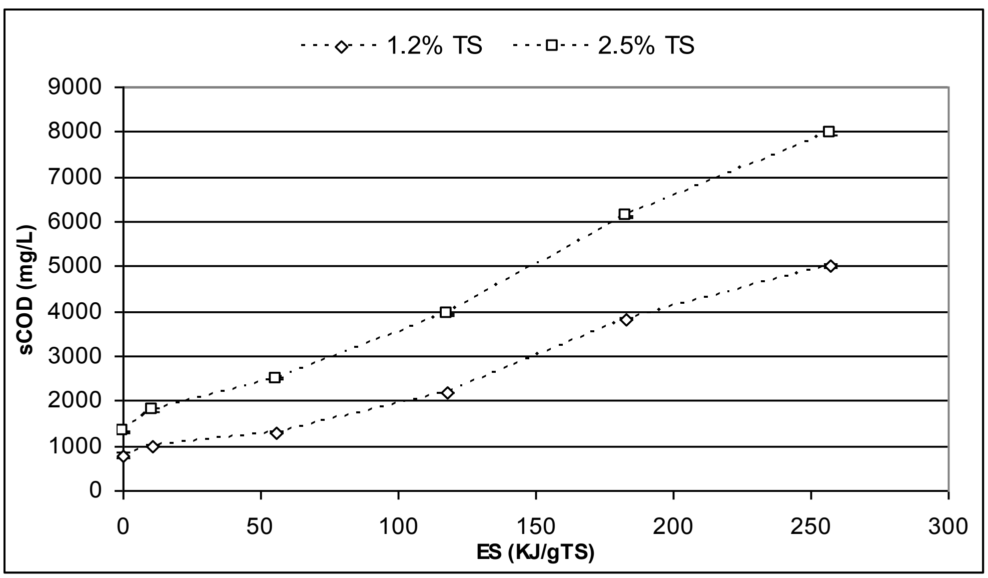
Table 3.2: One-way ANOVA for particle numbers analysis

Source	DF	SS	MS	F	P	F_{crit}
Factor	5	$3.4X10^{11}$	$6.9X10^{10}$	13.65	0.000	2.53
Error	30	$1.5X10^{11}$	$5.05X10^{9}$			
Total	35	$4.9X10^{11}$				

The results obtained in the present study are in contradiction with those presented by Show et al. (2007) in which, it was concluded that the most vigorous particle disruption occurred in the initial sonication period. After only 1 minute sonication, sludge particle size decreased by 78% compared to control and remained almost constant thereafter. Despite the differences in sonication measurement, equipment used and type of sample, such an effect was not found elsewhere in current literature. Results shown in Figure 3.1 indicated that at lower ES inputs (11 and 56 KJ/gTS) US energy was not sufficient to disrupt the flocs completely, but suggested that it eroded its perimeter contours, generating a more uniform floc size, thus higher particle numbers with a maximum at 56 KJ/gTS. It is believed that the decrease in particle numbers observed for ES input in the range of 183- 257 KJ/gTS compared to control and the other ES inputs was due to more severe disruption of the WAS resulting in total floc breaking and solubilization (particles < 0.45 μm). Figure 3.2 shows the effect of ES on WAS solubilization (measured as sCOD, mg/L). From Figure 3.2 it can be observed that WAS solubilization increased almost linearly with ES input. Corroborating the effectiveness of US pretreatment for WAS disruption at higher ES inputs and TS concentrations. The linear increase in solubilization with ES is clearer with the concentrated WAS samples as opposed to dilute samples where more of the US energy it is believed to be dissipated in the liquid phase and the contact between a bubble and a floc are less likely to occur (Dewil et al., 2006; Gonze et al., 2003).



a)



b)

Figure 3.2: Effect of US treatment on raw WAS solubilization for (a) dilute WAS and (b) sedimented WAS. Data represent the mean value and error bars the standard deviation from six replicates. In (b) errors bars are too small to be observed.

The following linear equation (obtained from the best subsets regression function in Minitab15, R^2-adj = 0.72) relates the sCOD concentration for a given ES (KJ/gTS) input and TS (%w/w) concentration for the WAS used in the present study. Due to the differences in WAS characteristics with sludge age, equation (3.2) is specific to the WAS used in the present study, but may have implications for young WAS (5-7d SRT).

$$sCOD = 8.8[ES] + 1497[TS] - 568 \qquad (3.2)$$

Based on the floc size reduction, the extent of WAS disruption can also be estimated by the ratio of the mean ECD at the different ES inputs (ECDt) to that of the control (non-sonicated) ECD_0.

$$ECD_d = \frac{ECD_t}{ECD_0} \qquad (3.3)$$

According to Raman and Abbas (2008) the degree of particle size reduction (determined in the present experiment as ECD_d) with ES input can be used to determine the rate of floc disruption according to:

$$K_d\, \delta\, t = 1 - ECD_d \qquad (3.4)$$

Where K_d is the rate of disruption (s^{-1}), δ is the amplitude ratio (dimensionless, applied amplitude/maximum equipment amplitude), t is the average sonication time (sec).

In the present experiment, US amplitude and time were adjusted in order to obtain a constant ES input, by taking the average values of both (δ ~0.8 and t ~1, 5, 10 15 and 20 min) it was possible to determine the extent of WAS disruption for the different TS concentrations tested. Figure 3.3 shows the average WAS K_d as a function of ES input for the four dilute WAS samples and the two sedimented WAS samples.

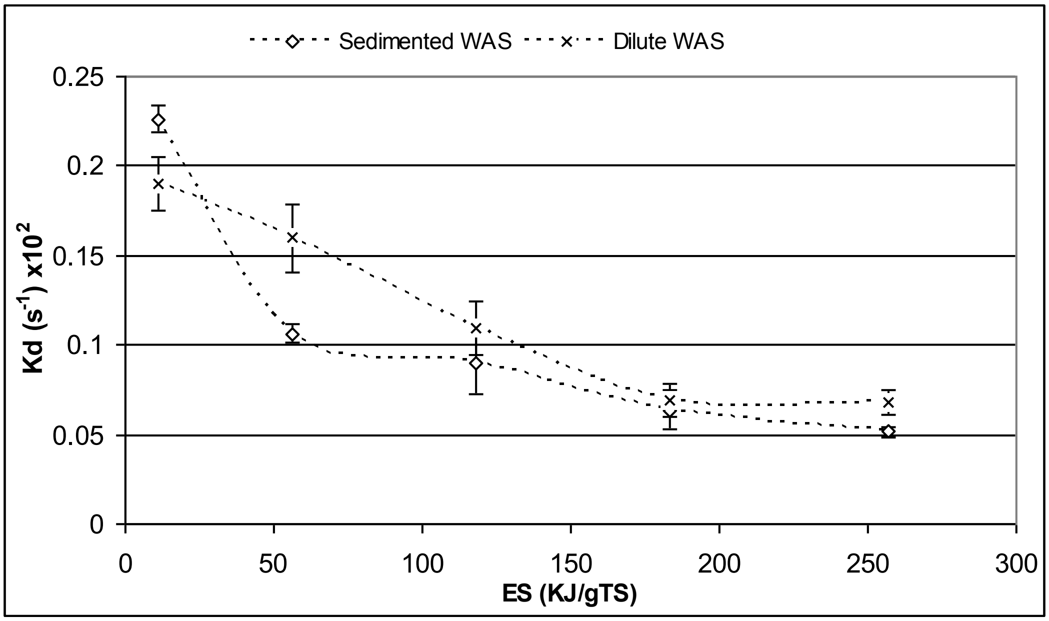


Figure 3.3: Raw disruption rate during sonication. For the dilute WAS data represents the mean value and error bars the standard deviation from the 4 solids concentrations tested, while for sedimented WAS data represents the mean value and error bars the SD from the two concentrations tested.

From figure 3.3 it can be observed that in both cases a decrease in floc particle size occurred up to ES inputs of 56 KJ/gTS, the fact that the disruption rate levelled-off for ES inputs > 118 KJ/gTS would indicate that floc solubilization was the main mechanism, which was also corroborated from Figure 3.2. Also from Figure 3.3 it can be observed that the TS concentration had a significant impact on WAS disruption. As WAS TS concentration increased the floc disruption rate also increased, indicating that the best conditions to enhance raw WAS dewaterability (enhanced bound water release) are at ES input higher than 118 KJ/gTS. While lower ES lead to the production of high number of particles but low solubilization. These experimental results add more weight to the claim that US pretreatment is enhanced at higher sample TS concentrations. Although, the degree of sample disruption, are specific for the WAS and Branson sonifier used in this experiment. However, this approach could be used to compare WAS disruption between different apparatus.

3.4.2 Effect of ultrasound on WAS dewaterability as measured by the CST method

Figure 3.4 shows the effect of ES input on the standardized CST for the WAS sample at the various TS concentrations tested. From Figure 3.4 it can be observed that for all WAS concentrations treated, CST increased compared to controls, suggesting that US treatment had a deleterious effect on WAS dewaterability.

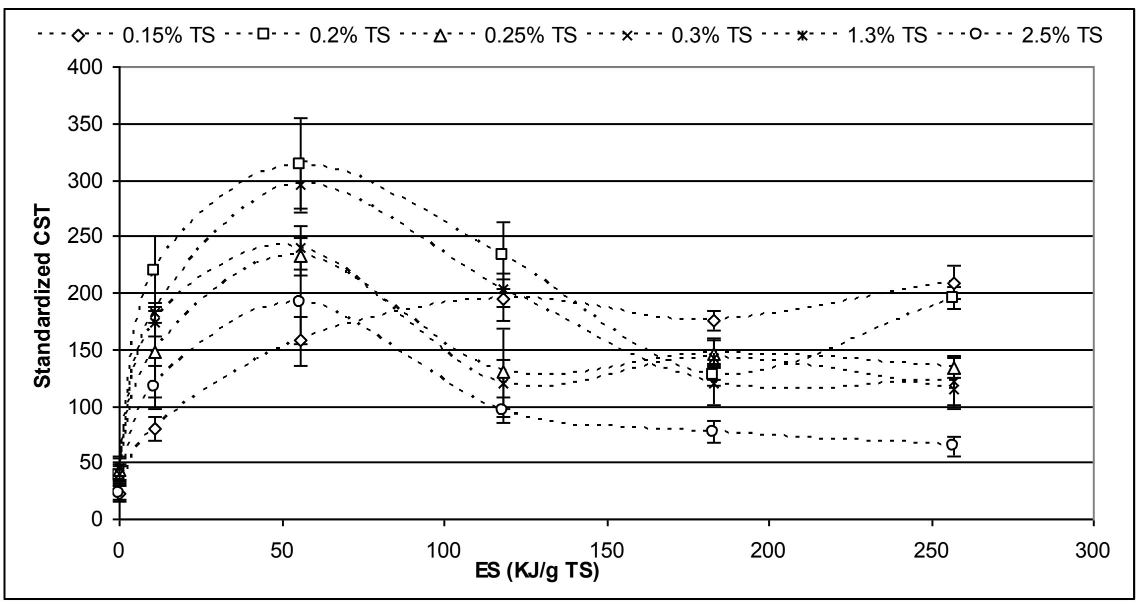


Figure 3.4: Effect of ES on standardized CST. Data represent the mean value and error bars the standard deviation from twelve replicates

Previous studies with TWAS have suggested that dewaterability deterioration increased with higher sonication times or applied ES. Such results were believed to be due to a substantial increase in fine particles which clogged the CST filtration paper (Skidmore, 2005; Wang et al., 2006; Dewil et al., 2006). In the present study a proportional increase in CST with greater ES input was not observed. CST increased and reached maximum values at 56 KJ/gTS, but declined as ES was increased to 118 KJ/gTS. At higher ES inputs up to 257 KJ/gTS the CST for each TS concentration tested stabilized at approximately 40-60% of the maximum CST achieved at 56 KJ/gTS. The results from the CST assay tend to correlate with particle number counts (Figure 3.1); the highest particle numbers were achieved at 56 KJ/gTS then diminished with increased ES input. Higgins and Novak (1997) reported that supracolloidal particles in the 1-100 µm range had the most adverse effect on sludge dewaterability (measured as RFT or CST). In the present study it was observed that particles in the size range of 1 to 240 µm correlated with high CST values ($R^2 = 0.92$) corroborating that the high number of fine particles was responsible for the increased CST, most likely due to CST papers clogging. In addition, Liao et al. (2001) indicated that the steric forces arising from EPS physically prevent the cells from close contact. Activated sludge flocs contain between 70-80% of its organic content as proteins and polysaccharides. Such compounds are believed to contribute significantly to the water binding capacity of the floc matrix (Dignac et al., 1998; Jin et al., 2004). Although it has been observed that high concentrations of proteins in the bulk water have a detrimental effect on WAS dewaterability (measured by CST and RTF) it is the high proteins/carbohydrates proportion rather that the total amount of EPS which is more important for WAS surface charge determination. Figure 3.5 illustrates the total protein/sugars (TPN/TS) ratio determined during the present experiment after WAS sonication. It can be observed that in general, the TPN/TS ratio remained almost constant, and at higher ES inputs greater release of proteins and sugars to the bulk. Similar trends have been observed in other studies (Feng et al., 2009). In the present study however, WAS dewaterability (CST) did not worsen after ES inputs > 118

KJ/gTS, but actually improved as compared to 56 KJ/gTS. These results suggested that at ES of 11 and 56 KJ/gTS WAS negative surface charge increased due to floc fragmentation, which in turn increased the electrostatic repulsion among WAS particles (i.e. more particles per mL) having a direct deleterious impact on the CST paper. While, at ES inputs > 118 KJ/gTS and due to floc total disruption, solubilization, and proteins denaturation the same effect was lower.

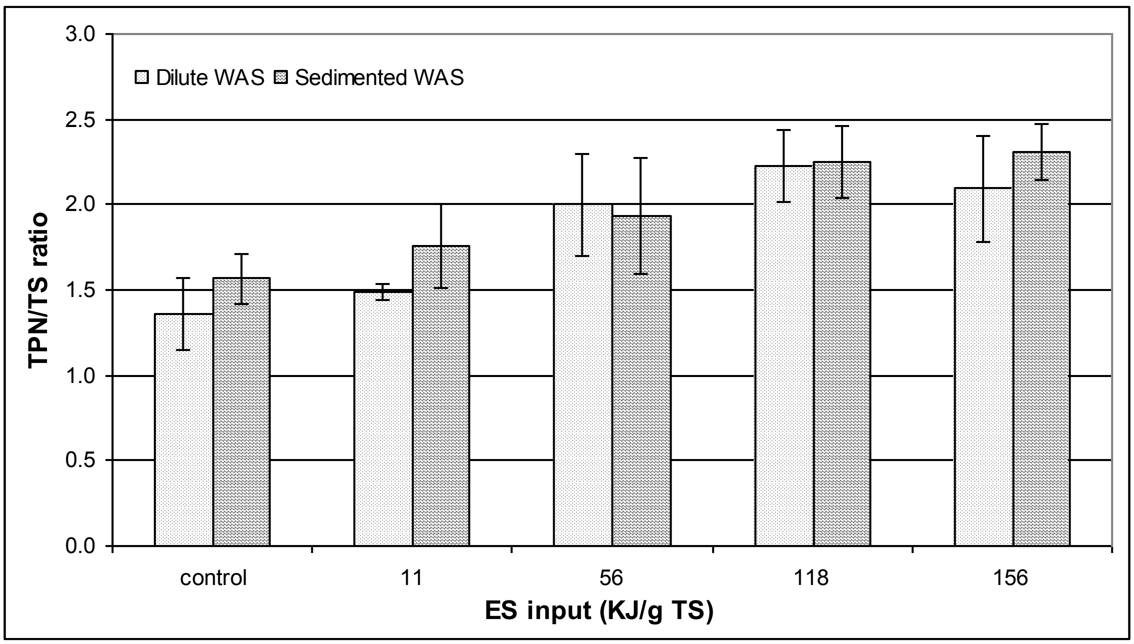


Figure 3.5: TPN/TS ratio during WAS sonication. Data presented are the mean value ± standard deviation between 6 measurements.

Although the rate of water release from a sludge can be quantitatively measured by the CST method, these results would suggest that the CST method if taken alone may underestimate the effect of US on raw WAS dewaterability and alternative methods alone or in conjunction with the CST should be used to provide unbiased results on the effect of specific emerging treatment technologies on sludge dewaterability, specially because many physical-chemical treatments inherently enhance floc disruption. Closer inspection of the results tends to indicate that as WAS concentration increases the increase in CST at a constant ES tends to be inversely proportional to the concentration. In fact for the lowest WAS concentration evaluated (0.15%) the CST increased with increased ES did not show a maximum at 56 KJ/gTS. This would suggest that a threshold concentration may exist that influences the CST response for the given treatment technology.

3.4.3 Effect of ultrasound on raw WAS dewaterability as measured by a centrifugal method

Sludge dewaterability has been defined either as the rate of free water filtration or as the volume of residual bound water after thickening and dewatering (Jin et al., 2003). Usually the CST has been the most common method used for sludge dewaterability determination. This empirical method is easy to perform, cheap and it is considered a good index of sludge filterability, which is a predominant parameter that controls the output of various types of dewatering equipment such as belt presses, filter presses and vacuum filters (Scholz, 2005). However, CST does not provide information on WAS water distribution. According to Erdincler and Vesilind (2003), moisture distribution is a more suited parameter for dewaterability assessment, because the interactions between water molecules and WAS organic components establish the different physical-chemical properties of water within and outside the flocs which are highly overlooked by the CST method. On the other hand, WAS water distribution is still a subject of debate in related literature; its broadest classification includes free and bound water (Erdincler and Vesilind, 2003). Since free water surrounds the flocs it can be easily removed by mechanical equipment. Bound water on the other hand, is considered to be attached to the flocs by physical or chemical forces making its removal possible only under thermal conditions (Jin et al., 2003). However, lessening WAS bound water content would actually improve its dewaterability as more water will be in the free form, boosting the total amount of water mechanical devices can remove.

In the present study, bound water content was defined based on the work of Vesilind (2004) and Jin et al. (2003) as the sum of interstitial water (water trapped in capillaries and void spaces between and inside flocs), surface water (water adhered or absorbed onto the floc surface) and internal water (water inside cells and chemically bound to cell components).

Figure 3.6 shows the changes in WAS bound water content (g H_20/gTS) as a function of ES input for the various WAS concentrations assayed.

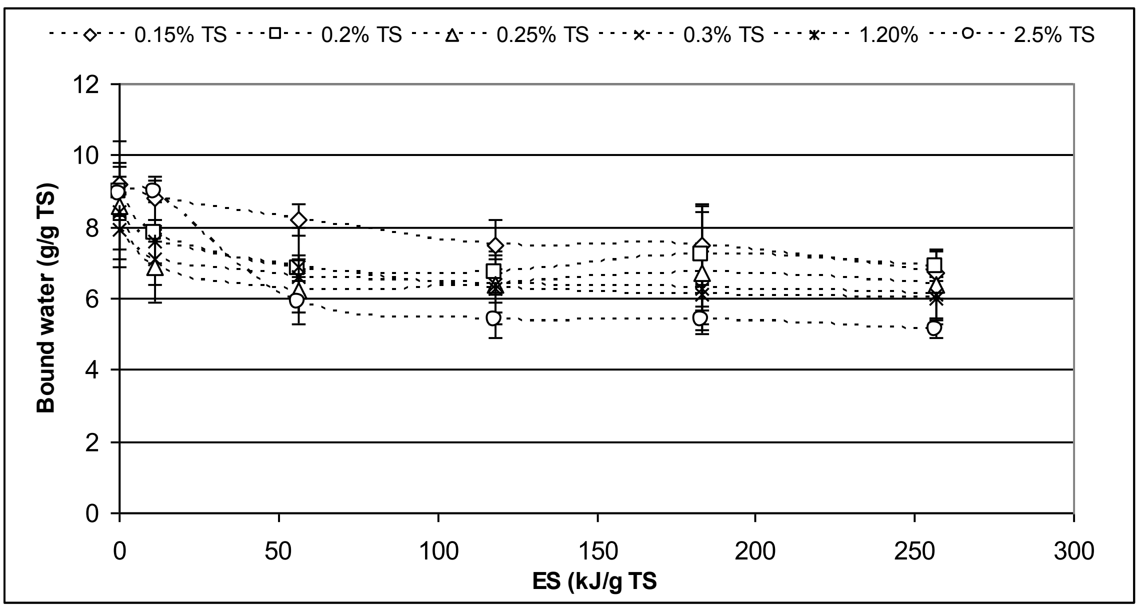


Figure 3.6: Effect of ES input on raw WAS water content. Data represent the mean value and error bars the standard deviation from six replicates, data are joined to indicate the trend.

It can be observed from Figure 3.6 that US treatment changed WAS water distribution by decreasing the bound water content up to 25% on average for all TS concentrations tested compared to the control. The maximum bound water release for all solids concentrations tested in the present experiment was observed at 118 KJ/gTS and remained almost constant thereafter. It can be speculated that release of bound water was due to various effects of US on the complex WAS structure: at lower ES (≤56 KJ/gTS) the floc matrix was disrupted leading to the release of mostly interstitial water, while at ES inputs higher than 118 KJ/gTS floc solubilization resulted in the release of the surface and internal water. This hypothesis is supported by the change in particle numbers with increasing ES exposure. Maximum increase in particle numbers (1-240 μm range) occurred at ES inputs of 56 KJ/gTS, indicating floc disruption only. While at higher ES inputs the particle number decreased; indicating solubilisation of the disrupted floc and concomitant release of surface and internal bound water. If this hypothesis is accepted the results shown in Figure 3.6 would tend to indicate that the majority of the bound water that is released can be attributed to the interstitial water trapped in capillaries and void spaces between and inside flocs. TS concentration also had an effect on WAS bound water release. The pattern observed in Figure 3.6 suggested that for dilute WAS concentrations after 11 and 56 KJ/gTS ES, changes in bound water were not substantial, while for the same ES and higher WAS TS concentrations bound water release was enhanced, corroborating again, that US is better suited for samples containing high TS concentrations (Gonze et al., 2003; Dewil et al., 2006).

3.4.4 Effect of ultrasound on raw WAS multivalent cation content

Table 3.3 shows the ratio of monovalent to divalent cations (M/D) for controls and the various concentrations of sonicated WAS at ES of 11 and 257 KJ/gTS. Since the major cations in WAS are sodium, potassium, ammonium, calcium, magnesium, iron and aluminum (Higgins and Novak, 1997), in Table 3.3 monovalent cations are the sum of sodium and potassium, while the divalent cations are the sum of calcium and magnesium.

It can be observed in Table 3.3 that the average M/D ratios and average ± standard deviation for either the soluble fraction or floc was never larger than 2 for the controls and for any of the experimental conditions tested.

Table 3.3: WAS monovalent to divalent cation ratio after US treatment

	M/D[1] soluble	M/D floc		
Control	**1.4**	**0.4**		
	11 KJ/gTS		**257 KJ/gTS**	
TS (%)	**M/D soluble**	**M/D floc**	**M/D soluble**	**M/D floc**
0.15	1.3 ± 0.2[2]	0.46 ± 0.2	1.5 ± 0.4	0.4 ± 0.2
0.2	1.3 ± 0.5	0.41 ± 0.4	1.6 ± 0.3	0.4 ± 0.2
0.25	1.4 ± 0.1	0.30 ±0.2	1.4 ± 0.2	0.5 ± 0.2
0.3	1.3 ± 0.3	0.43 ±0.4	1.4 ± 0.2	0.3 ± 0.2
1.2	1.5 ± 0.2	0.49 ± 0.1	1.5 ±0.3	0.3 ± 0.1
2.5	1.6 ± 0.3	0.38 ± 0.2	1.7 ±0.1	0.3 ± 0.1

[1] Monovalent to divalent cation ratio (M/D)
[2] Data represent the mean value ± standard deviation from 3 replicates

According to Higgins and Novak (1997) cations are not only a fundamental part of the aerobic floc but their presence in proper proportions (M/D ratio) has important implications for WAS dewaterability. M/D ratios greater than 2 are usually potential indicators of settling and dewatering problems. Although, no significant statistical correlation between dewaterability (measured by both CST and moisture distribution methods) and cation content was found in the present experiment, the M/D ratios shown in Table 3.3 would indicate that WAS settleability/dewaterability was not likely to deteriorate after sonication due to high M/D ratios. It has been suggested that multivalent cations interconnect negatively charged biopolymers and cells in the WAS floc matrix, contributing to the stable structure needed for good settling, dewaterability and concomitant effluent quality (Bruus et al., 1992; Urbain et al., 1993; Higgins and Novak, 1997). Hence, higher divalent cation concentrations and lower M/D ratios were expected to be found in the soluble phase at the higher ES as a result of floc disintegration and solubilisation. However this outcome was not observed under any of the experimental conditions tested. Only a minor increase (5-7%) in the soluble phase for divalent concentration was observed at certain combinations of ES inputs and WAS TS concentration. Although inconclusive, this result might suggest that only lower concentrations of divalent cations are weakly bound to the flocs, while the highest concentrations are attached to microbial structures, hence after cell break up the cations still remain in the cell debris (Table 3.3). Peters and Herman (2007) studied the cations contained in the inorganic fraction of aerobic flocs during industrial wastewater treatment and reported that Ca^{2+} was most likely present in the flocs as a precipitated salts, hence such enmeshment of solids within the flocs produced a heavier better settling flocs. On the other hand, Chu et al. (2001) obtained similar results as those obtained in the present study after WAS sonication of up to 40 min, it was concluded that floc breakdown is not necessarily directly associated with the release of divalent cations (Ca^{2+} + Mg^{2+}). Furthermore, using aerobic flocs under starvation conditions, Wang et al. (2007) produced aerobic granules and found high concentrations of Ca^{2+} and $CaCO_3$ in the granule core, while the granule shell was nearly calcium free. Other studies have also demonstrated that although divalent cations are determinant for aerobic granules formation only Ca^{2+} is believed to have relevant importance for the granule structure, while Mg^{2+} has a more important role on bacterial metabolism (Liu et al., 2010). This would suggest that in addition to divalent cations some other intermolecular forces (hydrogen bridges, hydrophobic interactions) might be as relevant for the floc structure and in consequence for WAS dewaterability.

3.5 Conclusions

- Moisture distribution based on the centrifugal method tended to indicate that US pretreatment enhanced raw WAS dewaterability. ES inputs of 118 KJ/gTS or higher decreased the WAS bound content by approximately 25% in average for all TS concentrations tested compared to controls.
- CST method is not well suited for WAS dewaterability determination after US treatment, or for any other treatment that disrupts the floc due to the methodology's shortcomings attributed to fine particles CST paper interaction and clogging.
- The role of divalent cations on WAS dewaterability was inconclusive in the present study as no significant correlation was found between M/D ratio and dewaterability measured by either method.

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Chapter 4

Effect of Low Frequency Ultrasound Treatment on Anoxic Biomass Metabolic Activity and Process Optimization Using Response Surface Methodology

Juan Marin and Kevin J. Kennedy

4.1 Abstract

Currently, tighter environmental regulations regarding final disposal of excess sludge are contributing to new modifications to the activated sludge process (ASP) in order to minimize sludge production during wastewater treatment. The present study proposes the use of a modified ASP for the treatment of municipal wastewater, in which the biomass will be recycled to the ASP through a controlled anoxic environment: the holding tank (HT) followed by ultrasound (US) treatment. It is a premise of the present research that both post ASP processes could act synergistically and contribute to a decrease in the amount of excess sludge production during wastewater treatment by enhancing biomass maintenance metabolism of the HT biomass. This manuscript presents results of the effect of low frequency ultrasound treatment on HT biomass activity and ultrasound treatment optimization for further continuous studies on ASP sludge reduction during treatment of real municipal wastewater. Biomass activity from the HT, measured as specific oxygen uptake rate (SOUR) and dehydrogenase activity (DHA) was enhanced by more than 50-200 % at US specific energy (ES) input of 11 KJ/gTS compared to control samples depending on the concentration of the HT biomass. Higher ES inputs (>56KJ/gTSS) had irreversible effects on the HT biomass such as total loss of biomass activity and floc structure. Low ES inputs had a predominantly morphological effect on

the floc structure, indicating that floc size reduction lead to an increased microbial-substrate interaction and enhanced biomass bioactivity. The US treatment process was further optimized using surface response methodology (RSM). Sonication optimization indicated that there is only a small range of ES inputs ($\leq$ 20KJ/gTS) in which HT biomass activity can be enhanced without compromising certain floc characteristics important for wastewater treatment. All improvements observed were realized with real primary effluent from an operating municipal wastewater treatment plant which demonstrates the applicability of the technology. Results from the sonication process optimization will be used in conjunction with continuous studies to evaluate excess sludge reduction during municipal wastewater treatment with the modified ASP.

***Key words: activated sludge process, biomass activity, DHA, municipal wastewater specific oxygen uptake rate, XTT**

4.2 Introduction

Due to its reliable operation, the activated sludge process (ASP) is still the globally preferred technology for the biological treatment of both municipal and industrial wastewaters. The ASP can achieve removal efficiencies of up to 80 and >90% of the incoming carbonaceous organic matter (measured as chemical oxygen demand, COD) and total suspended solids (TSS) respectively (Metcalfe and Eddy, 2003) depending on the characteristics of the wastewater. In the ASP, the removal of organic pollutants (soluble and colloidal) is achieved by the action of a heterogeneous group of aerobic microorganisms, which convert the incoming organic pollutants into CO_2, H_20 and a flocculent microbial suspension (excess sludge) that once settled, can be separated from the treated water and disposed of. Although a robust technology, the ASP is constantly evolving in order to improve/maintain organics removal while minimizing operational costs.

During the last 40 years, improvements were implemented mainly to correct process shortcoming and to enhance its general efficiency (Wiesmann et al., 2007), leading to the development of specific ASP designs (step aeration, tapered aeration, nutrient removal systems, high rate systems, etc). However, a common disadvantage to most ASP designs is still the production of large amounts of excess microbial sludge (EXS) that must be safely managed and disposed of. According to Ginestet (2007) EXS is an unavoidable by-product of wastewater treatment, since it is produced through the growth of the microorganisms that remove the organics. Currently, EXS post-production management costs which can be as high as 50% of a wastewater treatment plant operation (Andreottola and Foladori, 2006), added to more stringent environmental regulations regarding EXS final disposal, are spurring the development of new modifications to the ASP design towards addressing the reduction of EXS production during wastewater treatment (Deleris et al., 2002; Odegaard, 2004). Emerging technologies such as membrane bioreactors with long biomass residence times seem a promising option for EXS reduction. However, this process is energy intensive and still under development.

At present, some of the approaches used for EXS reduction at the source, rely on modifying the metabolism of ASP microorganisms in order to reduce the observed yield (Y_{obs}). Amongst them, disintegration and return to the aeration tank of a portion of EXS has been recognized as a promising option with potential for full-scale applications compared to other approaches such as membrane bioreactors, bacteria predation by higher microbial forms (protozoan) or the direct application of metabolic uncouplers to the ASP (Ginestet, 2007). EXS disintegration has been achieved by using ozone (Ahn et al., 2002; Yusai and Shibata, 1994; Yusai et al., 1996; Kamiya and Hirotsuji, 1998), other chemical treatments (Yamaguchi et al., 2006; Rocher et al., 2001) and mechanical disintegration (Muller, 2000; Zhang et al., 2007). Ultrasound (US) is a mechanical disruption technology that has been tested at full-scale wastewater treatment plants as a treatment to enhance the anaerobic digestion of municipal and industrial EXS (Nickel, 1999; Muller, 2000; Thiem et al., 2001; Saha et al., 2011). Additionally the potential of US for EXS reduction at the source (within the ASP) has been reported (Cao et al., 2006; Ginestet, 2007; Strunkman et al., 2006; Zhang et al., 2007). However, implementation of such an approach requires the addition of a separate unit process in the wastewater treatment train, since direct application of US in the aeration tank would be expensive. Currently, certain modified ASP processes have made use of such an approach i.e., the proprietary Cannibal Process^{TM} (SIEMMENS, water technologies). Another approach makes use of an anaerobic stage before the ASP biomass is recycled into the aeration tank (Chen et al., 2006). In both cases as high as 50% EXS reduction has been reported although specific details and their associated mechanisms are not available. In the present study, the proposed modification to the ASP includes the addition of a controlled environment: the anoxic holding tank combined with low frequency US treatment of the biomass prior to it being returned to the aeration tank. It is believed that the addition of the holding tank which will likely promote the development of a mainly facultative culture combined with ultrasound treatment at source will play an important role in the metabolic selection of ASP microorganisms. It is hypothesized that high biomass yield aerobes could be partially replaced by a more facultative microbial population with higher specific activity and lower biomass yield. Concomitantly the main objectives of the present study are to investigate the effect of low frequency US treatment on metabolic activity and morphological characteristics of the HT biomass as a prelude to further ASP sludge reduction studies. The present study also presents an alternative approach to assess biomass metabolic activity and stress using a total and extracellular dehydrogenase enzymatic assay which is correlated to respirometry measurements.

4.3 Methodology

4.3.1 Holding tank inoculation

The holding tank (HT) was constructed from a 5L Erlenmeyer flask (Figure 4.1). Portion A of the HT was removable to allow biomass feeding and withdrawal. The HT was operated as an intermittent semi-batch cycle system at room temperature (20 - 23 ºC) with 6 h of micro-aeration (8 ± 1 mL air/min or 0.002 v/v min) followed by 2 h of non-aeration with repeating intermittent cycles. Aeration in the intermittent cycles was controlled with outlet timers (Traceable^{R}, Fisher Sci. Canada) attached to a solenoid gas valve. Semi-batch feeding was achieved by removing 500 mL of effluent daily during the non-aeration period of the final cycle of the day then adding the same volume of fresh primary influent from the Robert O. Pickard Environmental Center (ROPEC, City of Ottawa, ON). ROPEC is one of the largest wastewater treatment plants (WWTP) in Canada, treating approximately 120 MGD of municipal wastewater (MWW), primary effluent is treated by a conventional activated sludge process (ASP) operated at an average sludge retention time (SRT) of 5 days (City of Ottawa, 2009). Since the MWW strength used varied in concentration the volumetric organic loading rate (VOLR) to the HT was in the range of 35 to 48 gCOD/m^3 d. This steady-state VOLR was chosen to achieve a moderate loading in order to maintain a relatively constant HT biomass concentration and healthy active biomass with constant metabolic activity. The mixed liquor suspended solids (MLTSS) were measured everyday to ensure concentrations in the range of 3000 to 4000 mg MLTSS/L; pH was monitored but not controlled. The HT was equipped with air injection and exhaust lines, aeration was provided from the in house filtered air line using stone diffusers. The HT was inoculated with aerobic biomass taken from the ASP aeration basin at ROPEC. After sampling at ROPEC, activated sludge samples were saturated with air using an aquarium pump to a dissolved oxygen (DO) concentration above 5 mg/L and transported directly (<1 h) to the laboratory.

