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Enhancement of Anaerobic Waste Activated Sludge Digestion by Microwave Pretreatment

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ENHANCEMENT OF ANAEROBIC WASTE ACTIVATED SLUDGE DIGESTION BY MICROWAVE PRETREATMENT

by

Cigdem Eskicioglu

Ph. D. Thesis

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Under the supervisions of

Prof. Ronald L. Droste

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Abstract

Improvement of biodegradability of waste activated sludge (WAS) depends on enhanced disintegration of the floc structure of sludge and increasing the accessibility to both intracellular (within the microbial cell) and extracellular (within the polymeric network) materials before WAS is sent to anaerobic digesters. This study proposes microwave (MW) technology as a new and an alternative pretreatment method to disintegrate the floc structure of secondary sludge, to enhance the hydrolysis and to improve the anaerobic digestion of WAS in comparison to existing pretreatment methods such as, chemical, mechanical and conventional heating (CH) techniques.

In the first stage of the study, the effects of MW pretreatment on disintegration and hydrolysis of WAS by soluble chemical oxygen demand (COD), soluble protein, soluble sugar and nucleic acid leakage detection experiments were investigated. The effects of three variables [MW temperature (T), MW intensity (I), WAS concentration (C)] and the effects of four variables [T, I, C and volume percentage of WAS pretreated (PT)] were investigated on WAS solubilization and biogas production in two multilevel factorial statistical designs containing 24 solubilization runs and 54 mesophilic batch reactors, respectively. In a low temperature range (50-96°C) using a household type (1250 W, 2450 MHz) MW oven, pretreated WAS samples resulted in 3.6 ± 0.6 and 3.2 ± 0.1 fold increases in soluble COD/ total COD ratios at high [5.4% total solid (TS), w/w] and low (1.4% TS, w/w) sludge concentrations, respectively. WAS, pretreated to 96°C, produced the greatest improvement in biogas production with 15 ± 0.5 and 20 ± 0.3% increases over the controls (unpretreated) after 19 d of digestion at low and high WAS concentrations.

In the second stage of the study, two different pretreatment temperatures (50 and 96°C) were further tested in a total of 10 semi-continuous digesters at sludge retention times (SRTs) of 5, 10 and 20 d. Digesters using CH WAS were also run to investigate *thermal* and *athermal* effects of MW pretreatment. In general, incremental increases in total solid (TS), volatile solids (VS) and total COD removal efficiency of pretreated digesters compared to controls dramatically increased as SRT was gradually shortened from 20 to 10 to 5 d. WAS pretreated to 96°C by MW and CH achieved 29 and 32% higher TS and 23 and 26% higher VS removal efficiencies compared to controls at SRT of 5 d, while similar reactors at SRT of 20 d had only 16% higher TS and 11 and 12% higher VS removals than those of controls, respectively.

Ultrafiltration (UF) was also used to characterize the soluble molecular weight (Mw) distributions of control, CH and MW irradiated WAS at 96°C. Soluble CODs of CH and MW irradiated WAS were 361 ± 45 and 143 ± 34% higher and resulted in 475 ± 3 and 211 ± 2% higher cumulative biogas productions relative to the control at the end of 23 days of mesophilic batch anaerobic digestion, respectively. Depending on the Mw fraction, the range of substrate volumetric utilization rate increases from anaerobic digesters was between 94-184% for the CH and 26-113% for the MW compared to the control for the first 9 days of the digestion. Digesters treating high Mw materials (Mw > 300 kDa) resulted in smaller first-order biodegradation rate constants, k, indicating that microorganisms require a longer time to utilize high Mw fractions which are most likely the cell wall fragments and exopolymers. MW studies under the boiling point (100°C at 1 atm) have promised a significant potential to disintegrate the floc structure and to enhance the hydrolysis and biodegradability of WAS in full-scale digesters.

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I dedicate this thesis to my husband, Murat Eskicioglu.

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CHAPTER 1

INTRODUCTION

Municipal wastewater treatment plants (MWTPs) generate large amounts of primary and secondary sludges that have high organic content. Therefore, sludge management has become a key factor in wastewater management during the last two decades. Anaerobic sludge digestion is often applied to WAS to reduce the mass of solids for disposal, to reduce the pathogen content and to generate biogas for energy recovery.

Rapid and complete stabilization of WAS via anaerobic digestion has not been achievable due to the rate limiting hydrolysis step of large organic molecules associated with microbial cells. Recent studies have indicated that activated sludge has a more complex floc structure than first realized. It is comprised of different groups of microorganisms, organic and inorganic matter agglomerated together in a polymeric network formed by microbial extracellular polymeric substances (EPS) and cations (Li and Ganzarczyk, 1990; Frølund *et al.*, 1996). It is believed that hydrolysis of EPS and/or microbial biomass together within the activated floc limits the rate and extent of degradation (Higgins and Novak, 1997). EPS does not only originate from the metabolism and cell autolysis associated with activated sludge bacterial cells but also originates in part from the raw influent wastewater coming into the treatment plant (Urbain *et al.*, 1993; Frølund *et al.*, 1994). According to the most recent WAS-floc agglomeration concept, EPS and divalent cations may be the most important parameters governing WAS hydrolysis. These two parameters rather than microbial cells represent the major organic fraction determining the floc structure, integrity and strength (Higgins and Novak, 1997; Novak *et al.*, 2003). Disruption of the EPS and divalent cation network

followed by subsequent enhanced stabilization of microbial biomass should result in enhancing the rate and extent of WAS biodegradability (Park *et al.*, 2003) and increase dewaterability (Ormeci and Vesilind, 2001) during and after anaerobic digestion.

Improvement of biodegradability of WAS via anaerobic digestion depends on enhanced disintegration of the floc structure of sludge and increasing the accessibility to both intracellular (within the microbial cell) and extracellular (within the polymeric network) materials before WAS is sent to the anaerobic digesters. There are many methods that have been studied and shown to be effective, such as; ***mechanical disintegration*** by ball milling (Baier and Schmidheiny, 1997; Muller *et al.*, 1998); a rotor-stator shearing device (Muller *et al.*, 2003), special thickening (Dohanyos *et al.*, 1997a) high pressure homogenizer (Muller *et al.*, 1998), ultrasound (Tiehm *et al.*, 1997; Schlafer *et al.*, 2000; Tiehm *et al.*, 2001; Chu *et al.*, 2001; Lafitte-Trouque and Forster, 2002; Onyeche *et al.*, 2002; Barber, 2002; Brown *et al.*, 2003; Gonze *et al.*, 2003), ***thermal disintegration*** by freezing and thawing of biomass (Dohanyos *et al.*,1997b; Wang *et al.*, 1999; Ormeci and Vesilind, 2001), thermal hydrolysis (Barnard *et al.*, 2002; Abraham and Kepp, 2003) and ***chemical disintegration*** by acid or caustic addition and sometimes chemical disintegration following mechanical pretreatment such as; high pressure homogenizer (Shaw *et al.*, 2002; Stephenson *et al.*, 2003) or ultrasound (Chiu *et al.*, 1997; Chu *et al.*, 2002). The full-scale application of pretreatment methods depends on technical and economic conditions.

Studies on WAS have indicated that any pretreatment to enhance floc disintegration and cell lysis is likely to improve anaerobic sludge digestion. MW technology is an attractive alternative heating method to CH due to its environmental and energy

conservation properties (Decareau, 1985; Kingston and Jassie, 1988). So far, publications regarding the effect of MW technology on anaerobic digestion efficiency are very limited (Park *et al.*, 2004) and mostly focused on fecal coliform destruction rather than treatibility analyses (Hong *et al.*, 2004; Hong *et al.*, 2006).

1.1 Hypothesis

The purpose of this thesis is to propose ***MW technology*** as a new and an alternative pretreatment method to enhance anaerobic digestion in comparison to existing pretreatment techniques summarized above. ***It is hypothesized that dipole rotation (explained in Section 2.2.2) and following heating (thermic) effects of MWs disintegrate the complex floc structure of WAS and make organic molecules unfold, denature and eventually more biodegradable.*** The following sections of this report contain an extensive literature survey on existing pretreatment methods and propose an experimental design to prove this hypothesis and to analyze the effects of MW pretreatment on anaerobic digestion of WAS.

1.2 Scope of the Research

The specific objectives of this research are:

- To evaluate the effects of pretreatment variables, such as MW temperature, intensity, WAS concentration and volume percentage of sludge pretreated on:
  - Disintegration and hydrolysis of WAS.
  - Anaerobic batch digestion of WAS.
- To verify the effects of MW pretreatment on WAS in continuous flow anaerobic digesters operating at SRTs of 5, 10, and 20 d.

- To characterize the distribution of soluble COD fractions with different molecular weights from raw and pretreated sludge and the overall anaerobic biodegradability and biodegradation rate of these soluble fractions by UF and BMP tests, respectively.

In order to achieve objectives outlined above, this thesis mainly focuses on the primary treatibility performance (increased rates and overall biogas production, COD and TS/VS destruction) and secondary performance (improved solubility/denaturation and dewaterability) of the batch and continuous flow sludge digesters after MW pretreatment.

1.3 Thesis Organization

This thesis is organized as a paper-format thesis; the main results presented in Chapters 3-6 were prepared in a journal manuscript format. Chapter 3 displayes the results on the effects of MW pretreatment from mesophilic batch anaerobic digesters. Batch reactors are often preferred for preliminary studies, since they are simpler and they avoid the hydraulic reactor problems. In Chapter 3, the effects of pretreatment temperature (T), intensity (I), WAS concentration (C) and volume percentage of WAS treated (PT) on WAS solubilization and anaerobic digestion of sludge was studied in two separate multilevel factorial designs containing 24 solubilization runs and 54 batch anaerobic digesters. The contents of chapter 3 were presented at WEFTEC 2005, 78^{TH} Annual Technical Exhibition and Conference in Washington DC, USA, October 29-November 2, 2005 and the manuscript shown as Chapter 3 was accepted by Journal of Water Environment Research (WEF) for publication.

In Chapter 4, biopolymer release from floc structure of WAS during MW irradiation was investigated by soluble protein, soluble sugar and nucleic acid leakage

detection tests. In addition, empirical modeling of data generated (in Chapter 3) from the effects of MW irradiation on COD solubilization and biogas production from batch digesters was conducted. A summary paper that contains a portion of results included in Chapter 4 was presented at WEFTEC 2006, 79TH Annual Technical Exhibition and Conference in Dallas, TX, USA, October 21-25, 2006 and it was accepted by Journal of Water Environment Research (WEF) for publication.

After preliminary batch studies, Chapter 5 verified the effects of MW pretreatment on continuous flow sludge digesters mostly used for full-scale applications. Two pretreatment temperatures (50 and 96°C) were tested in a total of 10 semi-continuous (SC) flow mesophilic digesters at sludge retention times (SRTs) of 5, 10 and 20 d. Digesters using conventionally heated (CH) TWAS were also run to investigate *thermal* and *athermal* effects of MW pretreatment. This manuscript was submitted to *Journal of Environmental Engineering,* ASCE for publication

The purpose of Chapter 6 was to conduct more in depth studies to analyze the size and impact of the soluble products produced by MW irradiation on anaerobic digestion of WAS in an attempt to obtain a better understanding of the advantages or disadvantages of various pretreatment strategies (i.e., why more pretreatment may or may not result in decreased rates or extent of WAS degradation). In this chapter, using ultrafiltration (UF) and biochemical methane potential (BMP) tests, the distribution of soluble COD fractions from raw and pretreated sludge and the overall anaerobic biodegradability and biodegradation rate of soluble fractions with different molecular weight cutoffs (MwCOs) were addressed. The manuscript shown as Chapter 6 was accepted by Water Research for publication.

CHAPTER 2

LITERATURE REVIEW

This section will discuss the effects of existing WAS pretreatment methods on conventional mesophilic anaerobic digestion (MAD).

2.1 Existing Sludge Disintegration Methods

2.1.1 Mechanical Disintegration

In mechanical disintegration, the breakup of cells occurs in minutes instead of days and intracellular components are released and readily available for biological degradation. Different mechanical techniques have been studied to create mechanical shear on sludge. In 1997, Baier and Schmidheiny applied solids disintegration by two different methods, ball milling and cut milling and they observed more effective solubilization in terms of increased soluble COD concentration by the ball milling technique. The overall degradation of volatile solids (VS) could be increased from 38 to 57% and biogas production was enhanced by 10%. Based on their observations, the composition of biogas was not influenced by the pretreatment, showing consistent methane content of 71-74%. Muller *et al.* (1998) studied four different pretreatment methods; disintegration by a stirred ball mill, a high pressure homogenizer, an ultrasonic homogenizer and a shear-gap homogenizer. The best results were obtained by using the stirred ball mill and high-pressure homogenizer. The degree of degradation was between 10 and 20% higher in comparison to the untreated sludge and therefore biogas production was increased and the digester volume needed was reduced. However, disruption of the

particle structure caused an increase in polymer-demand and no improvement in dewatering results was observed.

Another interesting pretreatment method was proposed by Dohanyos *et al.* (1997a). Their objective was to create partial destruction of excess sludge cells during a special centrifugal thickening with no additional energy demand, due to the dissipated kinetic energy generated by the centrifuge that incorporated a special impact gear in the thickening centrifuge. They concluded that improvement of methane yield and biodegradability was dependent on the quality of sludge (primary or secondary) and the efficiency of the thickening centrifuge. They achieved an average 13.6% improvement in methane yield (m^3 CH_4/kg VS added) from the mixture of thickened WAS (TWAS) and primary sludge (PS) and an average of 31.8% improvement from TWAS only.

Besides studies on increased biogas production and reduced volume required for anaerobic digestion, MWTPs are being pushed to generate better sludge, such as Class A biosolids, because of the risk that disease could be transmitted through Class B sludge. Without pretreatment, MAD falls short of an ideal biosolids management system due to high costs and the large quantities of unstabilized and pathogen rich sludge that still needs to be disposed. The new-patented *MicroSludge*™ process is claimed to result in complete and rapid VS destruction and near complete pathogen destruction (Class A biosolids) by alkaline pretreatment (with NaOH for 1 hour) to weaken the cell walls and a homogenizer (pressure ≈ 83,000 kPa) for a sudden pressure change to lyse the cells prior to MAD (Shaw *et al.*, 2002). Based on recent bench and pilot scale tests, within 8 weeks, soluble COD removal was improved from 5 to 96%, biochemical oxygen demand (BOD) removal was increased from 6 to 96% and VS removal increased from 41 to 73%

(Stephenson *et al.*, 2003) and first full-scale *MicroSludge*™ achieved VS reductions of up to 90% for secondary sludge alone (Rabinowitz and Stephenson, 2005).

