APPLICATION OF MICROWAVES AND THERMOPHILIC ANAEROBIC DIGESTION TO WASTEWATER SLUDGE TREATMENT

by

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Ph.D. Thesis

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Abstract

Anaerobic digestion of waste activated sludge can be improved if hydrolysis of particulate substrates is enhanced and available substrate is made more accessible by both breakup of the sludge matrix floc and rupture of the cell wall. Microwave (MW) pretreatment was suggested and studied as a way to improve digestion efficiency. The work done focuses on the effects of MW pretreatment on the characteristics of the sludge, due to thermal and athermal effects. It also evaluates the effects some process variables in the activated sludge process have on the pretreatment efficiency as well as the effect operating conditions in the downstream anaerobic digestion process have on the biodegradability efficiency of those sludges.

Effects of athermal and thermal MW radiation were measured by use of a customized MW oven capable of providing MW radiation with uncoupled thermal and athermal effects. Athermal radiation was capable of increasing substrate present in the soluble phase of sludge, and had a positive effect in the digestion of athermal samples. The increases in biogas production and substrate solubilisation were smaller in magnitude than the increases measured for MW thermal tests. Further refining of the tests with athermal and thermal sludge, involved separation by size class of the solubilized substrate by means of ultrafiltration (UF), and revealed that changes in particle size distribution were significant not only for MW thermal tests, but also for athermal tests, with a particular emphasis in proteins in athermal tests. These changes had an effect on the biodegradability of the sludges by class size, with thermally pretreated sludge producing more biogas for smaller particles size classes but also exhibiting more inhibition.

Tests were made with several combinations of sludge with different ages and subject to different MW pretreatment temperatures. The work showed that sludge age or solids retention time (SRT) has a significant effect on the pretreatment efficiency with maximum biogas improvements

measured at different MW pretreatment temperatures depending on the SRT of the sludge tested, and with different behaviour for mesophilic and thermophilic digestion. Mesophilic tests showed greater improvements in terms of digestion effiency on average, but thermophilic tests showed more uniform performance, with a higher baseline efficiency. The presence of an optimum of MW pretreatment temperature and sludge SRT for maximal biogas production is more defined for mesophilic conditions than for thermophilic conditions.

Semi-continuous studies were conducted with several combinations of single and two stage mesophilic and thermophilic digestors treating MW pretreated sludge and non-pretreated sludge. Staging and thermophilic digestion allowed the maintenance of a stable digestion process with high biogas productions and high solids removal efficiencies with production of sludge with good bacteriological characteristics for an very low residence time (5 d).

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Finally, I can never fully thank everything my family, specially my mother and father, have made for me. All the love, sacrifices, support and kind gestures and words, are an infinite source of encouragement for all I do in life. I dedicate this thesis to them.

“There are two types of Ph.D.thesis: perfect and submitted”

-The unknown student-

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Glossary of Terms

SRT	Solids Retention Time
WAS	Waste Activated Sludge
COD	Chemical Oxygen Demand
sCOD	Soluble Chemical Oxygen Demand
tCOD	Total Chemical Oxygen Demand
AS	Activated Sludge
BMP	Biochemical Methane Potential Assay
HRT	Hydraulic Retention Time
SBR	Sequencing Batch Reactor
SRT	Solids Retention Time
TSS	Total Suspended Solid
TWAS	Thickened Waste Activated Sludge
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compound
VSS	Volatile Suspended Solid
WAS	Waste Activated Sludge
PFRP	Process to Further Reduce Pathogens
TPAD	Temperature Phased Anaerobic Digestion
VS	Volatile Solids
TDS	Total Dry Solids
EPS	Extracellular Polymeric Substances
WWTP	Waste Water Treatment Plant
TS	Total Solids
MwD	Molecular Weight Distribution
AMwD	Apparent Molecular Weight Distribution

Chapter 1

Introduction

Wastewater treatment is an imperative in human ecosystems that intend to maintain a satisfactory balance between resource consumption and resource renewal. Growing pressure due to growing population means that more wastewater treatment plants are to be expected and the ones that already exist should experience an increase in wastewater volume to be treated. The treatment of that wastewater generates large amounts of sludge (biosolids), that need to be disposed and constitute a large portion of treatment plant operational costs, often as much as 50-60% of the total operational costs (Barret, 1996; Weemaes and Verstraete, 1998). Hence, wastewater treatment may convert a water pollution problem into a solid waste disposal problem. With the quantity of sludge to dispose of increasing, and the options to dispose decreasing (with bans on ocean and landfill disposal as examples), management of this residue is very important.

Due to their high nutrient content in an organic matrix, land application has been one of the options to dispose of these residues. However, the nature of the sludge residues, with high organic and inorganic fractions, but also hazardous contaminants such as bacteria, viruses, heavy metals and synthetic organic compounds, has forced authorities to strictly regulate this practice.

In the US, the sludge produced after treatment is classified as Class A or B depending on the pathogenic microorganism content of the sludge and its degree of stabilization. Class A is the more demanding class, and consequently, has less severe restrictions of application to land (EPA 40 CFR part 503) becoming an economical and environmental friendly way to dispose of biosolids.

Anaerobic digestion is a technology commonly used to treat wastewater sludges because it reduces the pathogen content, stabilizes it and reduces the volume of sludge to be disposed. Besides that, no oxygen is required and methane is generated, such that in certain cases, an energy surplus can be obtained.

However, some aspects of this technology are subject to improvement. Wastewater sludges, especially secondary waste activated sludge (WAS), are not very biodegradable, since a portion of them is comprised of microbial cells. These cells are resistant to biodegradation because they have walls that act as a physical and chemical barrier, preventing the action of exoenzymes and hydrolysis (Park *et al*., 2004). Usually, biodegradation of these sludges is limited to 35-45% reduction in volatile solids (VS) (Gosset and Belser, 1982).

The increase in biodegradability would be a very important improvement in the sludge treatment, because it would result in more methane produced per mass of sludge sent to the digester and less solids to ultimately be disposed. The benefits can be even more positive if the solids produced are easier to dewater and with a pathogen free quality that they can be classified as Class A.

The use of thermophilic anaerobic digestion was suggested by some researchers as a way to improve the process, since it should have higher degradation rates and greater solids reductions. It also removes more pathogens than the conventional mesophilic anaerobic process, and potentially produces a more dewaterable sludge. However, the added input of energy required for thermophilic treatment may not be compensated by the increased rates of methane production and solids reduction.

Some pretreatment techniques have been tested to improve the biodegradability of the sludges, mainly by disintegrating or solubilising cell walls prior to digestion, such as mechanical disintegration by various means (ball milling, special thickening, high pressure homogenizer), thermal disintegration (heating or freezing and thawing of biomass) or chemical disintegration

(acids, bases, oxidants) (Andreottola and Foladori, 2006). In some cases, a combination of more than one of the techniques was tested. These studies revealed that the breakup of cell walls does increase the biodegradability of the substrate and causes an increase in the rate of biodegradation.

The application of microwaves (MW) is a relatively recent sludge pretreatment technique. It was used by Hong (2004) to produce biosolids with low pathogen content with good results. Additionally, it was reported that biogas production increased with the application of microwaves and that it was higher than the gas production obtained in tests subjected to the same temperature but with conventional heating, suggesting that other effects besides the thermal effect would occur when using this technique. This effect is usually called the athermal effect; however, it is not clear if this effect really exists. So, this phenomena should be defined, along with its potential influence on WAS digestion performance.

The WAS sludge type also has an effect on sludge treatment performance. Some operational parameters in wastewater treatment affect the composition of the WAS generated and thus affect subsequent sludge treatment. An increase in activated sludge age or solid retention time (SRT) decreases its biodegradability in the anaerobic process, making this parameter important when considering the anaerobic process (Bolzonella *et al.*, 2005). The sludge age also affects the amount and composition of the exocellular polymeric substances produced by the bacteria, which can change the cell-floc matrix. These polymers may comprise up to 90% of the sludge organic mass (Bo Frølund *et al.* 1996, Neyens *et al.*, 2004; Per Halkjær Nielsen, *et al.*, 1997), and are potentially biodegradable, although some researchers consider that these polymers add considerable resistance to biodegradation of sludges (Zhang and Bishop, 2003; Boyd and Chakrabarty, 1994).

Since many wastewater treatment plants are designed and operated not only for carbon removal but also for nitrification, high sludge ages are used, producing a sludge that eventually will not

generate as much biogas and solids reduction in the later anaerobic digestion step. For this reason it is important to investigate the effect of MW pretreatment on WASs with high SRT.

1.1 Hypothesis

The purpose of this thesis is to explore some aspects regarding the application of MW technology as a process to further increase the performance of wastewater sludge anaerobic digestion. It is hypothesized that microwave radiation might have an effect not linked to temperature increase which is often called the athermal effect. The existence of this effect is disputed, and part of this uncertainty is likely to be connected with the difficulty to uncouple thermal and athermnal effects. Secondly, it was hypothesised that the effect of MW pretreatment in the improvement of activated sludge anaerobic digestion might be affected by several options regarding wastewater treatment process options upstream of the discard point (as is the SRT used in the aeration basin in activated sludge plants), and anaerobic digestion process options downstream of the waste activated sludge discharge point (as is the digestion temperature and reactor configuration). These options might then be manipulated or controlled to allow maximum improvement in digestion after MW pretreatment.

1.2 Research objectives

According to the premises above, and considering the previous work done in MW pretreatment technology in this same lab, and also considering the conditions available and the time frame available for the research, the objectives of this research were then to:

- Verify the influence in the biodegradability and activated sludge characteristics of exposure to the microwave electric field, not linked to heating effects (*athermal effect*).
- Investigate the thermophilic anaerobic digestion of sludge pretreated with MWs, in terms of biogas generation, solids reduction, and effluent quality using batch tests.

- Study the influence of low and high SRT pretreated sludges on the behavior and performance of the subsequent anaerobic digestion of pretreated sludge.
- Assess the applicability and performance of a continuous thermophilic process (as a single step, or as a separate preliminary step to mesophilic digestion, as Temperature Phased Anaerobic Digestion) in the anaerobic digestion of pretreated sludge.

1.3 Thesis organization

This thesis is arranged in a paper format thesis. Chapter 2 provides a brief introduction and literature review of fundamentals of anaerobic digestion, MW pretreatment technology, pretreatment mehods and changes in waste activated sludge characteristics due to process options in aerobic treatment in wastewater treatment plants, namely SRT. Results from the research are presented in Chapters 3-6 in a journal manuscript format. Overal conclusions and recommendations are given in Chapter 7.

Chapter 3 provides an analysis of the effects and changes measured in sludge that was subject to two different types of MW radiation, conventional MW radiation with consequent temperature increase in the medium subject to radiation, and MW radiation where the temperature increase was limited by a device built during this research work. It was verified that changes do exist in sludges subject to athermal radiation and control sludges, and that the magnitude of the changes due to this effect are significantly lower than changes induced by conventional MW radiation. The results of this work were published in the *Journal of Environmental Science and Engineering*.

Chapter 4 analyses the changes caused by both types of MW radiation in more detail, with the separation of solubilised substrate in several size classes through ultrafiltration and each analysed in terms of protein, sugar and chemical oxygen demand. Each fraction was also analysed in terms

of biodegradability and mathematical analyses were made as to measure activities, inhibition patterns and maximum biogas productions. This manuscript was submitted to *Water Research* and is awaiting peer review.

Chapter 5 studies combinations of different MW pretreatment temperatures, applied on sludges with different SRT and two different digestion temperatures. All these conditions are combined and analysed in terms of digestion improvement in terms of biogas production improvement. Several empirical models were developed and adjusted to adequaltely translate the influence of both SRT and MW pretreatment temperature on the digestion efficiency. The influence of digestion temperature is also analyzed. An analysis is performed to the biogas production curves and conclusions were drawn in terms of inhibition and maximum activities. This manuscript was also submitted to *Water Research* and is awaiting peer review.

Chapter 6 studies several combinations of digester configurations both mesophilic and thermophilic, treating pretreated and non pretreated sludge. Configuration options also include single-stage and two-stage, and effects of all these options were measured in the anaerobic digestion efficiency, as measured in biogas production and solids removal. The effect of these configurations on some important characteristics of final sludge were also measured such as pathogenic indicator microorganisms and dewaterability. The manuscript corresponding to this work was published in *Water Research*.

Chapter 2

Literature Review

2.1 Wastewater treatment and biosolids

All communities and industries produce liquid, solid and/or gaseous residues. The liquid portion – wastewater - if discharged in the environment without any type of treatment may cause exhaustion of the receiving environment natural regenerating capacity, with consequent detrimental effects such as the depletion of oxygen in receiving waters and formation of bad odours and death of aquatic life. Besides, wastewater frequently contains pathogenic bacteria, nutrients (that can cause eutrophization) and toxic compounds such as heavy metals. These reasons make wastewater treatment a necessity. A very important portion of this treatment is the removal of organic matter present in soluble and particulate form in wastewater. This removal is performed by biological processes that use bacteria to degrade and remove organic compounds from the water phase. These bacterial processes are normally included in a broader treatment process designed not only to remove organic matter, but also provide nutrient removal (in some cases), solids removal, disinfection, etc. All these processes are then assembled together in what are the present day wastewater treatment plants. The biological process, by using bacteria to consume organic matter present in the untreated effluent causes the generation of more bacteria, so a mass of excess bacteria is normally produced when operating these plants, and a line dedicated to process and dispose of this discarded sludge (called biosolids after being processed) is also part of a conventional treatment plant (Figure 1).

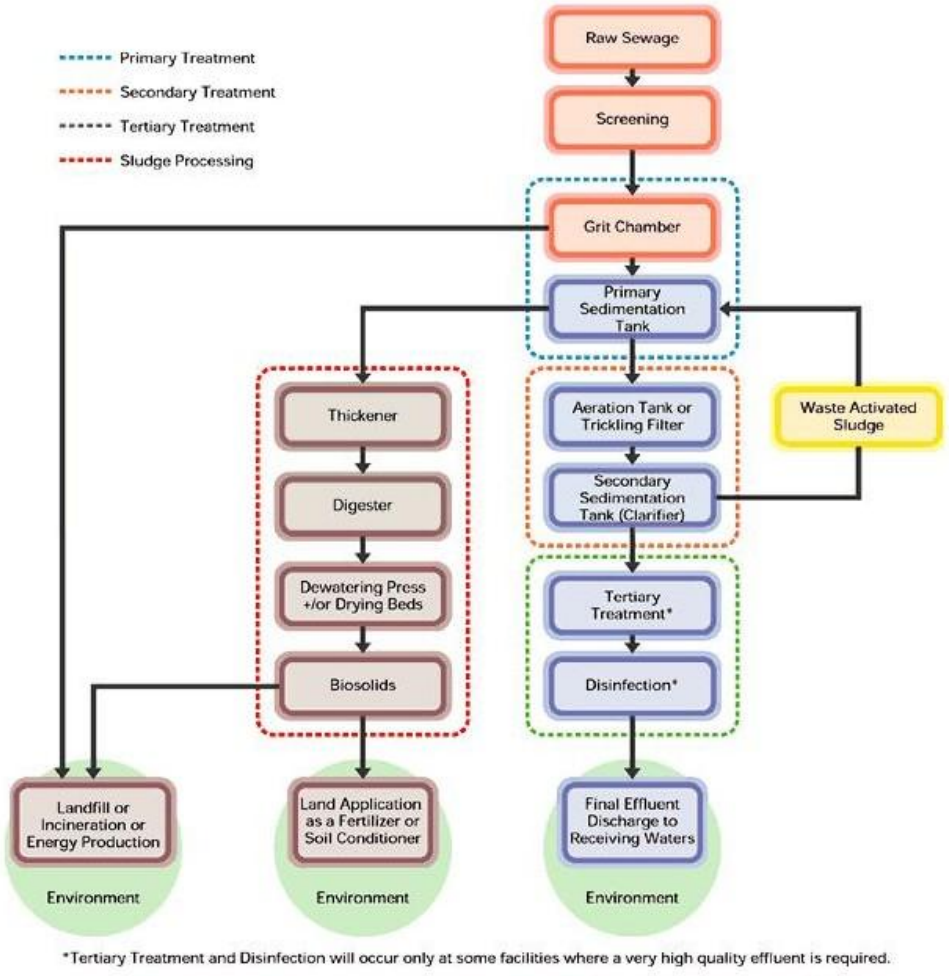


Figure 2.1 - Conventional wastewater treatment plant flow process (source: UNEP 2002)

The amount of sludge produced in a wastewater treatment plant is dependent on its operational parameters. Some of the characteristics of the sludge are also dependent on not only the type of wastewater being treated but also on the operational parameters applied in the process. In any case, sludge production is almost always significant, and a substantial portion of operating costs goes to process and dispose excess sludge, often as much as 50-60% of the total operational costs (Barret, 1996; Weemaes and Verstraete, 1998). Table 1 shows data for sludge production in several states in Europe and the US.

Table 2.1 - Sludge production and disposal methods in Europe and North America (source: UN 2002)

	Annual Production	**Disposal method (percentage of total)**			
	(10^3 dry tons)	Agriculture	Landfill	Incineration	Other
Austria	320	13	56	31	0
Belgium	75	31	56	9	4
Denmark	130	37	33	28	2
France	700	50	50	0	0
Germany	2500	25	63	12	0
Greece	15	3	97	0	0
Ireland	24	28	18	0	54
Italy	800	34	55	11	0
Luxembourg	15	81	18	0	1
Holland	282	44	53	3	0
Portugal	200	80	13	0	7
Spain	280	10	50	10	30
Switzerland	50	30	20	0	50
UK	1075	51	16	5	28
US	5357	36	38	16	10
Ontario	150	-	-	-	-

The volume of wastewater subjected to treatment has been increasing steadily in the last decade, due to both the growing population, and the increasing coverage of urban and rural areas with sewer drainage and treatment systems. In cities that show noticeable industrial growth, further increases of the volume of wastewater to be treated are measured. Hence, wastewater treatment may convert a water pollution problem into a solid waste disposal problem. With the quantity of sludge to dispose increasing, and the options to dispose of it decreasing (with bans on ocean and landfill disposal as examples), management of this residue is a very important concern.

Due to their high nutrient content in an organic matrix, land application has been one of the options of biosolids disposal. However, the nature of biosolids, with high organic and inorganic fractions, but also hazardous contaminants such as bacteria, viruses, heavy metals and synthetic organic compounds has forced authorities to strictly regulate this practice.

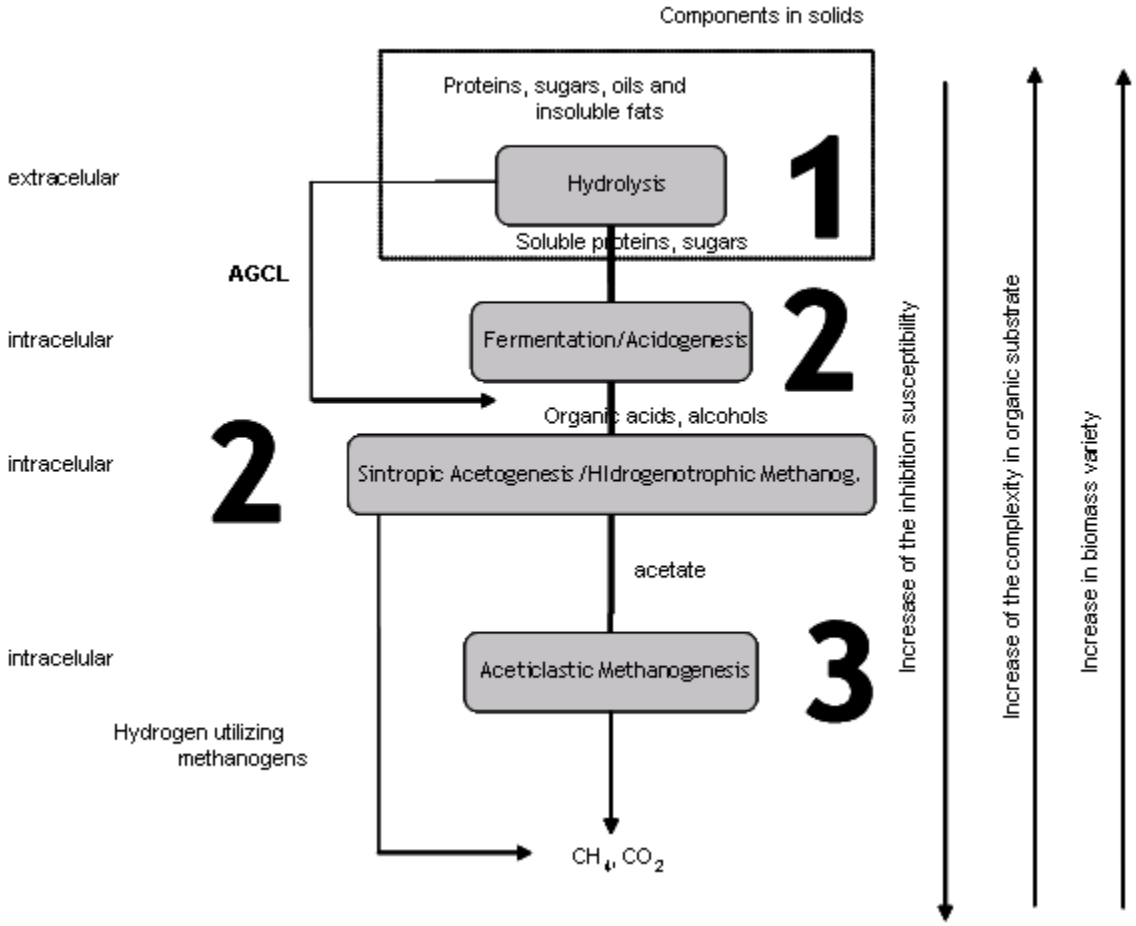
In the US, the biosolids produced after treatment is classified as Class A or B depending on the pathogenic microorganism content and its degree of stabilization. Class A is the more demanding class, and consequently, has less severe restrictions for application to land (EPA 40 CFR part 503) becoming an economical and environmental friendly way to dispose of biosolids. In Canada, regulations for disposal of biosolids are defined at the provincial rather than at the federal level, and some refer to part 503 of EPA for guidance. In Ontario, biosolids are subject to regulation 347 of the Environmental Protection Act that regulates disposal of biosolids and follows a similar characterization as the USA Class A and B designations.

2.2 Anaerobic digestion

One of the processes that is used to process sludge before disposal is anaerobic digestion. The process manages to stabilize sludge, decrease the solids content and, in certain cases, decrease or even eliminate pathogenic microorganisms, without any addition of chemicals, with only the action of other type of bacteria. Anaerobic digestion is the biological degradation, by a complex microbial consortium, of organic substrates in the absence of oxygen. During the process, organic matter is converted, mainly, to methane, carbon dioxide and more biomass. The nitrogen that is not used for growth is, usually, released as or reduced to, ammonia. This process is very attractive because besides not needing any added chemical reagents, it can produce a usable form of energy, as methane gas, and so reduce or eliminate (in optimal conditions) the need to supply energy to a wastewater treatment plant. Anaerobic digestion occurs by means of a series of parallel and sequential methabolic processes, performed by a variety of microbiological consortia. The compounds involved in anaerobic digestion can be grouped as primary substrates,

which are present in the effluent or residues to be treated, in intermediate substrates and in final products. The anaerobic process consists of four main steps (Batstone, 1999):

- Hydrolysis – it is a step mediated by extracellular enzymes, in which substrates and particles that can not be used directly by the microorganisms are solubilized (Fig 2.2, step 1);
- Acidogenesis or fermentation – is the degradation of soluble substrates, such as amino acids and sugars that can be degraded without an external electron acceptor. The products are organic acids and alcohols (Fig 2.2, step 2);
- Syntrophic acetogenesis and hydrogenophilic methanogenesis –acetogenesis is the degradation of the fermentation products to acetate, using hydrogen ions or bicarbonate as external electron acceptors. This process is coupled with the methanogenesis from hydrogen, which maintains a low concentration of hydrogen (necessary to keep the reaction thermodynamically favourable) (Fig 2.2, step 3);
- Aceticlastic methanogenesis – the degradation of acetate to carbon dioxide and methane, by highly specialized microorganisms (Fig 2.2, step 3).



1 Clostridium, Proteus vulgaris, Proteococcus, Bacteroides, Bacillus, Vibrio, Acetovibrio celluliticus, Staphylococcus, Bacteroides

2 Lactobacillus, Escherichia, Staphylococcus, MIcrococcus, Bacillus, Pseudomonas, Desulfovibrio, Selenomonas, Veillonella, Sarcina, Streptococcus, Desulfobacter, Desulfuromonas, Clostridium, Eubacterium llimosun, Syntrophomonas wolfeii, Syntrophobacter wolinii

3 Methanosaeta, Methanosarcina, Methanospirillum, Methanobacterium, Methanobrevibacterium, Methanoplanus

Figure 2.2 - Basic steps in anaerobic digestion showing the main substrates and the bacterial genera involved (Stronach et al., 1986; Van Haandel and Lettinga, 1994)

In the hydrolysis step, larger particles and or insoluble particles are degraded into smaller and more degradable forms. Microorganisms cannot use particulate substrates, or non-soluble ones that are too large to cross the cellular membrane; consequently extracellular enzymes are released outside of the cells so the polymers are transformed into smaller molecules. Hydrolysis is considered as the limiting step in the anaerobic degradation of particulate substrates, as is the case of the sludges (Pavlosthathis and Gomez, 1991).

The acidogenic bacteria comprise approximately 90% of the total bacterial population in an anaerobic digester (Zeikus, 1980). The acidogenic bacteria have short duplication times (Mosey, 1983), and it was verified that acidification is rarely limiting in the global anaerobic digestion process (Gujer and Zehnder, 1983).

Methanogenesis, is the final step of the process, and in some cases is the limiting step. The bacteria that degrade acetate to methane are the weakest link of the chain of reactions of the anaerobic process, when it comes to resistance to adverse conditions, such as organic and hydraulic load shocks and presence of toxic and inhibitory substances (Alves, 1998).

Although the majority of methane is formed from acetate, these bacteria can use other types of substrates to produce methane:

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$

$$CH_3COO^- + H^+ \longrightarrow CH_4 + CO_2$$

$$4HCOO^- + 4H^+ \longrightarrow CH_4 + 3CO_2 + 2H_2O$$

$$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$$

Approximately 70% of the methane formed in anaerobic digesters is formed from acetate (Jeris and McCarty, 1965), making this reaction the most important in anaerobic degradation of sludge.

In order for anaerobic digestion to occur stably and at the best possible rates, it is necessary to supply certain nutrients and conditions, such as micro- and macro-nutrients, pH and alkalinity and a controlled temperature.

The micro- and macro-nutrients are comprised of the elements that are found in the elemental composition of bacteria. Usually, these elements are found in sufficient quantities in the sludge.

Table 2.2 - Average elemental composition of methanogenic bacteria (Scherer et al., 1983; Takashima and Speece, 1990)

Element	µg/g (dry weight)
C	370000-440000
H	55000-65000
N	95000-128000
P	5000-28000
S	5600-12000
Na	3000-40000
K	1300-50000
Ca	1000-4500
Mg	900-530
Fe	700-2800
Ni	65-180
Co	10-120
Zn	50-630
Mo	10-70
Cu	<10-160
Mn	5-25

Alkalinity and pH are two factors that have a significant impact on the activity of the bacteria that operate in the anaerobic digestion process. The pH affects the metabolism of these mircroorganisms that have a range of pH values for optimum activity.

Values between 6.8 and 7.4 generally are the best conditions for the methanogenic bacteria, the most sensitive anaerobic bacteria to variations in pH, while values between 6.4 and 7.8 are considered adequate for the whole process of anaerobic digestion (Grady et al., 1999).

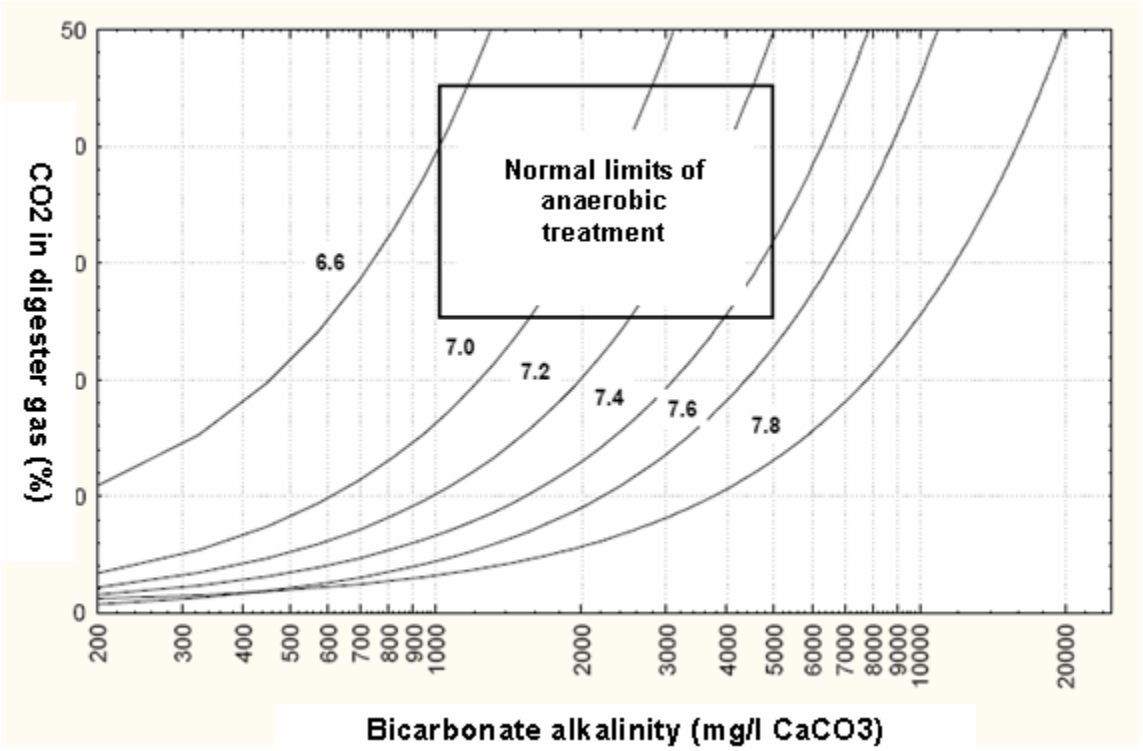


Figure 2.3 - Relationship between alkalinity, pH and CO2 composition in the gas phase in the anaerobic process (Parkin and Owen, 1986)

Also, alkalinity is important because it allows the maintenance of a stable pH, even with the high productions of volatile acids by the acetogenic bacteria. Values usually found for anaerobic reactors operating with sludges are 1000 – 5000 mg/L as $CaCO_3$. For solid waste reactors, these values can be higher.

The temperature, similar to the pH, is also one of the factors that most influence the process of anaerobic digestion. The rate of biochemical reactions is dependent on temperature, with the rates doubling at approximately every 10ºC increase in temperature, until a limiting temperature is reached. Usually, there are three main ranges of temperature used in anaerobic digestion, as seen in Table 2.3.

Table 2.3 - Temperature ranges for anaerobic digestion

Range	Temperature	Optimum
Psychrocrophilic	<20ºC	n.d.
Mesophilic	20 – 45ºC	30 – 40ºC
Thermophilic	>45ºC	50 – 60ºC

Thermophilic digestion occurs at a rate faster than mesophilic digestion, but, in most cases, the mesophilic temperature range is chosen for operation of anaerobic digesters. This happens because it is thought that thermophilic anaerobic digestion is more prone to phenomena such as increase in toxicity effects (Hwu, 1997), greater instability of the systems, greater problems with foaming and odours (Grady et al., 1999), lower quality of the effluent, mainly due to high volatile fatty acids (VFA) concentration (Kugelman and Guida, 1989), and greater energy costs (Bhur and Andrews, 1977).

However, other researchers applied this temperature range with positive results, obtaining significantly higher reductions in VS, and higher specific methane productions (Ghosh *et al.*, 1995, Ros and Zupancic, 2003). Zabranska et al. (2000) even reported that in a full-scale test

lasting one year in a large wastewater treatment plant, the energy balance for thermophilic digestion was positive and more favourable than at mesophilic conditions.

Although thermophilic digestion is not considered by EPA as a process to further reduce pathogens (PFRP), the reduction in pathogen content in thermophilic treatment is substantial, and it has been reported that the sludge produced consistently meets the requirements necessary for classification as a Class A biosolids (Witzgall et al., 2004, Iranpour et al., 2002, Han et al., 1997). The sludge produced in thermophilic anaerobic reactors was reported by some authors as easier to dewater (Bhur and Andrews, 1977, Cheunbarn and Pagilla, 2000).

In recent years, some researchers have been working on techniques to combine some of the advantages of thermophilic and mesophilic processes. The dual stage thermophilic/mesophilic process has gained some popularity due to the fact that it tries to combine the advantages of the thermophilic systems in terms of pathogen control and VS reductions with the advantages of mesophilic digestion and still is economical to operate because the bulk of the digestion takes place in the mesophilic stage (Carrimgton et al., 1991; Ghosh et al., 1995; Pagilla et al., 1996; Zhao and Kugel, 1996; Han and Dague, 1997). The dual system is normally comprised of the following elements:

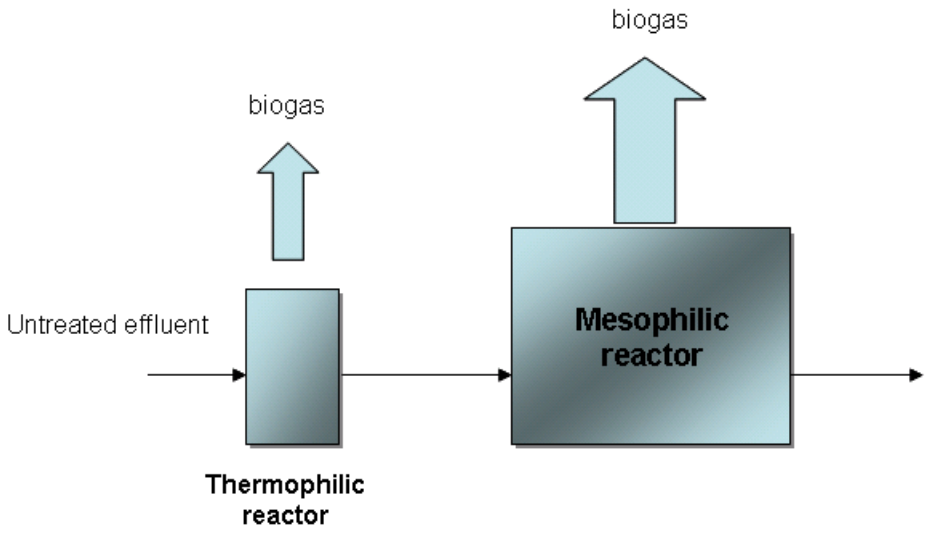


Figure 2.4 - Thermophilic/Mesophilic dual stage anaerobic treatment

The thermophilic reactor can be used as a first step in two stage anaerobic digestion, in a process called Temperature Phased Anaerobic Digestion (TPAD), as an acidifying reactor in the acid/gas phased digestion. In the mesophilic reactor, most of the VFAs that cause the odours characteristic of thermophilic digestion are degraded to methane, and a further stabilization of the effluent occurs, normally in a more easily controlled digester.

Both of these configurations have been shown to have greater efficiencies in reducing volatile suspended solids (VSS) compared to single stage mesophilic or thermophilic digestion or dual stage mesophilic digestion (Roberts et al., 1999, Azbar and Speece. 2001, Schafer and Farrel, 2000). Some of the reasons for the better performance of dual stage temperature phased digestion are:

- The dual phase configuration allows the setting of optimal conditions for two different bacterial populations. It is known that the methanogenic and hydrolytic/acidogenic microbes have different optimal pH, and the acidogenic growth rate is higher than methanogens. The setting of optimal conditions for each of these bacterial groups would maximize the performance of those two steps on the anaerobic digestion process;

- Some compounds that are inhibitory to methanogenesis are less inhibitory after being acidified, such as phenol or unsaturated fatty acids (Kobayashi et al., 1989, Komatsu et al., 1991);
- The lower pH in the first reactor may cause a different distribution of the VFA produced by the acidogenic bacteria, one that includes a smaller proportion of more difficult to degrade VFAs, such as propionate (Azbar and Speece, 2001, Breure and van Andel, 1984, Dohanyos et al., 1982).

The results obtained in these systems showed that this configuration can be more stable than the single stage system, reaching higher loadings and maintaining high removal efficiencies, with the ability to better absorb shock loadings than mesophilic or thermophilic single stage systems. VS reductions range from 50 - 60% in these systems, where in conventional ones, 40% is a satisfactory value (Schaffer and Farrell, 2000).

At this time, limited information is available for full-scale performance of dual stage acid/gas phased digestion. In 2001, only three plants were in operation in North America (Wilson and Dichtl, 2001). Table 2.4 summarizes the information and compares the efficiency in terms of VS destruction percentage.

Table 2.4 - Some recent published results regarding studies with dual-stage or thermophilic digestion.

Authors	Set-up	Temp. (ºC)	Max VS removal (%)	Retention time	Sludge type	Gas production (m3CH4/kgVSr)
Roberts et al., 1999	Thermo-Meso	55-35	45	4h +12d	Primary +WAS (1:1)	0.59
Han et al., 1997	Thermo-meso	55-35	50	8+20d	Primary +WAS (1:1)	
Han et al., 1997	Thermo-meso	55-35	54%	2+10d	Primary +WAS (1:1)	
Bhattacharya et al., 1996	Meso-meso	35-35	43%	2+10d	WAS	
Cheunbarn and Pagilla, 2000a	Thermo-meso	62-37	61%	1+14d	Primary +WAS (WAS)	0.48
Rubia et al., 2005	Thermo single stage	55	53%	27d	Primary+WAS (non specified ratio)	0.32
Song et al., 2004	Thermo single stage	55	47%	10d	Primary+WAS (non specified ratio)	0.416
Bousková et al., 2005	Thermo single stage	55	44%	20d	Primary+WAS (40:60)	0.51

2.3 Sludge pretreatments

WAS produced in wastewater treatment is mainly comprised of microbial cells and extracellular polymeric substances (EPS) produced by the cells as part of their metabolic activity. The organic content of WAS is approximately 59-88% (w/v) and the average general composition is shown in Table 2.5.

Table 2.5 - Average general composition of wastewater sludge (Weemaes et al., 1998)

Item	Primary sludge	Activated sludge
Total dry solids (TDS)%	2.0 - 8.0	0.83 – 1.16
Volatile solids (% of TDS)	60 - 80	59 – 88
Grease and fats(% of TDS)	13 – 65	5 – 12
Protein (% of TDS)	20 – 30	32 – 41
Nitrogen (N, of TDS)	1.5 – 4	2.4 – 5.0
Phosphorous (P,% of TDS)	0.17 – 0.6	0.6 – 2.3
Potash (K,% of TDS)	0 – 0.41	0.2 – 0.29
Cellulose (% of TDS)	8.0 – 15.0	-
pH	5 – 8	6.5 – 8.0
Alkalinity (mg/L $CaCO_3$)	500 – 1500	580 – 1100
Organic acids (mg/L as acetate)	200 – 2000	1100 – 1700
Energy content (MJ/kg)	23.2 - 29	18.6 – 23.2

The microbial cells and EPS form a matrix that is the substrate for the anaerobic digestion of WAS. Biological digestion techniques have traditionally been employed to reduce the volume and weight of sludge. Recent studies suggest that cations (ratio of divalent to monovalent cations) are central in the binding of biopolymers to microbial flocs. Most of the EPS (proteins and carbohydrates) is negatively charged, and the binding of EPS with positively charged cations increases the strength of the WAS floc structure. The type of cations (mono-, di- or trivalent) determine the biodegradability of the WAS and the best option (anaerobic versus aerobic) to digest the WAS (Novak et al., 2003). With respect to their physical state, microbial cells represent a relatively unfavourable substrate for subsequent microbial degradation. A large part of the organic matter in WAS is compartmentalized within the microbial cell membranes. The cell envelope of microorganisms is a semi-rigid structure which provides sufficient intrinsic strength to protect the cell from osmotic lysis. This microbial cell wall contains glycan strands cross-linked by peptide chains that give the walls resistance to biodegradation. Because of this,

conventional biological digestion techniques require long hydraulic retention times (HRT) on the order of 20 – 30 days to achieve acceptable WAS biodegradation rates. To improve digestion efficiency, the most logical approach is to disrupt the microbial cells in the sludge, to make the organic material inside the cell walls available (Pavlostathis and Giraldo-Gomez, 1991). The pretreatment also has the goal of decreasing the particle size, allowing a greater surface area per unit volume available for degradation (Muller et al., 2004).

Sludge disintegration was therefore introduced to solubilise and convert slowly degradable, particulate organic materials in the sludge, like the bacterial cells, and the high molecular weight biopolymers (protein, polysaccharide, humic and nucleic acids) of the extracellular polymeric network to low molecular weight, readily biodegradable compounds. The result of the pretreatments is an increase in the soluble chemical oxygen demand (COD) of the sludge, since most of the material released is soluble. Pretreatment also disintegrates the high molecular weight polymers that form the EPS matrix.

Disintegration may be performed by several different techniques:

- Chemical;
- Thermal;
- Mechanical;
- By a combination of the previous techniques.

The performance of these techniques is generally expressed as a solubilisation percentage, indicating the ratio of the soluble COD content over the total COD.

2.3.1 Mechanical pretreatments

Mechanical sludge disintegration methods are generally based on the disruption of microbial cell walls by shear stresses. In mechanical disintegration, the breakup of cells and floc structure occurs in minutes instead of days, and the intracellular components are released and readily available for biological degradation. Several techniques have been reported to apply mechanical shear to sludge. The disruption of cells by the colloid mill process was reported by Harrison (1991). Choi *et al*. (1997) reported a process in which a jet of WAS was aimed at a collision-plate at 30 bar pump pressure. The VS removal in the subsequent process was increased by 35- 50% and the digestion rate increased from 0.01 to 0.04 d^{-1}. Nah et al. (2000) reported that the mechanical treatment of WAS decreased the SRT in anaerobic digester from 13 to 6 days with the same efficiency and effluent quality.

Ball mill shakers were reported to efficiently disintegrate activated sludge bacteria, having a disintegration yield of 90%, but with high energy consumption (approximately 60 MJ/kg TDS) (Weemaes and Verstraete 1998).

Baier and Schmidheiny (1997) increased the VS removal by 38 – 57% and the methane production by 10% after the application of sludge disintegration by ball milling.

Another method developed was high pressure homogenization. In a high pressure homogenizer, the sludge is compressed to 60 MPa. The suspension then leaves the compressor through a valve at a high speed, smashing on an impaction ring. The cells are hereby subjected to turbulence, cavitation and shear stresses, resulting in cell disintegration. Cell disintegrations up to 85% were achieved at relatively low energy levels (30 – 50 MJ/m^3) (Harrison, 1991).

Also based on cavitation processes, ultrasound can be used to disrupt cell walls. Ultrasound uses sound waves that generates cavitation (implosion) processes in liquids giving rise to local high-temperature hotspots over 1000ºC and pressure increases up to 500 bar. Rivard and Nagle (1996)

found an enhancement in biodegradability of sewage sludge to 80 – 83% by a sonication treatment of 4 – 8 min duration at 55ºC. Wang *et al.* (1999) reported an increase of 64% in the production of methane in sludge subject to ultrasound treatment and indicated that the optimum pretreatment time was around 30 minutes. Sonication is one of the most powerful methods to disrupt cells. At high power levels, cell disintegrations of 100% can be reached with ultrasound. However, this method has a high power consumption on the order of 200 MJ/kg TDS.

Another type of mechanical pretreatment was reported by Dohanyos *et al*. (1997). This method uses a centrifuge equipped with a special impact gear, which uses the energy generated by the centrifuge to partially destroy sludge cells without any additional energy demand. The results showed an increase of 13.6% in methane yield from combined sludge (primary plus WAS) and an average 31.8% increase when digesting WAS only.

Although disintegration yields may be very satisfactory, the energy demand for these processes can be high. Farkade *et al*. (2006) reported that hydrodynamic cavitation was the most energy efficient process among different mechanical disintegration techniques. The values reported were:

Table 2.6 - Energy demand for mechanical pretreatment methods

Process	**Energy demand (MJ/m^3 sludge treated)**
Hydrodynamic cavitation	0.74[a]
High pressure homogenization	30 – 50[a] 2 – 7[b]
Sonication	1792[a] 200[b]

[a] – Farkade *et al.*(2006[b])- Dichtl *et al.* (1997)

Some authors suggest that the high energy levels required to mechanically pretreat sludges, are the main reason that the application of mechanical pretreatments is still limited.

2.3.2 Thermal pretreatments

Another technique to pretreat the sludges is the application of heat. Originally it was applied to improve the dewaterability of the sludges. Heat treatment results in the breakdown of the gel structure of the sludge and the release of intracellular bound water, additionally, cell walls are also damaged (Smith and Goransson, 1992). The release of intracellular compounds was seen as a drawback but now it is important in the application of this process to increase the degradation of sludges or to supply internal carbon sources for nutrient removal.

Thermal pretreatment usually involves heating of sludge to temperatures in the range of 150 – 200ºC. Pressures are usually in the range of 600 – 2500 kPa (Barlindhaug, and Ødegaard, 1996). The Cambi company in Norway developed a sludge treatment process that included thermal hydrolysis. It comprises heating sludge to 180ºC for 30 minutes, resulting in solubilization of approximately 30% of the sludge. An increase in biogas production of 150% was reported and a reduction of 50% in the solids volume was observed (Weemaes and Verstraete, 1998).