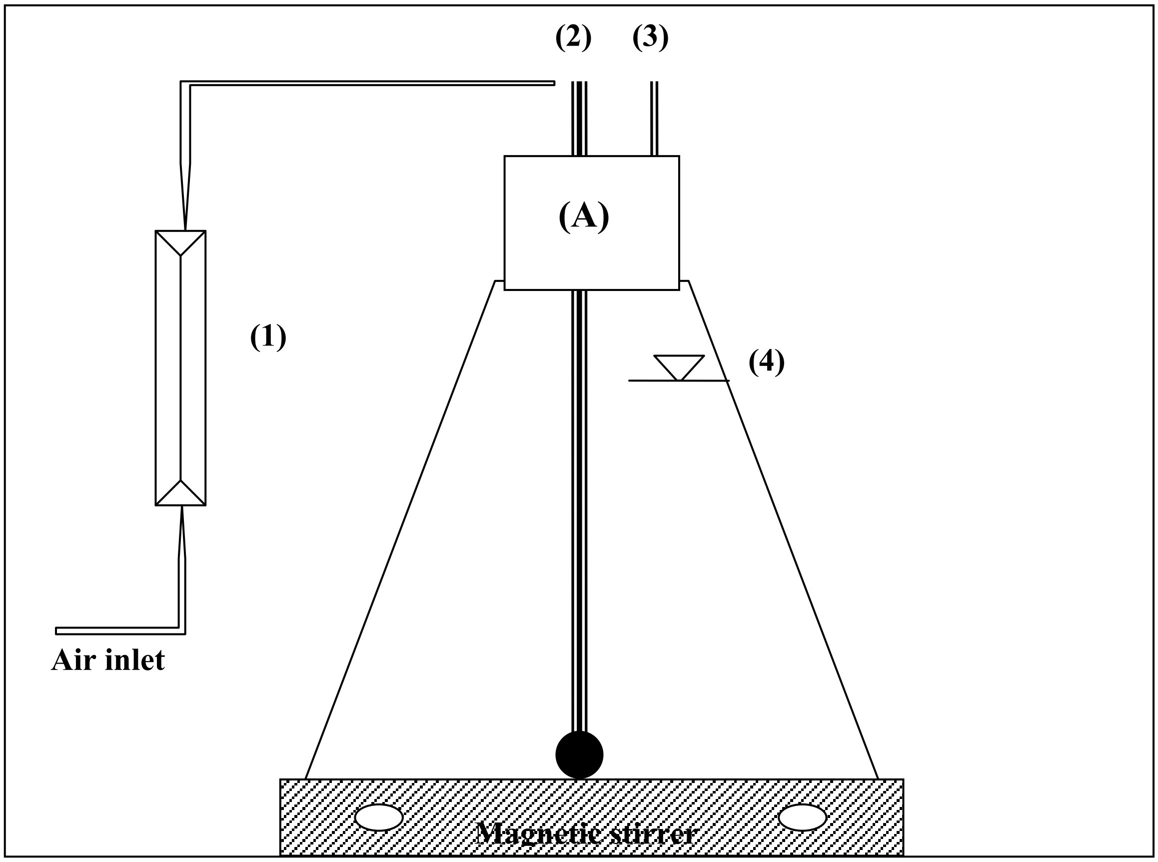


Figure 4.1: Experimental set up for holding tank. In the diagram: (1) air flow meter, (2) air line, (3) exhaust line and (4) liquid level

The aerobic biomass was concentrated to ~8000 mg TSS/L in an 8 L cylindrical glass reactor and from this the HT was inoculated to a final biomass concentration of 4000 ± 100 mg TSS/L. Once the HT was operating at steady-state in the intermittent cycle semi-batch mode (minimum of 5 cycles), biomass was withdrawn after the non-aeration phase as required and different MLTSS concentrations were prepared for US treatment. US treatment took place at least 36 h after a minimal of 5 cycles followed the HT inoculation. Table 4.1 shows the characterization of ROPEC activated sludge biomass as collected.

Table 4.1: ROPEC's activated sludge characterization

Parameter	Activated sludge
pH	6.7 ± 0.2[1]
MLTSS (mg/L)	1513 ± 88
MLVSS (mg/L)	1062 ± 39
Total COD (mg/L)	1456 ± 137
Soluble COD (mg/L)[2]	198 ± 10
Alkalinity (as mg $CaCO_3$/L)	207 ± 21
NH_3-N (mg/L)	31 ± 16

[1] Data indicates the mean value and ± the standard deviation from 3 replicates
[2] Filtered sample (<0.45 µm)

4.3.2 Experimental approach

The metabolic activity of aerobic biomass depends on certain physical/chemical parameters such as pH, temperature, and sludge age, amongst others. Additionally, and to a great extent it also depends on the specific substrate and process operational procedures. In the present study the concentration and activity of the initial HT biomass was fixed by the SBR operational strategy. Concomitantly, from an operational point of view for the proposed modified ASP that combines an HT step with US treatment to enhance EXS reduction, the two independent variables that can be relatively easy to manipulate and may have an important effect on post US biomass activity were identified as the US specific energy (ES) input and the MLTSS concentration. In the present study the effect of low frequancy US ES input (10, 56, 118 and 183 KJ/gTSS) on HT biomass metabolic activity was initially investigated based on a general factorial design using different concentrations of MLTSS (1500, 2000, 2500 and 3000 mg/L)). The MLTSS concentrations were chosen because these are typical concentrations encountered in most conventional ASP for municipal wastewater treatment. Regarding the ES input, a wide range was initially chosen to determine which conditions could be used for further US process optimization. After sonication, biomass activity was evaluated by respirometry and a colorimetric dehydrogenase enzymatic assay (DHA). In addition other parameters such as floc morphology, biomass settling characteristics, and degree of biomass solubilization were assessed. Statistical analysis was performed using Minitab15 (Minitab Inc., USA)

4.3.3 Apparatus and ultrasound treatment

Ultrasound treatment was performed using a Branson 102-CE (Branson Ultrasonics Corporation, USA) bench scale ultrasound system (20 kHz, tip surface area of 2.12 cm^2, peak power output of 276 ± 22.3 W). Specify energy (ES, KJ/gTS) input was calculated according to equation 4.1 (Khanal et al., 2007).

$$ES = \frac{P * t}{V * TS} \tag{4.1}$$

Where P was the ultrasonic power (KW), t the sonication time (sec), V the sample volume (L) and TS the total solids concentration (the sum of the TSS plus the total dissolved solids (TDS) and expressed as gTS/L). ES input was maintained constant by varying US intensity and sonication time at the different sample concentrations. Before US treatment, 500 mL of biomass from the HT was washed twice with equal volume of a buffer solution (42.5g KH_2PO_4 and 54.3 g K_2HPO_4 in 1L distilled water, pH adjusted to 7.2 with a 6M NaOH solution). For sonication, 500 mL of previously washed sample was placed in a 600 mL crystal beaker; the ultrasonic horn was centered and immersed to a fixed depth of 3.5 cm below the air-water interface inside the beaker. During sonication an ice bath was provided to minimize temperature increase. US treatment was performed in duplicate. US-mediated biomass degree of solubilization (DD_{COD}) compared to the ultimate degree of solubilisation was determined according to equation 4.2 (Khanal et al., 2007)

$$DD_{COD} = \left(\frac{COD_{US} - COD_{C}}{COD_{NaOH} - COD_{C}} \right) * 100 \qquad (4.2)$$

Where, DD_{COD} represented the degree of biomass disintegration (%), COD_{US} sample soluble COD after US treatment (mg/L), COD_{C} sample soluble COD without US treatment (mg/L) and COD_{NaOH} sample soluble COD (mg/L) chemically hydrolysed with a 0.5 M NaOH solution at 20 ºC for 24h.

4.3.4 Holding tank biomass metabolic activity evaluation by respirometry

A respirometric analysis provides valuable information about the metabolic activity/state of biomass. Batch specific oxygen uptake rate (SOUR) was determined based on Standard Method 2710A (APHA, 1998) for ROPEC ASP and HT biomass control and sonicated samples. During SOUR determinations, one of the most critical parameters is the initial substrate/biomass ratio (S/X). A high S/X ratio enhances biomass growth, leading to an overestimation of the kinetic parameters while at low S/X ratio; substrate is used too quickly due to the excess biomass leading to an underestimation or inaccurate kinetic values (Spanjers and Vanrrolleghem, 1995). Throughout this study, the S/X ratio for SOUR assays was kept constant at around 0.17 ± 0.02 (mgCOD/mgVSS), using filtered ROPEC wastewater (Whatman* glass microfiber filter GF/C, pore size 1.2 µm, Fisher Sci., Canada) as substrate. This is the optimum S/X ratio recommended for accurate determination of respirometric data (Spanjers and Vanrrolleghem, 1995). Control (ASP and HT) and sonicated HT biomass was transferred to the respirometry chamber (Figure 4.2). Once the chamber was sealed, air was introduced to achieve a dissolved oxygen concentration (DO) of 8.5-9 mg/L. Aeration was then stopped and DO consumption was recorded every 30 seconds until DO concentrations lower than 1 mg/L were reached. This process was repeated 2-4 times until all readily available extracellular substrate was consumed and pseudo steady-state (PSS) was achieved (constant DO consumption). PSS was the point at which the biomass was in an endogenous state. In the endogenous state the biomass consumes oxygen at a relatively low but constant rate over a determined period of time (Milenko, 1993). Due to endogenous biomass self consumption, a true steady-state is actually never attained because DO consumption is always present (Milenko, 1993).

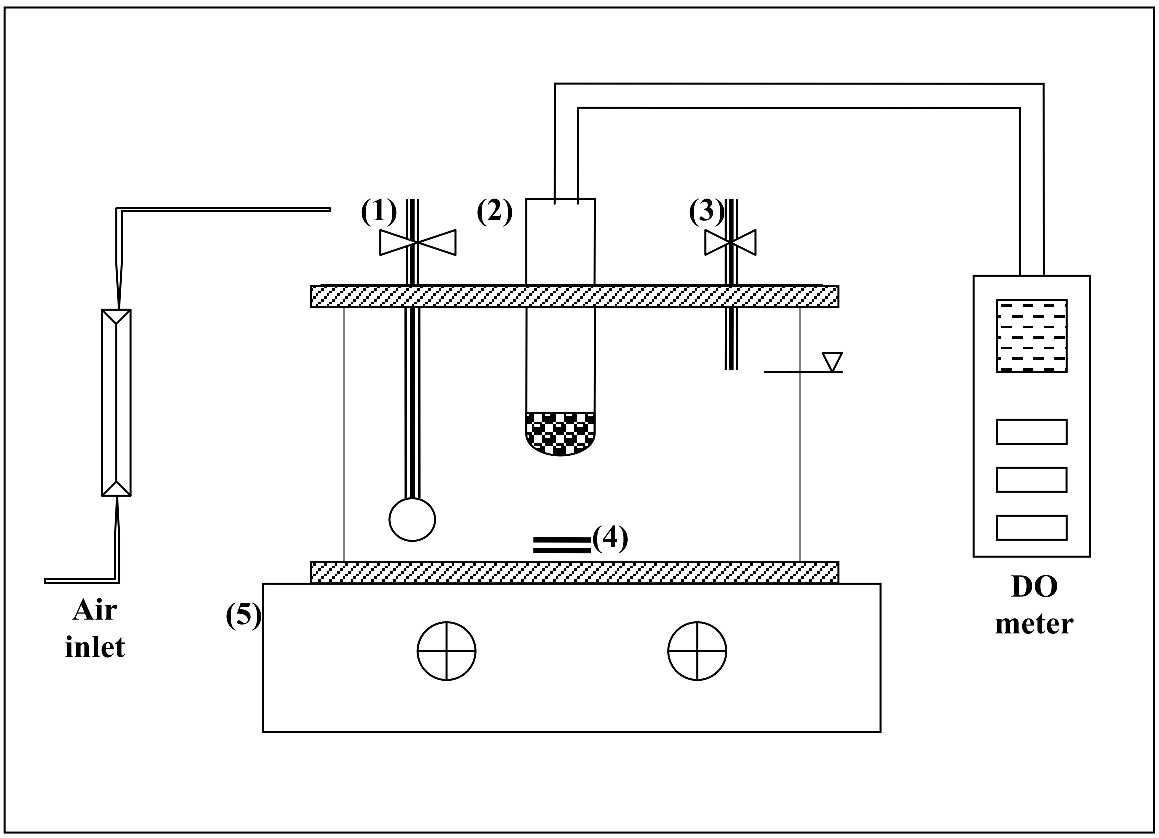


Figure 4.2: Set up for respirometric assessment of biomass activity. In the diagram: (1) air inlet, (2) dissolved oxygen meter, (3) feed inlet, (4) magnetic stirrer and (5) magnetic plate

After the PSS endogenous oxygen uptake was achieved, air was again introduced to achieve DO concentrations in the range of 8 - 9 mg/L and 50 mL wastewater previously aerated (DO ~ 8 mg/L) was added to the respirometric chamber to give an S/X ratio of 0.17 ± 0.02 mgCOD/mgVSS. DO consumption for the substrate starved biomass was again monitored until DO concentrations were lower than 1 mg/L. The observed DO concentrations (mg/L) were plotted versus time (min) and the Oxygen Uptake Rate (OUR) was calculated according to equation 4.3:

$$OUR = -\frac{DO_i - DO_{i-1}}{t_i - t_{i-1}} \tag{4.3}$$

Where DO_i and DO_{i-1} indicated the DO (mg/L) at time t_i and t_{i-1}(min) respectively. From equation (4.3) the SOUR (mg O_2/g h) was calculated according to equation 4.4

$$SOUR = \frac{OUR}{VSS} X 60 \tag{4.4}$$

Where, VSS is the total volatile suspended solids in the sample (g/L). SOUR determination was performed immediately after sonication at room temperature in duplicate.

4.3.5 Holding tank biomass metabolic activity evaluation by XTT enzymatic assay

Biomass activity of ROPEC ASP biomass, HT control and US treated samples was also evaluated by using the tetrazolium salt 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT). In metabolically active cells, XTT acts as an external acceptor for detecting dehydrogenase activity (DHA). After microbial degradation, XTT is reduced to a colored water soluble product that can be measured by spectrophotometry, with color production directly proportional to biomass DHA. XTT has been used to assess respiratory activity in natural waters, drinking water, aerobic activated sludge and anaerobic microorganisms (Wuertz et al., 1998; Roslev and King 1993; Marin et al., 2010; Marin et al., 2011), and can provide a qualitative measurement of biomass activity. Total DHA biomass activity was performed directly on 0.5-mL sample before and after sonication. For the extracellular DHA activity measurement which is a proportional indicator of microbial stress , 1-mL of sample was withdrawn and centrifuged for 5 min at 8000g in a micro centrifuge, subsequently from the supernatant, 0.5-mL was filtered through 0.22-μL filter (Milex® - GV, syringe driven filter, VWR, Mississauga, ON). Total and 0.22-μL filtered fractions were mixed with 40-μL of 6mM XTT (Sigma-Aldrich, Oakville, ON) and 120-μL of 10-mM Menadione (Sigma-Aldrich, Oakville, ON). Samples were incubated at 37 °C for 45 min, and at the end of the incubation period they were placed in an ice bath to stop the reaction. Absorbance at 470 nm was then measured. Specific DHA biomass activity (total and extracellular) was evaluated based on sample's VS content and reported as Δabs_{470} /VS. Measurements were performed in duplicate.

4.3.6 Biomass morphological evaluation

Biomass morphology was evaluated by micro-flow imaging (MFI) before and after US treatment using a Dynamic Particle Analyzer (DPA) 4100 series B (BrightWELL Technologies INC, Ottawa, Canada); equipped with a low magnification flow cell (sample channel depth of 400μm). MFI operates by capturing images from the sample as it passes through the flow cell's sensing zone (Figure 4.3). Particles' morphology is measured in situ and is displayed on the system monitor in real-time. The DPA4100B can be configured to store images upon detecting particles falling within predetermined size ranges. In the present experiment, the DPA4100B was set at an optical magnification of 4.9X, particle size range: 1µm to 400µm, display resolution: 0.25µm, field of view: 1960µm x 1570µm and pixel resolution of 1280 x 1024. For the test, a special 1 mL pipette tip (CPL model Neptune BT 1000, BrightWELL Technologies INC, Ottawa, Canada) was used for sample injection. The diluted sample volume was dispensed at a flow rate of 0.35 mL/min. Particle morphology analyses were performed immediately after sonication in duplicate.

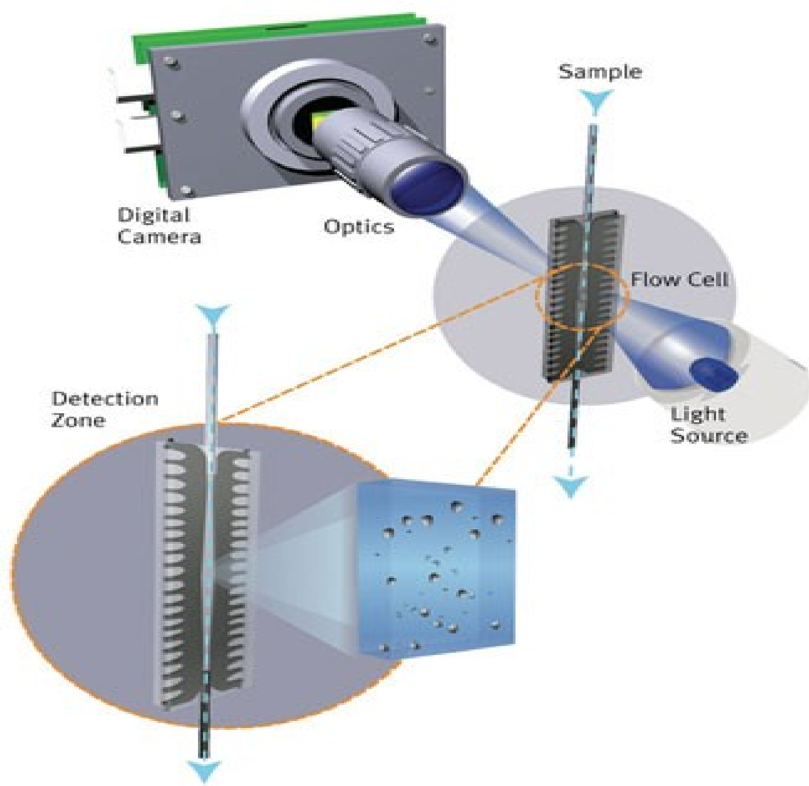


Figure 4.3: MFI system operation. The MFI operates by capturing images from the samples as it passes through the flow cell's sensing zone (picture adapted from www.brightwelltech.com).

4.3.7 Holding tank biomass reflocculation ability after ultrasound treatment

As a disruption technology, US treatment under certain operation conditions can lead to biomass solubilization and the decrease or total loss of specific metabolic activity. Furthermore, some biomass characteristics essential for effective ASP treatment of wastewater (once the biomass is recycled back to the aeration basin) can also be adversely affected. Activated sludge flocculation is one of the most critical parameters during ASP wastewater treatment due to its direct effects on secondary clarification that impact effluent quality and SRT control (Biggs and Lant, 2000). Flocculation is a very complex mechanism and its study is beyond the scope of the present research. However, it is important to determine optimum sonication conditions (increased specific metabolic activity with no or minimal impact on biomass flocculation and sedimentation) that would not have a concomitant detrimental effect on ASP performance when post sonicated HT biomass is returned following sequential HT and US treatment. In the present study the reflocculation procedure developed by Bigss and Lant (2000) was modified to evaluate the ability of the HT biomass to reflocculate after US treatment. Reflocculation was measured as the increase in floc mean diameter (measured as equivalent circle diameter (ECD)) with time. In addition the sludge volume index (SVI) in combination with the XTT enzymatic assay was used to better assess the effect of sonication on HT biomass reflocculation. For the reflocculation assay, 1.5 L biomass with MLTSS concentrations of 1500 and 3000 mg/L respectively were sonicated at different ES inputs. Following sonication 300 mL of ROPEC primary effluent was added under mild aeration conditions (0.01 vvm) to allow biomass reflocculation at room temperature. ECD, SVI and XTT activity were measured at regular time intervals. During reflocculation assays the ECD and biomass activity were determined in duplicate.

4.3.8 Analytical methods

Total and volatile suspended solids (MLTSS and MLVSS) concentrations were determined based on Standard Methods 2540D and 2540E respectively (APHA, 1998). Total dissolved solids (TDS) were determined according to Standard Method 2540C (APHA, 1998). Sludge volume index (SVI, mL/g) was determined according to Standard Method 2710D (APHA, 1998). Aerobic biomass was poured directly into a 1 L graduated cylinder and allowed to stand undisturbed for 30 min, after which settled sludge volume was recorded and divided by the MLTSS concentration. SVI was performed in duplicate for control samples. pH was measured with a Fisher Accumet® pH meter Model 925 (Fisher Sci., Ottawa, ON). Total and soluble chemical oxygen demand (COD) analyses were performed using the closed reflux colorimetric technique and the titrimetric closed reflux Standard Methods 5220B and 5220C respectively (APHA, 1998). For the colorimetric COD procedure a Perkin-Elmer spectrophotometer (Fisher Sci., Ottawa, ON) was used to measure the light absorbance of the samples at 600 nm. For sCOD determination, samples were centrifuged (Sorvall Legend™, T_+/RT_+ Thermo Scientific, Germany) for 20 minutes at 8,000g and the supernatant was then filtered through a 0.45 µm pore size membrane. Sample alkalinity was determined according to Standard Method 2320B (APHA, 1998). Dissolved ammonia ($NH_{3(aq)}$ and NH_4^+) concentration was determined with an Orion ammonia electrode (model 95-12) with a Accumet® model 750 pH/ion meter (Fisher Sci., Ottawa, ON) according to Standard Method 4500D (APHA, 1998). Total and soluble protein and humic-like substances concentrations were determined according to the modified Lowry method (Frolund et al., 1995), with bovine serum albumin (Fisher Sci., Ottawa, ON) and humic acids (H16752, Sigma-Aldrich, Canada, Ltd) used as the protein and humic-like substances standards respectively. Total sugar concentration was determined according to Dubois et al. (1956), using glucose as standard. A Beckman DU® 50 series spectrophotometer (Beckman Instruments, Mississauga, ON) was used to measure absorbance at 750 and 490 nm for proteins or humic-like substances and sugars, respectively. All tests were performed in duplicate.

4.4 Results and Discussion

4.4.1 Effect of ultrasound on holding tank biomass activity

The initial average SOUR activity of the ASP biomass and HT biomass without sonication were 12 ± 2 mg O_2/g_{VSS} h and 8 ± 1 mg O_2/g_{VSS} h respectively for the different MLTSS concentrations assayed in the present study. This initial baseline SOUR data indicated that the 20-25 % decrease in SOUR in the HT biomass was indicative of a shift in the microbial consortia in the HT from a predominately aerobic culture to one that had a higher proportion of facultative bacteria. Figure 4.4 shows the effect of ultrasound ES input on HT biomass activity determined by the SOUR. It can be observed that compared to the HT control the SOUR reached maximum increases ranging from 52 ± 6% to 225% ± 8% at ES input of 11 KJ/gTS for increasing MLTSS concentrations of 1500 to 3000 mg/L TSS respectively. As ES input increased from 11 to 56 KJ/gTS, SOUR began to decrease sharply to values slightly lower than the HT control, followed by a further decrease and total absence of respiratory activity as ES was further increased to 118-156 KJ/gTS. SOUR is used to assess how fast the biomass metabolizes a determined substrate (in this study real ROPEC primary effluent); hence it gives an indication of the actual bulk biomass metabolic activity for domestic primary effluent. Results shown in Figure 4.4 showed that biomass respiratory activity was enhanced only at low ES inputs and in a narrow range at around 11 KJ/gTS (between 0 and 50KJ/gTS).

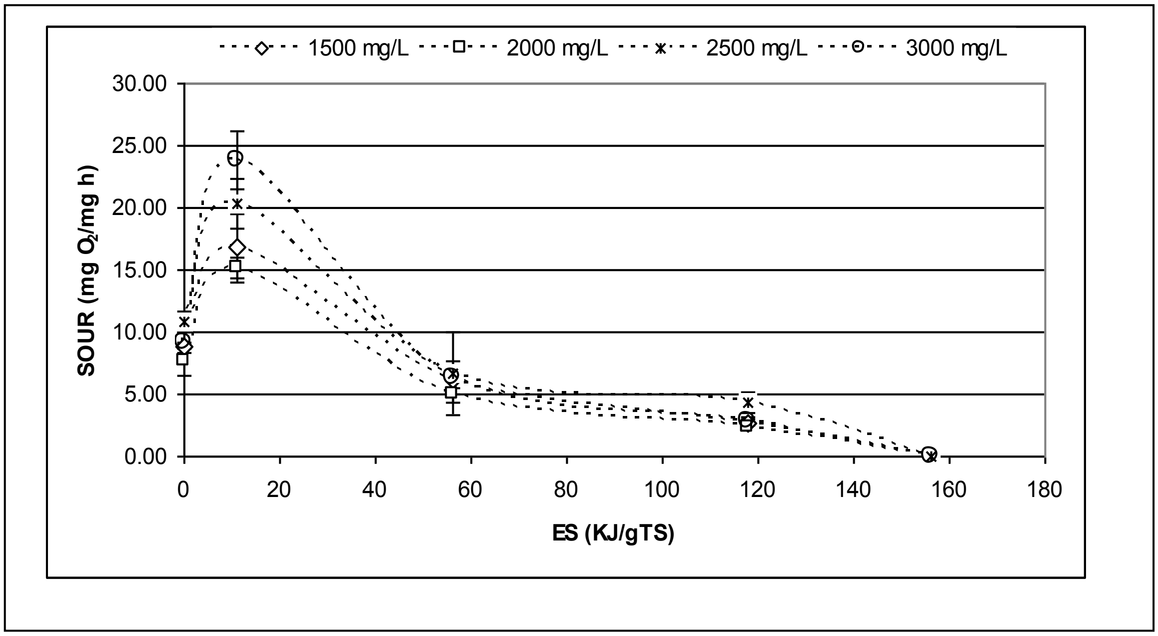
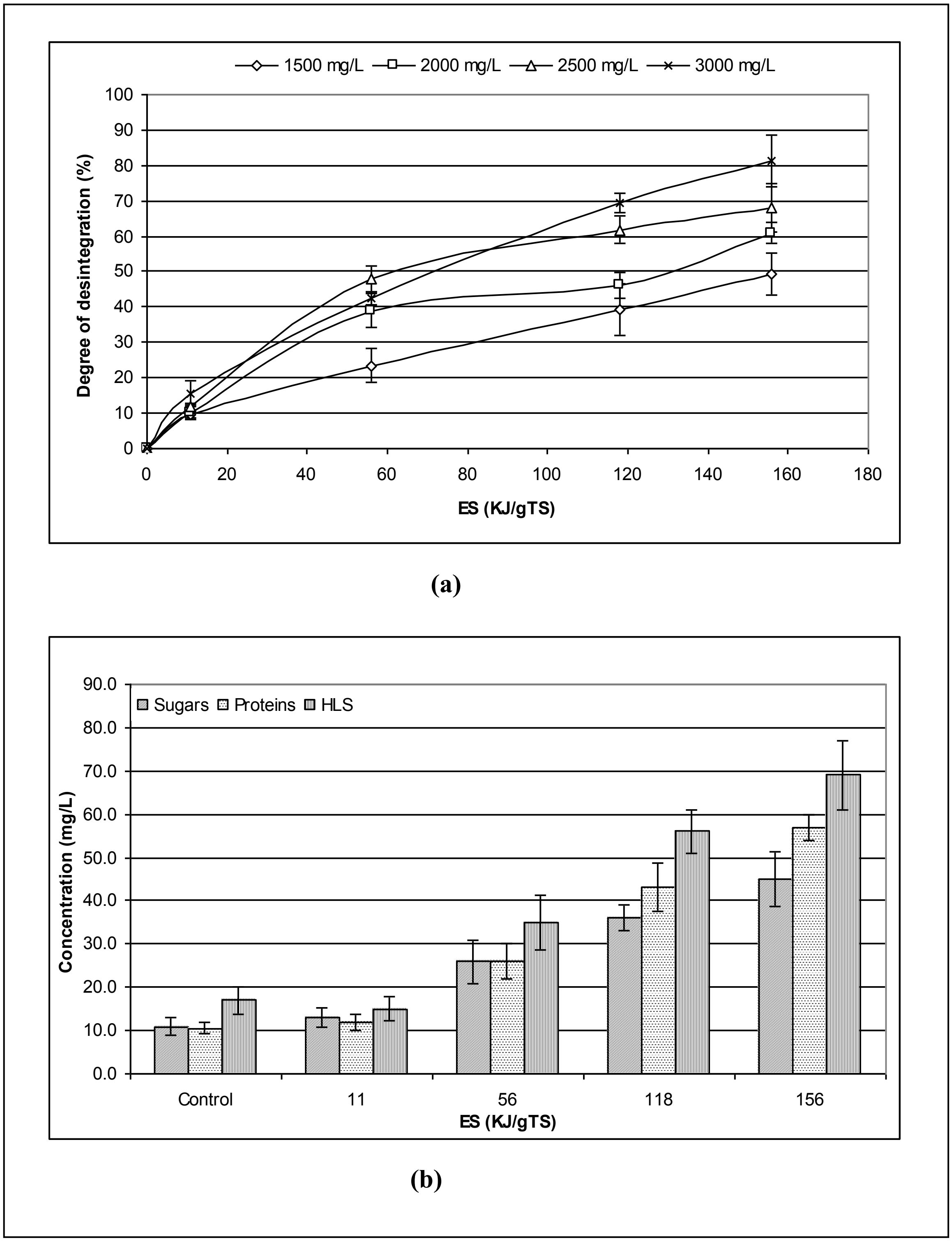


Figure 4.4: Effect of ES input on HT biomass activity as determined by respirometry. Data represent the mean value and error bars the standard deviation from 4 measurements. Data are joined to indicate the trend

In related literature US mediated biomass activity enhancement is an ongoing debate. Although several researchers have reported on the enhanced microbial activity of diverse types of biomass after sonication, the mechanisms that trigger such an effect are still not well understood. Since minimal increases in temperature or biomass solubilization have been observed at low ES inputs, Zhang et al. (2007) suggested that US mechanical effects on the floc rather than free radical formation was responsible for the enhanced SOUR of activated sludge biomass. If it is considered that flocs are organized into aggregates formed of numerous individual particles and micro-colonies encapsulated in the polymeric matrix (Scuras et al., 1998), then this most likely would encourage or inhibit the growth of different types of bacteria depending on oxygen availability and energy requirements within the floc structure. Most likely following this hypothesis Zhang et al. (2007) postulated that at low ES inputs the conglomerated flocs were mostly dispersed exposing more bacteria (most likely facultative bacteria) to the bio-available substrate media, which could contribute to the increased oxygen demand and substrate consumption observed after low ES sonication. Although, the data in Figure 4.4 at low ES input provides support for this theory it does not support the hypothesis in its totality. If only US mechanical effects were the predominant mechanism to enhance biomass activity then it would be expected to find elevated SOUR (biomass respiratory activity) over a wider range of ES after sonication between 11-56 KJ/gTS since at 56KJ/gTS more bacteria where being exposed to the media without being solubilized. However, the results observed in Figure 4.4 missed this effect and at 56 KJ/gTS there was a marked decrease in SOUR for all HT biomass concentrations assayed. This energy input may be sufficient to disrupt the connection between cells within the floc matrix as well as break cell walls and lyse membranes (Muller, 2000). Figure 4.5a shows the effect of ES input on biomass/floc disruption and biomass solubilization. It can be observed that biomass solubilization started to be significant at ES inputs higher than 56 KJ/gTS, with a net increase in biomass solubilization from 13 ± 2% to 36 ± 16% at 11 and 56 KJ/gTS respectively. Analysis of the proteins, sugars and humic-like substances released to the soluble phase (<0.45 μm) shown in Figure 4.5b also indicated that cell lysis and biomass solubilization most likely started at ES inputs greater than 56 KJ/gTS, where higher concentrations of proteins and total sugars were determined in the soluble phase as compared to control and at ES inputs of 11 KJ/gTS.