Recently, Muller and his colleagues performed a combination of bench and full-scale studies to test high intensity mechanical shear in an internal recycle loop MAD (Muller *et al.*, 2003). Increases of 46 and 15% in biogas production could be observed in short-term (56 h) and long-term (20 d) batch studies, respectively. Full scale applications were reported to have 21 and 11% increases in VS destruction for PS and WAS digesters, respectively. However, in terms of the dewatering characteristics, polymer demand was increasing (84% increase in polymer demand) for PS and there was no effect on pathogen reduction based on total and fecal coliforms.

Ultrasound is another mechanical sludge disintegration method considered as a new application in WAS management. The term "ultrasound" is used to describe sound energy at frequencies ranging from 20 to 10 MHz, which is outside the audible range. Especially at low frequencies from 20 to 40 kHz, cavitation (bubble formation and collapse) occurs by high pressure gradients causing an extreme increase in temperature inside and around the bubble, which results in cell lysis and concomitant increased rates and amount of anaerobic digestion.

Different ultrasound frequencies and acoustic intensities were studied to find the optimum sludge disintegration. Tiehm *et al.* (1997) used a frequency of 31 kHz and high acoustic intensity and observed increase of COD in the supernatant and size reduction of sludge solids. Semi-continuous anaerobic digesters experienced a 9% increase in VS destruction with a biogas production of 2.2 times (disproportional to VS removal) that of

the control digester. After 96 seconds of ultrasonic treatment, 30% more sludge disintegration was achieved than for chemical disintegration methods.

Recent ultrasound studies have shown that sludge disintegration was most significant at low frequencies due to larger cavitation exerting strong shear forces in the liquid. Tiehm *et al.* (2001) studied a frequency range between 41-3217 kHz and the disintegration of sludge was more effective at the lowest end, 41 kHz. They concluded that it would be possible to achieve better disintegration at the lower frequencies, such as 20 kHz, but lower frequency was not possible with the device available. While short sonication times caused sludge floc deagglomeration without the destruction of the cells, longer sonication resulted in release of dissolved organic matter after breakup of cell walls. Therefore, recent studies are mostly focused on low ultrasound frequencies in the range of 20-25 kHz (Chu *et al.*, 2001; Lafitte-Trouque and Forster, 2002; Onyeche *et al.*, 2002; Brown *et al.*, 2003; Gonze *et al.*, 2003). However, sonication did not affect the fecal coliform concentration significantly in the sludge (Lafitte-Trouque and Forster, 2002) and it just qualified as "Class B" sludge according to US sludge regulations (Chu *et al.*, 2001).

Almost all studies on ultrasound pretreatment have experienced an increase in VS destruction (in the range of 9-27%) as well as an increase in the biogas production (in the range of 22-120%) depending on numerous factors, such as duration and necessary power level of ultrasound application (sludge disintegration percentage), concentration of sludge samples and quality of the waste sludge (primary, secondary, or mixed). The latter was particularly important, since maximum biogas and dewatering results could be obtained when pretreating only a fraction of the secondary sludge line as opposed to pretreating

the full stream (Barber, 2002) as shown in Figure 2-1. According to Figure 2-1, biogas production was highest when only 60% of secondary sludge was being treated with ultrasound. Another important result from the same figure was that biogas production increased as percent of PS in digester feed increased as also seen in Figure 2-2.

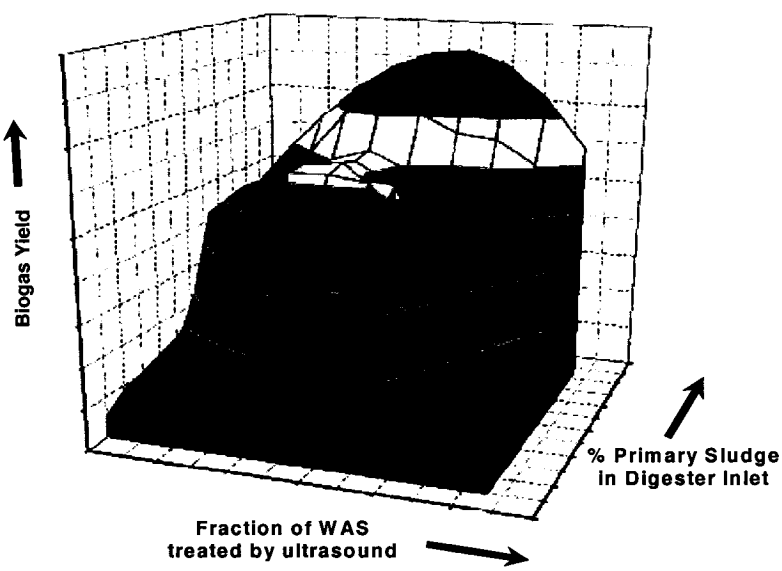


Figure 2-1 Effect of part-stream ultrasonic disintegration on biogas production from anaerobic digesters (Barber, 2002, reprinted with admission).

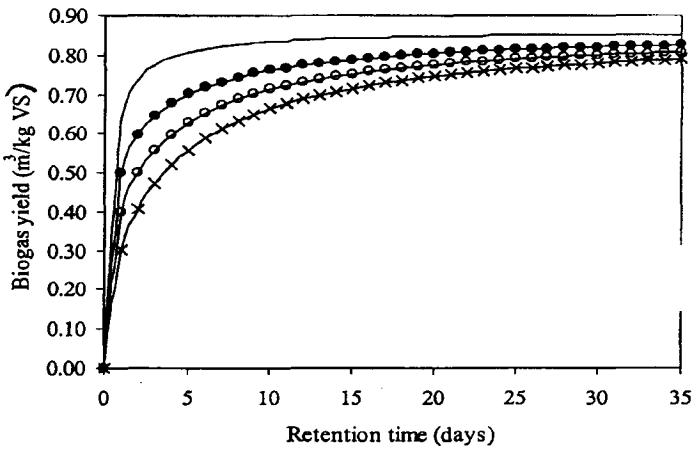


Figure 2-2 Effect of altering primary: secondary sludge influent ratio on expected biogas yield during digestion at different retention times. Key: (—) 100% PS, (•) 70% primary 30% secondary, (o) 50% primary 50% secondary, (x) 30% primary 70% secondary (adapted from Barber, 2002).

Biogas production also changes as a function of influent dry solids (DS) concentration subjected to ultrasound (Barber, 2002; Onyeche *et al.*, 2002; Gonze *et al.*, 2003). Figure 2-3 shows that the influence of ultrasound is greater as DS inlet concentrations increase. For example, if the influent has 3% DS, ultrasound pretreatment will produce almost 50 kW energy, which is higher than the energy produced if ultrasound is not applied (Barber, 2002).

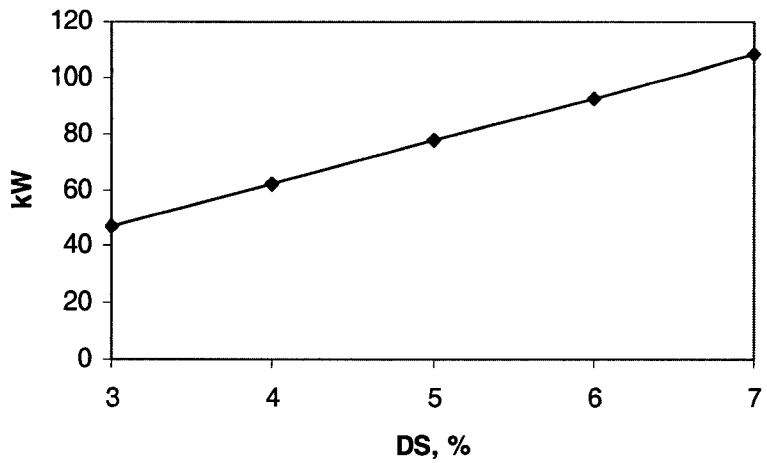


Figure 2-3 Effect of influent DS concentration in increase in energy generation due to ultrasound on anaerobic digester treating sludge at HRT of 20 days (adapted from Barber, 2002).

Ultrasound pretreatment was applied in two full scale sewage treatment plants (Mannheim and Wallerfangen) in Germany in 2001 and improvements in dewatering operations were reported by both treatment plants. In the Mannheim Sewage plant, which treats a population equivalent of 725,000 inhabitants, a reduction in polymer consumption of 1.5 kg/tonnes DS was observed. Before ultrasound application, belt presses, which gave cake DS between 16 to 17% (occasionally by addition of lime) was being used in Wallerfangen (90,000 inhabitants). After installation of an ultrasound system to pretreat a fraction of the secondary sludge, the plant experienced a 31%

decrease in polymer demand, which equaled a reduction of 40,000 kg polymer/yr. Recently, after ultrasound pretreatment, belt presses produced 23% DS without the usage of lime {1}. Similar dewatering results were reported by Brown *et al.* (2003) using a bench scale belt press. They observed improvements of 1.2 -2.6% (mean 1.64 ± 0.32%) in biosolids cake from an ultrasound test digester compared to the control at an identical polymer dosage.

2.1.2 Thermal Disintegration

Thermal disintegration is another way to promote cell lysis before anaerobic sludge digestion. Methods such as, repeated freezing and thawing of biomass, heating the biomass in an autoclave (120-170°C, 30 min), rapid thermal conditioning (about 10 s) at high pressure and temperature (180-200°C) were observed to enhance anaerobic sludge digestion. However, if pretreatment is applied for longer period than necessary, as a result of high pressure and temperature (>175°C), enzymes can be inactivated and toxics, such as ammonia, can be formed (Stuckey and McCarty, 1984). However, an interesting result from the literature survey was that although many of the researchers monitored the ammonia concentration in the anaerobic digesters after pretreatment, no definite ammonia inhibition case was reported.

Dohanyos *et al.* (1997) compared two different methods (repeated freezing and thawing of sludge and heating of anaerobic sludge to 100°C for 20 min). The first method was more successful in terms of increase in methane production (61.9%) and increase in VS removal (46.8%) than the second method (41.8 and 27.6%, respectively). Freeze-thaw conditioning of activated sludge caused cell disruption (higher DNA in supernatant of

sludge) and released intracellular materials to the sludge supernatant phase (Ormeci and Vesilind, 2001). On the other hand, results obtained by Wang *et al.* (1999) were somewhat contradictory. They applied three thermal pretreatments (one in an autoclave at 120°C, one in a hot water bath at 60°C and the third one freezing at −10°C) and a low frequency (9 kHz) ultrasound pretreatment. The maximal rates of methane generation (from 500 mL pretreated WAS in batch digesters with acclimatized inoculum at 35°C) with ultrasound, heating at 120°C, at 60°C and freezing treatment were 766, 737, 616, and 560 mL/d, respectively. Freezing pretreatment was least successful in terms of methane production.

In addition to increase in biogas production and VS destruction, thermal hydrolysis is also used to increase dewaterability of sludge and to produce "Class A" sludge, with existing and/or new MAD. One technology sold under CAMBI™ has been installed in different countries in Europe. This technology provides high temperature (160-170°C) and pressure (600-800 kPa) to hydrolyze and to pasteurize feed sludge to downstream reactors (Barnard *et al.*, 2002). CAMBI™ was expected to operate at an average DS concentration of 13.5% and after pretreatment, digested sludge can be centrifuged to 34%. This technology has also been used in the Ringsend wastewater treatment plant (WWTP) in Dublin, Ireland. Startup data indicated VS destruction 50% greater than conventional MAD (Abraham and Kepp, 2003). In another high temperature and pressure pretreatment study, semi-continuous digesters treating WAS pretreated by steam explosion at 220°C, 2068 kPa yielded 49 and 31% higher methane production at SRTs of 15 and 8 d compared to controls, respectively (Dereix *et al.*, 2005).

2.1.3 Chemical Disintegration

Acid and caustic addition to sludge samples can also be used to hydrolyze and decompose lipids and proteins to smaller soluble substances. A combination of chemical treatment with mechanical pretreatment methods can take the advantages of two methods and provide better disintegration. Therefore, Chiu *et al.* (1997) used an experimental set-up with three different combinations of alkaline treatment and ultrasonic treatment: (1) sludge pretreated with 40 meq/L of NaOH for 24 hours, (2) sludge pretreated with 40 meq/L of NaOH for 24 hours followed by low frequency ultrasonic vibration (20 kHz, 24 s/mL), (3) simultaneous ultrasound treatment (20 kHz, 14.4 s/mL) applied to samples dosed with 40 meq/L NaOH. NaOH was used, since it was reported to yield greater solubilization than lime. Pretreatment using simultaneous alkaline and ultrasonic treatment (3) was observed to be more effective in releasing both soluble COD and soluble organic nitrogen than alkaline treatment alone (1) and achieved results close to alkaline treatment followed by ultrasonic treatment (2). Pretreatment with NaOH only for 24 hours was not effective in hydrolyzing the particulate COD. Interesting results were reported when low frequency ultrasound (20 kHz, 20 min) treatment was applied to a sludge conditioned with a cationic polyelectrolyte flocculent (Chu *et al.*, 2002). The presence of the polyelectrolyte flocculants enhanced methane production within 6 days of digestion (phase I) but inhibited digestion thereafter (phase II).

2.2 Proposed Sludge Disintegration Method: Microwave Technology

MW power, which is created by radio waves in the decimeter area, is an alternative to CH due to its environmental and energy conservation properties. The development of

radar technology to detect aircraft during World War II stimulated the rapid growth of MW technology. The first MW heating applications soon followed, heating food with MW energy. However, because of the need for a better understanding of how MWs interact with different samples and for proper hardware, acceptance of this new technology was very slow (Decareau, 1985; Kingston and Jassie, 1988). The following sections of this report aim to give basic information on theoretical concepts and equipment design of MW technology. This information is essential for understanding the effect of MW technology on biological sludge treatment.

2.2.1 Microwave Theory

MWs are electromagnetic energy. In the electromagnetic spectrum, MW radiation occurs in an area of transition between infrared radiation and radio frequency waves, as shown in Figure 2-4. Frequency of MWs is between 30 GHz and 300 MHz with wavelengths of 1 cm and 1 m, respectively (Vollmer, 2004). To avoid interference with telecommunications and cellular phone frequencies, heating applications must use ISM (Industrial Scientific and Medical Frequencies) bands, which are 27.12, 915, and 2450 MHz with wavelengths of 11.05 m, 37.24 cm, and 12.24 cm, respectively (Kingston and Jassie, 1988). The frequency of 2450 MHz (maximum output achievable is 15 kW) can be freely used in industrial applications without requiring any permission. Frequencies around 900 MHz (in USA, 915 MHz and in UK, 896 MHz), which can produce up to 100 kW, are used for larger plants. Cellular telephone services also use this band (872-960 MHz), and very strict safety requirements exist for radiation from industrial equipment operating at 900 MHz. The choice of frequency may change for each plant. Lower

frequencies, such as 915 MHz, provide longer wavelengths (37.24 cm) for applications where deeper penetration into material is necessary. However, if the MW is used for heating or drying a product in thin layers with a large surface area, shorter wavelengths (2450 MHz-12.24 cm) could be adequate {2}.