Dohanyos et al. (1997b) tested a treatment that consisted in heating the sludge to 100ºC for 20 minutes. The results showed an increase of 41.8% in methane production and 27.6% in VS reduction. Tanaka et al. (1997) tested several temperatures for a pretreatment time of 1 hour and noticed that VSS solubilization was around 15% for temperatures between 115 and 150ºC and then increased further above 160ºC, reaching 30% at 180ºC. The sludge used was WAS of several origins [household, residential and industrial wastewater treatment plant (WWTP)].

Li and Noike (1992) tested several pretreatment options varying either the temperature (between 65 and 175ºC) and the duration of the pretreatment (between 15 and 120 minutes), they found that the maximum improvement occurred for temperatures of 170ºC and 60 minutes duration. Longer times did not result in better results. The retention time in the digester could be reduced by 5 days and methane production was twice as high as the control.

Stuckey and McCarty (1984) tested the biodegradability of WAS in temperatures between 150-275ºC and found that the maximum was attained at a temperature of 175ºC. At temperatures over 180ºC there appears to be formation of inhibitory compounds that reduce the degree of stabilization sludge. Most of the thermal pretreatments limit the temperature to 180ºC to avoid this phenomenon.

2.3.3 Chemical pretreatments

In chemical pretreatments, an acid or basic reagent is added to the sludge to solubilize the sludge floc and microbial cells. Other chemical compounds such as powerful oxidants can also be used in chemical pretreatments, with the conversion of some organic matter to carbon dioxide along with the break-up of cell walls and sludge flocs. The addition of acid improves sludge solubilization at ambient and elevated temperatures, while for alkaline pretreatment, variable results have been found. Some researchers report very good results while others report no effect on the solubilization, and subsequent digestion of the sludges. Alkaline pretreatments have the advantage of being compatible with the subsequent biological treatment, usually not requiring neutralization prior to the anaerobic digestor. Alkaline hydrolysis has been reported to significantly increase organic yield from acidogenesis (Hashimoto et al., 1991).

Tanaka *et al*. (1997) tested the addition of NaOH to WAS, and found a solubilization percentage of VSS of 15% for an alkaline dose of approximately 0.6 g NaOH/g VSS. The methane production was 50% higher compared to the control for a dose of 1 g NaOH/gVSS.

Lin *et al*. (1997) tested the addition of two different concentrations of NaOH (20 and 40 meq/L) to sludges with two different solids concentrations (1 and 2%). The methane production was between 19 and 286% higher in the sludge pretreated compared to the control sludge. The amount of soluble COD increased from a total COD/soluble COD ratio of 2 to 38% in the test with 1% TS sludge pretreated with 40 meq/L NaOH.

Ozone is one of the most powerful oxidant agents, and has the power to break microbial cell walls and convert some of the organic matter to carbon dioxide. This ability has been used by some researchers to improve the digestion of municipal sludges. Weemaes et al. (2000) applied ozone doses of 0.05 – 0.20 g O_3/g COD on a mixture of primary and secondary sludge. A large increase in the soluble COD concentration was noted (fraction of soluble COD increased from 0.8 to 47%) while the total COD decreased, an indication that some of the organic matter was being oxidized to CO_2. The methane production for the test pretreated with 0.10 g O_3/g COD was 283% higher on a COD basis than the production for the control test.

Yeom et al. (2002) tested several ozone doses on WAS and the percentage of COD solubilized increased from 0.8% in the control to 23.9% for an ozone dose of 0.1 g O_3/g total suspended solids (TSS) and 32.7% for a dose of 0.2 g O_3/g TSS. Further increases in ozone dose did not result in an increase in the solubilization. Methane production of the sludge was increased by 100% for the test with 0.2 g O_3/g TSS when compared to the control.

2.3.4 Combined techniques

In order to increase the degree of solubilization attainable and further increase the biogas yield and solids removal, some researchers combined more than one pretreatment technique.

Tanaka *et al*. (1997) tested a thermochemical pretreatment that combined the addition of NaOH and the heating of the sludge. For a NaOH dose of 0.3 g NaOH/g VSS and 5 min heating at 130ºC, the increase in solubilization was 45% for combined sludge and 70 – 80% for WAS. The methane production increased 220% for combined primary/WAS and 30% for WAS alone.

Smith and Goransson (1992) raised the pH to 12 with NaOH and submitted the sludge to heating up to 120 – 160ºC and reported that the solubilization reached 40 – 60%.

The patented process MicroSludge uses a combination of a chemical pretreatment (alkaline pretreatment) and a mechanical pretreatment (homogenizer) to reach almost complete VS destruction. The results reported showed an increase in soluble COD removal from 5 to 96% and the VS removal was increased from 41 to 73% (Shaw *et al*. 2002; Stephenson *et al*. 2003).

2.3.5 Evaluation of the different techniques

The sludge pretreatment techniques offer an increase in biogas yield that can be attained in the anaerobic digestion of the organic matter present in the sludge. However, until now, none of the techniques has found a real breakthrough. Most of the pretreatment techniques are energy intensive (such as the thermal pretreatments) or are not economically feasible, either because of the cost associated with chemical reagents in chemical pretreatment, or the cost associated with equipment, as in mechanical pretreatment. In some cases, pretreatments result in other nuisances. The occurrence of odour problems in thermal treatments is sometimes reported, while in other cases, increase in corrosion problems is an issue.

Weemaes *et al.* (1998) estimated the costs for several sludge pretreatment techniques, expressed on a dry weight sludge basis (Table 2.7).

Table 2.7 - Estimated costs of several pretreatment techniques (Weemaes *et al*. 1998)

Method	% cell disintegration	Estimated cost (€/tonTDS)	Major advantage	Major disadvantage
Seber colloid mill	50	-	Simple	Energy dissipation – suspension heating
Ball mill shakers	90	414 – 2500	High efficency, simple	Energy intensive
High pressure homogenization	85	42 – 146	High efficiency, low energy	Complicated
Hydrodynamic cavitation	75	3	Good energy efficiency	Very little information and experience
Ultrasound	100	8330	Complete disintegration	Energy intensive
Krepro (acid plus thermal hydrolysis)	55	224	Recycling of all waste products, flexibility	Corrosion and odour problems
Cambi	30	190		Relatively low yield, dependence on sludge type
Thermochemical treatments in general	15 – 60	-	Relatively simple	Corrosion, odour, subsequent neutralization
Biological	5 - 50	-	Simple operation, low cost	Very low yields, odour problems
Vertech (wet air oxidation)	95	450	High disintegration efficiency, no need for high pressure pumps	Corrosive, leakages, blockages in the shaft
Loprox (acid thermal oxidation)	90	800	High disintegration efficiency	Low pH, corrosive, high cost

Some authors directly compared different pretreatment techniques applied to the same WAS sludge. Kim *et al.* (2003) tested thermal (121ºC for 30 min), ultrasound (42 kHz for 120 min),

chemical (NaOH addition to pH 12, 7 g/L) and thermochemical (heating to 121ºC for 30 min plus NaOH addition, 7 g/L) pretreatments to the same kind of sludge (WAS) in order to make direct comparisons.

The results (Table 2.8) showed that thermochemical pretreatment was the most effective in reducing the size of the particles, closely followed by thermal pretreatment. The efficiency in the breakdown of particles resulted in increased production of methane in the subsequent anaerobic digestion. Both thermal and thermochemical pretreatments produced more methane per volume of sludge than any other pretreatment.

Table 2.8 - Results for several pretreatments applied to the same sludge (Kim *et al*, 2003)

Pretreatment	**Particle size after pretreatment (90^{th} percentile, µm)**	**VS removal (%)**	**Methane production (L/m^3 WAS)**
Control	450	20.5	2,500
Chemical	186	29.8	1,400
Thermal	153	32.1	3,400
Thermochemical	144	46.1	3,400
Ultrasonic	240	38.9	3,050

Muller *et al*. (2004) also tested different pretreatments with the same WAS sludge. The pretreatments that were tested as well as the effects on items studied are detailed in Table 2.9.

Table 2.9 - Effects of different pretreatment methods in sludge parameters (Muller *et al*., 2004)

	Pretreatments			
Item	**Stirred ball-mill**	**Ozonation**	**Lysate centrifugation**	**Ultrasounds**
Specific energy (kWh/m^3)	21.0	49.5	11.0	28.0
Degree of disintegration (%)	23.0	35.0	5.0	17.5
Increase in degradation degree (%)	14.0	20.0	8.0	10.0
Increase in soluble COD concentration (%)	19.0	59.0	8.5	2.0
Increase in ammonia concentration (%)	11.0	16.5	11.0	5.0
Increase in polymer demand (%)	7.5	29.0	6.0	10

The results show that there is a close link between the size of the particles and the increase in biodegradability of the sludge. In general, the higher the disintegration, the higher the production of methane and reduction of VS. The same trend can be observed in the energy consumption, since for high disintegration results, it is necessary to apply a high amount of energy. In some cases, the material resulting from the disintegration is not as biodegradable, so the type of substrate produced by the pretreatments is also an important factor. Along with these considerations, the influence of the pretreatments in dewatering and the amount of polymer necessary for dewatering is an important factor. High degree disintegration usually mean more mass of coagulant needed in the dewatering processes downstream.

The potential benefits obtained from increased production of methane and higher removal of solids might be offset by the increased needs of chemicals downstream, and the increased loads of nutrients in the recycle streams; therefore a global energy assessment should be done in order to evaluate the merits of a pretreatment.

The ideal pretreatment would then be the most advantageous match between an increase of methane and solids reduction and a low energy input per mass of sludge pretreated.

2.4 Microwave pretreatment

2.4.1 Microwave radiation

Microwaves are electromagnetic waves that lie in the region of 0.3 to 300 GHz of the electromagnetic spectrum (Figure 2.5). Other types of radiation include infrared, ultraviolet, radio waves, X-rays and gamma rays. All these waves travel at the speed of light and the only difference between them is their wavelength, which is inversely proportional to their energy. The shorter the wavelength of the radiation, the greater will be their energy.

Each of the wavelengths has specific characteristics and consequently different applications. Low frequency waves are useful in communications, MW and infra-red heating, visible light in photosynthesis, X-rays in the visualization of internal structures, etc. Research on the potential applications of MW started in the Second World War when the first MW generator was produced. Since then the industrial use of MWs has been increasing steadily. MWs are used primarily to heat materials. Microwave ovens are designed to produce waves that interact with polar materials.

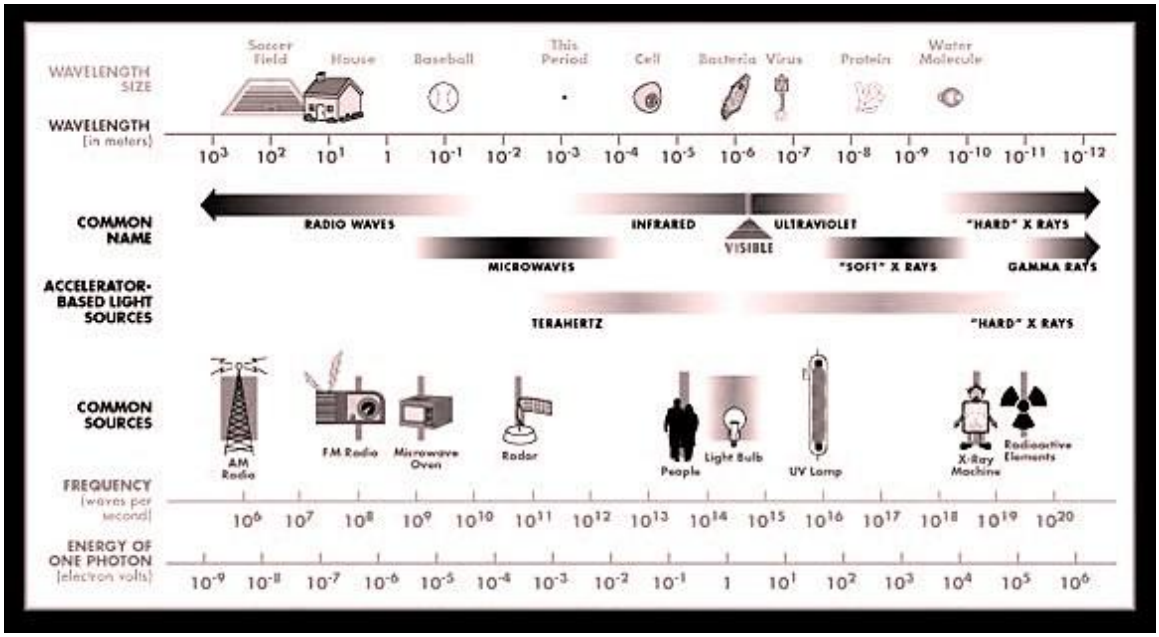


Figure 2.5 - The electromagnetic spectrum (http://lightsources.org)

Since water is the most abundant element in most foodstuffs, most ovens produce waves in the frequency of 2.45 GHz which is a frequency where water molecules absorb a large amount of energy, but still allow some to pass, in order to provide heating that is not limited to the surface in large samples. In this way, the heating is generated by the friction caused by rapid oscillation of water molecules, and the energy absorbed by the food is very high (Metaxas and Meredith, 1983).

The behavior of a sample subject to microwave heating is dependent on its chemical and physical properties. The most important properties are the dielectric loss factor, the dielectric constant and the dissipation factor (Table 2.10).

Table 2.10 - Important physical properties in microwave heating

Property	Symbol	Definition
Dielectric loss factor	ε´´	The amount of absorbed energy that is dissipated as heat
Dielectric constant	ε´	The amount of microwave energy absorbed by the material.
Dissipation factor	tan δ	The ratio between ε´´ and ε´. Measures the ability of a material to be heated by microwaves. Higher ratios mean higher heating rates.

The materials are then classified according to their characteristics when exposed to MWs. The materials can be:

- Absorbers – if they absorb a great amount of the energy irradiated. An example of an absorber material is water. These materials have high dielectric constants.
- Transparent – if they do not absorb energy. An example of this type of material is glass. These materials have very low dielectric constants.
- Reflectors – if they reflect the waves that are applied to them. No absorption or transmission occurs in these materials. An example is metals.

Microwave ovens are generally comprised of six components, the MW cavity, turntable, magnetron (the device that generates the MWs), wave guide (that directs the waves to the MW cavity), mode stirrer (that distributes the waves inside the MW cavity) and circulator (that directs the lost energy to a dummy load to protect the magnetron). A schematic of a commercial MW oven is shown in Figure 2.6.

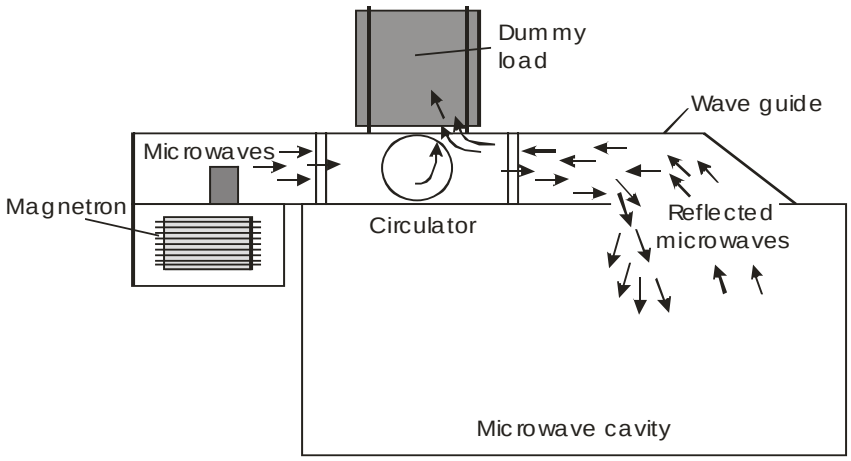


Figure 2.6 - Schematic of a microwave oven with the common components (Kingston and Jassie, 1988)

When MWs are adequately used, heating can be accomplished in shorter time and more economically when compared with conventional heating. Some of the advantages of MW heating compared to conventional heating are (Metaxas and Meredith, 1983; Hong 2002):

- Rapid and uniform heating. The heating occurs instantly and throughout the whole sample. Despite some temperature profiles in samples subject to MW treatment, heating can reasonably be considered uniform throughout the sample.
- Heating can be controlled instantly, and the power applied can be regulated accurately.
- Selective heating. The heat will concentrate in the materials that have a high dielectric factor.

At present, MW heating is used in many industries, besides its usual use in domestic households. It has been used in the food industry (baking, thawing, pasteurization, and drying), and in the medical industry (sterilization) among other areas (Hong, 2002).

2.4.2 Application of microwaves in sludge pretreatment

Microwaves have been used recently in municipal sludge pretreatment to improve the digestion and to decrease the pathogen content of these sludges. Hong (2002) applied MW radiation to different types of sludge in order to check the effect on biodegradability. The effect in solubilizing COD was effective in activated sludge since the fraction of soluble COD (sCOD) to total COD (tCOD) increased from 8.5 to 18%. The pretreatment consisted of heating the sludge to a temperature of 70ºC. The increase in this ratio for primary sludge was only 1%. For higher pretreatment temperature (100ºC) the digestion of sludge showed an increase in the amount of methane produced of 22.7% for primary sludge and 15% for activated sludge (Hong 2002).

Park et al. (2004) tested the use of MWs in the digestion of sludge. The ratio sCOD/tCOD on sludge irradiated to boiling point increased from 2 to 22%. Results for digestion of sludge pretreated with microwaves were compared with results obtained for digestion of non-pretreated sludge. The digestion was performed at two SRTs (10 and 15 d), and the results showed that the irradiated sludge improved the production of biogas and the removal of VS (Table 2.4).

Tests done at SRT of 8 days showed that the process was still stable, since the biogas production and VS removal rates were still high and the VFAs concentration in the effluent was low. In another study, Park *et al.* (2002), reported an increase of 57% in the amount of biogas produced in the anaerobic digestion of primary and secondary sludge pretreated with MWs at boiling temperature.

Table 2.11 - Results for microwaved sludge digestion (Park et al., 2004)

	SRT (d)	COD removal (%)	Biogas prod. rate (L/m^3.d)	methane prod. (L CH/kgVS)	VSS removal (%)
Pretreated sludge	15	23.6	117	314	25.9
	10	19.8	183	315	25.5
Control	15	14.4	94	242	23.0
	10	13.8	134	245	23.2
Maximum increase		64% (15d)	37% (10d)	30% (15d)	13% (15d)

Pino-Jelcic *et al.* (2006) applied MWs to primary and WAS prior to anaerobic digestion obtaining high degrees of solubilization. For the WAS, approximately 46% of the non-soluble COD was solubilized after irradiation. For the case of primary sludge, this increase was only 12%. The pretreatment consisted in microwaving the sludge to a temperature of 60ºC. The effect on the digestion of the sludge was measured in semicontinuous reactors with a SRT of 25 days. An increase of the biogas production of 16.4% compared to the control and of 6.3% as compared to sludge heated to the same temperature but using conventional heating. The MW heating also showed a higher inactivation of pathogenic microorganisms than sludge pretreated thermally by the conventional way (4.2 log units and 2.9 respectively).

Eskicioglou et al. (2004) investigated the effects of MW intensity, temperature and sludge concentration on the solubilization of WAS (taken from an activated sludge unit operating at 5 d SRT). It was reported that the MW intensity had a positive effect on the solubilization of the COD but negligible effect on the biogas production of the irradiated samples. However, sludge

concentration and temperature did show an influence on both parameters. The sludge irradiated at 96ºC had a greater production of biogas than the sludge irradiated at 75ºC and this sludge in turn produced more biogas than the sludge irradiated at 50ºC. The sludge pretreated to 96ºC showed an increase of 20% in biogas production compared to the control in the essays at 3% total solids (TS). For the assays at 1.4% TS the increase in biogas production was 15%. A differentiated effect in the solubilization was reported for samples pretreated with MW and conventional heating for the same temperature, with a greater fraction of total COD being solubilized by the conventional heating, a fact that was attributed to the longer time conventional heating requires to reach the same final temperature. The authors also performed a study based on the ultrafiltration membrane fractionation of the soluble fraction of the pretreated sludge that confirmed that digesters treating high molecular weight materials resulted in smaller biodegradation rate constants.

Thibault (2005) tested MW pretreatment of combined primary/WAS sequencing batch reactor sludge (15d SRT) and reported that applying MWs to 85ºC improved the biogas production by 16.2%. Multiple irradiation cycles to the same temperature did not improve results. The maximum sCOD/tCOD achieved in the tests using MW pretreatment was 7%.

2.4.3 Biological effects of microwaves

Microwaves have been used mainly to heat materials and inactivate bacteria. Today MWs are used to sterilize all kinds of equipment and materials. There is evidence that the MWs cause different biological effects depending on field strength, frequencies, wave forms, modulation and duration of exposure (Rai *et al*. 1994 a, b).

The exposure to certain frequencies of MWs was reported to increase up to 15% or decrease by 29% the growth rate of *Saccharomices cerevisiae* by certain frequencies of MW radiation within 41.8-42.0 GHz (Grundler *et al.,* 1977, 1982, 1988). The same effects were observed in the growth rate of *Candida albicans.* A 3 hour continuous irradiation at 72 GHz increased the growth rate by about 25% over the control (Dardanoni *et al.,* 1994).

Banik *et al*. (2006) reported the increase on the amount of methane produced by a strain of methanogenic bacteria (*Methanosarcina barkeri* –DSM 804) after the irradiation of the bacterial culture with MWs in the frequency ranging from 13.5 to 36.5 GHz for 2 hours. Another effect reported by the author was the increase in the growth rate of this methanogenic bacterium.

The majority of studies however report the killing and inactivation of bacteria by the action of MWs. These effects are mainly attributed to the heating caused by MW action, but in recent years some investigators suggested that other causes might be in action when exposing a sample to MW irradiation.

In general, the studies involving MW irradiation resulted in two conflicting conclusions, that the cell death and solubilization was solely the result of heat produced by MW action, and that death was not only the result of the heat produced but also from a MW electric field effect, that is commonly known as the athermal effect (Kenyon *et al*., 1971; Toishi and Muranaka, 1982; Dreyfuss and Chipley, 1980; Ishihara, 1992).

Much effort has been devoted to studies that have attempted to demonstrate the existence of non-thermal effects of MW irradiation by maintaining end-point temperatures below thermal death points of microorganisms under investigation. Culkin and Fung (1975) demonstrated that *E. coli* and *Salmonella typhimurium* could not survive in solutions irradiated at 915 MHz by MW irradiation. They found that microbial destruction occurred at lower temperature and shorter time

periods when compared to conventional heating methods. They postulated athermal factors other than thermal effects might be involved in the inactivation of the microorganisms.

Other authors tried to verify the existence or not of athermal effects by comparing the results of heating produced by MWs to the heating produced by conventional methods for the same temperatures. The difference in the results obtained - with a greater degree of bacterial inactivation in samples heated by MW - was, according to these authors, evidence that MWs have associated a biocidal effect not linked with the heating (Hu *et al*., 1996; Furia *et al*., 1986; Barnes and Hu, 1977).

However, opposite conclusions were drawn in other studies, where the researchers claimed that there was no evidence of an athermal MW effect and that the biocidal effects of MWs were either due entirely to heating or were indistinguishable from external heating (Fujikawa *et al*., 1992, Goldblith, 1967, Jeng *et al*., 1997, Lechowich *et al.*, 1969, Vela and Ju, 1979, Welt *et al*., 1994)

Some investigators claim that the different conclusions are due to the difficulty in dissociating the microwave irradiation and temperature increase (Banik et al., 2003, Sato, 1996).

Bearing this in mind, Sato (1996) developed a system that kept the temperature constant while being irradiated with MWs. After irradiating a culture of *E. coli* with MWs of different intensities but maintaining the same temperature as the cultures not subject to microwave irradiation, the results showed that the death rates were higher in the samples irradiated with the microwaves but kept at constant temperature than the cultures not subject to MW irradiation. Another interesting result was that increasing intensity of MW also caused higher death rates, although the temperature was kept the same.

The results seem to confirm that an effect other than the thermal effect exists. Barnes and Hu (1977) presented a mathematical model to show that athermal effects of MW irradiation could be due to ion shifts across membranes and reorientation of long-chain molecules. Straub and Carver

(1975) stated that increased active ion transport could be responsible for increase in potential differences and electrical current across the membranes. This conclusion was reached after detecting irreversible damage in the bacterial cell wall with a decrease in the ion transport increase in passive permeability.

Besides, it has been reported that the MW field can cause the polarized side chains of the molecules to line with the direction of the electric field, leading to a possible breakage of hydrogen bonds, and to alteration of the hydration zone (Teixeira-Pinto, 1960, Wilderbank, 1959). Such effects can cause denaturation or coagulation of molecules, and that was confirmed experimentally by Fleming (1961). Stuerga and Gaillard (1996a, b) reported that electromagnetic fields induce structuring and orienting effects within the irradiated medium. The magnetic energy was converted to heat by thermal conversion (Brownian movements), while allowing induced organization of the irradiated medium under the athermal condition. Although the mechanism of the athermal effect is unknown, violent motion of dipoles in molecules by MW field seems to destroy structures in polyatomic molecules such as proteins and phospholipids at high temperatures. Microwaves either cause ions to accelerate and collide with other molecules or cause dipoles to try to rotate and line up with the rapidly alternating electrical field (in commercial MW ovens at frequencies of approximately 2450 million times per second).

2.5 Sludge retention time

SRT on activated sludge is an important parameter in the biological treatment of wastewater. It is one of the main design parameters when dimensioning a wastewater treatment system and exerts a dominant effect in the capabilities and performance of a biological operation. As an example,

the SRT applied determines the type of microorganisms that are found in the system, as well as their activity, thereby determining effluent quality.

The normal practice has been setting the SRT in the activated sludge plants to between 3 and 6 days for the removal of carbonaceous organic matter and flocculent growth of heterotrophic bacteria (Grady *et al*., 1999). In recent years, though, stringent effluent standards require in many cases the removal of nutrients such as nitrogen; the biological removal of nitrogen needs the application of higher SRT (> 10 d), in order to allow the growth of nitrifiers. As a consequence of such high SRTs, partial stabilization of the sludge occurs in the activated sludge process and the following anaerobic stabilization of WAS can result in low efficiency both from a processing and economic standpoint (Nielsen and Petersen, 2000, Bolzonella *et al.*, 2002), since this substrate shows a low biomethanization potential (Jih-Gaw *et al*., 1999). The specific biogas production rate determined on the basis of the VS destruction when treating WAS is in the range 0.6 – 0.8 m^3/kg $VS_{destroyed}$ rather than a typical value of around 1 m^3/kg $VS_{destroyed}$ observed when digesting mixed sludge (Metcalf and Eddy, 2003). This fact results in a decrease in biogas production so that the energy balance of the anaerobic digester is often negative if sludges are not properly thickened, especially in winter (Bolzonella *et al*., 2002). Bolzonella *et al*. (2005) in a study where sludges of several different SRT activated sludge processes were studied, reported a decrease in the amount of biogas produced with the increase in SRT applied in the anaerobic treatment. According to the results, an increase in SRT from 10 to 20 d in the activated sludge process resulted in a decrease of 25% in the specific gas production (m^3/kg VS_{fed}).

The SRT on activated sludge processes also determines other characteristics of the sludge such as the floc structure and the composition of the EPS. These EPS form a three dimensional polymeric gel-like matrix in which the bacterial cells are embedded that originates both from the microorganisms (excretion and lysis) and wastewater (biosorption). The EPS contain variable

proportions of proteins, carbohydrates, nucleic acids (Pavoni *et al*., 1972; Urbain et al., 1993; Jorand *et al*., 1995), humic-like substances (Dewalle and Chian, 1974; Frolund *et al*., 1996), lipids (Goodwin and Forster, 1985) and heteropolymers such as glycoproteins (Horan and Eccles, 1986). The total mass of EPS in a sludge may vary significantly but has been reported to be around 15 – 25% of the sludge TS (Frolund *et al*., 1996; Urbain *et al*., 1993). The most common compounds in the EPS matrix are carbohydrates and proteins (Sponza, 2003, Zhang *et al*., 2003, Liao *et al*., 2001) with the relative amount of proteins and carbohydrates being variable. Some authors state that proteins are the main compound in EPS (Sponza, 2003, Urbain *et al*., 1993, Frolund *et al*., 1994 and Bura *et al*., 1998), which could be due to the presence of exoenzymes from bacterial excretions, but other authors report a higher amount of carbohydrates in certain cases (Azeredo *et al*., 1998, Horan and Eccles, 1986).

The ratio of proteins to carbohydrates in the EPS as well as the amount produced is dependent on the SRT. Liao *et al*. (2001) reported that the ratio of protein/carbohydrate changed from 1.3 to 5 when the SRT increased from 5 to 12 d. Sponza (2003) also reported an increase in the protein content with the increase in the SRT with the content of carbohydrates remaining constant. The increase in SRT was reported to cause an increase in the production of EPS (Liao *et al*., 2001, Pavoni *et al*., 1972, Chao and Keinath, 1979, Sheintuch *et al*., 1986, Sheintuch 1987). However, other authors state that the amount of EPS per unit mass of solids remains constant with different SRT (Brown and Lester, 1982, Liao *et al*., 2001).

Recent studies by Novak et al. (2003) and Zhang and Bishop (2003) have shown that EPS rather than cells undergo lysis and produce short chain organic compounds which are then converted to methane. So, in the absence of a mechanism to disrupt the bacterial cell walls, the majority of the substrate available in the anaerobic digestion of sludge will be the EPS. The EPS can be degraded

by its own producers or other bacterial cultures and the EPS that are mainly comprised of carbohydrates are degraded more rapidly than proteinaceous EPS (Zhang and Bishop, 2003).

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Chapter 3

Effect of Microwave Athermal and Thermal Radiation on Wastewater Sludge Properties

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3.1 Abstract

Samples of thickened waste activated sludge (TWAS) at two different concentrations were exposed to microwave (MW) radiation. Some of the samples were not allowed to heat up, to study the athermal effect of microwaves. The samples exposed to MWs where their temperature was allowed to increase showed a higher degree of chemical oxygen demand (COD), protein and carbohydrate solubilization compared to a control. The size distribution of particles was changed after exposure of TWAS to MWs. These results were also observed in the samples exposed to microwaves but kept at a constant temperature, suggesting the occurrence of a MW athermal effect. Thermally (samples experiencing a temperature increase) and athermally (samples that were maintained at ambient temperature) microwaved samples produced more methane than the non-microwaved controls in subsequent anaerobic biodegradation.

KEYWORDS: Athermal, Solubilization, Biodegradability, Microwave, Thermophilic, WAS

3.2 Introduction

Microwaves have been studied recently as a pretreatment in municipal sludge digestion in order to improve anaerobic digestion and to decrease the pathogen content of these sludges. These studies showed that generally MW exposure causes an increase in soluble substrate, in the amount of biogas produced from anaerobic biodegradation, and in pathogenic microorganism inactivation. These effects depend on intensity, temperature, sludge concentration, and type of sludge [1,2].

In general, studies involving MW irradiation resulted in two different conclusions, that cell death and solubilization were solely due to heat produced by MW action, and that death was not only the result of the heat produced but also from a MW electric field effect, that is commonly known as an athermal effect [3, 4].

Much effort has been devoted to studies that have attempted to demonstrate the existence of non-thermal effects of MW irradiation by maintaining end-point temperatures below thermal death points of the microorganisms under investigation. Increase in bacterial death rate, or complete inactivation of bacteria were interpreted as a sign of existence of athermal effects [5,6]. Differences in death rates of samples heated by MWs and conventional heating methods were also interpreted the same way [7,8]. However, opposite conclusions were reported in other studies, where researchers claimed that there was no evidence of an athermal MW effect and that the biocidal effects of MWs were either due entirely to heating or were indistinguishable from external heating [9,10]. Some investigators claim that the different conclusions are due to the difficulty in dissociating MW irradiation and temperature increase [11,12]. Bearing this in mind, Sato [12] developed a system that kept the sample temperature constant while being irradiated with microwaves. The results showed that microorganism death rates were higher in samples irradiated with MWs but kept at constant temperature than cultures not subject to MW irradiation.

Increasing intensity of radiation also caused higher death rates, although sample temperature remained constant. These results seem to confirm that an effect other than the thermal effect exists. Athermal effects of MW irradiation could be due to ion shifts across membranes and reorientation of long-chain molecules [8], increased active ion transport causing potential differences and electrical current across the membranes [13], and possible breakage of hydrogen bonds and alteration of the hydration zone [14]. Mertens and Knorr [15] stated that when a large number of magnetic dipoles are present in one molecule, enough energy can be transferred to the molecule to break a covalent bond, so that certain critical molecules in a microorganism, such as DNA, or proteins, could be damaged or broken by the action of the oscillating magnetic field, resulting in the inactivation of the microorganism and release of organic material to the medium.

In order to assess more precisely the existence of the athermal effect in sludge pretreatment, the studies reported herein were designed to uncouple the heating effect from the MW radiation. Some studies were done previously using conventional heating as a standard to compare the results with MW generated heat tests. These studies were helpful because they gave more insight in the changes that occur in both heating options. However, the single effect of the oscillating magnetic field on the sludge could not be observed and possible phenomena of interaction (e.g., synergies) between heat and magnetic field oscillations could have happened, making it difficult to quantify the magnitude of each parameter on the effect observed. This work was therefore designed to assess the presence of an athermal effect and, should it exist, the magnitude of it in terms of chemical oxygen demand (COD), protein, and sugar solubilization, and subsequent methane production in the anaerobic degradation.

3.3 Material and methods

3.3.1 Sample characterization and pretreatment

Samples of thickened activated waste sludge (TWAS) were collected from the thickener centrifuge at the Robert O. Pickard Environmental Centre (ROPEC), located in Ottawa (ON, Canada). This wastewater treatment plant has preliminary and primary treatment followed by a conventional activated sludge process, operated at an average solids retention time (SRT) of 5 days. The sludge was characterized by measuring several parameters (Table 3.1).

Table 3.1 - Characteristics of sludge from ROPEC

Parameter	Sampling date			
	17/03/2004[a]	20/06/2006[a]	13/07/2006[a]	07/06/2007
pH	7.5	7.8	7.1	7.9
TS (% w/w)	5.4 (0.01)	5.5 (0.01)	4.6 (0.02)	4.3 (0.2)
VS (% w/w)	3.7 (0.02)	3.7 (0.01)	3.1 (0.02)	2.9 (0.2)
VS/TS	0.69 (0.00)	0.67 (0.00)	0.67 (0.00)	0.67 (0.00)
TCOD (mg/L)	67,301 (5873)	55,786 (6498)	45,714 (0)	54,602 (2429)
SCOD (mg/L)	3957 (29)	2757 (1009)	4286 (0)	4331 (250)
SCOD/TCOD	0.06 (0.00)	0.05 (0.01)	0.09 (0.00)	0.08 (0.00)

[a]Data obtained by Eskicioglu et al, [15] for sludge in the same sampling point

The sludge obtained from ROPEC was diluted in order to obtain two different concentrations: 3% and 1% total solids (TS). Distilled water was used to dilute the original sludge to the desired concentration. Then samples were subjected to different types of MW pretreatment. The MW oven used for the pretreatment of the sludges was a conventional domestic oven (Sanyo EM-S759S P=1350 W, 2450 MHz) modified with a unit shown in Figure 3.1 to maintain constant temperature in the sample [10,17]. Sato *et al*. [12] also used a similar system. It consists of a loop in which a MW transparent apolar solvent (kerosene) was circulated and used as a coolant of the

samples while not interfering with the action of the MW field in the samples. Heat was removed from the coolant by passing it through an external (to the microwave oven) ice bath (Figure 3.1).

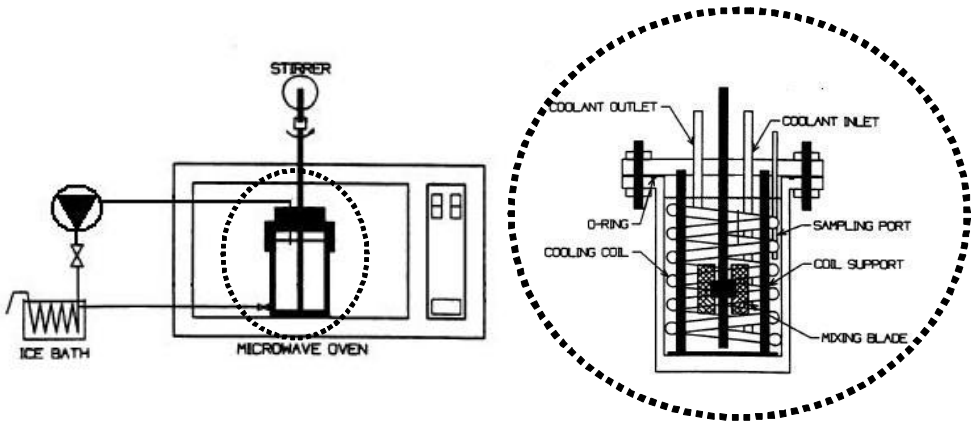

Figure 3.1 - System used to test athermal microwave effects (adapted from Welt *et al*, 1994[10])

This system was used to irradiate TWAS with or without the associated increase in temperature. The coil was placed close to the walls of the sample vessel, since most of the MW power is absorbed in the outer layers of the sample (1.1 cm), as determined by measurement of the effective penetration range in TWAS [1]. To avoid temperature gradients, the sludge vessel was continually mixed by a stirrer, made of MW transparent material. The stirrer was driven by a shaft coupled to an electrical motor, and was operated at 150 rpm. The electrical motor and part of the shaft were placed outside the MW cavity and the shaft was introduced through a hole in the metal cage of the oven. Microwave losses through the hole made in the cavity were minimized by using MW attenuators. Microwave losses were measured using a MW leak detector (EMF Inc. model MD-200) and were below the safety limit of 5 mW/cm^2 [18] at the surface of the oven.

In each of the thermal tests, 200 g of sludge was irradiated and allowed to heat, with the cooling system not functioning. The oven operated as a normal MW oven. The temperature was allowed to rise until a final temperature of 96ºC was reached to prevent sludge losses by boiling (Figure 3.2). A maximum temperature below boiling was chosen to minimize vaporization of liquid. After this temperature was reached, no further radiation was supplied. The samples were not covered during the MW exposure, but were covered while cooling, to reduce losses of water and volatile compounds. Water lost by evaporation was replaced by distilled water. The MW oven was operated with two different intensities, 100% and 50% of the total oven power. For the tests at 50% intensity, the radiation exposure time necessary to reach 96ºC was longer than for the tests made at 100% intensity, as expected.

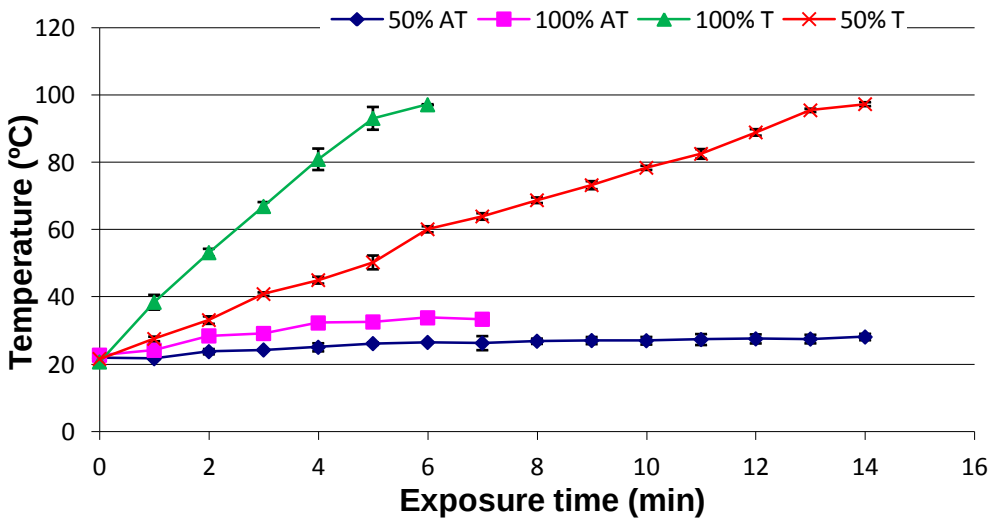

Figure 3.2 - Thermal profiles for the tests in the customized microwave oven (with 95% confidence intervals for each average temperature point)

For the athermal tests, the cooling fluid was circulated through the coil inside the vessel and the ice bath at a rate of 400 mL/min. The exposure time was the same as for the thermal tests; therefore, the MW exposure of the samples was the same as in the test where the sample

temperature was allowed to rise. Temperature was monitored at regular intervals using a thermocouple, to ensure athermal conditions were maintained. The temperature profiles in Figure 3.2 for the athermal tests confirm that sample temperature was maintained close to room temperature during these assays. The set of conditions tested in this work is given in Table 3.2.

Table 3.2 - Conditions tested for the pretreated TWAS

	Intensity (Power)	**Concentration (TS, % w/w)**	**Exposure time (min)**
TWAS (thermal, T)	50, 100%	1%, 3%	6 (for 100% tests) 14 (for 50% tests)
TWAS (athermal, AT)	50, 100%	1%, 3%	6 (for 100% tests) 14 (for 50% tests)
TWAS non-irradiated	-	1%, 3%	-

3.3.2 Sludge Analysis

Several parameters were measured in the pretreated samples. To compare the effects produced by the treatment, the same parameters were measured in samples not subjected to any radiation acting as control tests. Thermally pretreated samples were allowed to cool to room temperature before any analysis was performed. TS and volatile solids (VS) were determined based on Standard Methods procedure 2540G [19]. Colorimetric COD measurements were performed using Standard Methods procedure 5250D [19] with a Coleman Perkin-Elmer spectrophotometer Model 295 at 600 nm light absorbance. Samples on which soluble COD (sCOD) was measured were centrifuged for 20 min at 5856 RCF, and filtered with 0.45 μm pore size disc filters prior to the COD analysis. Total soluble protein (after filtration through 0.45 μm pore size filter) was measured according to the procedure described in Bradford [20], using bovine serum albumine (BSA) as the standard. Determination of total soluble sugars was performed using the phenol-

sulphuric acid test method proposed by Benefield and Randall [21], with glucose used as a standard. Particle size distribution was determined using DPA 4100 particle analyzer (Brightwell Technologies, Inc.) with a flow rate of 100 μL/min using diluted samples.

3.3.4 Biomethane potential tests

Biomethane potential tests (BMP) were performed in duplicate using 500 mL glass bottles capped with butyl rubber stoppers. In each bottle, 200 mL of pretreated or control sludge and 45 mL of thermophilic inoculum (acclimatized to microwaved sludge for 8 months at a solids retention time of 20 d) were added. After addition of a mixture containing equal parts of $NaHCO_3$ and $KHCO_3$ to achieve an alkalinity of 4000mg/L (as $CaCO_3$), the bottles were bubbled with N_2 and sealed. Reactor pH, total volatile fatty acids (VFAs; summation of acetic, propionic, and butyric acids) and biogas composition (nitrogen, methane, and carbon dioxide percentages) were monitored weekly during the batch anaerobic digestion. Total VFAs were measured by injecting supernatants into an HP 5840A GC with glass packed column (Chromatographic Specialties Inc., Brockville, ON, Canada, Chromosorb 101, packing mesh size: 80/100, column length x ID: 304.8cm x 0.21 cm) and a flame ionization detector (oven, inlet and outlet temperatures: 180, 250, and 350^{o}C, respectively, carrier gas flowrate: 25 mL helium/min) equipped with HP 7672A autosampler. Biogas composition was determined with an HP 5710A GC with metal packed column (Chromatographic Specialties Inc., Brockville, ON, Canada, Porapak T, packing mesh size: 50/80, column length, OD: 304.8cm, 0.635 cm) and thermal conductivity detector (oven, inlet and outlet temperatures: 70, 100, and 150^{o}C, respectively) using helium as the carrier gas (flowrate: 25 mL/min). Serum bottles were kept in a darkened temperature-controlled incubator shaker at 55 ± 2^{o}C and 90 rpm until they stopped producing biogas. Biogas volumetric

production was measured daily by puncturing the rubber septum with a thin needle and measuring displacement in a water column manometer.

3.4 Results and discussion

3.4.1 Effect on COD, protein and carbohydrate solubilization

Results show that the amount of soluble organic matter is affected by the exposure to radiation. The most important fact is that solubilization increases at thermal and athermal tests, which is the evidence of the existence of athermal effects. The organic matter in athermal tests was solubilized without the need of a temperature increase. Changes in sCOD are shown in Figure 3.3 for the various pretreatment conditions. It is observed that samples have higher solubilization percentages after being irradiated when the TWAS was at the higher concentration of 3%. In the 1% TWAS concentration tests no statistically relevant change was detectable, which is consistent with observations made by Eskicioglu et al. [2] who showed that solids concentration was one of the most important parameters influencing changes detected after microwave pretreatment. The increase is significant for 3% TWAS concentration made at both thermal and athermal conditions using 100% intensity, but for pretreatment at 50% intensity, only the thermal test produced an increase in the amount of sCOD. This suggests that intensity is a more significant factor in athermal conditions. This observation is also supported by the fact that sCOD is approximately the same for the thermal tests at 50 and 100% intensity for 3% sludge.