(a)

(b)

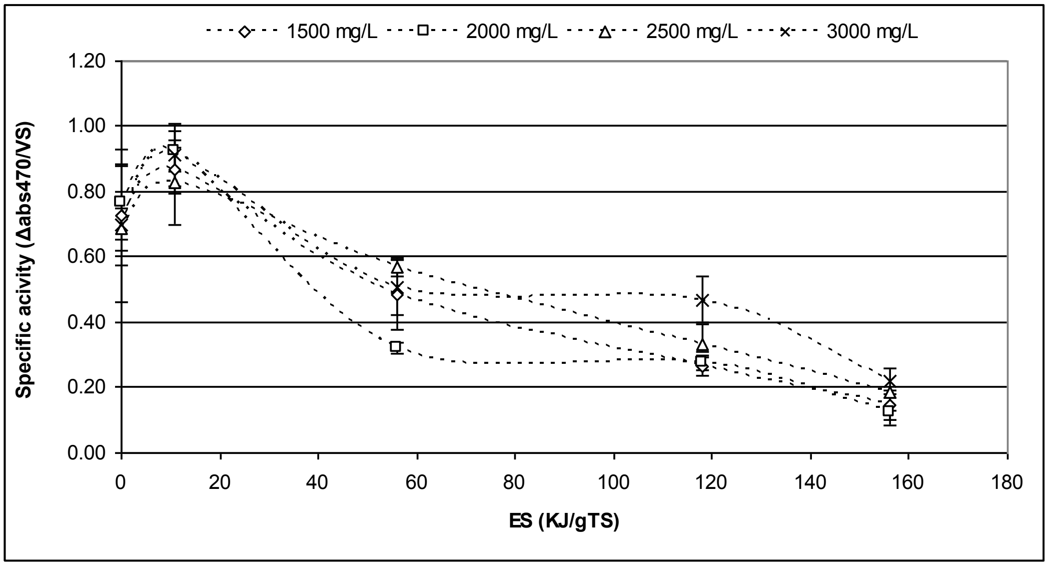
Figure 4.5: Effect of ES input on (a) biomass degree of solubilization and (b) release of macromolecules. Data represent the mean value and error bars the standard deviation from 4 measurements

If the increased SOUR was only a consequence of the exposure of more free cells to the media (direct bacteria-media interaction or shorter oxygen diffusion path), then it may have been expected to still find a high SOUR at 56KJ/gTS or at least equal to the control biomass SOUR, as more free cells were being exposed to the media without being solubilized. In the present study, the results shown in Figures 4.4 and 4.5 suggested that the enhanced HT biomass activity at low ES inputs was not just a consequence of the conformational changes of the floc structure induced by US mechanical effects. It has also been suggested that biomass exposure to stress induced environmental conditions, can also stimulate biological activity (Rai et al., 2004). Most likely exposure to the US shock wave as well as high localized pressures and temperatures can result in microbial stress. SOUR increase or decrease from its baseline conditions after sonication might also have indicated that the biomass was partially wounded and more cellular energy was being directed to maintenance and repair metabolism rather than normal reproductive functions. Xie and Lui (2010) also reported that activated sludge bioactivity improved significantly under the proper sonication conditions. In their study they also found a rather narrow range of bioactivity enhancement at approximately 10 min sonication, after which, biomass activity deteriorated. While specific energy was not actually reported they concluded that the observed increase in biomass enzymatic and metabolic activity was a response to 'microbial wounding' induced by US, however after a threshold sonication or excessive wounding, biomass activity stopped. In the present study, similar effects as Xie and Lui (2010) were observed, but with more supporting information on SOUR as well as solubilization data. The trends followed in both studies suggested that initially US low ES inputs caused structural changes in the floc matrix in addition to induced biomass wounding to such an extent that cell repair mechanisms were triggered (higher SOUR). However, as the biomass exposure to the sonication source increased, US cavitation (high localized temperatures and pressures) most likely damaged microbial cell walls beyond repair, accelerating cell lysis and halting biomass viability as indicated by the sharp SOUR decrease with increasing ES input (Figures 4.4 and 4.5). At ES inputs of 11KJ/gTSS enhanced biomass activity as determined by SOUR was enhanced by 50-225 % compared to control samples. While at higher ES inputs most likely the HT biomass was damage to an extent that its respiratory activity not only decreased but it was totally interrupted. The degree of biomass solubilization shown in Figure 4.5 also corroborates this hypothesis. It is still not totally clear if the enhanced biomass activity resulting from low US exposure (11 KJ/gTS) was predominately the result of increased substrate/microbe interaction or increased cellular maintenance due to cell wounding or a possible synergistic combination of both phenomena, However, comparison of SOUR results as well as disintegration and solubilizaion results at 11 vs. 56 KJ/gTS suggests that the decrease in activity at the higher ES input is likely related to excessive wounding such that the maintenance activity mechanisms within the cellular biomass were more negatively affected compared to any advantages resulting from increased substrate/microbe interaction as a result of increased contact of substrate with increased microbial floc area.

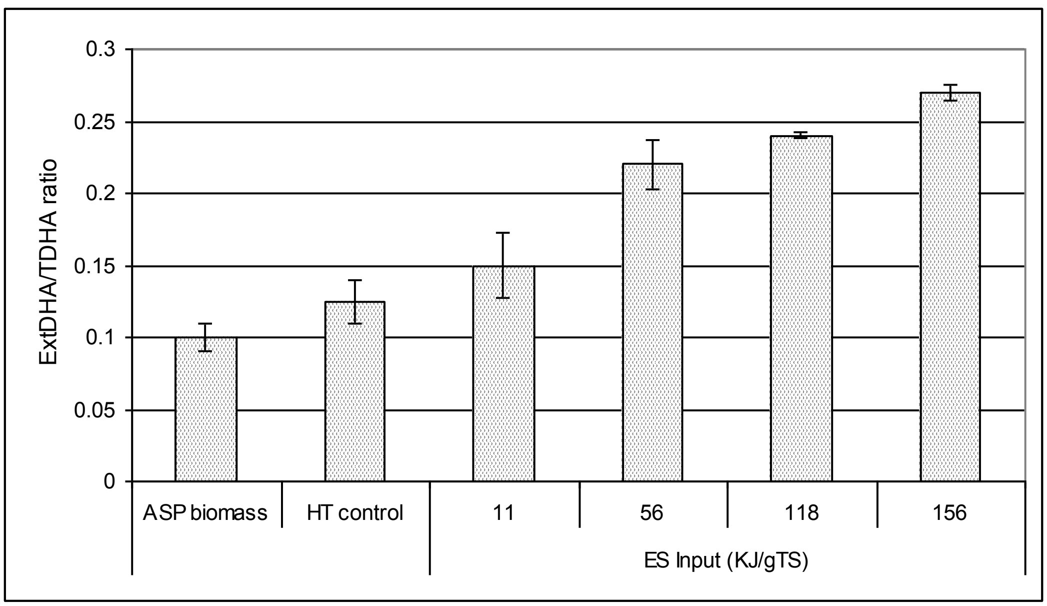
4.4.2 Effect of ultrasound on holding tank biomass activity as measured by an enzymatic assay

Dehydrogenases comprise a large group of endocellular enzymes responsible for driving the electron transfer chain reaction in respiring cells (Roslev and King, 1993).

They are essential in the biological oxidation of organic compounds by transferring hydrogen and electrons from substrates to acceptors. Therefore, DHA measurement represents an immediate assessment of biomass metabolic activity. Previous studies have reported on the effectiveness of using tetrazolium salts such as 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) to measure DHA in activated sludge samples (Griebe et al., 1997). In active electron transport systems, CTC is reduced to a colored insoluble intracellular formazan product. However, the time consuming extraction step makes the routine use of such a procedure difficult to implement. The tetrazolium salt XTT on the other hand, produces a water-soluble formazan which does not require further extraction before its colorimetric determination as such the activity can be measured quickly. The rapid response of the XTT test allows for the concurrent determination of both intra and extra cellular DHA activity. It has been reported that when microbial systems are in the presence of difficult to degrade substrates or environmental stressors microbial cells exhibit higher extracellular DHA (Wuertz et al., 1998; Marin et al., 2010; Marin et al., 2011). The DHA test allows for the determination of the total and the extracellular biomass metabolic activity which makes the XTT a fast and reliable indicator of microbial stress. Concomitantly, XTT has the potential to be correlated to biomass activity enhancement (SOUR) and potentially differentiate between SOUR attributable to a cellular defence response of the biomass to environmental stress (microbial wounding) versus increased substrate/microbe interaction as a result of increased contact of substrate with increased microbial floc area. In the present study the ratio of extracellular DHA to total DHA (EDHA/TDHA) was considered as an indicator of the biomass internal bulk stress as a result of US treatment. The higher the EDHA in respect to the TDHA at a determined ES input, the higher the biomass stress. The applied stress response as measured by the EDHA/TDHA ratio can also be regarded as a quantitative indicator of the amount of cellular activity being devoted to maintenance metabolism. Figure 4.6a shows the effect of ES input on biomass total DHA (intra and extracellular). From Figure 4.6b it can be observed that there was a small difference in the EDHA/TDHA ratio between the HT biomass and the ASP biomass from ROPEC. The EDHA/TDHA difference was less than 20% but the TDHA of the HT biomass was 25% higher, corroborating that the change in environmental conditions from aerobic to anoxic imposed a certain degree of stress in the HT biomass, or was an indicator of the change in the constituents of the microbial consortia in the controlled anoxic HT tank. From Figure 4.6a it can be observed that the trend in TDHA was closely related to SOUR (Figure 4.4), following the same general trend. A maximum TDHA was achieved at approximately 11 KJ/gTS, after which it started to decrease in proportion to the increase in ES input. In the present study, total DHA assessment and likewise SOUR results, indicated the existence of a critical ES input, after which biomass activity was not further enhanced. In figure 4.6b which shows the EDHA/TDHA ratio of the HT biomass, it can be observed that as ES input increased the EDHA/TDHA ratio also increased indicating that the biomass was under stress. At low ES input of 11 KJ/gTS the degree of stress increased by 16% to an EDHA/TDHA ratio from 0.12 in the HT. As the ES input increased to 56 KJ/gTS the degree of stress on the HT biomass was further increased from 16 to 43% compared to HT control. However it must be realized that increased EDHA/TDHA ratio with increasing ES input was also occurring with a steady decrease in TDHA. This indicated that little bioactivity was present but most likely the biomass was under extreme stress.



(a)



(b)

Figure 4.6: Effect of ES input on HT biomass activity as determined by the XTT test (a) and Effect of ES input on EDHA/TDHA ratio (b). Data represent the mean value and error bars the standard deviation from 4 replicates. Data points are joined to indicate the trend.

Based on combining the observation of TDHA and EDHA/TDHA ratio results with the SOUR and the degree of biomass solubilization provides a better understanding of the effects of increased substrate/microbe interaction or increased cellular maintenance due to cell wounding or a possible synergistic combination of both phenomena is possible. It would seem that at EDHA/TDHA ratio of less than 0.2 that increased substrate/microbe interactions likely predominate attributing to the higher SOUR while at EDHA/TDHA ratio higher than 0.2 it would indicate that increased cellular maintenance due to cell wounding was predominating. However, the increase in EDHA/TDHA ratio occurred concurrently with a decrease in TDHA and increase in solubilisation indicating that the ES input applied was not only disruptive to the floc but also to the microbial cell structure. It should be noted that based on the present results the EDHA/TDHA ratio of >0.2 is still somewhat speculative as a precise measure of the degree of cell stress and it should only be used as an indicator, which could be directly influenced by the type of biomass and the specific treatment used. In a related study, Yan et al. (2010) also reported a maximum in enzyme activity enhancement for ASP biomass at ES inputs in the range of 30 KJ/gDS for four hydrolytic enzymes followed by a decrease at higher ES inputs. Although they did not report comprehensive SOUR or solubilization results as in the present study it was concluded that activated sludge bacteria was disrupted at ES inputs higher than 30 KJ/gTS. These results in conjunction with results from the present study which was conducted with real primary effluent show that HT biomass activity is enhanced only at low ES inputs. However, the ES range to achieve such enhanced biomass activity is narrow and has to be experimentally determined based on biomass characteristics, biomass concentration and source of the biomass.

4.4.3 Effect of ultrasound treatment of holding tank biomass morphology

Figure 4.7 illustrates typical changes in HT biomass floc structure after sonication. It can be observed that although lower ES inputs (11-56 KJ/gTS) caused floc size reduction, a clear floc structure could still be observed but which was lost at 118 and 156 KJ/gTS. After sonication and during reflocculation test at the different ES inputs, the sonicated biomass was placed in a 1.5L flask with 300 mL ROPEC primary effluent wastewater with mild aeration, followed by floc morphology reassessment. After 2h of mild aeration the biomass sonicated at 11 and 56 KJ/gTS had almost recovered its original floc size (82% ± 9); in terms of ECD while biomass sonicated at higher ES inputs (>118 KJ/gTS), lost completely and irreversibly its floc structure and ability to reflocculate. Previous research, also working toward reducing excess sludge minimization via increasing microbial activity due to enhanced aeration (Friedrich, 2002; Abbasi et al., 2002) suggested that increased biomass activity after floc reduction was due to better substrate and oxygen diffusion within the floc. While they did not use any treatment nor reported on floc morphology changes after increased aeration under ambient conditions, they concluded that by increasing the oxygen concentration alone, a deep diffusion of oxygen and enlargement of the aerobic volume inside the floc could be achieved which increased SOUR and reduced excess sludge production. From the results obtained in the present study it is believed that in the Abbasi et al. (2002) study, improved biomass activity was a result of the combination of increased deep oxygen diffusion as well as increased microbial floc area resulting from the mechanical aeration turbulence and subsequent increased substrate/microbe interaction.

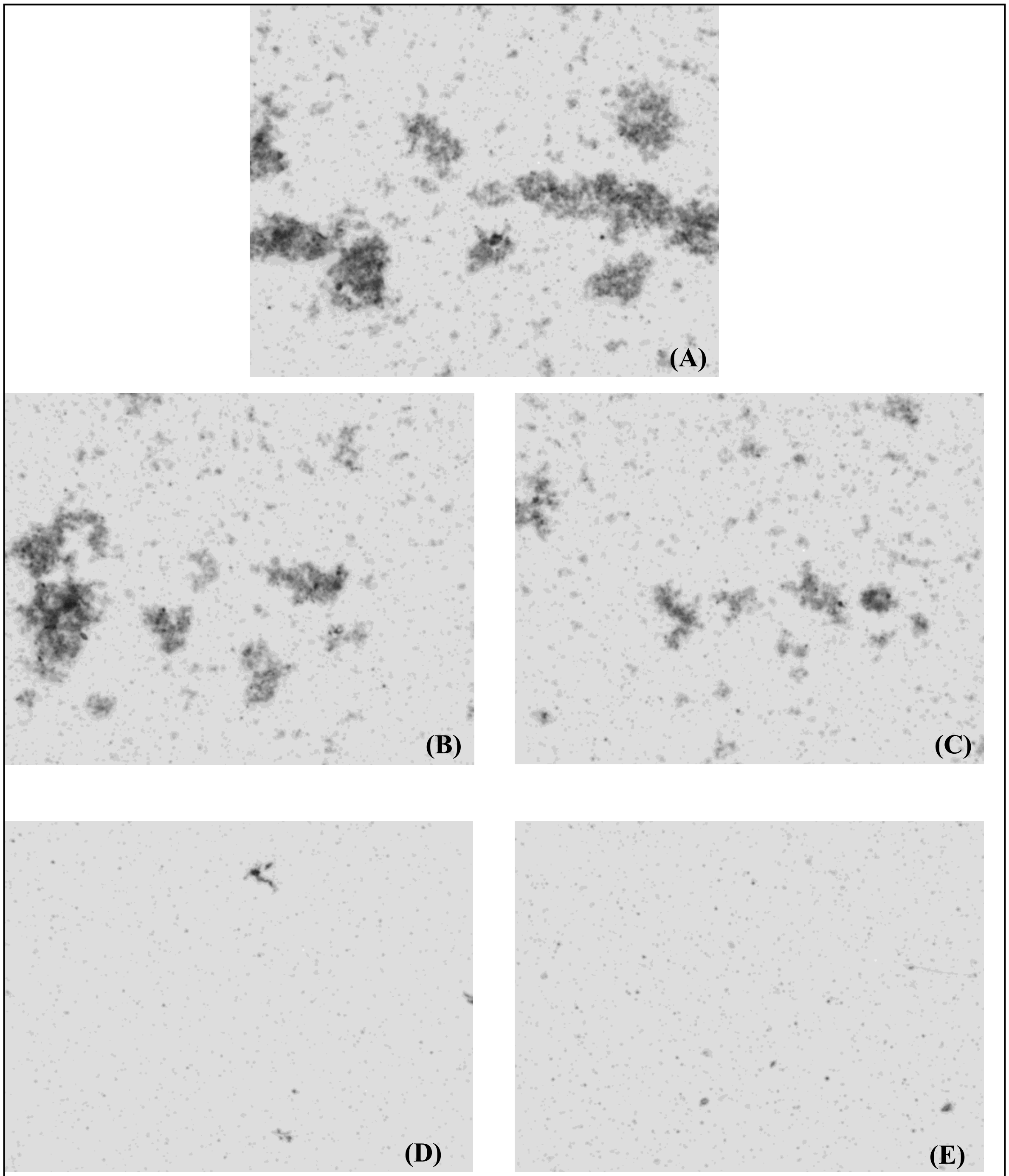


Figure 4.7: Effect of ES input on floc morphology for (A) HT control and (B), (C), (D), and (E) for ES inputs of 11, 56, 118 and 156 KJ/gTS respectivaly. Images presented are from HT biomass concentrations of 3000 mg/L. similar results where observed for all MLSS concentrations tested.

Therefore, the increased biomass activity and eventually excess sludge reduction could have been due to changes in the floc structure (i.e., decreased floc size). In the present study, it was observed that while the floc size decreased at low ES (11 KJ/gTS) both the SOUR and total DHA activities increased and then they both decreased with increasing ES input. From the previous section in the present study the decrease in SOUR and TDHA was linked to excessive microbial stress and cell lysis. If one now considers that as a floc particle gets smaller with increased ES then the coefficient of molecular diffusion for oxygen and substrates should increase (Metcalfe and Eddy, 2003) leading to increased activity. Combining SOUR, TDHA as well as floc morphology data now suggest the existence of a break-point in size reduction with US treatment, beyond which biomass activity no longer increases and subsequently decreases and becomes irreversibly lost. If the average SOUR for the HT biomass at all MLTSS concentrations is considered for every ES input, there was an associated percent reduction in floc size versus the control. By plotting the average biomass activity (measured as SOUR) versus relative percent floc size reduction as illustrated in Figure 4.8 it can be observed that enhanced biomass activity was only achieved with a reduction in floc size in a range of between 5 to 12%. At higher relative reductions in floc size which occur at higher ES inputs there was a loss in HT biomass activity compared with controls. In terms of the floc morphology, decreases in floc size greater than approximately 12 % have negative impacts on enhanced SOUR or TDHA.

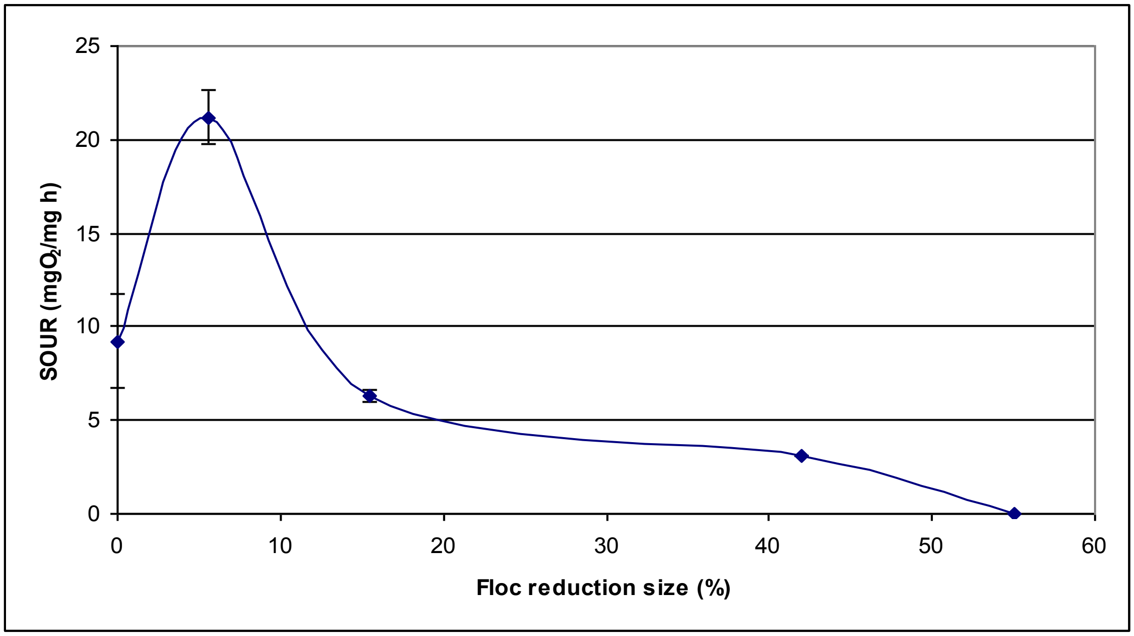
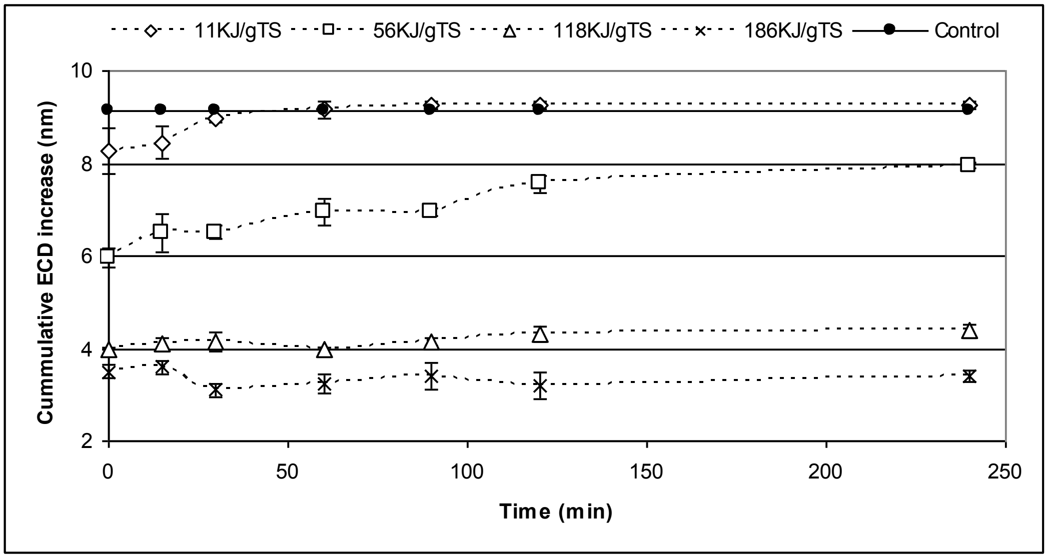


Figure 4.8: HT biomass activity (SOUR) observed during floc size reduction during US treatment. Data presented are the mean value and error bars the standard deviation between eight measurements.

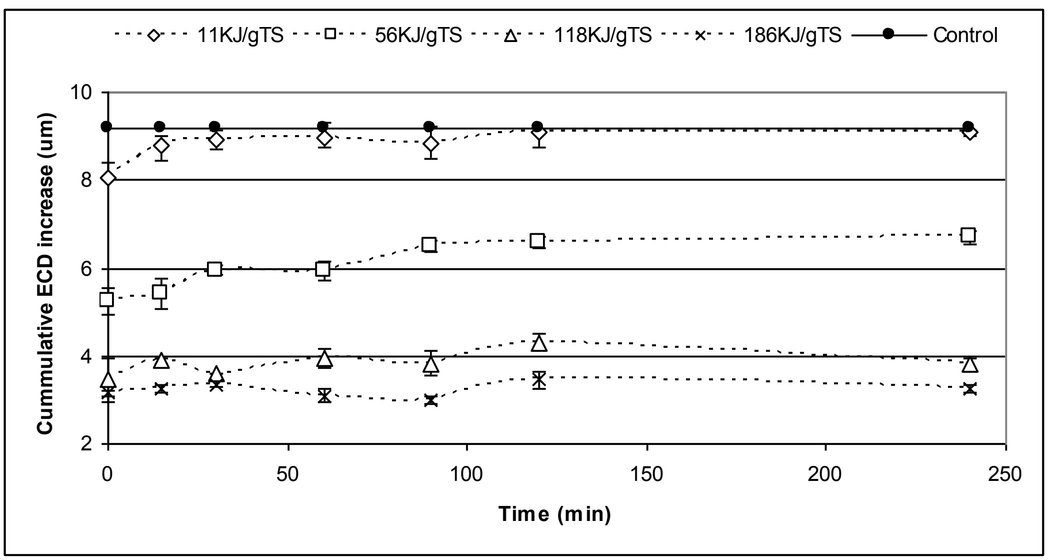
Based on the results from the present study, it can also be postulated that the enhanced biomass activity at low ES inputs is the result of several combined mechanisms: 1) the improved diffusion of nutrients and oxygen through the matrix floc as a result of a mild floc size reduction. 2) US ES input energy at such conditions most likely also caused a slight increase in substrate biomass interaction of organic materials in the contours of the floc making this substrate readily available to the bulk microbial cells and finally 3) as ES input increased it also wounded the microbial cells without causing a significant degree of lysis (Figure 4.5b) Hence those 'wounded' cells required more oxygen and nutrients for repair/maintenance functions. Additionally, as a method to set the maximum enhanced increase in specific activity, percent floc size reduction can be optimized to a desired size range using US, which is usually at lower ES inputs. This finding illustrates the potential economical advantage of incorporating low intensity US treatment as a potential method to increase enhanced sludge reduction in conventional ASPs compared to other technologies including longer SRT processes and/or increased aeration.

4.4.4 Effect of US pretreatment on holding tank biomass reflocculation ability

During aerobic biological treatment of municipal wastewater, biomass flocculation is a critical parameter that has direct impact on biomass settling characteristics and effluent quality. Biomass flocculation mechanisms are very complex and are beyond the scope of the present study. However, as the results from this research will be used in conjunction with an ASP and due to the potential disruptive effect of US on HT biomass at high ES inputs, it is important to observe the effect of sonication on HT tank biomass reflocculation before being recycled (post-sonication) to the ASP. Although there is no agreement in the current literature on which parameters can give a direct measurement of flocculation in complex biological matrices, several studies conducted on flocculation of inorganic particles have used the increase in floc size with time under specified conditions to assess particle reflocculation. Additionally, floc size growth has also been used to assess the extent of reflocculation in biological samples following a deflocculating event (Spicer et al., 1998; Biggs and Lant, 2000). Figure 4.9a and 4.9b shows reflocculation of HT biomass (in the presence of municipal primary effluent) via the floc size increase (expressed as ECD) with time after US floc dispersion at various ES inputs for HT biomass concentrations of 1500 and 3000 mg TSS/L respectively. From Figure 4.9 it can be observed that HT reflocculation was shown to be dependent on the ES input. For ES inputs of 11 KJ/gTS at both HT biomass concentrations it was observed that rapid reflocculation occurred and the floc diameter increased to control values within 30 minutes and remained stable for the remainder of the reflocculation test.



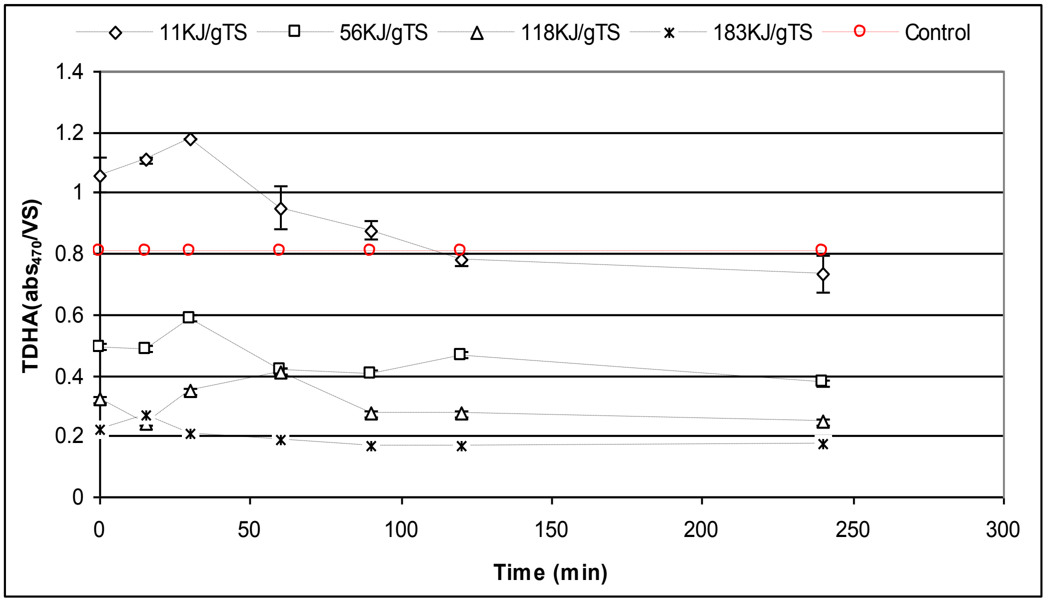
(a)



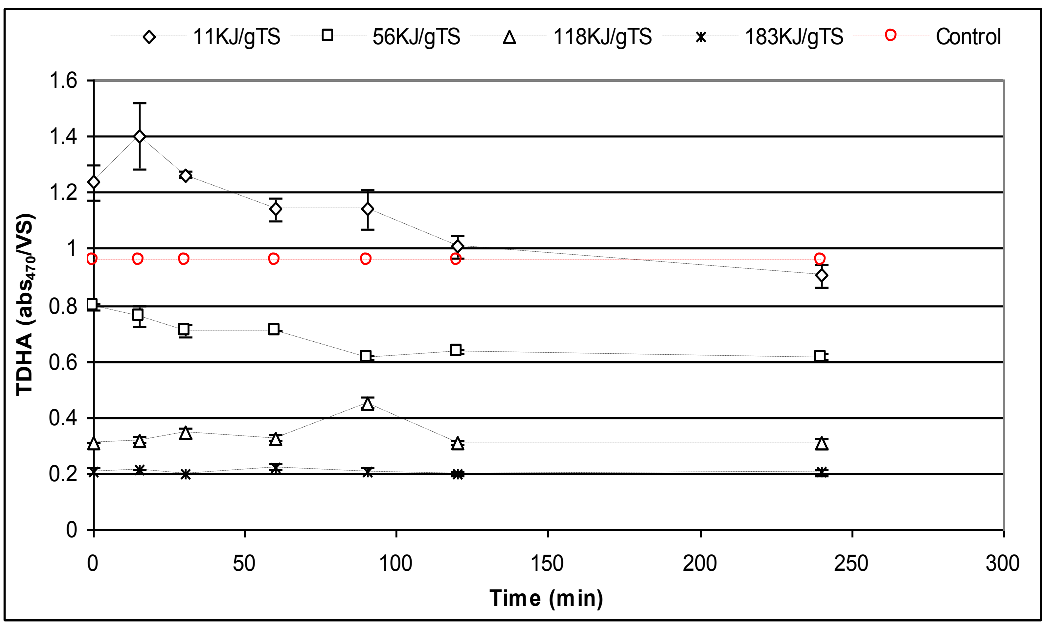
(b)

Figure 4.9: Percent (%) increase in floc size for HT biomass at concentrations of (a) 1500 mg/L and (b) 3000 mg/L. Data represent the mean value and error bars the standard deviation from duplicate measurements. Data points are joined to indicate the trend

At US input of 56KJ/gTS initial floc size was initially disrupted to ECD of about 5-6 nm for both HT concentrations from the control value of about 9 nm. During reflocculation floc size growth increased marginally in the first 60-90 minutes but ECD values were in the range of 6.5 and 7.5 nm for HT biomass concentrations of 3000 and 1500 mg/L respectively which corresponded to a approximately 65 and 75% of the control ECD diameter. With additional reflocculation time there was no further increase in ECD diameter. While for higher ES inputs (118 and 186 KJ/gTS) floc size was originally disrupted to ECD values of 3-4 nm from control ECD diameters of 9 nm then remained unchanging with time for both HT biomass concentrations tested. These results suggest that the sonicated HT biomass was only able to fully refloccualate (without any flocculent agents) at specific ES inputs lower than 56 KJ/gTS for the two different HT biomass concentrations tested (only partial reflocculation at 56 KJ/gTS). The results from the present study at low ES inputs are in agreement with those presented by Biggs and Lant (2000). In their study, aerobic biomass sonicated for 3 min (50W energy input), showed rapid reflocculation with increase in floc size to its original control size after 30 min reflocculation (without the addition of flocculent agents). They concluded that activated sludge reflocculation after floc dispersion occurred due to the presence of polymeric materials (EPS) in the dispersed floc. In the present study, although sonication at ES inputs higher than 56KJ/gTS resulted in higher concentrations of proteins and sugars into the soluble phase (Figure 4.5) biomass reflocculation was not observed during the test, indicating that the formation of the polymeric matrix and concomitantly reflocculation required metabolically active bacteria. In the present experiment, reflocculation ability of HT biomass was correlated with the activity of the biomass. Due to the rapid response of the XTT test it was used as the measure of activity during reflocculation assays. This is not possible with SOUR which is more time consuming and cannot be measured at discrete time intervals. It was observed that as ES input increased above 56KJ/gTS, the initial biomass metabolic activity determined by TDHA also decreased for both HT biomass concentrations tested compared to HT control biomass activity. This decrease in TDHA was a definite indication that HT biomass was wounded or possibly lyzed as a result of the increased ES input. Following TDHA over time with real primary effluent substrate during reflocculation indicated that only at the lowest ES input was there an increase in TDHA activity as the HT biomass metabolized the primary effluent substrate during reflocculation. For ES input of 11 KJ/gTS TDHA increased by 20% and 30% during the first 25-30 minutes of the reflocculation assay for HT biomass concentrations of 1500 and 3000 mg TSS/L respectively (Figure 4.11), then decreased with time. The increase in TDHA correlated with the ability of the HT biomass to refloccualate, likely due to the reformation of the polymeric matrix by metabolically active bacteria. However, at 11 and 56 KJ/gTS the TDHA metabolic activity decrease after 60 minutes was most likely associated with substrate limiting conditions within the reflocculation assay system as no further primary effluent was added to the assay. In the case of ES inputs higher than 56KJ/gTS, TDHA initial biomass metabolic activity decreased by more than 60% for both HT biomass concentrations and remained low over time for the whole reflocculation test. These results indicated that for the HT biomass generated in this study at ES input conditions above 118 KJ/gTS, reflocculation was not possible, most likely due to the irreversible loss of biomass metabolic activity (measured as TDHA) induced by exposure to the high energy sonication source.



(a)



(b)

Figure 4.10: Effect of ES input on HT biomass activity (TDHA) during reflocculation tests. For HT concentrations of (a) 1500 and (b) 3000mg/L. Data represent the mean value and error bars the standard deviation between replicate measurements

The loss in metabolic activity and inability to reflocculate at ES inputs higher than 56KJ/gTS was further supported by the analysis of the EDHA/TDHA ratio during the reflocculation assay. Not only did TDHA decrease with increased ES input but also the EDHA/TDHA ratio increased an average for both solids concentrations tested by 15 ± 3% as ES increased from 11 to 118 KJ/gTS respectively. As mentioned previously the increase in EDHA/TDHA ratio is especially in combination with a decrease in TDHA a strong indication of severe wounding and lysis of the microbial biomass. Also from Figures 4.9 it can be observed that solids concentration did not seem to have a large effect on HT biomass reflocculation. For both HT biomass concentrations exposed to the lowest ES input (11 KJ/gTS) full reflocculation to control values occurred within approximately 30 minutes. The reflocculation results tend to indicate that reformation of the polymeric matrix is not a passive process but rather an active biologically mediated one. This suggests that although both ES input and biomass concentration are highly correlated in terms of energy consumption, samples with high solids concentrations would require less ES input, which can be beneficial in terms of sonication associated costs.