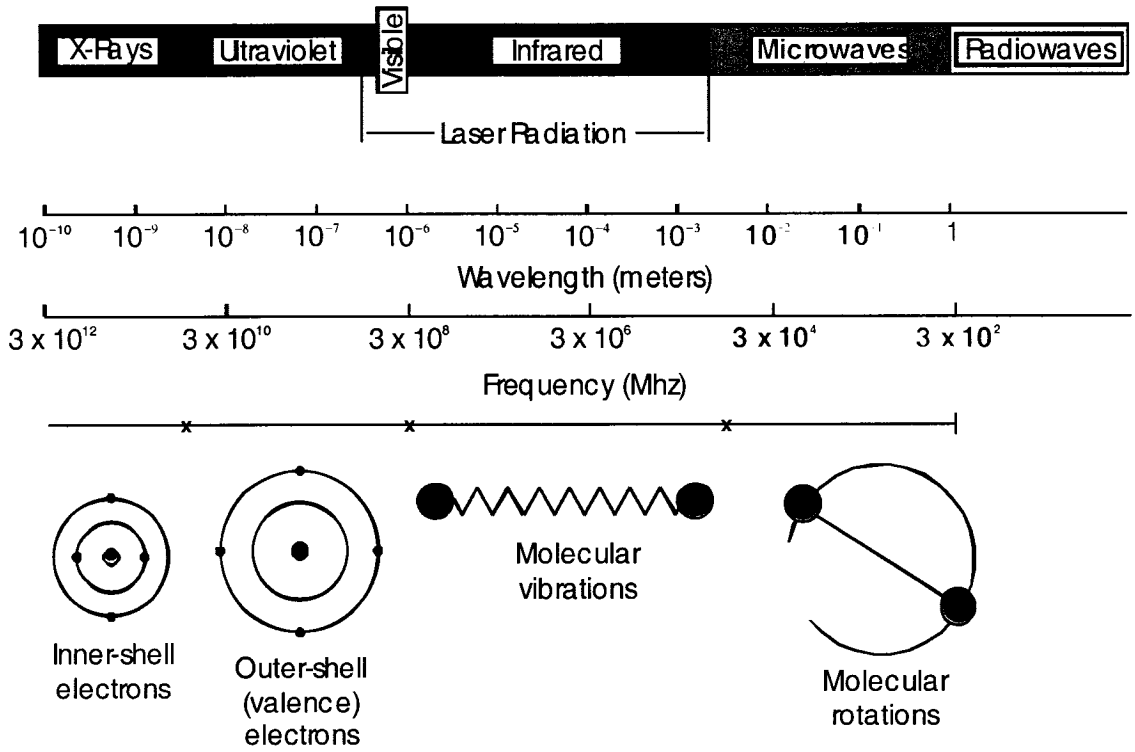


Figure 2-4 Electromagnetic spectrums (adapted from Kingston and Jassie, 1988).

2.2.2 Interaction between Electromagnetic Field and Sample

MW heating results from interactions of the chemical constituents of the substrate with the electromagnetic field. Molecular friction, primarily because of the disruption of weak hydrogen bonds associated with the dipole rotation of free water molecules, and migration of the free salts in an electrical field are two important results of these interactions (Decareau, 1985). Therefore ionic and solid properties of samples play important roles in an electromagnetic field as shown in Table 2-1.

Table 2-1 Effect of ions and solids in samples on dielectric properties (adapted from Decareau, 1985).

Constituents	Relative Dielectric Activity
Water, bound	Low
Water, free	High
Salts, associated	Low
Salts, dissociated	High
Colloidal solids	Low

Dipole rotation refers to the alignments that result because of the electric field. As the electromagnetic field increases, it aligns the polarized molecules (see Figure 2-5a) and as the field decreases, disorder is restored (see Figure 2-5b). There is stored energy associated with the preferred orientation and when the magnetic field is removed, in the relaxation time, t, molecules return to disorder and thermal energy is released. At the frequency of 2450 MHz, alignment of molecules followed by returning to disorder happens 4.9×10^9 times per second and results in very fast heating (Kingston and Jassie, 1988; Loupy, 2002).

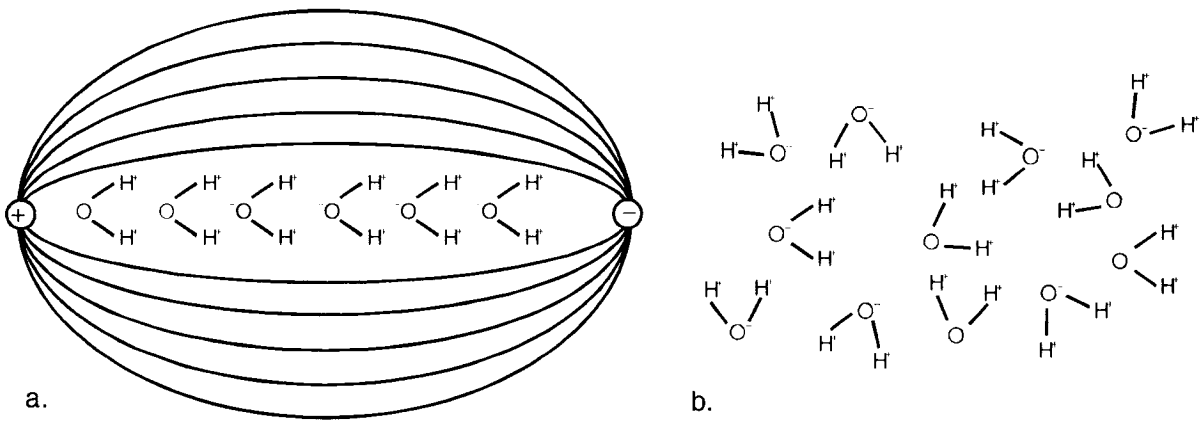


Figure 2-5 Schematic of the molecular response to an electromagnetic field. (a) polarized molecules aligned with the poles of the electromagnetic field; (b) thermally induced disorder as electromagnetic field is removed (adapted from Kingston and Jassie, 1988).

Chemical and physical properties determine the dissipation factor (tan δ) of a sample. The dissipation factor is a ratio of the sample's dielectric loss or loss factor (ϵ'') to its dielectric constant (ϵ'); tan $\delta = \epsilon''/\epsilon'$. While the dielectric constant, ϵ', represents the measure of the ability ***to obstruct*** MW energy as it passes though the sample, the loss factor, ϵ'', indicates amount of the input power ***lost (absorbed)*** by the sample by being dissipated as heat. The higher the loss factor of a substance is, the better the substance can be heated in a MW field. The rest of the energy, which is not absorbed, penetrates to deeper sections of the sample (Kingston and Jassie, 1988). Depending on their MW radiation absorption behavior, materials can be separated into three categories.

- *absorbers*, such as water (ϵ'' = 12 at 25°C), aqueous substances (practically all foodstuffs)
- *transparents*, such as porcelain quartz glass (ϵ'' = 0.0023), teflon
- *reflectors*, such as metal, graphite

Penetration is considered infinite in materials that are transparent and it is considered zero in reflective materials, such as metals. A useful way to characterize penetration is by the half-power depth ($P_0/2$) for a given sample at a given frequency. The half power depth is that distance from the surface of the sample at which power density is reduced to one-half that at the surface (Kingston and Jassie, 1988). Figure 2-6 illustrates the relationship between input MW frequency and penetration depth. The half power depth for water at 25°C is about 10.16 cm (4 in.) for 915 MHz and about 2.54 cm (1 in.) for 2450 MHz.

Lambert's law, which uses an attenuation factor (α), is also used to describe penetration phenomena by equation (2-1):

$$P_Z = P_0 e^{-2\alpha Z} \tag{2-1}$$

where P_Z is power at depth Z, P_0 is power at the surface

According to Lambert's law, penetration depth (Z in cm) is the depth from the surface of the sample at which 1/e of the power (P_0 in Watts) at the surface is not absorbed. If the dielectric properties (such as; tan δ, ε', dimensionless) of a sample at a certain temperature are known, Z can be calculated by using wavelength (λ in cm) at a certain frequency by the equation (2-2):

$$Z = \frac{1}{\alpha} = \frac{\lambda}{2\pi}\left[\frac{2}{\varepsilon'\left[\left[1+\tan^2\delta\right]^{0.5}-1\right]}\right]^{0.5} \tag{2-2}$$

Table 2-2 illustrates approximate penetration depths in a semisolid at high, intermediate, and low moisture contents for arbitrary values of dielectric constant and loss tangent.

Table 2-2 Effect of moisture content of semisolids on penetration (adapted from Decareau, 1985)[a].

			Penetration Depth (cm)	
Moisture	**ε'**	**tan δ**	**915 MHz**	**2450 MHz**
High	60	0.25	8.4	3.1
Intermediate	20	0.20	11.7	4.4
Low	10	0.15	22.1	8.2

[a]ε'= dielectric constant of the sample; tan δ = dissipation factor of the sample.

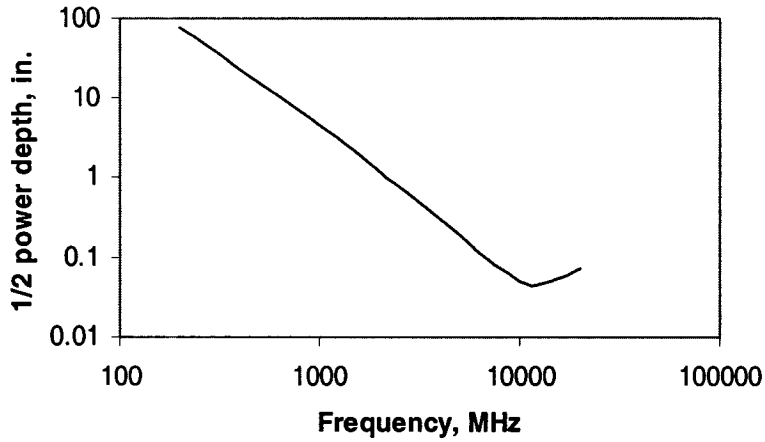


Figure 2-6 Variation of penetration with MW frequency for water at 25°C (adapted from Kingston and Jassie, 1988).

2.2.3 Microwave Instrumentation

The typical MW instrument used for heating has six major parts (shown in Figure 2-7): MW generator (magnetron), wave guide, MW cavity, mode stirrer, a circulator and a turntable. MW energy is produced by the magnetron, propagated by the wave guide and injected into the MW cavity where the mode stirrer distributes the incoming energy in different directions. The cuboid cooking chamber has metallic walls, which act as a Faraday cage. MWs are effectively reflected by the metallic walls and form standing waves. The glass front door and the light bulb cavity are covered by metal grids (Vollmer, 2004). Some percentage of incoming power from the wave guider is absorbed by the sample depending on size (see Figure 2-8) and dielectric properties (ϵ'', ϵ') of the sample (see Figure 2-9) in the MW oven. A sample with a high dissipation factor would absorb nearly 100% of the input power but generally MW samples contain some amount of acids that do not absorb the MW energy at 2450 MHz. This situation causes some MW reflection and mismatch between the magnetron and MW cavity and can damage the

magnetron because of excessive heating. A device called a terminal circulator, which uses ferrites and static magnetic fields, was developed to divert the reflected waves into a dummy load (Figure 2-7) where the reflected energy is safely dissipated (Kingston and Jassie, 1988).

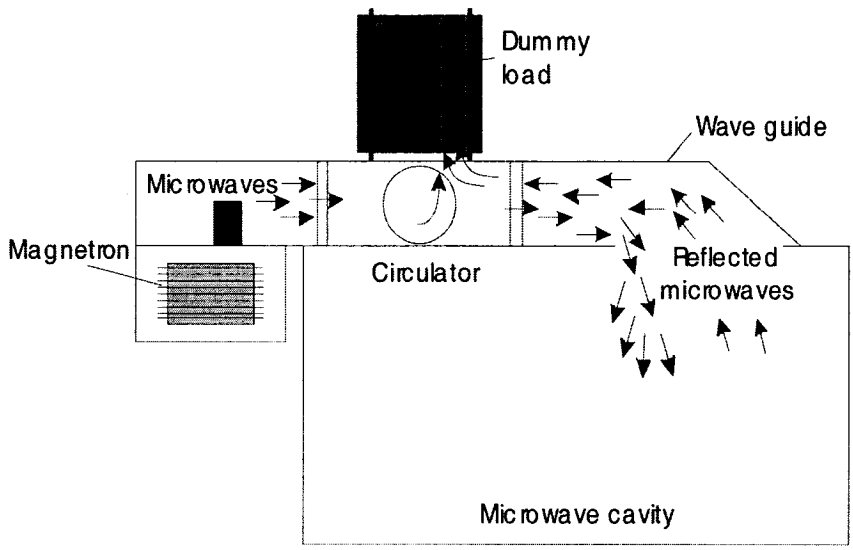


Figure 2-7 Schematic of the MW Cavity, wave guide and magnetron (adapted from Kingston and Jassie, 1988).

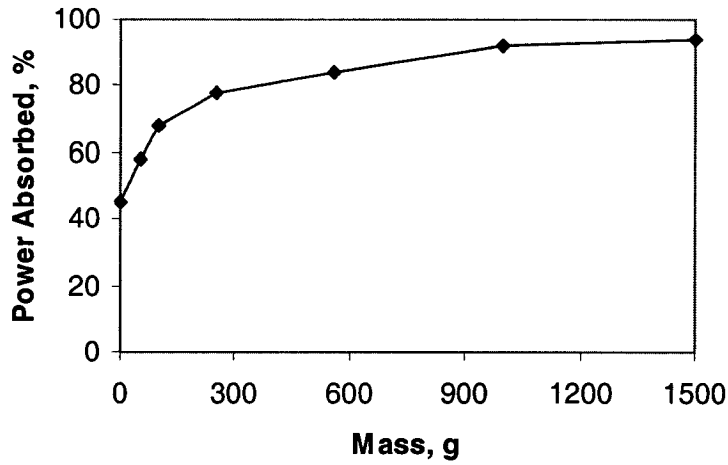


Figure 2-8 Percent MW power absorbed by water (adapted from Kingston and Jassie, 1988).