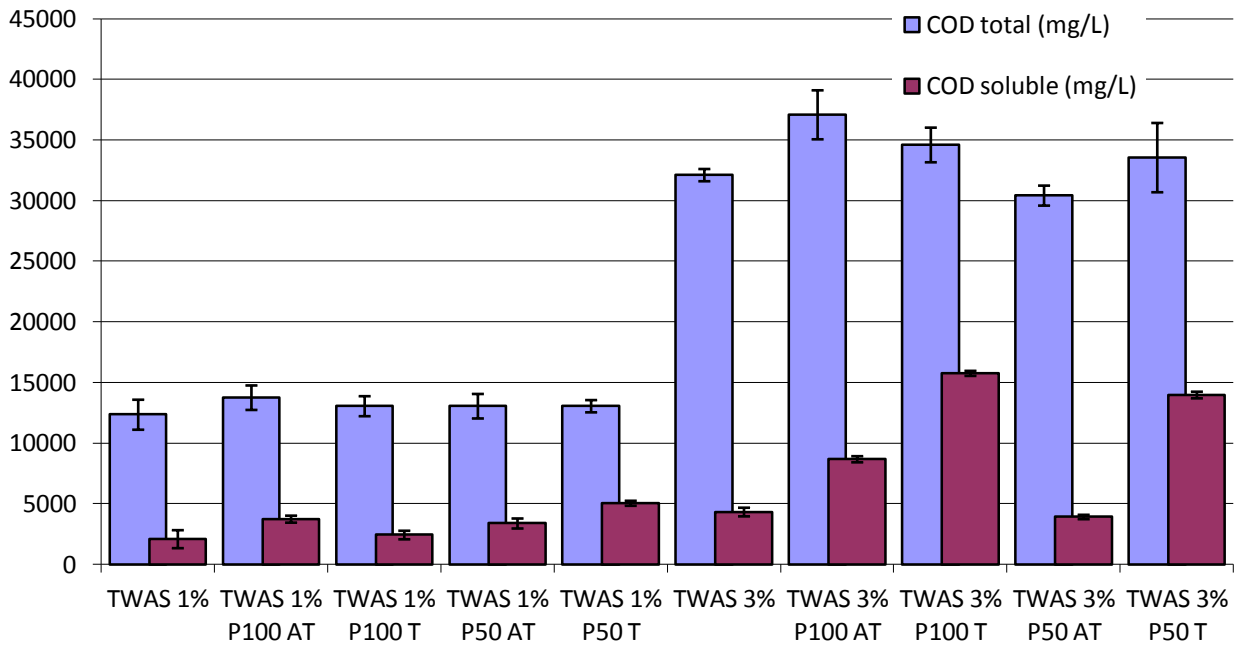

Figure 3.3 - Total and soluble COD change in sludge after pretreatment (error bars indicate confidence interval of 95%)

The maximum increase in sCOD occurred in thermal tests. Relative changes show that the highest increases occur for the two tests at thermal conditions, with the test at 100% intensity reaching a slightly higher value. For the athermal conditions, the increase in sCOD was only visible in the 100% intensity test and the value reached was significantly lower (less than half) compared to values recorded for thermal tests (Table 3.3).

Table 3.3 - Solubilization ratios for 3% tests

	Solubilization ratio (sCOD/tCOD) (%)	Increase relative to control (TWAS 3%) (%)
TWAS 3%	13.5	-
TWAS 3% 50 AT	12.9	-0.04
TWAS 3% 100 AT	23.4	73.3
TWAS 3% 50 T	41.7	209
TWAS 3% 100 T	45.6	237

Flocs in activated sludge are comprised of a polymeric matrix made up of variable quantities of extracelular polymeric substances (EPS) such as proteins, carbohydrates, humic substances, glycoproteins, lipids, and nucleic acids with the bacterial cells embedded in the mesh [22,23]. However, the most prevalent substances are proteins and carbohydrates [24,25]. Results for proteins and carbohydrates show that there is solubilization of these compounds after exposure to radiation (Fig. 3.4).

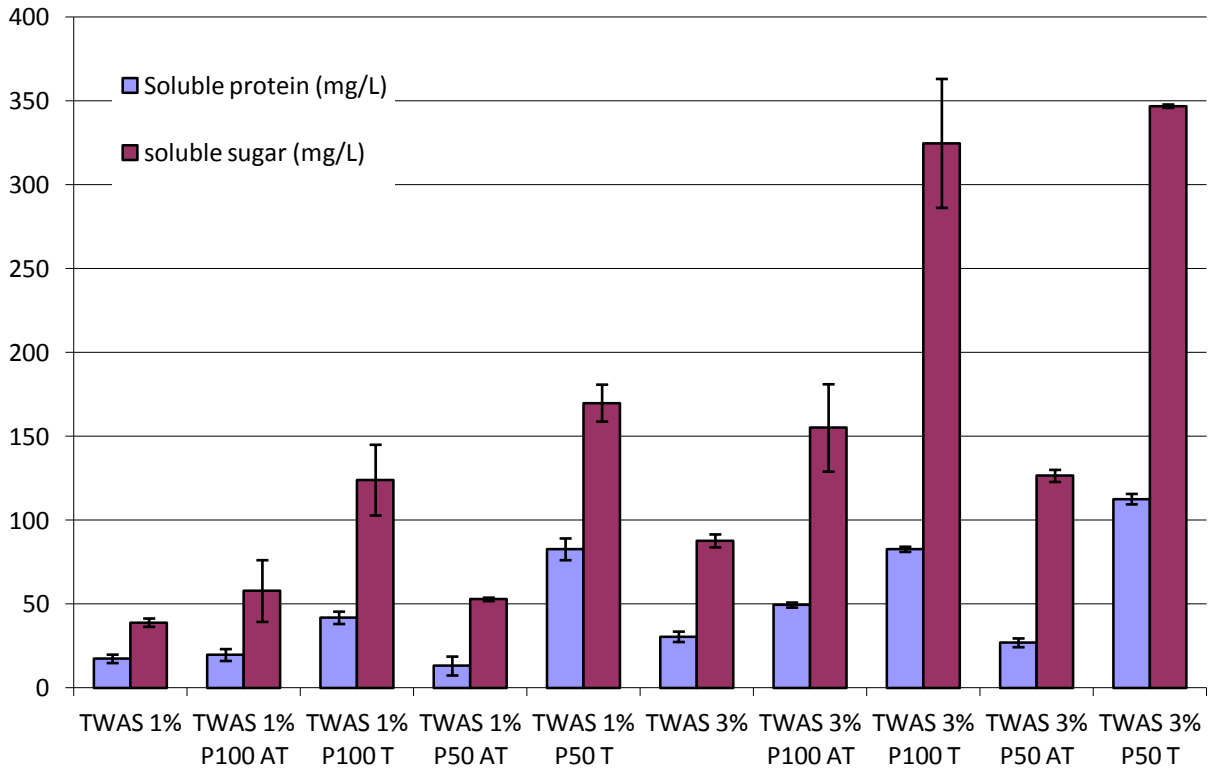

Figure 3.4 - Soluble protein and sugar in sludge subject to pretreatment (Error bars indicate confidence interval 95%)

Measurements indicated that the amount of soluble sugars is higher than soluble protein before and after pretreatment in all tests, which agrees with results by Azeredo et al. [26] that reported that EPS is mainly comprised of carbohydrates. Protein measurements revealed that irradiation

coupled to thermal variation causes an increase in the amount of protein released at both TWAS concentrations. For athermal tests, there is no perceptible change in soluble protein in the lower TWAS concentration tests (1%). However, this change is statistically significant in the test performed at 3% solids and 100% intensity. The protein concentrations measured in the tests were low and the expected change was not high in the athermal tests at 1% solids or 50% intensity (both at 1% and 3% solids). Additionally, the method is not highly sensitive for changes at low values, which could have prevented accurately measuring changes for these tests. The magnitude of the change is greater in thermal tests compared to athermal tests for the same concentration and power applied, a result that was expected, since the heat generated in the process is the main physical factor causing the solubilization of sludge flocs. One important aspect noticed in the experiments was the apparent correlation between increase in soluble COD and protein solubilization. Increase in soluble COD was detected in all tests that also showed an increase in soluble protein, the only exception was the test performed at athermal condition and 50% intensity. In this test, no significant increase was noticed in the soluble protein and also in the soluble COD, suggesting proteins either have a greater impact in the amount of organic soluble matter present in sludge, or are indicative of a process that releases more amounts of soluble COD, as is the lyse and consequent release of intramollecular compounds of bacterial cells.

The soluble sugar concentration increases after exposure to MW radiation either in athermal or thermal tests. Increases were statistically significant for both TWAS concentrations. For 1% solids, the athermal tests showed an increase relative to the control at 50% and 100% intensity with the degree of solubilization being approximately the same for both cases. In the 3% solids tests, the degree of solubilization is slightly higher in the 100% intensity AT test compared to the 50% intensity AT test. In the thermal tests, all tests show increases in sugar solubilization

compared to controls, with the final solubilization being higher in the 50% intensity tests. The increase due to vaporization losses was ruled out since the mass of water lost was replaced after cooling of the sample. This may have something to do with the exposure time, since 50% intensity tests were irradiated for 14 minutes compared to 6 minutes used in the 100% intensity tests. Another explanation to the higher degree of soluble sugars measured might be the occurrence of Maillard reactions. These reactions occur between amino acids and sugars and cause the polymerization of these compounds, reducing the soluble fraction. These reactions occur mainly above 80ºC and may have been responsible for the lower concentrations at higher applied power.

Although sludge exposed at two different intensities absorb similar total final amount of energy, since the final temperature is the same, it was noticed that results for athermal tests were dependent on radiation intensity. MW penetration depth is only dependent on the frequency, and the difference in the irradiation power and time did not produce significant change in the vaporization of liquid. So, the differences should be consequence of the athermal effect mechanism. Bohr and Bohr [27] suggest that certain reactions, e.g, protein denaturing, involve crossing an energetic barrier, and this barrier is lowered when certain molecular movements are coherent, and sufficient energy is transmitted to molecular dipoles. These events cause a shift in the kinetics of the reaction increasing protein unfolding. Higher power of the electrical field involves higher energy transference to molecular dipoles and more extensive alignment effects increasing the chance of otherwise less likely reactions to occur, this way explaining the differences detected with different applied intensities.

3.4.2 Effect on particle size distribution

Since the change in soluble COD and protein was not significant in some of the tests performed at 50% intensity, it was assumed that the change in size distribution of particles would not be measurable with the type of equipment available. For this reason, only the sludge exposed to 100% intensity radiation was analysed for particle size distribution.

There are changes in the relative size distribution of particles with pretreatment as can be seen in Figure 3.5.

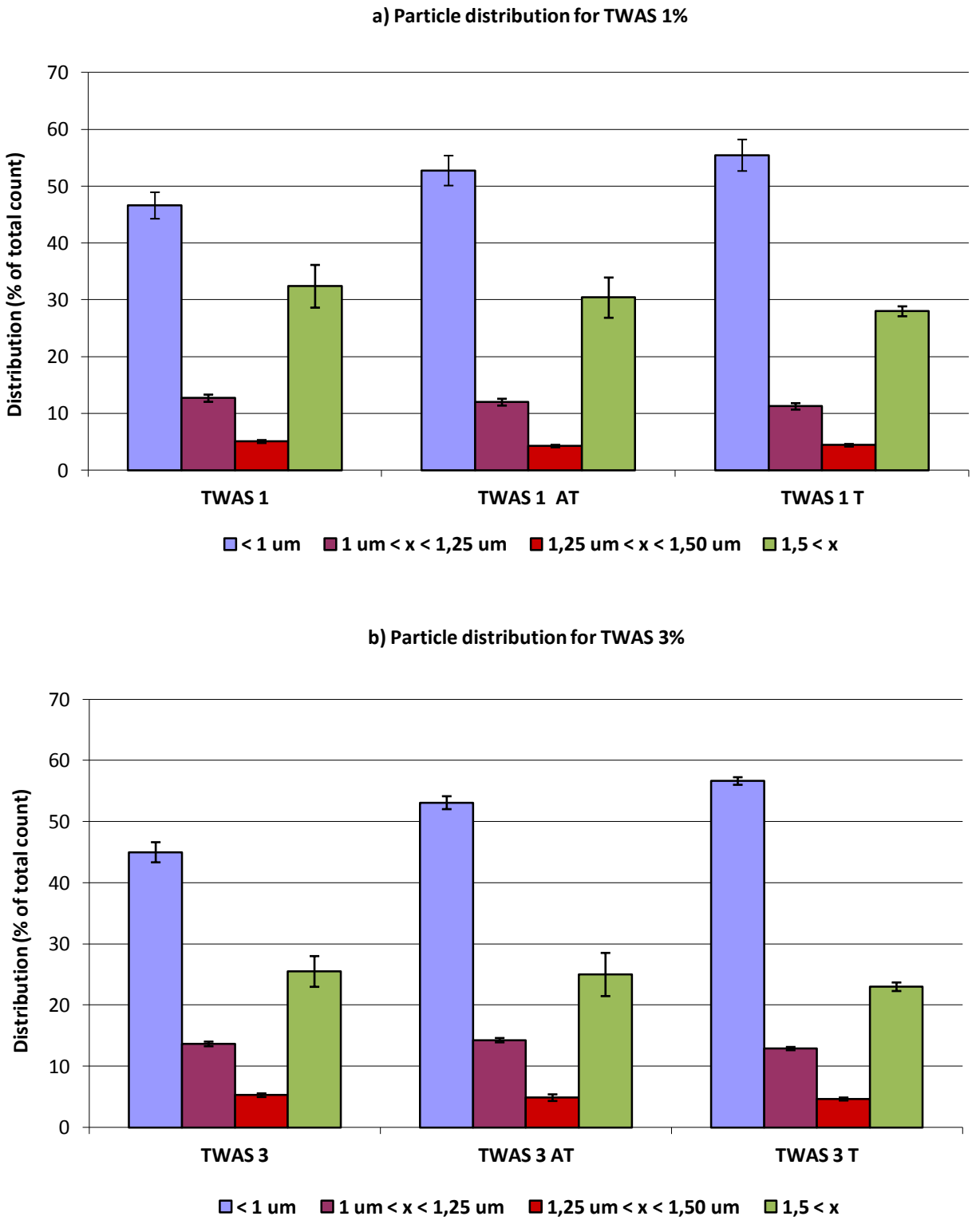

Figure 3.5 - Particle size distribution for a) sludge at 1% total solids and b) sludge at 3% total solids exposed at 100% power (Error bars indicate confidence interval 95%)

In all tests, the fraction with the highest particle count corresponds to smaller size (< 1 μm) particles. Changes are detectable when sludge is exposed to MW irradiation. There is an increase in the smaller particle size fraction, while the fraction including particles larger than 1.5 μm shows a slight decrease. These changes occur in athermal and thermal tests, and the changes are significant for both solids concentrations tested. As in the previous tests, the change - in this case the increase in the fractional distribution of particles of smaller size - is greater at thermal conditions. Other fractions show no significant change for all tests. Sludge flocs have a size that can range from as small as 10 μm up to 200 μm or larger, with the particles that comprise the extracellular polymeric matrix having a smaller size. Disruption of some of the hydrogen bonds and protein structures can be caused by the oscillating electrical field and lead to destabilization and liberation of particles and breakage of the matrix into smaller particles. The increase in number of smaller size particles, is most likely due to MW action on the floc matrix. This phenomenon may have occurred here, since the increase is noticed both in athermal and thermal tests. In thermal tests this effect is coupled with temperature rise and increase in the fraction of smaller particles is higher as expected.

3.4.3 Effect on methane production potential

Sludge digestion potential was assessed by measuring methane production in tests performed at thermophilic conditions. Thermal microwave pretreatment caused an increase in the amount of methane obtained (Fig. 3.6). In the case were sludge was exposed to radiation but not allowed to heat, there was also an increase in the amount of methane produced in tests performed at 3% solids.

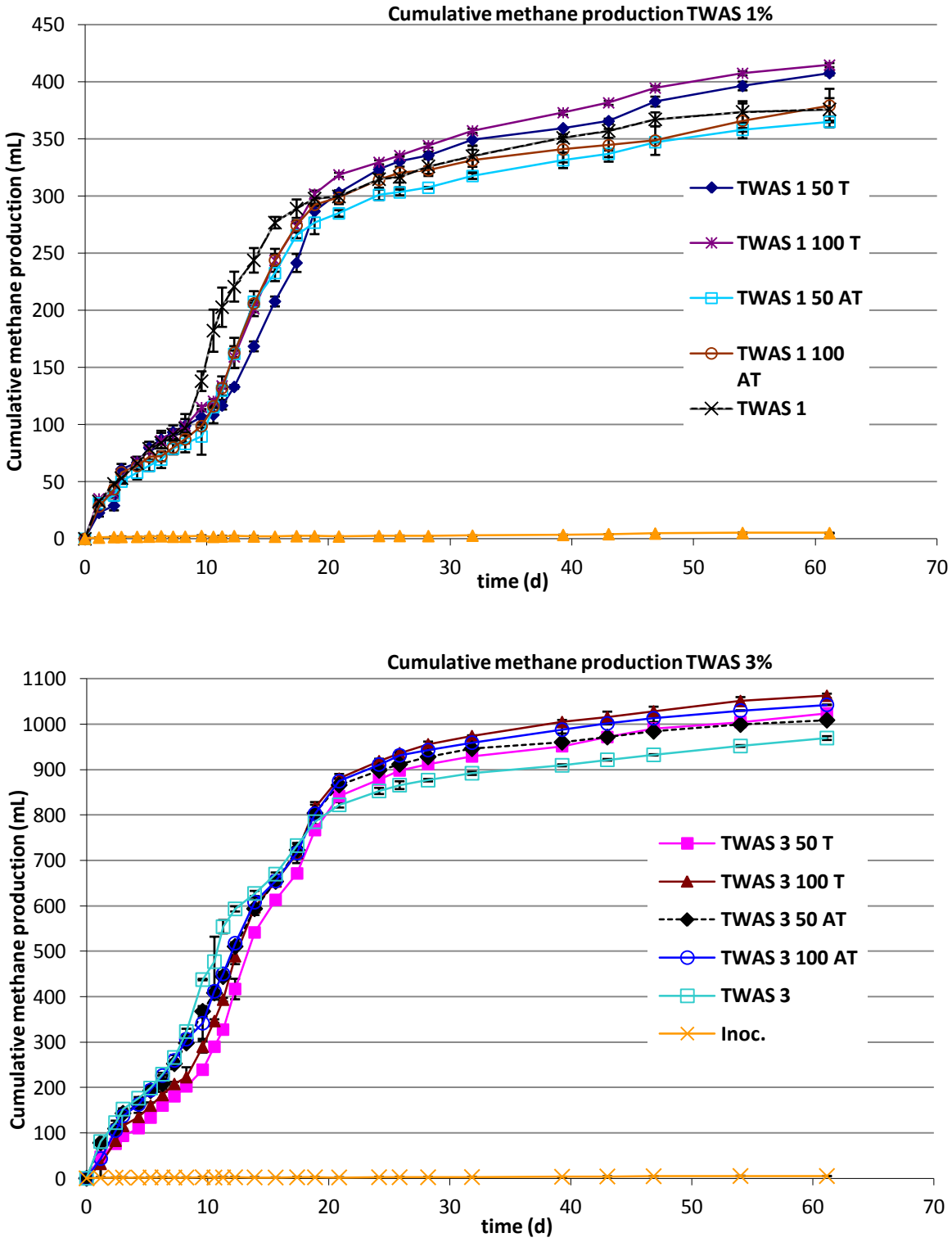

Figure 3.6 - Cumulative methane production for 1% and 3% total solids sludge (error bars indicate variability between duplicates)

The amount of methane produced was higher in the 100% power tests compared to the control, both at thermal and athermal conditions. It is possible that longer exposure time at high temperatures in the 50% intensity thermal test caused greater losses of biodegradable volatile compounds that could explain the smaller amount of methane produced (Table 3.4).

For the tests at 1% solids, the difference between athermal tests and the controls is not statistically significant. However, both tests at thermal conditions produced more methane than the controls. In the cumulative curves for the two sets of tests, it is noticeable that methane production is higher for the control in the initial phase of the digestion. Microwaved sludge only starts producing more methane after a period of approximately 20 days either in 1% and 3% solids tests. This phenomenon also occurred in studies by Eskicioglu et al. [16], who attributed it to inhibition, although not identifying the inhibition agent. Microwave action involves solubilization of particle substrate and breakage of the polymer matrix surrounding the bacterial cells, and it is hypothesized that this matrix has an important role in inhibition prevention since it can function as a barrier to toxins and inhibitory substances through sorption and/or reaction with the matrix components, as well as retarding the penetration of toxins [28,29]. Other authors state that polymeric matrix rather than cells undergoes lysis and produces short-chain organic compounds which are then converted to methane in anaerobic digestion [25]. In these tests, it is likely that toxins adsorbed in the extracellular matrix were released after exposure to the MW field due to breakage of the matrix structure. This could explain the degree of inhibition that is observed when digesting microwaved sludges, even though ultimately more methane is produced, due to more extensive solubilization of flocs and their matrix. Other possible explanation might be the lethal action of the MWs that inactivate bacteria in sludge. The 20 days might then be a necessary time period to regenerate bacterial flora, and obtain an intensive digestion process. Further work is being carried on to provide insight on this aspect.

Table 3.4 - Relative methane production increase in tests at 1% and 3% total solids sludge

Test	Total methane production (mL)	% increase	Test	Total methane production (mL)	% increase
TWAS 1%	375,95	0,0	TWAS 3%	969,38	0,0
TWAS 1% 50 AT	365,04	-2,9	TWAS 3% 50 AT	1008,82	4,1
TWAS 1% 100 AT	379,29	0,9	TWAS 3% 50 T	1023,86	5,6
TWAS 1% 50 T	407,65	8,4	TWAS 3% 100 AT	1042,5	7,5
TWAS 1% 100 T	414, 74	10,3	TWAS 3% 100 T	1062,44	9,6

3.5 Conclusions

The results clearly show that MW pretreatment increases the solubilization of organic material, as seen by solubilization of COD, proteins, and sugars, and the increase in small size particle fraction. These effects are more easily measured in the highest sludge concentration tested, because the magnitude of change is sufficiently high to make them statistically significant. Biodegradability of sludge exposed to MW increases, and more methane can be obtained from pretreated substrate.

Microwave athermal effects were observed. There was an increase in the amount of sCOD present in some samples. Soluble protein and sugar also show increases, with the intensity being an influential parameter in the athermal tests, since only tests with 100% intensity showed increases in soluble protein and sugar. Athermal effects are also detectable in the size distribution shift. The fraction of smaller particles increases relative to the control with exposure to radiation uncoupled from heating effects.

Inhibition phenomena seem to occur in the anaerobic degradation of microwaved sludge, both athermally or thermally pretreated. Although ultimately the yield in methane may be greater, methane is initially produced at a higher rate in tests with non-pretreated sludge.

3.6 Acknowledgements

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Chapter 4

Thermal and Athermal Microwave Radiation Effect on Soluble Organic Matter Distribution and Thermophilic Digestibility of Activated Sludge

Nuno M. Coelho, Kevin J. Kennedy, Ronald L. Droste

4.1 Abstract

Waste activated sludge was subjected to microwave (MW) pretreatment and athermal irradiation. The soluble phase of each type of sludge pretreatment was used in order to detect changes in the composition of the sludge as a result of the heating and athermal effect of MW pretreatment. Each soluble fraction was subject to ultrafiltration (UF) in series using progressively smaller pore sizes membranes (300, 100, 10 and 1 kDa) and each separate size fraction was further evaluated based on its anaerobic biodegradability. Results show that MW pretreatment solubilises a considerable amount of the suspended organic substrate, but athermal irradiation also causes solubilisation of organic matter, although at a smaller scale than MW. Proteins are particularly sensitive to athermal irradiation and both MW and athermal irradiation are capable of changing the size distribution of dissolved organic matter. Athermal irradiation and MW have a substantially different effect on thermophilic anaerobic biodegradability of the various size fractions obtained after UF. Slight inhibition and decrease in total biogas production was measured in some MW tests. Athermal irradiation does not cause a decrease in maximum biogas production rate in any test and slightly increases biogas production.

KEYWORDS: Athermal, Solubilization, Inhibition, Microwave, Thermophilic, Ultrafiltration

4.2 Introduction

Microwave (MW) heating is a relatively new method to thermally pretreat excess activated sludge prior to anaerobic digestion. In comparison with other thermal pretreatment methods, MWs can heat sludge faster and more economically than conventional heating methods, since heating occurs instantaneously and throughout the whole sample. Despite a moderate temperature profile, heating can be considered uniform in the whole sample. Heating can be controlled instantly, and the power regulated accurately. Finally, heating using MW is selective. The energy and heat will concentrate in the materials that have a high dielectric factor, reducing energy loss (Hong, 2002a; Metaxas and Meredith, 1983).

Thermal pretreatment causes disintegration of the matrix that comprises bacterial cell and extracellular polymeric substances (EPS) that are produced by the cells as part of their metabolic activity. This matrix, along with material retained inside cell walls are the substrate used in anaerobic digestion that undergo lysis and produces short chain organic compounds which are then converted to methane (Novak et al., 2003; Zhang and Bishop, 2003). Furthermore, breakdown of the matrix releases water entrapped in the floc, increasing the dewaterability of pretreated sludges (Neyens and Baeyens, 2003).

It was shown by several authors that the use of MW pretreatment significantly increases the soluble fraction of chemical oxygen demand (COD), and biogas production along with volatile solids (VS) removal in the anaerobic digestion process, with the added benefit of eliminating pathogens present in the sludge (Toreci et al., 2009; Eskicioglu, et al,, 2007; Hong, 2002, Coelho et al., 2011b). Previous works reported improvements in biogas production of 20 - 37% for sludge pretreated to the boiling point (96-100˚C) (Eskicioglu et al., 2007; Park et al., 2004), for higher temperatures some authors report higher biogas yields, in some cases reaching 60-70% more than non-pretreated sludge, while others report smaller increases (30%) with increasing

inhibition phenomena attributed to the formation of inhibitory compounds in the heating phase (Neyens and Baeyens, 2003; Toreci, et al., 2009).

MW pretreatment causes the release of organic matter enclosed inside bacterial cells but also transformation of macromolecules and aggregates of molecules into smaller units. Since hydrolysis of microbial mass and EPS within the activated sludge floc is believed to limit the rate and extension of degradation, changing colloidal and particulate organic matter into soluble substrate effectively causes an improvement in the rate of digestion (Kim et al., 2003). Activated sludge contains soluble organic products that are a result of the following events: bacterial metabolism of available substrate; excretion by bacteria in response to environmental conditions; release during lyse and degradation of bacterial cells; compounds that were present in the influent that were not subject to bacterial action. The size of the soluble products can vary over a wide range between 1 to 100 kDa. These soluble organic products often show a non-normal skewed molecular weight distribution (MwD) with a predominance of a very low Mw fraction in the case of the influent. However, after treatment (activated sludge or anaerobic digestion), a bimodal distribution is observed, with a large quantity of soluble matter with low Mw (< 1kDa) along with a large quantity of large Mw products with cut-off sizes that can vary between 50 - 200 kDa, and small quantities of soluble matter in between these two peaks (Barker and Stuckey, 1999; Boero et al., 1996).

MW pretreatment as well as the majority of pretreatments, causes a shift in the MwD of organic substrate, increasing the fraction of smaller sizes. Smaller soluble organic compounds are often correlated with an easier or faster degradation since less or no hydrolysis is required. In the same way, different biodegradabilities of similar sludges may be partially explained by the distribution of particle sizes for the particular sludge (Dulekgurgen et al., 2006, Karahan et al., 2008, Leiviskä et al., 2008). A standardized method for measuring the particle size distribution/MwD is not yet

available, making it difficult to compare results. Two main techniques are presently used to determine MwD of wastes, either as a continuous distribution determined by gel permeation chromatography (GPC) or a discrete distribution determined by ultrafiltration (UF). UF shows some advantages compared to GPC, as it does not require evaporation or freeze-drying to concentrate samples, which could alter the size distribution. UF also allows the use of larger volumes of sample, with consequent larger volumes of filtrate that can be characterized. Additionally, UF units can be operated in series or in parallel. Parallel operation can reduce the time to filter a sample through several pore sizes filters; however, series filtration shows less problems with clogging when using small pore size filters (Barker and Stuckey, 1999).

The improvement in the digestion process after MW pretreatment is mainly due to the heating effect of microwaves (Coelho et al., 2011a, Shazman et al., 2007), nevertheless, some effects not directly related with heat are routinely reported by several authors. Some authors reported changes in physiological characteristics of microorganisms after exposure to MW (Rai et al., 1995; Singh et al., 1994), increases in the inactivation rate of microorganisms in comparison with conventionally treated samples (Dreyfuss and Chipley, 1980; Hong et al., 2004), increases in microbial biological activity such as biogas production or growth rate (Grundler et al., 1992; Grundler and Keilmann, 1983; Banik et al., 2006). These phenomena are commonly explained by the existence of an athermal effect, because it occurs independently of temperature rise in media irradiated with MW. Despite these studies, the existence of an athermal effect caused by MW is far from consensual. Several studies claim that there is no evidence of an athermal MW effect and that the biocidal effects of MW are either due entirely to heating or are indistinguishable from external heating (Fujikawa et al., 1992; Jeng et al., 1987; Welt et al., 1994) . An explanation for the differences in results regarding the athermal effect is that it is difficult to dissociate MW irradiation and temperature increase, making it difficult to isolate thermal and potentially

athermal effects (Banik et al., 2003, Sato et al., 1996). This difficulty was circumvented by Sato et al. (1996) who devised a system that isolated the MW irradiation and the increase in temperature in the irradiated medium. This allows more reliable studies about the presence or not of athermal effects since the magnitude of the thermal effect -the changes it causes in physical, chemical and biological properties - sometimes hides the presence of athermal effects that have a considerably smaller impact in those same properties (Coelho et al., 2011b).

Considering some of the theories regarding MW athermal effect, it is possible that this effect alone might cause some changes in the MwD of soluble substrate. Ion-shifts across membranes and reorientation of long-chain molecules coupled with movements of the polarized side chains of large molecules with consequent breakage of hydrogen bonds and alteration of hydration zones can cause destruction of structures in polyatomic molecules such as proteins and phospholipids, leading to division of macromolecules in smaller units (Barnes and Hu, 1977, Straub and Carver, 1975, Stuerga and Gaillard, 1996a; 1996b).

In order to investigate the influence of thermal and athermal effects in MwD plus the influence of pretreatment in the biodegradability of several fractions when digested in thermophilic conditions, pretreated, non-pretreated and athermally pretreated sludges were ultrafiltered and the various fractions were characterized and compared in terms of biodegradability and biogas production modelled.

4.3 Materials and methods

4.3.1 Sample preparation and MW pretreatment

Thickened waste activated sludge (TWAS) was obtained from the Ottawa municipal wastewater treatment plant, situated in Gloucester, ON. This wastewater treatment plant performs

preliminary and primary treatments followed by a conventional activated sludge process, at an average sludge retention time (SRT) of 5 d. Ferric chloride is added to the sludge for phosphorus removal prior to thickening. Sludge characteristics at the time of sampling are given in Table 4.1:

Table 4.1 - Sludge characteristics at the time of sampling

Parameter	[a]
pH	7.9
TS (% w/w)	4.3 (0.2)
VS (% w/w)	2.9 (0.2)
VS/TS	0.67 (0.00)
TCOD (mg/L)	54,602 (2429)
SCOD (mg/L)	4331 (250)
SCOD/TCOD	0.08 (0.00)

[a]Data represent arithmetic mean of duplicates (absolute difference between mean and duplicates)

Sludge collected at the treatment plant was subjected to MW pretreatment and MW athermal irradiation without any dilution. Previous studies demonstrated that MW pretreatment has a higher efficiency when sludge concentration is high (Eskicioglu et al., 2007). Higher concentrations of sludge were also thought to be more favourable in order to detect athermal effects, given the smaller magnitude of these effects.

Two types of MW pretreatment were tested in this experiment. MW conventional pretreatment was performed using a commercial domestic MW oven (Sanyo EM-S759S P=1350 W, 2450 MHz). Samples were weighed and in each of the thermal tests, 200 g of sludge were irradiated and allowed to heat. The temperature was allowed to rise until a final temperature of 96ºC was reached to prevent sludge losses by boiling. To avoid temperature gradients, the sludge vessel was continually mixed by a stirrer, made of MW transparent material. The stirrer was driven by a shaft coupled to an electrical motor and was operated at 150 rpm. The electrical motor and part of the shaft were placed outside the MW cavity and the shaft was introduced through a hole in the

metal cage of the oven. MW losses through the hole made in the cavity were minimized by using MW attenuators. MW losses were measured using a MW leak detector (EMF Inc. model MD-200) and were below 5 mW/cm^2 at the surface of the oven (safety limit recommended by USFDA). A maximum temperature immediately below the boiling point was chosen to minimize vaporization of liquid. After this temperature was reached, no further radiation was supplied. The samples were not covered during MW exposure, but were covered while cooling, to reduce losses of water and volatile compounds. Water lost by evaporation was replaced by distilled water.

In the case of MW athermal tests (AT), the same MW oven was used but with modifications in order to maintain the temperature low and constant in the sludge being irradiated (Welt et al., 1994; Sato et al., 1996; Coelho et al., 2011b). Changes to the oven consisted of installing a loop in which a MW transparent apolar solvent (kerosene) used as coolant was circulated which ensured cooling of the samples while at the same time not interfering with the action of the MW field in the samples. Heat was removed from the coolant by passing it through an external ice bath. The set-up of the AT system was identical to the one used in previous tests (Coelho et al., 2011b). This modification allowed temperature profiles in tested sludges to remain practically unchanged during the whole irradiation period as reported in a previous experiment (Coelho et al., 2011b). The remaining procedures were similar to the ones used in the MW conventional test viz., the volume of sludge used in each batch and control of the weight of the sample before and after treatment to check for any substantial loss of water.

4.3.2 Sludge Supernatant Ultrafiltration

The pretreated samples [conventional MW (CMW) and athermal MW (AMW)] plus the control sample (non microwaved sludge (NMW)) were centrifuged at 6300g (International Refrigerated Centrifuge, Model B-20, International Equipent Co., USA) to separate the supernantant, and this supernatant was subsequently filtered using 0.45 µm pore size disc filters (GN-6 Metricel S-Pack membrane). Previous assays with UF membranes showed that dilution was advisable to avoid immediate clogging of the pores. Supernatant filtered at 0.45 µm was then diluted with distilled water in a ratio of 6.25:1 before performing UF assays. The supernatant filtered at 0.45 µm was characterized prior to UF tests by measuring soluble COD (sCOD), soluble protein and soluble sugars. Colorimetric COD measurements were performed using Standard Methods procedure 5250D (APHA, 1995) with a Coleman Perkin-Elmer spectrophotometer Model 295 at 600 nm light absorbance. Total soluble protein was measured according to the procedure described in Bradford (1976) using bovine serum albumine (BSA) as the standard. Determination of total soluble sugars was performed using the phenol-sulphuric acid test method proposed by Benefield and Randall (Benefield and Randall, 1976), with glucose used as a standard.

After filtration, and dilution, the samples were then subject to UF. To perform these assays, Amicon model 8400 stirred cells (Amicon Corp., MA) with a 400 mL reservoir were used, along with high recovery, low organic adsorption hydrophobic membranes (Millipore, MA). Four different types of membranes with different cut-off sizes were used. The Mw cut-off sizes used were 300, 100, 10, and 1 kDa. These membranes were used in a cascade series (Figure 4.1) to provide a UF process with smaller risks of clogging membranes with low cut-off sizes.

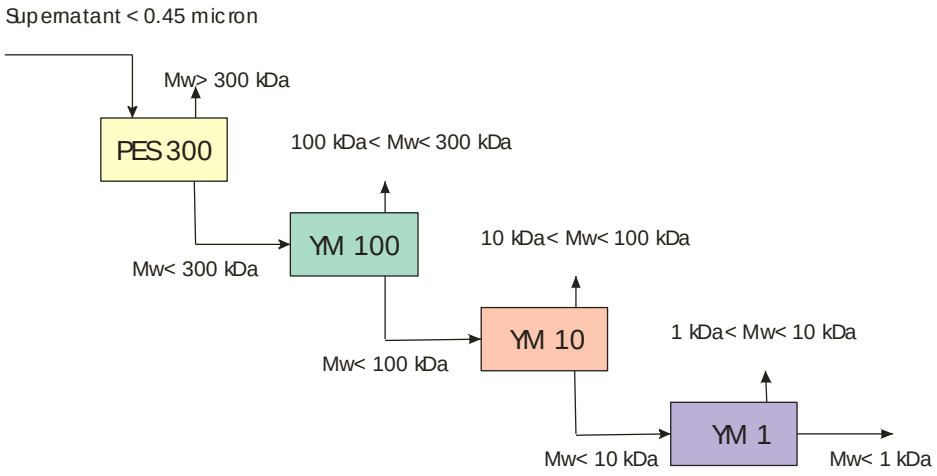


Figure 4.1 - Cascade series set-up of UF units for determination of apparent molecular weight distribution (AMwD).

The membranes used in the UF process were first rinsed with Mili-Q water for 60 minutes with 3 water changes in that period. The membranes were then placed in the UF units and the unit was loaded with 300 mL of liquid to filter. After the unit was closed, pressure was supplied in the form of nitrogen gas to create a driving force to allow UF to occur. The pressure applied was 10 psi for membrane number PES300 and 20 psi for the remaining three as recommended by the manufacturer. After UF had started, the process was stopped when the liquid remaining inside the UF unit was 30 mL (10% of the original volume). The unit was depressurized and the cell was stirred for 15 additional minutes to improve recovery of molecules adsorbed on the membrane surface. After this procedure, the 30 mL remaining inside the cell was collected and labelled as retentate. The used membranes were soaked in a bath of 0.1 M NaOH solution for 1 hour and then rinsed with Milli-Q water before being used in another UF cycle. These cycles were repeated until sufficient volume of filtrate and retentate was obtained to perform all the assays and measurements. Membranes were kept in a 10% (v/v) ethanol-water solution at 4˚C when not in use as recommended by the manufacturer.

4.3.3 Biodegradability tests

The measurement of biodegradability of each UF fraction of retentate and permeate was performed using biological methane potential tests (BMP). These tests were done using 125 mL serum bottles (Wheaton borosilicate glass, VWR, Montreal, Canada), sealed with butyl rubber stoppers and crimped with aluminum caps. To test all permeates and retentates produced by the three different types of sludge [CMW, AMW and NMW (control sludge)], and using duplicates in all the tests, a total of 50 serum bottles was necessary. The conditions tested in each bottle are shown in table 4.2.

Table 4.2 - Test conditions for UF biodegradability test

Test*	Phase	Inoculum (mL)	Membrane**	Sludge type	Phase vol. (mL)
1	Perm.	15	M1	Control	70
2	Perm.	15	M2	Control	70
3[a]	Perm.	15	M3	Control	70
4	Perm.	15	M4	Control	70
5	Ret.	15	M1	Control	70
6	Ret.	15	M2	Control	70
7	Ret.	15	M3	Control	70
8	Ret.	15	M4	Control	70
9	Perm.	15	M1	MW	70
10	Perm.	15	M2	MW	70
11	Perm.	15	M3	MW	70
12	Perm.	15	M4	MW	70
13	Ret.	15	M1	MW	70
14	Ret.	15	M2	MW	70
15	Ret.	15	M3	MW	70
16	Ret.	15	M4	MW	70
17	Perm.	15	M1	AT	70
18	Perm.	15	M2	AT	70
19	Perm.	15	M3	AT	70
20	Perm.	15	M4	AT	70
21	Ret.	15	M1	AT	70
22	Ret.	15	M2	AT	70
23	Ret.	15	M3	AT	70
24	Ret.	15	M4	AT	70
25	Inoc.	15	-	-	-

*all tests were made in duplicate; M1 (PES 300) MwCO = 300 kDa; M2 (YM 100) MwCO = 100 kDa; M3 (YM 10) MwCO = 10 kDa; M4 (YM 1) MwCO = 1 kDa; [a] test 3 was not performed.

In each serum bottle, 15 mL of inoculum was added to 70 mL of permeate or filtrate depending on the case. The inoculum was obtained from thermophilic sludge collected from the Annacis Island Wastewater Treatment Plant (Vancouver, BC). After addition of a mixture containing equal parts of $NaHCO_3$ and $KHCO_3$ to achieve an alkalinity of 4000 mg/L (as $CaCO_3$), the bottles were sparged for one minute with nitrogen gas and sealed. Biogas volumetric production was measured daily by puncturing the rubber septum with a thin needle and measuring displacement in a water column manometer, and its composition was determined with an HP 5710A GC with metal packed column (Chromatographic Specialties Inc., Brockville, ON, Porapak T, packing mesh size: 50/80, column length, OD: 304.8cm, 0.635 cm) and thermal conductivity detector (oven, inlet and outlet temperatures: 70, 100, and 150^{o}C, respectively) using helium as the carrier gas (flowrate: 25 mL/min). Serum bottles were kept in a darkened temperature-controlled incubator shaker at 55 ± 2ºC and 90 rpm until they stopped producing biogas. The data obtained in the biodegradability tests were used to calculate biodegradation rates of permeate and retentates for the different AMW fractions and adjust kinetic models to biogas production. To perform this, Microsoft Excel© Solver was used, along with Aquasim (Reichert, 1998) modelling software.

4.4 Results

4.4.1 Effect of pretreatments on solubilization

It was shown previously that MW pretreatment causes the breakdown of isolated particulate organic matter from sludge, but also causes the destabilization and breakdown of the EPS matrix that comprises the activated sludge floc, releasing soluble compounds to the bulk of the liquid (Coelho et al., 2011b; Toreci et al., 2008). The bacterial cells that form the flocs are also affected, with the breakdown of the cellular wall and subsequent release of intracellular material. Even

though some of the released matter still can be considered colloidal, a significant portion of this matter is transformed into molecules small enough to be considered soluble organic matter. The measurements made after exposing sludge to MW pretreatment confirm the solubilising effects of MWs since a great increase of sCOD (< 0.45 µm) was measured as shown in Figure 4.2. The relative increase in the amount of sCOD is approximately 230% compared with the control (non pretreated sludge). The breakdown of Mw fractions after athermal and normal MW pretreatment is given in Table 4.3.

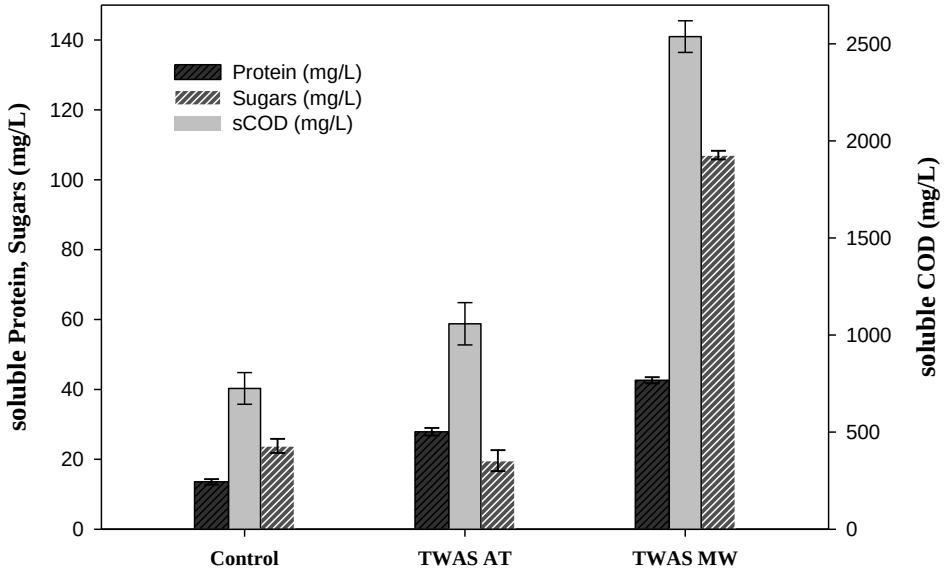


Figure 4.2 - Sludge characteristics after pretreatment and before UF fractionation (error bars indicate 95% confidence interval).

This increase was achieved by release and breakdown of organic molecules that include proteins (total soluble protein after MW pretreatment of 42.6 ± 0.89 mg/L, relative increase of 213%) but mainly by the increase in soluble sugars (final concentration of 107.1 ± 1.2 mg/L, relative increase: 349%). It is generally agreed that proteins and carbohydrates are the main components

of the EPS matrix; however, the main component or the ratio at which they are present is variable, and operating conditions can affect which component is predominant (Sponza, 2003). In this case, the amount of carbohydrates is substantially more than the amount of proteins, assuming all or at least most substances present in the EPS matrix were released, which agrees with observations done previously that found a higher ratio of carbohydrates/proteins for young sludges (SRT $\leq$ 5 d) compared to older sludges (Liao et al., 2001).

The presence of an athermal effect is detectable by measuring the changes that occurred after athermal irradiation tests. Soluble COD increases after the test compared to the control (approximately 38%), and this difference is statistically significant (*t-test*; $\alpha = 0.05$, $P = 0.0253$ for $\mu 1 = \mu 2$). The amount of sugars measured in the soluble phase actually decreased after ATs. This decrease was approximately 18%, but the difference between values before and after athermal irradiation is not enough to be considered significant (*t-test*; $\alpha = 0.05$, $P = 0.0634$ for $\mu 1 = \mu 2$). However, for soluble proteins, a noticeable increase in their soluble concentrations was detected after exposing sludge to athermal tests. This increase doubled the concentration of soluble protein present (increase of 105%), to a final concentration of 27.9 ± 1.1 mg/L, and the change is statistically significant (*t-test*; $\alpha = 0.05$, $P = 0.0139$ for $\mu 1 = \mu 2$). The different behaviour of sugars and proteins in athermal tests might be linked to their structural properties. It was reported that the MW field causes polarized chains of molecules to align with the direction of the electrical field and this violent motion of dipoles can lead to breakage of hydrogen bonds that are very important in acquiring and maintaining the folding structure of macromolecules such as proteins and phospholipids (Fleming, 1961; Stuerga and Gaillard, 1996b). This effect can occur without any rise in temperature, thus it can be one of the sources of changes (viz., denaturation and solubilisation of protein) in characteristics of sludge due to the athermal effect

of MW radiation. Another conclusion that can be drawn from solubilisation data is that the magnitude of the athermal effect by itself (especially for sCOD and sugars) is significantly smaller than the effects detected when using MW treatment with temperature increase. The differences in the magnitude of the different effects can also contribute to the difficulty in determining and detecting the presence of athermal effects, since the thermal effects can in some way hide or mask small changes caused by other effects occurring in sludge irradiated by MWs, as reported in previous works (Coelho et al., 2011b).