Since biomass reflocculation ability has a direct impact on biomass settleability the sludge volume index (SVI) was also determined during the reflocculation assay at different time intervals. Although SVI is an empirical test, it is routinely used for the determination of sludge settling ability and as an indicator of sludge bulking. SVI values less than 150 mL/g usually indicate activated sludge with good settling characteristics, while values higher than 150 mL/g are associated with bulking sludge (Metcalfe and Eddy, 2003). Figure 4.10 shows the average SVI measurement from both HT biomass solids concentrations tested. It can be observed from Figure 4.10 that there was a steady decrease in SVI with increasing ES down to 40 mL/g for the highest ES input from 112 mL/g for the control, suggesting potential improvements in sludge settling and possible dewaterability over time. This finding is not that unexpected based on the solubilization data and increased lysis and fragmentation of the HT biomass with increased ES input. However, it should be recognized that at the highest ES inputs there was little TDHA and no biological activity present in the biomass.

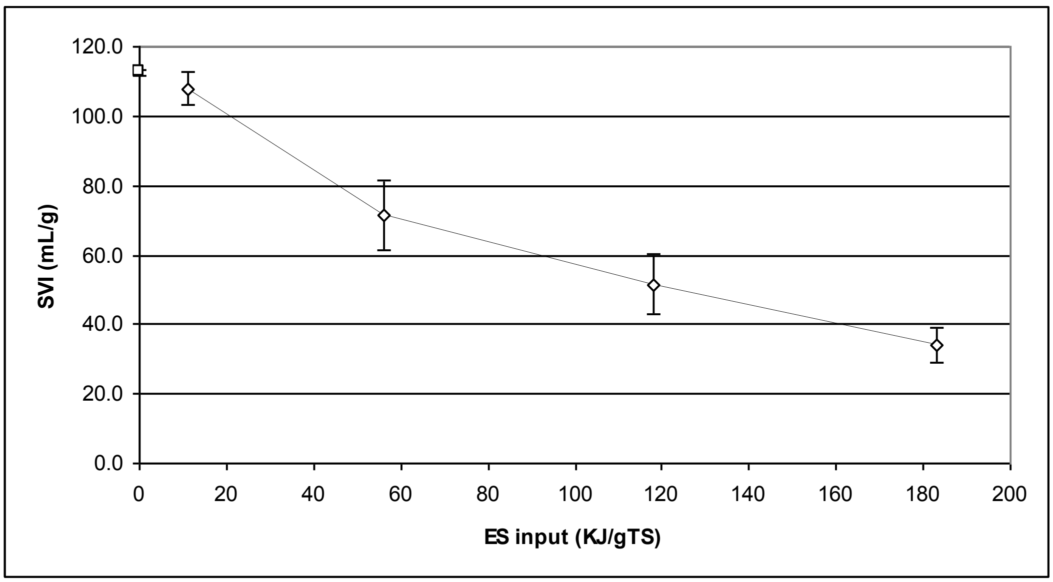


Figure 4.11: SVI determination during reflocculation tests. Data presented are the mean values between HT biomass concentrations of 1500 and 3000 mg/L and error bars the standard deviation between two measurements. Data point are joined to indicate the trend

In terms of development of an HT biomass to potentially decrease excess sludge production by increased maintenance metabolism while also retaining certain physical characteristics such as microbial activity, flocculation and settleability the data from the present study tends to indicate that the lowest ES input is best suited to enhance HT biomass activity as well as maintaining physical characteristics such as flocculation and settleability to accomplish this task. At higher ES inputs the loss of biological activity and reflocculation ability would tend to indicate that this higher energy input strategy would be better suited to reduce excess ASP biomass production via the cryptic growth approach. If the cryptic growth approach was to be adopted as the sludge minimization strategy it follows that there would be no need for the controlled anoxic HT, however energy requirements and costs would be substantially higher than the metabolic maintenance approach that uses much lower ES inputs. .

4.5 Ultrasound treatment optimization using response surface methodology and HT biomass activity empirical modelling

The intricate relation between physical and chemical parameter characteristics of most biological processes makes deterministic modeling very difficult. Although probabilistic approaches also present certain disadvantages, they result from the experimental data, and under the proper assumptions they can describe the phenomena of interest. Response surface methodology (RSM) is a collection of statistical and mathematical tools used to define the effect of the independent variables (alone or in combination) on the process response of interest (Bas and Boyaci, 2007). RSM experimental methodology can be used to analyze the effects of the independent variables and generate an empirical mathematical model to describe the process of interest (Meyers et al., 2009). The relationship between the response and the independent variables is given by equation 4.5

$$Y = f(x_{1,} x_2, ..., x_n) + \varepsilon \qquad (4.5)$$

Where Y is the response of interest, f is the unknown function of the response, $x_1, x_2, \ldots, x_n$ are the independent variables, n is the number of variables and ε is the statistical error, which represent other sources of variation non accounted for by f. In general, it is assumed that ε has a normal distribution with mean zero and variance σ^2 (Meyers et al., 2009). According to Khuri and Cornell (1996) RSM is a process that involves: (1) Setting up a series of experiments that will yield measurements of the response of interest, (2) determination of the mathematical model that best fits the collected data from the design chosen in (1) by conducting appropriate tests of hypotheses concerning the model parameters and (3) determination of the optimal settings of the experimental factors that produce the maximum (or minimum) value of the response. When the best values for optimization are beyond the available resources of the experiment, RSM is aimed at obtaining at least a better understanding of the overall system. In RSM, regression analysis helps to establish empirically (by fitting some form of mathematical model) the type of relationship that is present between the response and the independent variables (Khuri and Cornell, 1996). The recent development in statistics software has made possible fast and accurate regression analysis, experimental design and RSM. Minitab15 (Minitab Corp., USA) is one of many suites of programs that is used by industry and research alike. Minitab15 has many features that make the design of experiments and its analyses very reliable. In this study, all statistical analysis, from experiment design through analysis of results was carried out using Minitab 15.

Initially in the present study a general full factorial design was used to determine the influence of ES input energy (0, 11, 56, 118 and 156 KJ/gTS) and HT biomass concentration (1500, 2000, 2500 and 3000mg/L) on biomass activity (measured as SOUR and DHA). Minitab15 was used to identify the best fitting regression models that can be constructed with the specified variables. Hence, all possible subsets of the predictors, beginning with all models containing one predictor, and then all models containing two predictors and so on can be examined. Once a potential model has been identified further analysis is needed in order to determine if the potential explanatory model does not violate any of the regression assumptions. Table 4.2 shows the Minitab15 output for the best models fitting the data from the full factorial experimental runs.

Table 4.2: Best subsets regression from the initial full factorial design

Vars	R-Sq	R-Sq (adj)	Mallow's Cp	Variance S	MLTSS x1	ES x2	Int x1*x2	$x1^2$	$x2^2$
1[1]	63.7	62.8	0.4	4.21		X[2]			
1'	57.1	55.9	7.1	4.58					X
2	64.8	62.9	1.3	4.20	X	X			
2'	64.8	62.9	1.3	4.20		X		X	
3	65.5	62.7	2.6	4.21	X	X	X		
3'	65.5	62.6	2.7	4.22		X	X	X	
4	66.1	62.3	4	4.24	X	X	X		X
4'	66.1	62.1	4.1	4.24		X	X	X	X
5	66.1	61.2	6	4.3	X	X	X	X	X

[1] Potential explanatory model in duplicates
[2] X indicates a particular predictor to be included in the explanatory model

In Table 4.2, Vars indicates the number of predictors or variables to be included in the model. The test statistic Mallow's Cp is an estimate of the total mean square error of the 'n' fitted values using 'p' parameters (Larose, 2006). The estimate is

$$Cp = \frac{SSE(p)}{MSE(k+1)} + 2p - N \qquad (4.6)$$

Where: SSE(p) is the error sum of squares (SSE) for the model with p parameters, MSE(k+1) is the mean squared error (MSE) for the full model, p is the number of variables in the regression model and N is the number of observations. Cp is approximately p when the regression model has an adequate number of parameters. The best fitted model is determined by plotting Cp for all possible combinations of variables against 'p', the model with the lowest Cp approximately equal to 'p' is considered the most adequate. Fitted models with large Cp values most likely have large bias and low statistical significance. In addition, an adequate explanatory model should have high values of R^2(adj) and low variance (S). From Table 4.2, it can be observed that any of the potential models was adequate to fit the experimental data. The variables in all possible combinations presented in Table 4.2 had almost the same low values for the variance (S), R and R^2(adj). Table 4.3 shows the ANOVA for the full model (Model 5 Table 4.2), including the quadratic terms and interactions. Table 4.3 also includes the variance inflation factor (VIF) for the parameters. The VIF is the reciprocal of the tolerance and indicates how much the variance of the coefficient estimate is being inflated by multicollinearity (Larose, 2006). When multicollinearity is present the strong linear association between the independent variables makes the process difficult to model, as it is not possible to determine the effect of one specific parameter on the response without the presence of the other. VIF values > 10 indicate that the individual parameter should not be included in the model. Inspection of the VIF and p-values for the predictors in Table 4.3 indicated that VIF values for all variables including interactions are > 10 and from the general factorial design it is not possible to obtain a statistically significant model to fit the experimental data.

Table 4.3: Analysis of variance and variable coefficients from initial full factorial design

Predictor	Coef	SE Coef	T	P	VIF
Constant	8.21	13.62	0.6	0.55	
ES	-0.08768	0.06488	-1.35	0.186	33.22
MLTSS	0.00288	0.01238	0.23	0.818	103.52
ES*MLTSS	$-1.68X10^{-5}$	$2X10^{-5}$	-0.84	0.408	18.47
$(MLTSS)^2$	$-1X10^{-7}$	$2.7X10^{-6}$	-0.04	0.971	102.25
$(ES)^2$	$2.3X10^{-4}$	$2.9X10^{-4}$	0.79	0.435	17.02
S = 4.302	R-sq = 66.1%	R-sq(adj)	= 61.2		
PRESS	= 846.96	R-sq(pred)	= 54.4%		

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	5	1229.92	245.98	13.29	0.000
Residual error	34	629.41	18.51		
Lack of Fit	14	625.73	44.69	242.91	0.000
Pure error	20	3.68	0.18		
Total	39	1859.33			

Source	DF	Seq SS
MLTSS	1	20.35
ES	1	11.85
ES*MLTSS	1	13
$(MLTSS)^2$	1	0.02
$(ES)^2$	1	11.54

Lack of Fit test
Possible curvature in variable ES (p-value = 0.0001)
Possible interaction in variable ES*MLTSS (p-value = 0.002)
Possible interaction in variable ES*MLTSS (p-value = 0.0001)
Possible curvature in variable $(ES)^2$ (p-value = 0.05)
Overall Lack of Fit test is significant at p = 0.05
p-value < 0.05 is significant at $\alpha = 0.05$

In addition, Table 4.3 also indicated the possible interaction between the independent variables as well as the presence of curvature in the response. According to Montgomery (1997) when the model is fitted over wide ranges or levels of the independent variables (as was the case for ES) higher terms and their interactions become apparent and they exert great influence on the response (as can be observed in Table 4.2). Furthermore, Figure 4.12 also indicated the presence of curvature in the low range of ES input, suggesting that a maximum was most likely found around the low ES input range. General factorial designs are frequently used (conditions permitted) as screening tools which allow the examination of various independent variables and their potential degree of interaction on the response of interest (Montgomery, 2007). However, when an approximation to the true response is not possible most likely due to the presence of curvature or higher order terms, the model needs to be upgraded to a second order model of the form presented in equation 4.7

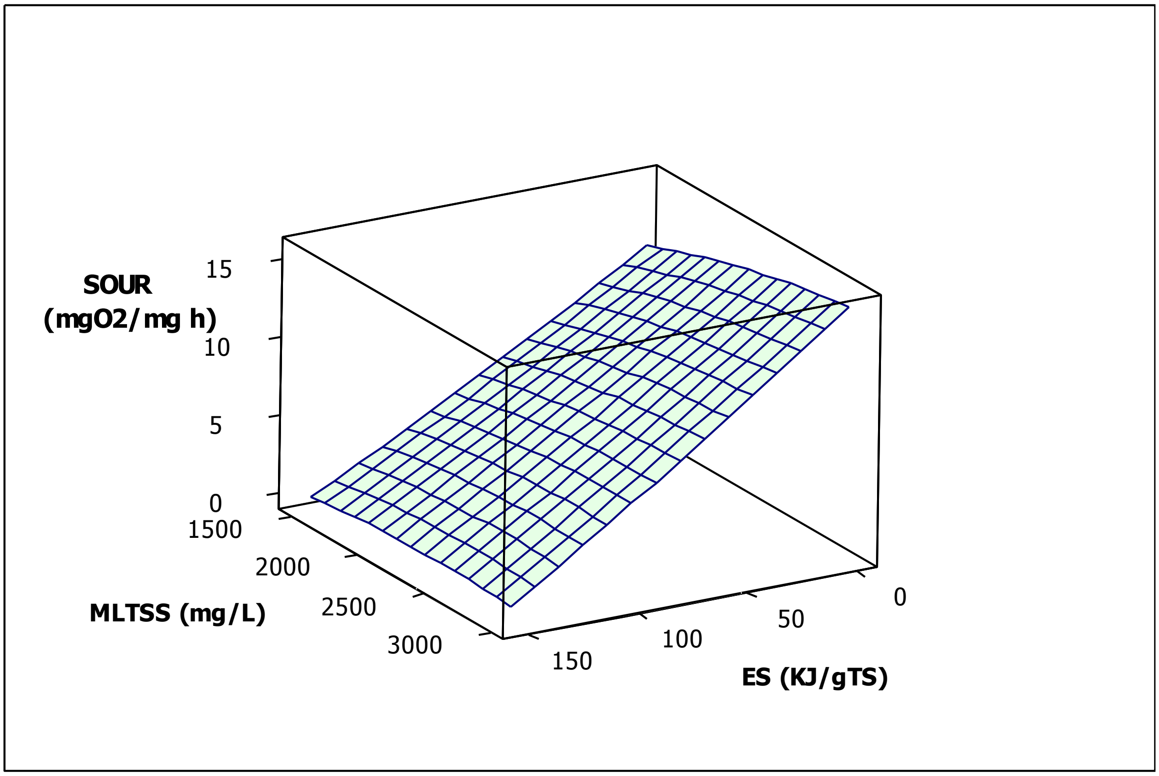


Figure 4.12: Response surface from general full factorial design

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (4.7)$$

Where Xi, Xj,…, Xk are the input variables which influence the response Y; and β_0, βi (i= 1, 2, …,k), βij(i=1,2,…k; j=1, 2, …,k) are unknown parameters and ε is the random error.

In the present study from the initial general full factorial design the average HT biomass SOUR showed three distinctive regions of biomass activity after ultrasound treatment (Figure 4.13). In area (I) a maximum SOUR (and DHA) was reached (compared to control sample) indicating that ES inputs lower than ~40 KJ/gTS could be the most suitable to wound the aerobic biomass and the slight floc dispersion could increase the flux of nutrients and oxygen through the floc matrix resulting in an increased biomass respiratory activity. In area (II), even though the floc structure was still maintained to a great extent and reflocculation was possible under certain conditions, the HT biomass activity was lower than the control sample. Finally, in region (III) longer exposure of the HT biomass to the US source most likely in combination with the high temperature produced lead to floc solubilization and the total irreversible loss of biomass metabolic activity. Therefore, the HT can be operated either at region I, in which a more active biomass once returned to the aeration tank would consume more oxygen and nutrients for maintenance functions rather than reproduction or it can also be operated under ES input conditions in region (III) which clearly leads to the production of an autochthonous substrate which once returned to the aeration tank would increase in general the metabolic activity of the ASP biomass.

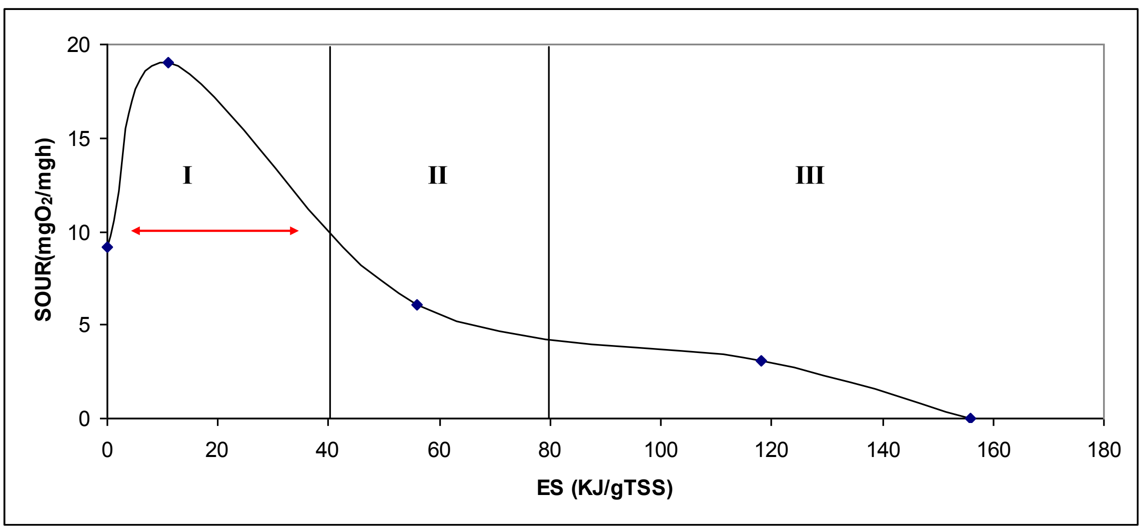


Figure 4.13: General effect of ES input on HT biomass activity (SOUR). The red line indicates the range of ES input which produced the maximum SOUR

However, HT operation in region (III) presents additional challenges. It is believed that soluble products of cell lysis such as sugars and proteins will contribute to activated sludge biomass cryptic growth once they are returned to the aeration tank. However, this increase in soluble COD can generate other problems in the ASP in general, such as enhancing the growth of free cells, which in turn can lead to an increased TCOD in the effluent. It can also enhance the growth of fast growing filamentous microbes with potentially detrimental effects on effluent quality and finally, it can also contribute to the accumulation of non-biodegradable COD in the effluent (Metcalfe and Eddy, 2003; Ginestet, 2006). Although no further research was performed on the operation of the holding tank under conditions (II) it is believed that due to the lower biomass activity and the partial loss of floc structure the ASP operation would require certain modifications to the modified ASP once the sonicated HT biomass is returned to the aeration tank, i.e increased sludge age or increased settling time in SBR. For the present study it was decided to do further research under ES input conditions in region I (Figure 4.13), which in addition to being more economically favourable in terms of energy utilization, elevated temperature in the biomass is not of concern since at such low ES the potential net increase in sample temperature is low (~10 ºC maximum) which can be beneficial in cold climates or it can be easily controlled if necessary, eliminating the effect of this variable on the process. Finally, US operation at lower ES inputs is less severe on the US horn tip assembly which will extend horn life.

4.5.1 Central composite design

In order to better access the effect of the operational variables (ES input and HT solids concentration) on biomass activity, a central composite design (CCD) experiment with two replicates was performed in the region of maximum SOUR (also maximum TDHA) which is in the low ES input range for region I (Figure 4.13). The simplest of the

CCD can be used to fit a second order model to a response with 2 factors (Montgomery, 2001). In the specific case of the present study, a two level full factorial (2^2) design was augmented with runs at central and axial points. The number of axial points having 2 factors is given by 2^2 (Table 4.4). Table 4.5 shows the CCD in terms of the actual and coded variables for ES (low =10 and high = 40 KJ/gTS) and for MLTSS (low =1500 and high= 3000 mg/L) with the respective response (SOUR and DHA). In order to minimize potential biases in the response, the experimental runs were randomized.

Table 4.4: Central composite design to optimize US as applied to HT biomass

Factors	2
Base runs	13
Total runs	26
Cube points	8
Center points in cube	10
Axial points	8
α value	1.41

Variables were coded according to Meyers et al. (2009)

$$X_i = \frac{x_i - \left[\dfrac{x_{high} + x_{low}}{2}\right]}{\dfrac{x_{high} - x_{low}}{2}} \tag{4.8}$$

Where x_i is the natural variable, X_i is the coded variable and x_{high} and x_{low} are the maximum and minimum values of the actual variables. Tables 4.6 and 4.7 show the potential explanatory models for SOUR and TDHA respectively. The explanatory models presented in Tables 4.6 and 4.7 were analyzed considering the number of potential variables added to the explanatory models starting with the constant model (Vars of 1), the mean response plus the linear components and their interactions and finally the addition of the quadratic effects.

Table 4.5: Experimental design data from the CCD with response variable values

		Actual units		Coded units			
Obs	**Run**	**ES (KJ/gTS) x1**	**MLTSS (mg/L) x2**	**X1**	**X2**	**SOUR (mgO_2/mg h)**	**DHA (Δabs_{470}/VS)**
1	5	3.8	2250	-1.41	0	17.7	1.27
2	11	25	2250	0	0	16.4	1.22
3	8	25	3310	0	1.41	15.7	0.94
4	10	25	2250	0	0	16.8	1.17
5	12	25	2250	0	0	16.1	1.21
6	9	25	2250	0	0	16.2	1.19
7	7	25	1189	0	-1.41	14.2	0.89
8	13	25	2250	0	0	16.6	1.15
9	3	10	3000	-1	1	22.8	1.42
10	2	40	1500	1	-1	7.8	0.82
11	1	10	1500	-1	-1	19.6	1.12
12	4	40	3000	1	1	7.8	0.81
13	6	46.2	2250	1.41	0	4.2	0.84

As it can be observed from Tables 4.6 and 4.7 the addition of variables improved the statistical significance of both the SOUR and TDHA models (high R-Sq, R-Sq(adj)) values and low S and Mallows Cp).

Table 4.6: CCD best subsets regression for HT biomass activity (SOUR)

Vars	R-Sq	R-Sq (adj)	Mallow's Cp	S	ES x1	MLTSS x2	$(x1)^2$	$(x2)^2$	x1*x2
1	83.5	82.8	80.7	2.1	X				
1'	11.5	7.9	529.1	4.88			X		
2	95	94.6	10.8	1.18	X		X		
2'	84.4	83.1	77	2.09	X	X			
3	**96**	**95.4**	**7.1**	**1.08**	**X**	**X**	**X**		
3'	95.7	95.1	9.1	1.12	X		X		X
4	96.6	90.9	71.9	1.63	X		X	X	X
4'	**96.2**	**95.5**	**7.8**	**1.08**	**X**	**X**	**X**	**X**	
5	**96.8**	**96**	**6**	**1.01**	**X**	**X**	**X**	**X**	**X**

Table 4.7: CCD best subset regression for HT biomass activity (TDHA)

Vars	R-Sq	R-Sq (adj)	Mallow's Cp	S	ES x1	MLTSS x2	$(x1)^2$	$(x2)^2$	x1*x2
1	55.1	53.2	100.2	0.13	X				
1'	22.7	19.5	188.3	0.17				X	
2	77.8	75.8	40.5	0.09	X		X		
2'	59.2	55.7	90.9	0.12	X				X
3	84.9	82.9	23	0.09	X		X	X	
3'	81.9	79.2	31.2	0.08	X			X	X
4	89.1	87	13.7	0.07	X		X	X	X
4'	88.5	86.3	15.3	0.07	X	X	X	X	
5	**92.6**	**90.8**	**6**	**0.05**	**X**	**X**	**X**	**X**	**X**

Furthermore, the model fitting procedure outlined in Table 4.8 (low PRESS values and high R-Sq(pred)) supported the selection of the full CCD model (SOUR and TDHA model Vars 5) in both cases. Further model adequacy determination was accomplished by eliminating from the full models the terms that were not significant (p-value > 0.05) and then considering the PRESS and R-Sq(pred)). As mentioned earlier models were considered adequate for prediction when PRESS had a low value and R-Sq(pred)) is comparable to R-Sq.

Table 4.8: Sequential model sum of squares

	Source	DF	Seq SS	Pred R-sq	PRESS	p-value*
SOUR	Linear	2	545.84	79.2	134.4	0.000
	Interaction	3	3.92	79.5	132.4	0.000
	Quadratic	5	75.94	93.3	43.4	0.000
DHA	Linear	2	0.554	47.6	0.5	0.000
	Interaction	3	0.039	50.9	0.46	0.000
	Quadratic	5	0.281	85.9	0.13	0.000

*** p-value <0.05 is significant at α = 0.05**

The ANOVA for the explanatory models for SOUR and TDHA (Table 4.9) and the 95% confidence interval for each parameter, calculated as $b_i \pm t_{\alpha/2,n-p} * s_{bi}$ (Table 4.10) suggested that in the case of SOUR the quadratic term ($X2^2$) and the interaction term

(X1*X2) could be eliminated from the explanatory model (p>0.05 and zero as a plausible value in the confidence interval).

Table 4.9: Analysis of variance and regression coefficients for the prediction models (coded units)

Source	SOUR				DHA			
	Coefficient	**Sum of squares**	**p-value**	**VIF**	**Coefficient**	**Sum of squares**	**p-value**	**VIF**
Constant	16.46	0.3221	0.000		1.19930	0.0186	0.000	
X1	-5.809	0.2547	0.000	1.00	-0.1803	0.0147	0.000	1.00
X2	0.611	0.2547	0.026	1.00	0.0459	0.0147	0.000	1.00
$X1^2$	-2.336	0.2731	0.000	1.00	-0.0696	0.0158	0.000	1.00
$X2^2$	-0.311	0.2731	0.268	1.02	-0.1321	0.0158	0.000	1.02
X1*X2	-0.07	0.3602	0.066	1.02	-0.7000	0.0208	0.003	1.02
Lack of fit		19.511	0.000			0.0167	0.000	
R-sq(adj)	0.9599				0.9081			
R-sq(pred)	0.9328				0.8560			
PRESS	43.45				0.14			

p-values < 0.05 is significant at α = 0.05
Lack of fit is not significant at p-values > 0.05

Table 4.10: 95% confidence intervals for model parameters for SOUR and TDHA

Parameter	95% confidence interval	
	SOUR	**DHA**
β_0 (constant)	16.46 ± 0.67	1.199 ± 0.039
β_1 (X1)	-5.808 ± 0.53	-0.1803 ±0.030
β_2 (X2)	0.611 ± 0.16	0.0459 ± 0.0307
β_{11} ($X1^2$)	-2.336 ± 0.57	-0.0696 ± 0.033
β_{22} ($X2^2$)	-0.311 ± 0.57	-0.132 ± 0.132
β_{12} (X1*X2)	-0.700 ± 0.75	-0.700 ± 0.035

While for TDHA, although the 95% confidence interval indicated zero as a plausible value for the quadratic parameter ($X2^2$), from Table 4.9 it can be observed that the parameter was significant (p-value <0.05). Furthermore elimination of $X2^2$ from the explanatory model increased the VIF of the remaining parameters and decreased in general the statistical significance of the model (R-sq, R-sq(adj) and R-sq(pred) decreased to values of 0.669, 0.606 and 0.512 respectively) and it also had a negative effect on the statistical significance of the remaining parameters (p-values > 0.05). This result indicated that the independent variables presented a high degree of correlation. It has been suggested that the elimination of variables with VIF > 10 might remediate the predictive capability of the model, however, if the variable is fundamental to explain the response, its elimination from the explanatory model would make such a model completely inaccurate (Larose, 2006). Although the relationship between the experimental variables on the response is complex, in the literature there is evidence that sonication effects depend not only on the specific energy input (ES) but also on the sample's solids concentration; therefore, the full model (Vars of 5) as outlined in Table 4.9 was retained for TDHA. On the other hand, the elimination of the non significant parameters from the explanatory model for SOUR did not change the general statistical significance of the model. However, the terms that included the variable MLTSS became statistically non significant (p-values > 0.05) and when the interaction term was included, the model predictive capability increased (R-sq, R-sq(adj) and R-sq(pred) to 0.962, 0.933 and 0.955 respectively). This corroborated again the high correlation between the independent variables (ES input and HT solids concentration). According to Raymond et al., (2009) multicollinearity might arise from errors during analysis; however in some cases the independent variables might be highly correlated. Under such conditions if the proposed explanatory model does not violate the regression assumptions, the estimates can still be considered as the best linear unbiased estimators. Figure 4.14 shows the normal probability plots and the residual plots versus the fitted values for the SOUR and TDHA Vars-5 models. From Figure 4.14, it can be observed that the normal probability plot follows a straight line with no outliers present, indicating that the normality assumption for the model adequacy is met. Additionally from Figure 4.14 it can be observed that the residuals are randomly scattered, with no specific trend, suggesting that the variance of the original observations is constant. Therefore in the explanatory model for SOUR the interaction term was also kept. Hence, the final fitted models for SOUR (Equations 4.9 and 4.10) and TDHA (Equations 4.11 and 4.12) in coded and uncoded units respectively correspond to:

$$SOUR = 16.24 - 5.81X_1 + 0.62X_2 - 2.31X_1^2 - 6.2X10^{-5}\,X_1X_2 \qquad (4.9)$$

$$\text{SOUR} = 14.2 + 0.26(\text{ES}) + 2.4\text{X}10^{-3}(\text{MLTSS}) - 0.01(\text{ES})^2$$
$$-6.2\text{X}10^{-5}\,(\text{ES})(\text{MLTSS}) \qquad (4.10)$$

$$TDHA = 1.2 - 0.18X_1 + 0.046X_2 - 0.07X_1^2 - 0.13X_2^2 - 0.07X_1X_2 \qquad (4.11)$$

$$\text{TDHA} = -0.37 + 1.8\text{X}10^{-2}(\text{ES}) + 1.3\text{X}10^{-3}(\text{MLTSS}) - 3.1\text{X}10^{-4}(\text{ES})^2 - 2.3\text{X}10^{-7}(\text{MLTSS})^2$$
$$-6.2\text{X}10^{-6}(\text{ES})(\text{MLTSS}) \qquad (4.12)$$

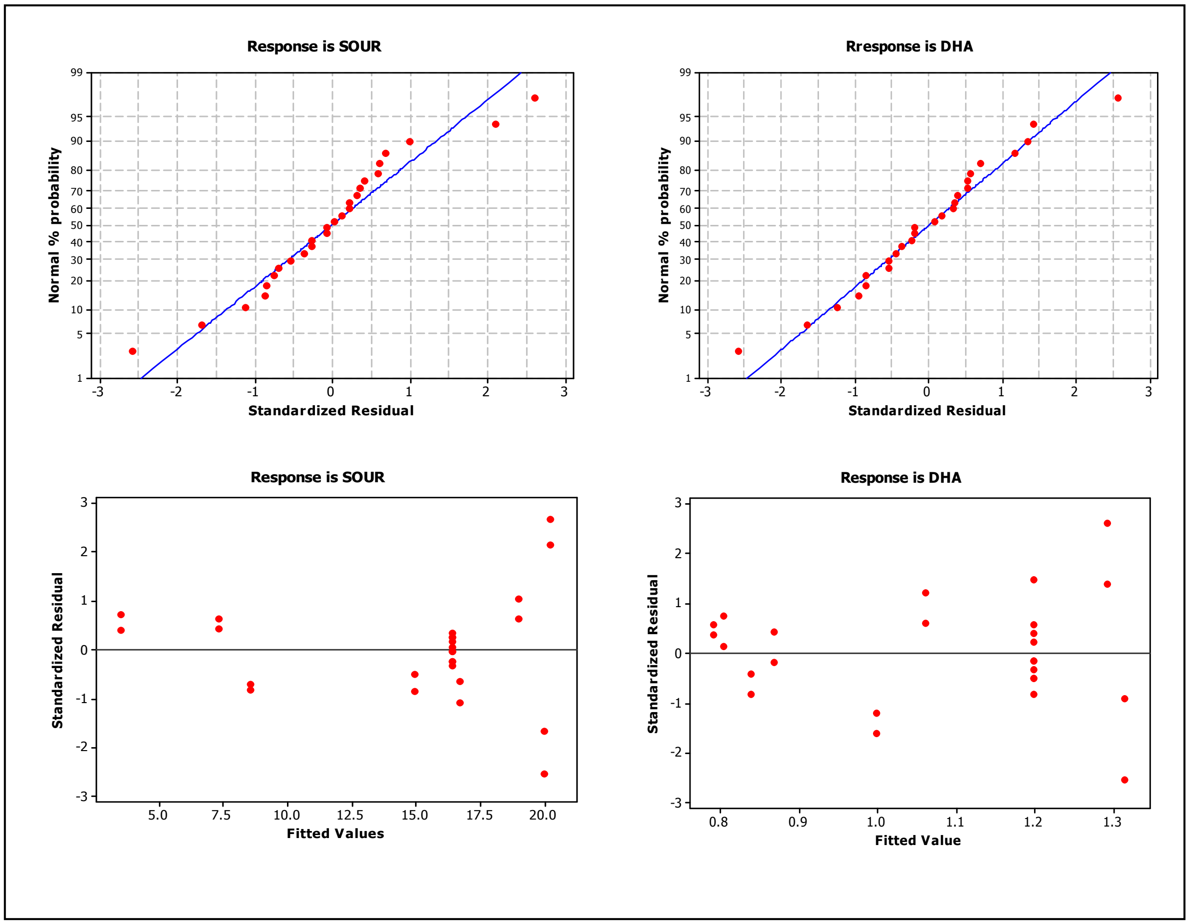


Figure 4.14: Normal probability and residual plots for the fitted models for SOUR and TDHA

In order to visualize the combined effects of ES input and solids concentration on HT biomass activity contour plots and response surfaces were generated for the corresponding Vars-5 models for SOUR and TDHA (Figures 4.15 and 4.16 respectively). The response surfaces in both cases indicated the presence of maximum activities within the experimental range. Also from the response surfaces (Figures 4.15 and 4.16) it can be observed that SOUR and TDHA biomass activity followed similar trends and both showed higher sensitivity to changes in the ES input variable as compared with changes in the HT biomass concentrations.