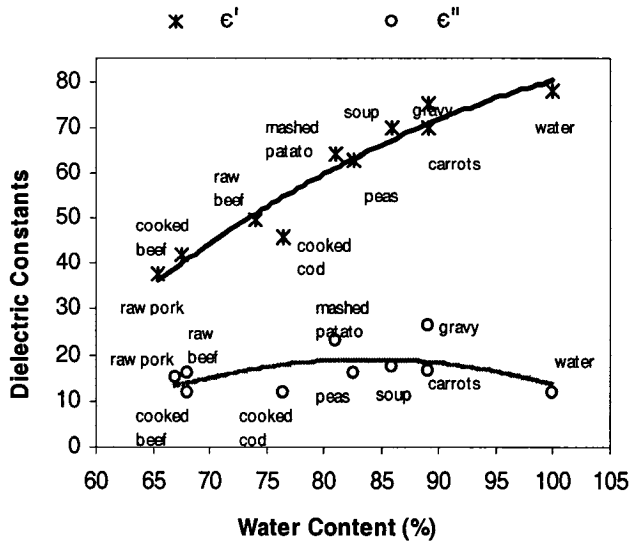


Figure 2-9 Dielectric Constants of Various Kinds of Food (ϵ' = dielectric constant; ϵ'' = loss factor) (adapted from Vollmer, 2004).

The MW heating technique is totally different from CH and results in dramatic reduction of reaction time. For example, typical time required to complete a thermal digestion by CH is 1-2 h while it can be completed in a MW oven in 5-15 min. In CH, heat transfers from the heating device to the medium; therefore, heating performance depends on thermal conductivity, temperature difference across the material and convection currents (Plazl *et al.*, 1995). Since vessels used for CH are poor conductors of heat, it takes a longer time to heat the vessel and transfer heat to the solution. Besides that, due to the vaporization at the surface of the liquid, a thermal gradient is developed by convection currents, and only a portion of the fluid is at the temperature of the heat applied to the outside of the vessel (Figure 2-10). On the other hand, in a MW oven, heating of the entire sample happens simultaneously and the solution reaches its boiling point very rapidly (Kingston and Jassie, 1988). Because of this fast heating process, localized superheated regions (hot spots, Figure 2-10) can be observed in a MW system.

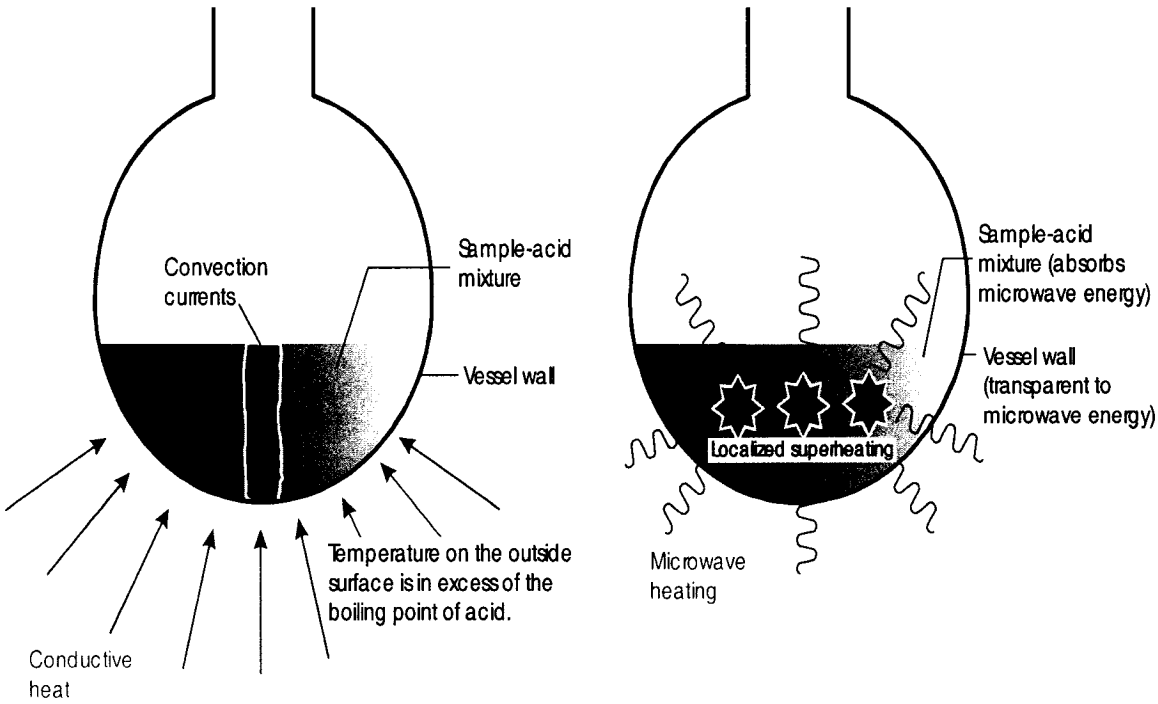
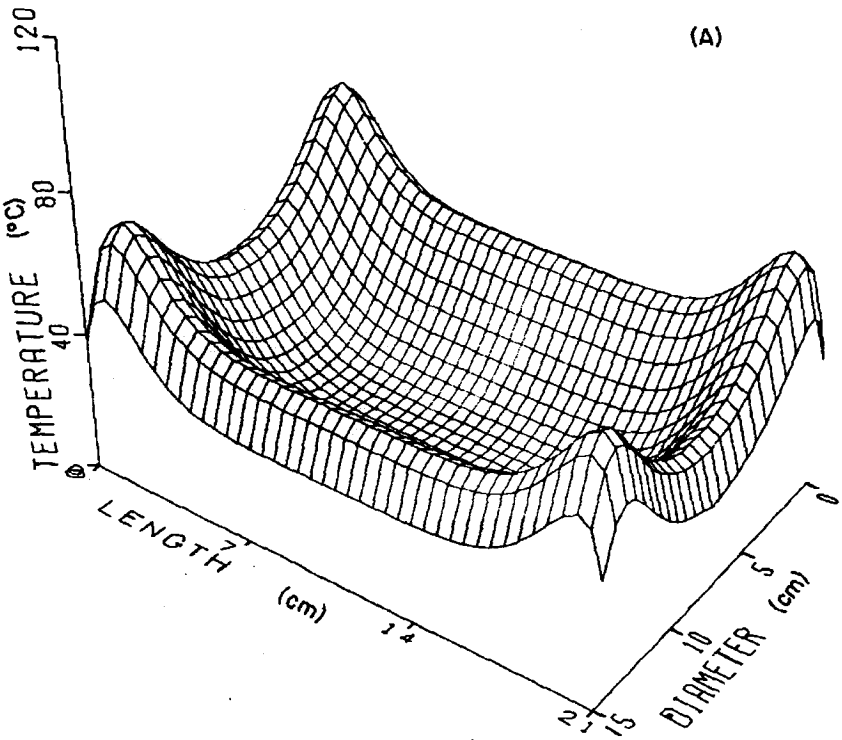
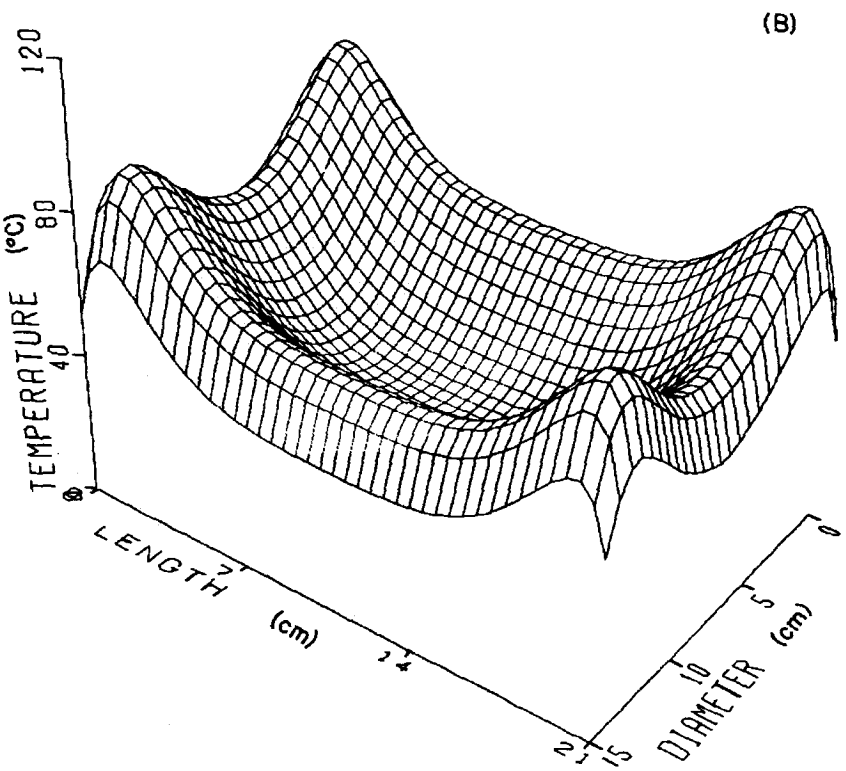


Figure 2-10 Schematic of sample heated by CH (on the left) and by MW technology (on the right) (adapted from Kingston and Jassie, 1988).

Observation of time-temperature profiles within a sample during MW heating (Figure 2-11) is fundamental, since it gives important information on how a sample behaves at certain frequencies and durations. However, conventional temperature sensors (thermistors or thermocouples) cannot provide accurate temperature measurements, since they interact with the magnetic field (act as antennae and cause local heating within the sample) component. Recently developed fiber optic temperature probes can be used inside MWs; since they are not affected by the field but such measurements are very expensive (Decareau, 1985; Kingston and Jassie, 1988; Lorenz, 1999; Loupy, 2002). In a homogenous sample, the temperature measurement with an infrared radiation (IR) pyrometer is also possible, but an IR pyrometer measures the temperature of the sample surface rather than temperature inside of the sample. Loupy (2002) used thermocouples at the end of the microwaving applications and observed that temperature inside graphite

powder samples was 20 to 50°C higher than indicated by the IR pyrometer. As a practical solution, time-temperature profiles can be measured by conventional sensors after the microwaving time is over by inserting probes at various positions within the products, since the response time of the thermocouples are relatively short compared to the time constant for conventional heat transfer (Decareau, 1985).


(A)
TEMPERATURE (°C)
120
80
40
0
LENGTH (cm)
7
14
21
DIAMETER (cm)
0
5
10
15


(B)
TEMPERATURE (°C)
120
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LENGTH (cm)
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21
DIAMETER (cm)
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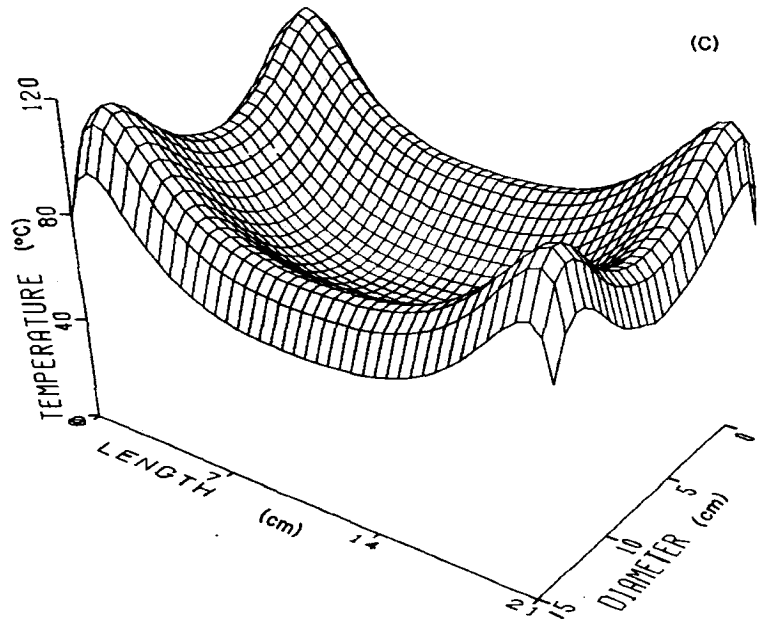

Figure 2-11 Computer – generated temperature pattern across the diameter of a cylinder beef roast cooked at 300 W of MW power at 2450 MHz after (A) 30 min, (B) 60 min, and (C) 90 min (adapted from Decareau, 1985).

2.2.4 Industrial Microwave Applications

Nowadays, MW technology is being used in many different areas, such as, the food industry (baking, thawing, tempering, pasteurization and sterilization), ceramic manufacturing, pulp drying, sludge dewatering, carbon reactivation, solvent waste management, incineration, biomedical waste and sterilizing wet organic waste. Continuous MW application equipments in food industry generally contain metal band conveyor belts for food transfer (Decareau, 1985). In the sterilization process, microorganisms are destroyed at 100°C for 30 min and denaturation of enzymes and structural proteins is observed. Analysis of protein digestion by using MW technology has shown that the time needed for a conventional acid digestion of peptide bonds could be reduced from 18-24 to 2 h when MWs are applied (Kroll *et al.*, 1998). On the other hand, Plazl *et al.* (1995) compared the reaction kinetics of hydrolysis of sucrose to

fructose and glucose under strong catalysis of acidic caution exchange resin by conventional and MW heating (2450 MHz kitchen type MW oven) and they observed no difference in terms of the reaction rate.

2.2.5 Microwave Pretreatment Studies on WAS

Initial MW pretreatment studies have been conducted at temperatures less than 100°C and focused on understanding basic phenomena occurring during MW irradiation of municipal sludge, such as MW penetration depths, temperature depth profiles and death of coliforms in order to produce Class A sludge. Using MW irradiation, fecal coliforms were not detectable in PS and WAS pretreated to 65 and 85°C, respectively (Hong, 2002). Solubilization of WAS due to MW irradiation have also been reported and SCOD/TCOD ratios of WAS increased from 0.08 (control) to 0.18 after MW to 70°C (Hong, 2002). SCOD/TCOD ratios of 0.19 and 0.21 were also reported for WAS MW-irradiated to 91°C and boiling temperatures, respectively (Park *et al.*, 2004).

The publications on the effects of MW irradiation on anaerobic digestion of WAS are very limited. Park *et al.* (2004) studied semi-continuous (SC) anaerobic digesters using pretreated WAS microwaved to 91°C at 8, 10, 12 and 15 d SRTs and reported 64 and 31% higher COD removal and methane production, respectively, compared to controls at SRT of 15 d. Hong (2002) also studied performance of SC digesters at SRTs of 20, 15, 10 and 5 d after microwaving sludge; however, results related to biogas production, VS/TS, and TCOD treatment efficiencies were not reported since their study focused on fecal coliform destruction in continuous flow digesters (Hong *et al.*, 2004).