4.4.2 Molecular weight distribution of soluble and solubilised matter

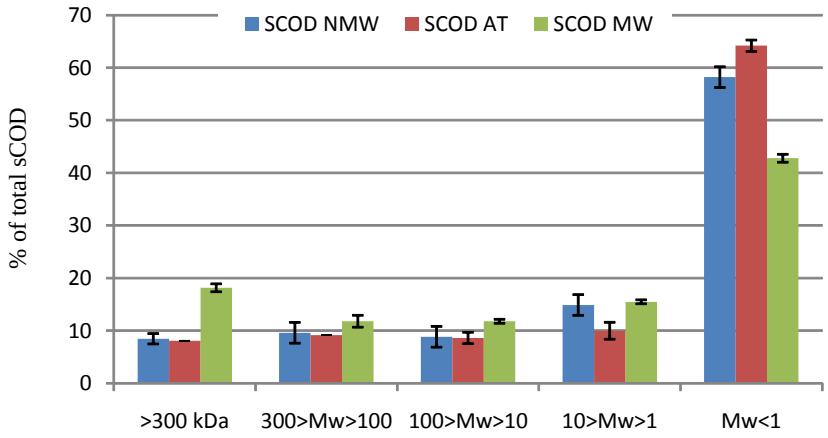
The results obtained after UF of the soluble fractions of the three different sludges used in this study (Figure 4.3 a-b, Table 4.3), show that MW pretreatment has a significant effect on MwD of sCOD, protein, and sugars, but athermal pretreatment also causes a noticeable change in the way a portion of soluble compounds is spread over the Mw cut-off sizes that were used.

In control sludge (NMW), most of sCOD is comprised of small particles, since close to 60% of all sCOD was measured below Mw<1 kDa. The MwD of dissolved organic matter (DOM) is skewed to the interval of smaller size, since all fractions above 10 kDa are relatively similar in value and each is always below 10% of the total sCOD, while the sum of all DOM < 10 kDa (adding Mw<1 kDa and 10 > Mw > 1 kDa) accounts for 73.2% of the total sCOD.

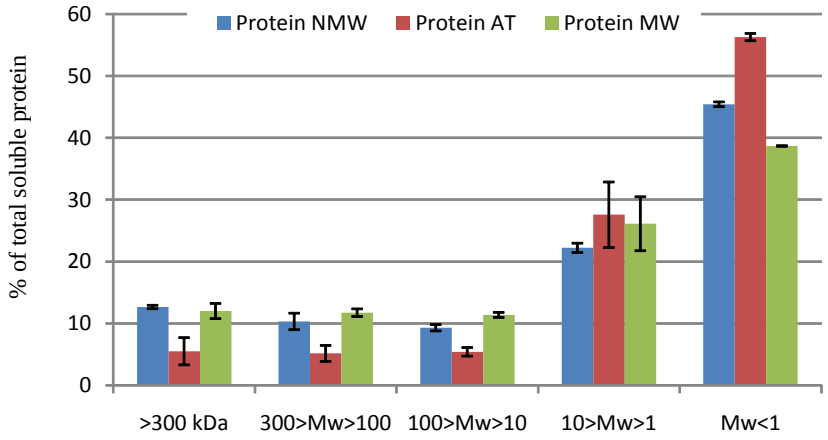
Table 4.3 - Apparent molecular weight distribution (AMwD) of soluble substrate in UF samples.

	Before UF (mg/L)	After UF (mg/L)	Mw>300 kDa (% w/w)	300>Mw>100 (% w/w)	100>Mw>10 (% w/w)	10>Mw>1 (% w/w)	Mw<1 (% w/w)
sCOD							
Control	767±33	827±34	8.5±1.0	9.6±2.0	8.8±2.0	14.9±2.0	58.2±2.0
TWAS AT	1058±133	1519±46	8.1±1.1	9.2±1.2	8.6±1.1	10±1.6	64.2±1.1
TWAS MW	2162±82	2538±36	18.2±1.0	11.8±1.1	11.8±0.4	15.5±0.4	42.8±0.8
Sugars							
Control	24±2.3	23±1.9	15.3±0.8	9.1±1.0	8.3±1.5	67.3±8.2	0.0±0.4
TWAS AT	20±1.0	20±0.8	13.0±0.4	9.3±3.4	9.4±1.0	65.4±2.3	2.8±1.0
TWAS MW	108±1.2	81±0.4	15.1±0.0	13.1±0.5	13.1±0.1	48.0±0.2	10.8±0.0
Protein							
Control	14±0.3	26±0.2	12.7±0.3	10.3±1.3	9.3±0.5	22.2±0.8	45.2±0.4
TWAS AT	28±5.4	33±0.9	5.5±2.2	5.2±1.3	5.4±0.7	27.6±5.3	56.3±0.6
TWAS MW	43±0.9	68±2.0	12.0±1.2	11.8±0.6	11.4±0.4	26.1±4.4	38.7±0.1

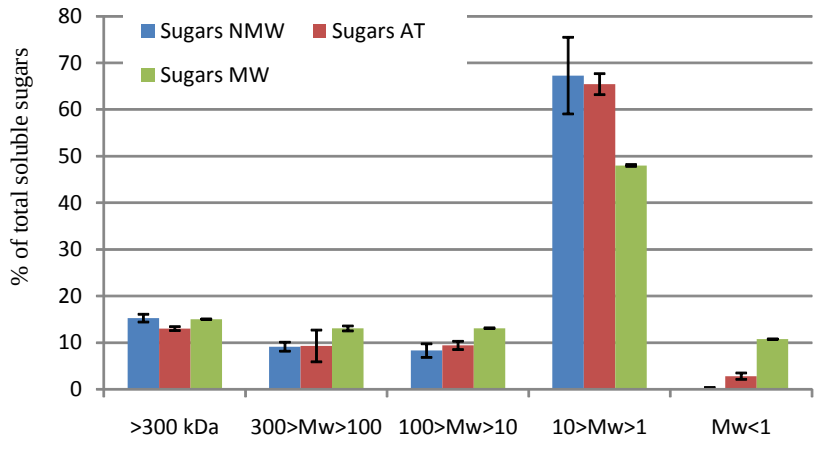
MW – Microwave pretreatment; Mw – Molecular weight



(a)



(b)



(c)

Figure 4.3 - AMwD of soluble dissolved matter after ultrafiltration (a) – total soluble COD, (b) – soluble protein, (c) – soluble sugars. Mw expressed in kDa.

This result is in contrast with previous reports indicating bimodal distributions with a significant or prevalent fraction of high MW compounds (Manka and Rebhun, 1982; Grady et al., 1984; Namkung and Rittmann, 1986). However, it is consistent with results obtained by Toreci et al. (2009) which used sludge from the same origin. Other researchers also reported that small Mw compounds were predominant in the soluble phase of the sludge (Levins, 1971; Pribyl et al., 1997). MwD varies significantly with the type of process being used in secondary treatment (namely suspended biomass or fixed biomass) and also with operating conditions such as SRT. The sludge used in this study was a young sludge (SRT = 5 d), and young sludges are reported to produce a high amount of soluble microbial products of low Mw, in part because endogenous decay is not the main process occurring in the aeration tank when low SRTs are applied (Boero et al., 1996). MW pretreatment causes a noticeable increase in the relative amount of sCOD with higher Mw, more than doubling the relative amount of sCOD for Mw > 300 kDa. The total increase in this interval was 460% compared to the concentration measured before MW pretreatment. The intermediate Mw intervals distribution between the larger and smaller sizes also had a small increase relative to the control, but not as significant as for the largest Mw, making the distribution after MW pretreatment bimodal. An explanation for the large increase in the higher Mw sCOD fraction might be due to breakage of bacterial cell walls and sludge flocs that release large compounds as fragments of the cell wall, nucleic acids and polymers attached to the sludge floc matrix. The increases in the intermediate sizes indicate that part of the material released when flocs and cells are destroyed does not have high Mw, or that part of the high Mw material is further divided into smaller units. Absolute increases in all fractions above 1 kDa in the total sCOD in each interval were 460, 221, 249 and 172% for Mw>300,300>Mw>100, 100>Mw>10 and 10>Mw>1 kDa, respectively, with the smaller increase measured for the smaller Mw (< 1kDa). The smaller increase for Mw<1kDa means that the percentage of sCOD

with Mw smaller than 1 kDa actually showed a decrease in its weight relative to the total even though there was a increase in the total amount of sCOD in this interval, since 42.8 ± 0.8% of sCOD of the MW sludge is contained in this size class, compared to 58.2 ± 2.0% for the control sludge.

MW pretreatment also causes soluble protein concentration to rise in all Mw intervals tested, with significant increases specially for all intervals above 1kDa; however, the distribution does not change significantly compared to the control sludge except for the smaller Mw class. The change measured in the class 10>Mw>1 (immediately above the smaller one) is not statistically significant (*t-test*; $\alpha = 0.05$, $P = 0.1823$ for $\mu 1 = \mu 2$). The percentage of total soluble protein present in the interval Mw < 1 kDa, is smaller than the control sludge. This interval, similarly as for sCOD, showed the smallest increase in total soluble protein concentration of all intervals (145%).

MW also increased the amount of sugars solubilized in every size class, and changed the way sugars were distributed; however, in this case, initially, there were no sugars measured in the control sludge at the smaller size category, and there seems to have been a displacement of some particles from the second smallest size class to the smallest, since after MW pretreatment, 10.8% of total sugars ended up in the smaller membrane filtrate, while the fraction immediately above registered the smallest increase in soluble sugar from the 5 that were measured.

Athermal pretreatment also causes changes not only in the amount of total sCOD present, but also in the AMwD. The increase in total sCOD present after athermal pretreatment itself is a strong indicator of the presence of phenomena that are not related to temperature increase during MW irradiation. However, beyond the increase in sCOD there were also changes in the distribution of DOM. There is an increase of the concentration of sCOD in all intervals, but

increase is more pronounced for Mw < 1 kDa. This percentage increase is similar in magnitude to the one measured for MW pretreatment, which hides the changes in the AMwD of sCOD for higher Mw. Only for the smaller Mw fraction, is the AMwD for athermal assays greater than for the control. The athermal effect is more evident when analyzing the results obtained for soluble protein, since, when comparing dissolved protein and sCOD, the difference between control and athermal sludge AMwD is more pronounced for Mw < 1 kDa (10.86% for protein, 5.98% for sCOD). There is also a noticeable increase in the value for 10>Mw>1, in contrast to what was measured for sCOD, but the difference in the average value for athermal and control is not statistically significant (*t-test*; $\alpha = 0.05$, $P = 0.0508$ for $\mu 1 = \mu 2$). Contrary to sCOD, not all Mw intervals had increased protein concentration. Higher Mw intervals (> 300 kDa and 300>Mw>100) had a significant decrease in the soluble protein concentration (–20.4 and –8.6%, respectively), which naturally were reflected in the AMwD for those intervals, concomitantly showing a smaller value than what was calculated for control or MW sludge. Athermal pretreatment does not seem to significantly affect the AMwD for sugars in the soluble phase. Even though the amount of sugars solubilized after athermal pretreatment was negligible, it was hypothesized that athermal radiation could still change the AMwD of sugars already solubilized. When analyzing the results, it is clear that changes in AMwD for sugars due to athermal radiation did not occur. All size classes above the smallest one (Mw < 1 kDa) did not change significantly. For Mw < 1 kDa, there is a change in AMwD but the amount is minimal (only 3%).

This indicates that, although athermal radiation can cause disruption of the matrix, or at least partial disruption of the compounds surrounding bacterial cells that aggregate particles like proteins, sugars or humic substances, and can also break higher Mw particles into molecules of smaller Mw, this effect seems to apply specially to proteins, or compounds that include proteins

(as glycoproteins) since no change was detected in the total concentration of dissolved sugars or in the AMwD of those sugars. Aditionally, athermal radiation *per se* does not seem to be able to break cellular walls or large flocs, since no particles of Mw > 100 kDa were released after athermal pretreatment.

4.4.3 Biodegradability of filtered fractions

Each of the fractions produced using UF (permeates and retentates) were subject to a biodegradability test, in duplicate, using thermophilic sludge inoculum, with the average cumulative biogas production used to calculate ultimate biodegradabilty and substrate removal rates. When calculating substrate utilization rates and biogas production rates, in anaerobic biodegradability tests, several models are available, but first-order kinetics is normally used since it requires much less information than structured models (such as ADM1) and it describes the process reasonably well. First-order models were used previously to model biogas production and substrate utilization rate with MW pretreated sludge (Toreci et al., 2009; Eskicioglu et al., 2006), with the substrate utilization rate expressed as:

$$r_s = \frac{dS}{dt} = -kS \qquad (1)$$

And organic substrate removed as:

$$Y_t = L(1 - e^{-kt}) \qquad (2)$$

With Y_t as the organics removed (mg COD/L) at time t and L being the ultimate biodegradable organics (mg COD/L). However, the first-order model, despite returning regression coefficients (r^2) above 0.9 for all tests, failed to adequately describe the complete biogas production curve for the assays performed in this study, especially for the initial stage of digestion, where frequently a lag period with no or very little production of biogas was observed, but also in the maximum biogas production stage, with calculated biogas production rates lower than the ones observed.

This type of biogas production pattern, characterized by a lag period in the initial stage of digestion, followed by a substrate degradation activity similar to control sludge, is typical of inhibition phenomena that are temporary and reversible. Microorganisms manage – after a lag time that can be longer or shorter depending on the inhibition compound or its concentration – to acclimate to the inhibitory compound and regain metabolic activity that is very similar to the activity measured when degrading substrate that is not inhibitory. In most cases, all the substrate initially degraded without the presence of inhibition is also degraded when this type of inhibition occurs (Rozzi and Remigi, 2004).

In order to better adjust the predicted curve to the observed values, the Gompertz equation was used. This equation was used to describe the growth of *Lactobacillus plantarum* and *Lactobacillus acidophilus* (Cho et al., 1996; Zwietering et al., 1990). The equation is:

$$N = \int_0^t r_g \, dt = A * exp\left(-exp\left[\frac{\mu_m * e}{A}(\lambda - t) + 1\right]\right) \quad (3)$$

This expression can be transformed to calculate cumulative methane production by substitution and modification of the original terms of the Gompertz equation as demonstrated by Lay et al. (1998).

Bacterial growth rate (r_g) and substrate utilization rate (r_{su}) are related according to:

$$r_g = Y_1 * (-r_{su}) \quad (4)$$

While substrate utilization and methane production are related according to the following expression:

$$-r_{su} = Y_2 * r_m \quad (5)$$

Given equations 4 and 5, methane production rate can be defined as:

$$-r_m = \frac{r_g}{Y_1 * Y_2} \tag{6}$$

So the cumulative methane production rate can be expressed as:

$$M = \int_0^t r_m \, dt = \int_0^t \frac{r_g}{Y_1 * Y_2} dt = \frac{1}{Y_1 * Y_2} \int_0^t r_g \, dt \tag{7}$$

Now replacing the term for $\int_0^t r_g \, dt$ in equation (3) we have the resulting expression for cumulative methane production:

$$M = \frac{A}{Y_1 * Y_2} * exp\left\{-exp\left[\frac{\frac{\mu_m}{Y_1 * Y_2} * e}{\frac{A}{Y_1 * Y_2}}(\lambda - t) + 1\right]\right\} \tag{8}$$

$\frac{A}{Y_1 * Y_2}$ can be replaced by the term *P* (methane production potential) and $\frac{\mu_m}{Y_1 * Y_2}$ is the maximum methane production rate that is equal to the specific methane production rate (*SMA*) multiplied by the biomass present in the vial *(B)*. These substitutions result in a final expression for cumulative methane potential:

$$M = P * exp\left\{-exp\left[\frac{B * SMA * e}{P}(\lambda - t) + 1\right]\right\} \tag{9}$$

With *P* being the methane/biogas production potential (mL CH_4 or mL biogas), *B* the biomass concentration (g VSS), *SMA/SBA* the specific methanogenic/biogas activity (mL CH_4/g VSS.d) and λ the lag-phase time duration (d). Equation 9 was then used as an alternative expression to model methane production on biodegradability tests, with better results than those obtained when using a first-order model.

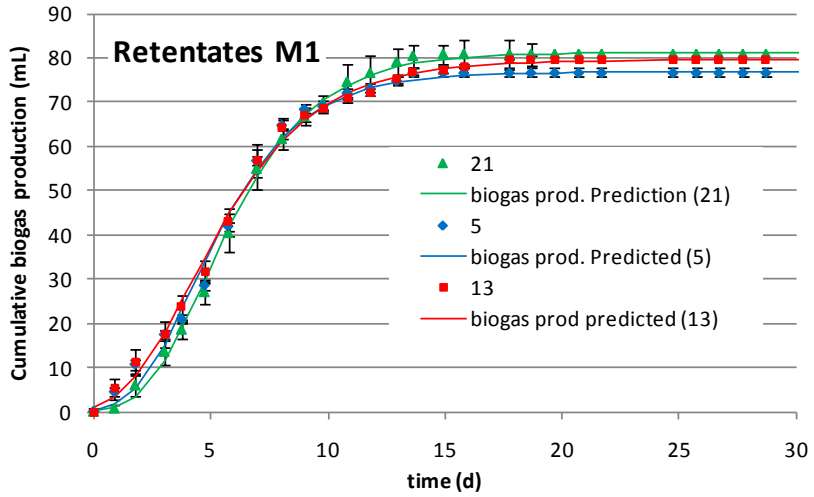
Previous works with MW pretreated sludge biodegradation assumed biogas production as a first-order reaction and returned correlation coefficients (r^2) between 0.90-0.98 for Toreci et al. (2009) and 0.97 -0.99 for Eskicioglu (2006). Some of those tests even if they had high correlation coefficients, showed a clear mismatch between model and experimental values when visually

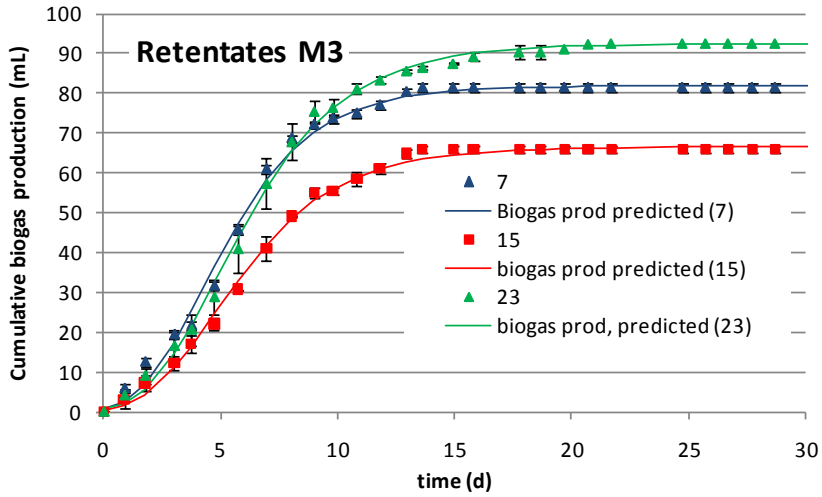
compared, especially in the initial days of digestion, despite the use of acclimated sludge This forced the use of another kinetic model (zero-order) for the exponential phase of biogas production (Toreci et al.,2009) to return biodegradation rates not affected by lag periods. In this study however, all the tests returned a correlation coefficient (r^2) above 0.99 and the visual match between observed and model values is very good. The experimental results are shown in Table 4. The results are shown along with the confidence interval (95%) of each parameter estimate.

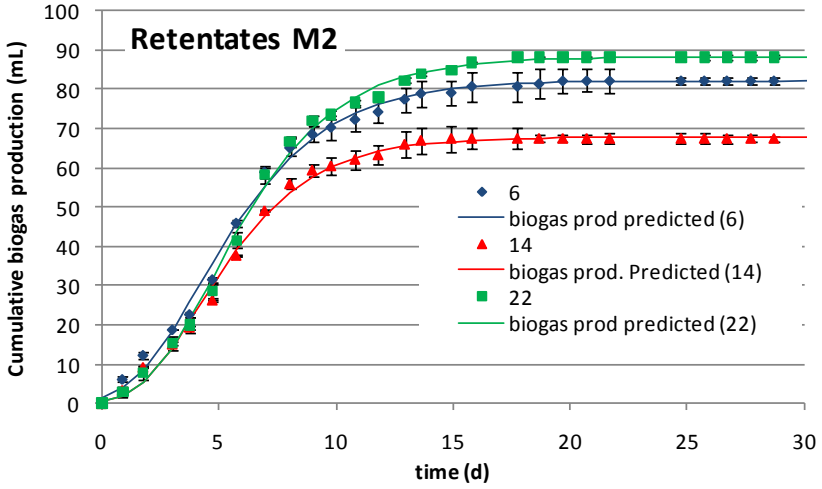
Table 4.4 - Calculated biodegradation rates for retentates and permeates from UF tests.

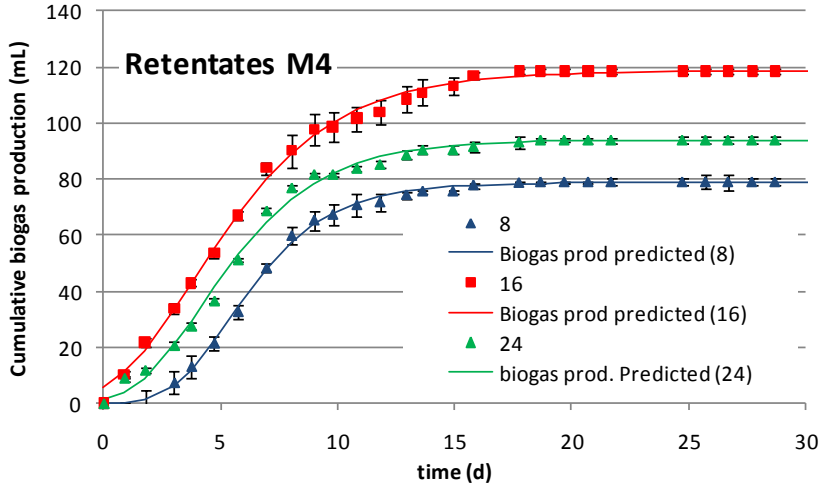
Test	SBA (mLbiog/gVSS.d)	P (mL biogas)	λ (d)	r^2	D^*	X^{2**}	df	goodness of fit
1	28.75 ± 0.76	78.3 ± 0.63	2.30 ± 0.12	0.9984	0.97	10.41	13	Accepted
2	32.02 ± 0.47	74.4 ± 3.03	2.47 ± 0.25	0.9975	0.84	28.81	18	Accepted
4	29.09 ± 0.35	67.6 ± 0.12	3.16 ± 0.14	0.9981	1.09	11.74	16	Accepted
5	30.26 ± 1.26	76.8 ± 0.24	1.74 ± 0.11	0.9952	0.88	12.49	15	Accepted
6	27.26 ± 0.26	82.2 ± 5.67	1.23 ± 0.10	0.9966	1.08	5.63	15	Accepted
7	30.92 ± 0.89	81.8 ± 1.05	1.56 ± 0.03	0.9946	0.90	12.74	14	Accepted
8	32.15 ± 1.66	78.7 ± 2.10	2.87 ± 0.30	0.9981	1.37	2.08	15	Accepted
9	27.83 ± 0.07	74.7 ± 0.12	1.89 ± 0.05	0.9959	1.03	12.33	14	Accepted
10	26.90 ± 0.50	66.5 ± 0.03	2.65 ± 0.08	0.9985	1.21	4.46	15	Accepted
11	21.68 ± 1.42	54.9 ± 2.47	3.25 ± 0.26	0.9978	1.14	1.28	14	Accepted
12	27.59 ± 0.04	89.2 ± 3.94	1.28 ± 0.30	0.9992	1.85	2.00	16	Accepted
13	27.04 ± 2.19	79.5 ± 1.68	1.29 ± 0.19	0.9975	1.11	4.18	16	Accepted
14	25.39 ± 0.77	67.8 ± 4.55	1.58 ± 0.08	0.9970	0.97	5.05	13	Accepted
15	22.52 ± 0.59	66.4 ± 1.17	1.81 ± 0.20	0.9971	0.83	5.23	14	Accepted
16	34.42 ± 2.58	118.6 ± 2.25	0.44 ± 0.21	0.9976	0.94	6.53	15	Accepted
17	37.55 ± 0.24	79.3 ± 0.22	2.66 ± 0.22	0.9960	0.72	42.12	15	**Rejected**
18	37.83 ± 0.45	77.6 ± 0.22	2.73 ± 0.22	0.9961	0.83	24.37	15	Accepted
19	37.00 ± 0.41	79.1 ± 1.93	2.16 ± 0.04	0.9950	0.82	26.11	16	Accepted
20	36.54 ± 1.43	72.2 ± 6.17	2.96 ± 0.25	0.9963	0.77	33.25	15	**Rejected**
21	30.99 ± 1.33	81.1 ± 3.13	2.16 ± 0.15	0.9988	1.15	2.87	14	Accepted
22	30.04 ± 1.82	88.2 ± 2.00	1.89 ± 0.30	0.9979	0.98	3.42	15	Accepted
23	30.29 ± 3.30	92.4 ± 2.75	1.86 ± 0.25	0.9977	0.85	5.87	15	Accepted
24	32.71 ± 1.50	93.9 ± 6.25	1.35 ± 0.30	0.9958	1.06	10.75	14	Accepted

*Durbin-Watson test; **Chi-square test assumes 95% confidence level


Retentates M1
Cumulative biogas production (mL)
time (d)
21
biogas prod. Prediction (21)
5
biogas prod. Predicted (5)
13
biogas prod predicted (13)


Retentates M3
Cumulative biogas production (mL)
time (d)
7
Biogas prod predicted (7)
15
biogas prod predicted (15)
23
biogas prod, predicted (23)


Retentates M2
Cumulative biogas production (mL)
time (d)
6
biogas prod predicted (6)
14
biogas prod. Predicted (14)
22
biogas prod predicted (22)


Retentates M4
Cumulative biogas production (mL)
time (d)
8
Biogas prod predicted (8)
16
Biogas prod predicted (16)
24
biogas prod. Predicted (24)

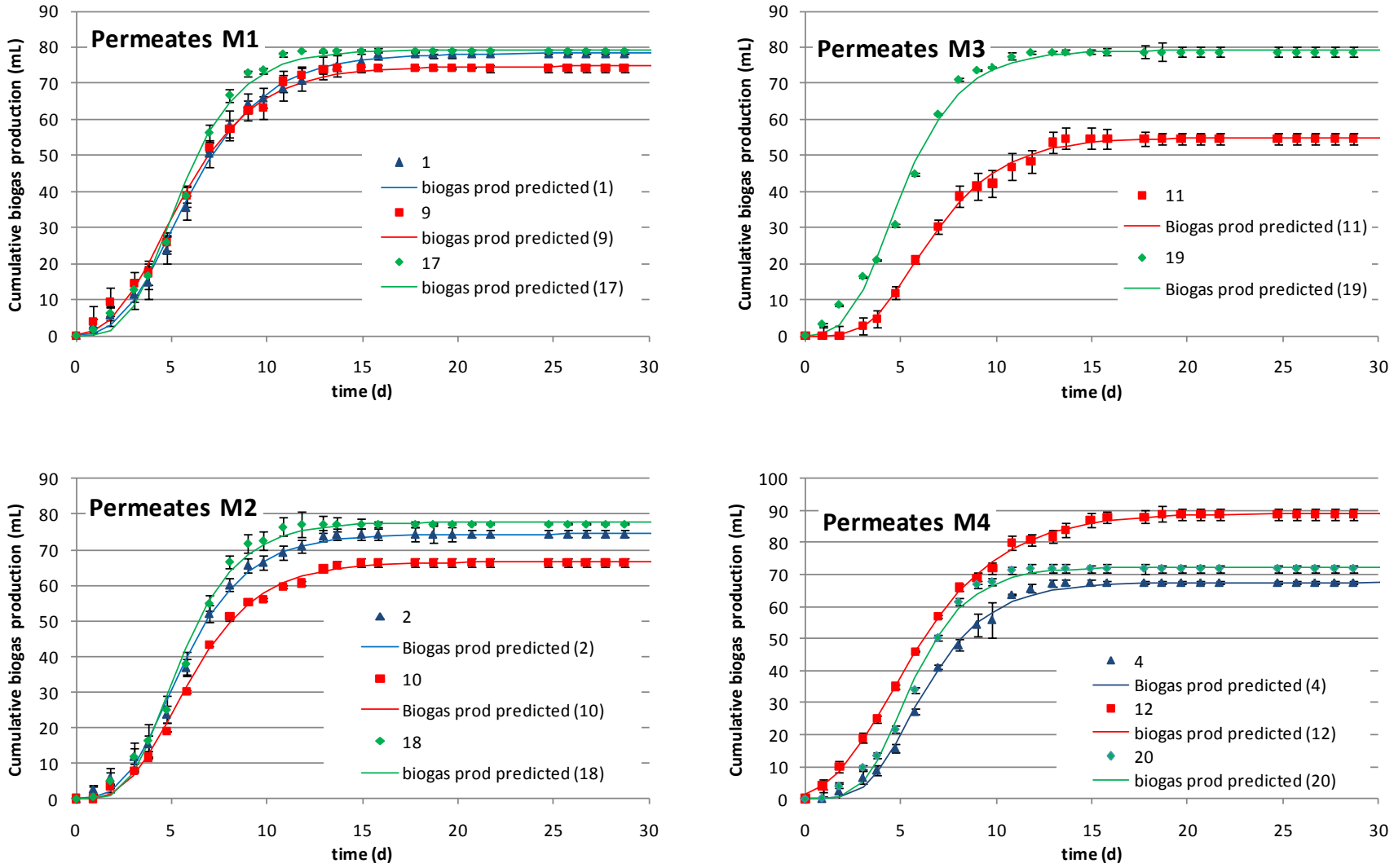


Figure 4.4 - Observed and predicted cumulative biogas production curves for Mw fractions that were produced by UF membranes (M1 (300 kDa); M2 (100 kDa); M3 (10 kDa); M4 (1 kDa))

When analyzing the curves and data obtained, all the curves show a noticeable delay in biogas production in the first days of digestion. MW pretreatment influences the duration of the lag period, since for all the retentates and permeates tested, the MW fraction has a smaller or similar lag period (difference not statistically significant using t-test and α =0.05) when comparing it with the same Mw fraction for the control sludge (it was not possible to verify if this was true for M3 permeate, since the control permeate from M3 was lost). MW pretreatment causes not only an increase of the concentration of substrate in each of the intervals tested but also causes complex macromolecules to transform into smaller entities making them easier to be immediately used by bacteria. In the control sludge, it is likely that some time had to pass before all the enzymes necessary for hydrolysis of substrate were present in order for biogas production to reach its maximum. It is noteworthy that the test with the smallest lag-time was the test where retentate from M4 was digested (test 16). This fraction contained the smallest substrate particles, from all the 4 retentate fractions separated for each sludge, since M4 has a Mw cutoff size of just 1kDa, and it was the sludge subject to MW pretreatment, increasing the concentration of readily degradable substrate in that size class. Interestingly, the permeate fraction of M4 of the same sludge (also with substrate < 1kDa) also had a very small lag time. Probably the difference between the values obtained comes in part from the difference in concentration (the permeates were not subject to an increased concentration of substrate as were the retentates due to substrates larger than the membrane pores being retained in a smaller volume). Contrastingly if the MW pretreatment effect is positive for lag-time, the same comment cannot be made for the maximum activity achievable during digestion. All tests for MW sludge, either permeates or the retentates, returned specific biogas production rates smaller than those calculated for the equivalent test using control sludge, with the notable exception of retentate M4 (test 16). A smaller maximum activity in comparison with the control is a signal of some inhibitory phenomena occurring when

MW pretreated sludge is being digested. The fact that the difference between control and MW sludge tests is greater in retentates than in permeates, can also be related in part to a higher concentration of substrate and inhibitory compounds, suggesting inhibition is dependent on concentration. MW pretreatment disrupts the EPS matrix that surrounds bacterial cells. This matrix is known to prevent bacterial cells to be inhibited because that EPS matrix can adsorb, complex and/or sequester compounds, elements or molecules (such as heavy metals, or chemical toxins) that cause inhibition if they are allowed to freely access bacteria (Henriques and Love, 2007). When MW disrupts this EPS matrix, some of the compounds retained in the EPS matrix are released to the bulk liquid, causing a potential for inhibition in the biomass that will degrade the pretreated sludge. Additionally, the high temperature of MW pretreatment favours the occurrence of Maillard reactions that cause low weight sugars and proteins to react and form inhibitory and hard to degrade end products. However, it is not likely that inhibitory substances are present at all Mw class intervals tests, since retentate for M4 has the highest activity of all the retentates tested. This is likely a sign that substances, or compounds, that caused the inhibition were not present, or at least were removed to a great extent (since separation in UF is never complete) in the previous membrane UF step (M3), suggesting inhibiting compounds in this case have a Mw size greater than 1kDa. Leiviska et al. (2008) reported the same phenomena when separating and analysing biodegradabilities of pulp and paper effluents. MW also has a mixed effect on total biogas production. Biogas production in MW retentates tests is smaller in intermediate sizes (300>Mw>100 kDa (M2) and 100>Mw>10 kDa (M3)) when compared to the control, but is noticeably higher in the smaller Mw class [10>Mw>1 kDa (M4)], while at the highest size class [Mw>300 kDa (M1)], there is no statistically significant difference. Given that soluble substrate concentration is higher in all class sizes for MW tests, it must be assumed that even though MW pretreatment solubilises a substantial part of particulate COD, not all of that

solubilised substrate will be easily degraded, or degraded at all, at least for class sizes above the smaller one.

Athermal radiation is able to change (although not to the same extent as MW radiation) soluble COD and protein concentrations and their respective AMwD, and, when analyzing the results, also has an effect in the biodegradability of some of the fractions tested. Athermal radiation, curiously, seems to have an effect opposite to MW, since it increases lag-time, but also increases the specific activity when compared to control tests. This can be seen both in permeate and in retentate fractions. Specific biogas activity is higher in athermal tests for all retentates and permeates (even though the difference is not statistically significant for the retentates, t-test, α = 0.05), with the exception of the retentate of M3, where the activity for athermal test is slightly less than for the control, but statistically not different. For lag-time, retentates for athermal tests had longer lag for all but M4 retentate, and all the permeates for athermal tests also had a longer lag except for M4 when compared with control test. Apparently, athermal radiation is capable of destabilizing the floc structures, but not to the same extent as MW radiation. This partial destabilization causes an increase in soluble protein and soluble COD in the sample, and thus increases the amount of easier to degrade substrate in the smaller size class. In the other size classes, either the substrate released is not as depolymerised as in MW tests and thus requires some time to degrade, or that partial destabilization of the floc also releases a small part of the toxins and inhibitors that are enmeshed in the EPS matrix, causing some delay in biomass response. However, if this is the case, the amount of inhibitory substances released by athermal radiation must be substantially smaller than in MW tests, since the inhibition is temporary and does not affect the maximum biogas production rate. Contrary to MW tests, athermal tests have a positive effect on cumulative biogas production. All tests irradiated athermally had greater production of biogas for every class size tested, though the difference is not statistically

significant for permeate tests. The sum of all the permeate and retentate for each type of sludge reveals that athermal tests even though having a smaller biogas production for the smaller size membrane retentate and permeate manage as a whole to obtain a higher amount of biogas in the sum of all the fractions, in comparison with the MW sludge (Table 4.5). MW increases significantly biogas production from the smaller fraction but the effect on the other fractions, both on the permeate and retentates is not positive. The result for AT tests is not totally unexpected, since substrate solubilisation increased, even if slightly, in all the size classes. However, contrary to MW pretreatment, the absence of released inhibitory compounds, or the non-production of refractory organic compounds due to the MW pretreatment might be an explanation for the higher biogas production especially for the intermediate size classes [300>Mw>100 kDa (M2) and 100>Mw>10 kDa (M3)].

Table 4.5 - Sum of the biogas production for all fractions and for each type of sludge tested.

Sludge type	Fraction	M1	M2	M3	M4	Total (mL)
NMW	Permeate	78.3	74.4		67.6	220.3
	Retentate	76.8	82.2	81.9	78.8	319.6
MW	Permeate	74.7	66.6	54.9	89.2	285.4
	Retentate	79.6	67.8	66.5	118.7	332.5
AT	Permeate	79.3	77.7	79.1	72.3	308.4
	Retentate	81.2	88.3	92.5	94.0	355.9

4.5 Conclusions

MW pretreatment is capable of solubilising particulate organic matter present in waste activated sludge, however, athermal MW irradiation also causes solubilisation of part of the particulate organic matter, and this effect seems to be particularly effective for proteins. The magnitude of

changes caused by athermal effects (namely solubilisation of COD and sugars) is significantly smaller than MW, which can explain in part the difficulty in detecting athermal effects.

Both MW and athermal irradiation cause a change in the AMwD of soluble substrate, and athermal irradiation seems to be particularly focused on protein. MW pretreatment causes substantial increase in soluble substrate concentration in all size intervals tested; however, both the smallest size and the largest size class have the greatest increases, creating a bimodal distribution when initially soluble substrate was distributed in a skewed distribution towards the smaller size molecules. For athermal tests, an increase in sCOD in all intervals is detected, and a change in the distribution of soluble substrate also noticed with a increase of the smaller class sizes. Soluble protein showed an increase in the smaller and immediately adjacent size class and sugars did not apparently change their AMwD.

MW and athermal irradiation have different effects on soluble substrate biodegradability. MW decreases lag-time but also decreases the maximum biogas production rate, suggesting that inhibition occurs, and this inhibition is removed if the sludge is filtered below 10 kDa. Athermal radiation does not shorten lag-time but allows biomass to attain higher maximum biogas production activities.

MW pretreated soluble fractions produced more biogas than the control only in certain tests (the smaller size class retentate and permeate and the retentate of the higher size class). In addition to inhibition phenomena, some of the solubilized substrate is not biodegraded or has low biodegradability. For athermal tests, all tests produced more biogas than the comparable control test (Table 4.5).

First-order reaction kinetics did not adequately fit biogas production, especially in the initial phase of the biodegradability tests, where lag periods were detected. The Gompertz equation provided a more accurate description of the biogas production over the course of digestion.

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Chapter 5

Sludge age and pretreatment condition effects on mesophilic and thermophilic sludge digestion

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5.1 Abstract

Waste activated sludge produced in processes with different average sludge retention times, or sludge age (SRT), namely 4, 7 and 15 d were subject to microwave (MW) pretreatment at different temperatures (100, 150 and 175˚C). MW pretreatment efficiency in terms of solubilization of substrate showed a dependence on temperature but also on SRT, with higher increases in solubilization ratios for high SRT sludge. Results show that MW pretreatment improves digestion, measured in biogas production for all SRTs tested and for both mesophilic and thermophilic digestion. Relative increases are higher for mesophilic tests but absolute biogas production is higher for thermophilic tests for the majority of conditions tested. The kinetic analysis showed that thermophilic digestion was able to return higher specific biogas activity (SBA), but also showed that thermophilic digested sludge has a higher sensitivity to MW pretreatment, with longer adaptation periods, and a greater inhibition. Thermophilic digested sludge is able to produce significantly more biogas for conditions outside the optimum area calculated for mesophilic digestion. When operating at the optimal conditions for mesophilic sludge, the difference between mesophilic and thermophilic digestion performance is small.

KEYWORDS: Sludge age, Microwave, Thermophilic, Mesophilic, BMP Modelling

5.2 Introduction

Wastewater treatment plants have long been used to remove carbonaceous organic matter from liquid effluents of domestic or industrial nature. Increasing knowledge about treatment processes and pollutant impacts in the environment have led to more stringent standards applied to wastewater treatment. Many wastewater treatment plants are now required to remove nutrients (nitrogen and/or phosphorus), in addition to organic carbonaceous matter. Nutrient removal can be accomplished biologically at conventional wastewater treatment plants; as long as some changes in operating parameters and/or process diagram are implemented. One of the main parameters in an activated sludge treatment plant operation is sludge retention time (SRT). Usual SRT values in wastewater treatment plants that use conventional activated sludge process are between 3 to 6 days for carbonaceous matter removal, but for biological nitrogen removal to occur, SRT values need to be higher (>10 d) to allow the growth of nitrifying bacteria, since nitrifying bacteria have lower growth rates than carbon oxidizing bacteria (Metcalf and Eddy, 2003). However, changing SRT in an activated sludge process has several impacts on the physical, chemical and biological characteristics of the sludge produced in the treatment process, as well as in the downstream anaerobic digestion of the excess sludge to be discarded. One of the factors affected by SRT is the production of exocellular polymeric substances (EPS). These EPS form a three-dimensional polymeric gel-like matrix in which the bacterial cells are embedded that originates both from the bacteria (lysis and excretion) and from the wastewater (by adsorption), and form the flocs observed in normal activated sludge biomass. It was reported that sludge with high SRT has a higher amount of EPS (Pavoni et al, 1972; Chao and Keinath, 1979; Sheintuch, et al, 1986; Sheintuch, 1987; Ng and Hermanowicz, 2005). But other reports state that EPS remains constant for different SRT per unit mass of biosolids (Brown and Lester, 1982; Liao et al, 2001). These EPS contain proteins, carbohydrates and nucleic acids and the relative amount of each is

dependent on the SRT applied. The dominant compound present in EPS is protein, followed by carbohydrates, but some authors report that carbohydrates are in some cases the main compound (Sponza, 2003; Liao et al, 2001; Azeredo et al, 1998; Horan and Eccles, 1986). The change in SRT can also affect the relative composition of EPS, with proteins/carbohydrates ratio increasing with SRT (Sponza, 2003; Liao et al, 2001). EPS and the compounds that make up EPS are important substrates for bacteria in anaerobic digestion of excess sludge produced in aerobic biological treatment, and the prevalence of either protein or carbohydrates and the abundance or scarcity of the EPS itself can have a important effect on the anaerobic digestion process efficiency downstream (Novak et al, 2003; Zhang and Bishop, 2003).

The increase in SRT applied in activated sludge processes has another important effect on the characteristics of excess sludge since it can also cause a decrease in the biomethanization potential of excess discarded sludge in subsequent anaerobic digestion, due to partial stabilization of sludge (Bolzonella et al, 2005; Nielsen and Petersen, 2000; Bolzonella et al, 2002; Karlsson et al, 2011; Muller et al, 1998).

To address low biogas production of sludge digestion, microwave (MW) pretreatment has been tested to increase the biogas yield and reduce pathogen content. MW pretreatment causes a disruption of the flocs and cell walls of the activated sludge facilitating the hydrolysis of substrate that would be difficult to degrade because of cell and substrate stability. Results show a positive correlation between MW pretreatment temperature, chemical oxygen demand (COD) solubilisation and biogas production for low SRT sludge (Toreci et al, 2009). However, excessively high pretreatment temperatures (170-200˚C), can cause the appearance of compounds that are difficult to degrade or even inhibitory. These compounds can originate from Maillard reactions occurring between proteins and carbohydrates at high temperatures, thus decreasing the overall biodegradability of the sludge, or from the release of refractory compounds or cellular

debris enmeshed in the floc matrix before pretreatment that are liberated after the floc is destabilized by that same pretreatment (Penaud et al, 2000). Some authors suggest that the optimum for COD solubilisation is not necessarily the optimum for biodegradability because of these factors; thus a temperature lower than the one where maximum solubilisation is achieved should be used in order to obtain optimal results in terms of biodegradability of substrate or biogas production (Dwyer et al, 2008; Penaud et al, 2000).

Conventional anaerobic digestion is performed at mesophilic temperatures, but thermophilic anaerobic digestion has been shown to have the capacity to reach greater reaction rates, with significant improvements both in volatile solids (VS) reduction and specific biogas production, due either to increased hydrolysis rates or increased extent of hydrolysis (Song et al, 2004). Thermophilic digestion also was reported as providing complete elimination of pathogenic microorganisms, conducive to the production of Class A biosolids, and significant reductions to very short retention times (Riau et al, 2010; Coelho et al, 2011). However, some literature also indicates that thermophilic anaerobic digestion is more susceptible to instability, inhibition phenomena, toxicity effects, foaming and odours which can prevent its application (Grady et al, 1999; Hwu and Lettinga, 1997; Marneri et al, 2009).

The goals of this work were first, to determine the impact of MW pretreatment at different pretreatment temperatures on the characteristics of sludge and its biodegradation. Taking into account that MW pretreatment increases solubilisation but also increases the formation of inhibitory or recalcitrant compounds, specially at high temperatures (>160℃) there is a possibility that the digestion optimum in terms of biogas production or solids removal is not at the same point were maximum solubilisation is measured. Secondly, different SRT sludges may behave differently when biodegraded after MW pretreatment, since soluble organic content, EPS composition and quantity, and floc morphology changes with SRT. It was particularly important

to study the effect pretreatment like MW could have on a type of waste activated sludge (high SRT activated sludge) that was partially stabilized, difficult to degrade and consequently with an *a priori* lower biodegradability compared to a already relatively low biodegradable material as is activated sludge with low SRT. Thirdly, the influence of the digestion temperature was also studied, since at thermophilic and mesophilic temperatures, the dominating bacterial genera are different so that can cause a difference in the response of the systems to changes on SRT and/or MW pretreatment temperature.