Also from Figures 4.15 and 4.16 visually it can be observed that the optimum conditions for high specific TDHA response were low ES inputs (~10 kJ/gTS) and high HT biomass concentration (~3000 mg/L). On the other hand visual inspection of Figure 4.15 indicates that maximum SOUR response was achieved as well at low ES input (~10 KJ/gTS) and was not so sensitive to HT biomass concentration (~1500-3000 mg/L). However, when the Solver (Excel, Microsoft) was used to find the solutions to equations (4.10) and (4.12), the program was not able to converge to a solution. As mentioned previously the high correlation of the independent variables produced explanatory models that need a different approach in order to find the optimum operation conditions. Concomitantly at this stage visual inspection of Figures 3.15 and 3.16 would seem to indicate that TDHA was more sensitive than SOUR and if selecting conditions for optimum Region I operation for post HT biomass treatment that it would be best to operate at low ES and high solids concentration of ~10 kJ/gTS and ~3000 mg TS/L respectively.

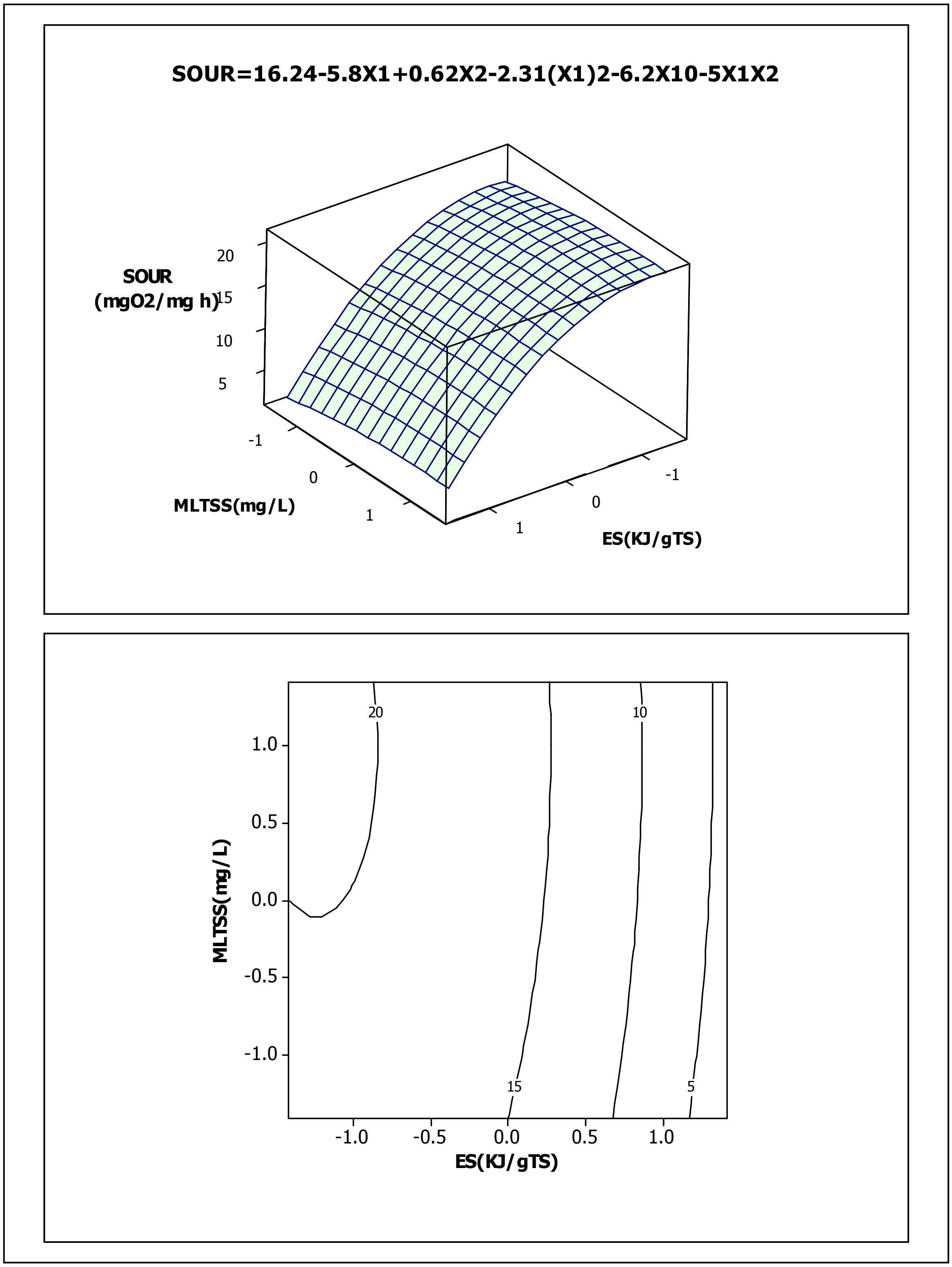


Figure 4.15: Response surface and contour plots for SOUR

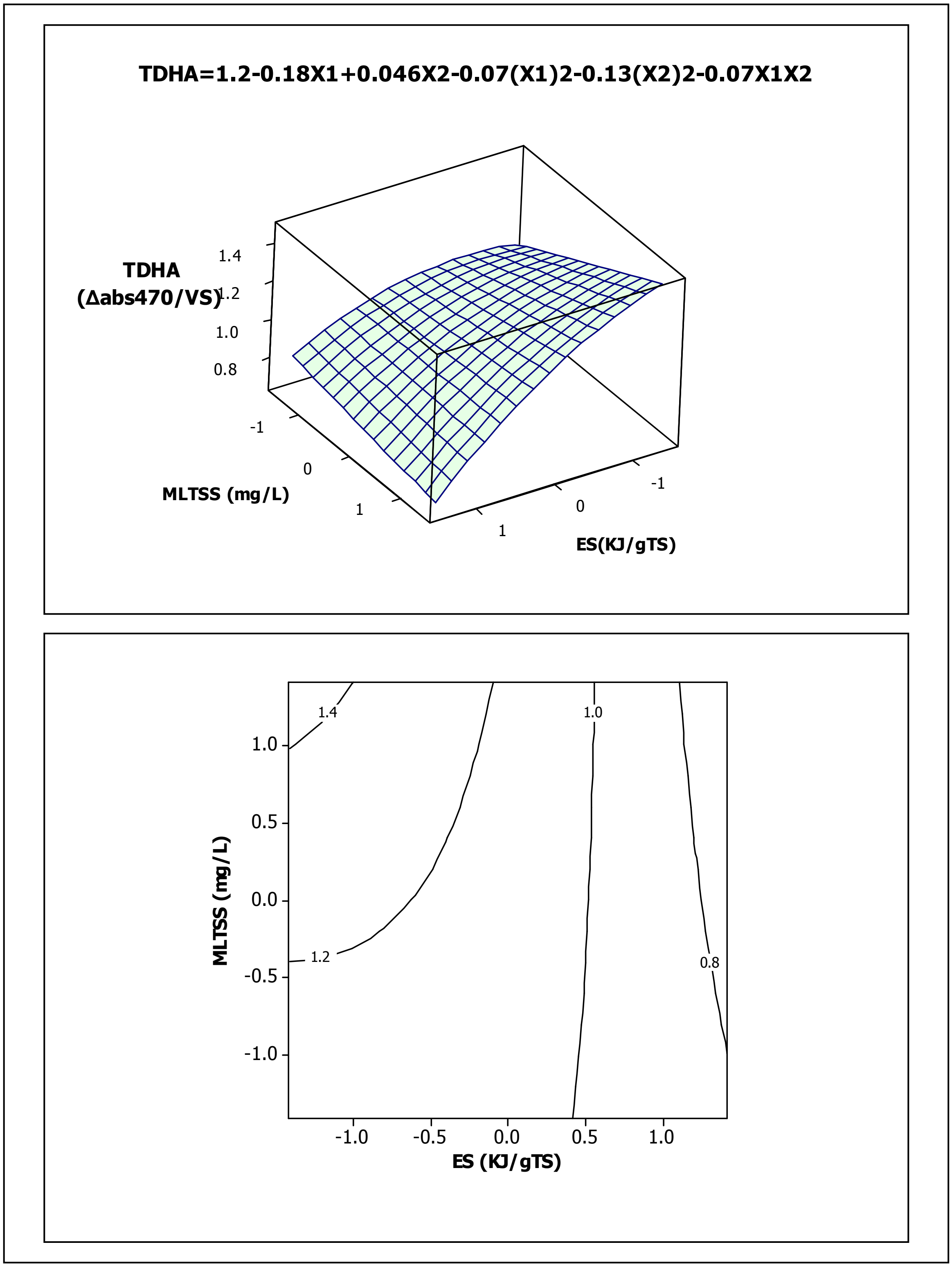


Figure 4.16: Response surface and contour plot for TDHA

4.5.2 Model verification

After the Vars-5 explanatory models for SOUR and TDHA were obtained four additional runs were carried out in duplicate for model verification purposes to assess their predictability. Table 4.11 outlines the run conditions and the results from the measured and the predicted values for SOUR and TDHA. From Table 4.11 it can be observed that the measured and the predictive values are very close indicating that the explanatory models obtained for SOUR and TDHA had good predictability in the experimental range. From Table 4.11 it can be observed that when ES input was 15 KJ/gTS the SOUR predicted and measured response was fairly accurate at both high and low HT biomass concentrations. However for TDHA when ES input was 15 KJ/gTS the model over predicted at low HT biomass concentrations and under predicted for higher ones. However in both cases the values were relatively close and were less than 5% and 10% different respectively. In all cases the validation of the Vars-5 explanatory models for SOUR and TDHA were deemed to be appropriate.

Table 4.11: Comparison of measured/predicted SOUR and TDHA obtained during model verification runs

ES (KJ/gTS)	MLTSS (mg/L)	SOUR ($mg\ O_2/mg\ h$)		TDHA ($\Delta abs_{470}/VS$)	
		Measured[1]	Predicted	Measured	Predicted
15	1500	16.3 ± 1.2	18.1	1.18 ± 0.11	1.12
15	3000	19.3 ± 2	20.3	1.22 ± 0.16	1.38
20	1500	15.8 ± 0.8	17.4	1.12 ± 0.14	1.11
20	3000	16.7 ± 1.6	18.9	1.29 ± 0.12	1.32

[1] **Data indicates the mean value and ± indicates the standard deviation between duplicates**

4.5.3 Ultrasound process optimization

According to Myers et al. (2007) one of the most useful approaches to optimize problems with more than 3 design variables is the procedure developed by Derringer and Suich (1980) which makes use of desirability functions. The general approach is to convert each response y_i into an individual desirability function d_i that varies in the range

$$0 \le d_i \le 1 \qquad (4.13)$$

Where if the response y_i is at its goal, then $d_i=1$ and if the response is outside an acceptable region, $d_i = 0$. The design variables are chosen to maximize the overall desirability

$$D = (d_1 d_2 ... d_n)^{\frac{1}{m}} \qquad (4.14)$$

When there are m responses the individual desirability functions are structured depending on what the research objective is: a maximum, a minimum, or a target point on the surface response. In the present experiment the desirability functions approach was used to find the experimental conditions that could lead to the maximum SOUR or TDHA. Hence the function takes the form:

$$d = \begin{cases} 0 \\ \left(\dfrac{y-L}{T-L}\right)^r \\ 1 \end{cases} \qquad (4.15)$$

In equation (4.14) d = 0 if y < L, d = $\left(\dfrac{y-L}{T-L}\right)^r$ if $L \le y \le T$ and d = 1 if y > T, where T an L are the constraints of the response and r is the weight of the response. When r =1 the desirability function is linear. A high value of D is considered the optimal solution of the system. In Minitab15 using the command response surface optimizer makes it possible to search for a combination of input variables that jointly optimize a single response or a set of responses by satisfying the requirements of each response in the set. The optimization is accomplished by obtaining the individual desirability (d_i) for each response, combining the individual desirabilities to obtain the combined or composite desirability (*D*) and maximizing the composite desirability and identifying the optimum input variables settings. Minitab15 makes it possible to calculate an optimal solution and produces an optimization plot. The optimization plot allows changes to the input variable settings to perform sensitivity analyses and improve the initial solution. Table 4.12 shows the results from the optimization for SOUR and TDHA.

Table 4.12: HT ultrasound optimization process to achieve maximum SOUR and TDHA

Composite desirability (D)	Predicted SOUR (mg O_2/mg h)	Global solution (coded units)	Predicted TDHA (Δabs_{479}/mgVS)	Global solution (coded units)
0.98	20.3	X1=-1.242 X2= 0.797	1.377	X1=-1.339 X2= 0.875
1	20.37	X1= -1.242 X2=0.985	1.447	X1= -1.339 X2=1.378
1	20.37	X1=-1.242 X2= 0.985	1.462	X1=-1.414 X2= 1.419

From Table 4.12 it is important to observe that as the acceptable composite desirability (D) reached 1; the predicted SOUR and TDHA (from equation 4.10 and 4.12) reached the maximum value of 20.37 mg O_2/mg h and 1.4447 Δabs_{470}/VS respectively. Hence according to this approach the optimum conditions to enhance the HT biomass SOUR and TDHA activity are achieved at an ES input of 6.5-8.7 KJ/gTS and HT biomass concentrations of 2848-2989 mg/L, taking the average of both values the optimum values for the independent variables to enhance bioactivity of the HT biomass post US treatment are ES of 7.6 KJ/gTS and HT biomass concentrations of 2920 mg/L. It should be noted that the optimum values for ES and HT biomass concentration for optimum post HT biomass treatment to achieve maximum activity in region 1 based on the visual inspection of the RSM curves for TDHA and SOUR are in excellent agreement with the desirability function approach.

4.6 Conclusions

Based on the previous results it can be concluded that

- HT biomass activity enhancement without causing cell lysis was achieved at ES inputs in the range of 6.5 to 8.7 KJ/gTS. The effect of ultrasound was more pronounced with samples that contained higher solids concentrations with an optimum HT biomass concentration in the range of 2848-2989 mg/L. This enhancement in activity was not at the expense of deterioration in the reflocculating ability of the biomass or its settleability.
- ES inputs higher than 118 KJ/gTS lead to the irreversible loss of biomass settleability and bioactivity due to extensive cell lysis and sample solubilization.
- The results of this study have potential to be transferable to an actual pilot MWWTP as all results were developed using real domestic primary effluent.
- It is hypothesised that biomass activity enhancement after US pretreatment was the result of a series of mechanisms. Although the mechanical effects associated with low ES inputs seemed to have played a mayor role more research is needed for this to be a conclusion.
- The use of the tretrazolium salt XTT for enzymatic activity determination proved to be a fast and reliable method to assess bioactivity. However, DHA

determination alone can not replace the more cumbersome determination of biomass activity by respirometry.

- The use of response surface methodology and the desirability functions approach proved to be a fast and effective way to optimize the US pretreatment of HT biomass which had highly correlated variables.
- The results of the present study set the conditions for the modified activated sludge process proposed in this research to study excess sludge reduction during municipal wastewater treatment. The modified ASP will include a controlled holding tank followed by ES treatment at ES input of 8 KJ/gTS and total HT biomass concentration of 3000 mg/L.

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Chapter 5

Excess Sludge Reduction and Process Performance for Activated Sludge Municipal Wastewater Treatment by Integrating an Anoxic Holding Tank with Post Ultrasound Treatment to Enhance Maintenance Metabolism

Juan Marin and Kevin J Kennedy

5.1 Abstract

This manuscript presents results on excess sludge minimization during activated sludge processing (ASP) of real municipal wastewater by exposing different portions of the ASP biomass to a controlled anoxic tank (HT) followed by low intensity ultrasound (US) post-treatment in order to enhance microbial maintenance metabolism prior to recycling to the ASP. Biomass exchange between the HT and the ASP was controlled by varying the stress factor (SF, biomass exchanged from the HT relative to the total biomass in the ASP). The operation of the ASP under the different SF had direct effects on effluent quality, residual sludge characteristics, and ASP biomass observed sludge yield (Y_{obs}). It was observed that operation of the ASP at SF of 1 (biomass exchanged without post-sonication) decreased the Y_{obs} by 20% compared to control ASP. On the other hand, SF increase from 0.5, 1 and 1.5 (biomass exchanged with post-sonication) further decreased the Y_{obs} by 33, 25 and 44% respectively as compared to control ASP. The results indicate that combining anoxic HT exposure with US biomass post-treatment before being exchanged to the ASP enhanced the degree of sludge reduction by increasing microbial maintenance metabolism in combination with microbial flora shift in the ASP depending on SF. The ASP organic removal efficiency in terms of effluent quality (total chemical oxygen demand (TCOD) and total suspended solids (TSS)) had removal efficiencies of 88 and 91% respectively for the control ASP which remained high and unchanged for SF

of 1 for ASP + anoxic HT no sonication and for SF of 0.5 and 1 with ASP + anoxic HT + USPT. At SF of 1.5 for the ASP + anoxic HT + USPT the tCOD and TSS removal efficiencies decreased by 12 and 41% respectively compared to ASP control. Under all SF conditions tested the residual ASP sludge characteristics such as sludge volume index and dewaterability (16% decrease in ASP residual biomass bound water) improved with respect to the continuous ASP control. Additionally, it was observed that the ASP biomass activity and microbial stress measured as specific oxygen uptake rate (SOUR) and dehydrogenase activity (DHA) respectively changed as different SF and US treatments were applied to the HT biomass. On average SOUR and DHA increased for ASP + anoxic HT no sonication and for ASP + anoxic HT + USPT by 23% and 39% respectively as compared to control, indicating that the different SF combined with the HT and US PT induced additional metabolic stress on the ASP biomass which resulted in lower Y_{obs}. Integration of the two processes (anoxic exposure + USPT) at SF of less than 1 had a synergistic effect on excess sludge minimization without having detrimental effects on ASP effluent quality and improved dewaterability while increasing SF to 1.5 increased excess sludge minimization but resulted in a decrease in sludge settling and dewaterability.

*Key words: dehydrogenase activity, excess sludge minimization, sludge volume index, specific oxygen uptake rate, stress factor, XTT

5.2 Introduction

Worldwide, the safe disposal of excess municipal wastewater treatment sludge (EXS) faces many challenges as urbanization and volume capacity of centralized wastewater treatment facilities continues to increase. In addition to the high costs associated with some of the methods used for final sludge disposal such as landfilling and incineration, there is also an increasing awareness from the population at large towards the drawbacks of such methods resulting in the not in my backyard syndrome (NIMBY). In part, the concern arises from the definition of sewage sludge. According to the USEPA (2010) EXS is a mud-like residue (solid, semisolid or liquid) that remains after treatment of human and other wastes from households and industries at a sewage plant. In addition, the United States Department of Agriculture (USDA, 2010) states that EXS also includes: domestic septage as well as scum and solids removed in primary, secondary, or advanced wastewater treatment processes. Due to the high content of organic matter, nitrogen and phosphorus, EXS has also been marketed as a fertilizer or soil amendment agent. However, such practice is also being challenged due to the fact that sludge can also contain volatile organics, disease-causing pathogenic microorganisms (virus, bacteria), heavy metals and inorganic ions, along with toxic chemicals from industrial wastes, household chemicals and pesticides, which can be transmitted and/or accumulated in plants, livestock and humans (USEPA, 2010). Since EXS is produced through the growth of the microorganisms that remove the organics during aerobic biological treatment of wastewater its production is unavoidable (Ginestet, 2006). Once the EXS is removed from the ASP system, post treatment handling is aimed mostly towards reducing its mass, volume and environmental impact before final disposal. Typical EXS post-treatment might include: conditioning, thickening, dewatering, drying, storage, transportation and final disposal (Metcalfe and Eddy, 2003), and it can account for as much as 30 to 60% of the total operational costs of a wastewater treatment plant (Canales et al., 1994, Androletta and Fedory, 2006). Although, post-treatment processes such as anaerobic digestion (35ºC and 15 days solids retention time (SRT)) can achieve between 30 to 40% solids reduction and a high level of pathogen destruction, the process implementation is expensive and more suitable for wastewater treatment plants with capacity of 40,000 population equivalent (PE) and higher (Metcalfe and Eddy, 2003; Ginestet, 2006). Hence, there is a pressing need to explore and develop new technologies for sewage sludge minimization and/or its safe reuse for all sizes of urban communities. According to Odegaard (2004) an ideal approach to minimize EXS production would be to target the aeration basin, where sludge production predominantly occurs. In this way, preventative measures could be put in place minimizing to a certain extent the formation of biosolids and would be more economically sound than instituting processes for the destruction of already formed solids. During municipal wastewater treatment, the removal of organic compounds and cell growth take place simultaneously, and the ratio of biomass produced relative to the amount of substrate consumed is directly linked and defined as the growth yield. For aerobic microorganisms, the growth yield is very high and varies between 0.4 to 0.8 grams of biomass produced per gram of biochemical oxygen demand (BOD) removed (Metcalfe and Eddy, 2003). From the assimilated energy, a portion is used for microbial growth and the remaining portion is used to maintain the cell activity and integrity (maintenance). Consequently, enhancing the biomass non-growth activities or maintenance during wastewater treatment could decrease the overall growth yield.

According to Ginestest (2006) this can be accomplished during aerobic wastewater treatment by increasing biomass maintenance requirements (less energy for growth), by enhancing biomass lysis and decay or by increasing the mineralization of slowly or recalcitrant biodegradable organics. In these approaches the released products could be reutilized by other bacteria in the system. Since such approaches are directly applied in to the aeration basin, the chosen strategy must not have any detrimental effects on the overall efficiency of the activated sludge process (ASP), which is to retain the same quality criteria for the effluent (carbon, nutrients and solids removal) and the residual sludge (settleability, dewaterability). The partial disintegration of a portion of the EXS sludge and its return to the aeration tank is one of the proposed approaches with high potential for EXS reduction. Sludge disintegration has been achieved using mechanical, chemical, thermal and hybrid technologies (Muller, 2000). Ultrasound (US) is a disruption technology that has already been applied at full-scale wastewater treatment plants, mostly to enhance EXS reduction via anaerobic digestion (Nickel, 1999; Muller, 2000; Thiem et al., 2001). More recently, the potential of applying high intensity US for EXS sludge reduction at the source in the ASP aeration basins by enhancing cryptic growth has also been reported (Cao et al., 2006; Strunkmann et al., 2006, Zhang et al., 2007). However, US EXS reduction mechanisms and effects on the activated sludge process performance are still not clear and some times contradictory. Additionally, most of the reported studies have used synthetic wastewaters with no inorganic solids present, giving a biased account on the extent of sludge reduction as well as final effluent quality and residual sludge settleability and dewaterability. Therefore; more research on sludge minimization is needed using real municipal wastewater. The present research proposes a new approach to enhance EXS minimization. During municipal wastewater treatment a portion of the aerobic biomass from the ASP would be first exposed to an anoxic controlled environment (the HT) followed by post sonication at low specific energy inputs (ES) before being returned to the aeration basin. It is hypothesized that this synergistic and novel approach could enhance EXS reduction by making use of two important mechanisms: a process that enhances biomass maintenance with concomitant reduction of the biomass yield coefficient (low intensity ultrasound) and a process that enhances the selection of a microbial consortia with a low yield coefficient (the controlled anoxic HT). Results from previous research (chapter four) on the optimization of US treatment of anoxic HT biomass to enhance maintenance metabolism will be integrated in a modified continuous activated sludge process. The main objective of this research is to study EXS reduction with this modified ASP that exploits enhanced microbial maintenance and to determine the effect of operating conditions on final effluent quality as well as residual sludge settleability and dewaterability while treating real municipal wastewater.

5.3 Material and methods

5.3.1 Activated sludge sequencing batch reactor start-up and operation

The three activated sludge sequencing batch reactors (SBRs) used in the present experiment were made of Plexiglas (60X20, 0.5cm thick) with a total working volume of 6 L. Aeration and mixing were provided with stone diffusers located in the base of the SBR and air was dispensed through a solenoid pump connected to a calibrated air flow meter to ensure dissolved oxygen (DO) concentrations higher than 3 mg/L (Figure 5.1).

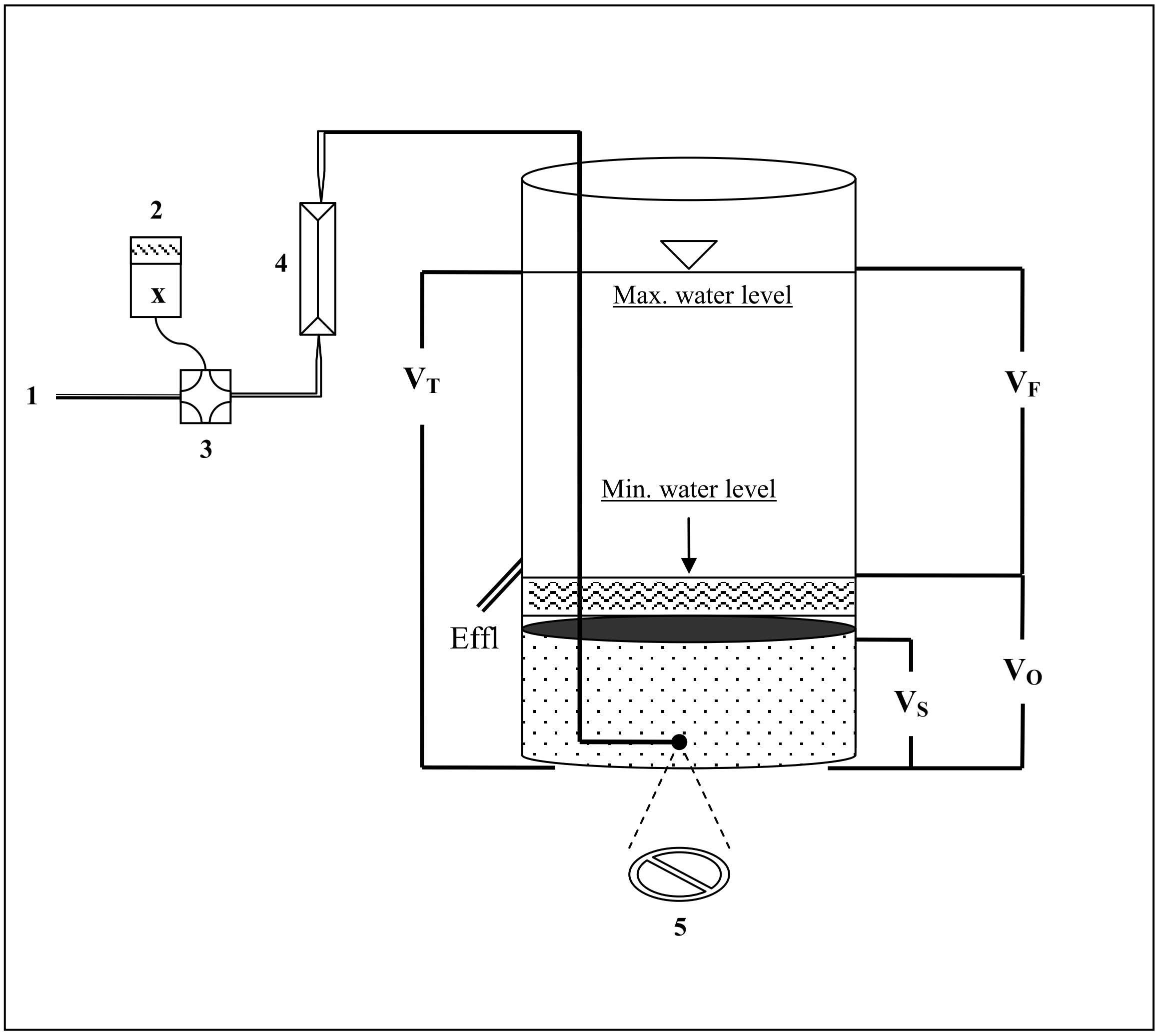

Figure 5.1: SBR schematic for excess sludge reduction continuous studies. In the diagram (1) is the compressed air outlet, (2) timer, (3) the selonoid pump, (4) the air flow meter and (5) the stone diffuser arrangement to provide air and mixing

Table 5.1 indicates the volume distribution in the SBRs as well as the most important hydraulic parameters used during the present study. The ASP SBRs were operated at the same hydraulic conditions for 3 cycles of 8h every day. A typical cycle (T_C) included: 40 min feeding (T_F, static fill), 4h aeration (T_A), 2h settling (T_S), 40 min effluent withdrawal (T_D), and 25 minutes of total idle time (T_I). T_F, T_A, T_S and T_D were controlled with timers (Traceable Fisher Sci., Canada).

Table 5.1: Activated sludge SBR operation parameters

SBR volume distribution	Symbol	Volume (L)	Parameter	Value
Total volume[1]	V_T	6	Volume exchange Ratio ($VER=V_F/V_T$)	0.53
Variable volume	V_F	3.2		
Stationary volume[2]	V_0	2.8	Cycles per day (m)	3
Sludge volume	V_S	1.5	Fill time ratio ($FTR=T_F/T_C$)	0.08
Clear zone	C_Z	1.3	Nominal hydraulic retention time HRT[3] (h)	15

[1] $V_T=V_F + V_0$
[2] $V_0=V_S + C_Z$
[3] nHRT $= \left(1+\frac{V_0}{V_F}\right)T_C$ (Artan and Orhon, 2006)

The ASP SBRs were inoculated with activated sludge biomass (Table 5.2) taken from the aeration basin at the Robert O. Pickard Environmental Center (ROPEC, Ottawa, ON). This is one of the largest wastewater treatment plants (WWTP) in Canada, treating approximately 120 MGD of municipal wastewater. At ROPEC, primary effluent is treated in an aerobic conventional activated sludge reactor (ASP) operated at an average SRT of 5 days. The WWTP at ROPEC through a series of physical, biological and chemical processes meets and exceeds all provincial guidelines for treated secondary effluent as defined by the Ministry of Environment (City of Ottawa, 2009).

Table 5.2: ROPEC's activated sludge characterization

Parameter[1]	Value[2]
pH	7 ± 0.15
Mixed liquor total suspended solids (MLTSS)	1150 ± 50
Volatile suspended solids (VSS)	847 ± 24
Total COD (TCOD)	3089 ± 221
Soluble COD (SCOD)	318 ± 36
Sludge volume index (SVI)	118 ± 8
Alkalinity (as mg CaC03/L)	205 ± 16
Ammonia	66 ± 5

[1] All parameter units in mg/L, except for SVI with units of mL/g
[2] data presented is the mean value ± standard deviation between 4 measurements

The initial sludge concentration in the ASP SBRs was set at 1500 ± 80 mg MLTSS/L. Since only carbon removal and excess sludge production were of interest in the present study, the SRT was set at 5 days. Additionally, there was no need for aerobic biomass acclimation as the activated sludge and municipal wastewater to be treated were both taken from ROPEC. The SBRs were operated at room temperature (21 ± 2 ºC) for a minimum of 3 consecutive SRTs (45 cycles in total for each SBR and 4 to 5 cycles during transition state) at the operation conditions given in Table 5.3. In Table 5.3 the stress factor (SF) is the average volume of biomass treated and exchanged per day relative to the total SBR biomass inventory. The average SF (1/d) was determined according to equation 5.1 (Ginestest, 2006) assuming constant MLTSS concentrations in the anoxic holding tank (HT) and AS SBR and based on biomass exchange occurring twice a day.

$$SF = \frac{Amount\ of\ \ HT\ treated\ \ per\ day\,(g\,/\,d)}{Amount\ of\ \ sludge\ in\ SBR\,(g)} \quad (5.1)$$

SBR1 was run as a conventional ASP control to set baseline conditions for the determination of specific effluent quality parameters (TSS, TDS, tCOD), excess sludge production as well as for further comparisons with the rest of the experimental runs. SBR2 included biomass exchanged from the HT only (without sonication), while SBR3 to SBR5 included both different proportions of sonicated biomass exchanged from the HT with post sonication. Every day during the study period, fresh primary effluent wastewater (WW) was manually placed in the influent tank. Timers controlled at every cycle the mixing of the WW before being fed to the SBRs as well as the peristaltic pumps (Masterflex, Cole-Parmer, Chicago, IL) used for WW feeding. After SBR static filling, another timer controlled the solenoid pump that supplied aeration. Once aeration stopped the activated sludge was allowed to settle for 2 h, then another peristaltic pump withdrew the treated effluent (Figure 5.2). Anoxic HT biomass withdrawal was performed manually before sonication according to the specific proportions shown in Table 5.3.

Table 5.3: SBR operation conditions during the present study

Reactor	SF (1/d)	Exchanged volume (L)	Observations
SBR1	0	0	Control SBR
SBR2	**1**	**½**	**HT biomass exchanged but no sonication applied**
SBR3	0.5	¼	HT biomass sonicated before exchange
SBR4	1	½	HT biomass sonicated before exchange
SBR5	1.5	¾	HT biomass sonicated before exchange

After sonication, the anoxic biomass from the HT was diluted with distilled water to produce the same average MLTSS concentration as in the AS SBR and it was exchanged manually to the SBRs within 2 h of the beginning of the aeration period. A portion of the exchanged biomass from the SBRs was allowed to sediment and used to replenish the HT to the initial 5 L volume and biomass concentration. Since one of the AS SBR cycles ran at night, biomass exchange was only performed during the two day time cycles. During SBR operation several parameters were measured in the influent primary effluent, the final effluent and in the AS SBRs as depicted in Table 4.4. Despite the broad application of SBR technology for municipal wastewater treatment, a unified basis for design and operation is still lacking (Artan and Orhon, 2005; Wilderer et al., 2003). Although, the SBR presents some inherent constraints that demand different design and operation considerations, similar to the conventional continuous-flow ASP, a steady-state SBR approach can be adopted. In both cases, the same mass balances for sludge production and substrate removal are applied and SBR long term operation under constant influent conditions has been shown to result in identical and consistent cycle profiles and secondary effluent quality characteristics (Artan and Orhon, 2005). Hence, SBRs can be considered as a continuous activated sludge process that achieves, if properly operated, a cyclic steady-state for a given set of operating conditions such as feed, structure of the cycles and temperature of operation, (Artan and Orhon, 2005).