2.2.6 Microwave Absorbing Materials

Materials, that absorb MW radiation, contain dipoles. When MWs are applied to a material, these dipoles align and flip around. The simplest MW absorber is water. Many electrically insulating materials, such as oxides, are electrically transparent to MWs at room temperature, causing inefficient operation. However, if powders of these materials can be mixed with polar liquids or electroconductive particles of Fe_3O_4 (iron oxide or magnetite), MnO_2 (manganese dioxide), NiO, calcium aluminates, they can be effectively heated (National Research Council, 1994). Magnetite is the most common mineral, which is known to be a hyperactive MW absorber and therefore used as thermal seed. Studies done by the Ministry of Northern Development and Mines (1990) indicated that 35 g of magnetite sample interacted strongly with a MW field and the sample reached about 730°C in two minutes. Table 2-3 shows the results of MW heating studies on other rocks and minerals. Another study by Uslu and Atalay (2003) reported an enhancement on reduction of sulfur content of coal by addition of magnetite. Initially, coal was subjected to a magnetic field in a MW oven (850 W and 2450 MHz) but the magnetic field could not significantly reduce the sulfur content (only 22.3% removal). After addition of 5% by weight magnetite, sulfur content of coal was reduced by 55.1%. MW absorbers were also tested in a pyrolysis study on sewage sludge in a quartz reactor, which in turn was placed inside a multimode resonant MW cavity (frequency of 2450 MHz). Researchers found that if only the raw sludge is treated in the MW, only drying takes place. On the other hand, if sludge is mixed with a small amount of a suitable MW absorber (such as char produced from pyrolysis itself), temperatures up to 900°C can be achieved (Menendez *et al.*, 2002). Other types of MW absorbers, such as fine powders of ferrite dispersed in a

polymer, have also been studied and absorption kinetics could be modeled. From these studies, two important questions arise. Do MW absorbers work as efficiently in a solution of WAS or TWAS as they work in solid samples (such as; coal)? Is it possible to remove and recycle these materials after MW treatment before or after anaerobic digestion? Extensive research is necessary on MW pretreatment of WAS in order to answer these questions.

Table 2-3 MW heating rates (at 2450 MHz) on materials (adapted from Ministry of Northern Development and Mines, 1990).

Material Classification	Heating Rate Reported	Maximum Temperature	Notes
a)Hyperactive Materials	**(~°C/sec)**	**(°C)**	
UO_2	200	1100	Go black with high thermal conductivities
MoS_2	150	900	
C (Charcoal)	100	1000	
Fe_3O_4	20	500/1000	Heating efficiency depends on Fe_3O_4/Fe_2O_3 ratios
FeS_2	20	500	
CuCI	20	450	
MnO_2	-	-	Probably > 20°C/sec
b)Active Materials	**(~°C/min)**	**(°C)**	
Ni_2O_3	200	1300	Violent
Co_2O_3	150	900	Violent
CuO	100	800	
Fe_2O_3	20	1000	
FeS	20	800	
CuS	20	600	
b)Difficult to Heat Materials	**(~°C/min)**	**(°C)**	
Al_2O_3	80	1900	
PbO	70	900	
MgO	33	1300	
ZnO	25	1100	
MoO_3	15	750	
d)Inactive Materials	**(~°C/min)**	**(°C)**	
CaO	5	200	
$CaCO_3$	5	130	
SiO_2	2-5	70	

2.3 Conclusions from the Literature Survey

The conclusions from the literature survey related to existing sludge pretreatment methods are summarized in Table 2-4.

Table 2-4 Summary on existing pretreatment techniques.

Improvements → / Method ↓	VS Destruction	Dewaterability	Pathogen Destruction	Energy Saving	Inhibition Observed
Mechanical Disintegration	(+)	(-)	(-)	(-)	[a]n/a
Ultrasound	(+)	(+)	(-)	(+)	n/a
Thermal Hydrolysis	(+)	(+)	(+)	(-)	n/a
Chemical + Mechanical	(+)	(-)	n/a	(-)	(+)
MW Treatment	(?)	(?)	(+)	(+)	(?)

[a]n/a = not available.

MW technology has not been fully tested as a pretreatment for anaerobic sludge digestion. Advantages of MW technology over CH give it great potential for waste sludge applications. This report proposes MW technology as an energy efficient alternative to existing pretreatment techniques for enhancing anaerobic sludge digestion.

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CHAPTER 3

Enhancement of Batch Waste Activated Sludge Digestion by Microwave Pretreatment

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3.1 Abstract

Batch anaerobic digesters were used to stabilize microwave (MW)-irradiated waste activated sludge (WAS) at mesophilic temperature. A low temperature range (50-96°C) MW irradiation was applied by a household type MW oven at a frequency of 2450 MHz. Effects of four different variables [pretreatment temperature (T) and intensity (I), sludge concentration (C) and percentage of sludge pretreated (PT)] were investigated in a multilevel factorial type of statistical design containing 54 mesophilic batch reactors, including controls (no pretreatment) and duplicates by monitoring cumulative biogas production (CBP). A multifactor analysis of variance (ANOVA) determined that the most important factors affecting WAS solubilization were T, I, and C while improvements in biogas production from WAS were significantly affected by PT, T, and C at the 95% confidence level, respectively. MW pretreatment of WAS samples resulted in 3.6 ± 0.6 and 3.2 ± 0.1 fold increases in soluble chemical oxygen demand (SCOD)/total chemical oxygen demand (TCOD) ratios at high and low sludge concentrations, respectively. Solubilization was always slightly higher at 50% than at 100% MW intensities for both high and low sludge concentrations at the same MW temperatures due to longer exposure time to the MW field at low MW intensities. WAS, pretreated by microwaving to 96°C, produced the greatest improvement in biogas production with 15 ± 0.5% and 20 ± 0.3% increases over the controls after 19 d of digestion at low and high WAS concentrations. Dewaterability of microwaved sludge was also enhanced after anaerobic digestion compared to controls.

KEYWORDS: Anaerobic sludge digestion, microwave, pretreatment, solubilization.

3.2 Introduction

Municipal wastewater treatment plants (MWTPs) generate large amounts of primary and secondary sludges that are high in organic content. Sludge management has become a key factor in wastewater management during the last two decades. Anaerobic sludge digestion is often applied to waste sludge to reduce the mass of solids for disposal, to reduce the pathogen content and to generate biogas for energy recovery. However, secondary sludge consists of cellular material (microbial cells) that are resistant to direct anaerobic degradation, since cell walls are physical and chemical barriers against exoenzyme degradation (Baier and Schmidheiny, 1997). Besides the microbial cells, exocellular polymeric substances (EPS) comprise a major organic fraction in activated sludge floc structure and binding mechanisms of EPS to cations appeared to be a significant factor determining the dewaterability and digestibility of activated sludge. Several studies have suggested that divalent cations can bind to negative sites on EPS (such as polysaccharides and proteins) which increase the floc size, floc strength and dewaterability (Eriksson and Alm, 1991; Andreadakis, 1993; Park *et al.*, 2003). More specifically, polysaccharides were found to be associated with lectin-like compounds and bind to divalent cations (such as calcium and magnesium), while proteins contain two fractions, one binding to iron and possibly aluminum and the other associated with divalent cations and polysaccharides (Novak *et al.*, 2003). Depending on the ratio of divalent/monovalent cations present, bound proteins with divalent cations improve settling and dewaterability, while sludges with high monovalent cations can be difficult to settle and dewater (Higgins and Novak, 1997). The link between the type of cations, biopolymers and sludge digestibility was also examined. Studies suggested that anaerobic

digestion would yield the best solid destruction for sludges with high iron content, and aerobic digestion can release high magnitude of divalent cations required for floc integrity (Novak *et al.*, 2003). As a result of these factors, hydrolysis becomes the rate-limiting step and degree of degradation achieved is limited to 30-35% COD reduction in conventional anaerobic sludge treatment (Parkin and Oven, 1986).

Improvement of biodegradability via anaerobic digestion depends on enhanced disintegration of the sludge floc structure and increasing accessibility to the extracellular and intracellular components. There are many methods that have been studied and shown to be effective, such as; mechanical disintegration by ball milling (Baier and Schmidheiny, 1997; Muller *et al.*, 1998); a rotor-stator shearing device (Muller *et al.*, 2003; Cartmell *et al.*, 2004), special thickening centrifuge (Dohanyos *et al.*, 1997a), high pressure homogenizer (Muller *et al.*, 1998), and ultrasound (Brown *et al.*, 2003; Gonze *et al.*, 2003), thermal disintegration by freezing and thawing of biomass (Dohanyos *et al.*,1997b; Wang *et al.*, 1999) or thermal hydrolysis (Abraham and Kepp, 2003; Jolis *et al.*, 2004; Skiadas *et al.*, 2004) and chemical disintegration by acid or caustic addition and combinations such as chemical disintegration followed by mechanical pretreatment such as; high pressure homogenizer (Stephenson *et al.*, 2003) or ultrasound (Chiu *et al.*, 1997; Chu *et al.*, 2002). Studies on WAS have indicated that all the pretreatment methods above are likely to improve solids destruction. However, mechanical pretreatment methods caused deterioration in WAS dewaterability resulting in increased polymer demand for sludge dewatering after anaerobic digestion and there was no effect on pathogen reduction based on total and fecal coliforms (Muller *et al.* 1998; Muller *et al.*, 2003). Ultrasound studies also indicated that sonication did not affect the fecal coliform

concentration significantly after digestion (Lafitte-Trouque and Forster, 2002) and digested sludge only qualified as "Class B" sludge according to US regulations (Chu *et al.*, 2001). Thermal disintegration methods with high temperatures (160-170°C) and pressures (600-800 kPa) appear to be superior to other methods with a surplus energy gain due to higher biogas production compared to control reactors. Because of the high temperatures reached during pretreatment, pathogen removal efficiency was also higher (Barnard *et al.*, 2002; Abraham and Kepp, 2003).

MW technology is an attractive alternative heating method to conventional heating (CH) due to its environmental and energy conservation properties (Decareau, 1985; Kingston and Jassie, 1988). So far, publications regarding the effect of MW technology on anaerobic sludge digestion are limited (Park *et al.*, 2004) and mostly focused on fecal coliform destruction rather than treatibility analyses (Hong *et al.*, 2004). The purpose of this research is to investigate the effect of MW dose on anaerobic treatment efficiency of WAS in comparison to existing pretreatment techniques summarized above.

3.3 Background Information on Microwaves

MWs are electromagnetic energy with frequencies between 30 GHz and 300 MHz and wavelengths of 1 cm and 1 m, respectively (Vollmer, 2004). To avoid interference with telecommunications and cellular phone frequencies, heating applications must use ISM (Industrial Scientific and Medical) frequency bands, which are 27.12, 915, and 2450 MHz with wavelengths of 11.05 m, 37.24 cm, and 12.24 cm, respectively (Kingston and Jassie, 1988). The frequency of 2450 MHz (maximum output achievable is 15 kW) can be freely used in industrial applications without requiring any permission. Frequencies

around 900 MHz (in US, 915 MHz and in UK, 896 MHz), which can produce up to 100 kW, are used for larger plants. Cellular telephone services also use this band (872-960 MHz), and very strict safety requirements exist for radiation from industrial equipment operating at 900 MHz. Lower frequencies, such as 915 MHz, provide longer wavelengths (37.24 cm) for applications where deeper penetration into material is necessary. However, if the MW is used for heating or drying a product in thin layers with a large surface area, shorter wavelengths (2450 MHz-12.24 cm) could be adequate.

MW heating is totally different from CH and results in dramatic reduction of reaction time and energy requirements. In CH, heat transfers from the heating device to the medium; therefore, heating performance depends on thermal conductivity, temperature difference across the material and convection currents (Plazl *et al.*, 1995). On the other hand, in a MW oven, heating of the entire sample happens simultaneously and the solution reaches its boiling point very rapidly. Because of this rapid heating process, localized superheated regions (hot spots) can be observed in the matrix heated in the MW system (Kingston and Jassie, 1988). These hot spots are the reason for non-homogeneous temperature profiles observed in most of the MW heating applications.

MW heating results from interactions of the chemical constituents of the substrate with the electromagnetic field. Molecular friction, primarily because of the disruption of weak hydrogen bonds associated with the dipole rotation of free water molecules and migration of the free salts in an electrical field is an important result of these interactions (Decareau, 1985). It is believed that dipole rotation or orientation (athermic) and subsequent heating (thermic) effects of MW break apart the weak hydrogen bonds and

make complex organic molecules unfold, smaller, all of which contribute to making them more biodegradable. To be able to prove this hypothesis, this study was focused on analyzing the effects of MW irradiation on both solubilization and anaerobic digestion of WAS by monitoring biogas productions from mesophilic batch sludge digesters.

3.4 Materials and Methods

Thickened WAS (TWAS) used in the study was obtained from the thickener centrifuge with total solid (TS) concentration of 5.4% (w/w) at the *Robert O. Pickard Environmental Center (ROPEC)* located in Gloucester (ON, Canada). ROPEC has preliminary and primary treatment followed by a conventional aerobic activated sludge unit operated at an average sludge retention time (SRT) of 5 d. Ferric chloride is added to WAS for P removal prior to WAS thickening. TWAS and primary sludge (PS) are blended in a 58:42 v/v ratio and undergo mesophilic anaerobic sludge digestion to produce a stabilized biosolids product for disposal. MW irradiation was applied by a 0.045 m^3 capacity household type MW oven [Panasonic NNS53W + inverter, 1250 W, 2450 MHz frequency and 12.24 cm wavelength].

3.4.1 Experimental Design

Previous studies on other types of pretreatment techniques such as, thermal treatment, mechanical treatment and ultrasound, indicated that temperature (Wang *et al.* 1999; Barnard *et al.*, 2002) and intensity of the pretreatment method (Tiehm *et al.*, 2001; Muller *et al.*, 2003), sludge concentration, and percentage of sludge pretreated (Barber, 2002) affect efficiency of the pretreatment. Therefore, in this project, the effects of these variables were investigated on:

(1) Disintegration and hydrolysis of WAS.

(2) Anaerobic digestion of WAS.

A multilevel factorial design, as shown in Figures 3-1A and B and Table 3-1 was applied. The main advantages of *multilevel designs* are the flexibility to test different variables at different levels (Montgomery, 1983). A study on ultrasound pretreatment showed that biogas production was the highest when only 60% of WAS was subjected to ultrasound (Barber, 2002). This interesting result on partial treatment (PT) was the starting point of this statistical design and other variables, such as; pretreatment temperature (T), WAS concentration (C), and MW intensity (I) were nested under the partial treatment (PT) variable. Studies on other pretreatment methods indicated that efficiency of the pretreatment increases with TS concentration of WAS (Barber, 2002). For samples with high TS content, this result is not unexpected since the power supplied by the equipment, whether it is an external heating source or an ultrasound generator, will be used by the solids rather than the water content of the sludge samples. However, in a MW pretreatment study, water in WAS samples would be the main polar element exposed and affected by MWs since MWs make dipoles rotate and line up rapidly with alternating electric field resulting in a change in tertiary or secondary structure of proteins (Kingston and Jassie, 1988; Banik *et al.*, 2003).