5.3 Material and methods

Activated sludge produced from systems treating municipal wastewater at different SRTs, namely 4, 7 and 15 days was collected after secondary settling, respectively from Ottawa's municipal wastewater treatment plant, the Robert O. Pickard Environmental Centre (ROPEC, Ottawa), and pilot plants at the University of Waterloo (SRT 7 and 15 d). ROPEC has a conventional aerobic activated sludge process with an SRT of 4 days and a primary settling step prior to the activated sludge aerobic tank. Ferric chloride is added for phosphorus removal. Excess sludge from ROPEC is centrifuge thickened before being sent to anaerobic digesters and has approximately 4-5% total solids (TS) concentration. The thickened waste activated sludge (TWAS) from ROPEC was diluted to approximately 2% TS so as to reach similar total solids (TS) concentration found in sludges obtained from the pilot plants from the University of Waterloo.

The characteristics of the sludges used in this study are shown in Table 5.1:

Table 5.1 - Properties of sludge used in this test

	SRT 4 d	**SRT 7 d**	**SRT 15 d**
TS (%)	2.34 ± 0.02	2.53 ± 0.12	2.42 ± 0.04
VS (%)	2.01 ± 0.05	1.98 ± 0.09	1.92 ± 0.08
VS/TS	0.86 ± 0.03	0.78 ± 0.07	0.79 ± 0.04
tCOD (g/L)	11.42 ± 0.31	14.52 ± 0.42	16.16 ± 0.25
sCOD (g/L)	1.40 ± 0.04	1.55 ± 0.01	1.56 ± 0.07
sCOD/tCOD	0.12 ± 0.04	0.11 ± 0.03	0.10 ± 0.05
NH_3-N (mg/L)	313 ± 21.3	407 ± 14.0	425 ± 11.1

These sludges were subject to MW pretreatment at three different temperatures (T) each (100, 150 and 175 ˚C), using a MW oven with pressurized cells and pressure control. Sludge pretreatments were carried out with a Mars 5© (MW Accelerated Reaction System; CEM Corporation) MW oven. Mars 5© can supply 1200W ± 15% MW energy at 2450 MHz frequency and has a controllable operating range of up to 250˚C and 3.45 kPa. In each pretreatment round, 500 g of sludge was inserted into the 24 air sealed and pressure controlled plastic vessels and distributed uniformly in the circular motorized platform of the MW oven. MW intensity was controlled by adjusting the temperature ramping time to achieve the set temperature. Each MW pretreatment was performed using a 3.75˚C/min temperature ramp and sustaining the final temperature for 1 minute before turning off and cooling the MW oven. The pretreated sludge was allowed to cool to room temperature by exposure of the sealed vessels to air without pressure release. The temperature increase rate was tested previously along with other values and was shown to result in the greatest solubilisation of COD and greatest improvements in biogas production (Toreci et al, 2007). The total MW pretreatment heating time for the highest pretreatment temperature was 40 min. TWAS concentration was maintained constant for all tests

and water lost during the pretreatment was replaced by distilled water. A factorial design was used in order to test all the conditions and possible interactions between the type of sludge (SRT), pretreatment condition (MW T) and digestion temperature (mesophilic and thermophilic) (Table 5.2). Three extra conditions were tested to be used as control tests and correspond to the sludges without any pretreatment.

Table 5.2 - Factorial design of the experiment

Factors / **Levels**	**SRT (d)**	**MW T (˚C)**	**Digestion Temp**
1	4	100	Mesophilic (35˚C)
2	7	150	Thermophilic (55˚C)
3	15	175	

Before and after pretreatment, several parameters were measured to evaluate the efficiency of MWs to solubilise and destroy the sludge cells and sludge flocs (total and soluble COD, soluble protein and soluble sugar). Colorimetric COD measurements were performed using Standard Methods procedure 5250D (APHA, 1995) with a Coleman Perkin-Elmer spectrophotometer Model 295 at 600 nm light absorbance. Total soluble protein was measured according to the procedure described in Bradford (Bradford, 1976), using bovine serum albumine (BSA) as the standard. Determination of total soluble sugars was performed using the phenol-sulphuric acid test method proposed by Benefield and Randall (Benefield and Randall, 1976), with glucose used as a standard. TS and VS were determined based on Standard Methods procedure 2540G (APHA, 1995). Ammonia measurements were perforemd using an ORION Model 95-12 ammonia gas

sensing electrode connected to a Fisher Accumet pH meter model 750. The analysis was conducted according to Standard Methods 4500D procedure (APHA, 1995) and reported as ammonia-N. Pretreated sludges were subjected to anaerobic digestion at mesophilic and thermophilic temperatures and monitored with biological methane potential tests (BMP tests). These tests were done using 125 mL serum bottles (Wheaton borosilicate glass, VWR, Montreal, Canada), sealed with butyl rubber stoppers and crimped with aluminum caps. To test all conditions using duplicates in all tests, a total of 52 serum bottles were necessary. In each serum bottle, 15 mL of thermophilic or mesophilic inoculum was added to 70 mL of MW pretreated sludge or non-pretreated sludge (in the case of controls). The thermophilic inoculum was obtained from thermophilic sludge collected in Annacis Island Wastewater Treatment Plant (Vancouver, BC) while mesophilic inoculum was obtained from ROPEC anaerobic digestors. Both inocula were acclimatized using two completely mixed reactors, the mesophilic reactor at 35 ± 1˚C and the thermophilic reactor at 55 ± 1˚C, both operating at a SRT of 20 d, and fed everyday with microwaved sludge for more than a year. After addition of a mixture containing equal parts of $NaHCO_3$ and $KHCO_3$ to achieve an alkalinity of 4000 mg/L as $CaCO_3$, the bottles were bubbled with N_2 and sealed. Biogas volumetric production was measured daily by puncturing the rubber septum with a thin needle and measuring displacement in a water column manometer, and its composition was determined with an HP 5710A GC with metal packed column (Chromatographic Specialties Inc., Brockville, ON, Canada, Porapak T, packing mesh size: 50/80, column length, OD: 304.8 cm, 0.635 cm) and thermal conductivity detector (oven, inlet and outlet temperatures: 70, 100, and 150˚C, respectively) using helium as the carrier gas (flowrate: 25 mL/min). Serum bottles subjected to thermophilic or mesophilic BMP were kept in two darkened temperature-controlled incubator shakers (Phycro- Therm, New Brunswick Scientific Co. Inc., NB), one at 55 ± 1ºC and another at 35 ± 1˚C, and were both shaken at 90

rpm, until they stopped producing biogas. All statistical analyses were performed using either Microsoft Excel© or STATISTICA version 8.0, StatSoft, Inc. (2007).

5.4 Results

5.4.1 Effect of SRT and MW pretreatment temperature on substrate solubilisation

MW pretreatment was able to increase soluble COD (in comparison with soluble COD of non-pretreated sludge, which is the control) a significant amount in all the sludges and at all the temperatures applied as expected. Increase in temperature caused a significant increase in soluble COD, with the influence of temperature being statistically significant (ANOVA, α=0.05). However, the effect of sludge SRT was also calculated to be significant, as well as the interaction of SRT and MW temperature at the level of significance used (α=0.05) (Tables 5.3 and 5.4).

Table 5.3 - Soluble COD concentration after pretreatment for the tested sludges (gCOD/L), with solubilisation ratios (sCODsample/sCODcontrol) in parenthesis.

	MW T (˚C)*		
SRT (d)	**100**	**150**	**175**
4	2.83 ± 0.41 (2.02 ± 0.29)	3.02 ± 0.16 (2.16 ± 0.12)	3.13 ± 0.33 (2.23 ± 0.02)
7	2.83 ± 0.20 (1.83 ± 0.13)	2.68 ± 0.17 (1.68 ± 0.11)	2.76 ± 0.54 (1.79 ± 0.35)
15	2.52 ± 0.08 (1.63 ± 0.05)	3.52 ± 0.45 (2.28 ± 0.29)	5.16 ± 0.43 (3.34 ± 0.28)

*average values ± 95% confidence interval

Table 5.4 - ANOVA table for sCOD

Source	SS	df	Mean Squares	F	p-value
Sludge SRT	4915333	2	2457666	30.074	0.0000020
MW Temp	4260791	2	2130396	26.069	0.0000050
Interaction	6647022	4	1661756	20.334	0.0000020
Error	1470981	18	81721		

Further refining of the ANOVA test was made using *Duncan's* test method of *post- hoc* paired comparisons. This procedure is based on the general notion of studentized range. The range of any subset of *p* sample means must exceed a certain value before any of the *p* means are found to be different. This value is called the least significant range for the *p* means and is denoted by R_p, where:

$$R_p = r_p \sqrt{\frac{s^2}{n}}$$

.

The values of the quantity r_p, called the least significant studentized range, depend on the desired level of significance and the number of degrees of freedom of the mean square error. These values may be obtained from tables for *p* =2, 3,..., n means (Walpole et al, 2011).

The Duncan test shows that temperature has a positive and significant effect at the lower and upper SRT values used in this study. Values for SRT 4 and 15d sludge pretreated at 175˚C are significantly different (p=0.05), and higher, than the same sludges pretreated at 100˚C. For the specific case of SRT 15 d this statistical difference extends to tests at 150˚C, while the same is

not true for SRT 4d sludge. Differences across the interval of temperatures for the tests performed on sludge with SRT 7d are not sufficient to dictate that increasing temperature from 100˚C has an effect on solubilisation ratio. The effect of SRT is also visible in the average solubilisation but only for higher temperatures (150 and 175˚C), since for 100˚C, the ratios are not statistically different. At 150 and 175˚C the 15 d SRT sludge exhibits a higher ratio of solubilisation. In the case of 7 d SRT sludge, even though the average value of solubilisation ratio is lower than the corresponding value for SRT 4 d sludge, these differences are not statistically significant.

Soluble protein follows closely the behaviour of soluble COD and increases with SRT, as well as with the increase in pretreatment temperature. Temperature, as well as SRT have a significant effect on the soluble protein concentration (ANOVA, α=0.05), with the interaction between SRT and MW T also being significant. For all types of sludge, increase of MW temperature caused a statistical significant increase in soluble proteins both from 100 to 150˚C and from 150 to 175˚C (Figure 5.1). Increase in SRT generally resulted in an increase in soluble protein after pretreatment. Change was not significant at 175˚C for SRT 4 and SRT 7 sludges and SRT 7 had a decrease in average soluble protein at 150˚C compared with the SRT 4 sludge.

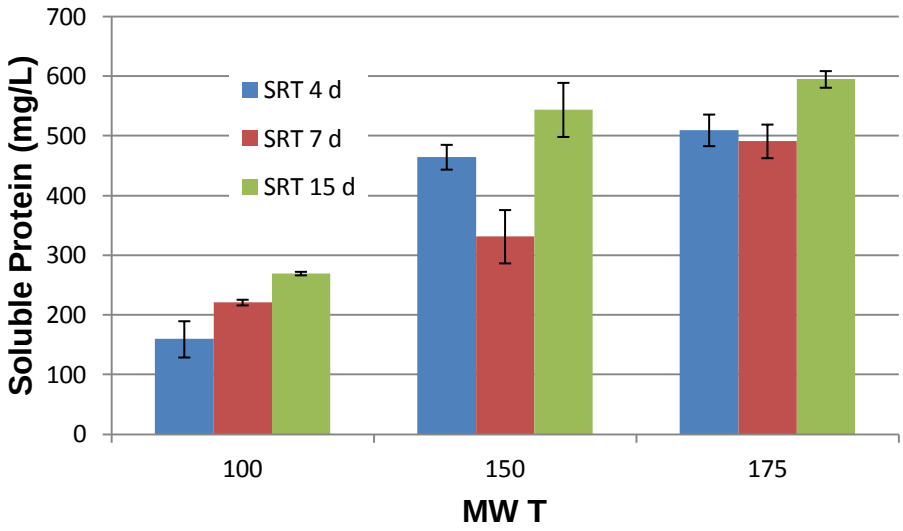

Figure 5.1 - Soluble protein concentration in sludge after MW pretreatment

Solubilisation of soluble sugars (Figure 5.2) is also dependent on both MW temperature and SRT of the sludge tested. Both effects are significant (ANOVA, α=0.05). However, in this case, the temperature effect seems more consistent than the SRT effect, since all sludges showed significant differences when MW temperature increased, with the exception of SRT 7 at 150˚C compared to 175 ˚C.

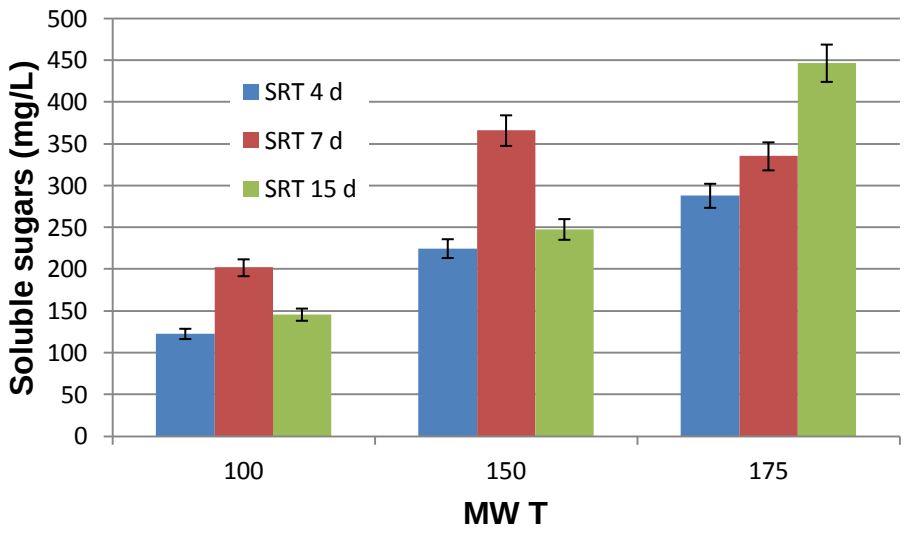

Figure 5.2 - Soluble sugars in sludge after pretreatment

The effect of SRT is somewhat less straightforward. There is no statistically significant difference between SRT 4 and SRT 15 sludge for 100 and 150˚C with SRT 7 sludge showing a maximum in soluble protein. For the highest pretreatment temperature (175˚C), it is apparent that soluble sugars in SRT 7 sludge did not change significantly from the test at 150˚C, while on the other sludges there was still a measured increase in solubilisation of sugars, with the increase being greater for SRT 15.

An empirical linear model was developed to characterize COD solubilisation as a function of both MW pretreatment temperature and sludge SRT in the area bordered by the tested conditions. All the factors that were calculated as being statistically significant using ANOVA (Table 4) were included in the model expression, and different model structures (from simple zero-order to more complicated third-order) were tested and evaluated. Model complexity and adequacy of its function to predict the amount of variability of the response measured is a function of variables included in said model. The coefficient of determination (R^2) is sometimes used to evaluate the

quality of the model in terms of its ability to explain the variability of measured data. However, the coefficient of determination is positively correlated with the quantity of variables included in the model, so this criterion is not adequate to evaluate competing models for the same data set. Adding additional terms without checking its significance to the model increases R^2, can lead to a model that is *overfitted* (inclusion of too many unnecessary model terms). A more useful evaluation tool is the adjusted coefficient of determination (R^2_{adj}):

$$R^2_{adj} = 1 - \frac{n-1}{n-(k+1)}(1-R^2) = 1 - \frac{SS_{err} * df_t}{SS_{tot} * df_e} \quad (1)$$

R^2_{adj} is formulated as a variation of R^2 that provides an adjustment for degrees of freedom of the model. R^2 cannot decrease with the addition of model terms and consequent decrease of degrees of freedom, but R^2_{adj} decreases its value when non-significant terms are added to the model expression and, in conjunction with F-tests and t-tests can be used as a tool to detect the best subset of parameters that minimize the use of unimportant terms that can cause the variance of the estimated response to increase.

The previous statistical tools (F-tests and t-tests, R^2 and R^2_{adj}) can be used simultaneously in a sequential method of selection of model terms called *Forward Stepwise* regression. The algorithm is as follows (Walpole et al, 2011):

1. The variable (x_1) with the largest increase in R^2 is chosen from the pool of initial variables, and its significance is tested using an F-test. If the variable is not significant the algorithm is terminated.
2. A second variable (x_2) is chosen that returns the largest R^2 increase with the presence of (x_1) over the R^2 found in step 1. The variable (x_2), is the one, from the remaining pool of variables, that has the highest value of regression sum of squares adjusted for the other variables, that is, that maximizes expression (2).

$$R(\beta_2|\beta_1) = R(\beta_1, \beta_2) - R(\beta_1) \quad (2)$$

2.1 The model with (x_1) and (x_2) is fitted, and (x_2) is tested for significance, as well as the increase in R^2 using the appropriate F-test as expressed in (3):

$$f = \frac{R(\beta_1, \beta_2)}{s^2}; \quad f_{crit} = f_\alpha(1, n-k) \quad (3)$$

If f fails the significance test, the algorithm is terminated. s^2 is the mean square error of the model containing the variables added so far.

2.2 Since it is possible that the addition of a new variable might render a previous existent and significant variable redundant and unimportant because of relationships existent between it and other variables entering at later stages, a F-test is performed to all the variables added at this stage, and the ones that do not show a significant f-value are deleted. The procedure is continued until a stage is reached where no additional variables can be deleted.

3. A third variable is added (x_3) that maximizes expression (4):

$$R(\beta_3|\beta_1, \beta_2) = R(\beta_1, \beta_2, \beta_3) - R(\beta_1, \beta_2) \quad (4)$$

The same tests of step 2 are performed and the process is repeated until the last variable added fails to induce a significant increase in the explained regression.

In the cases where more than one subset of variables was chosen by the algorithm (algorithm depends partially on the initial pool of available variables defined), the final model expression was chosen using the R^2_{adj} criteria (highest R^2_{adj}), since it favours models with lower complexity and equivalent predictive potential. Regression algorithms are more robust, precise and show fewer round off and multicollinearity errors when variables are coded and centered, so MW T

and SRT values were codified (MW T' and SRT') according to expressions (5) and (6), even though they could not be exactly centered:

$$SRT' = \frac{SRT - 9.5}{5.5} \quad (5)$$

$$MW\ T' = \frac{MW\ T - 137.5}{37.5} \quad (6)$$

Coded variables levels were then (-1, -0.455, 1) for SRT' and (-1, 0.333, 1) for MW T', and the regression model initially considered as (7), becomes (8), using the coded variables:

$$Y = \beta_0 + \beta_1 SRT + \beta_2 MW\ T + \cdots + \varepsilon \quad (7)$$

$$Y = \beta_0{}^* + \beta_1{}^* SRT' + \beta_2{}^* MW\ T' + \cdots + \varepsilon \quad (8)$$

Results of the forward stepwise method of parameter selection are presented in Table 5, along with regression results for the whole model:

Table 5.5- Estimated coefficients (along with 95% confidence intervals) for COD solubilisation as a function of SRT and MW T

Coefficients	**Value**	**Std.Error**	**t**	**p-value**
$\beta_0{}^*$	*1.7554±0.2045*	*0.0986*	*17.7998*	*0.0000*
$\beta_1{}^*$	*0.1882±0.1089*	*0.0525*	*3.5841*	*0.0017*
$\beta_{11}{}^*$	*0.5625±0.2438*	*0.1175*	*4.7855*	*0.0001*
$\beta_2{}^*$	*0.3757±0.1078*	*0.0520*	*7.2301*	*0.0000*
$\beta_{22}{}^*$	*Pooled*			
$\beta_{12}{}^*$	*0.4186±0.1256*	*0.0606*	*6.9092*	*0.0000*

	SS	***df***	***MS***	***F***	***p-value***	R^2	R^2_{adj}
Whole model	6.0772	4	1.5193	31.1180	0.0000	0.8498	0.8225

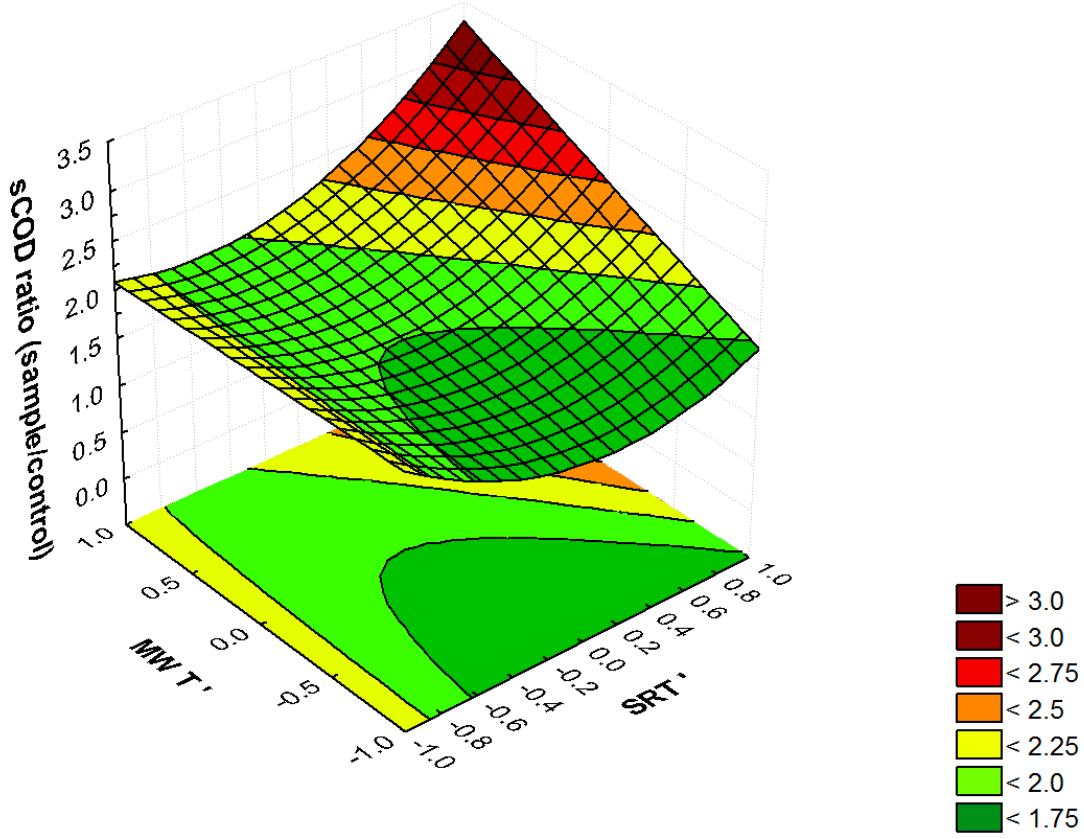

Figure 5.3 - Solubilization ratio for COD (sCODsample/sCODcontrol) as a function of SRT and MW pretreatment temperature.

The graph in Figure 5.3 shows that both SRT and MW T have a significant effect on the increase of soluble COD (sCOD) after pretreatment. Increasing temperature has a positive effect on solubilisation, as expected, and already previously reported, but this effect is dependent on the SRT of the sludge. For low SRT sludge (4 d), the increase in sCOD along the temperature interval used is significantly less pronounced as the increase measured for SRT 15d. It is known that changes in SRT cause changes in sludge floc morphology and substrate utilization (Liao et

al, 2001; Grady et al, 1999). Low SRT sludge does not consume all the available substrate even though it accumulates intracellular storage granules or extracellular polymers. Some available substrate remains as soluble substrate, decreasing the efficiency of pretreatment when measured as sCOD increase (Liao et al, 2001). Also, low SRT sludge also produces a higher fraction of low molecular weight soluble microbial products that are also measured as soluble COD (Barker and Stuckey, 1999). For high SRT sludge, soluble biodegradable undigested substrate is present in a much lower concentration, and the products of bacterial metabolism tend to be retained in the mesh of EPS surrounding the floc, so higher increases in sCOD are observed when these flocs are destabilized by MW pretreatment. High SRT also show a steeper increase in soluble COD when MW temperature increases and that might also be a consequence of morphological changes that happen in sludge and sludge flocs due to changes in SRT. Flocs in high SRT sludge are more compact, compared with the relatively loose structure of low SRT flocs, with less bound water, more uniform shape and possess a thicker layer of EPS on its surface (Liao et al, 2000; Liao et al, 2006; Liss et al, 2002; Saunders and Dick, 1981). These changes increase the resistance and stability of the floc to adverse conditions and thus make it necessary to apply more energy to disrupt it. These reasons might explain why the ratio of COD solubilisation at the lowest MW temperature (100˚C) is significantly smaller when compared to MW pretreatment temperature of 175˚C, and also why the ratio increases more in sludge with SRT 15 d than at SRT 4 d. The ratio is practically the same for all sludges at 100˚C, and does not change much with increase in temperature for SRT 4 d, suggesting that easily solubilisable material is solubilised at this temperature, and is present at roughly the same proportion in all the sludges tested. It is difficult to draw any conclusions from the differences in solubilisation between SRT 4 d and SRT 7 d because the difference between SRT 4 d and SRT 7 d does not seems to be great enough to provide statistically significant differences for soluble COD. SRT 7 d is less than one doubling

time for SRT 4 d, while SRT 15 d is more than a doubling time for SRT 7 d and almost two doubling times for SRT 4 d.

5.4.2 Effect of SRT and MW pretreatment temperature on biogas production

MW pretreatment was shown previously to be able to increase biogas in anaerobic digestion of activated sludge, but most studies, at the time of writing this thesis, conducted MW sludge pretreatment at or below boiling point (Park et al, 2004; Pino-Jelcic et al, 2006) with positive results. More recently, (Toreci et al, 2009) tested MW pretreatments with temperatures up to 175˚C and noticed that despite solubilisation increasing with temperature, some inhibition phenomena and formation of recalcitrant compounds were also observed when applying such high temperatures. The test used young sludge (SRT 5 d) and pretreated sludge was digested using mesophilic temperatures only. In this study, both SRT and MW pretreatment temperature were varied and two digestion temperatures were used, in order to supply a broader base of knowledge about the effect MW pretreatment might have on different types of sludge.

MW pretreatment managed to increase biogas production in all tests in comparison with the respective control (non-pretreated sludge with SRT 4, 7 or 15 d) at mesophilic tests (Table 5.6).

Table 5.6 - Average cumulative biogas production (CBP) of mesophilic BMP tests (mL) for each condition. The relative increase to control test is given in parentheses.

		MW T (˚C)*		
SRT (d)	**Control**	**100**	**150**	**175**
4	279.3 ± 2.7	311.0 ± 7.8 (1.11 ± 0.03)	346.5 ± 6.9 (1.24 ± 0.02)	318.74± 6.9 (1.14 ± 0.02)
7	269.2 ± 2.5	314.5 ± 4.5 (1.17 ± 0.02)	359.1 ± 7.8 (1.33 ± 0.03)	336.0 ± 5.9 (1.25 ± 0.02)
15	270.8 ± 6.6	296.9 ± 3.7 (1.10 ± 0.01)	344.4 ± 5.0 (1.27 ± 0.04)	312.0 ± 6.0 (1.15 ± 0.04)

*average values ± 95% confidence interval

The increases in biogas production compared with the control are always superior by at least 10% and reach a maximum of 33% for SRT 7 and 150˚C. The fact that for SRT 4 a greater proportion of the substrate is in an easily biodegradable form can help explain a smaller percentage of biogas increase after pretreatment when comparing SRT 4 and SRT 7 sludge. The improvements measured for sludge with SRT 4 d correlate satisfactorily with previous results obtained with sludge from the same origin (ROPEC) and with similar SRT (4-5 d). An improvement of 10% was measured for sludge pretreated at 100˚C (Coelho et al, 2011), while the same approximate increase was also measured by Toreci et al, (2009) for similar sludge but pretreated at 175˚C. For SRT 15d improvements are also significant, but less than for SRT 7d. Even though more soluble COD is released after pretreatment for the older sludge, the fraction of this COD that is easily biodegradable is most likely smaller than for younger sludges, thus explaining a decrease in biogas production improvement in comparison with SRT 7d.

Both SRT and MW pretreatment temperature have a statistically significant effect on the increase of biogas production relative to control for mesophilic digestion as can be seen in the ANOVA Table 5.7:

Table 5.7 - ANOVA table for relative increase in CBP for mesophilic tests.

Source	SS	df	Mean Squares	F	p-value
Sludge SRT	0.0262	2	0.0131	48.7341	0.0000
MW Temp	0.0753	2	0.0377	139.9052	0.0000
Interaction	0.0022	4	0.0006	2.0514	0.1704
Error	0.0024	9	0.0003		

However, interaction between SRT and MW T was not found to be significant. In the development of the model to describe variation of the relative biogas production, the same algorithm applied for COD solubilisation was used in order to find the best model, and since the interaction between SRT and MW T was not statistically significant, it was naturally pooled with the other coefficients by the regression routine.

Table 5.8 - Estimated coefficients (along with 95% confidence intervals) for CBP increase relative to control as a function of SRT and MW T for mesophilic tests.

Coefficients	**Value**	**Std. Error**	**t**	**p-value**
β_0^*	*1.3831 ± 0.0265*	*0.012334*	*112.1356*	*0.0000*
β_1^*	*Pooled*			
β_{11}^*	*-0.1016 ± 0.0252*	*0.011734*	*-8.6621*	*0.0000*
β_2^*	*0.0271 ± 0.0115*	*0.005375*	*5.0427*	*0.0002*
β_{22}^*	*-0.1552 ± 0.0229*	*0.010666*	*-14.5531*	*0.0000*
β_{12}^*	*Pooled*			

	SS	***df***	***MS***	***F***	***p-value***	R^2	R^2_{adj}
Whole model	*0.1013*	*3*	*0.0338*	*97.4244*	*0.0000*	*0.9543*	*0.9445*

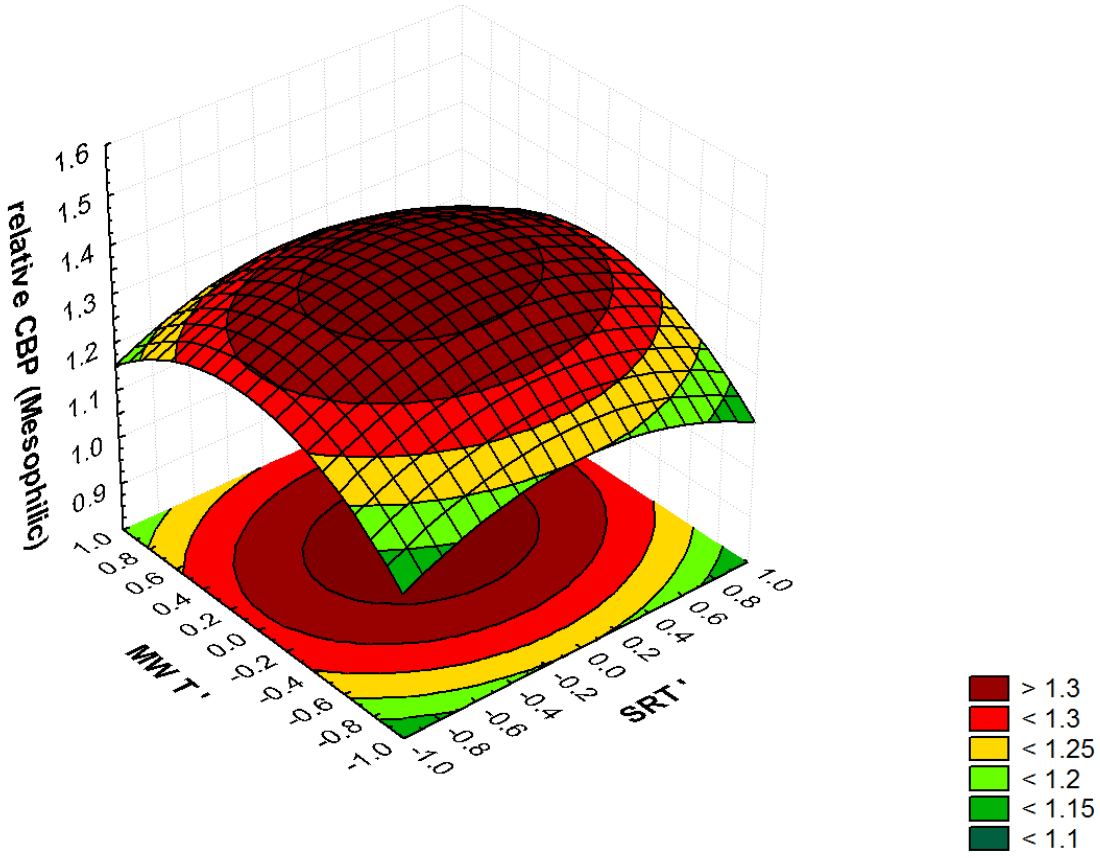

Figure 5.4 - CBP (relative to control) as a function of SRT and MW T for mesophilic digestion tests.

Biogas increase relative to control has a clear maximum increase zone around a MW pretreatment temperature of approximately 140˚C, a value also reported previously to be the temperature around where maximum increase in biodegradability was achieved (Dwyer et al, 2008). Maximum increase calculated by the regression model was around 38%. Temperatures above 140˚C where reported to increase soluble COD concentration, but also to increase significantly the concentration of inhibitory compounds, or inhibitory effects that keep biodegradability of dissolved substrate below that measured at 140˚C (Toreci et al, 2009; Dwyer et al, 2008). In the case of Toreci et al (2009), even though reporting an increase in inhibition

effects, cumulative biogas productions were still significantly greater for sludge pretreated at 175˚C (approximately 30%), than at lower MW pretreatment temperatures (110˚C). In this study, the increase in biogas production for sludge pretreated at the highest MW temperature (175˚C) is much smaller, for all SRT sludges tested. The use of inocula that was only acclimatized to sludge pretreated at 100˚C could have been a factor to cause a greater degree of inhibition or decrease in biodegradability of sludge exposed to 175˚C MW pretreatment. The importance of acclimatization when digesting MW pretreated sludge in the improvement of digestion outcome was demonstrated in previous works (Toreci et al, 2009). The SRT that shows the maximum increase in CBP compared to control is calculated by the regression model at around 9.5 d. This can be explained by the fact that very young sludge has a proportion of substrate that is either still soluble in the bulk liquid around the flocs, or is contained in the structure of flocs that are looser, with a more irregular shape, less compact and with a higher amount of bound water as was reported by other studies (Liao et al, 2006; Liss et al, 2002), thus older sludge might benefit more from the effects of pretreatment enhancement, thus increasing the biodegradability more when compared with the control.

Results for thermophilic tests are given in Table 5.9.

Table 5.9 - Average CBP of thermophilic BMP tests (mL) for each condition. The relative increase to control test is given in parentheses.

		MW T (˚C)		
SRT (d)	**Control**	**100**	**150**	**175**
4	311.6 ± 3.5	347.5 ± 4.9 (1.11 ± 0.02)	352.5 ± 4.9 (1.13 ± 0.02)	348.5 ± 2.9 (1.1 ± 0.01)
7	321.6 ± 2.4	353.5 ± 5.9 (1.10 ± 0.02)	363.1 ± 6.1 (1.13 ± 0.02)	343.0 ± 2.0 (1.07 ± 0.01)
15	295.8 ± 3.0	334.2 ± 3.6 (1.13 ± 0.01)	343.5 ± 6.8 (1.16 ± 0.02)	316.0 ± 2.0 (1.07 ± 0.01)

MW pretreatment also increased CBP for all tests made at thermophilic temperature when compared to the control, but increases are in general smaller than those measured for mesophilic tests. The range of relative increase has a minimum of 7% and a maximum of 16% (Table 5.9). For mesophilic tests the range is significantly greater, varying between 10% and a maximum of 33% (Table 5.6). However, absolute biogas production was higher in the majority of thermophilic tests, with a single exception for (SRT 15 d; MW T 150) where the values were almost identical. ANOVA results for CBP thermophilic tests are given in Table 5.10.

Table 5.10 - ANOVA table for relative increase in CBP for Thermophilic tests.

Source	**SS**	**df**	**Mean Squares**	**F**	**p-value**
Sludge SRT	0.0023	2	0.0012	9.8136	0.0055
MW Temp	0.0095	2	0.0047	40.1895	0.0000
Interaction	0.0037	4	0.0009	7.8315	0.0053
Error	0.0011	9	0.0001		

In thermophilic tests, both SRT and MW temperature have an effect on relative CBP increase, and, contrary to what was calculated in mesophilic tests, there is a significant presence of interaction effects, which was reflected in the regression results since the regression coefficient was not pooled as can be seen in the results table (Table 5.11):

Table 5.11 - Estimated coefficients (along with 95% confidence intervals) for CBP increase relative to control as a function of SRT and MW T for thermophilic tests.

Coefficients	**Value**	**Std.Error**	**t**	**p-value**
β_0^*	*1.1195 ± 0.0190*	*0.008778*	*127.5396*	*0.000000*
β_1^*	*Pooled*			
β_{11}^*	*0.0292 ± 0.0181*	*0.008353*	*3.4957*	*0.003947*
β_2^*	*-0.0174 ± 0.0084*	*0.003882*	*-4.4860*	*0.000613*
β_{22}^*	*-0.0408 ± 0.0164*	*0.007588*	*-5.3789*	*0.000126*
β_{12}^*	*-0.0166 ± 0.0095*	*0.004412*	*-3.7580*	*0.002391*

	SS	***df***	***MS***	***F***	***p-value***	R^2	R^2_{adj}
Whole model	*0.0143*	*4*	*0.0036*	*20.3070*	*0.0000*	*0.8620*	*0.8196*

The surface obtained for thermophilic tests is much flatter than the equivalent for mesophilic tests, showing a smaller influence of both SRT and MW temperature on the outcome of the digestion process, as measured through biogas production. Thermophilic digestion is known to have greater reaction rates than mesophilic digestion, as well as higher bacterial activities. This can lead to, when comparing digestion using the same retention times, a more complete degradation of all the biodegradable substrate in pretreated sludge digested thermophilically. In the case of the thermophilic tests, there is still some influence on the biogas production when

changing SRT and MW temperature and this influence seems to be different from that measured in mesophilic tests for the SRT parameters. Greater increases are seen at the extremes of SRT tested, (4 and 15 d) and not, as for mesophilic tests, at an intermediate value. Also, the pretreatment temperature where the greatest increase in biogas production occurs is dependent on SRT, and is a smaller value with higher SRT. MW pretreatment temperature for SRT 4 d, where CBP increase is maximal, is 137 ˚C, while at SRT 7 d this value decreases to 133˚C and at SRT 15 d this value decreases further to 122˚C. One possible explanation is that the increase in SRT causes a change in the composition of the sludge flocs with a higher relative amount of proteins and cellular debris in the floc matrix, less bound water and a higher proportion of recalcitrant substrate for older sludge. The higher SRT also increases the amount of sCOD released after pretreatment. This might increase the rate of formation of inhibitory compounds at a given temperature, for sludge with higher SRT due to greater concentration of both biodegradable substrate and refractory compounds. Similar to mesophilic tests, there is a noticeable decrease in the biogas production for the highest temperature tested. In the case of thermophilic tests, the relative biogas production decreases to a value as low as 1, not offering any advantage over non-pretreated sludge.

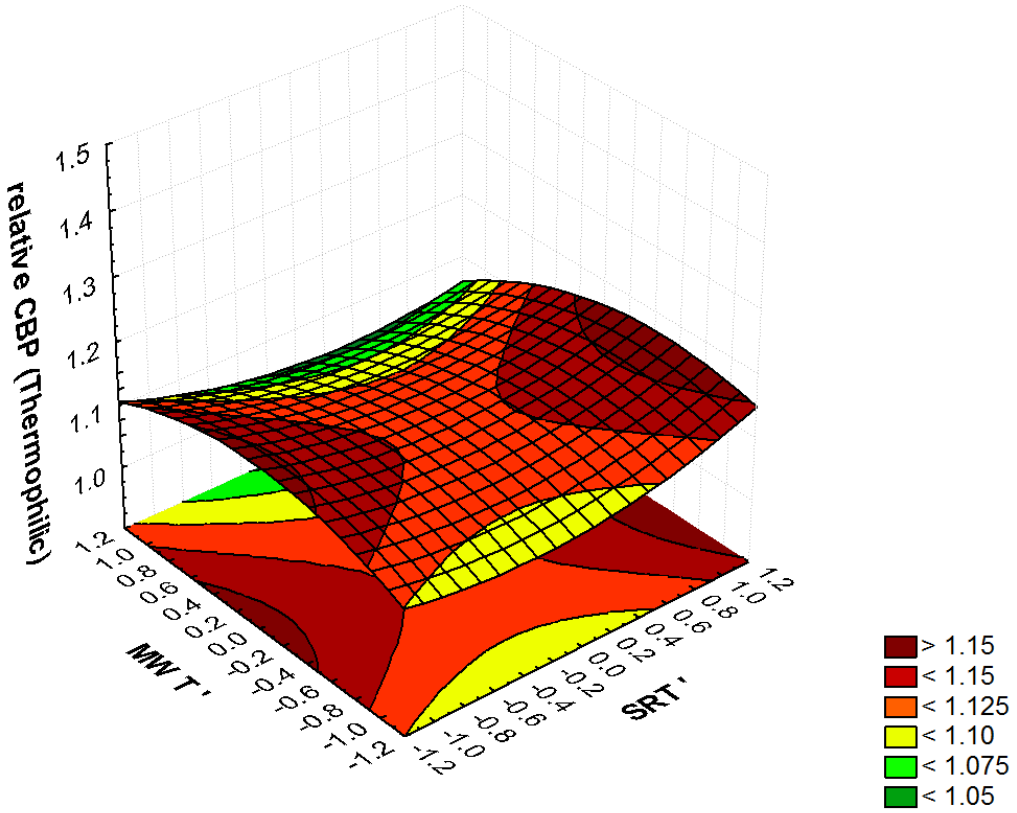

Figure 5.5 - CBP (Relative to control) as a function of SRT and MW T for thermophilic digestion tests.

Thermophilic digestion is referred in several works as having the potential to produce more biogas when digesting similar substrates compared to mesophilic digestion. To compare the results obtained in the digestion of pretreated sludge at both temperatures, a new regression was made with the difference in total biogas production between thermophilic and mesophilic tests of the same type of sludge (SRT and MW T). The new variable was then:

$$\Delta CBP = CMP_{Therm.} - CMP_{Meso.} \quad (9)$$

The results (Table 5.12 and Figure 5.6) show that thermophilic digestion produces more biogas in the majority of tests, however this difference is minimal for regions around the optimal pretreatment temperature at every SRT. For the highest SRT, this difference is even slightly

negative. The greatest improvements are seen at the extremes of temperature tested, either at 100 or 175˚C. Mesophilic tests had a greater influence of either SRT and MW T on the biogas production with a noticeable maximum around 140^{o}C and SRT of approximately 9 d and sharp decrease of the relative increase in CBP outside this region, contrasting with the broader area of maximum biogas production that thermophilic tests exhibit (appendix A), so making the differences larger outside the MW T optimum point region for mesophilic digestion. The difference is greater at 100˚C due to the fact that at this temperature much of the substrate is dissolved but not transformed into inhibitory or recalcitrant material as happens when temperatures above 150˚C cause Maillard reaction products to be generated. Model coefficients are given in Table 5.13.

Table 5.12 - Average difference in CBP (ml) for thermophilic and mesophilic tests for the tested conditions. The relative increase in thermophilic biogas production compared to mesophilic is given in parentheses.

	MW T (˚C)		
SRT (d)	**100**	**150**	**175**
4	36.5 ± 9.3 (11.7%)	6.0 ± 8.4 (1.7%)	30.0 ± 7.5 (9.4%)
7	38.5 ± 7.4 (12.2%)	4.1 ± 9.9 (1.1%)	7.0 ± 6.2 (2.1%)
15	37.3 ± 5.2 (12.7%)	-1.0 ± 8.4 (-0.002%)	4.0 ± 6.3 (1.3%)

Table 5.13 - Estimated coefficients (along with 95% confidence intervals) for ΔCBP as a function of SRT and MW T.

Coefficients	**Value**	**Std. Error**	**t**	**p-value**
β_0^*	*-5.0294 ± 7.2836*	*3.371459*	*-1.4918*	*0.159624*
β_1^*	*-5.3053 ± 4.3181*	*1.998781*	*-2.6543*	*0.019850*
β_{11}^*	*Pooled*			
β_2^*	*-12.7062 ± 4.4932*	*2.079829*	*-6.1093*	*0.000037*
β_{22}^*	*29.7739 ± 8.7806*	*4.064384*	*7.3256*	*0.000006*
β_{12}^*	*-5.4421 ± 5.1475*	*2.382706*	*-2.2840*	*0.039821*

	SS	***df***	***MS***	***F***	***p-value***	R^2	R^2_{adj}
Whole model	*4266.1*	*4*	*1066.5*	*21.1844*	*0.0000*	*0.8670*	*0.8261*

Some authors defend that thermophilic bacteria are more sensitive to inhibition than mesophilic bacteria (Angelidaki and Ahring, 1994; Wilson et al, 2008) and that can be the reason behind the fact that the difference measured was smaller for 175˚C than at 100˚C. The difference is also smaller for higher SRT for tests at 175˚C, which is not noticeable for tests done with sludge pretreated at 100˚C. The same reason that explains the decrease in optimum MW pretreatment temperature for thermophilic tests coupled with the potentially higher sensitivity of thermophilic bacteria to inhibitory substances might be the reason behind the influence of high SRT and high MW T in the smaller difference of biogas productions between mesophilic and thermophilic tests.