Table 5.4: Parameter determination schedule for SBR's operation

Parameter	Frequency of measurement			Observations
	Influent WW	SBR Effluent	SBR	
pH	ED[1]	ED	ED	2-3 times/day
Dissolved Oxygen	ED	ED	ED	2-3 times/day
tCOD and sCOD	ED	ED		2 times/day
WW and effluent fractioning	2/MT[2]	1/MT		
MLTSS, MLTVSS	ED	ED	ED	2 times/day
Alkalinity	1/WK	1/WK[3]		
Ammonia	1/WK	1/WK		
Total phosphorus	1/WK	1/WK		
Dissolved solids		2/WK		3 times/day
SVI			ED	2 times/day
Batch SOUR			2/WK	
Biomass activity			2/WK	

[1]ED = everyday, [2]2/MT = twice every month, [3]1/WK once a week. The above mentioned parameters were determined in duplicates, except for SVI and batch SOUR.

In addition, SBR operation also relies on the common approach of assessing selected volumetric and organic loadings and sludge age values (Metcalfe and Eddy, 2003).

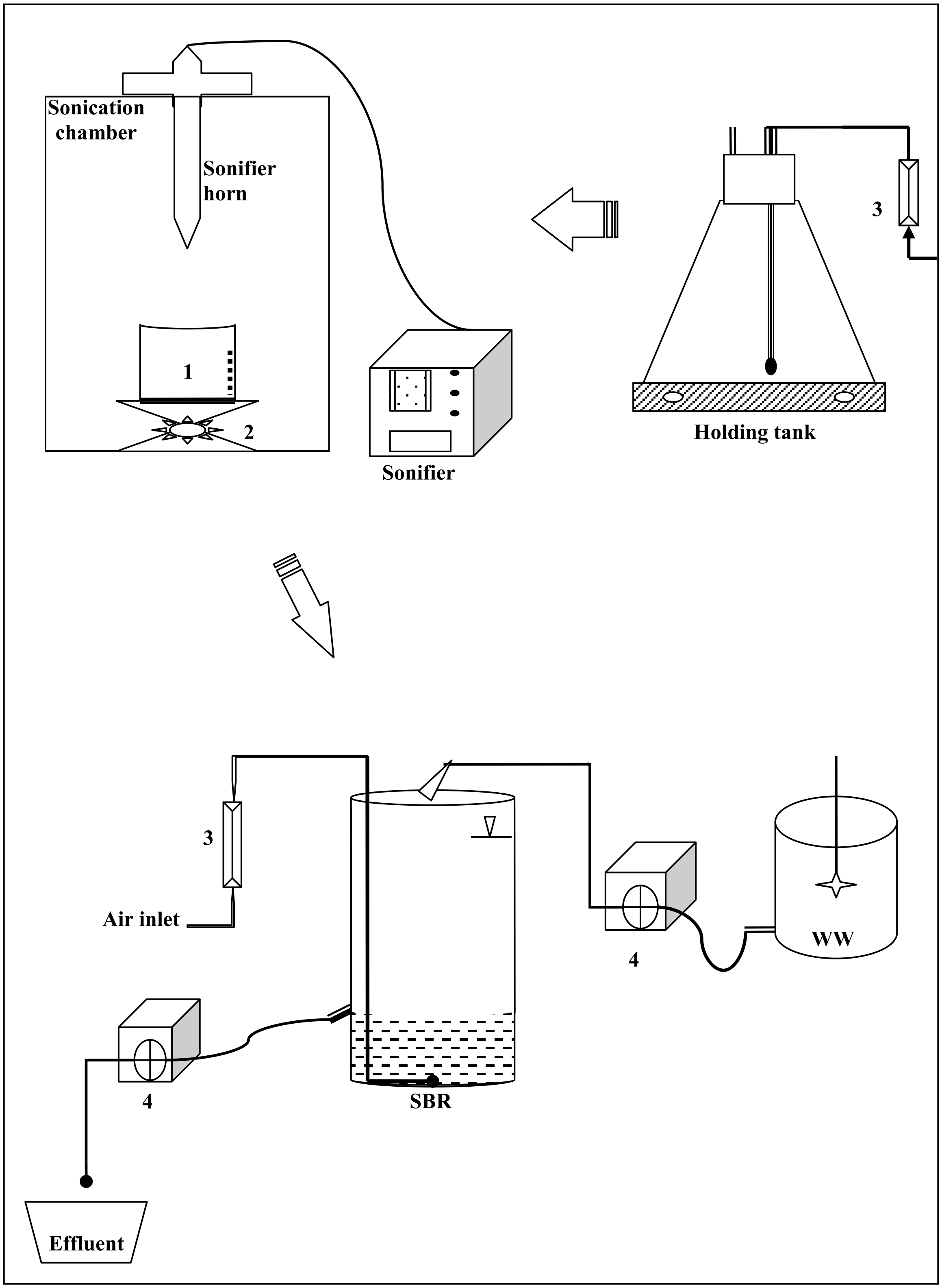

Figure 5.2: Experimental set up for excess sludge reduction continuous studies. In the diagram; (1) is the beaker used for HT sonication, (2) lab-jack, (3) air flow meter and (4) the peristaltic pump for WW feeding and withdrawal

In the present study, steady-state (SS) conditions were considered to be achieved for the control AS SBR (no biomass exchange from the HT and no sonication) based on tCOD and TSS effluent parameters as well as sludge production: a difference in effluent tCOD and TSS and sludge production of less than 5% over 2 days (6 cycles). Since the AS SBRs were also operated under different proportions of returned sonicated biomass from the HT, it was expected they would differ in terms of effluent quality parameters (TSS, tCOD, TDS) and biomass characteristics (sludge production, biomass activity) as compared to the control AS SBR (SBR1). Hence, under the different operation conditions, the AS SBRs were assumed to be at steady-state when a difference of less than 5% between 2 days of operation for the same parameters as the control SBR1 (tCOD, TSS) were achieved.

5.3.2 Observed yield determination

In order to maintain a relatively constant biomass concentration in the SBRs, daily sludge production in terms of TSS (P_{XT}, g/d) was measured and the required amount was wasted. Excess sludge wasting depended on sludge production under the different operation conditions outlined in Table 5.3. The average sludge production was the slope of the straight line obtained by plotting the cumulative sludge production against the days of SBR operation at the determined conditions. The average substrate removal (ΔCOD, g/L) was obtained in a similar way, by plotting the cumulative ΔCOD removal versus SBR operation days. The observed yield (Y_{obs}, gTSS/gCOD) under the different AS SBR operational conditions was calculated as the ratio between the cumulative sludge production and cumulative COD removal over the determined period of time using equation 5.2 (Camacho et al., 2005).

$$Y_{obs} = \frac{\sum_d P_{XT}}{\sum_d \Delta COD} \qquad (5.2)$$

Based on the Y_{obs} for control AS SBR (SBR1) and the AS SBRs at the different operational conditions, the degree of excess sludge reduction was calculated according to equation 5.3 (Ginestet, 2006).

$$DSR = \left[1 - \frac{Y_{obs,OC}}{Y_{obs,control}}\right] * 100 \qquad (5.3)$$

Where DSR is the degree of sludge reduction (%),$Y_{obs,contol}$ and $Y_{obs,OC}$ is the Y_{obs} of the control SBR and the SBRs at the different operation conditions tested, respectively. DSR would yield positive values when excess sludge reduction occurred compared to the control and negative values would indicate an actual net increase in sludge production compared to control.

In order to calculate Y_{obs} the determination of the sludge production per day was necessary. In an AS SBR the sludge retention time (SRT, day) is defined as in the conventional continuous-flow ASP, as the mass of sludge contained in the reactor (M_X, g), divided by the sludge produced per day as given in equation 5.4.

$$\theta_X = \frac{M_X}{P_{XT}} \qquad (5.4)$$

SBRs offer the advantage of sludge wasting from the mixed liquor, which provides a direct and effective means of controlling SRT (Henze et al., 2008). Therefore, θ_X can be defined in terms of the total reactor volume (V_T, L), the volume of excess sludge wasted from the mixed liquor in each cycle (V_W, L) and the total cycle time (T_C, d) as shown in equation 5.5.

$$\theta_X = \left(\frac{V_T}{V_W}\right) T_C \qquad (5.5)$$

Another important aspect to consider in an AS SBR is the aerobic sludge age (θ_{XA}), which is defined as a function of the aerated periods within the cycle (equation 5.6).

$$\theta_{XA} = \theta_X \frac{T_A}{T_C} \qquad (5.6)$$

For carbon removal, it is generally assumed that the growth of heterotrophs only occurs during the aerated period, which is called the effective period (T_E). Hence, in the absence of anaerobic/anoxic periods (T_{AOX}), equation (5.7) also defines the effective sludge retention time (θ_{XE}).

$$\theta_{XE} = \frac{\theta_{XA}}{\left(1 - \frac{T_{AOX}}{T_E}\right)} \qquad (5.7)$$

Once the θ_X has been selected, based on the particular characteristics of the municipal wastewater and microbial kinetics, the excess sludge production can be calculated based on equations 5.8 and 5.9 (Artan and Orhon, 2005).

$$Y_{NH} = (1 + f_E b_H \theta_{XE}) \frac{Y_H}{1 + b_H \theta_{XE}} \qquad (5.8)$$

Where, Y_{NH} is the net heterotrophic biomass yield (gCOD/gCOD), f_E is the particulate inert fraction of biomass, b_H is the endogenous respiration rate (1/day) and Y_H is the heterotrophic yield coefficient (gCOD/gCOD)

$$P_{XT} = i_{TSS,COD}\left(Y_{NH} Q C_{bCOD} + Q C_{pnbCOD}\right) + Q X_{FSI} \qquad (5.9)$$

Where, P_{XT} is the sludge produced (g TSS/d), $i_{TSS,COD}$ is the coefficient to convert COD to TSS (0.9 gTSS/gCOD), Q is the daily volumetric flow rate (m^3/d), C_{bCOD} is the influent total biodegradable COD (g/m^3), C_{pnbCOD} is the influent inert particulate COD (g/m^3) and X_{FSI} is the influent fixed solids concentration (g/m^3).

In the present experiment, the kinetic and stoichiometric coefficients for heterotrophic biomass used were the average values reported in Artan and Orhon (2005) and Henze et al. (2008) and are shown in Table 5.5.

Table 5.5: Kinetic coefficients used to determine sludge production during SBR operation

Parameter	Symbol	Value	Units
Yield coefficient	Y_H	0.65	gCOD/gcellCOD
Endogenous respiration rate	b_H	0.15	1/day
Biomass particulate inert fraction	f_E	0.20	-

5.3.3 Wastewater characterization and chemical oxygen demand fractioning

The municipal wastewater (MWW) used during the present study was taken from the primary effluent line at ROPEC (Table 5.6). In order to keep MWW quality as constant as possible, Primary effluent sample collection was undertaken 3 times a week during the morning hours (7-7h30 am) and stored at 4ºC. MWW was only used within two days of storage.

Table 5.6: ROPEC's primary effluent characterization

Parameter[1]	Value[2]
pH	7 ± 0.1
cBOD5	108 ± 4
TCOD	219 ± 15
Total suspended solids (TSS)	88 ± 7
Volatile suspended solids (VSS)	75 ± 5
Organic nitrogen (TKN)	24 ± 6
Total Phosphorous (TP)	6.8 ± 2
Alkalinity (as mg $CaCO_3$/L)	220 ± 23
Ammonia (NH_3-N)	26 ± 2

[1] All parameter units in mg/L, except for pH

[2] Data represent the mean value ± standard deviation between 4 measurements, except for $cBOD_5$ which is the mean ± standard deviation between 3 measurements.

In the present study, the chemical oxygen demand (COD) was used as the parameter to assess primary effluent strength. According to Metcalfe and Eddy (2003) the use of COD requires an elementary characterization of the MWW organic content (i.e. soluble, biodegradable, non-biodegradable and particulate COD concentrations), as they each play important roles during biological MWW treatment using the ASP. The non-biodegradable particulate COD concentration strongly affects sludge accumulation in the aeration basin and daily sludge production. On the other hand, the soluble non-biodegradable COD concentration fixes the minimum possible filtered effluent COD concentration that could be achieved from the system (Henze et al., 2008). According to Henze et al. (2008), when nutrient removal (nitrogen, and phosphorous) is of interest then a thorough WW characterization is also required in terms of phosphorous and nitrogen. In this study, the ASP was essentially operated for carbon removal and concomitant sludge production. Therefore, only the MWW COD fractions as outlined in Figure 5.3 were determined. Total and soluble COD were determined according to Standard Method 5220C (APHA, 1998). After addition of the digestion solution and sulphuric acid reagent, the sample was placed in an oven at 150 ºC and digested for two hours. Once the samples cooled down, they were titrated with ferrous ammonium sulphate (FAS) 0.1M and ferroin indicator solution was used to indicate the titration end point. Samples were diluted as needed. Total COD was calculated according to equation 5.10:

$$COD(mgO_2/L) = \frac{(A-B)*M*8000}{mL_{sample}} \qquad (5.10)$$

Where A was the mL FAS used for blank, B was the mL of FAS used for the sample, M the molarity of FAS and 8000 is the milliequivalent weight of Oxygen per 1000 mL/L. For the soluble COD determination, samples were first filtered through a 0.45μm pore size membrane. Determination of the bCOD fraction required the sample biochemical oxygen demand assessment (BOD_5). In this study the BOD_5 was determined based on Standard Method 5210B (APHA, 1998). Primary effluent, dilution water and acclimated inoculum were mixed and placed in a 300 mL BOD bottle and saturated to a dissolved oxygen (D_1) concentrations of ~8.5 mg/L.

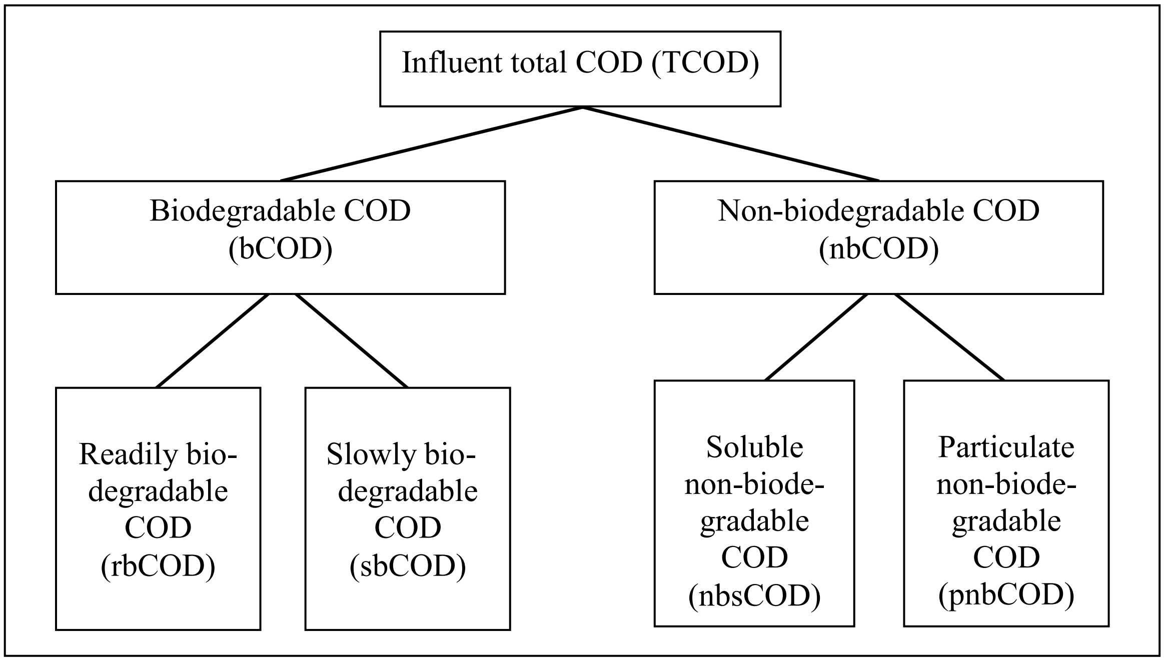

Figure 5.3: ROPEC primary effluent COD fractioning

After the bottles were sealed, they were placed in an incubator for 5 days at 20 ºC. The bottles were reopened and the DO was measured again (D_2) using an Accumet DO meter (Fisher Sci., Canada) equipped with a self-stirring BOD probe. BOD_5 was calculated using equation 5.11:

$$BOD_5 = \frac{(D_1 - D_2) - (B1 - B2)f}{P} \qquad (5.11)$$

Where D_1 was the DO of the diluted sample immediately after preparation (mg/L), D_2 was the DO of the diluted sample after 5 days (mg/L), B_1 was the DO of the inoculum before incubation (mg/L), B_2 the DO of the inoculum after incubation (mg/L), f the fraction of seeded dilution water volume in sample to volume of seeded dilution water in seed control, and P the fraction of wastewater sample volume to total combined volume. BOD_5 tests were determined in triplicate. From the BOD_5 results the bCOD was calculated according to Metcalfe and Eddy (2003) as

$$\frac{bCOD}{BOD_5} = 1.64 \qquad (5.12)$$

The soluble rbCOD and the soluble nbsCOD were determined according to the flocculation method developed by Mamais et al. (1993). According to Metcalf and Eddy (2003) determination of the bCOD by the flocculation method gives a reasonable estimate of the bCOD. For the test, 100 mL of primary influent were mixed with 1 mL of a 100g/L zinc sulphate solution and mixed vigorously for 1 min. The sample pH was then adjusted to 10.5 with a 6M solution of sodium hydroxide and left to settle for 15 min. The supernatant was carefully withdrawn and filtered through a 0.45µm pore size membrane (GN-6 Matricel, Pall Corporation, Canada). The COD of the supernatant filtrate was determined following the same procedure as the total COD. In the case of nbsCOD, the final effluent from ROPEC (before disinfection) was used for the determination. The nbCOD was determined by subtracting the bCOD from the TCOD concentration. sbCOD and the pnbCOD were determined according to equations 5.13 and 5.14 (Pasztor et al., 2009)

$$sbCOD = bCOD - rbCOD \qquad (5.13)$$

$$pnbCOD = TCOD - nbCOD - nbsCOD \qquad (5.14)$$

During AS SBR operation, ROPEC primary effluent parameters such pH, and strength parameters were regularly monitored.

5.3.4 Activated sludge activity determination during SBR operation

In order to determine if the control and modified SBR operational conditions (stress factor) would have a permanent effect on activated sludge activity and characteristics, the specific oxygen uptake rate (SOUR) and dehydrogenase activity (DHA) were monitored regularly during continuous treatment of primary effluent. Biomass activity determination by batch SOUR and DHA were determined according to section 4.2.4 and 4.4.5 respectively. Due to the length of time needed for the SOUR test, it was only performed once at random cycles at steady state during SBR operation (see Table 5.4). DHA was determined in duplicate.

4.3.5 Dewaterability determination

During SBR operation, residual sludge (sedimented to ~1%TS) dewaterability was measured using two methods: capillary suction time (CST) and a centrifugal method. CST determination was based on Standard Method 2710G (APHA, 1998), using a Capillary Suction Timer (Model 440, Fann Instrument Co., TX, USA) with an 18mm sludge reservoir and chromatography grade paper No 17 (Venture Innovation, INC, Lafayette, LA, USA). In order to maintain the same humidity through CST measurements the chromatography paper was kept in a desiccator. For the test, 5 mL of sample was poured into the reservoir with a modified open mouth syringe, a digital timer was used to determine the time (sec) required for water released from the sample to travel between two contact points on the chromatography paper. Measurements were performed in quadruplicate. Dewaterability determination by the centrifugal method was based on Skidmore (2005) using a Sorvall Legend™ T_+/RT_+ Thermo Scientific Tabletop centrifuge (Fisher, Canada) operated at ~8000g for 1 hr. 50 mL of sedimented sludge samples were placed in pre-weighted and marked centrifuge tubes. After centrifugation the supernatant was removed and the tubes weighed again. This gave the volume of free water in grams. The remaining cake was carefully removed from the centrifuge tubes and its water content determined by the standard evaporation technique at 105 ºC. This was assumed to be the bound water (percentage of water content per gram of WAS cake). The total water content was determined by the addition of free and bound water.

5.4 Analytical methods

5.4.1 Sludge Volume Index (SVI)

During SBR operation, SVI was determined according to Standard Method 2710D (APHA, 1998). 1 h before the aeration period was stopped, aerobic biomass from the SBRs was directly poured into a 1 L graduated cylinder and allowed to stand undisturbed for 30 min, after which the settled sludge volume was recorded and divided by the MLTSS concentration. SVI (mL/g) was then calculated according to equation 5.15:

$$SVI = \frac{settled\ sludge\ volume\ (mL/L)\ X\ 1000}{MLTSS\ (mg/L)} \qquad (5.15)$$

Where MLTSS was the sample total suspended solids content (mg/L).

5.4.2 pH

pH was measured with a Fisher Accumet® pH excel meter Model 925 (Fisher Sci., Ottawa, ON). pH meter calibration was performed routinely using standard solutions provided with the unit.

5.4.3 Mixed liquor total suspended solids (MLTSS)

Mixed liquor total suspended solids content was determined according to Standard Method 2540D (APHA, 1998) using glass fibre filters (Grade GF 52, VWR International Ltd Ottawa, Canada). The filters were pre-ashed at 550 ºC for 20 min and kept in desiccators. Before the test, the filters were weighted and a volume of sample (dilution depended on the solids concentrations) was filtered through under vacuum. The filters were then placed in an oven at 105 ºC for a minimum of 5 h and placed again in the desiccator until dried and re-weighted. MLTSS was determined according to equation (5.16)

$$MLTSS = \frac{(A - B)}{C} \qquad (5.16)$$

Where MLTSS was the totals suspend solids concentration (g/mL), A was the weight of the filter plus dried residue (g), B was the weight of the ashed filter (g) and C the sample volume (mL).

5.4.4 Mixed liquor volatile suspended solids (MLVSS)

Volatile suspended solids were determined according to Standard Method 2540E (APHA, 1998). The fibre glass filter holding the total suspended solids residue was ashed at 500 ºC in a furnace oven for 1h. After cooling in a desiccator the filters were reweight and the MLVSS concentration was calculated according to equation 5.17:

$$MLVSS = \frac{(A - B)}{C} \qquad (5.17)$$

Where MLVSS (g/mL) was the total volatile solids, A the weight of the fibre filters before ignition (g), B was the weight of the fibre filter after ignition (g) and C the sample volume (mL).

5.4.5 Total dissolved solids (TDS)

TDS concentration was determined according to Standard Method 2540C (APHA, 1998). Porcelain dishes were ignited in a furnace oven for 1 h and stored in a desiccator. Usually the filtrate obtained from the MLTSS test was used for TDS determination. A determined volume of filtrate was poured into the already dried and weighted porcelain dish and placed in an oven at 105 ºC for 12 h. The porcelain dishes were then transfer to a desiccator and allowed to cool down before being reweighted. TDS concentration (g/mL) was determined according to equation 5.18:

$$TDS = \frac{(A - B)}{V_{sample}} \qquad (5.18)$$

Where A represented the weight of the dish plus dried residue (g), B the initial weight of the porcelain dish (g) and V was the sample volume used (mL).

5.4.6 Ammonia (NH_4^+)

Dissolved ammonia concentration was determined according to Standard Method 4500D (APHA, 1998) using an Orion ammonia electrode (model 95-12) with a Accumet® model 750 pH/ion meter (Fisher Sci., Ottawa, ON)

5.4.7 Alkalinity

Sample alkalinity (mg $CaCO_3$/L) was determined according to Standard Method 2320B (APHA, 1998). The sample volume was titrated with 0.1N sulphuric acid to a pH of 4.6. pH monitoring during titration was performed with a Fisher Accumet® pH excel meter Model 925 (Fisher Sci., Ottawa, ON). Alkalinity was calculated according to equation 5.19:

$$Alk = \frac{A * N * 50,000}{V_{sample}} \qquad (5.19)$$

Where A was the mL of sulphuric acid consumed during titration, N was the normality of the sulphuric and, V was the sample volume (mL).

5.4.8 Total phosphorus (TP)

TP was determined according to Standard Methods 4500-PB5 and 4500-PC (APHA, 1998). The glass material was exclusively used for TP determination and was only rinsed with an 8% solution of sulphuric acid (V/V). Sulphuric acid solution and ammonium persulfate were added to the sample volume and placed in a hot plate for 40 min; distilled water was used as blank. After digestion the sample and blank were filtered through a 0.45 μm pore size membrane filter already soaked in an 8% (v/v) solution of sulphuric acid. The filtrate was then transfer to another flask and the strong acid solution plus molybdate reagent and stannous chloride were added. Flaks were mixed and allowed to react for 10 min. Samples and blank absorbance were measured using a Spectronic $20D^+$ (Thermo Sci., USA) at 690 nm. The sample TP concentration was then determined from a calibration curve prepared with a standard phosphate solution.

5.4.9 Total Kjeldahl nitrogen (TKN)

TKN was determined based on Standard Method 4500-N_{org} (APHA, 1998). The digestion reagent and the sulphuric acid were added to the sample volume and allowed to digest for 1h. After digestion the ammonia was distilled into a boric acid solution and the titrated with a standardized 0.02N sulphuric acid solution.

5.5 Statistical analyses

The multiple comparisons Tukey's honestly significant difference (HDS) test was applied to assess if the differences for the SBRs with different treatments (SF) were significant. Tukey's HSD makes used of one-way ANOVA and provides comparison intervals. A difference between sample means is considered to be significant if its confidence interval does not contain zero. All tests were performed using Minitab15 (Minitab Corporation, USA).

5.6 Results and discussion

5.6.1 Effect of SBR operation conditions on effluent quality

In the present study, it was observed that SBR operation under the different conditions of sonicated sludge exchange or stress factor (SF) at the same ultrasound specific energy (ES) input had several effects on effluent quality, activated sludge activity and on the characteristics of the residual sludge as compared to the control (SBR1). Figure 5.4a and 5.4b shows the effect of SF on effluent quality in terms of total chemical oxygen demand (tCOD) and total suspended solids (TSS) respectively. In Figure 5.4 the presented data are the daily average of the 3 respective SBR cycles run at the different SF. While Figure 5.5 shows the trend the SBRs operation had on effluent quality during the total time of the present study. From Figure 5.4 and 5.5 it can be observed that under SF of 0 to 1 (not sonicated and sonicated sludge exchanged) the SBR achieved on average, tCOD removal efficiencies of 88%. It was also observed that a large proportion of the effluent tCOD at SF of 0-1 (78 ± 4 %) was soluble (filtered through 0.45μm pore size membranes).

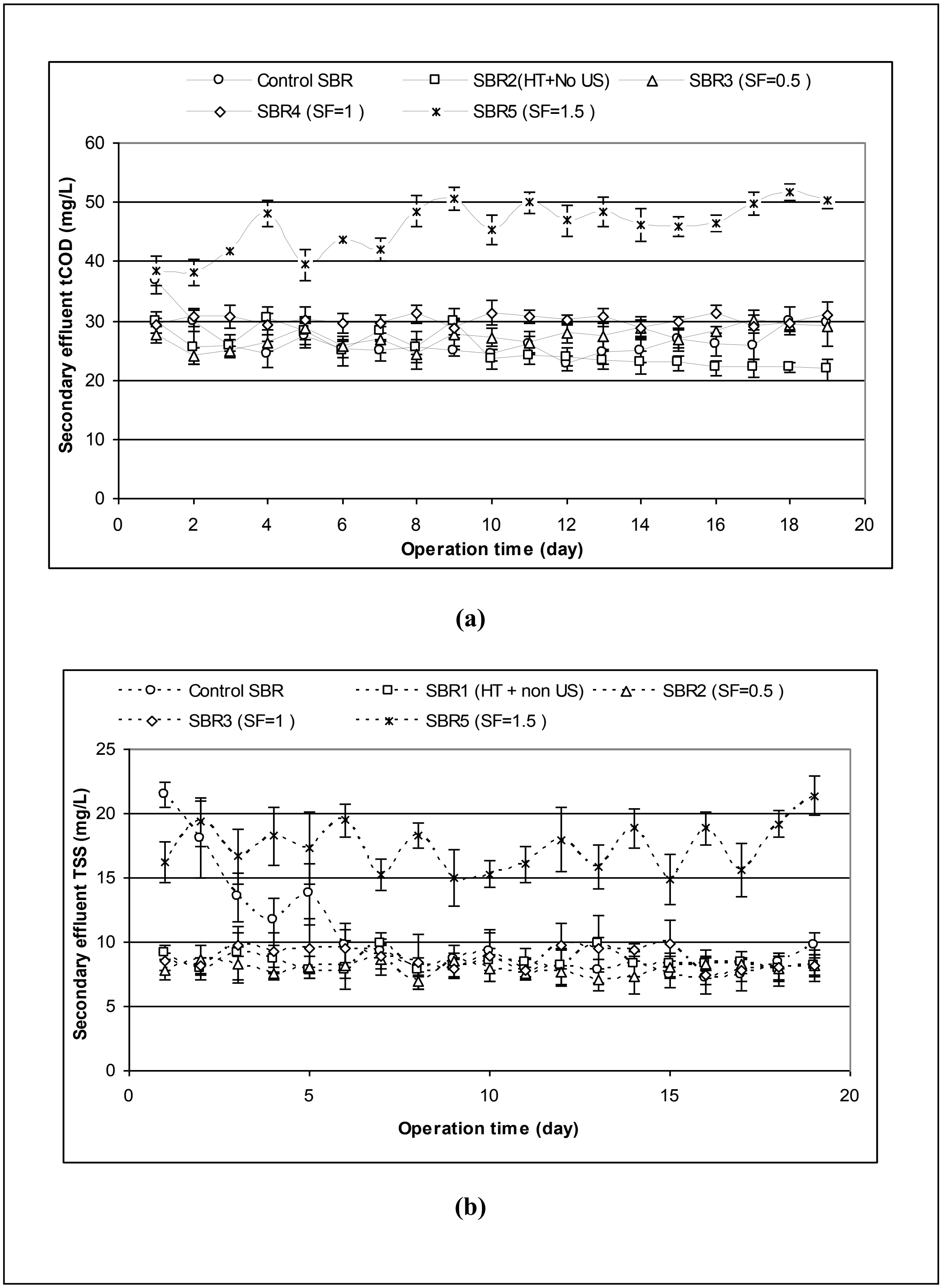

Figure 5.4: Effect of SF on AS SBR effluent quality for (a) tCOD and (b) TSS. Data represent the mean value ± standard deviation between 4 to 6 measurements taken daily.

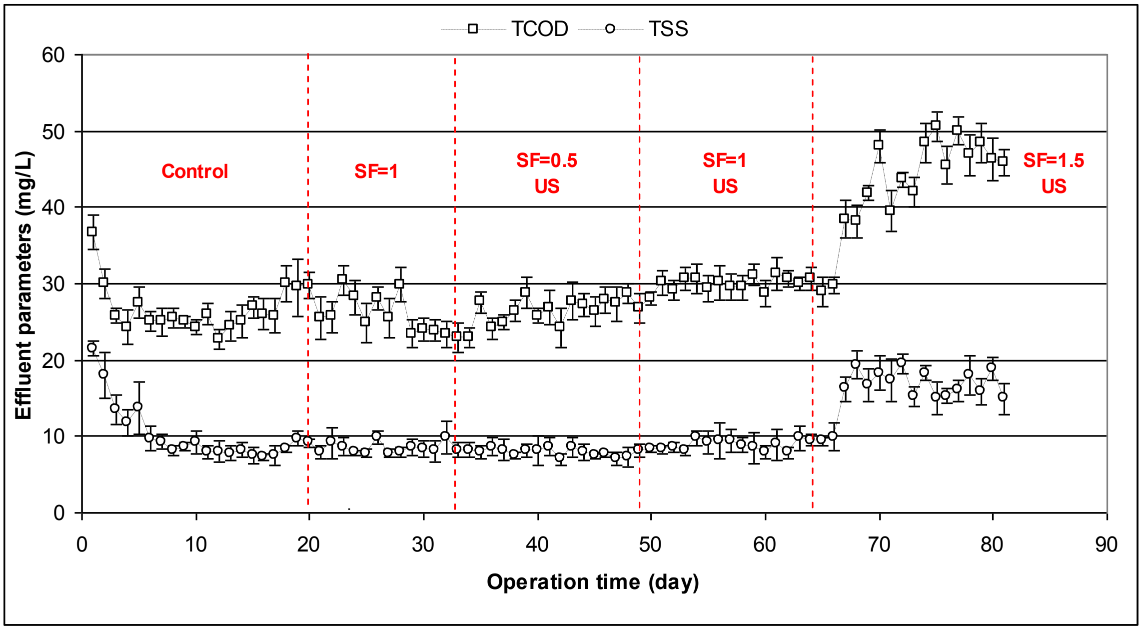

Figure 5.5: Effect of SF on AS SBR effluent quality during steady state operation. Data represent the mean value ± standard deviation between 6 measurements taken at steady state. US indicate that sonication was applied.

As such increasing the SF from 0 to 1 with or without sonication did not seem to have any negative impact on ASp SBR effluent quality. However, as the SF increased to 1.5 with sonication, SBR tCOD removal efficiency decreased to 79%. This result indicated that a SF of 1.5 (higher volume of stressed biomass exchanged) which included sonication and the turbulence created during aeration could have had a synergistically detrimental effect on floc structure and concomitantly on SBR performance as determined by the tCOD and TSS removal data. The increase in effluent tCOD at SF of 1.5 was mostly due to the presence of floc debris and most likely free cells produced during exposure to the stressful conditions in the holding tank plus US treatment. The effluent TSS concentration (Figure 5.4 and 5.5) also indicated that at SF of 1.5, SBR performance in terms of TSS removal decreased from 90% to 56%. During SBR operation at SF of 1.5 with sonication it was observed that there was an increased number of protozoa that could be observed with the naked eye, closer inspection by light microscope (X40) showed the presence of high numbers of most likely bristle worms (Figure 5.6) compared to the control and SF of 0.5 and 1.