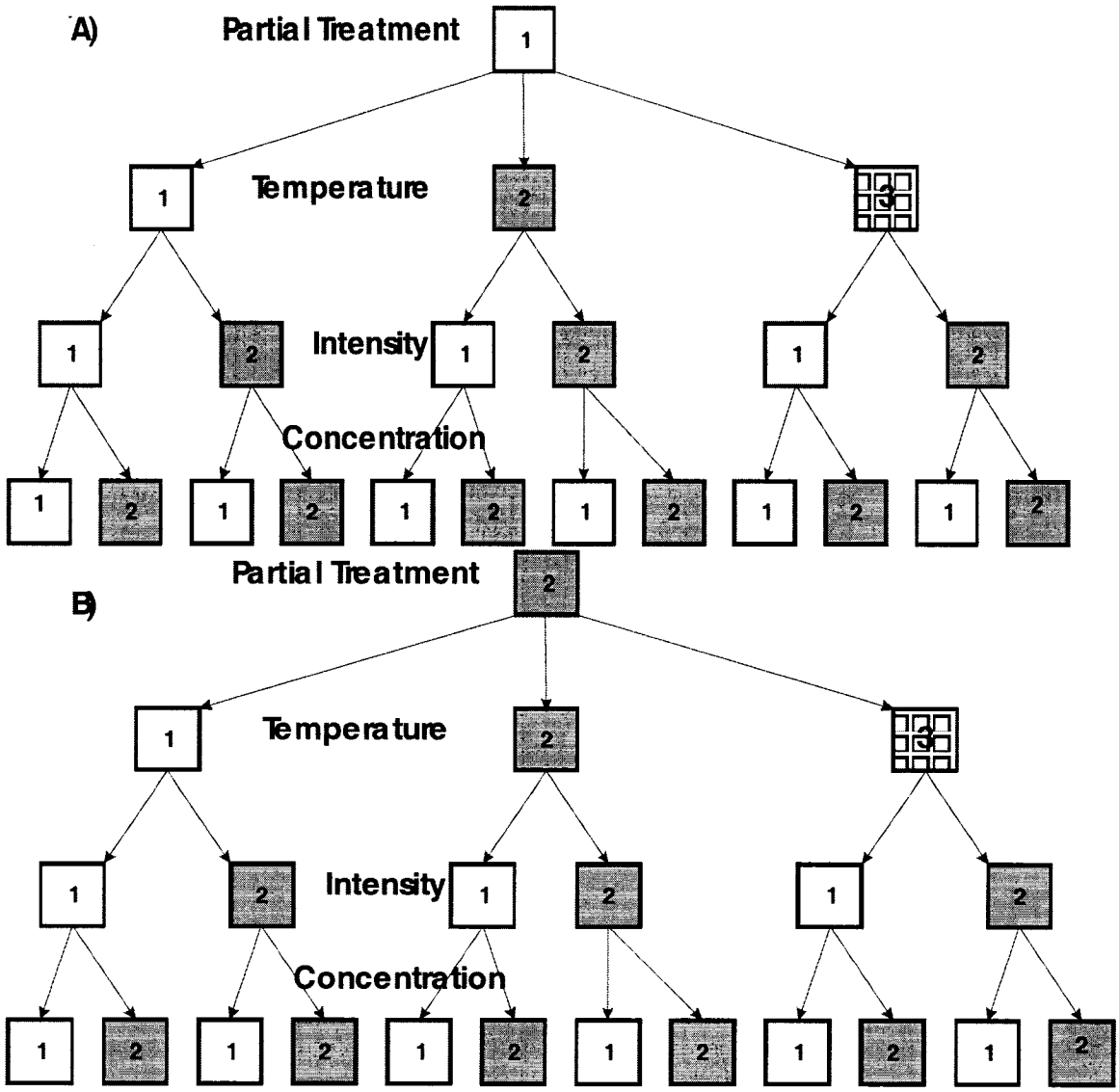


Figure 3-1 Multilevel factorial design for A) partial treatment of 50%; B) partial treatment of 100% (1, 2 and 3 indicate the level of the variables).

As seen in Table 3-1, sludge concentration variable was studied at two different levels for the pretreatment step. While low-level concentration [1.4% TS (w/w)] represents a typical sludge concentration after secondary sedimentation, high-level concentration [5.4% TS (w/w)] can be considered as the highest TS achievable after thickening before dewatering in a typical municipal treatment plant. In a full-scale treatment plant, pretreatment will mostly likely be applied to only WAS line since

hydrolysis is not a rate limiting step for the PS. After pretreatment of secondary sludge, both waste sludge streams will be mixed prior to digestion which will lead to dilution of the TWAS to a typical digester concentration around 3% TS (w/w). Therefore, in this study, the inoculum was acclimatized to 3% TS (w/w) and biochemical methane potential (BMP) tests were run for WAS with maximum 3% TS (w/w) concentrations. Due to lower energy consumption at low pretreatment temperatures, this study focused on a temperature range of 50-96°C, which was achievable with a kitchen type MW oven and a plastic MW transparent container designed for food applications. If the boiling point of water was to be exceeded, an industrial type of MW and a properly sealed vessel designed for high temperatures and pressures would be required.

Table 3-1 Variables and levels in the multilevel factorial design[a].

Variables	PT (%)	T (°C)	I (%)	C [% TS (w/w)]
Levels				
1	50[l]	50	50[n]	1.4
2	100	75	100	5.4
3	n/a	96	n/a	n/a

[a]PT = partial treatment, T = temperature, I = intensity, C = sludge concentration, n/a = not available.
[l]50% of total volume of sludge was being microwaved and the other 50% was non-pretreated.
[n]50% of maximum MW power (1250 W) was used.

3.4.2 Microwave Calibration

Different samples absorb different quantities of MW energy depending on their concentration and characteristics as well as total mass present. Therefore, MW calibration curves were prepared for two different sludge concentrations [1.4 and 5.4% TS (w/w)] at two different MW intensities (50 and 100%) to reach desired pretreatment temperatures (50, 75 and 96°C). For each pretreatment, 500 g of WAS sample at room temperature ($T_{initial}$ = 20 ± 1°C) was transferred to a 1 L (19 x 12 x 4.4 cm) plastic MW resistant

container and subsequently exposed to a MW field for different durations of times while using the turning table in the MW oven. The container was covered with a plastic lid to prevent evaporation during the irradiation. Due to problems related to monitoring temperature profiles inside the MW oven, such as interaction with the MW field causing local heating within the sample (Lorenz 1999; Loupy, 2002), temperature measurements were done outside the MW oven as soon as microwaving was terminated. After the container was removed, the sample was vigorously stirred and the maximum temperature was recorded within 10 s. Temperature readings were taken with fast response insulated thermo-couple probes [Cole-Parmer (P-08506-75), T-type, fine-gauge Teflon PTFE response time of 0.5 s, Labcor Technical Sales Inc., ON, Canada (Appendix A.1, Figure A-1)] connected to a module for analog-to-digital conversion and recorded by a laboratory computer system [LabVIEW Software Version 6, National Instruments Co., Austin, TX, USA (Appendix A.1, Figure A-2)]. Experimentally prepared calibration curves and derived models were used to pretreat WAS for the biodegradability assays. Figures 3-2a, b display the MW calibration curves for WAS with concentration of 5.4% TS (w/w) under and at boiling points, respectively.

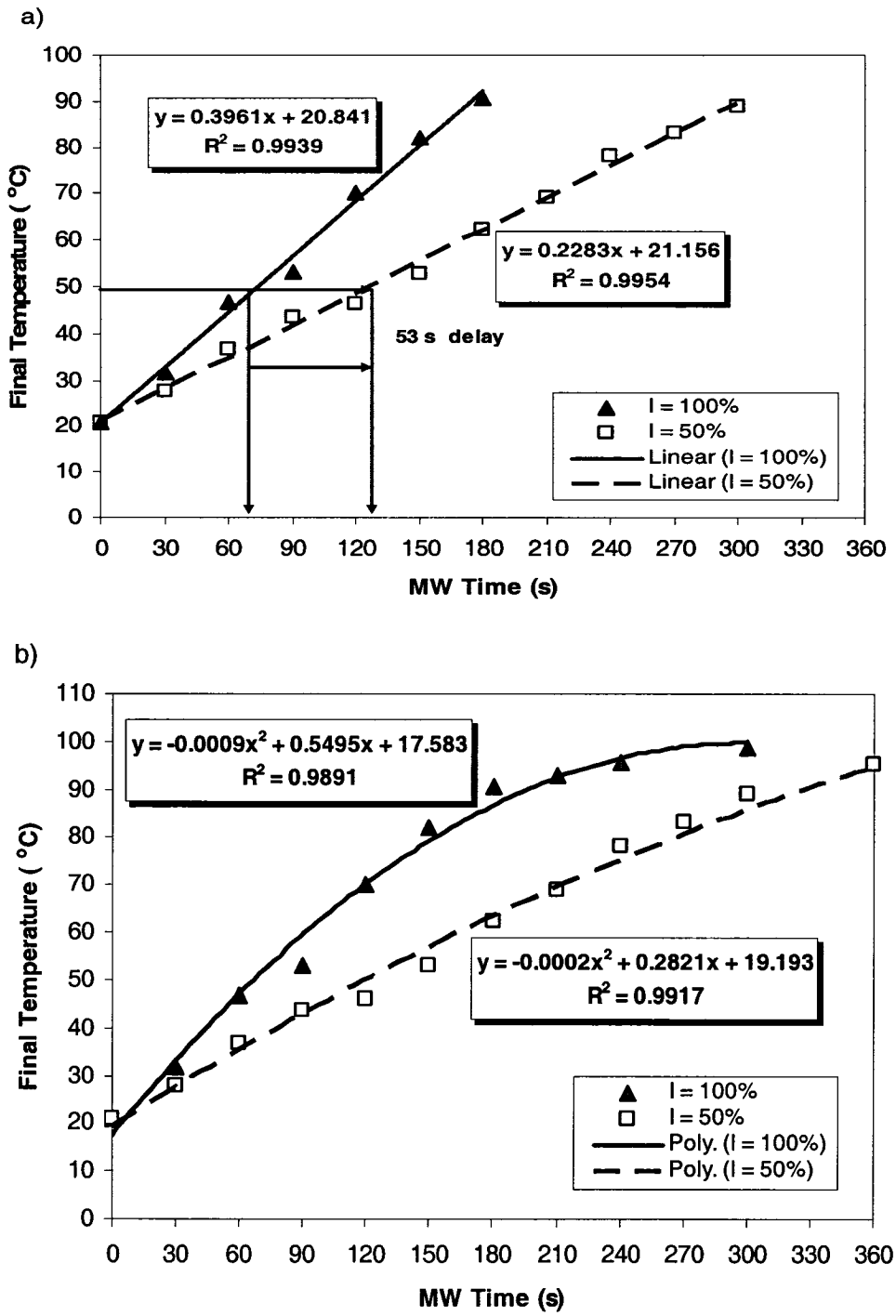


Figure 3-2 Microwave calibration curves for WAS (5.4% TS) a) under boiling point; b) at the boiling point (MW: microwave; I: intensity).

In Figure 3-2a, only temperature measurements up to 90°C were considered. In this range, it was clear that there was a linear relationship between MW time and temperature increase of WAS at both MW intensities. Due to the lower amount of power applied at low intensities, 50% MW intensity resulted in a longer exposure time for the same sludge sample to reach the same temperature as a sample microwaved at a 100% MW intensity. On the other hand, when a larger range (up to 100°C) was covered, the calibration curve was transformed to a second-order function (Figure 3-2b). The logical explanation for this result is that as temperature values closer to the boiling point were reached, some of the heat energy added to the system was used to change state (from liquid to gas) instead of raising the temperature of the system. The calibration curve results for WAS with 1.4% TS (w/w) (Appendix B, Figure B-1) were quite similar to results presented for 5.4% TS (w/w).

3.4.3 Biochemical Methane Potential (BMP) Test

The statistical design explained above resulted in 54 batch reactors, including controls and duplicates. 500 mL glass bottles [Wheaton (219919) bottles with polypropylene caps (240746), screw cap size of 45 mm, VWR, Montreal, QC, Canada] with butyl rubber stoppers [Wheaton (22400-503) lyophilization stopper, screw cap size of 43 mm, VWR, Montreal, QC, Canada (Appendix A.2, Figure A-3)] were used for the BMP test. Acclimated inoculum (70 mL) was transferred into the bottles and then pretreated WAS samples (280 mL) were added according to Figures 3-1A and B. Nitrogen sparging was applied to batch reactors when WAS and inoculum were mixed to prevent exposure to air and reactors were sealed after addition of an equal mixture of

$NaHCO_3$ and $KHCO_3$ to achieve an alkalinity of 4000 mg/L as $CaCO_3$. Batch reactors were kept in a temperature controlled incubator shaker [PhycroTherm, New Brunswick Scientific Co. Inc., NB, Canada (Appendix A.1, Figure A-4)] at 33 ± 1°C and mixed at 90 rpm to keep the bacteria - sludge mixture in suspension.

After the target pretreatment temperatures were reached, samples were removed from the heat source and cooled down to room temperature in sealed plastic containers. All analysis was done on control and pretreated samples at room temperature. TS, volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), pH, alkalinity, TCOD, SCOD and ammonia-N analysis were done on raw, pretreated and digested WAS and inoculum (both acclimatized and un-acclimatized) samples. TS and VS were determined based on Standard Methods procedure 2540G (APHA, 1995). For TSS and VSS analysis, it was impossible to filter TWAS samples with 5.4% TS, therefore centrifugation [for 20 min at 5856 relative centrifugal force (RCF) in a Dupont instruments Sorvall SS-3 automatic centrifuge] was used to separate suspended solids from dissolved solids. Dissolved ammonia concentrations were measured in the supernatants of sludge obtained by centrifuging the sludge samples under similar conditions. 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 procedure 5250D (APHA, 1995) with a Coleman Perkin-Elmer spectrophotometer Model 295 at 600 nm light absorbance. Before SCOD determination, sludge samples were centrifuged (20 min at 5856 RCF) and filtered through GN-6

Metricel S-Pack membrane disc filters with 0.45 μm pore size. Biogas production was measured daily by inserting a needle into the reactors attached to a manometer. Reactor pH, total volatile fatty acids (TVFAs; summation of acetic, propionic and butyric acids) and biogas composition (nitrogen, methane and carbon dioxide percentage) were monitored weekly during anaerobic digestion. TVFAs were measured by injecting supernatants to a HP 5840A GC with glass packed column (Chromatographic Specialties Inc., Brockville, ON, Canada, Chromosorb 101, packing mesh size: 80/100, column length x ID: 10 ft x 2.1 mm) and a flame ionization detector (oven, inlet and outlet temperatures: 180, 250 and 350°C, respectively, carrier gas flowrate: 25 mL helium/min) equipped with HP 7672A autosampler. Biogas composition was determined with a HP 5710A GC with metal packed column (Chromatographic Specialties Inc., Brockville, ON, Canada, Porapak T, packing mesh size: 50/80, column length x OD: 10 ft x 0.25 inches) 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).