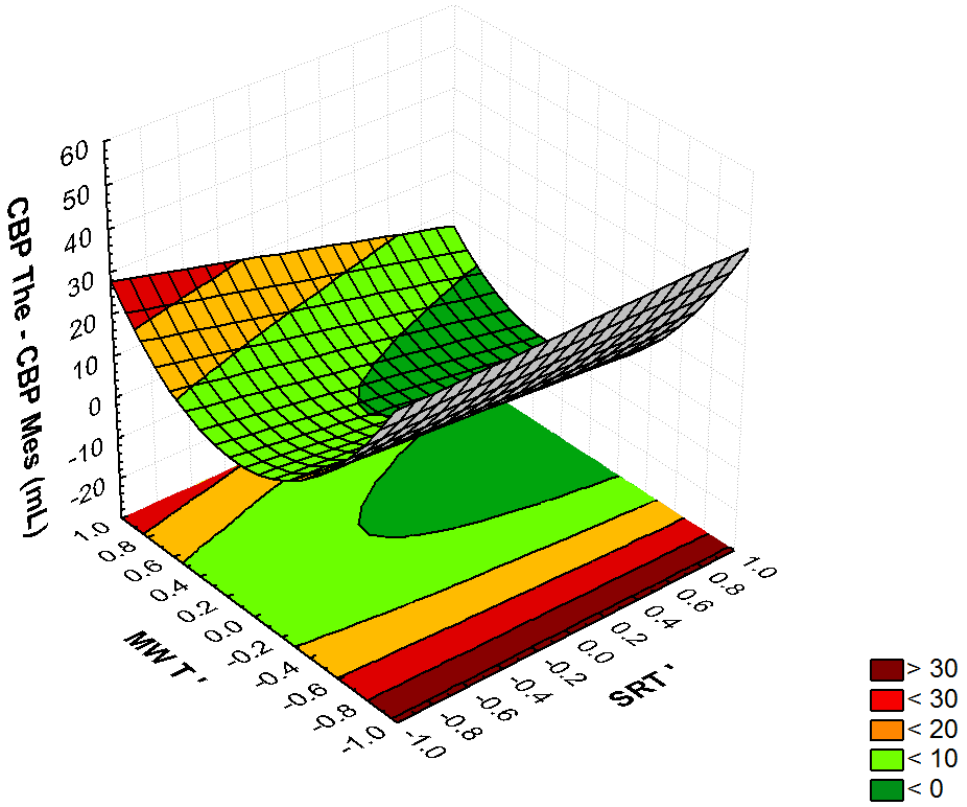

Figure 5.6 - ΔCBP as a function of SRT and MW T.

The results show that MW pretreatment has the capacity to improve solubilisation and to improve digestion efficiency, but these two factors are correlated only until a certain temperature. Above an optimal pretreatment temperature, MW pretreatment causes the onset of other phenomena besides COD solubilization, such as the formation of inhibitory compounds or liberation of recalcitrant substrate that adversely affect digestion efficiency. Thermophilic digestion also has a beneficial effect in digestion efficiency but this effect is not uniform. A more beneficial effect is measured with MW temperatures away from the optimum area (around 140˚C). High SRT sludge

also benefits from MW pretreatment but at very high temperatures, inhibition phenomena limits the improvement.

5.4.3 Kinetic analysis of BMP test curves

Biogas production data were used to analyse kinetic parameters in the degradation of substrate using the Gompertz equation. This equation was used to describe the growth of *Lactobacillus plantarum* and *Lactobacillus acidophilus* (Cho et al, 1996; Zwietering et al , 1990) and is equal to:

$$N = \int_0^t r_g \, dt = A * exp\left(-exp\left[\frac{\mu_m * e}{A}(\lambda - t) + 1\right]\right) \quad (10)$$

Expression (10) can be transformed to calculate cumulative methane production by substitution and modification of the original terms of the Gompertz equation as demonstrated by Lay et al (1998). The final equation is thus:

$$M = P * exp\left\{-exp\left[\frac{B * SMA * e}{P}(\lambda - t) + 1\right]\right\} \quad (11)$$

With P being the methane/biogas production potential (mL CH_4 or mL biogas), B the biomass concentration (g VSS), *SMA/SBA* the specific methanogenic/biogas activity (mL CH_4/ g VSS.d) and λ the lag-phase time duration (d). Equation 11 was then used as an alternative expression to model methane production on biodegradability tests that includes a parameter to measure lag-time duration and a direct method to estimate true SMA/SBA. First-order models were previously used to model biogas production in BMP tests of MW pretreated sludges (Toreci et al, 2009 ; Eskicioglu 2006). In these tests however, a lag period before exponential phase was not accounted for, and different models were applied for the exponential phase data in order to have

an estimate of SMA that was not skewed by previous data points. SMA or SBA can be a measure of the non-temporary and non reversible inhibition that affects a microbial community, while lag-time can be used as a measure of the reversible and temporary inhibition (Rozzi and Remigi, 2004). All the curves were adjusted with the Gompertz model and SBA and λ were estimated using Excel Solver. The results are expressed in Table 5.14:

Table 5.14 - Parameter estimation results for BMP curve modelling.

SRT (d)	MW T (˚C)	SBA (mLbiog/gVSS.d)	λ (d)	r^2	χ^{2**}	df	goodness of fit
Mesophilic tests							
4	Control	134.8 ± 15.5	0.000 ± 0.000	0.9885	0.2171	18	Accepted
4	100	138.0 ± 5.6	0.054 ± 0.019	0.9910	0.2477	18	Accepted
4	150	179. 4 ± 18.0	0.101 ± 0.026	0.9925	0.2727	18	Accepted
4	175	156.2 ± 14.3	0.712 ± 0.106	0.9896	0.2337	18	Accepted
7	Control	120.6 ± 2.0	0.000 ± 0.000	0.9715	0.0475	18	Rejected
7	100	140.9 ± 16.3	0.000 ± 0.000	0.9799	0.0349	17	Rejected
7	150	228.9 ± 48.7	0.115 ± 0.024	0.9943	0.4365	18	Accepted
7	175	159.9 ± 19.7	0.433 ± 0.019	0.9882	0.3948	18	Accepted
15	Control	123.0 ± 1.2	0.000 ± 0.000	0.9732	0.0601	18	Accepted
15	100	124.5 ± 2.9	0.000 ± 0.000	0.9856	0.1064	18	Accepted
15	150	161.6 ± 7.9	0.000 ± 0.000	0.9841	0.0868	18	Accepted
15	175	153.9 ± 15.8	0.282 ± 0.044	0.9921	0.7431	18	Accepted
Thermophilic tests							
4	Control	185.3 ± 25.3	0.809 ± 0.128	0.9982	0.8641	18	Accepted
4	100	155.8 ± 22.7	1.041 ± 0.555	0.9929	0.2115	18	Accepted
4	150	163.8 ± 22.4	1.577 ± 0.875	0.9930	0.2012	16	Accepted
4	175	165.5 ± 31.2	1.605 ± 0.080	0.9893	0.1345	18	Accepted
7	Control	182.2 ± 10.9	1.079 ± 0.255	0.9973	0.9810	18	Accepted
7	100	196.5 ± 16.7	0.823 ± 0.020	0.9967	0.3890	18	Accepted
7	150	172.6 ± 11.5	1.990 ± 0.203	0.9891	0.0011	19	Rejected
7	175	144.1 ± 14.7	1.792 ± 0.355	0.9770	0.0014	18	Rejected
15	Control	144.3 ± 22.9	1.506 ± 0.575	0.9922	0.4431	18	Accepted
15	100	178.0 ± 12.6	1.646 ± 0.256	0.9923	0.1423	17	Accepted
15	150	163.8 ± 16.4	2.382 ± 1.430	0.9861	0.0002	18	Rejected
15	175	149.4 ± 26.7	1.594 ± 0.588	0.9873	0.0119	18	Rejected

** - Chi-square test at 95% confidence level.

The adjusted models show that thermophilic inoculum shows greater activity than mesophilic inoculum (measured by the specific biogas activity SBA) when biodegrading control sludge, that is, non-pretreated sludge for all the types of sludge tested (SRT 4, 7 and 15 d). The increase in activity for thermophilic sludge relative to mesophilic is highest for SRT 7 d sludge (increase of 51%), and smallest for SRT 15 d sludge (17% increase). Thermophilic sludge is reported by several authors to be able to convert fatty acids more quickly and also be able to hydrolyze particulate substrate at a faster rate (Puchajda and Oleszkiewicz, 2006; Gavala et al, 2003), so the higher overall activity is both a result of the higher rate of conversion of fatty acids into methane but also due to the higher availability of substrate for methanogens due to increased rate of hydrolysis. The introduction of MW pretreatment causes two effects in pretreated sludge digestion both at mesophilic and thermophilic temperatures: one is that it increases the soluble substrate already hydrolyzed, but the other effect is that it also changes slightly the nature of that substrate, causing the appearance of inhibition phenomena, temporary and reversible and/or irreversible and permanent. For mesophilic tests, all tests using pretreated sludge showed a higher SBA, and this activity was dependent on the MW pretreatement temperature as can be seen in Figure 5.7. All SBA measured by the model on the MW pretreated tests were higher than the SBA of the control, suggesting that hydrolysis was an important limiting step in the degradation of substrate. The SBA increases with the increase of soluble substrate (due to higher MW pretreatment temperature) suggesting that methanogens in the mesophilic inoculum have a large activity potential that was not completely used for control tests and MW test at the lowest temperature. For MW temperatures higher than 150˚C, it is observed that there is a sharp decrease in the activity, most likely due to the presence of the inhibitory compounds formed by reactions between soluble sugars and proteins at high temperatures. There is a decrease in SBA, but also the appearance (in the case of SRT 15 d) or sharp increase (in the case of SRT 4 d and

SRT 7 d) of a lag period. Taken as a reference the activity of the control test at 150˚C, this shows that mesophilic bacteria spend some time adapting to the substrate that causes an acute inhibition and manage to recover, but not completely since the SBA measured is lower than the maximum measured at 150˚C. For lower temperatures, there is some lag period for SRT 4 and 7 d, but this inhibition is reversible, since the activity after that lag period is higher than the one measured for the control. Despite the reversible and irreversible inhibition at 175˚C, MW still has a positive effect in the biodegradation of sludge at mesophilic temperatures, since SBA is still higher than the one measure for the control. The inhibition measured is not so great that it cancels the effect of the elimination (or attenuation) of the hydrolysis limiting step.

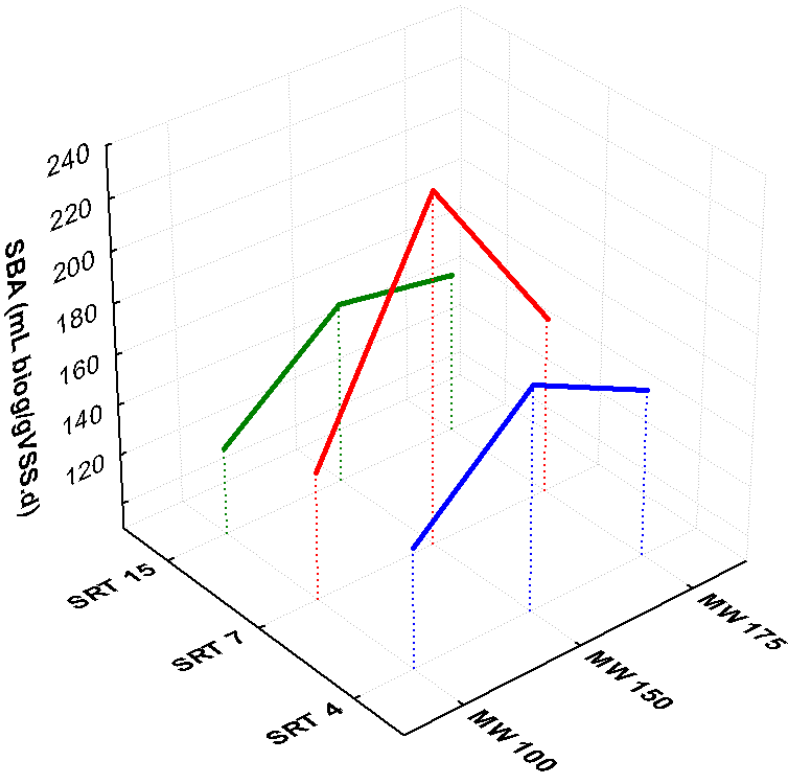

Figure 5.7 - SBA for mesophilic MW pretreated sludge tests.

For thermophilic tests, MW has a somewhat different effect. Despite the fact that a large increase in soluble substrate is seen after pretreatment, this is not directly transformed into a higher SBA. In the tests for SRT 4d and SRT 7 d, the activity is actually lower than the activity measured for the control sludge. And the values are smaller for all the tests (with the exception of MW 100 SRT 7 d) for the younger sludges. The increase in MW pretreatment temperature has the effect of decreasing noticeably the activity for all but SRT 4 d sludge, where a statistically significant change was not measured in all the MW temperatures tested (Figure 5.8). The lag period also increases in the MW tests relative to the control tests and lag periods were detected in all tests, contrary to the mesophilic case. This suggests that inhibition is a phenomenon that thermophilic sludge is more sensitive to than mesophilic sludge. This assumption correlates with the observation that the average levels of volatile fatty acids, and especially propionic acid are generally higher in the thermophilic tests, and tend to increase with the MW pretreatment temperature (Appendix D). However, since the control activity for thermophilic sludge is higher than the corresponding mesophilic test, thermophilic tests still show a higher SBA in most cases, especially for lower MW pretreatment temperatures. For the highest MW pretreatment temperature, mesophilic tests showed a higher SBA in certain conditions (SRT 7 and 15 d).

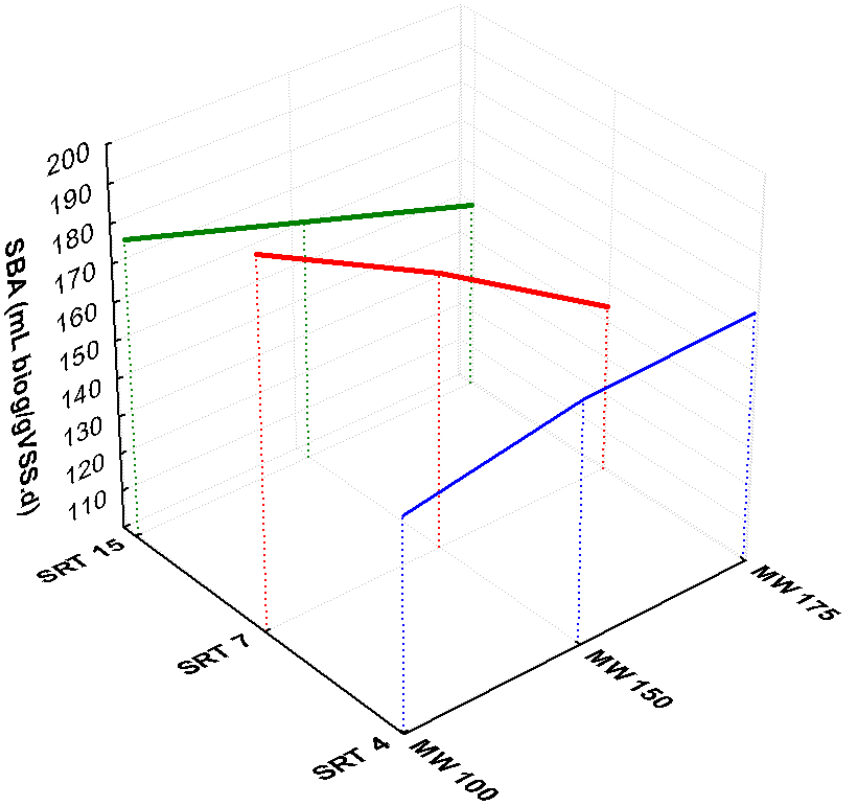

Figure 5.8 - SBA for thermophilic MW pretreated sludge tests.

The values for SBA and lag-period, show that although thermophilic sludge has a higher activity than mesophilic sludge, it also is more susceptible to inhibition phenomena caused by the MW pretreatment temperature, and that the inhibition is detected at a temperature less than the one that caused a decrease in SBA for mesophilic tests. For mesophilic tests, the main cause of inhibition seems to be the formation of inhibitory compounds at high temperatures, but for thermophilic tests, the decrease in SBA relative to control at lower temperatures seems to be caused by other factors. It is true that for thermophilic tests, the limiting factor of hydrolysis rate is less visible and a less masked behaviour of methanogenic thermophilic bacteria is seen since,

more frequently than for mesophilic tests, methanogens at thermophilic temperatures are exposed to a higher concentration of soluble substrate because thermophilic hydrolytic bacteria degrade substrate faster. This leaves less room for improvement of SBA by means of improvement of hydrolysis rates because thermophilic methanogens are closer to their maximum specific conversion rate. This fact can leave thermophilic bacteria more exposed to the influence of inhibitory substances not produced by high MW temperatures but pre-existent in the sludge floc matrix and released with the pretreatment. It is noteworthy that one of the functions or consequences of the existence of a floc matrix around the bacterial cells is that the matrix can and does adsorb compounds and elements such as heavy metals, or toxin that otherwise would cause inhibition of the bacterial functions, (Henriques et al, 2007; Henriques et al, 2005).

5.5 Conclusions

MW pretreatment is able to solubilise a significant portion of sludge COD. The amount of COD solubilized is generally dependent on the pretreatment temperature, with higher temperatures causing an increase in sCOD. Sludge SRT influences the amount of COD that is solubilized and the pretreatment temperature at which a certain degree of solubilisation is reached. In this study, a high SRT sludge showed a greater increase in solubilisation ratio with increased MW temperature than young sludge.

MW pretreatment has a beneficial effect on biogas production when digesting pretreated sludges, both at mesophilic and thermophilic temperatures; however, the degree of improvement is significantly different in each case. Mesophilic digestion was capable of reaching higher improvements relative to control tests, but total biogas production was higher in thermophilic tests. While for mesophilic tests, there seems to be a clear area where maximum improvements

are achieved (around 140˚C for all kinds of sludge), the relative increases attained in thermophilic tests seem much more stable and constant across the spectrum of conditions tested.

In both cases, MW pretreatment has a maximum beneficial effect and a sharp decrease in those beneficial effects, (measured as increase in biogas production) for high MW pretreatment temperature, a decrease more significant for thermophilic digestion since at the highest MW temperature, the increase in biogas production relative to control is almost negligible.

For conditions close to the optimum point measured in this work for mesophilic digestion, the difference between thermophilic and mesophilic is very small, but this difference increases significantly for MW pretreatment temperature values outside this area.

Thermophilic bacteria have higher biodegradation activity, but also are more susceptible to inhibition phenomena when digesting MW pretreated sludge. Mesophilic bacteria can show some signs of inhibition in certain conditions, but inhibition is reversible and mesophilic sludge regains all the activity prior to exposure to MW pretreated sludge. In the thermophilic case, a period of adaptation was observed for all the tests, but in some cases, the inhibition was not reversible and activity after adaptation did not reach the same values as observed before exposure to MW pretreated sludge.

5.6Acknowledgements

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Chapter 6

Evaluation of Continuous Mesophilic, Thermophilic and Temperature Phased Anaerobic Digestion of Microwaved Activated Sludge

Nuno M. Coelho, Kevin J. Kennedy, Ronald L. Droste

6.1 Abstract

The effects of microwave (MW) pretreatment, staging and digestion temperature on anaerobic digestion were investigated in a set-up of ten reactors. A mesophilic reactor was used as a control. Its performance was compared to single-stage mesophilic and thermophilic reactors treating pretreated and non-pretreated sludge, temperature-phased (TPAD) thermophilic-mesophilic reactors treating pretreated and non pretreated sludge and thermophilic-thermophilic reactors also treating pretreated and non-pretreated sludge. Four different sludge retention times (SRTs) (20, 15, 10 and 5 d) were tested for all reactors. Two-stage thermo-thermo reactors treating pretreated sludge produced more biogas than all other reactors and removed more volatile solids. Maximum volatile solids (VS) removal was 53.1% at an SRT of 15 d and maximum biogas increase relative to control was 106% at the shortest SRT tested. Both the maximum VS removal and biogas relative increase were measured for a system with thermophilic acidogenic reactor and thermophilic methanogenic reactor. All the two-stage systems treating microwaved sludge produced sludge free of pathogen indicator bacteria, at all tested conditions even at a total system SRT of only 5 d. MW pretreatment and staging reactors allowed the application of very short SRT (5 d) with no significant decrease in performance in terms of VS removal in comparison with the control reactor. MW pretreatment caused the solubilisation of organic material in sludge but also allowed more extensive hydrolysis of organic

material in downstream reactors. The association of MW pretreatment and thermophilic operation improves dewaterability of digested sludge.

KEYWORDS: Single stage**,** Two-stage digestion, Microwave, Mesophilic, Thermophilic

6.2 Introduction

Anaerobic digestion is commonly used in wastewater sludge treatment. However, low biodegradability of sludges, particularly waste activated sludge (WAS) is an issue. Hydrolysis is a rate limiting step when degrading this type of complex organic material, and most of the biodegradable material is either enclosed inside the microbial cell wall (Park et al., 2004) or enmeshed in a extracellular polymeric matrix (Neyens and Baeyens, 2003), which further contributes to limit the biodegradability of these sludges to 35-45% reduction in volatile solids (VS) (Bolzonella et al., 2005; Bhattacharya et al, 1996).

Microwaving is a novel method to thermally pretreat sludges that increases digestion efficiency and decreases pathogen content. It is an energy efficient method, since it eliminates heat losses that occur in energy transmission in conventional heating. MWs can also provide rapid increases in the inner temperature of bulk liquids, decreasing pretreatment time (Metaxas & Meredith, 1983). Hong (2002) applied MW radiation to different types of sludge in order to check the effect on biodegradability. The effect in solubilizing chemical oxygen demand (COD) was effective in activated sludge since the fraction of soluble COD (sCOD) to total COD (tCOD) increased from 8.5 to 18%. The pretreatment consisted of heating the sludge to a temperature of 70ºC. The increase in this ratio for primary sludge was only 1%. For higher pretreatment temperature (100ºC) the digestion of sludge showed an increase in the amount of methane produced of 23% for primary sludge (PS) and 15% for activated sludge (Hong 2002).

Eskicioglu et al. (2007) investigated the effects of MW intensity, temperature and sludge concentration on the solubilization of WAS (taken from an activated sludge unit operating at 5 d SRT). It was reported that the MW intensity was not a significant factor influencing digestion but temperature of pretreatment and sludge concentration did show an influence on both WAS solubilisation and biogas production. Sludge irradiated to 96ºC had a greater production of biogas than sludge irradiated to 75ºC and this sludge in turn produced more biogas than sludge irradiated to 50ºC. Sludge pretreated to 96ºC showed an increase of 20% in biogas production compared to the control in the assays at 3% total solids (TS). For the assays at 1.4% TS the increase in biogas production was 15%. The authors also performed a study based on the ultrafiltration membrane fractionation of the soluble fraction of the pretreated sludge that confirmed that digesters treating high molecular weight materials resulted in smaller biodegradation rate constants. Toreci et al. (2009) tested MW pretreatment at temperatures above the boiling point (175^{o}C) and reported increases of 31% in biogas production in mesophilic anaerobic digestion compared to controls without pretreatment. The authors noted also the occurrence of inhibition in the early stages of digestion. In previous experiments the same authors reported higher percentages of solubilisation of tCOD at MW pretreatment temperatures of 175^{o}C than those obtained at pretreatment below boiling point (Toreci et al. 2008).

The dual-stage thermophilic/mesophilic process or temperature-phased anaerobic digestion (TPAD) has gained some interest due to the fact that it tries to combine the advantages of thermophilic systems in terms of pathogen control and VS reduction, makes use of process optimization due to staging, and it is still economical to operate because the bulk of the digestion takes place in the mesophilic stage (Han et al. 1997, Sung and Santha 2003). Some of the reasons proposed to explain better performance of dual-stage TPAD include the setting of optimal conditions for two different bacterial populations (mesophilic methanogens and thermophilic

hydrolytic/acidogenic) in terms of pH, temperature and residence time. It is known that the methanogens and hydrolytic/acidogenic bacteria have different optimal pH, and the thermophilic acidogens growth rate is higher than mesophilic methanogens (Kiyohara et al, 2000). Also, some compounds that are inhibitory to methanogenesis such as phenol or unsaturated fatty acids, are less inhibitory after being acidified (Kobayashi et al,1989). Finally, a lower pH in the first reactor may cause a different distribution of the VFA produced by the acidogenic bacteria, one that includes a smaller proportion of more difficult to degrade VFAs, such as propionate (Breure and van Andel 1984, Azbar and Speece 2001).

Very few studies have been published that report the use of pretreatment methods prior to TPAD or two-stage digestion. Toreci et al. (2009) tested high temperature MW pretreatment (175^{o}C) combined with two-stage mesophilic digestion for three different SRTs (20, 10 and 5 d) with somewhat inconclusive results. Although MW pretreatment alone improved biogas production and VS removal for all SRT in comparison with non-pretreated sludge, and dual-stage digestion alone showed greater biogas production and higher VS removal, MW pretreatment associated with dual-stage digestion did not show any advantages regarding VS removal and biogas production. Variations on the composition of sludge, the type of sludge being tested, viz., the SRT and MW pretreatment process, particularly MW intensity and pretreatment duration, may have interacted and caused the observed results.

The combination of two different techniques or two different pretreatment methods is not original. However, microwaving has not yet been used in combination with other methanization enhancement techniques (either other pretreatment options, or digestor set-ups other than mesophilic single or two stage). So, there is an interest to evaluate what a novel pretreatment technology that is energy efficient and has proved to increase digestion efficiency can provide in

terms of methane production or solids reduction when combined with another pretreatment technique or variations in digestion set-up from the conventional mesophilic digestor .

Given the aforementioned results by previous authors and in order to tackle insufficient or nonexistent experience and results with MW pretreatment and TPAD, a set of tests was devised to evaluate the influence of these parameters in global digestion performance.

6.3 Materials and methods

A total of 10 semi-continuous reactors were setup to study the effect of MW pretreatment, staging, digestion temperature and SRT on process performance. The experimental setup is depicted in Figure 6.1.

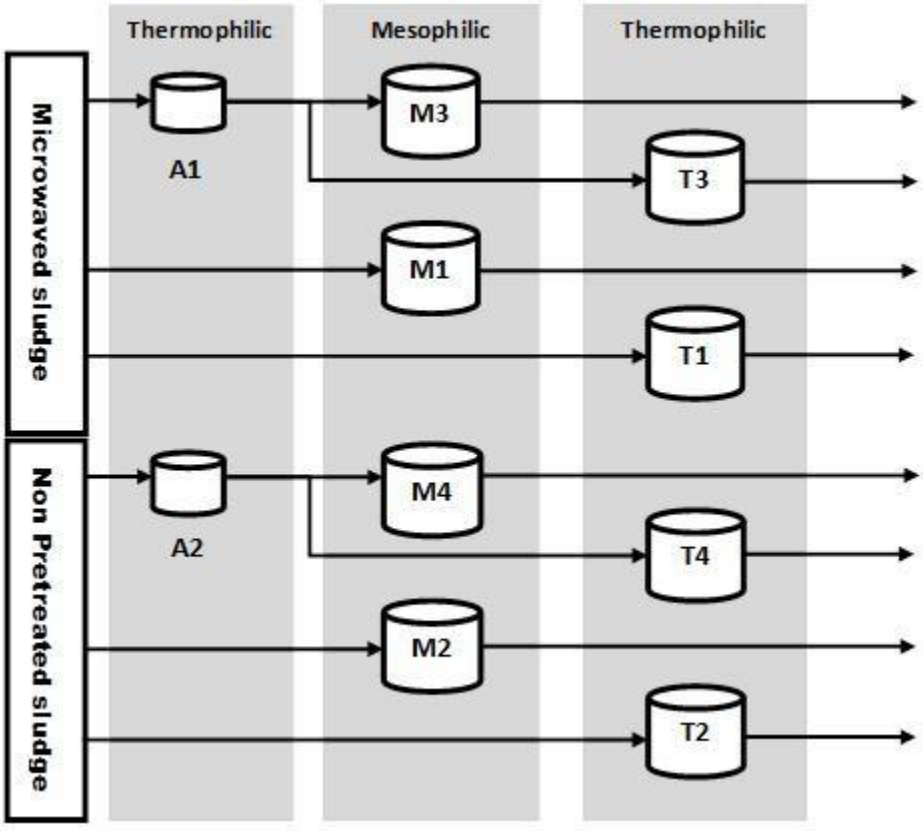


Figure 6.1 - Experimental setup of reactors

The reactors used were 1000 mL Schott borosilicate glass bottles, with a useful volume of 800 mL. The reactors were sealed with black butyl rubber stoppers (VWR, Montreal, QC) with two holes: one to sample, waste and feed the reactors and the other to collect and measure the biogas. Biogas was collected in 2 L Tedlar bags. The Tedlar bags (Chromatographic Specialties Inc., ON) were equipped with on/off valves and a septum fitting that was used for gas composition sampling. The volume of biogas produced daily was measured using a manometer.

The mesophilic reactors receiving pretreated sludge were inoculated with acclimatized sludge. This sludge was taken from the anaerobic reactors of the Ottawa, ON municipal wastewater treatment plant [Robert O. Pickard Environmental Centre (ROPEC)] that digest primary and secondary sludges at a feed ratio of 48:52. This seed sludge was acclimatized using a completely mixed reactor operating at 20 d SRT fed with microwaved sludge for more than a year. The remaining mesophilic reactors were inoculated directly using sludge from ROPEC mesophilic digesters. Thermophilic reactors testing pretreated sludge were inoculated using thermophilic sludge collected in Annacis Island Wastewater Treatment Plant (Vancouver, BC) that was acclimatized for more than one year using a 20 d SRT mixed reactor heated at 55º C fed everyday with microwaved sludge. The remaining thermophilic reactors were directly inoculated with non-acclimatized Annacis Island WTP thermophilic sludge. The use of thermophilic sludge to inoculate thermophilic reactors is based on the fact that thermophilic sludge provides a faster start-up to thermophilic reactors, along with a more stable operation since it avoids a rapid temperature change from mesophilic to thermophilic that may bring about a population shift if the groups are not compatible, specially a decrease in thermophilic methanogens, crucial to digestion stability (Mata-Alvarez, (2002, Nozhevnikova et al. 1999).

Feed sludge was comprised of thickened WAS collected at ROPEC. ROPEC has a conventional aerobic activated sludge process with a SRT of 5 days and a primary settling step prior to the

activated sludge aerobic tank. Ferric chloride is added for phosphorous removal and biosolids are stabilized by anaerobic digestion. Feed sludge was divided into two types, a non-pretreated sludge (NPT), and a microwave pretreated sludge (PT). MW pretreatment was performed by heating 500 mL sludge samples in a closed plastic container in a conventional domestic MW oven (Panasonic NNS53W + inverter, 0.045 m^3 capacity, 1250 W, 2450 MHz frequency and 12.24 cm wavelength), working at 100% MW intensity up to the boiling point (around 96 ºC). The closed container was used to minimize evaporation of water and volatile compounds. Sludge and container were weighed before and after pretreatment and distilled water was added in case weight was lost during MW pretreatment. A thermal profile of sludge samples was determined and a temperature ramp of 14.4 ºC/min was calculated when heating sludge samples of approximately 500 g at full power. The heating time required to reach boiling point from a room temperature of approximately 20ºC was 6 min. This pretreatment time was used for all microwaved samples.

Four different SRTs were tested in all the setups 20, 15, 10 and 5 d, with the total SRT of two-stage systems being equal to the single-stage SRT. For two-stage systems, the SRT applied in the acidogenic thermophilic stage (reactors A1 and A2) was 2 d, to avoid excessive methanization in that stage. The subsequent SRT used in the methanogenic stages were 18 d (for a total SRT of 20 d), 13 d (total SRT 15 d), 8 d (total SRT 10 d), and 3 d (total SRT 5 d).

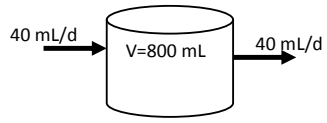
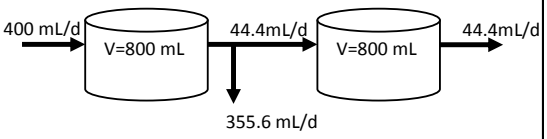
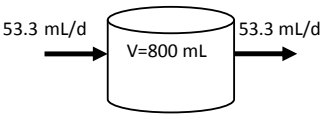
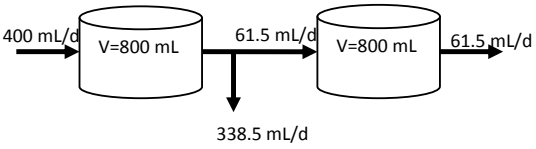
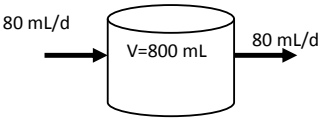
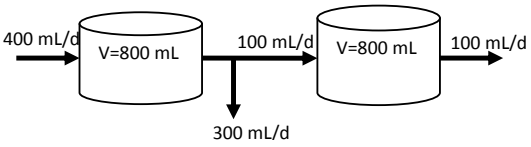
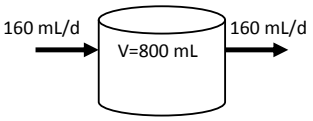
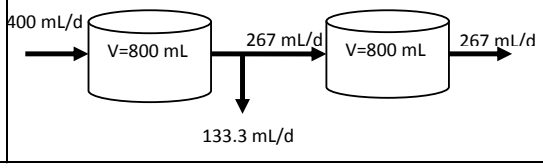
Single stage reactors	SRT (d)	Two-stage reactors	Total SRT (d)
	20		2 + 18 = 20
	15		2 + 13 = 15
	10		2 + 8 = 10
	5		2 + 3 = 5

Figure 6.2 - Conditions of each test period and SRT distribution on two-stage systems.

The mesophilic reactors were kept in a constant temperature controlled shaker at 35 ± 1ºC and 90 rpm (PhycroTherm, New Brunswick Scientific Co. Inc., NB), while the thermophilic ones were kept at 55 ± 1ºC and 90 rpm in a similar controlled temperature shaker. Reactors were fed semi-continuosly once a day, with non pretreated sludge and microwaved sludge being fed to the mesophilic single-stage, thermophilic single-stage and acidogenic reactors and with the effluent of the acidogenic thermophilic reactors being fed to the second reactor of the two-stage systems. All reactors were started with a SRT of 20 d, (18 d for the two-stage ones plus 2 d for the acidogenic reactors), and were operated with the same SRT until two conditions were met: a) fluctuation of less than 10% in the biogas production was observed and b) the fluctuation of less than 10% over an average value was observed over a period of at least three SRTs. To

characterize reactors performance, several parameters were measured during operation. tCOD, sCOD, TS, VS and pH were measured twice a week; ammonia, VFA) alkalinity, dewaterability and biogas composition were measured once a week. Bacterial count tests were also performed every two weeks. TS and VS were determined based on Standard Methods procedure 2540G (APHA, 1995). Ammonia measurements were carried out using an ORION Model 95-12 ammonia gas sensing electrode connected to a Fisher Accumet pH meter model 750. The analysis was conducted according to Standard Methods 4500D procedure (APHA, 1995) and reported as ammonia-N. Colorimetric COD measurements were done based on Standard Methods with a Coleman Perkin-Elmer spectrophotometer Model 295 at 600 nm light absorbance. Before sCOD determination, sludge samples were centrifuged and filtered through membrane disc filters with 0.45 µm pore size. Total VFA were measured by injecting supernatants to a HP 5840A GC with glass packed column and a flame ionization detector. Biogas composition was determined using a HP GC model 5710 equipped with a thermal conductivity detector. Dewaterability was determined using a capillary suction timer (Fann Instrument Company, Model 440, TX) without polymer addition, according to procedure 2710G (APHA, 1995). For bacterial enumeration, namely *E. Coli* and total coliforms, a semi-automated test for presence and quantification based on MPN after incubation for 24 h was used (IDEXX Colilert® Quanti-tray 2000). The method provides 95% confidence limits comparable to the membrane filtration method and can count up to 2000 CFU/mL without dilution.

6.4 Results and Discussion

Previous studies have shown that MW pretreatment is more effective for sludge with high solids concentration (Eskicioglu et al. 2007), so no dilution was made to sludge collected at ROPEC before feeding the reactors. The operation of all the reactors started with the highest SRT (20 d),

in order to minimize instability due to high organic load. The time required to obtain a stable 3 hydraulic retention time period (meaning a period where no more than 10% variation was observed in biogas production), was not longer than two to three weeks, except when a 5 d SRT was applied which resulted in some reactors (M1, M2 and M4) not reaching stable operating conditions.

The average properties of feed sludge used during the different periods are shown in Table 6.1. The values are averages calculated in each of the periods, along with the confidence interval assuming a normal distribution of the values around the mean and a confidence level of 95%.

Table 6.1 - Properties of sludge fed at the different SRTs tested.

	SRT 20		SRT 15		SRT 10		SRT 5	
Properties	**NMW**	**MW**	**NMW**	**MW**	**NMW**	**MW**	**NMW**	**MW**
TS (%)	5.14±0.09	5.80±0.06	5.41±0.03	5.70±0.04	4.32±0.04	5.73±0.06	4.81±0.06	5.89±0.06
VS (%)	3.61±0.08	3.94±0.09	3.96±0.04	3.91±0.04	3.23±0.02	3.94±0.04	3.14±0.03	3.92±0.04
VS/TS	0.70±0.02	0.68±0.01	0.73±0.01	0.69±0.01	0.76±0.06	0.69±0.01	0.68±0.12	0.67±0.01
tCOD (g/L)	60.26±0.36	69.55±0.45	63.39±0.41	69.54±0.40	52.90±2.90	68.24±0.32	61.17±3.35	68.91±0.73
sCOD (g/L)	3.82±0.32	13.94±0.44	3.91±0.34	13.82±0.40	3.94±0.40	13.00±0.65	3.67±0.47	13.80±0.81
sCOD/tCOD	0.06±0.01	0.20±0.01	0.06±0.01	0.20±0.01	0.07±0.01	0.19±0.01	0.06±0.01	0.20±0.01
Alkalinity	1578±123	1755±201	2135±114	1699±99	1651±102	1418±112	2004±201	1989±135
NH_3-N	751±185	853±102	887±114	1023±195	702±102	1203±112	874±124	1320±119
TVFA	228±128	560±132	441±130	712±211	197±172	702±155	225±99	802±131

The use of pretreatment markedly increased the amount of soluble organic matter, as measured by soluble COD, with solubilisation as a fraction of tCOD that is in soluble form increasing from around 0.06 to 0.2, indicating a potentially easier or faster digestion of organic matter present in the sludge. There is also a noticeable increase in ammonia on microwaved sludge, most likely due to release and breakdown of proteinaceous material due to MW and temperature effects during pretreatment.

Sludge fed to the reactors during the test periods had slightly different characteristics depending on the period and pretreatment applied. Solids concentration is higher for the SRT 20 and 15 d periods, due most likely to seasonal variations in sludge properties in ROPEC. Solids concentration is also generally a bit higher after MW pretreatment even though deionised water was added to compensate for evaporation. The same trend is visible for COD (Figure 6.3), with tCOD for microwaved samples generally being higher than in non-microwaved samples. sCOD concentration is significantly higher in all microwaved sludges. The decrease in tCOD during SRT 10 d is a reflection of the seasonal variations of ROPEC waste sludge characteristics. Even though the average values for VS and COD are different for MW and non MW sludge for each period, statistically, the difference is not significant, as can be seen by the error bars in the total COD values in Figure 6.3.

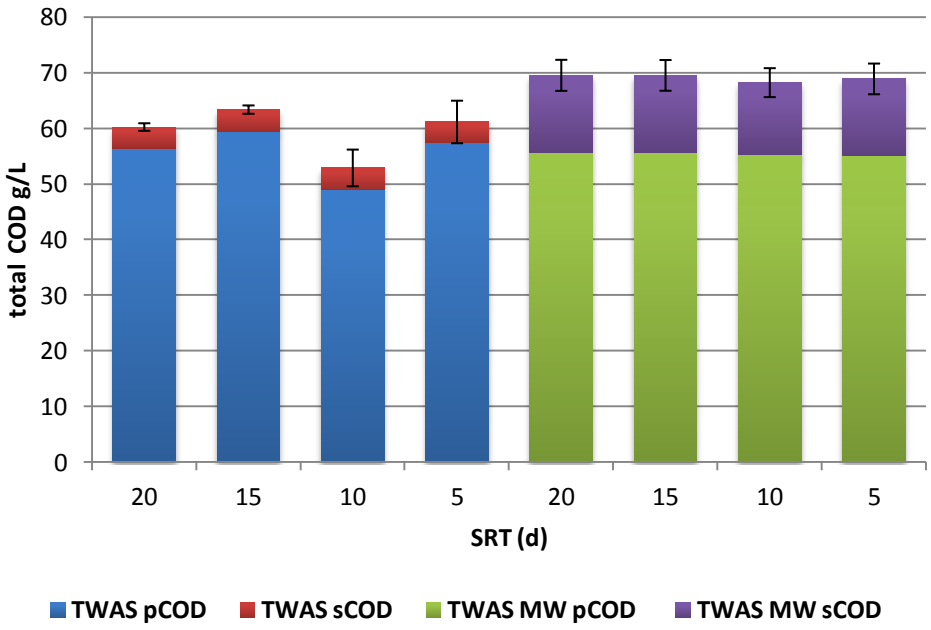


Figure 6.3 - COD distribution (particulate (pCOD) and soluble COD (sCOD)) in feed sludge during the tested periods.

Hydrolysis of substrates that contain large percentages of particulate matter, such as wastewater sludge, was identified as a limiting step in anaerobic digestion of these types of substrates (Eastman and Ferguson 1981, Miron et al. 2000). All the subsequent processes in anaerobic digestion occur at faster rates; thus an increase in hydrolysis results in more solubilised substrate ready to be acidified and transformed into methane and a more efficient and fast digestion. Particulate COD hydrolysis is generally considered a first-order process and can be calculated using a COD mass balance (Puchajda and Oleszkiewicz 2006, Schmit and Ellis 2001) according to the following equations:

$$Specific\ hydrolysis\ rate = \frac{\left(\frac{mass\ pCOD}{d}\right)_{influent} - \left(\frac{mass\ pCOD}{d}\right)_{effluent}}{mass\ of\ volatile\ solids\ within\ reactor} \quad (1)$$

$$pCOD = particulateCOD = totalCOD - solubleCOD \quad (2)$$

MW pretreatment increased sCOD but also caused the particulate COD that did not solubilize to be more easily hydrolysed in the following stage. As observed from the hydrolysis rates in Table 6.2, rates were higher in reactors fed with microwaved sludge as is the case of A1 in comparison with the other acidification reactor fed with non-microwaved sludge, A2. The same observation occurs when comparing reactor M1 with M2; both the hydrolysis and specific hydrolysis rates are higher in the reactor fed pretreated sludge in comparison with reactor M2, in the same operating conditions except the pretreatment applied to sludge. For T1 and T2 this is true for all but the highest SRT (20 d) and it can be argued that thermophilic sludge has higher intrinsic reaction rates due to higher temperature compared with mesophilic reactors, so a difference in performance between pretreated and non pretreated sludge is only observable when

the organic load is not too low. The MW pretreatment, besides solubilizing organic material, may have caused a partial hydrolysis that, despite not creating soluble material, modified the solid substrate to such an extent as to make its solubilisation easier in the following stage. The occurrence of partial hydrolysis has already been observed in the context of two-stage digestion (Watts et al. 2006), with the authors attributing it to a combination of both a heating process as well as chemical and biological activity.

Table 6.2 - Rates of hydrolysis for all reactors in the SRT's tested.

	A1	A2	M1	T1	M2	T2	M3	T3	M4	T4
Specific Hydrolysis rate mgCOD/mgVS.d										
20	0.595	0.405	0.049	0.074	0.048	0.074	-0.021	-0.011	0.042	0.021
15	0.540	0.497	0.054	0.077	0.050	0.067	-0.038	-0.027	0.007	0.005
10	0.403	0.301	0.063	0.095	0.045	0.071	-0.027	-0.006	0.008	0.024
5	0.546	0.460	0.087	0.148	0.048	0.129	-0.160	-0.102	-0.026	-0.003
Hydrolysis rate mgCOD/L.d										
20	18685.6	12161.9	1082.9	1548.5	1094.7	1555.2	-440.7	-212.3	428.4	424.4
15	15945.1	13866.6	1242.5	1591.9	1183.5	1572.6	-785.6	-488.2	-37.9	112.5
10	10969.4	7496.7	1473.4	2197.8	995.1	1516.7	-532.7	118.9	-89.6	481.6
5	14598.5	11446.3	2244.4	3496.2	1135.0	2971.9	-3161.6	-1951.0	-1487.5	-71.5

Rates of hydrolysis in reactors M3 and T3 are either significantly lower than those for single-stage reactors or negative, showing that most, if not all, of the hydrolysable substrate was solubilized either in the microwaving process or the acidifying reactors. The negative numbers are a consequence of production of cellular material that is washed out in the effluent. This washout occurs in all reactors to some extent, but for reactors M3 and T3, all the hydrolysable substrate is hydrolyzed prior to entering T3 and M3, so when calculating the balance, there is no hydrolysis occurring inside the reactor to compensate for the loss of bacterial cell mass as happens is all the other reactors. So for T3 and M3, the particulate fraction of COD is greater in the exit than in the entrance of the reactor due to biomass production inside the reactors using soluble COD. In the case of M4 and T4 some hydrolysis still occurs given that they show positive

values (though smaller) for all the periods tested (except in the case of 20 d SRT). This shows that reactor A2 does not solubilises organic material to the same extent as A1 and some of it is still solubilised in the methanogenic reactor.