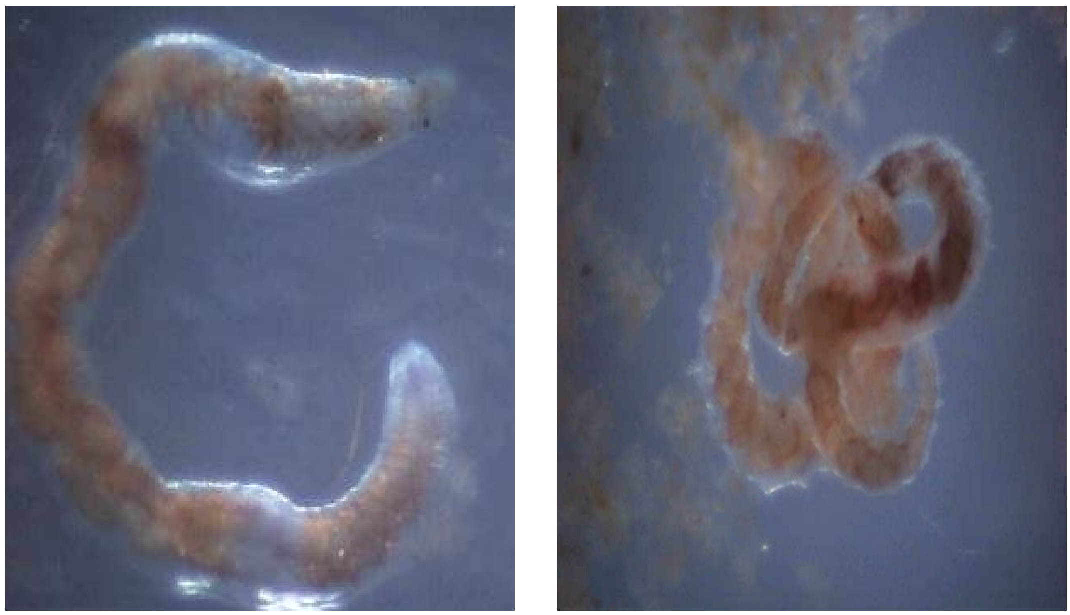

Figure 5.6: Bristle worms (Aelosoma) observed during AS SBR operation (SF = 1.5) Picture taken with a Hearth digital microscope (40X)

According to Jenkins et al. (1993) protozoa and other higher life forms constitute approximately 5% of the ASP biomass. These organisms are useful for influent toxicity assessment and for the removal of non-flocculated bacteria by phagocytises, contributing in this way to effluent clarification. Bristle worms in high numbers however, have been associated with activated sludge plants operated at long SRT and under high concentrations of nitrates (Jenkins et al., 1993). In the present study the SBRs were not operated under such conditions and since high numbers of bristle worms were observed only for the SBR operated at SF 1.5, it can be speculated that due to the high biomass exchange ratio the SRT in the HT decreased and denitrification was incomplete resulting in higher concentrations of nitrates in the SBR with SF of 1.5 after biomass exchange. In addition, it seemed that at SF of 1.5 plus ultrasound treatment the growth of free bacteria was enhanced. In the present experiment, it can be speculated that SBR operation at SF higher than 1.5 (plus sonication) would not be optimal and would produce an effluent of poor quality. In a pilot or at plant scale, the remediation of such effects would most likely require the addition of further operational units to the treatment train to achieve effluent quality standards. Since the detrimental effects were observed after only two SRTs it is highly possible that the negative effect on effluent was significant and SBR failure would have been the next logical consequence if SF was increased to values of 2 or higher. It should be emphasised that for the other operation conditions (SF 0-1 with and without sonication), SBR effluent was acceptable and even exceeded current Ontario (Canada) regulations for secondary effluent quality (MOE, 1993). Additionally, other SBR effluent parameters such as pH (7 ± 2), alkalinity (74 ± 19 mg $CaCO_3$/L) and total dissolved solids (647 ± 43 mg/L) remained fairly constant during all SBR operational conditions evaluated (included SBR operation at SF of 1.5).

Although nutrient removal was not thoroughly investigated in this study, some important aspects were observed during SBR operation at the different SF conditions. Table 5.7 presents the results from ammonia-nitrogen and total phosphorous (TP) for SBR effluent at the different operational conditions. It can be observed from Table 5.7 that the SBR control produced an effluent with ammonia concentrations that in general can be considered as low (81% removal efficiency).

Table 5.7: Effect of SF on nutrients removal (nitrogen and phosphorous)

Run	SF (1/d)	Ammonia (mg/L)[2]	Total phosphorous (mg/L)
SBR1	Control	5.2 ± 0.7	2.2 ± 0.2
SBR2	1[1]	4.3 ± 1.2	1.9 ± 0.1
SBR3	0.5	1.5 ± 0.3	1.8 ± 0.2
SBR4	1	1.1 ± 0.2	3.6 ± 0.3
SBR5	1.5	2 ± 0.4	3.8 ± 0.3

[1] HT biomass not sonicated
[2] Data presented are the mean ± standard deviation between 18 measurements taken during SBR steady-state operation

In addition, once the different SF (sonication and non-sonication) conditions were applied during AS SBRs operation, the effluent ammonia concentration decreased further and the SBRs with sonication reached on average 94% ammonia removal. It is well established that the introduction of anoxic periods before aerobic treatment of municipal wastewater generally helps in decreasing the ammonia effluent concentration (Henze et al., 2008). Most likely, the non sonicated HT biomass and feeding strategy used in the present study had an effect on lowering the effluent ammonia concentration after biomass exchange, but definitely the combination of SF between 0.5 and 1.5 plus ultrasound treatment enhanced the ammonia removal even further during SBR operation, even at the highest SF. However, SBR operation at SF of 1.5 resulted in lower effluent quality in terms of TSS and tCOD. According to Wilderer et al. (2008) one of the advantages of the aerobic AS SBR is that it can be operated at high MLTSS concentrations and the SRT can be easily controlled hydraulically, conditions that have a positive effect on the nitrifiers population even at short retention times such as the one used in the present study (5 d). Although ammonia removal in the SBR was somehow constant during the present research and even improved when the different SF were applied, the TP removal on the other hand presented a different profile and it was only improved with respect to control

SBR by 18% on average for SF of 1 and 0.5, but deteriorated further when ultrasound and high biomass exchange ratios (SF >1) were applied. According to Henze et al. (2008) to enhance the growth of microorganisms that accumulate phosphorous (PAOs) during municipal wastewater treatment requires a specific sequence of operational conditions: an anaerobic stage followed by an aerobic (or anoxic) stage plus the addition or formation of volatile fatty acids (or high concentrations of rbCOD).

In the present study, the HT cannot be considered as an oxic-settling-anaerobic system (OSA) since the biomass concentration was rather low (3500 ± 400 mg/L) and the SRT was short (1 to 1.5 days, depending on operational conditions). However the HT can be considered more a Cannibal-type reactor (SIEMMENS Technology) operated at intermittent micro-aeration periods. Furthermore, due to the low loading rate it can be assumed that the HT operated under a very low S_0/X_0 ratio and low rbCOD. However, it is possible that the HT biomass was able to hydrolyze the influent particulate fraction to some extent and produce VFAs. Therefore it is not likely that the anoxic HT would support a higher population of PAOs. Another reason for the rather higher TP concentrations in the SBR effluent could be that during the present study, TP was measured; hence a portion of the P contained in the effluent biomass (higher effluent TSS at SF of 1.5 plus US) could have been hydrolysed and was added to the soluble P. Although the mechanisms of nutrient removal in Cannibal-type configurations is not yet understood, it has been established that such technology does enhance nutrient removal during municipal wastewater treatment as compared to the conventional activated sludge process (Goel and Noguera, 2006; Novak et al., 2007). Due to the increasing efforts to reduce nutrient emissions to the aquatic environment, Cannibal-type configurations such as the ones in the present study (SBRs R2-R4) warrant more research with special attention to the combination of anoxic HT and SF with and without sonication.

5.6.2 Effect of SF and AS SBR operational conditions on residual biomass characteristics

The effect of the SF and SBR operational conditions on SBR residual biomass characteristics was assessed by closely monitoring the sludge volume index (SVI) and sludge dewaterability (by the capillary suction time (CST) and a centrifugal method) under steady-state conditions. Although both determinations are somewhat empirical they are routinely used at wastewater treatment plants for monitoring proper conventional ASP operation. Due to its simplicity, the SVI is the most common parameter used to quantify the settling characteristics of activated sludge biomass (Bye and Dold, 1998). According to Dick and Vesilind (1969), SVI can also be used to compare the effect of biological or physical/chemical treatments on the settling characteristics of sludge and for comparisons between different types of sludge. Figure 5.7 shows the average SVIs over 3 SRTs during SBR steady-state operation. It can be observed form Figure 5.7 that the settling properties of the sludge did not change substantially during the different SBR operational conditions. In fact, for all SF conditions tested the SVI decreased an average of 12.5 ± 6% compared to the SBR control, suggesting that under the different SF and US operating conditions sludge settleability was improved.

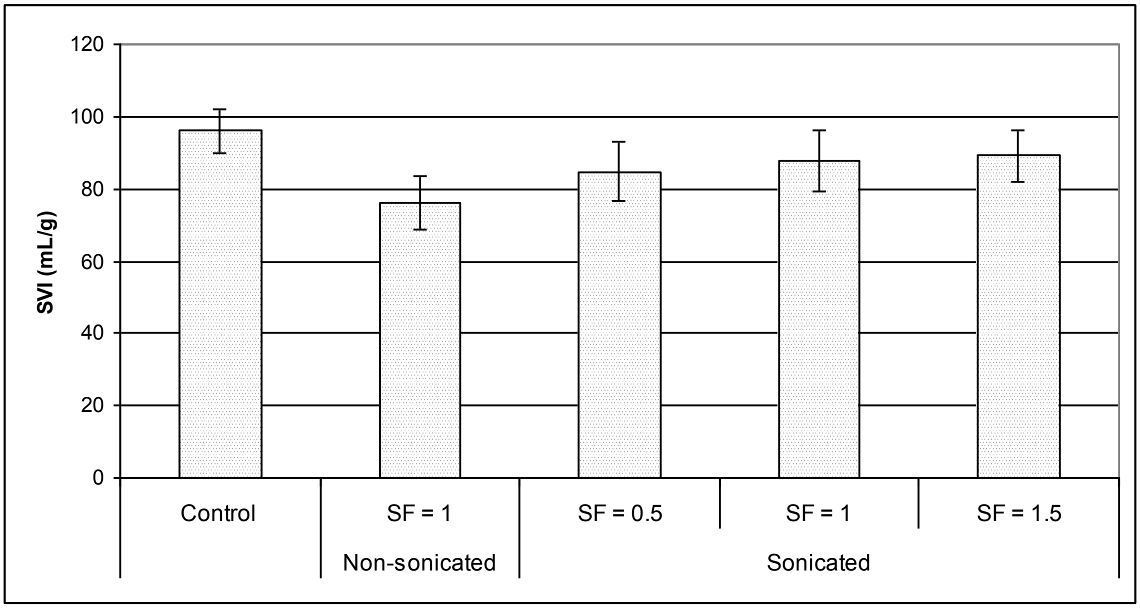

Figure 5.7: Effect of SBR operation on SVI index. Data represent the mean value (average of the SVIs) and error bars the standard deviation between 30 measurements

Similar results were observed by Camacho et al. (2002) and Cao et al. (2007) who worked on aerobic SBRs but without anoxic HT. On the other hand, it has been suggested that US can lead to the deterioration of sludge settling properties by promoting changes in the floc surface characteristics such as abundance of polymeric substances (Low and Chase, 1999; Zhang et al., 2007). Although the difference in SVI was not statistically significant based on Tukey's HDS test, most likely such effects can be encountered at higher ES inputs as previously observed in chapter 4 (4.3.4). According to Xin et al. (2008) introduction of biological selectors and SBR operation mode have significant effects on activated sludge settleability. Such conditions generally yield a biomass with superior settling characteristics due to the stress that the selectors impose on bulking bacteria growth rate. Therefore, it can be speculated that the exchange of biomass from the HT enhanced the growth of floc forming bacteria, as the lowest SVI was observed for the non-sonicated biomass exchanged (Figure 5.7). On the other hand, even though the settling characteristics of the biomass did not deteriorate with the incorporation of biomass exchange plus ultrasound (at constant ES), it is likely that high SF enhanced the introduction of fine particles produced during sonication (directly proportional to the volume of sludge exchanged) and likely more debris and free bacteria were exchanged to the aerated SBR. This would in part also explain why for the SBR operated at SF of 1.5 sludge settleability also improved. This result might contradict previous results observed in the effluent quality, in which the highest concentrations of TSS and tCOD were observed for this particular SBR operation condition. However, it has to be considered that the SVI test was performed during the aeration period and only considers the final sludge volume which after 30 min the sludge sample was returned to the corresponding SBR.

5.6.3 Effect of SBR operation conditions on sludge dewaterability

Table 5.8 shows the results for CST and %bound water content from the SBRs residual sludge at the different operational conditions. It can be observed that during the present study, sludge dewaterability (measured by CST method) did not present any particular trend, remaining almost constant for all SF tested during AS SBR operating conditions. It has been reported that low ES inputs have a deleterious effect on excess sludge dewaterability due to the increased number of fine particles that might clog the filtering paper. During SBR operation such an effect was not observed. Most likely, the fine particles produced during sonication at short SF were enmeshed within the floc once the biomass was exchanged to the SBRs. Additionally, it was observed in chapter 4 that under low ES inputs that anoxic HT biomass not only retained its biological activity but as well its ability to reflocculate which likely occurred once it was returned to the aerobic SBR.

Table 5.8: Effect of SF on residual sludge dewaterability from continuous SBR operation

		SRT 1		SRT 2		SRT3	
Run	**SF**	**CST[2]**	**Bound water[3]**	**CST**	**Bound water**	**CST**	**Bound water**
R1	Control	9 ± 4	8.6 ± 2	12 ± 3	9.2 ±1.2	7.5 ± 2	8.7 ± 2.2
R2	1[1]	10.2 ± 6	7.2 ± 0.5	7.4 ± 3	8.3 ± 0.5	6 ± 3	7.9 ± 1
R3	0.5	7.8 ± 2	7.4 ± 1	7.2 ± 4	7.6 ± 2	7.3 ± 1.5	8 ± 0.7
R4	1	10.2 ± 3	6.9 ± 1.3	9.7 ± 4	7 ± 1.5	10.8 ± 4	7.2 ± 0.4
R5	1.5	13 ± 8	7 ± 1	14.4 ± 7	6.7 ± 0.6	16 ± 4	6.8 ± 0.2

[1] HT biomass not sonicated
[2] Data represent the mean value ± standard deviation from twelve measurements at each SRT.
[3] Bound water content (g/gTS), data represent the mean value ± standard deviation from six measurements at each SRT

Only for the SBR operated at SF of 1.5 plus sonication a slight increase in CST was observed as compared to control SBR and the other experimental conditions. This was believed to be due to the high volumes of sonicated biomass exchanged, which might have increased the amount of fines in the SBR that could not refloccualate and consequently resulted in a direct detrimental effect on dewaterability as measured by the CST method. On the other hand, sludge bound water (BW) content was in general lower (16 ± 7% in average) for all the SBRs operated under the different SF conditions compared to SBR control. According to Higgins and Novak (1997) reactor operation is amongst the many parameters that have a direct impact on sludge dewaterability, as the way the aeration basin is operated determines to a great extent the dynamics of microbial population development, enhancing the growth of certain groups of microorganisms over others, consequently having a direct impact on sludge settling and dewaterability. Liao et al. (2000) suggested that the nature of the microbial community and the physical characteristic of the floc can explain the distribution of BW in sludge. In their study, they found that SRT or sludge surface charge were not correlated to BW content but as the floc size decreased the BW content increased as well as the SVI.

Additionally, experimental evidence has shown that excessive growth of bulking bacteria in ASP can cause problems associated with floc settling and dewatering (Jenkins et al. (1993). In the present study it was not possible to observe the microbial population dynamics after SBR operation conditions changed with SF. Hence, it is not possible to correlate the enhanced settling properties of the SBR residual sludge to the predominance of a microbial group(s) at any particular SF with or without sonication. And although three SRTs can not be considered long term operating conditions, the results seemed to indicate that SBR operation under the different SF enhanced sludge dewaterability, as the lower volumes of BW were found at conditions when biomass was exchanged (sonicated and not sonicated) compared to the control. Therefore, it can be speculated that the anoxic HT as well as SF and sonication played key roles on the enhancement of SBR settling and dewaterability properties compared to the AS SBR control.

5.6.4 Effect of the stress factor on activated sludge biomass activity

The specific oxygen uptake rate (SOUR) is commonly used to assess aerobic biomass activity. During normal ASP operation, the biomass consumes oxygen at a specific rate and the SOUR gives a measurement of how fast or slow the organics are being degraded (Metcalfe and Eddy, 2003). Additionally, SOUR variations from a predetermined average value can give insights into effluent toxicity or other ASP operational problems. Usually, when the ASP has been in operation for long periods of time with the same influent wastewater, SOUR variations can be mainly attributed to changes in biomass dynamics (changes in the microbial consortia). Figure 5.8 shows the average values for the SOUR under the different SBR operating conditions.

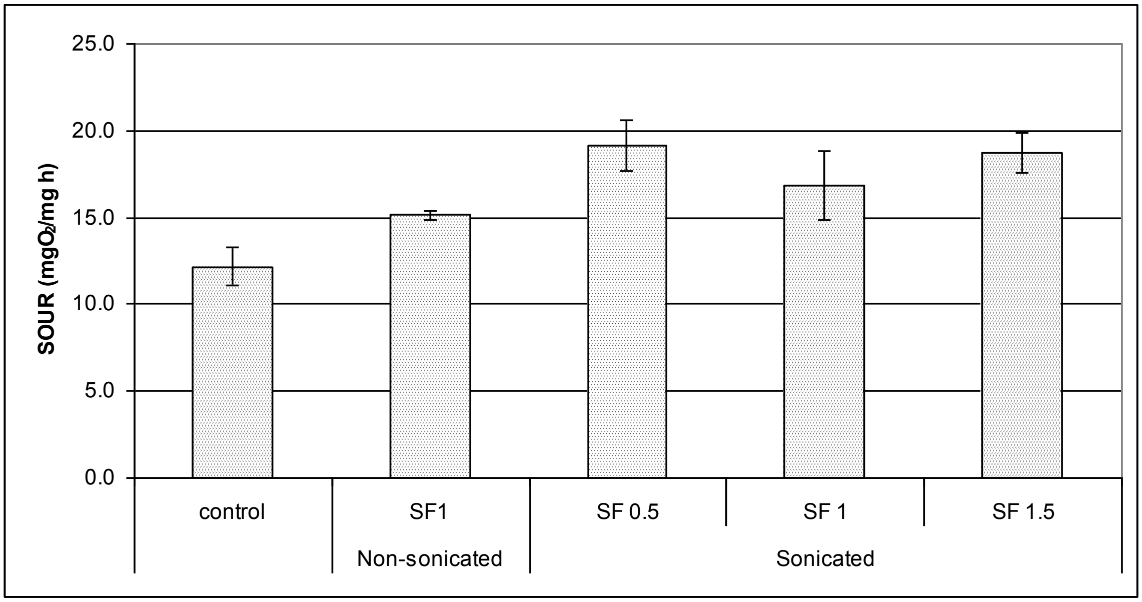

Figure 5.8: Average SOUR for ASP SBRs operated at the different SF. Data represent me mean value ± standard deviation between 6 measurements taken at steady-state.

From Figure 5.8 it can be observed that the SBRs SOUR increased an average of 25 ± 3% for an SF of 1 without sonication compared to the control. Furthermore the highest increases in the average SOUR occurred for all SF when sonication was applied and ranged from 41-59 ± 7%. The highest increase in the average SOUR was 59% for the SBR operated at SF of 0.5. The multiple comparison Tukey HDS test indicated that such differences were significant at $p<0.05$. Since the influent wastewater remained relatively constant during the present study, these results indicated that the increased SOUR was due exclusively to changes in the SBR biomass activity. It has been observed that changes in biomass activity, i.e initial decrease in SOUR followed by recovery of the initial activity might be due to increased maintenance metabolism (Camacho et al., 2002; Rai et al., 2004). Therefore, it can be postulated that the biomass exchanged from the anoxic HT under ultrasound and no-ultrasound conditions had a direct effect on SBR biomass dynamics and activity. In addition, it can be observed in Figure 5.9 that although the anoxic HT effluent alone (path a) also increased biomass activity (SOUR) its contribution was marginal when compared to paths b and c (Figure 5.9) which exposed non-sonicated (path b) and sonicated (path c) biomass to real primary effluent at So/Xo of 0.09. According to Vuellimin et al. (2003), anoxic periods could induce the decay of a percentage of heterotrophic bacteria incapable of adapting to low dissolved oxygen conditions. Furthermore, the same anoxic periods can cause the decay of a greater percentage of the bacterial grazers. Under such conditions a significant amount of organic matter can be produced, and although a portion is consumed within the anoxic HT (cryptic growth) a portion will be recycled to the SBR after biomass exchange. Hence, this can help explain the lower SOUR (3.2 mgO_2/mg h) when only the HT effluent was used. However, when the HT biomass was fed with ROPEC filtered wastewater, in both cases (non sonication/sonication) the SOUR increased to 13.5 and 16.8 mgO_2/mg h which was well above the control and for the latter indicative of stressed biomass with higher maintenance metabolism compared to the same HT biomass without sonication. This result again indicated that the HT anoxic biomass under the HT conditions used once exchanged to the SBR was a major contributor to the enhanced bioactivity in the SBR. It can also be speculated that the already stressed biomass in the anoxic HT suffered yet another additional environmental stressor when sonication was applied. Hence, once the sonicated HT biomass was returned to the SBR, most likely, the system would demand more oxygen for maintenance metabolism and repair functions (Rai et al., 2004). From an operational point of view, the increased SOUR combined with the best SF conditions

(SF=0.5) would consequently demand that more oxygen be supplied to the system in order to maintain the required minimum concentration (>2 mg/L) for aerobic municipal wastewater treatment.

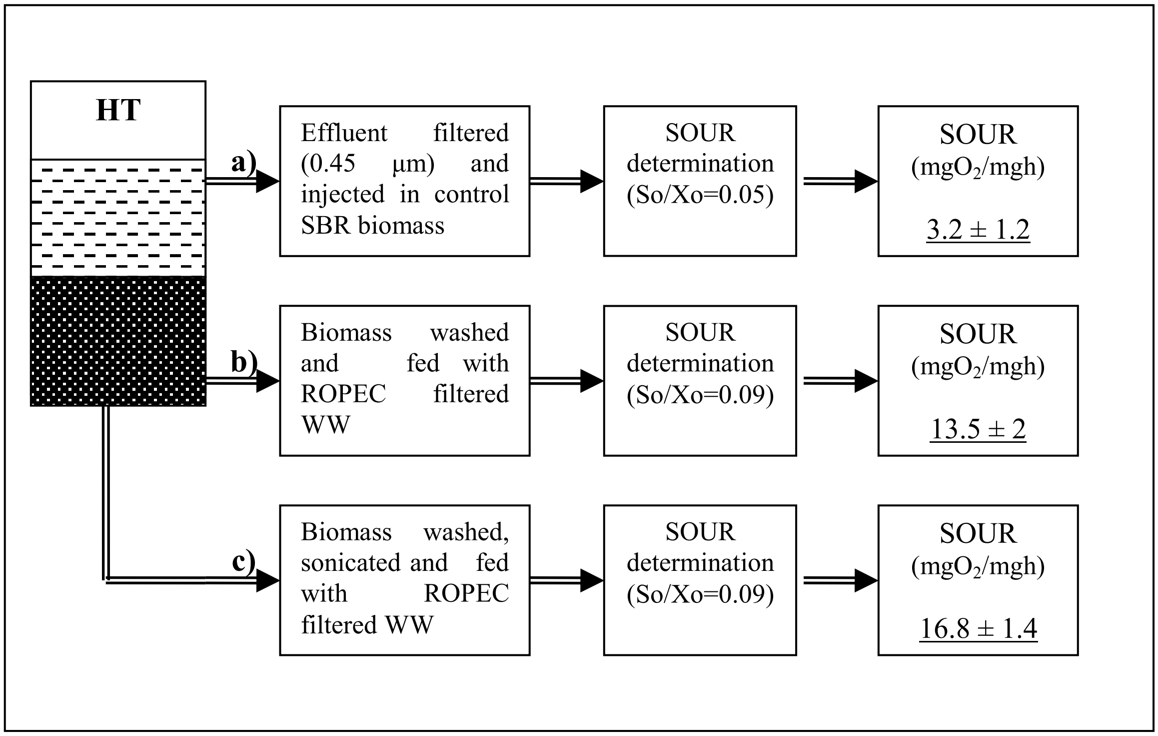

Figure 5.9: Respirometric assessment of the HT. In the diagram: a) effluent only, b) biomass only and c) sonicated biomass. SOUR data presented is the mean value ± standard deviation between two measurements.

In the present experiment, it was observed that after HT biomass (sonicated) was exchanged the DO in the SBR decreased. For the SF of 0.5 and 1, it took in average 15 min to achieve DO concentrations similar to those before the biomass was exchanged (5-6 mg/L). However, in the case of the SBR at a SF of 1.5 (sonicated), it took approximately 30 min for the DO concentration to be re-established, which required an increase in the aeration supply. Low and Chase (1999) observed similar behaviour in their studies, and concluded that cryptic-growth was associated with an increase in oxygen requirements. Although it is believed than in this study the main mechanism for enhanced biomass activity was not cryptic-growth, maintenance metabolism demands additional oxygen supply as well. Ginestest (2007) concluded after analyzing several routes for excess sludge reduction that most routes present the same behaviour in terms of increased oxygen demand in the aeration basin during application of the different routes. However in most cases the aeration capacity of most existing plants would be most likely sufficient to accommodate such increased oxygen demand. Most likely plant aeration capacity should be reassessed under the specific route if modifications to the plant are being considered for reduction in excess sludge production.

In the present study, activated sludge activity was also investigated by monitoring the dehydrogenase activity (DHA) at regular time intervals for the various SBR operating scenarios. Microbial activity is of importance in wastewater treatment because the organic matter degradability and biomass turnover depend on metabolically active microorganisms (Griebe et al., 1997). Anaerobic and aerobic heterotrophic bacteria alike possess electron transport systems, and DHA is central to oxidative substrate removal by transferring electrons to an acceptor, usually $NAD^{\pm}/NADP^{\pm}$ or a flavin coenzyme (Griebe et al., 1997; Stryer, 1988). To better understand biomass activity under experimental conditions; it is possible to use respirometry in combination with tetrazolium salts that can be used as artificial electron acceptors in metabolically active bacteria (Chapter 4). DHA detection is based on the reduction of the water soluble and colorless tetrazolium salts to a colored water soluble product with color development being proportional to DHA activity (Griebe et al., 1997; McClusky et al., 2005). In the present study, DHA activity was assessed by using the tetrazolium salt 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT). The great advantage of XTT over other tetrazolium salts is that it does not require product extraction; as the sulfonate groups in XTT structure make their corresponding formazan products water soluble (McClusky et al., 2005). Figure 5.10 shows the effect of SF on the average DHA activity during SBR operation.

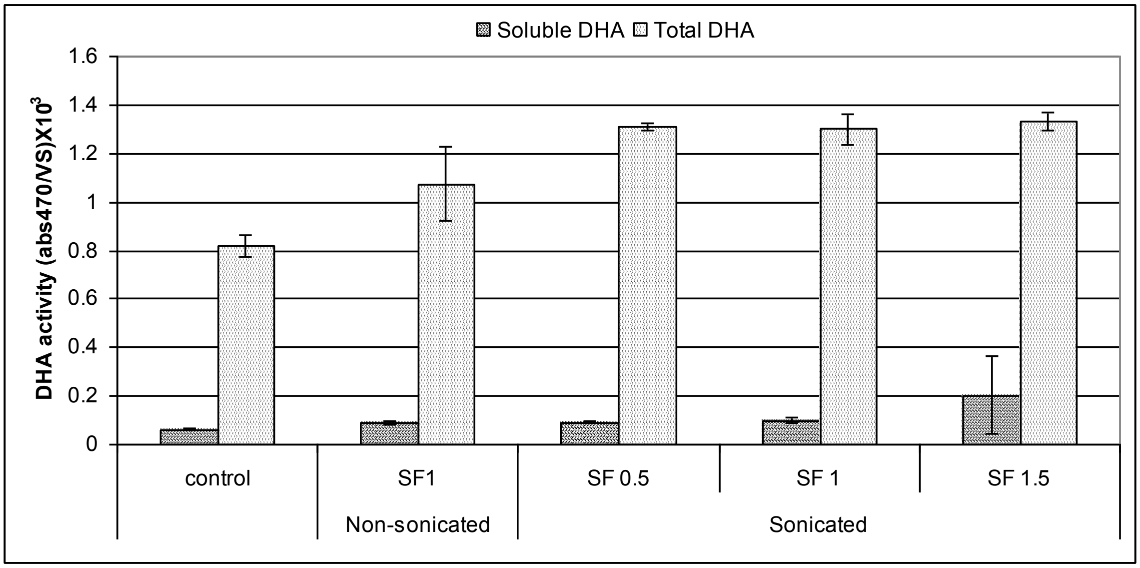

Figure 5.10: DHA (extracellular and total) for the different SF during AS SBR operation. Data presented are the mean value and error bars represent the standard deviation between 12 measurements at steady state.

Figure 5.10 shows that total TDHA activity also increased by 39% on average for an SF=1 and non-sonicated operating conditions compared to the SBR control. Following the same trend as SOUR enhanced TDHA bioactivity increased by about 62% compared to SBR control for SF of 0.5-1.5 when combined with sonication. As SOUR, DHA is substrate dependent and due to the rapid results turnover its use has been shown to be effective in the assessment of influent wastewater toxicity or stress (Wuertz et al., 1998; McCluskey et al., 2005). Wuertz et al. (1998) reported that activated sludge DHA treating wastewater containing recalcitrant materials was 4 times higher than during treatment of easily degradable organics. In addition, Marin et al. (2011a) reported higher DHA during anaerobic digestion of kitchen waste pretreated with microwaves than for the control samples, correlating the increased DHA with the production of enzymes that help the microbial consortia to degrade certain types of difficult to degrade substrates or as adaptation to a new environment (Marin et al., 2011b). According to Bodegom (2007) maintenance metabolism includes all the non-growth microbial functions such as shifts in metabolic pathways, energy spilling reactions, cell motility, and changes in stored polymeric carbon, synthesis and turnover of macromolecular compounds such as enzymes and RNA.

Therefore, it can be assumed that the increased TDHA in the ASP microbial community was triggered due to the shifting from one environmental condition (changing SF) to another in addition to the stress induced by HT biomass sonication. This can explain the highest value for the soluble DHA observed for the SBR operated at the highest SF of 1.5 with sonicated HT biomass (more volume of sonicated biomass exchanged). According to Bodegom (2007) determination of the dominant non-growth component of microbial maintenance metabolism is very difficult and certainly dependent on each particular group of microorganisms, but it is nonetheless a very dynamic process. The activated sludge biomass, presents the additional challenge of being a consortia of diverse groups of microorganisms which is further exacerbated in the present experiment. Real primary effluent is itself very heterogeneous and should result in even more microbial diversity when compared to other studies that are more idealistic and use a single homogeneous model substrate. The results form the present study indicated that although cryptic-growth to a certain extent might have also contributed to the higher TDHA during SBR operation, most likely the increased biomass activity can be attributed to biomass maintenance metabolism as the ES input conditions applied did not induce excessive biomass lysis or floc destruction (Chapter 4), and the wastewater while complex remained relatively constant during the study.

5.6.5 Effect of SBR operation conditions on excess sludge production

Daily sludge production was calculated according to equation 5.8 and 5.9. The daily flow (Q) was the total from the 3 cycle periods during which the SBR was operated (9.6 L/d). The other terms of equations 5.8 and 5.9 are provided in Table 5.9 and were calculated from fractioning and characterization of ROPEC primary effluent wastewater. It is important to mention that during the present study, it was expected to find day to day variations in influent wastewater characteristics. According to Metcalfe and Eddy (2003) despite daily variations, the C:N:P (100:5:1) ratio in municipal wastewaters remains fairly constant as well as some other important parameters required for biological treatment such as nutrients, pH, and alkalinity.

Table 5.9: ROPEC's primary effluent COD fractioning

ROPEC primary effluent COD fraction[1]	Concentration (mg/L)
Biodegradable COD (bCOD)	177 ± 9
Non-biodegradable COD (nbCOD)	42 ± 12
Readily biodegradable COD (rbCOD)	53 ± 5
Slowly biodegradable COD (sbCOD)	122 ± 4
Soluble non-biodegradable COD(nbsCOD)	11 ± 1
Particulate non-biodegradable COD (pnbCOD)	31 ±10
Influent fixed solids (X_{FSI})[2]	13 ± 0.86

[1]ROPEC wastewater fractioning was in total performed 7 times during this study. Data presented are the mean value ± standard deviation from 14 measurements
[2] Data presented are the mean value ± standard deviation from 15 measurements

In addition to variability, municipal wastewaters are very complex in composition and depend to a great extent on the regional weather as well as industrial economical activities. In the present study the observed biomass yield (Y_{obs}) was calculated taking into account the daily sludge production (wasted sludge, sludge lost in the effluent and the sludge in the SBR) and the cumulative substrate removal rate (tCOD) during SBR operation at the different SF conditions with and without HT biomass sonication. Due to the wastewater variability it was deemed necessary to calculate the sludge production every day and it is shown in Figure 5.11. From Figure 5.11, the net sludge production was calculated as the slope of the linearization of the correlation between cumulative excess sludge produced and the time (days) the SBR was operated at the determined SF. The linearization was calculated, but is not presented in Figure 4.10 for clarity. The Y_{obs} was then calculated by dividing the net sludge production by the COD removal rate as depicted in Table 5.10. From Figure 5.11 and Table 5.10 it can be observed that the Y_{obs} decreased for all the SF tested during this study compared to control SBR. The degree of sludge reduction (DSR) is shown in Table 5.11.