3.4.4 Inoculum Acclimation for BMP Test

Inoculum for the BMP test was taken from the effluent line of the anaerobic sludge digesters treating a mixture of PS and TWAS [58:42 (v:v)] at ROPEC. In order to eliminate gross underestimation of the BMP of pretreated sludge due to possible lag-phase and/or inhibition at the early stages of testing, inoculum was acclimatized to the harshest MW pretreatment condition prior to the BMP test. For acclimation, one 5 L anaerobic semi-continuous reactor (Appendix A.3, Figure A-5), fed with MW-irradiated sludge (96°C), was run at approximately 20 d SRT by gradually increasing the influent

flowrate over a period of approximately three SRTs (Ekama *et al.*, 1986). Organic loading rate (OLR) of the acclimation reactor was 2.0 ± 0.04 g TCOD/ L. d. MW toxicity effect on secondary sludge was expected to increase with MW temperature (Hong, 2002); therefore 96°C was used to pretreat feed sludge fed to the acclimation reactor. The concentration of feed was 3% TS (w/w), which can be considered as a typical sludge concentration in a full-scale sludge digester. When the inoculum was being acclimatized to the MW-irradiated WAS, daily biogas production, biogas composition and VFA readings reached a stable value and BMP reactors were set-up and used this acclimatized inoculum which had a specific activity of 0.12 ± 0.01 g TCOD/ g VSS. d.

Dewaterability of WAS digested in control and MW pretreated reactors was tested by a Capillary Suction Timer [Model 440, Fann Instrument Company, TX, USA] without polymer addition based on Standard Methods Procedure 2710G (APHA, 1995). The method consists of injecting a sludge sample to a small cylinder placed on a sheet of chromatography paper. While the paper extracts liquid from the sludge by capillary suction, water released from sludge travels between two contact points on the chromatography paper and the travel time or capillary suction time (CST in seconds) is recorded by a digital timer. In this project, sludge temperature and volume were kept constant (22 ± 1°C and 5 mL, respectively) since variations in temperature and sample volume can affect CST results. At the end of experiments, CST values indicated by the timer were divided by TS concentration of sludge samples in order to prevent bias among samples with different solid concentrations.

3.5 Results and Discussions

3.5.1 Effect of Microwaving on Disintegration and Hydrolysis of WAS

Results from analysis on characterization of raw and microwaved WAS samples with 5.4 and 1.4% TS (w/w) are presented in Tables 3-2 and 3-3, respectively. Inoculum characterization before and after acclimation was presented in Table 3-4. In order to avoid bias among control and microwaved samples due to evaporation of water following microwaving, solid and COD analysis results were reported as VS/TS, VSS/TSS and SCOD/TCOD ratios. Original data on ROPEC TWAS characterization was also tabulated in Appendix C, Table C-1. During pretreatment, all sludge samples experienced reduction in VS/TS and VSS/TSS ratios possibly due to volatilization of organics. VS/TSS and VSS/TSS ratios were almost identical at both 50 and 100% MW intensities, when samples were irradiated to the same temperatures.

Table 3-2 Characteristics of untreated and MW-irradiated WAS samples [5.4% TS (w/w)][a].

	Untreated WAS	100% MW-irradiated WAS					
		T = 50°C		T = 75°C		T = 96°C	
Parameter		**I = 50%**	**I = 100%**	**I = 50%**	**I = 100%**	**I = 50%**	**I = 100%**
pH [-]	6.49	6.92	6.81	6.78	6.84	7.31	7.27
VS/TS*100 [%]	69.8 (0.0)†	65.9 (0.1)	65.8 (0.1)	67.8 (0.4)	66.5 (0.0)	68.0 (0.4)	69.8 (1.0)
VSS/TSS*100 [%]	69.4 (0.2)	66.4 (0.1)	65.6 (0.2)	64.8 (0.0)	65.2 (0.2)	66.3 (0.1)	66.9 (0.1)
NH_3-N [mg/L]	536 (8)	1028 (12)	999 (0)	576 (0)	999 (0)	410 (2)	446 (0)
[2]Alkalinity [mg/L]	919 (0)	2583 (41)	2714 (41)	1819 (3)	2614 (3)	1224 (2)	1140 (2)
[3]TVFA [mg/L]	913	2665	2534	1611	2264	1278	1508
		50% MW-irradiated + 50% Untreated WAS					
pH [-]	6.49	6.71	6.65	6.64	6.67	6.90	6.88
VS/TS*100 [%]	69.8 (0.0)	67.9 (0.0)	67.8 (0.1)	68.8 (0.2)	68.2 (0.0)	68.9 (0.2)	69.8 (0.5)
VSS/TSS*100 [%]	69.4 (0.2)	67.9 (0.2)	67.5 (0.0)	67.1 (0.1)	67.3 (0.2)	67.9 (0.1)	68.2 (0.1)
NH_3-N [mg/L]	536 (8)	782 (10)	768 (4)	556 (4)	768 (4)	473 (5)	491 (4)
[2]Alkalinity [mg/L]	919 (0)	1751 (21)	1817 (21)	1369 (1)	1766 (11)	1071 (1)	1029 (1)
[3]TVFA [mg/L]	913	1789	1724	1262	1589	1095	1210

[a]T = temperature; I = intensity; WAS = waste activated sludge; TS = total solids;
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements).
[2] Bicarbonate alkalinity in units of mg/L as calcium carbonate ($CaCO_3$).
[3]TVFA; total volatile fatty acids (summation of acetic, propionic, butyric acids), no duplicate measurements.

Table 3-3 Characteristics of untreated and MW-irradiated WAS samples [1.4% TS (w/w)][a].

	Untreated WAS	100% MW-irradiated WAS					
		T = 50°C		T = 75°C		T = 96°C	
Parameter		**I = 50%**	**I = 100%**	**I = 50%**	**I = 100%**	**I = 50%**	**I = 100%**
pH [-]	6.78	6.78	6.74	6.41	6.91	6.61	6.65
VS/TS*100 [%]	69.5 (0.4)†	67.5 (0.6)	66.5 (0.1)	67.8 (0.2)	67.5 (0.5)	69.5 (0.2)	69.3 (0.3)
VSS/TSS*100 [%]	69.4 (0.2)	67.1 (0.1)	67.3 (0.1)	66.9 (0.0)	64.6 (0.2)	67.6 (0.5)	66.8 (0.2)
NH_3-N [mg/L]	139 (2)	232 (0)	225 (2)	114 (2)	185 (0)	88 (1)	410 (2)
[2]Alkalinity [mg/L]	238 (0)	618 (5)	603 (0)	341 (28)	439 (8)	262 (4)	230 (0)
[3]TVFA [mg/L]	237	0	768	228	379	0	154
		50% MW-irradiated + 50% Untreated WAS					
pH [-]	6.78	6.78	6.76	6.60	6.85	6.69	6.71
VS/TS*100 [%]	69.5 (0.4)†	68.5 (0.1)	68.0 (0.1)	68.6 (0.1)	68.5 (0.0)	69.5 (0.3)	69.4 (0.3)
VSS/TSS*100 [%]	69.4 (0.2)	68.2 (0.1)	68.3 (0.2)	68.1 (0.1)	67.0 (0.0)	68.5 (0.3)	68.1 (0.0)
NH_3-N [mg/L]	139 (2)	186 (1)	182 (0)	126 (2)	162 (1)	114 (1)	275 (2)
[2]Alkalinity [mg/L]	238 (0)	428 (2)	420 (0)	290 (14)	338 (4)	250 (2)	234 (0)
[3]TVFA [mg/L]	237	118	502	232	308	118	195

[a]T = temperature; I = intensity; WAS = waste activated sludge; TS = total solids;
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements).
[2] Bicarbonate alkalinity in units of mg/L as calcium carbonate ($CaCO_3$).
[3]TVFA; total volatile fatty acids (summation of acetic, propionic, butyric acids), no duplicate measurements.

Table 3-4 Inoculum characterization.

Parameter	Before acclimation	After acclimation
pH [-]	7.65	8.08
TS [%, (w/w)]	2.13 (0.01)†	1.96 (0.00)
VS [%, (w/w)]	1.21 (0.01)	1.02 (0.00)
VS/TS*100 [%]	57.0 (0.1)	52.1 (0.0)
TSS [%, (w/w)]	1.99 (0.01)	1.79 (0.02)
VSS [%, (w/w)]	1.10 (0.00)	0.90 (0.02)
VSS/TSS*100 [%]	55.2 (0.0)	50.6 (0.3)
TCOD [mg/L]	21,429 (857)	18,514 (343)
SCOD [mg/L]	383 (46)	557 (14)
SCOD/TCOD [-]	0.02 (0.00)	0.03 (0.00)
NH_3-N [mg/L]	937 (0)	1280 (0)
[a]TVFA [mg/L]	0	0
[2]Alkalinity	4239 (10)	5824 (5)
Specific Activity [g TCOD/g VSS.d]	n/a	0.12 (0.01)

†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements); n/a: not available.
[a]TVFA = summation of acetic, propionic and butyric acids (no duplicate measurements).
[2]Bicarbonate alkalinity in units of mg/L as calcium carbonate ($CaCO_3$).

The effects of WAS pretreatment temperature and intensity on solubilization of two different sludge concentrations [1.4 and 5.4% TS (w/w)] were investigated by SCOD experiments. The level of particulate COD solubilization in sludge was evaluated by SCOD/TCOD ratios (Figure 3-3) which resulted in 3.6 ± 0.6 and 3.2 ± 0.1 fold higher SCOD/TCOD values at high and low TS concentrations at a pretreatment temperature of 75°C, respectively. This result supports the strong solubilization effect of MWs on WAS previously reported by Hong (2002). SCOD/TCOD values were the highest when samples were irradiated up to 75°C at 50%. As expected, MW intensities for both sludge concentrations indicated that volatilization of organics during pretreatment had the

highest effect on SCOD at 96°C. In general, WAS with 5.4% TS (w/w) yielded higher SCOD/TCOD ratios at all three pretreatment temperatures than those of WAS with 1.4% TS (w/w). Furthermore, solubilization was always slightly higher at 50% than at 100% MW intensities for both sludge concentrations at the same pretreatment temperatures possibly due to longer exposure time to the MW field at low MW intensities. It appeared that WAS pretreatment temperature, MW intensity and WAS concentration had an effect on solubilization ratios; however a statistical analysis was necessary to prove this hypothesis. Data in Table 3-2 also indicate an increase in ammonia concentrations of pretreated WAS samples at 50 and 75°C resulting from the hydrolysis of organic nitrogen present in the raw sludge and a volatilization of released ammonia at the pretreatment temperature of 96°C along with the TVFAs.

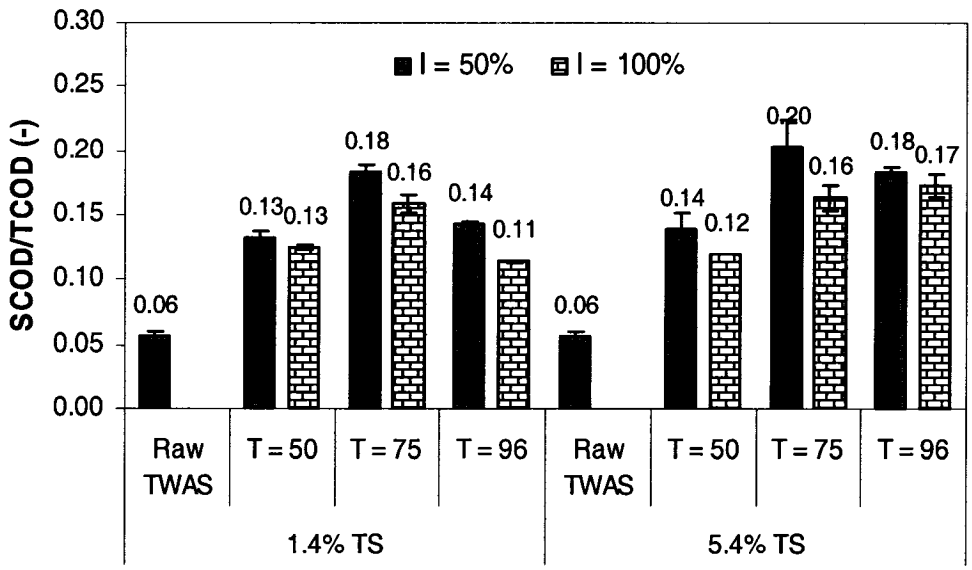


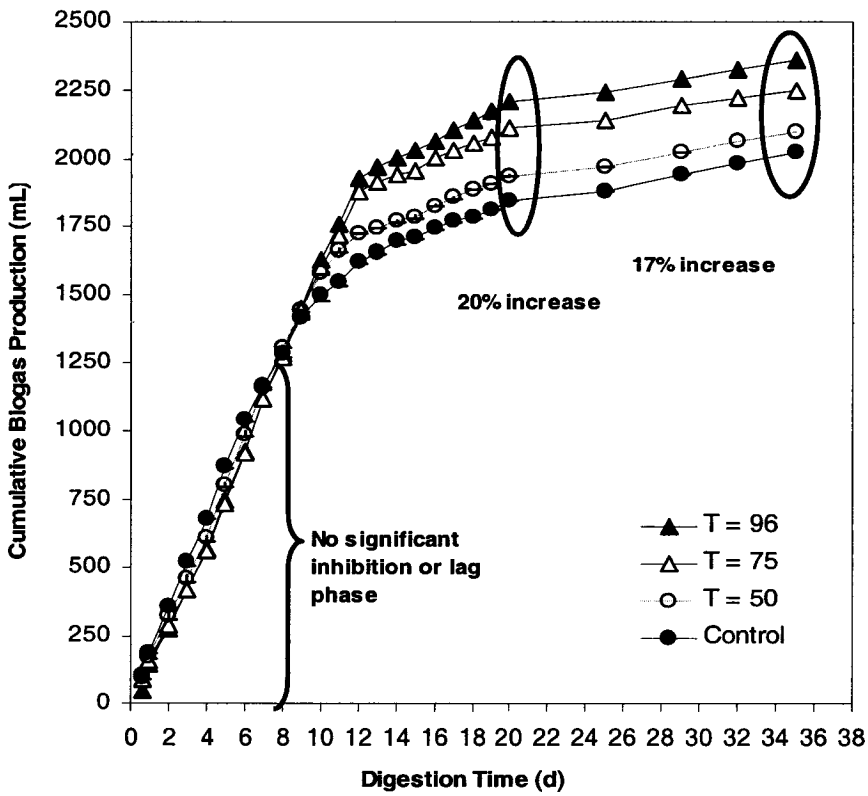
Figure 3-3 Solubilization effect of microwaves (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; I: intensity).