Table 6.3 displays a summary of steady state characteristics for all reactors after being fed pretreated and non-pretreated sludge. Values are the means calculated for each period after steady state was achieved, along with the 95% confidence interval values.

Table 6.3 - Steady state characterization of reactors at tested SRTs .

SRT = 20 d										
Parameters	**A1**	**A2**	**M1**	**M2**	**M3**	**M4**	**T1**	**T2**	**T3**	**T4**
OLR (kg VS/m^3.d)	26.27±0.57	24.06±0.53	1.97±0.04	1.80±0.04	1.75±0.05	1.67±0.06	1.97±0.04	1.80±0.04	1.75±0.05	1.67±0.06
OLR (kg COD/m^3.d)	40.17±0.47	46.37±0.30	3.86±0.02	3.35±0.02	2.60±0.23	2.62±0.24	3.86±0.02	3.35±0.02	2.60±0.23	2.62±0.24
VS rem %	20.3±3.7	16.7±4.7	44.1±3.9	37.4±5.8	47±5.9	43.5±4.1	47.0±6.6	42.0±5.9	50.2±6.5	45.1±5.4
TS rem%	13.4±3.3	5.6±3.2	29.8±4.6	26.8±5.0	34.0±5.7	23.1±4.6	35.5±5.0	28.9±6.9	32.9±6.0	26.9±6.7
tCOD rem%	32.3±2.9	21.7±2.0	50.4±1.8	47.0±2.1	60.5±2.7	59.3±1.2	55.6±2.5	55.3±4.7	61.9±2.8	56.2±2.7
Biogas prod. L/d	0.38±0.01	0.29±0.01	0.40±0.01	0.51±0.01	0.40±0.01	0.37±0.01	0.51±0.01	0.35±0.01	0.53±0.01	0.34±0.01
L/kg VS added	18±0.61	15±0.61	254±8.18	192±5.69	287±10.90	277±12.45	321±9.06	244±8.83	383±13.11	256±11.89
L/kg tCOD added	20±1.9	6±0.6	144±13.9	100±9.5	231±22.3	208±20.1	182±17.3	127±12.4	302±29.4	194±18.9
CH_4 %	43.5±4.2	43.1±3.6	53.8±1.9	52.8±4.0	52.6±4.1	54.5±2.9	64.1±1.9	57.9±2.9	63.2±2.7	62.7±2.1
L_{biogas}/kg VS rem	89±4.7	90±5.4	576±30.9	514±29.3	612±31.5	636±33.0	684±42.7	580±33.2	720±43.9	568±40.1
L_{CH4}/kg VS rem	38.7±4.3	38.8±4.0	309.9±19.9	271.4±25.7	321.9±30.1	346.6±25.8	438.4±30.3	335.8±25.5	455.0±33.9	356.1±27.8
TVFA (mg/L)	4118±457	4876±554	228±138	100±39	108±38	309±18	2013±430	1864±469	1799±472	2410±634
sCOD (mg/L)	28543±662	15043±338	575±34	136±24	438±34	968±45	3362±181	1610±454	3986±363	2763±428
NH_3-N (mg/L)	1745±102	1634±144	805±214	854±110	1237±155	1124±154	1200±124	984±114	1541±225	1347±117
pH	6.31±0.09	6.40±0.08	7.21±0.08	7.32±0.08	7.36±0.12	7.11±0.14	7.62±0.09	7.64±0.08	7.53±0.09	7.73±0.09
SRT = 15 d										
Parameters	**A1**	**A2**	**M1**	**M2**	**M3**	**M4**	**T1**	**T2**	**T3**	**T4**
OLR (kg VS/m^3.d)	26.09±0.25	26.37±0.27	2.61±0.02	2.64±0.03	2.27±0.03	2.14±0.04	2.61±0.02	2.64±0.03	2.27±0.03	2.14±0.04
OLR (kg COD/m^3.d)	42.26±0.27	46.36±0.26	5.35±0.03	3.22±0.03	4.23±0.43	3.64±0.24	5.35±0.03	3.22±0.03	4.23±0.43	3.64±0.24
VS rem %	24.5±4.4	29.5±4.7	40.7±4.4	39.9±5.4	46.7±4.8	45.7±6.7	47.5±7.3	40.6±6.6	53.1±6.9	45.4±3.5
TS rem%	11.1±5.2	21.7±5.4	31.3±5.3	27.5±5.2	34.5±5.3	31.4±5.3	36.3±5.7	27.4±5.4	40.4±6.3	31.2±4.3
tCOD rem%	21.0±2.2	25.4±1.7	36.4±1.2	33.9±2.6	48.3±2.5	48.5±3.7	41.9±1.5	37.8±3.5	50.4±3.7	47.5±3.7
Biogas prod. L/d	0.65±0.01	0.63±0.01	0.50±0.01	0.39±0.01	0.56±0.01	0.48±0.01	0.65±0.01	0.48±0.01	0.68±0.01	0.54±0.01
L/kgVS added	31±0.56	30±0.57	239±5.12	183±2.08	310±6.89	280±7.84	309±5.31	228±5.41	372±6.91	315±8.29
L/kg tCOD added	20±1.34	18±1.23	143±9.96	111±7.93	229±15.81	229±16.00	186±12.73	139±9.71	270±18.44	255±17.65
CH_4 %	44.3±2.9	39.8±2.7	52.5±1.8	54.4±3.2	56.6±2.9	52.5±3.0	64.0±1.9	62.0±1.6	63.1±1.9	61.3±1.7
L_{biogas}/kg VS rem	127±3.0	101±2.9	585±13.8	459±13.8	665±18.4	612±19.2	651±20.6	563±16.0	718±18.7	695±19.8
L_{CH4}/kg VS rem	56.3±3.9	40.2±3.0	307.1±12.8	249.7±16.5	376.4±21.9	321.3±20.9	416.6±18.1	349.1±13.4	453.1±18.0	426.0±16.9
TVFA (mg/L)	4431±451	5788±756	247±159	211±137	110±35	398±134	1361±754	2005±1063	1186±862	2129±599
sCOD (mg/L)	31116±6379	15555±536	241.4±22.9	150.0±56.1	337.4±43.6	321.9±4.19	3458±245	3551±668	3360±404	3248±336
NH_3-N (mg/L)	1415±54	1354±110	1024±125	1044±147	1430±152	1124±123	1333±321	1035±114	1445±141	1256±132
pH	6.21±0.10	6.31±0.10	7.36±0.08	7.32±0.08	7.20±0.10	7.25±0.12	7.70±0.13	7.77±0.10	7.63±0.12	7.72±0.14

SRT = 10 d										
Parameters	**A1**	**A2**	**M1**	**M2**	**M3**	**M4**	**T1**	**T2**	**T3**	**T4**
OLR (kg VS/m^3.d)	26.25±0.28	21.52±1.30	3.94±0.04	3.23±0.20	3.41±0.14	3.11±0.02	3.94±0.04	3.23±0.20	3.41±0.14	3.11±0.02
OLR (kg COD/m^3.d)	35.27±1.94	45.49±0.22	8.53±0.04	6.61±0.36	7.32±0.99	6.10±0.93	8.53±0.04	6.61±0.36	7.32±0.99	6.10±0.93
VS rem %	30.8±4.3	22.9±4.4	40.9±5.0	31.5±5.0	50.0±5.6	36.1±4.6	41.2±4.7	33.8±4.1	51.8±6.6	37.9±4.3
TS rem%	12.5±1.2	14.5±2.2	35.1±5.4	20.2±2.8	40.5±5.4	20.8±3.0	36.1±3.2	16.5±1.4	39.9±3.6	18.8±2.6
tCOD rem%	14.2±1.9	7.7±1.3	22.6±2.4	26.0±2.4	43.0±3.9	33.5±2.2	37.7±2.7	27.4±2.6	44.5±2.4	36.0±2.2
Biogas prod. L/d	0.83±0.02	0.68±0.01	0.60±0.01	0.55±0.01	0.68±0.01	0.65±0.01	0.83±0.01	0.55±0.01	0.79±0.01	0.66±0.01
L/kgVSadded	40±1.05	40±2.48	192±3.74	212±13.68	249±10.86	261±4.35	265±4.18	214±13.81	291±12.50	263±4.33
L/kg tCOD added	31±4.80	20±3.07	111±17.08	100±15.40	96±14.76	194±29.83	153±23.48	101±15.56	197±30.24	196±30.13
CH_4 %	42.0±3.3	40.7±3.4	53.9±3.9	53.7±3.9	56.7±4.8	51.6±5.1	59.9±3.5	59.0±2.4	61.7±3.9	62.8±2.5
L_{biogas}/kg VS rem	129±6.53	173±14.98	468±24.64	672±59.96	499±22.54	724±52.55	643±15.25	633±56.06	561±25.08	694±43.64
L_{CH4}/kg VS rem	54.2±5.1	70.4±8.5	252.3±22.6	360.9±41.5	282.9±27.1	373.6±45.8	385.2±24.3	373.5±36.4	346.1±26.8	435.8±32.4
TVFA (mg/L)	3459±931	4213±1057	329±117	550±54	440±60	437±53	2099±907	1837±136	1903±166	2592±1193
sCOD (mg/L)	25268±4227	14862±544	248.4±16.5	120.7±48.2	244.0±39.7	289.6±40.5	4030±304	4603±687	3388±434	4698±537
NH_3-N (mg/L)	1832±156	1554±222	1420±247	1544±161	1998±111	1234±215	1557±226	1452±286	1444±236	1963±269
pH	6.41±0.11	6.11±0.13	7.24±0.10	7.21±0.18	7.12±0.12	7.42±0.10	7.65±0.12	7.75±0.11	7.54±0.13	7.71±0.14
SRT = 5 d										
Parameters	**A1**	**A2**	**M1†**	**M2†**	**M3**	**M4†**	**T1**	**T2**	**T3**	**T4**
OLR (kg VS/m^3.d)	26.13±0.29	20.95±1.72	7.84±0.09	6.29±0.52	8.91±0.63	8.30±0.07	7.84±0.09	6.29±0.52	8.91±0.63	8.30±0.07
OLR (kg COD/m^3.d)	40.78±2.23	45.94±0.48	22.97±0.24	20.39±1.12	17.56±1.95	16.57±1.98	22.97±0.24	20.39±1.12	17.56±1.95	16.57±1.98
VS rem %	31.8±2.3	20.8±1.7	34.4±3.0	24.9±2.2	49.6±4.6	23.6±2.2	39.6±2.9	26.8±2.2	51.4±4.7	29.6±2.4
TS rem%	13.6±2.3	14.3±1.8	28.6±2.8	20.2±2.5	42.1±3.4	18.1±2.3	33.0±3.0	18.4±2.3	40.8±3.7	22.8±2.8
tCOD rem%	23.6±2.6	18.7±2.5	21.7±2.0	14.9±1.6	39.0±3.7	30.5±3.1	30.9±2.0	22.9±1.3	43.1±2.1	35.3±2.0
Biogas prod. L/d	0.83±0.01	0.69±0.01	0.62±0.04	0.60±0.06	0.90±0.01	0.76±0.04	1.00±0.01	0.70±0.01	0.98±0.01	0.85±0.01
L/kg VS added	40.0±0.66	41.0±3.42	100±6.55	120±15.57	126±9.02	114±6.08	159±2.42	139±11.66	137±9.79	129±1.87
L/kg tCOD added	27±3.23	20±3.40	57±7.72	61±9.48	138±16.49	123±16.00	90±10.75	72±8.63	147±17.56	135±16.14
CH_4 %	41.1±2.0	40.0±1.9	50.9±3.9	45.9±3.9	55.9±2.2	53.0±2.1	59.0±1.5	61.0±1.4	62.8±1.7	63.5±2.1
L_{biogas}/kg VS rem	125±9.15	199±23.30	289±20.71	480±74.92	255±18.51	482±51.05	401±11.47	521±61.29	266±19.36	435±36.41
L_{CH4}/kg VS rem	51.4±4.5	79.6±10.1	147.1±15.4	220.3±39.2	142.5±11.8	255.5±28.9	236.6±9.1	317.8±38.1	167.0±13.0	276.2±24.9
TVFA (mg/L)	3753±900	4143±1046	415±215	640±49	559±265	507±182	1349±474	1431±838	1766±886	2781±980
sCOD (mg/L)	26754±908	15105±932	305±46	200±47	312±52	482±52	4874±300	4540±661	3567±486	4636±534
NH_3-N (mg/L)	1920±167	1699±177	1023±120	1478±165	1144±121	1564±321	1778±113	1132±323	1560±235	1657±265
pH	6.32±0.21	6.24±0.15	7.01±0.32	6.99±0.29	7.32±0.13	7.54±0.13	7.61±0.10	7.55±0.12	7.61±0.11	7.72±0.09

† Steady state was not observed during this period

6.4.1Biogas production

The results obtained show that MW pretreatment has a positive effect on digestion, both in a single- or two-stage process. Single-stage reactors fed with microwaved sludge, both meso and thermophilic (M1 and T1) produced more biogas than reactors fed with non-microwaved sludge (M2 and T2) for all the SRT tested, with the exception of SRT 5 d where the difference between biogas production for M1 (0.62 ± 0.04 L/d) and M2 (0.60 ± 0.06 L/d) is not statistically significant (t-test, α=0.05, P=0.580 for $\mu_1 = \mu_2$). The maximum biogas production for single-stage reactors for each SRT occurs always in the thermophilic reactor T1. Reactor M1 shows the second best biogas production rates with rates statistically superior to T2 for SRT 20 (t-test α=0.05, P=6.72E-29 for $\mu_1 = \mu_2$), 15 (t-test, α=0.05, P=8.68E-5 for $\mu_1 = \mu_2$) and 10 d (t-test, α=0.05, P=4.77E-14 for $\mu_1 = \mu_2$). At SRT 5 d, the thermophilic reactor fed with non MW sludge T2 produces a higher amount of biogas than M1 (fed with MW sludge); however, the average for M1 was calculated without reaching steady state, since a stable state was not achieved during the period. Two-stage reactors also show that MW pretreatment has a positive effect in the digestion of sludge. Reactors digesting sludge from A1 (that acidifies sludge after MW pretreatment) had more biogas production than reactors fed with sludge from A2, that acidifies sludge not pretreated with MWs. Among reactors fed by A1, more biogas was produced in the thermophilic reactor T3 than the mesophilic reactor M3. The maximum biogas production for two-stage reactors was observed for T3 at the shortest SRT tested, 5 d with a value of 1.24 ± 0.01 L/d, (value calculated adding T3 biogas production plus A1 corrected for sludge volume fed). Reactor T3 produced more biogas in every SRT tested than any other single- or two-stage reactor tested. Also noticeable is biogas production from two-stage mesophilic reactor M3 which was always

higher than two-stage M4 as somewhat expected, but also higher than T4. In both cases, the difference is statistically significant (t-test, α=0.05, $P < 0.05$ for all the pairs for $\mu_1 = \mu_2$).

Reactor M2 (mesophilic without MW pretreatment) can be used as a control reactor to evaluate the relative improvements obtained since the majority of anaerobic reactors in use today are mesophilic digesters digesting sludge with no pretreatment (De Baere, 2000). Biogas production improvements for two stage reactors, (M3, M4 T3 and T4) were calculated including the contribution of the respective acidifying reactor (A1 for M3 and T3; A2 for M4 and T4). Improvements are visible for all reactors except for T2 at 10 d SRT where the difference was not significant (t-test, α=0.05, P=0.315 for $\mu_1 = \mu_2$), with the higher improvements being recorded in reactor T3. It shows higher improvements at all SRTs when compared with the other two-stage reactors (M3, M4 and T4) and with all the single-stage reactors. The highest improvement occurs at SRT 5 d where T3 shows an increase of 106% compared to biogas production in M2. When considering only single-stage reactors, thermophilic reactor T1 showed higher improvements for all SRT in comparison with the other single-stage reactors (M1 and T2). Thermophilic operation alone made T2 perform better in terms of biogas production compared to the control; however, performance was not as good as mesophilic reactor M1 digesting microwaved sludge for all SRTs except at an SRT of 5 d.

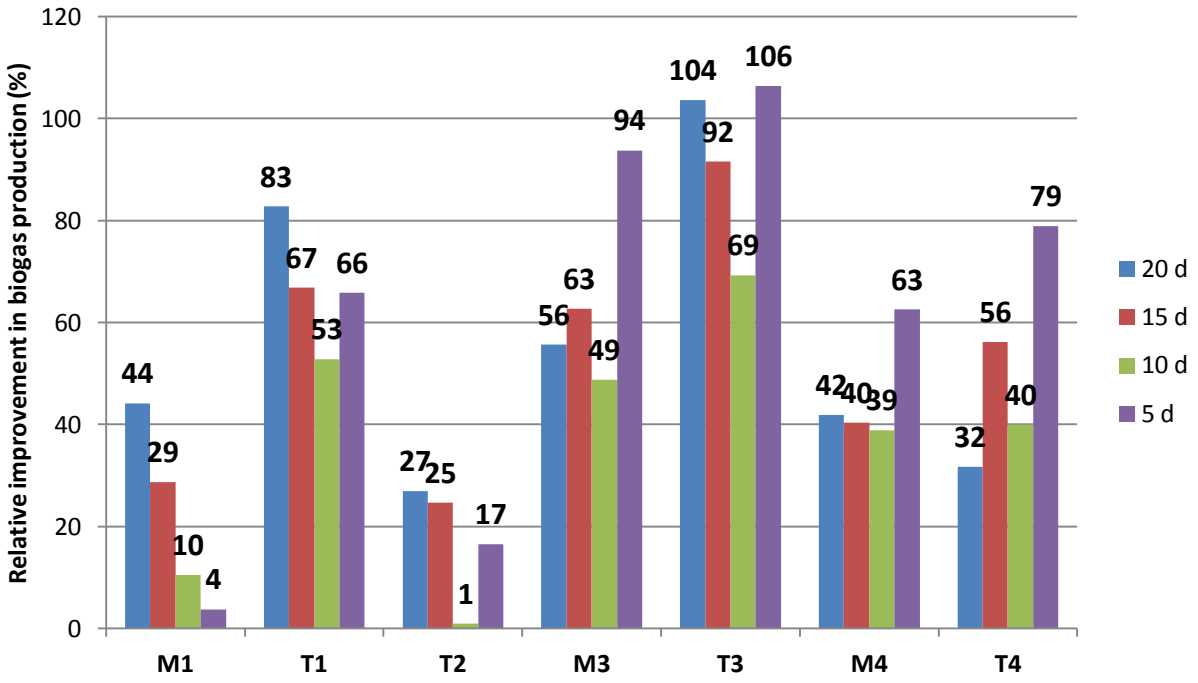


Figure 6.4 - Improvement percentages on biogas production relative to control reactor (M2).

Previous studies observed that MW pretreatment efficiency (degree of improvement over a control) increased with smaller SRT, or higher loads applied (Toreci et al.2009, Eskicioglu et al. 2007), and that differences between reactors digesting pretreated and non pretreated sludges were not significant at high SRT (20 d). In contrast, in the results obtained in this study, improvements were measured at all SRTs. It seems logical that pretreated sludges, in which the material available to digestion is comprised of extracellular polymeric substances (EPS) plus all the material that is released after cell wall breakdown due to pretreatment has a higher biodegradable potential than sludges where bacterial cell walls are intact, reducing the pool of easily biodegradable material to EPS. In the case of single-stage reactors, particularly for M1, the degree of improvement seems to decrease with higher SRT applied, while an opposite trend is visible in two-stage digesters. One should not rule out the fact that biogas production

measurements, at least for M1 at low SRT, could have underestimated the real value of biogas production, since biogas yield for M1 at SRT 5 was 289 ± 20.71 L/d, which is a relatively low value compared to biogas yields of 576 ± 30.9, 585 ± 13.8 and 468 ± 24.64 L/d for SRT 20, 15 and 10 d, respectively. Gas leaks were detected and repaired for measuring gas production. Other authors also reported the same problems with leaks especially when applying high loads (Eskicioglu et al. 2007). For the 4 two-stage digesters tested, biogas production improvement seems to increase with lower SRT, since for all reactors biogas production improvement is higher at SRT 5 d than at 20 d, with this trend particularly visible in reactors M3 and T4. The results for SRT 10 d were somehow dissonant of this trend most likely because they were affected by the composition of the original sludge collected in the wastewater plant. The average tCOD and sCOD of untreated sludge was noticeably lower than corresponding values measured in the three other periods, which might have lowered substantially the improvement measured at this SRT.

When comparing the effects of staging and microwaving it is interesting to note that for mesophilic conditions, staging alone increases more the biogas production than microwaving alone (M4 produces more biogas than M1) however, for thermophilic conditions, the opposite happens, since microwaving alone has a greater positive effect on biogas production than just staging (T2 produces more biogas than T4 in three of the four SRT tested).

6.4.2 VS removal

Microwaved sludge provides for greater VS reduction than non-microwaved sludge, given that single-stage reactors fed with microwaved sludge exhibit higher removal percentages than reactors fed with non-microwaved sludge, as is the case of single-stage reactor M1 compared with M2 and T1 compared with T2. For both cases (M1-M2 and T1-T2) the values are

statistically different except for the longest SRT (20 d) ($P<0.05$ for pairs at SRT 15, 10 and 5d, $P>0.05$ for SRT=20d). It is likely that for such a long retention time, bacteria are capable of biodegrading all biodegradable solids, so the difference between pretreated and non pretreating performance is not as pronounced. For single-stage reactors, thermophilic conditions resulted in higher removal than corresponding mesophilic operated reactors. T1 performs better than M1 for all SRTs except SRT 10 d where removal percentage is statistically not different (t-test, $\alpha=0.05$, $P=0.863$ for $\mu_1= \mu_2$), and T2 performs better than M2 for SRT 20 and 10 d, while at SRT 15 d (t-test ,$\alpha=0.05$, $P=0.101$ for $\mu_1= \mu_2$) and 5 d (t-test ,$\alpha=0.05$, $P=0.126$ for $\mu_1= \mu_2$), although the average value is higher, the difference is not statistically significant. Again, the change in the characteristics of feed sludge for the period tested at SRT 10 d may explain the lack of statistical relevancy of the difference calculated for SRT 10 d. And non attainment of stable conditions at SRT 5 d for M2 and consequent high variance could explain the lack of statistical significance in the difference between the means.

Two-stage reactors generally achieve higher VS removals than the correspondent single-stage reactors (M3 in comparison with M1, T3 with T1, M4 with M2 and T4 with T2). The removal efficiency of two stage reactors was calculated based on the VS concentration before the acidifying reactor and VS concentration after the methenogenic reactor, treating then the two stage reactors as a single system.

For SRT 5 d, despite average removal being higher for M2 compared to M4, the difference is not significant (t-test ,$\alpha=0.05$, $P=0.489$ for $\mu_1= \mu_2$). The highest VS removal for all reactors was obtained at SRT 15 d for T3 (53.1 ± 6.9%), a value that is relatively high considering that the feed sludge was comprised of activated sludge only. Sludge used in this test was young (SRT 5 d) which means it contained a higher proportion of biodegradable organic matter compared with

older sludge, particularly sludge produced in processes where nutrient removal is performed. The most striking fact from the values for VS removal calculated for two-stage reactors is that solids removal percentage did not significantly decrease when SRT was decreased for T3 and M3, in contrast with M4 and T4 that had their removal percentages decrease from 44 to 24% and 45 to 30% ,respectively, when SRT decreased from 20 to 5 d. Pretreatment causes a large part of influent feed to be easily digestible so the decrease of time available to bacteria to metabolize them apparently is not limiting in these reactors. Two-stage reactors M3 and T3 show consequently more solids removal than M4 and T4, for all but the higher SRT (20 d), where the difference between M3 and M4 is not significant (t-test ,α=0.05, P=0.359 for $\mu_1 = \mu_2$), as well as T3 and T4 (t-test ,α=0.05, P=0.773 for $\mu_1 = \mu_2$). Digestion temperature also had an effect in solids removal, since reactor T3 removes more solids at all SRTs than similarly fed M3. In the case of two-stage reactors fed with non pretreated sludge, the effect of digestion temperature is only visible at SRT 5 d since it is the only condition where the difference is statistically significant (t-test ,α=0.05, P=0.004 for $\mu_1 = \mu_2$).;

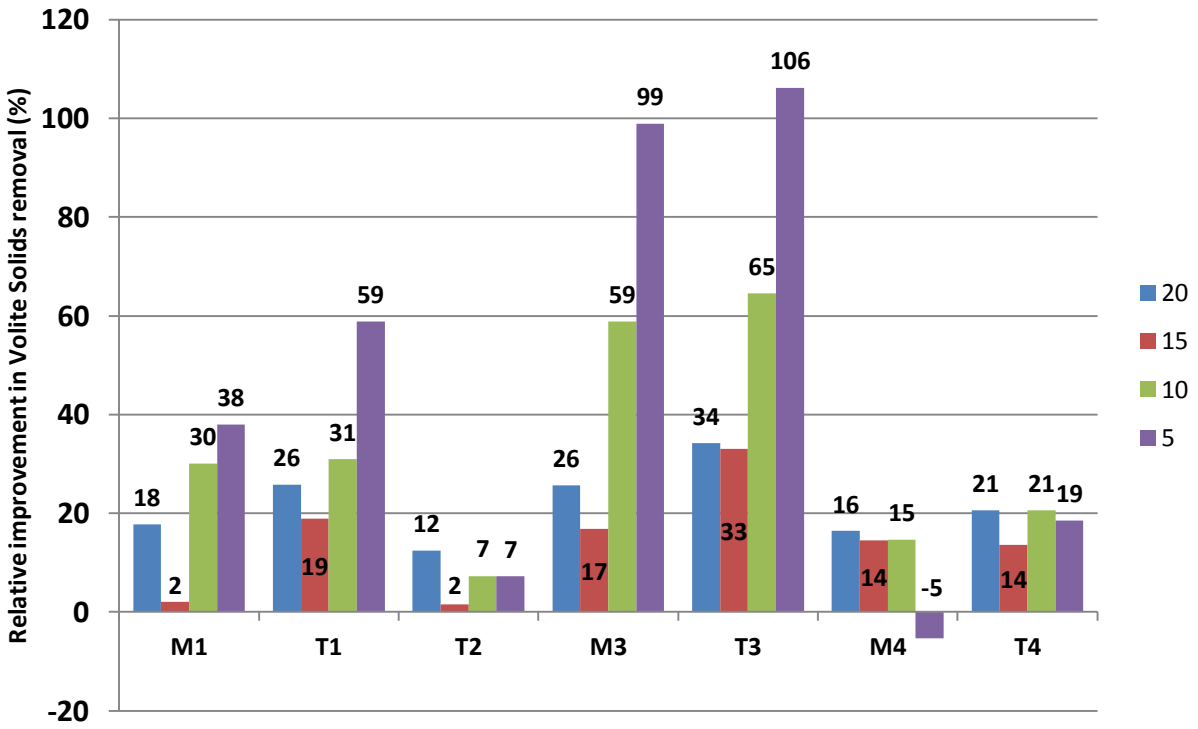


Figure 6.5 - Improvement percentages on VS removal relative to control reactor (M2).

Improvements in VS removal relative to control reactor, as shown in Figure 6.5, show that pretreatment is more effective for short SRT. All the reactors fed with pretreated sludge had increased relative improvements for SRT 10 and 5 d, compared with the initial SRT of 20 d. The increase is more pronounced in two-stage reactors fed pretreated sludge, because these reactors (M3 and T3) retained high solids removal efficiencies while the control reactor showed a drop in performance. Staging increases solids removal capacity; therefore high removal efficiencies are maintained in a high level even at SRTs where single-stage reactors show signs of overloading. Han et al, (1997) state that staging alone reduces the volume necessary for a removal efficiency of 60%; therefore it is not suprising that T3 and M3 (and to a lesser extent M4 and T4) showed such high removal efficiencies. Microwaving alone, though, seems to have a more beneficial effect in terms of solids removal than just staged digestion. M1 shows higher improvements

percentages for SRT 20, 10 and 5 d than M4 and the same happens with T1 for all SRTs in comparison with T4. This seems reasonable since microwaving has a high and direct impact on VS because it solubilises particulate matter to a greater extent allowing it to be easily transformed into methane and carbon dioxide.

It can be hypothesized that having a two-stage system provided better conditions to accommodate higher loadings in comparison with the single-stage systems, particularly the control, and microwaving increased the fraction of those higher loadings that were readily usable by bacteria. Microwaving feed sludge allowed two–stage reactors to use all the optimized capacity staging provides with increased proportion of methanogenic bacteria in the second reactor allowing it to handle higher substrate loading without decreasing performance.

6.4.3 VFA, sCOD and pH

Effluent characteristics for thermophilic reactors show a markedly higher concentration of VFA, both for single and two-stage reactors which was already reported as occurring for thermophilic digestion in steady state in previous studies (Moen et al. 1997a). One of the reasons for this might be that thermophilic methanogens have higher half-velocity constants compared to mesophilic methanogens (Gavala et al. 2003, Moen et al. 1997b). Also, thermophilic methanogens are generally thought to be more susceptible to inhibition and toxicity effects which can explain in part the accumulation of these methane precursors.

The same happens with sCOD, reflecting partially what happens with VFA. However, VFA alone does not account for all the sCOD difference between thermophilic and mesophilic reactors. One likely reason might be that part of the hydrolysates produced in thermophilic second stage reactors are not easily biodegradable, and subsequently are included in the effluent. Another

reason might be that thermophilic sludge seems to produce much more EPS than mesophilic sludge, and part of that EPS will be accounted when measuring the soluble fraction of tCOD.

For the acidification reactors, it was already shown that hydrolysis rates in the reactors fed microwaved sludge were higher, resulting in a significantly higher concentration of sCOD in the effluent of A1 at all periods. Total VFA is higher in A2 which can be a consequence of lower biogas production observed in that reactor that can cause a higher buildup of VFA. Interestingly, pH in these two reactors was never below 6, most likely due to the buffering capacity provided by the significant biogas production with a reasonable methane content.

Thermophilic pH values are generally, slightly higher than those measured for mesophilic reactors. This difference in pH can be attributed in part to the higher temperature in thermophilic reactors. Gas solubility in liquid is described using the Henry's Law that can be expressed as follows:

$$k_{H,pc} = \frac{p}{c} \qquad (3)$$

$k_{H,pc}$=Henry's constant (L.atm/mol);

c = amount concentration of gas in solution (in mol/L)

p = partial pressure of gas above the solution (in atm)

and temperature has an effect on the Henry`s constants for carbon dioxide, according to the expression:

$$k_{H,pc}(T) = k_{H,pc}(T^{\theta})e^{\left[-C\left(\frac{1}{T}-\frac{1}{T^{\theta}}\right)\right]} \qquad (4)$$

$$C_{(CO2)} = 2400\ K$$

Henry's constant is 29.41 L·atm/mol at 298 K, so, using eq (4), $k_{H,pc}$ (35ºC) = 38.34 L.atm/mol and $k_{H,pc}$ (55ºC) = 61.64 L.atm/mol. The ratio of concentration of CO_2 for these two temperatures is:

$$\frac{k_{H,pc}\,(55°\text{C})}{k_{H,pc}\,(35°\text{C})} = \frac{\frac{p}{c(55°\text{C})}}{\frac{p}{c(35°\text{C})}} \rightarrow \frac{61.64}{38.34} = \frac{c(35°\text{C})}{c(55°\text{C})} \rightarrow c(55°\text{C}) = \frac{c(35°\text{C})}{1.61}$$

Since CO_2 is an acidic gas, lower concentrations in the liquid phase at 55 ºC results in a higher pH, when alkalinity values are similar which can explain the difference.

6.4.4 Pathogen removal

Total coliforms and *E.coli* were measured and the results were used to assess the adequacy of the processes to produce Class A sludge biosolids according to the requirements laid down in 40 CFR Part 503 regulations (EPA, 1994), meaning that total fecal coliforms cannot be above a value of 1000 CFU/gTS. Total coliforms are a broader class of coliforms that include (but are not limited to) fecal coliforms and because of that, are always more numerous or, in limited cases, equal to the fecal coliforms present in the sample. Results are summarized in Figure 6.6.

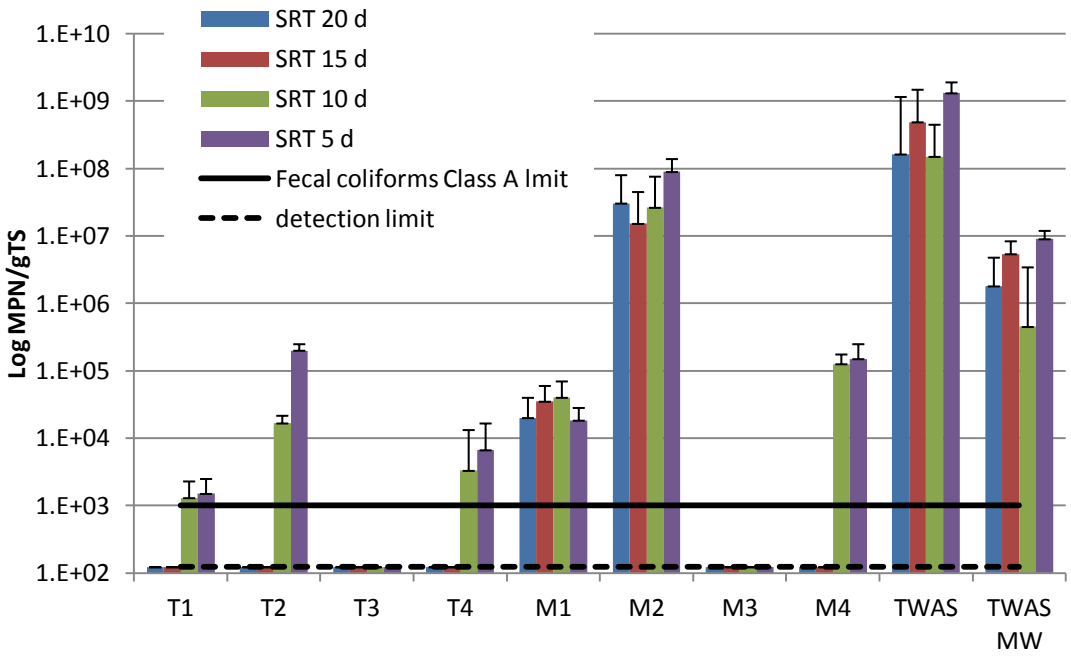


Figure 6.6 - Total coliforms in each reactor effluent for the tested periods.

Microwaving coupled with two-stage digestion produced sludge with pathogen indicator content below the detection limit for all SRTs tested (3.7 CFU/g TS), and consequently with quality to be classified as Class A. Microwaving alone is capable of an average 2.1 log reduction (approximately 99%) in total coliform population. (Hong et al. 2006) determined that a temperature of 86^{o}C would be sufficient to eliminate all coliforms from WAS; however, a smaller penetration depth of MWs in activated sludge (1.11 cm in WAS; 2.16 cm in tap water) combined with a lack of homogenization in the heating of sludge samples (the vessel used to heat sludge in the MW oven had no mixing mechanism) may have created spots where temperature did not rise above the necessary temperature to achieve inactivation; therefore, removal of coliforms was not complete. Conventional mesophilic digestion was also capable of some indicator removal but final density of coliforms is still very high (> 1x10^{7}). Mesophilic single stage digestion with MW

pretreatment was also not sufficient for complete removal of coliforms below the limit of 1000 CFU/g TS; however, due to MW pretreatment, the coliform values in effluent sludge are significantly lower than mesophilic single-stage reactor (M2), used as a control. Both thermophilic single-stage reactors removed coliforms below detection levels for SRT 20 and 15 d, with T1 having a higher reduction, even marginally approaching the required limit. Reactor T4 also shows the same behaviour suggesting that a minimum retention time of more than 10 d in thermophilic conditions is necessary to eliminate all pathogenic bacteria present. Staging alone was able to produce sludge with coliforms below the maximum level provided that a total SRT for the system was above 10 days. The thermophilic temperature combined with high VFA concentration in the acidogenic reactor provided enough reduction in coliform density to obtain compliant sludge even when using mesophilic second stage reactor for SRTs of 20 and 15 d. Minimum retention times in thermophilic conditions for coliform inactivation were also reported in other studies with variable results. Riau and De La Rubia (2010) reported minimum retention time of 4 d in a TPAD of a system total of 19 d, and Han et al. (1997) obtained Class A sludge with 4 days thermophilic reactor SRT plus 10 days in a mesophilic second stage reactor, while Cheunbarn and Pagilla (2000) reported also sludge compliant with the limit using thermophilic SRT of just 1 d plus 15 d SRT in a second stage mesophilic reactor. In this study, MW pretreatment allowed a TPAD system with a total SRT of just 5 d, with 2 d thermophilic SRT to consistently produce Class A sludge.

6.4.5 Dewaterability

Sludge flocs contain a high amount of free or bounded water that is attached to the sludge floc structure EPS by electrostatic interactions and hydrogen bonds. These flocs can then retain large amounts of water, negatively affecting sludge dewaterabilty. Thermophilic digestion is thought to

produce up to 10 times more the amount of EPS than is observed in mesophilic sludge (Zhou et al. 2002) and, consequently, with higher amounts of water retained in the EPS mesh in the floc, thermophilic sludge is thought to be more difficult to dewater (Bivins and Novak, 2001). MWs, on the other hand, have a direct effect on the bounded water, since they destabilize the floc structure, breaking hydrogen bonds between hydroxyl groups of EPS polymers and water molecules and electrostatic interactions between water molecules and induced dipoles of other functional groups in the EPS structure. This can lead to the release of bounded water, increasing the dewaterabilty of sludge. Dewaterabilty was tested using capillary suction time (CST) testing and results (Figure 6.7) show that thermophilic reactors without MW pretreatment (T2 and T4) show worse dewaterabilty properties (higher CST values) than T1 and T3. Reactors T3 and T4 had improved dewaterabilty compared with the control reactor (M2), particularly for lower SRTs. Staging also seems to have an effect since values for T3, are generally smaller than values for T1. The lowest values however, were measured for mesophilic reactors digesting pretreated sludge (M1 and M3). Staging seems to have a more significant effect in thermophilic reactors than in mesophilic ones, since CSTs tend to be lower in two-stage T3 and T4 in comparison with T1 and T2, respectively. T3 and T1 are both fed microwaved sludge and differ only in the staging setup, and the same happens with T4 and T2, with the difference that are both fed non-pretreated sludge. The results show that thermophilic reactors have higher CST values than the corresponding mesophilic reactors (ex: T1 in comparison with M1), however, some thermophilic reactors (T1, T3 and T4) are able to improve dewatering characteristics in comparison with the control reactor.

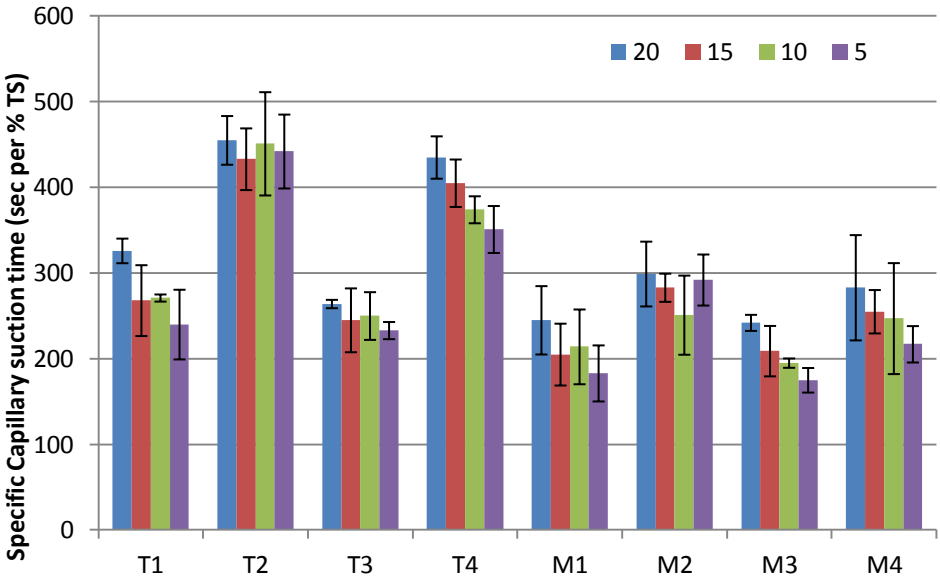


Figure 6.7 - Specific capillary suction time for all tested periods.

6.5 Conclusions

MW pretreatment increases solublization of organic matter and increases hydrolysis of matter not solubilized in the pretreatment step, thus causing a partial hydrolysation, that despite not creating soluble COD, facilitated solubilisation of solid substrate in anaerobic reactors, consequently increasing production of sCOD in acidogenic reactors.

MW has a positive effect in biogas production and VS removal, since reactors fed with microwaved sludge produced more biogas and removed more solids. The improvement seems to be not only in terms of speed of reaction but also in extent, since even at the highest SRT, more biogas and solids are removed in comparison with the control.

Digestion temperature was an important factor in digestion, since thermophilic reactors produced more biogas and removed more solids than mesophilic reactors in similar conditions.

Staging allows the maintenance of a longer interval of observed high biogas and solids removals (similar to those observed at the highest SRT) of microwaved sludge even at organic loadings where single stage reactors are not capable of reaching stable operation.

Microwaving coupled with staged digestion and thermophilic acidogenisis is capable of eliminating pathogen indicators completely even for total retention times of 5 d.

Although thermophilic operation alone decreased dewaterabilty of sludge, the association of MW pretreatment and thermophilic operation produced sludge that dewaters better than control sludge.

Even though the association of two techniques to maximize digestion performance is not a novel technique it was proved that combining MW pretreatment and TPAD has the effect of decreasing volume requirements for digestors, while at the same time, maintaining or in some cases even increasing digestion performance in terms of solid removal and biogas production.

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Chapter 7

Overall Conclusions and Recommendations

7.1 Conclusions

The research developed under this thesis reached the following general conclusions:

- Microwave athermal effect does indeed exist. It has an effect on the distribution of particles in sludge and has an effect on the soluble fraction of COD, soluble proteins and soluble sugars. The magnitude of this effect is significantly smaller than the effect conventional MW radiation has when samples are allowed to heat. This difference is one of the reasons why it might be difficult to detect athermal effects in MW experiments.
- Athermal radiation is capable of solubilising part of the organic matter present in sludge samples, as conventional MW does, and this effect is particularly focused on proteins.
- The solubilized substrate by athermal and thermal MW radiation behaves differently when biodegraded. MW thermal radiation manages to solubilise more substrate, but also produces more permanent inhibition, measured by decreased maximum substrate degradation activity in anaerobic digestion, while athermal radiation, also causing some delay in degradation, manages to increase maximum substrate degradation activity.
- MW thermally pretreated sludge produces soluble substrate that can cause significant inhibition. The inhibitory substrate seems to be limited to substrate with sizes above 10 kDa which can be reversed, provided these inhibitory compounds are removed from the matrix to be biodegraded.

- Athermally pretreated soluble fractions do not seem to be affected by inhibition phenomena detected for thermally pretreated samples, suggesting that solubilizaed subtrate by athermal processes is of a different nature.
- First-order reaction kinetics is not a satisfactory model when adjusting cumulative biogas production curves, since a period of latency is observed and first order underestimates maximum activity rates, and fails to measure the extension of the lag period. An alternative model (Gompertz model) was tested with better results.
- MW pretreatment temperature has a positive effect on COD solubilisation for all conditions tested but with a maximum improvement in biogas production for temperatures below the maximum tested (175 ºC) for all types of sludge. The maximum improvement in COD solubilisation point does not correspond to the conditions in which maximum improvement in biogas production was measured.
- Sludge SRT influences pretreatment efficiency in terms of solubilisation efficiency. Higher SRT sludge benefits more from the pretreatment than younger sludge, measured as relative increase in soluble substrate.
- Mesophilic digestion shows a greater improvement in digestion performance after sludge pretreatment, but thermophilic digestion still manages to reach higher efficiencies in most cases because it has higher baseline digestion efficiency, and more uniform performance throughout all the conditions tested (MW pretreatment temperatures and sludge types).
- Thermophilic digestion showed higher activity than mesophilic digestion but also is more prone to inhibition phenomena, both reversible and irreversible.
- Thermophilic digestion is able to produce more biogas and remove more biosolids from both single stage and two-stage reactor configurations. The combination of staging and

thermophilic reactors allows high yields in terms of biogas production, solids reductions and production of pathogen free microorganisms for very short retention times (5 d).

7.2Recommendations

The research made under this thesis work not only allowed some conclusions to be made but also opened the door to some questions that are worth considering.

The athermal radiation managed to cause an effect that was measurable both on the characteristics of the sludge but also in the way that sludge was biodegraded. A more in depth study of what are the actual mechanisms taking place at the molecular level when athermal MW effects take place would allow a better understanding of the process. It is apparent from this thesis that athermal effects seem particularly focused on proteins. Does the athermal radiation actually cause a complete breakup of all the hydrogen bonds, of other types of bonds present in the molecular structure? Is this effect limited to proteins present in the sludge matrix, or does it affects proteins present inside the bacterial cells too? Is it more prevalent in certain types of proteins than others? Is it permanent or reversible? Some of these questions are important since MWs not only are used in MW ovens but also are used in other devices commonly used by humans, like cell phones.

Since energy provided with MW pretreatment is dependent on radiation frequency, and also taking into account that molecular bonds behave differently when exposed to different frequencies, it could be an interesting option to analyze if the athermal effect is dependent on the frequency of the radiation used. Also, the impacts of MW radiation at other frequencies on the digestion efficiency of pretreated sludge could also be interesting to study.