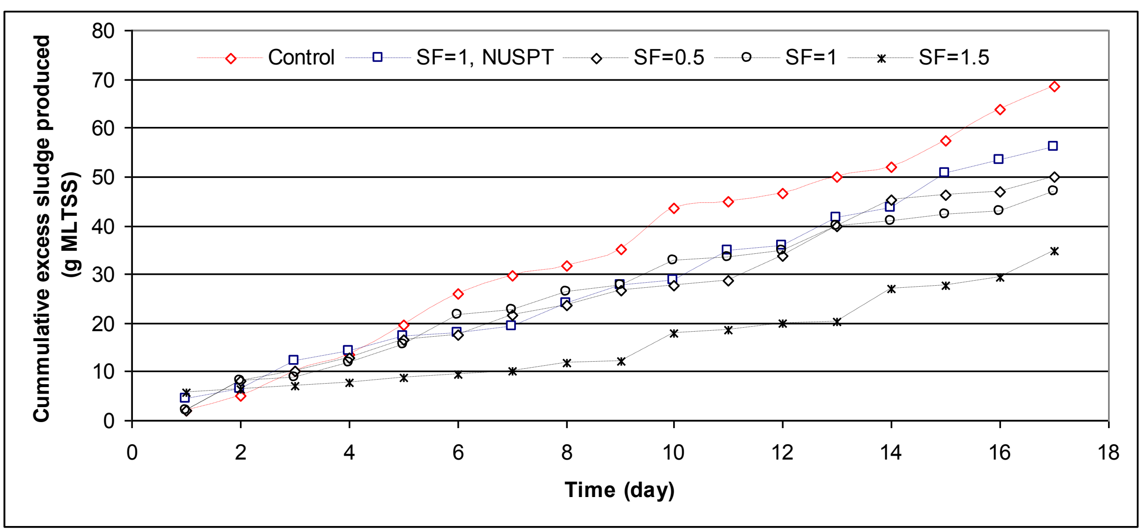

Figure 5.11: Cumulative excess sludge produced at the different SF during municipal wastewater treatment using the ASP SBR. In the Figure SF=1, NUSPT indicates biomass exchanged from the HT without USPT

Table 5.10: Observed growth yield for SBR at the different SF operation conditions

Run	SF (1/d)	Sludge production (g MLTSS/d)	TCOD removal rate (g COD/d)	Y_{obs} (gMLTSS/gCOD
R1	Control	4.02	10.3	0.39
R2	1[1]	3.20	10.1	0.31
R3	0.5	2.92	9.8	0.26
R4	1	2.73	9.3	0.29
R5	1.5	1.84	8.1	0.22

[1] HT biomass not sonicated

Table 5.11: Degree of sludge reduction achieved during primary effluent treatment at the different SF compared to SBR control

Run	SF (1/d)	DSR[2] (%)
R1	Control	-
R2[1]	1	21
R3	0.5	26
R4	1	30
R5	1.5	44

[1] HT biomass not sonicated
2 Calculated from equation 4.3

From Table 5.11 it can be observed that there was on average a 21% DSR compared to the SBR control for SF of 1 when the exchanged HT biomass was non-sonicated. The DSR increased to 26% when the SF was either 0.5 or 1 and exchanged HT biomass was sonicated. The highest DSR (44%) was achieved when a SF of 1.5 was applied with sonicated HT biomass. However, at SF of 1.5 with sonication the effluent quality deteriorated in terms of tCOD and TSS removals (Figure 5.4). Most likely the high DSR at SF of 1.5 was due to a combination of factors such as high TSS in the effluent and although it was not formally investigated, it can also be assumed that a high degree of biomass predation by protozoan had an effect on the lower sludge determined at SF of 1.5, since it was at this SF that an explosion in the number of *Aelosoma* was observed. These results indicated that a high degree of sludge reduction can be achieved in the aerobic ASP SBR by exchanging biomass (SF=1) from a stressful environment the anoxic HT. It is important to mention that the high SFs used in the present study were possible due to the biomass concentration maintained in the anoxic HT. Most likely, using higher solids concentration or the concentrations commonly used in the OSA system (5-7 g TS/L) would not allow for such high SF without the introduction of high amounts of solids to the aeration tank after biomass exchange. In addition, it is difficult and unreasonable to compare the results from the present study with other ASP modifications such as the OSA system, since in all of the reported studies membranes were used for treatment of a synthetic wastewater (Cheng et al., 2003). Also from Table 5.11, it can be observed that combining biomass exchange from the anoxic HT with ultrasound treatment further increased the DSR, with a net increase of 5.5 and 23% to about 26 and 44 % for SF of 0.5, 1.0 and 1.5 respectively. This was obtained with additional benefits in terms of effluent quality in particular for SF of 0.5 and 1.0 such as improved nitrogen and phosphorus removal, dewaterability and settleability. These results warrant future research for this integrated type of operational configuration (anoxic HT plus low level sonication) to enhance excess sludge reduction. In related studies at low US energy inputs, Rai et al. (2004) reported a 29% sludge reduction compared to control system. They also suggested that working under low energy inputs might not enhance cryptic growth, but rather increased maintenance energy requirements on the activated sludge biomass triggering the use of the a larger portion of the incoming substrate for repair functions. However, it should be noted that they used a simple homogenous substrate and did not incorporate an anoxic HT. Additionally; no information was provided on effluent quality and other important biomass ASP characteristics such as metabolic activity, settleability and dewaterability. Although enhanced biomass metabolism has not been accepted as the main mechanism for the reduced sludge production at low ES input energies, some other studies have indicated that it plays an important role (Cao et al., 2006). In addition, in all the above mentioned studies no biomass solubilization was observed. The trend in the present study suggested that through enhanced maintenance metabolism a high degree of excess sludge reduction during aerobic treatment of municipal wastewater treatment is possible. Additionally ASP operation at such conditions (HT plus sonication at low ES inputs) can be controlled to minimize deleterious effects on effluent quality and residual sludge. Furthermore, such operation conditions are likely more economically feasible for full scale wastewater treatment compared to cryptic-growth scenarios that require high specific energy inputs using any emerging technology. Cell lysis demands high amounts of energy input (Muller, 2000), which also usually results in high temperatures that must be considered. During the present experiment it was observed that at high ES inputs (Chapter 4) the equipment required constant exchange of horn tips which corroded very fast, and the unit automatically shut down after temperatures increased above 78 ºC, requiring rigorous temperature control. The effects of temperature and cavitation are also debatable and not yet clear. While some studies have concluded that high temperatures and cavitation enhance sample solubilization by weakening membrane cells (Chu et al., 2001) others have suggested that as temperature increases the viscosity and vapour pressure in the liquid change, generating larger microbubbles and less shear shock (Raman and Abbas, 2008). Nevertheless, implementation of US technology to induce cryptic-growth at full scale would demand high investment costs, not only in terms of equipment but also in energy needs, maintenance and repair which has to be balanced with the degree of sludge reduction achieved. Focusing on enhanced maintenance metabolism could be a more viable option although a lower degree of sludge reduction might be achieved.

5.7 Conclusions

Based on the presented results it can be concluded:

- SOUR and DHA activity tests show that incorporation of an anoxic HT with ultrasound treatment of HT biomass prior to exchanging it back to an aerobic SBR ASP process increases maintenance metabolism and the microbial dynamics within the aerobic SBR
- Introduction of an anoxic holding tank without incorporating sonication impacted the dynamics of the biomass within the aerobic SBR increasing maintenance metabolism that contributed to a 21% decrease in excess sludge production at an SF of 1.0 without compromising effluent quality parameters or residual sludge characteristics during treatment of real primary effluent municipal wastewater.
- Incorporation of low intensity ultrasound treatment of anoxic HT biomass in addition to HT biomass recycling at SFs of 0.5, 1.0 and 1.5 further enhanced changes in microbial dynamics in the aerobic SBR which enhanced maintenance metabolism even further with concomitant increase in excess sludge reduction by up to 44% compared to the control during aerobic treatment of real primary effluent municipal wastewater. However there is a specific stress factor after which effluent quality deteriorated. For this study SF higher than 1.5 combined with HT biomass sonication resulted in decrease in final effluent quality but not for residual sludge characteristics (settleability or dewaterability).

- Low SF of 0.5 and 1.0 enhanced the activated sludge settleability and dewaterability, although the mechanisms are not clear.
- The operation of the activated sludge process under low ES and low SF of 0.5 and 1.0 did not present any operational problems in terms of temperature increase or ASP control and such conditions are most likely economically attainable for an integrated sludge reduction process at pilot and full scale operation wastewater treatment plants.
- All conclusions were obtained with complex real primary effluent wastewater from a full scale MWW plant.

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Chapter 6

Overall Conclusions

6.1 Conclusions

This study proposed a specific modification to the activated sludge process for excess sludge minimization during municipal wastewater treatment. Based on the presented results of the combined effects of the anoxic holding tank plus sonication on residual sludge characteristics and the activated sludge effluent quality it can be concluded that

1) Through low frequency ultrasound (US) pretreatment it is possible to induce a series of different effects on activated sludge biomass. Such effects depend on ultrasound specific energy (ES) input and can go from enhanced biomass activity without producing cell lysis (ES inputs < 56KJ/gTS) to complete biomass solubilization at ES inputs higher than 118 KJ/gTS. US proved to be an adaptable and controllable technology to induce the desired effects on the biomass.
2) Although not yet clear biomass activity enhancement after sonication was believed to be the result of a series of combined mechanisms. This study added more weight to the claim that the mechanical effects associated with ultrasound at low ES inputs play an important role for bioactivity enhancement. However, the effects of localized temperatures and pressures induced by US are also important to consider. The ES input to enhance biomass activity has to be determined experimentally taking into consideration the origin and the particular characteristics of the biomass.
3) The use of XTT to determine biomass enzymatic activity proved to be a fast and reliable method. However, due to its qualitative nature DHA determination alone

can not replace the more cumbersome determination of biomass activity by respirometry.

4) The use of response surface methodology and the desirability functions approach proved to be a fast and effective way to optimize the ASP-HT-USPT processes. With optimum conditions at 8KJ/gTS and biomass concentration of 2900 mgTSS/L.
5) The introduction of the anoxic holding tank without sonication enhanced biomass characteristics and contributed to a 22% decrease on excess sludge production without compromising effluent quality parameters or residual sludge characteristics during real municipal wastewater treatment. The addition of low intensity US pretreatment further enhanced sludge reduction by an additional 20 to 26% under the different SF applied during real municipal wastewater treatment. However as SF increased to 1.5 + USPT effluent quality started to deteriorate as compared to control ASP.
6) Low SF (SF< 1) enhanced in general activated sludge biomass settleability and dewaterability as compared to ASP control. Although SF > 1.5 + USPT showed to be detrimental for ASP secondary effluent quality, the residual sludge characteristics were improved respect to control ASP.
7) The operation of the activated sludge process under low ES inputs and low SF did not present operational problems such as temperature increase or control. Additionally, such conditions are most likely attainable and more economically feasible at pilot and full scale wastewater treatment plants.

Chapter 7

Future studies and Recommendations

7.1 Future studies

Based on the presented results and on observations made during this research, the following topics of study are recommended to better understand the mechanisms of excess sludge reduction during municipal wastewater treatment:

1) Although the concept of maintenance metabolism is still a topic of debate, it is necessary to develop techniques that can offer an insight into this important process. The use of XTT could provide a fast and useful tool for the study of bacterial maintenance metabolism if the technique was made quantitative and standardized.
2) Investigate more thoroughly the effect of the anoxic holding tank on nutrient removal (nitrogen and phosphorous) as well as on excess sludge reduction with typical municipal wastewater raw and primary effluent.
3) Although ultrasound is still an expensive technology to implement at full scale, low ES processes like the presented in this study seemed to lower the operational costs associated with the technology. But it is necessary to investigate at a pilot scale excess sludge minimization with this approach to determine its potential for full-scale applications.
4) Integrate into this type of research molecular and culture techniques that can allow observing the microbial population dynamics, both in the activated sludge process and in the holding tank.

5) Development of a mathematical model suited for this process which can integrate the anoxic holding tank and the effect of ultrasound on biomass activity at low ES inputs. In the current literature most of the mathematical models are based on total cell lysis before being recycled to the aeration tank.

Appendix A

A.1 Ultrasound specific energy input calculation

From the literature review it is clear that the ultrasound (US) input energy calculation is a subject of high debate. US effects depend on many physical/chemical samples' characteristics (i.e. solids content, viscosity, pH, samples type and origin amongst others). Additionally, the design of the ultrasound system: horn, booster and converter (usually proprietary) is also vey important for US energy delivery. Currently, no deterministic model is available that can account for all factors. Hence, US systems need to be calibrated 'on-site' for the specific application and type of sample. The power or energy supplied for sludge disintegration can be expressed in a number of ways as shown in Table A1.

Table A.1: Ultrasound energy input measurement

	Equation[1]	Units	Definition	Remarks
Specific energy	$ES = \frac{P * t}{V_{sample} * TS}$	$\frac{KJ}{kgTS}$	Energy supplied per unit of sample mass	It relates sonication time and sample solids content
Ultrasound dose	$UD = \frac{P * t}{V_{sample}}$	$\frac{KJ}{L}$	Energy supplied per unit of sample volume	It cant be used to compare samples with different solids contents
Ultrasonic density	$Ud = \frac{P}{V_{sample}}$	$\frac{W}{mL}$	Energy supplied per sample volume	Similar to UD, but sonication time is not taken into account
Ultrasonic intensity	$UI = \frac{P * t}{A_{converter}}$	$\frac{W}{cm^2}$	Energy supplied per surface area of the converter	Reflects the generating power of the converter

[1] in the equation P = power (W), t = time (sec), V_{sample} (L/mL) and TS = solids content (kg/L)

From Table A1, the single parameters that can account for some of the most important operational variables, is the specific energy (ES). Also, from its equation it can be observed that ES is proportional to the sonication time. By keeping the ultrasound density constant, the ES input can be calculated if the sonication time and the solids content of the sample are known. On the other hand, the sonication time can be determined based on the desired ES input to the system once it has been calibrated. In this experiment the different solids concentrations were sonicated for different time intervals and the power recorded. The digital sonifier Branson S-450D allows sonication times as low as 1 second and produces a report after every experiment showing the date and time of the experiment and the parameters in effect: power input (in watts and in Joules), amplitude and the final temperature of the sample. Figure A1 shows the average ES for the different solids concentrations.

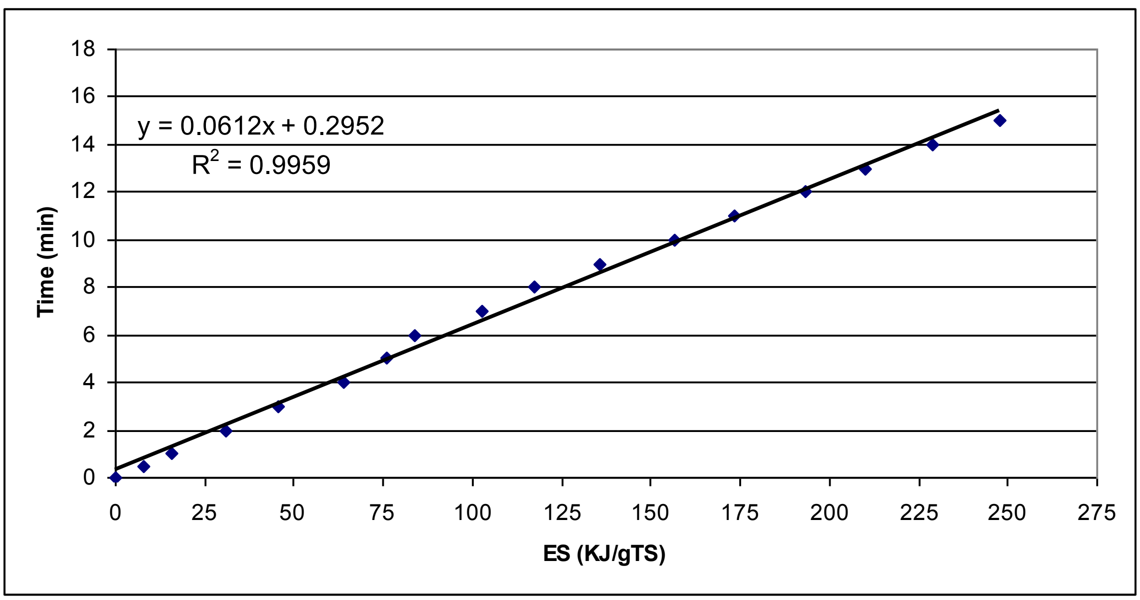

Figure A.1: Example of US machine calibration to obtain a constant ES input. The same procedure was repeated for all solids concentrations tested during the present study

From Figure A1 the sonication time needed to produce the desired ES input was calculated. Since the range of ES inputs used in this experiment were low, the sonication time and the sonication amplitude where modified when needed to keep the ES input as constant as possible. One aspect that has been highly overlooked in related literature is the horn tip corrosion during sonication. Tip corrosion is unavoidable and depends on sonication conditions. As the horn tip wears out, ES input decreases. During the initial calibration of the Branson sonifier it was observed up to 50% difference in ES input and consequently high variations on US effects. Therefore it is important to keep record of the date a new tip was changed and observe the tip reduction with time. In this experiment tip wearing out was determined based on tip weight. Once a new tip was installed its weight was recorded after visible erosion/wearing out the tip was removed and reweight again. It was observed that tip weight reduction beyond 80% produced high differences in ultrasound ES delivery, as well as some other operational problems with the Branson sonifier unit. Therefore, this study recommends a constant observation of the tip and the weight approach followed in this experiment proven to be an effective and easy way to ensure a constant ES input.

Appendix B

This appendix presents the summary of some important data obtained experimentally which were used during the analysis and discussion of results of the different chapter of the present study.

Table B.1: Particle numbers in the range of 1 to 227 µm after ultrasound treatment

ES	Sample solids concentration (%, w/w)											
KJ/gTS	0.15		0.20		0.25		0.3		1.3		2.5	
	Particles per mL (#/mL)											
0	46578	40606	58316	50158	51735	48967	49943	50801	62821	63817	70986	69224
11	182947	174189	114063	125635	184598	205958	193897	200597	249875	244610	332179	337675
56	221591	178821	219862	220116	284598	275854	300451	300332	449710	459717	578961	575875
118	141123	126655	130185	126081	177894	173492	220726	213818	207596	212102	220164	214628
183	58312	57452	78097	78923	124520	120364	109843	123865	138329	139984	100169	100012
257	67910	68662	66150	69496	110021	100275	92981	93575	32036	33321	52081	51697

Table B.2: Steady-state data for control AS SBR (SBR1)

Parameter	→	**pH**[1]		**tCOD**[2]		**TSS**[3]		**TDS**[4]		**Ammonia**[5]		**SVI**[6]		**SOUR**[7]	
Units	→	**-**		**mg/L**		**mg/L**		**mg/L**		**mg/L**		**mL/g**		**mgO_2/mgh**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Jan-4	7.1	0.1	36.8	2.2	21.5	1					119	5.7	12.7	1
2	Jan-5	7.2	0.2	30	1.8	18	2.9	633.5	9.2			128.5	9.2		
3	Jan-6	7	0	25.8	1	13.5	1.9			3.98	0.4	94	11.3		
4	Jan-7	7.1	0.2	24.3	2.2	11.8	1.7					91.5	2.1		
5	Jan-8	7.2	0.1	27.5	2.1	13.8	3.4					98	15.6		
6	Jan-9	7.2	0	25.1	1.3	9.8	1.7	619	26.9			92	8.5		
7	Jan-10	7	0.2	25	1.8	9.3	1			3.59	0.9	104	8.5	12.9	0.1
8	Jan-11	6.9	0.1	25.5	1.3	8.1	0.7					96	1.4		
9	Jan-12	6.8	0	25	0.8	8.7	0.5	596	2.8			105	4.2		
10	Jan-13	7.2	0.1	24.3	1	9.3	1.5					82.5	6.4		
11	Jan-14	6.9	0.2	26	1.4	7.9	0.9			2.6	0.9	97	1.4		
12	Jan-15	7.3	0	22.8	1.3	8	1.4	606	5.7			98	1		
13	Jan-16	7	0.1	24.5	1.9	7.8	1					100	2.8		
14	Jan-17	6.8	0.1	25	2.2	8.3	1					94	21.2	11.9	2
15	Jan-18	7.2	0	27	1.4	7.5	1	609.5	16.3			92.5	7.8		
16	Jan-19	7.1	0.2	26	2	7.3	0.5			2.14	0.3	105	9.9		
17	Jan-20	7	0.2	25.8	2.2	7.5	1.3					92	8.5		
18	Jan-21	6.9	0.2	30	2.3	8.4	0.5					124	36.8		
19	Jan-22	7	0.1	29.5	3.7	9.7	1					96.5	3.5		

1 average of the day (4 measurements)
2 average of the day (6 measurements)
3 average of the day (6 measurements)
4 average of the day (4 measurements)
5 average of the day (4 measurements)
6 average of the day (3 measurements)
7 average of the day (3 measurements)

Table B.3: continuation from Table B.2

Parameter	→	TDHA[8]		ETDHA[9]		Alkalinity[10]		TP[11]	
Units	→	Δabs_{470}/VS		Δabs_{470}/VS		mgCaCO3/mL		mg/L	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Jan-4	0.78	0.06	0.05	0.01				
2	Jan-5							2.2	0.21
3	Jan-6					58.6	12		
4	Jan-7								
5	Jan-8								
6	Jan-9								
7	Jan-10	0.87	0.3	0.07	0.015				
8	Jan-11					63.8	5.2		
9	Jan-12							2.1	0.21
10	Jan-13								
11	Jan-14								
12	Jan-15								
13	Jan-16					61.7	4		
14	Jan-17	0.81	0.04	0.61	0.012				
15	Jan-18								
16	Jan-19							2.2	0.2
17	Jan-20								
18	Jan-21					71.3	6.1		
19	Jan-22								

8 average of the day (6 measurements)
9 average of the day (6 measurements)
10 average of the day (6 measurements)
11 average of the day (6 measurements)

Table B.4: Steady-state data for SBR 2 (SF=0.5, non-sonicated biomass exchange)

Parameter	→	**pH[1]**		**tCOD[2]**		**TSS[3]**		**TDS[4]**		**Ammonia[5]**		**SVI[6]**		**SOUR[7]**	
Units	→	**-**		**mg/L**		**mg/L**		**mg/L**		**mg/L**		**mL/g**		**mgO_2/mgh**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Jan-23	7	0.2	29.8	1.7	9.2	0.5	645.8	26	3.5	0.33	66.5	2.1		
2	Jan-24	7		25.5	2.8	8	0.9					70	4.2		
3	Jan-25	7	0.1	25.7	2	9.2	2					77	7.1	15.1	1.6
4	Jan-26	7	0.1	30.5	1.9	8.7	1.1					71	2.8		
5	Jan-27	7.2		28.3	2.2	7.8	0.2	641	13			73	7.1		
6	Jan-28	6.9	0.2	25	2.6	7.8	0.6			4	0.28	81	2.8		
7	Jan-29	7	0.1	28.1	1.5	9.9	0.8					88.5	11		
8	Jan-30	7.1	0.1	25.6	2.6	7.8	0.5	667	12			80	1.4		
9	Jan-31	7.2	0.1	29.9	2.2	8	0.7					88	17	14.9	1.4
10	Feb-1	6.9	0.1	23.5	1.7	8.6	1					73	11		
11	Feb-2	7	0.2	24	1.4	8.4	1.1			1.8	0.7	74	4		
12	Feb-3	7.1		23.9	1.5	8.1	1.4	664	26			84	12		
13	Feb-4	6.8	0.1	23.4	1.6	9.8	2.2					71	2.1		
14	Feb-5	7	0.1	22.9	2	8.3	1					82	11.3		
15	Feb-6	6.9	0.2	22.9	1.3	8.4	1.2	691	11			64	1.3	15.5	1.1
16	Feb-7	6.9	0.1	27.6	1.4	7.9	2			1.7	0.21	83	6		
17	Feb-8	7	0.1	25.3	1.8	8.2	0.8					72.7	3.1		

1 average of the day (4 measurements)
2 average of the day (6 measurements)
3 average of the day (6 measurements)
4 average of the day (4 measurements)
5 average of the day (4 measurements)
6 average of the day (3 measurements)
7 average of the day (3 measurements)

Table B.5: continuation from Table B.4

Parameter →		**TDHA**[8]		**ETDHA**[9]		**Alkalinity**[10]		**TP**[11]	
Units →		**Δabs_{470}/VS**		**Δabs_{470}/VS**		**mgCaCO3/mL**		**mg/L**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Jan-23					46.8	5		
2	Jan-24								
3	Jan-25	1.28	0.07	0.093	0.03				
4	Jan-26							1.9	0.2
5	Jan-27								
6	Jan-28					62.5	10		
7	Jan-29								
8	Jan-30								
9	Jan-31	1.12	0.06	0.081	0.01				
10	Feb-1							1.98	0.08
11	Feb-2					50.3	2		
12	Feb-3								
13	Feb-4								
14	Feb-5								
15	Feb-6	0.9	0.1	0.089	0.01				
16	Feb-7					58.2	6.4		
17	Feb-8							1.89	0.1

8 average of the day (6 measurements)
9 average of the day (6 measurements)
10 average of the day (6 measurements)
11 average of the day (6 measurements)

Table B.6: Steady-state data for SBR 3 (SF=0.5 with sonicated biomass exchange)

Parameter	→	**pH[1]**		**tCOD[2]**		**TSS[3]**		**TDS[4]**		**Ammonia[5]**		**SVI[6]**		**SOUR[7]**	
Units	→	**-**		**mg/L**		**mg/L**		**mg/L**		**mg/L**		**mL/g**		**mgO_2/mgh**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Jan-23	7	0.1	27.6	1.4	7.9	0.8	696	9	2.7	0.7	79	7		
2	Jan-24	7	0.1	24.2	1.4	8.6	1.2					80	13		
3	Jan-25	6.9		24.9	0.4	8.3	1.4					84	3	20	1.4
4	Jan-26	7		26.3	1.6	7.6	0.4					80	1.4		
5	Jan-27	7.2	0.2	28.8	2	8.1	0.8	724	17			98.5	9.2		
6	Jan-28	7	0.1	25.8	1	8.2	1.9			3.7	0.3	71	16		
7	Jan-29	7.2		26.9	2.3	8.6	1.2					91.5	13		
8	Jan-30	7		24.3	2.5	7	0.8	752	42			98	16		
9	Jan-31	7.1	0.3	27.8	2.5	8.5	1.3					71.5	9	17.5	0.7
10	Feb-1	7		27.2	1.5	8	1					90	1.4		
11	Feb-2	7	0.1	26.3	1.9	7.6	0.5			4.7	0.8	80	2.8		
12	Feb-3	7	0.2	28	1.7	7.6	0.3	731	21			90.5	3.5		
13	Feb-4	7.1		27.3	2.3	7.1	0.8					84.5	8		
14	Feb-5	6.8		28.8	1.3	7.3	1.2					83.5	15		
15	Feb-6	7.2	0.1	26.7	1.9	8.1	0.6	746	40.3			87	3	20	1.3
16	Feb-7	7.1		28.1	0.8	8.4	0.5			4.4	1.2	89.5	5		
17	Feb-8	7.2		29.8	1.6	8.3	1.2					93	4.1		

1 average of the day (4 measurements)
2 average of the day (6 measurements)
3 average of the day (6 measurements)
4 average of the day (4 measurements)
5 average of the day (4 measurements)
6 average of the day (3 measurements)
7 average of the day (3 measurements)

Table B.7: Continuation from Table B.6

Parameter →		TDHA[8]		ETDHA[9]		Alkalinity[10]		TP[11]	
Units →		Δabs_{470}/VS		Δabs_{470}/VS		mgCaCO3/mL		mg/L	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Jan-23					52	2.3		
2	Jan-24								
3	Jan-25	1.32	0.07	0.098	0.007				
4	Jan-26							1.83	0.19
5	Jan-27								
6	Jan-28					54.5	8		
7	Jan-29								
8	Jan-30								
9	Jan-31	1.30	0.13	0.09	0.008				
10	Feb-1							1.81	0.2
11	Feb-2					60	6.4		
12	Feb-3								
13	Feb-4								
14	Feb-5								
15	Feb-6	1.34	0.04	0.1	0.01				
16	Feb-7					58	3		
17	Feb-8							1.7	0.2

8 average of the day (6 measurements)
9 average of the day (6 measurements)
10 average of the day (6 measurements)
11 average of the day (6 measurements)

Table B.8: Steady-state data for SBR 4 (SF=1, sonicated biomass exchange)

Parameter	→	**pH**[1]		**tCOD**[2]		**TSS**[3]		**TDS**[4]		**Ammonia**[5]		**SVI**[6]		**SOUR**[7]	
Units	→	**-**		**mg/L**		**mg/L**		**mg/L**		**mg/L**		**mL/g**		**mgO_2/mgh**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Feb-9	7.1	0.2	29.2	1.3	8.5	0.6	762	2.1	4.2	0.4	95.5	9.2		
2	Feb-10	7		30.6	1.5	8.2	0.8					89.5	3.5	14.7	0.9
3	Feb-11	7		30.7	2	9.8	0.9					96	2.8		
4	Feb-12	7		29.4	1.8	9.2	1.5	768	32			90	1.4		
5	Feb-13	7	0.1	30.1	2.2	9.5	2.4					95	10		
6	Feb-14	7	0.1	29.6	1.7	9.5	1.5			3.6	0.5	97.5	10.6		
7	Feb-15	7.1	0.2	29.5	1.6	8.9	1					80	1.4	17.5	1.1
8	Feb-16	7.2		31.1	1.4	8.5	2.1	703	18.4			95.5	9.2		
9	Feb-17	7		29	2	8	1					89.5	3.5		
10	Feb-18	7		31.3	2.1	9	2					93	3		
11	Feb-19	7		30.6	1.1	7.8	0.8	763	25	2	0.3	82	4.4		
12	Feb-20	6.9	0.2	30	0.8	9.8	1.6					74	1		
13	Feb-21	6.9	0.1	30.6	1.5	9.5	1					70	4	18.4	0.8
14	Feb-22	7.1		28.9	1.9	9.4	0.5					84.5	8		
15	Feb-23	7.1	0.1	30	1	10	1.8	725	15	2.6	0.5	85	13		
16	Feb-24	7	0.2	31	2.5	10.3	1.5					87	2		
17	Feb-25	7	0.1	30.2	1.5	9.7	1.2					91.4	7		

1 average of the day (4 measurements)
2 average of the day (6 measurements)
3 average of the day (6 measurements)
4 average of the day (4 measurements)
5 average of the day (4 measurements)
6 average of the day (3 measurements)
7 average of the day (3 measurements)

Table B.9: Continuation from Table B.8

Parameter →		TDHA[8]		ETDHA[9]		Alkalinity[10]		TP[11]	
Units →		**Δabs_{470}/VS**		**Δabs_{470}/VS**		**mgCaCO3/mL**		**mg/L**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Feb-9					73.4	5		
2	Feb-10	1.28	0.12	0.94	0.01				
3	Feb-11								
4	Feb-12							3.54	0.36
5	Feb-13								
6	Feb-14					68.3	2.5		
7	Feb-15	1.25	0.14	0.112	0.02				
8	Feb-16								
9	Feb-17							3.6	0.32
10	Feb-18								
11	Feb-19					66	4		
12	Feb-20								
13	Feb-21	1.37	0.1	0.097	0.009				
14	Feb-22								
15	Feb-23					69.5	3.2	3.71	0.25
16	Feb-24								
17	Feb-25								

8 average of the day (6 measurements)
9 average of the day (6 measurements)
10 average of the day (6 measurements)
11 average of the day (6 measurements)

Table B.10: Steady-state data for SBR 5 (SF=1.5, sonicated biomass exchange)

Parameter	→	**pH**[1]		**tCOD**[2]		**TSS**[3]		**TDS**[4]		**Ammonia**[5]		**SVI**[6]		**SOUR**[7]	
Units	→	**-**		**mg/L**		**mg/L**		**mg/L**		**mg/L**		**mL/g**		**mgO_2/mgh**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Feb-9	7.1		38.5	2.5	16.2	1.6	843	41	1.83	0.8	96.5	2.1		
2	Feb-10	6.9	0.1	38.1	2.2	19.4	2					91.5	9.2	20	1.3
3	Feb-11	6.8		42	1	16.3	2.2					92	2.8		
4	Feb-12	6.9	0.1	48.1	2.2	18.3	2	908	3			90	17		
5	Feb-13	7		39.5	2.6	17.3	3					77.5	5		
6	Feb-14	7		47.3	1	19.5	1.3			4	1.1	86.5	7.8		
7	Feb-15	7		42	2	15.3	1.3					91.5	10.6	18.5	0.7
8	Feb-16	6.9		48.5	2.6	18.3	1	866	37			85.5	11		
9	Feb-17	7.1		50.6	1.8	15.1	2.2					98	13		
10	Feb-18	7.1		45.5	2.5	15.3	1					79.5	13.4		
11	Feb-19	7		50	1.8	16.1	1.4	902	18	5.2	0.5	91	1.4		
12	Feb-20	6.9	0.2	46.9	2.7	18	2.5					103.5	1		
13	Feb-21	7.2	0.1	48.4	2.6	16	1.7					89.5	12	18	1.1
14	Feb-22	6.9	0.2	46.2	2.8	19	1.5					79.5	11		
15	Feb-23	6.8		45.9	1.7	15	2	912	49			86	17		
16	Feb-24	7	0.1	50.3	1.3	15	3			4.6	1.3	98.3	8		
17	Feb-25	7.1	0.1	49	3	18.6	3.4					96.4	12		

1 average of the day (4 measurements)
2 average of the day (6 measurements)
3 average of the day (6 measurements)
4 average of the day (4 measurements)
5 average of the day (4 measurements)
6 average of the day (3 measurements)
7 average of the day (3 measurements)

Table B.11: Continuation from Table B.10

Parameter →		**TDHA**[8]		**ETDHA**[9]		**Alkalinity**[10]		**TP**[11]	
Units →		**Δabs_{470}/VS**		**Δabs_{470}/VS**		**mgCaCO3/mL**		**mg/L**	
Run day	Date	avg	stdv	avg	stdv	avg	stdv	avg	stdv
1	Feb-9					57.1	4		
2	Feb-10	1.33	0.12	0.13	0.05				
3	Feb-11								
4	Feb-12							3.8	0.4
5	Feb-13								
6	Feb-14					64.7	6.1		
7	Feb-15	1.4	0.1	0.11	0.02				
8	Feb-16								
9	Feb-17							4	0.2
10	Feb-18								
11	Feb-19					78.5	2		
12	Feb-20								
13	Feb-21	1.37	0.06	0.11	0.3				
14	Feb-22								
15	Feb-23					67	9	3.93	0.7
16	Feb-24								
17	Feb-25								

8 average of the day (6 measurements)
9 average of the day (6 measurements)
10 average of the day (6 measurements)
11 average of the day (6 measurements)