Microwave pretreatment improves digestion efficiency but less for thermophilic than for mesophilic digestion. Microwave pretreatment also manages to improve digestion of partially stabilized sludge (sludge with high SRT). It could be a feasible and more economical option to test the staging of sludge digestion by using thermophilic digestion of non pretreated sludge as the first step and as a second step of the digestion process, use a mesophilic reactor digesting sludge from the thermophilic reactor subject to MW pretreatment. The first stage could rapidly degrade the easily degradable substrate and the second one more resistant and recalcitrant substrate produced in the thermphilic digestion and in the microwaving process.

Inhibition is a phenomena present when digesting MW pretreated sludge, and this inhibitory effect is removed when separating size fractions of the solubilized substrate. Conversely, the inhibition exhibited by larger fractions is attenuated when all fractions are mixed. This suggests that co-digesting other substrates (preferably easily digestible substrates) with microwaved sludge could be a feasible way to eliminate or attenuate the inhibitory effects of microwaved sludge, by way of synergistic or dilution effects.

It was shown that the combination of more than one option for digestion improvement (MW pretreatement and staging, plus different digestion temperatures) had a positive outcome on the digestion process efficiency. Another option to further improve digestion efficiency would be to add to the combinations tested in this study another of the pretreatments that are being tested or used already in sludge digestion. A chemical or mechanical pretreatment coupled with MW pretreatment before digesting sludge in any of the reactor configurations tested in this thesis work could be an interesting topic of research. A better digestion efficiency would not necessarily mean a more economical process, but economical analyses cannot be done without data obtained from these experiments.

APPENDIX A

Experimental set-up

A.1 Microwave athermal radiation oven set-up

The microwave oven used for the pretreatment of the sludges was a conventional domestic oven (Sanyo EM-S759S P=1350 W, 2450 MHz) modified with a unit shown in Fig. 1 to maintain constant temperature in the sample [10,17]. It consists of a loop through in which a microwave transparent apolar solvent (kerosene) was used as coolant and was circulated that provided cooling of the samples while not interfering with the action of the microwave field in the samples. Heat was removed from the coolant by passing it through an external (to the microwave oven) ice bath.

Figure A.1 - Athermal microwave radiation oven set-up.

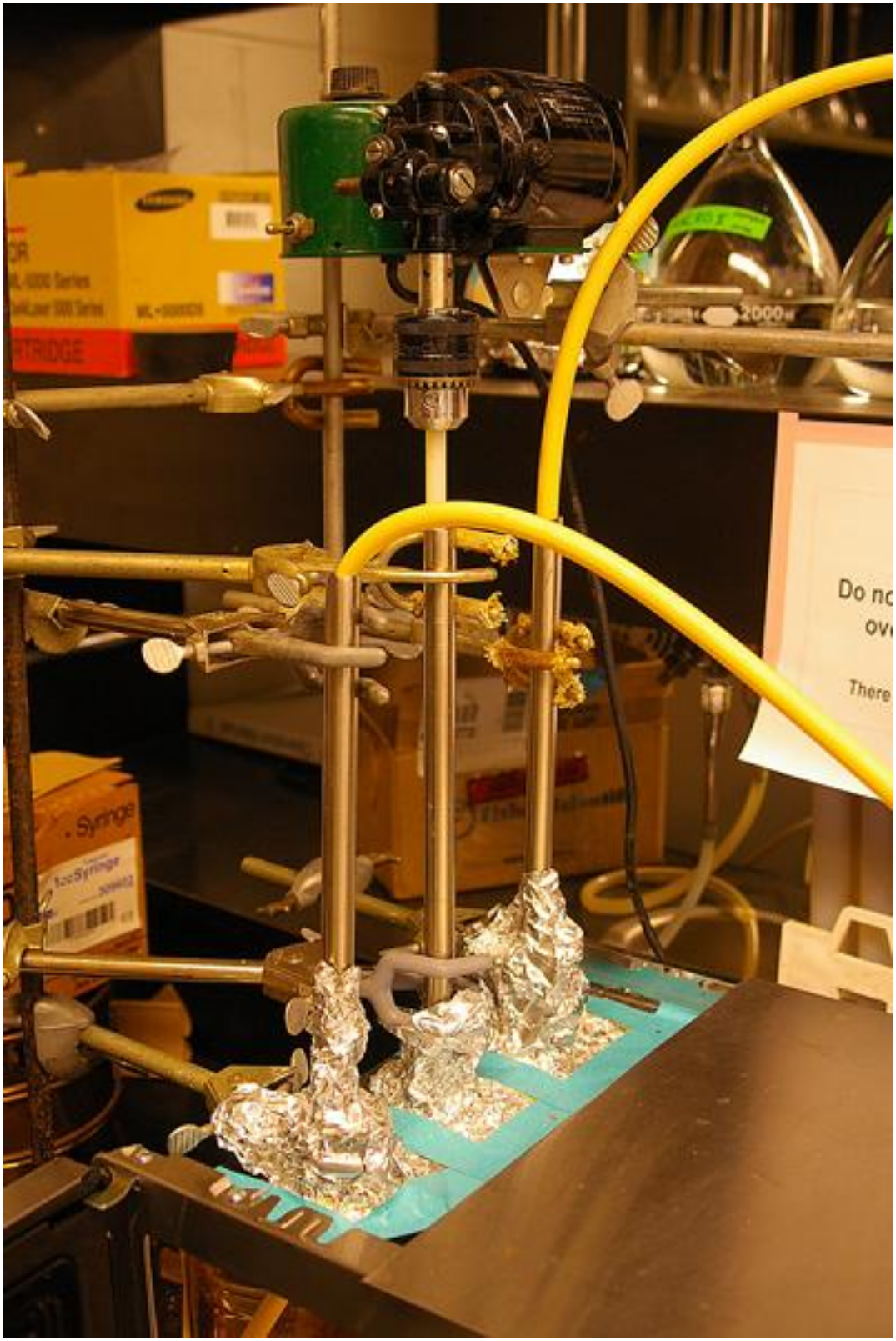


Figure A.2 - Detail of the coolant influow and outflow ports, and rotating shaft. All the ports are protected with microwave attenuators.

A.2 Microwave oven for thermal pretreatment of samples.

Sludge pretreatments with temperature ramp control were carried out with a Mars 5© (MW Accelerated Reaction System; CEM Corporation) MW oven. The oven can supply 1200W ± 15% MW energy at 2450 MHz frequency and has a controllable operating range of up to 250˚C and 3.45 kPa. An optic fiber temperature and electronic pressure sensor makes it possible to monitor the temperature and the pressure up to 250 ˚C and 34.5 kPa.

Figure A.3 - Microwave oven with pressure and temperature control

Figure A.4 - Closed vessel container units for sludge pretreatment.

Figure A.5 - Vessel ready for pretreatment assembled in the rotating carrousel with probes connected.

A.3 Batch anaerobic digestion

BMP tests were performed using either 500 mL Kimax glass bottles with butyl rubber stoppers, or 125 mL serum bottles (Wheaton borosilicate glass, VWR, Montreal, Canada), sealed with butyl rubber stoppers and crimped with aluminum caps. The bottles were incubated in a temperature controlled rotary shaker.

Figure A.6 - 500 mL bottles used for BMP tests.

Figure A.7 - 125 mL serum bottles used for BMP tests.

Figure A.8 - BMP bottles were incubated upside down to minimize biogas losses.

A.4 Semi-continuous reactors

The reactors used in the work reported in Chapter 6 were 1000 mL Schott borosilicate glass bottles, with a useful volume of 800 mL. The reactors were sealed with black butyl rubber stoppers (VWR, Montreal, QC) with two holes: one to sample, waste and feed the reactors and the other to collect and measure the biogas. Biogas was collected in 2 L Tedlar bags. The tedlar bags (Chromatographic Specialties Inc., ON) were equipped with on/off valves and a septum fitting that was used for gas composition sampling. The volume of biogas produced daily was measured using a manometer.

Figure A.9 - Schott borosilicate 1000 mL bottles used in the continuous reactors study.

Figure A.10 - Erlenmeyer with the Tedlar gas bag system used to measure produced gas in the Chapter 6 work.

A.5 Ultrafiltration devices

Amicon model 8400 stirred cells (Amicon Corp., MA) with a 400 mL reservoir were used to perform Ultrafiltration assays, along with high recovery, low organic adsorption hydrophobic membranes (Millipore, MA). Four different types of membranes with different cut-off sizes were used. The molecular weight cut-off sizes used were 300, 100, 10, and 1 kDa. These membranes were used in a cascade series (Figure 1) to provide a UF process with smaller risks of clogging membranes with low cut-off sizes. Pressure was supplied ny nitrogen gas.

Figure A.11 - Ultrafiltration cell.

APPENDIX B

Empirical models for Cumulative Biogas production for Mesophilic and Thermophilic tests for Chapter 5

B. 1 – Mesophilic biogas production

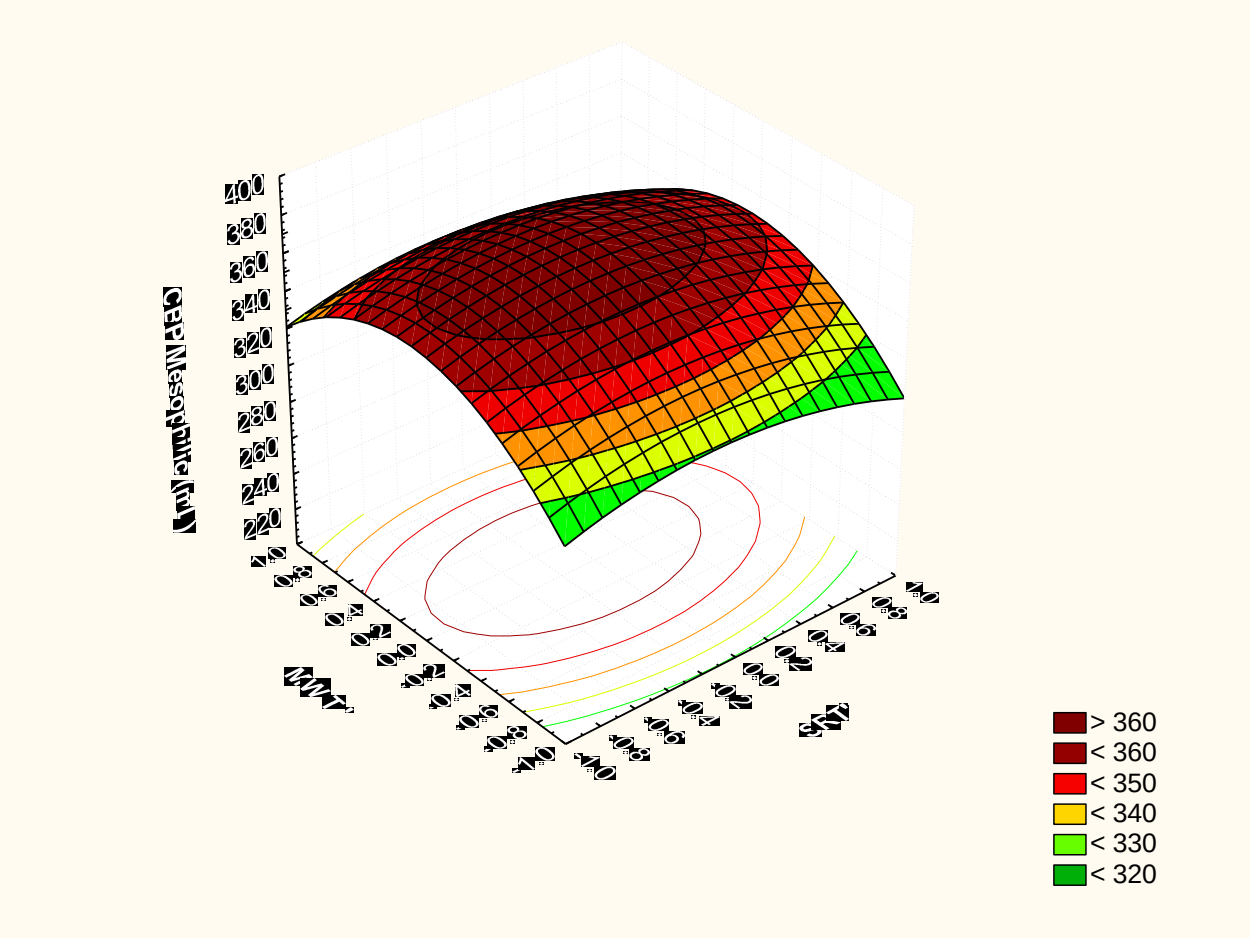


Figure B.1 – Empirical model for biogas production for mesophilic tests

APPENDIX B

Effect	Univariate Tests of Significance for CBP Mesophilic (Spreadshee Forward stepwise solution Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	291803.(	1	291803.(	11511.2	0.00000
SRT	171.8	1	171.8	6.78	0.02188
SRT^2	655.(	1	655.(	25.84	0.00021
MW T	649.4	1	649.4	25.62	0.00021
MW T^2	5459.(	1	5459.(	215.37	0.00000
SRT*MW T		0			
Error	329.5	13	25.3		

Effect	Parameter Estimates (Spreadsheet1) Sigma-restricted parameterization Comment (B/Z/P)	CBP Mesophilic Param.	CBP Mesophilic Std.Err	CBP Mesophilic t	CBP Mesophilic p	-95.00% Cnf.Lmt	+95.00% Cnf.Lmt
Intercept		368.827	3.43765	107.290	0.00000	361.400	376.254
SRT		-3.783	1.45343	-2.603	0.02188	-6.923	-0.643
SRT^2		-16.675	3.28043	-5.083	0.00021	-23.762	-9.588
MW T		7.356	1.45343	5.061	0.00021	4.216	10.496
MW T^2		-42.325	2.88406	-14.675	0.00000	-48.555	-36.094
SRT*MW T	Poole						

Dependnt Variable	Test of SS Whole Model vs. SS Residual (Spreadsheet1) Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
CBP Mesophilic	0.97615	0.95287	0.93837	6662.96	4	1665.74	329.543	13	25.3495	65.7109	0.00000

Dependnt Variable	Test of Lack of Fit (Spreadsheet1) SS Residual	df Residual	MS Residual	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit	F	p
CBP Mesophilic	329.543	13	25.3495	181.746	9	20.1940	147.797	4	36.9493	1.82971	0.20736

Dependnt Variable	Test of SS Whole Model vs. SS Pure Error (Spreadsheet1) SS Model	df Model	MS Model	SS Pure Err	df Pure Err	MS Pure Err	F	p
CBP Mesophilic	6662.96	4	1665.74	181.746	9	20.1940	82.4868	0.00000

Case number	Observed, Predicted, and Residual Values (Spreadsheet Sigma-restricted parameterization (Analysis sample) CBP Mesophilic Observed	CBP Mesophilic Predictd	CBP Mesophilic Resids
1	315.000	306.253	8.7467
2	307.000	306.253	0.7467
3	343.000	348.780	-5.7801
4	350.000	348.780	1.2198
5	322.000	320.966	1.0333
6	315.000	320.966	-5.9666
7	317.000	317.419	-0.4199
8	312.000	317.419	-5.4199
9	363.000	359.946	3.0532
10	355.000	359.946	-4.9467
11	339.000	332.133	6.8667
12	333.000	332.133	0.8667

B. 2 Thermphilic biogas production

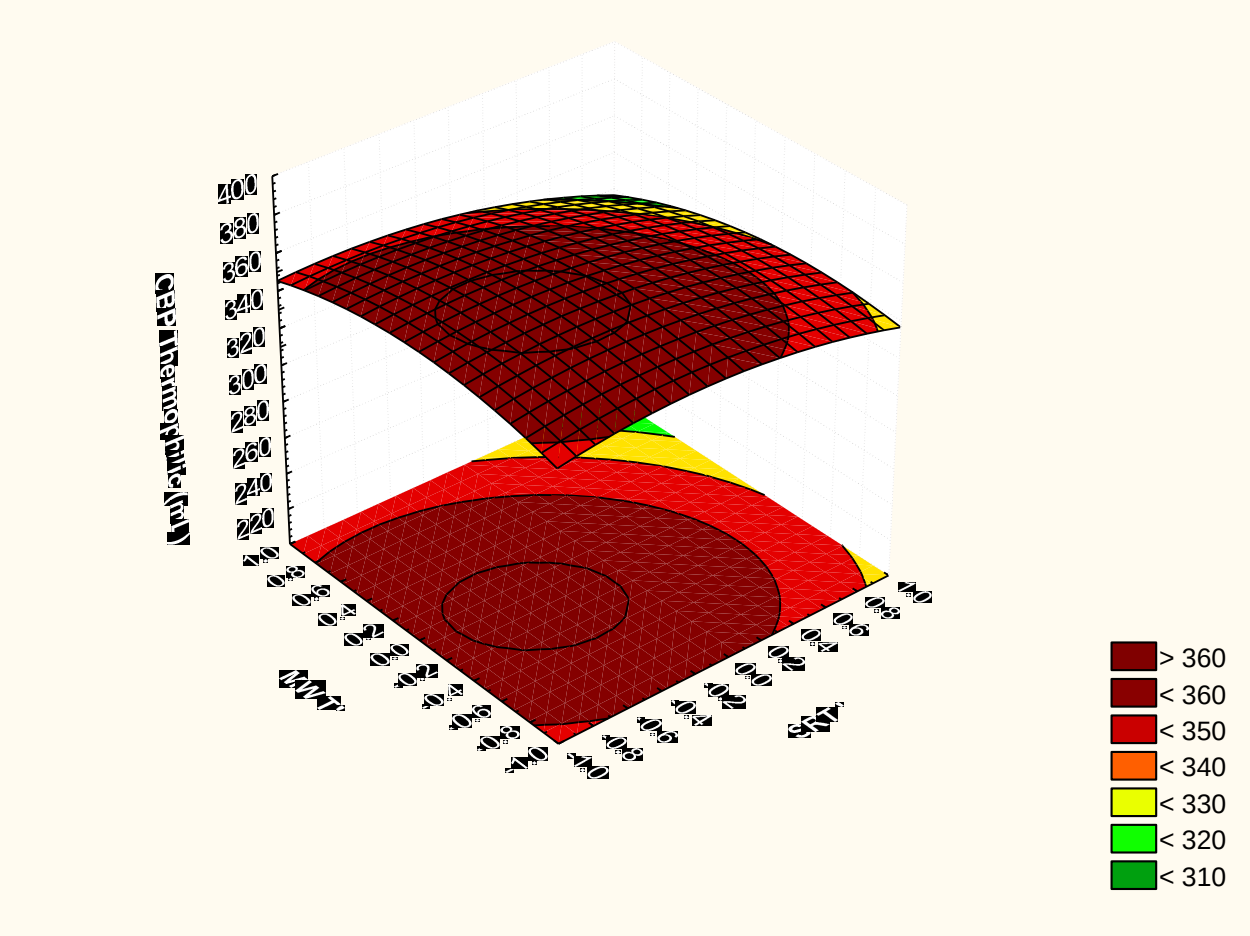


Figure B.2 - Empirical model for biogas production for Thermophilic tests.

Effect	Univariate Tests of Significance for CBP Thermophilic (Spreadsh Forward stepwise solution Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	276981.	1	276981.	15304.1	0.00000
SRT	1108.3	1	1108.3	61.24	0.00000
SRT^2	272.3	1	272.3	15.05	0.00219
MW T	322.2	1	322.2	17.80	0.00119
MW T^2	480.1	1	480.1	26.53	0.00024
SRT*MW T	209.6	1	209.6	11.58	0.00524
Error	217.2	12	18.1		

APPENDIX B

Effect	Parameter Estimates (Spreadsheet1) Sigma-restricted parameterization			
	CBP Thermophilic Param.	CBP Thermophilic Std.Err	CBP Thermophilic t	CBP Thermophilic p
Intercept	359.350	2.90478	123.709	0.00000
SRT	-9.690	1.23830	-7.825	0.00000
SRT^2	-10.752	2.77183	-3.879	0.00219
MW T	-5.261	1.24702	-4.219	0.00119
MW T^2	-12.551	2.43691	-5.150	0.00024
SRT*MW T	-4.861	1.42861	-3.402	0.00524

Dependnt Variable	Test of SS Whole Model vs. SS Residual (Spreadsheet1)									
	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	
CBP Thermophilic	0.96265	0.92669	0.89615	2745.68	5	549.136	217.181	12	18.0984	

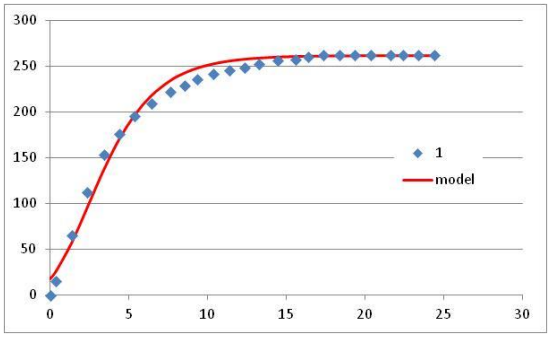
Dependnt Variable	Test of Lack of Fit (Spreadsheet1)								
	SS Residual	df Residual	MS Residual	SS Pure Err	df Pure Err	MS Pure Err	SS Lack of Fit	df Lack of Fit	MS Lack of Fit
CBP Thermophilic	217.181	12	18.0984	101.618	9	11.2909	115.562	3	38.5209

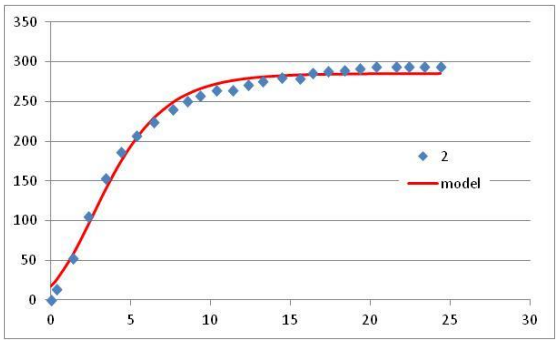
Dependnt Variable	Test of SS Whole Model vs. SS Pure Error (Spreadsheet1)							
	SS Model	df Model	MS Model	SS Pure Err	df Pure Err	MS Pure Err	F	p
CBP Thermophilic	2745.68	5	549.136	101.618	9	11.2909	48.6349	0.00000

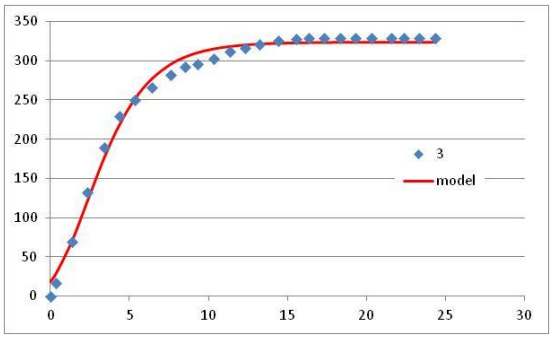
Case number	Observed, Predicted, and Residual Values (Spreadsheet1) Sigma-restricted parameterization (Analysis sample)		
	CBP Thermophilic Observed	CBP Thermophilic Predictd	CBP Thermophilic Resids
1	345.000	346.137	-1.1370
2	350.000	346.137	3.8629
3	350.000	357.026	-7.0267
4	355.000	357.026	-2.0267
5	347.000	345.336	1.6637
6	350.000	345.336	4.6637
7	350.000	352.033	-2.0339
8	356.000	352.033	3.9660
9	366.240	361.156	5.0840
10	360.000	361.156	-1.1559
11	342.000	345.930	-3.9300
12	344.000	345.930	-1.9300

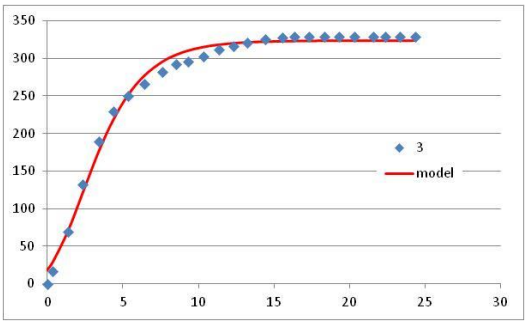
APPENDIX C

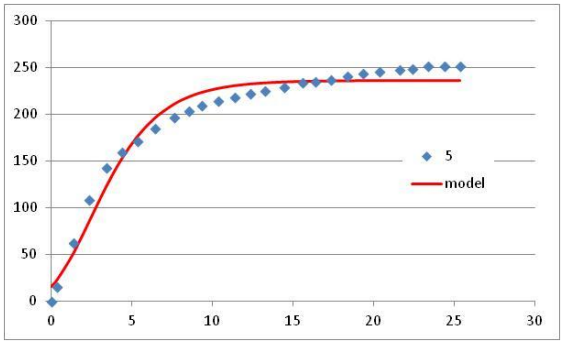
Cumulative biogas production curves and model adjusted curves for Chapter 5

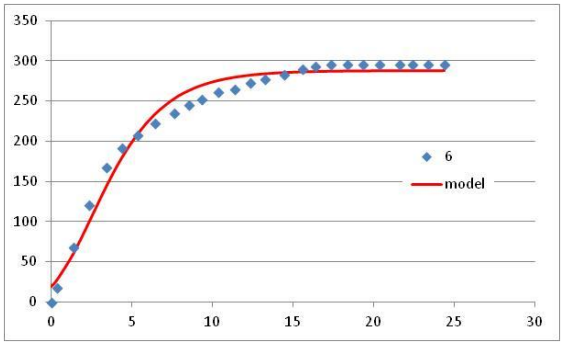


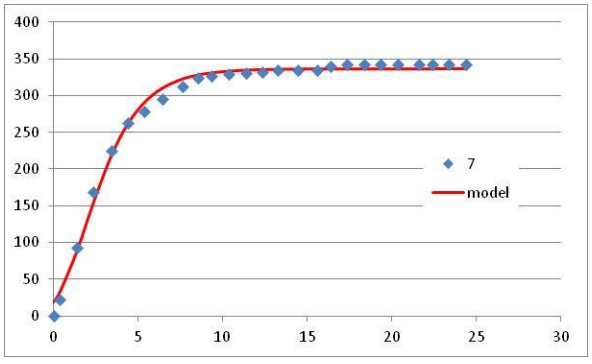


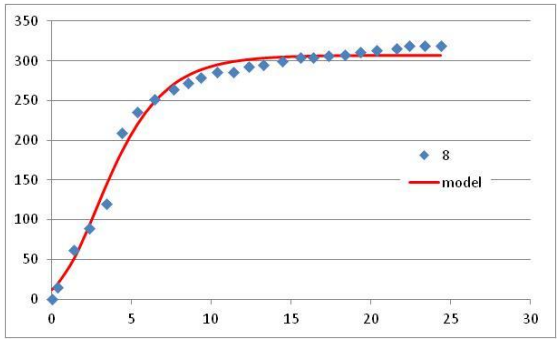




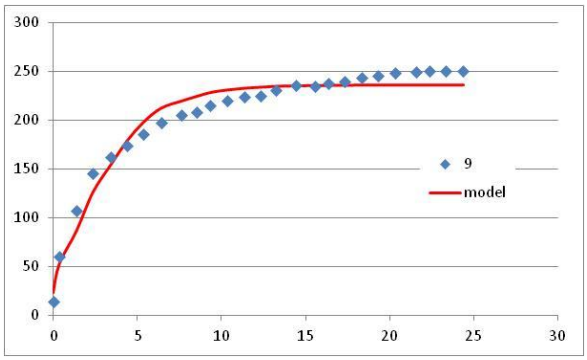








APPENDIX C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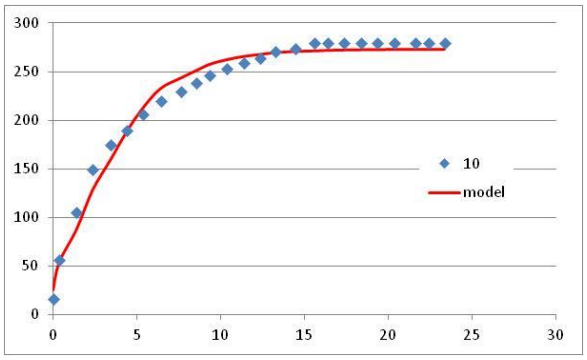
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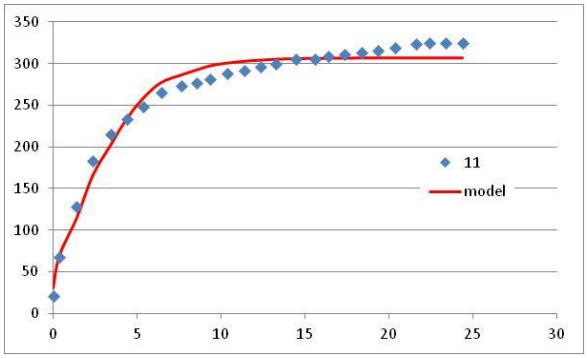
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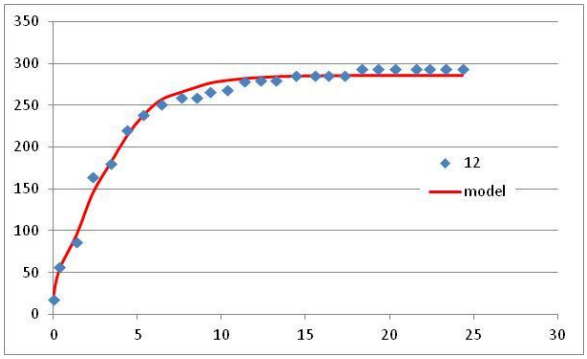
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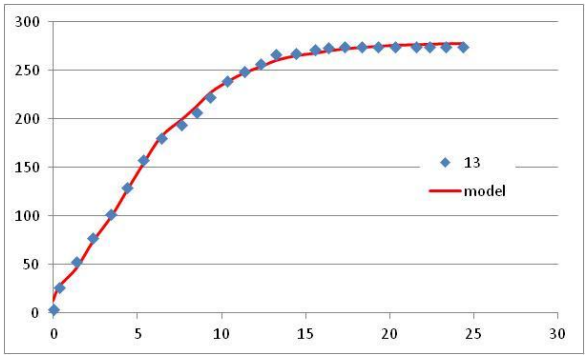
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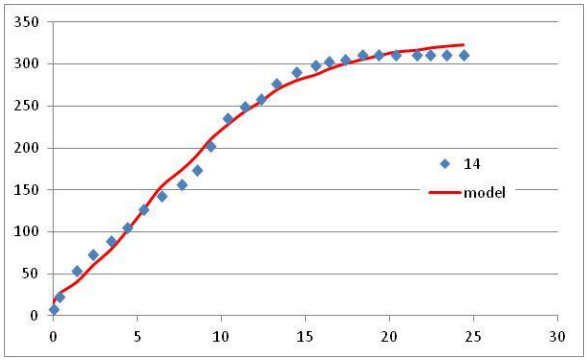
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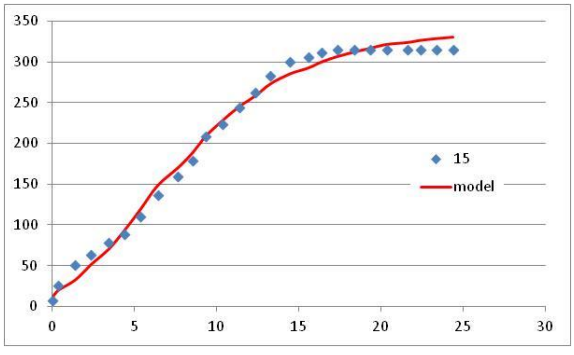
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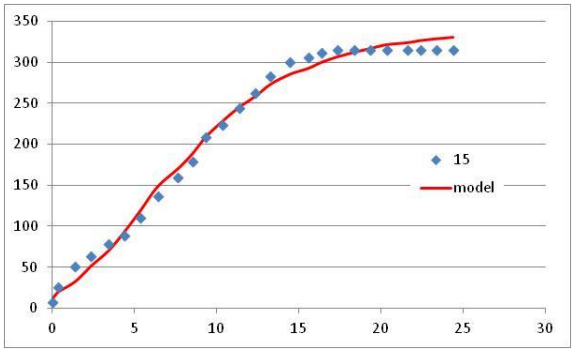
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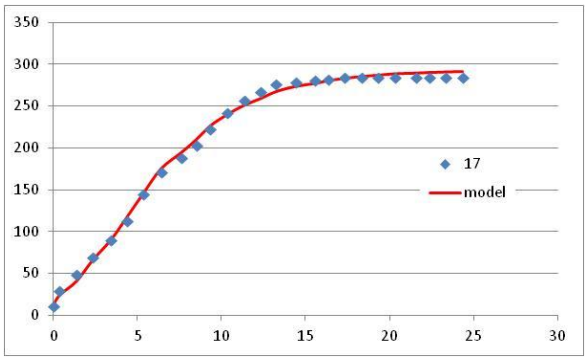
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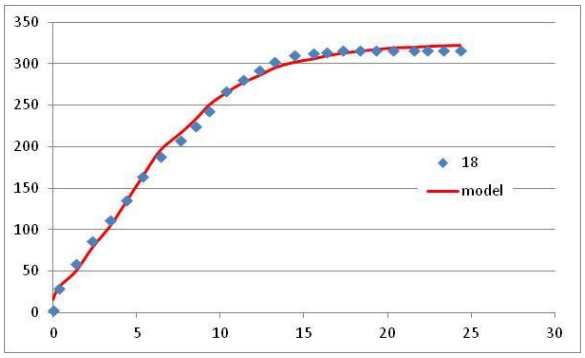
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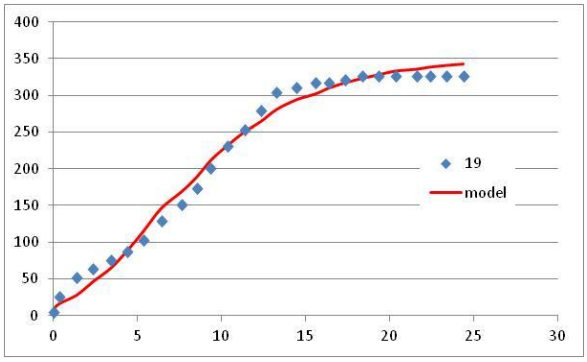
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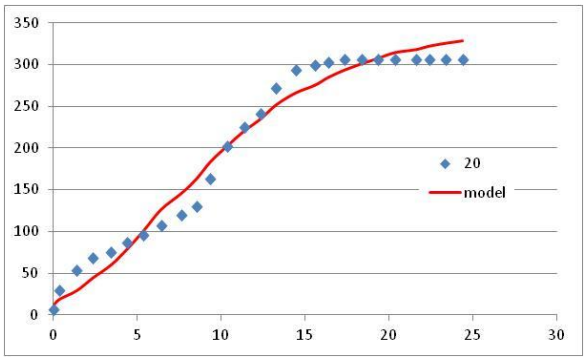
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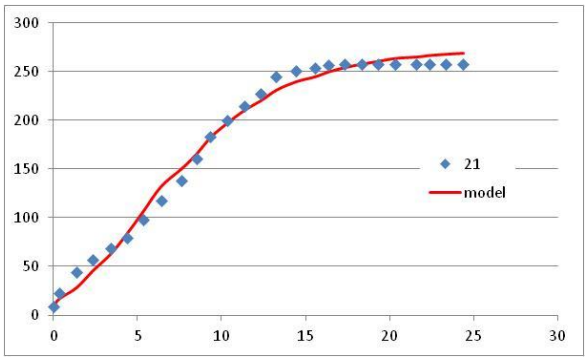
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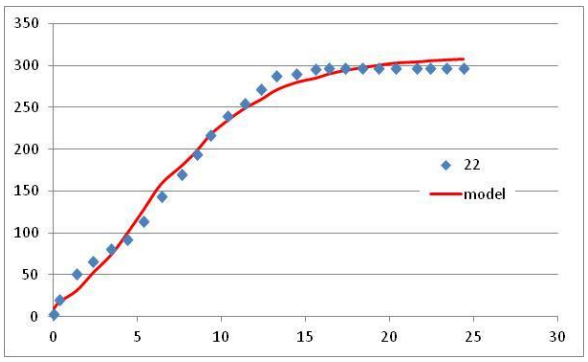
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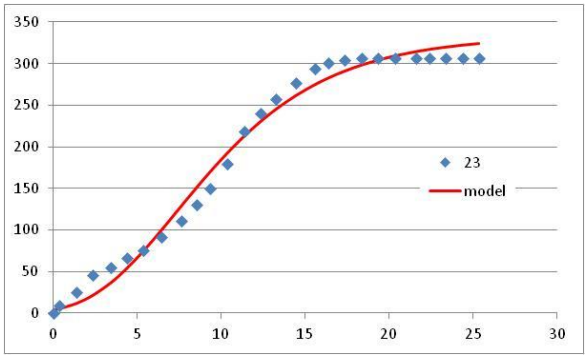
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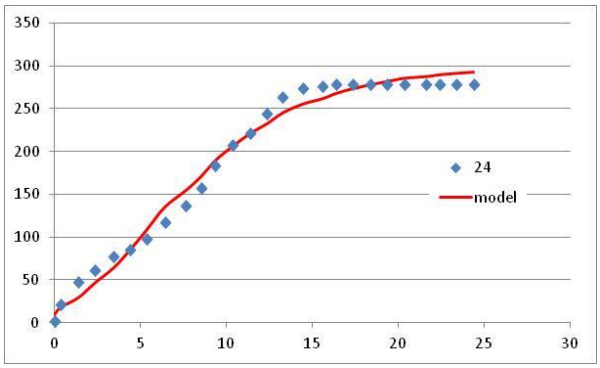
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APPENDIX D

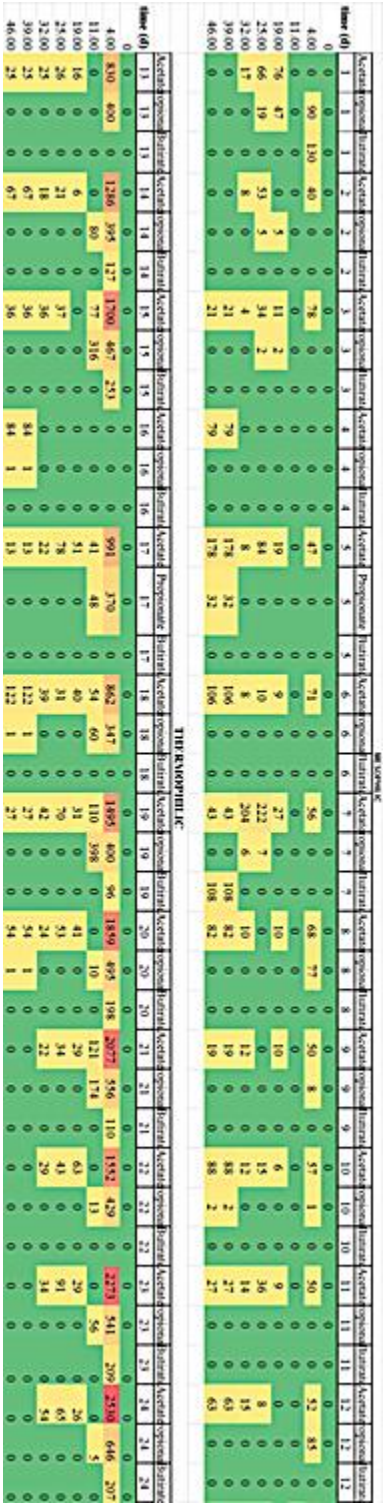

Figure D.1 - Average volatile fatty acids concentrations on BMP tests for Chapter 5.

APPENDIX E

COD mass balances for continuous reactors

		A1	A2	M1	M2	M3	M4	T1	T2	T3	T4
SRT 20d	Feed COD g/L	69.55	60.26	69.55	60.26	69.55	60.26	69.55	60.26	69.55	60.26
	COD rem%	32.3	21.7	50.4	47	60.5	59.3	55.6	55.3	61.9	56.2
	COD out g/L	47.08535	47.18358	34.4968	31.9378	27.47225	24.52582	30.8802	26.93622	26.49855	26.39388
	Biogas L/d	0.38	0.29	0.4	0.51	0.4	0.37	0.51	0.35	0.53	0.34
	% CH4	43.5	43.1	53.8	52.8	52.6	54.5	64.1	57.9	63.2	62.7
	CH4 L/d	0.1653	0.12499	0.2152	0.26928	0.2104	0.20165	0.32691	0.20265	0.33496	0.21318
A	CH4 COD (g)	0.472286	0.357114	0.614857	0.769371	0.601143	0.576143	0.934029	0.579	0.957029	0.609086
	sludge COD in g	27.82	24.104	2.782	2.4104	27.82	24.104	2.782	2.4104	27.82	24.104
B	sludge COD out (g)	18.83414	18.87343	1.379872	1.277512	17.95233	17.85762	1.235208	1.077449	17.90949	17.93981
A+B	sludge +CH4 COD	19.30643	19.23055	1.994729	2.046883	19.02576	18.79087	2.169237	1.656449	19.3388	18.90601
ratio	total CODout/total CODin	0.693976	0.797816	0.717013	0.849188	0.683888	0.779575	0.77974	0.687209	0.69514	0.784352

		A1	A2	M1	M2	M3	M4	T1	T2	T3	T4
SRT 15d	Feed COD g/L	69.55	63.39	69.55	63.39	69.55	63.39	69.55	63.39	69.55	63.39
	COD rem%	21	25.4	36.4	33.9	48.3	48.5	41.9	37.8	50.4	47.5
	COD out g/L	54.9445	47.28894	44.2338	41.90079	35.95735	32.64585	40.40855	39.42858	34.4968	33.27975
	Biogas L/d	0.65	0.63	0.5	0.39	0.56	0.48	0.65	0.48	0.68	0.54
	% CH4	44.3	39.8	52.5	54.4	56.6	52.5	64	62	63.1	61.3
	CH4 L/d	0.28795	0.25074	0.2625	0.21216	0.31696	0.252	0.416	0.2976	0.42908	0.33102
A	CH4 COD (g)	0.822714	0.7164	0.75	0.606171	0.9056	0.72	1.188571	0.850286	1.225943	0.945771
	sludge COD in g	27.82	25.356	3.707015	3.378687	27.82	25.356	3.707015	3.378687	27.82	25.356
B	sludge COD out (g)	21.9778	18.91558	2.357662	2.233312	20.81009	18.01503	2.153776	2.101543	20.72027	18.05401
A+B	sludge +CH4 COD	22.80051	19.63198	3.107662	2.839484	22.5384	19.45143	3.342347	2.951829	22.76892	19.71618
ratio	total CODout/total CODin	0.819573	0.774254	0.838319	0.84041	0.810151	0.767133	0.901628	0.873662	0.818437	0.777575

		A1	A2	M1	M2	M3	M4	T1	T2	T3	T4
SRT 10d	Feed COD g/L	68.24	52.9	68.24	52.9	68.24	52.9	68.24	52.9	68.24	52.9
	COD rem%	14.2	7.7	22.6	26	43	33.5	37.7	27.4	44.5	36
	COD out g/L	58.54992	48.8267	52.81776	39.146	38.8968	35.1785	42.51352	38.4054	37.8732	33.856
	Biogas L/d	0.83	0.68	0.6	0.55	0.68	0.65	0.83	0.55	0.79	0.66
	% CH4	42	40.7	53.9	53.7	56.7	51.6	59.9	59	61.7	62.8
	CH4 L/d	0.3486	0.27676	0.3234	0.29535	0.38556	0.3354	0.49717	0.3245	0.48743	0.41448
A	CH4 COD (g)	0.996	0.790743	0.924	0.843857	1.1016	0.958286	1.420486	0.927143	1.392657	1.184229
	sludge COD in g	27.296	21.16	5.4592	4.232	27.296	21.16	5.4592	4.232	27.296	21.16
B	sludge COD out (g)	23.41997	19.53068	4.225421	3.13168	21.45466	18.16586	3.401082	3.072432	21.3523	18.03361
A+B	sludge +CH4 COD	24.41597	20.32142	5.149421	3.975537	23.55226	19.91489	4.821567	3.999575	23.74095	20.00858
ratio	total CODout/total (	0.894489	0.96037	0.943256	0.939399	0.862846	0.941157	0.8832	0.945079	0.869759	0.945585

APPENDIX E

		A1	A2	M1	M2	M3	M4	T1	T2	T3	T4
SRT 5d	Feed COD g/L	68.91	61.17	68.91	61.17	68.91	61.17	68.91	61.17	68.91	61.17
	COD rem%	23.6	18.7	21.7	17.9	39	30.5	30.9	22.9	43.1	35.3
	COD out g/L	52.64724	49.73121	53.95653	50.22057	42.0351	42.51315	47.61681	47.16207	39.20979	39.57699
	Biogas L/d	0.83	0.69	0.62	0.6	0.9	0.76	1	0.7	0.98	0.85
	% CH4	41.1	40	50.9	45.9	55.9	53	59	61	62.8	63.5
	CH4 L/d	0.34113	0.276	0.31558	0.2754	0.5031	0.4028	0.59	0.427	0.61544	0.53975
A	CH4 COD (g)	0.974657	0.788571	0.901657	0.786857	1.437429	1.150857	1.685714	1.22	1.7584	1.542143
	sludge COD in g	27.564	24.468	11.0256	9.7872	27.564	24.468	11.0256	9.7872	27.564	24.468
B	sludge COD out (g)	21.0589	19.89248	8.633045	8.035291	18.24125	17.98167	7.61869	7.545931	17.48847	17.19772
A+B	sludge +CH4 COD	22.03355	20.68106	9.534702	8.822148	20.65333	19.9211	9.304404	8.765931	20.22153	19.52843
ratio	total CODout/total (	0.79936	0.845229	0.864779	0.901397	0.749287	0.81417	0.843891	0.895653	0.733621	0.798121