3.5.2 Effect of Microwaving on Batch Anaerobic Digestion of WAS

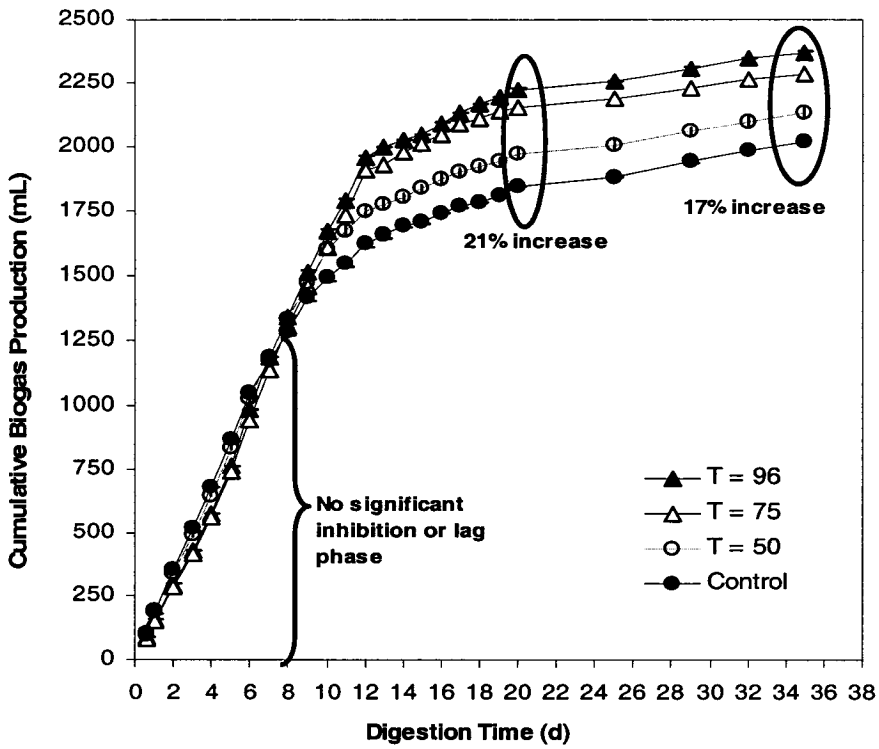
The effects of MW irradiation on anaerobic digestion of WAS were investigated by batch scale mesophilic anaerobic digesters and cumulative biogas productions (CBPs) are shown for both high and low sludge concentrations in Figures 3-4a-d, respectively. Figures 3-4a-d only indicate CBPs from digesters receiving 100% MW pretreated WAS. CBP results from batch digesters receiving 50% untreated and 50% MW pretreated WAS are displayed in Figures 3-7a and b. Inside the anaerobic batch digesters, the highest sludge concentration was 3% TS (w/w), since the inoculum was acclimatized to this concentration. In Figures 3-4a-d, biogas production contributed by inoculum itself was ignored since blank bottles produced only 62.1 ± 0.4 mL (less than 5%) CBP over 35 d of digestion. As observed from Figures 3-4a and b, due to the proper acclimation of inoculum, none of the reactors experienced an apparent lag phase at the beginning of the batch test. Until the 11^{th} d, control and pretreated bottles had very similar CBP rates; subsequently CBP rates in the control were the first to slow down. Around the 19^{th} d, CBP differences between the controls and the best pretreatment reactors (96°C) were around 20 ± 0.3 and 21 ± 0.5% for WAS microwaved at 100 and 50% intensities, respectively. As digestion continued further, the difference began to decrease to 17 ± 0.0 and 17 ± 0.9% at 100 and 50% intensities by day 34. This result is logical since given sufficient time; more difficult to degrade organics in the controls would continue to be degraded. However, the results also indicate that within a practical digestion time of 19 to 34 days that MW pretreatment enhanced the ultimate degradability of the WAS. The fact that the rate of biogas production for all samples were approximately equal during the

exponential phase but the duration of this rate was extended for MW pretreated samples also suggests that difficult to degrade organics in the WAS were converted into a more readily degradable substrate for anaerobic digestion.

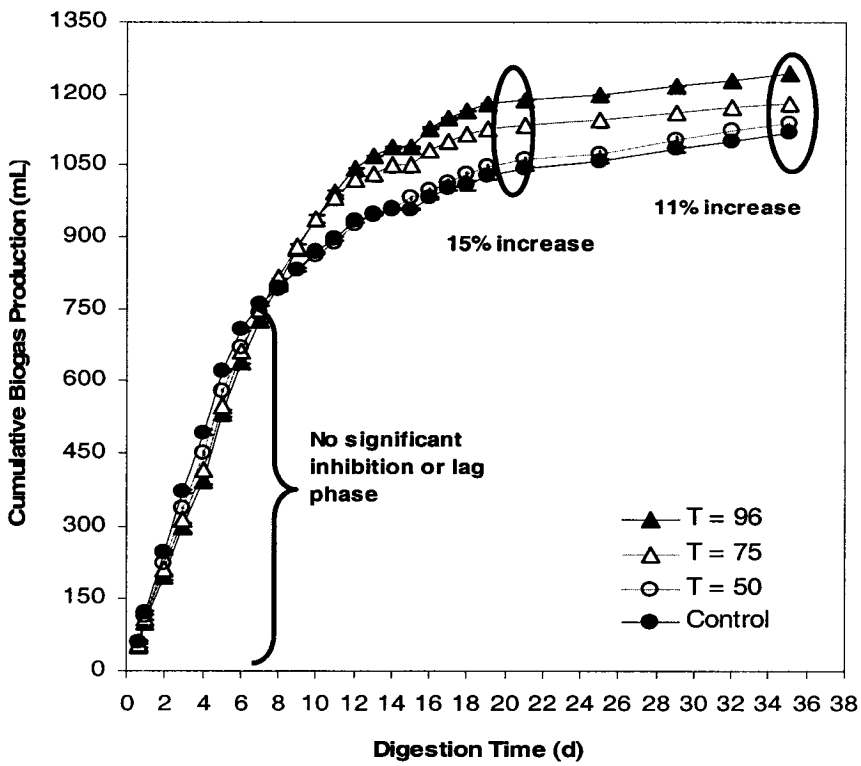
a)



b)


Cumulative Biogas Production (mL)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Digestion Time (d)
21% increase
17% increase
No significant inhibition or lag phase
T = 96
T = 75
T = 50
Control

c)


Cumulative Biogas Production (mL)
1350
1200
1050
900
750
600
450
300
150
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Digestion Time (d)
15% increase
11% increase
No significant inhibition or lag phase
T = 96
T = 75
T = 50
Control

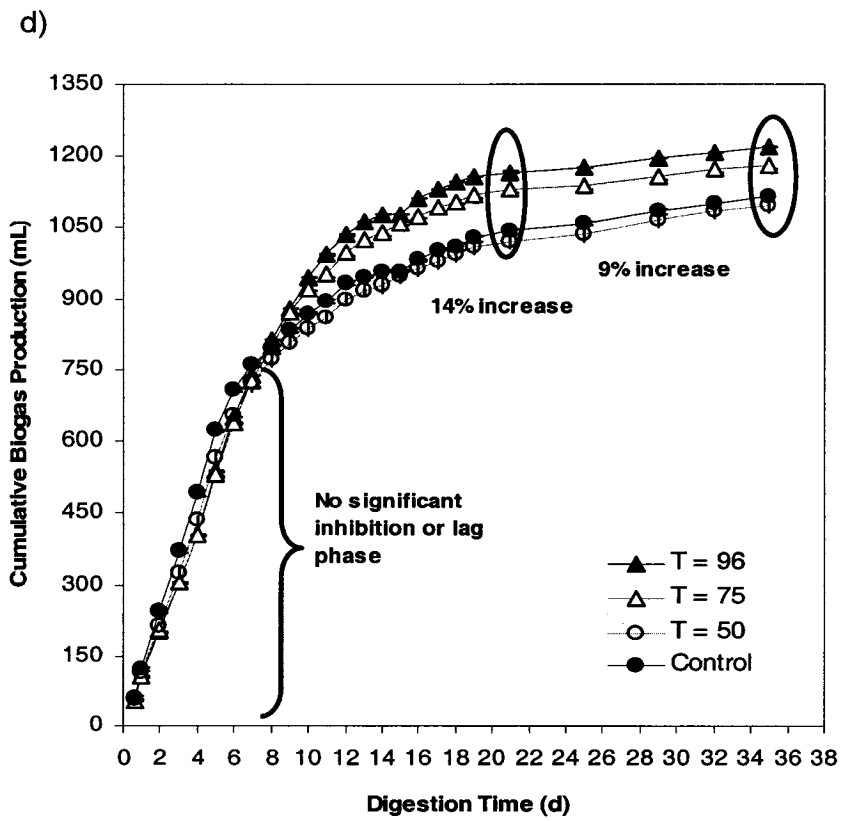
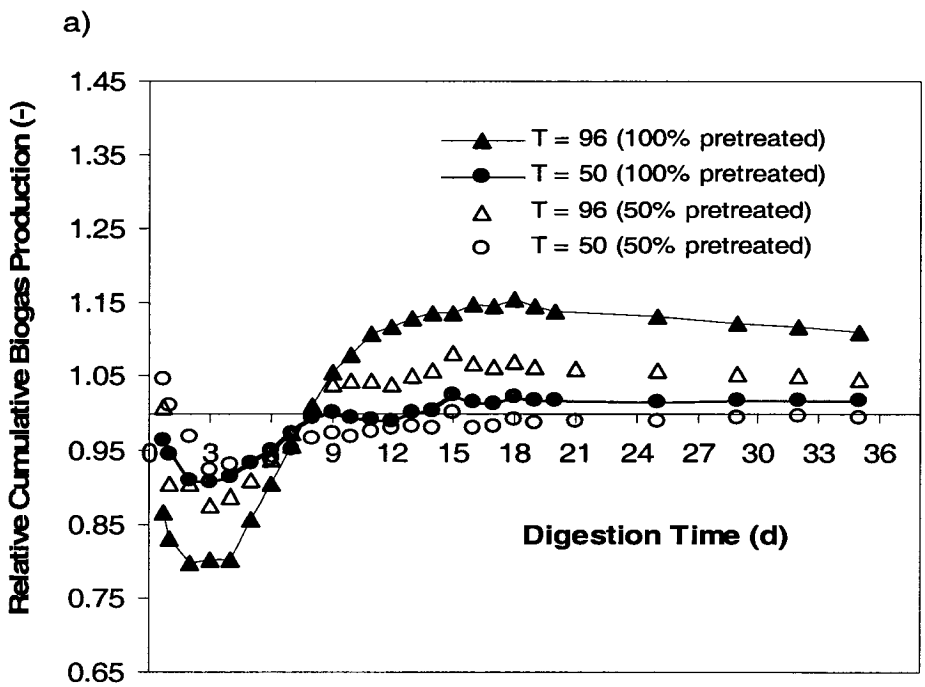


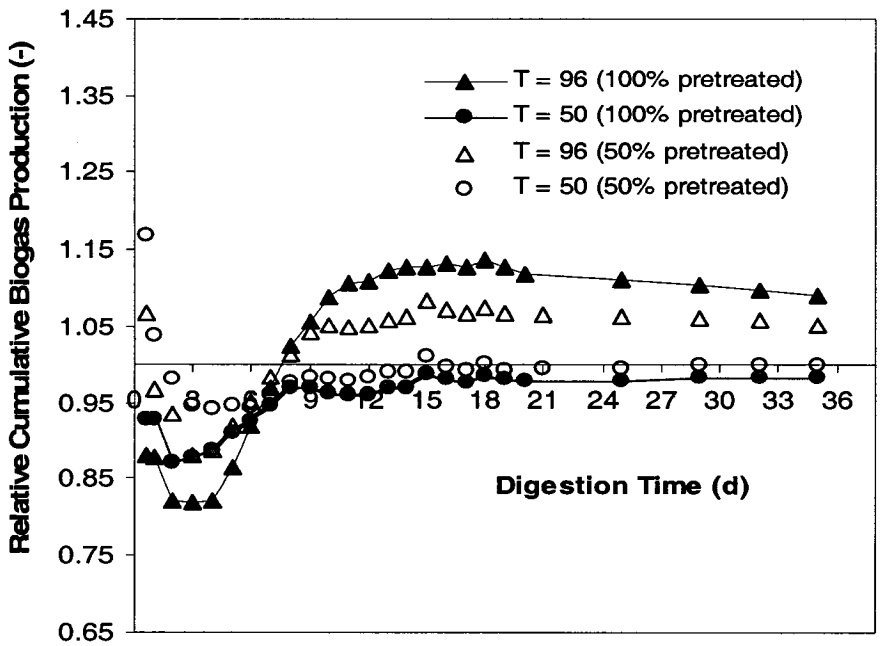
Figure 3-4 Cumulative biogas productions from a) WAS with 3% TS (Intensity: 100%); b) WAS with 3% TS (Intensity: 50%); c) WAS with 1.4% TS (Intensity: 100%); d) WAS with 1.4% TS (Intensity: 50%); (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively).

Interestingly, another important and reproducible response was observed when CPB results were converted to relative CBP (CBP from pretreated digesters over controls). Although the inoculum used in batch bottles was acclimatized to the highest MW temperature (96°C), control digesters initially had the fastest CBP (relative CBP = 1 in Figures 3-5a-d) followed by the digesters treating WAS pretreated to 50°C. In general, until the 7th day, reactors with 100% pretreated WAS at 96°C had the lowest CBP, and reactors with 50% untreated and 50% pretreated WAS at 50°C produced the highest amount of biogas. Obviously, the severity of the pretreatment temperature and the volume percentage of MW pretreated WAS present in the reactors influenced the biogas

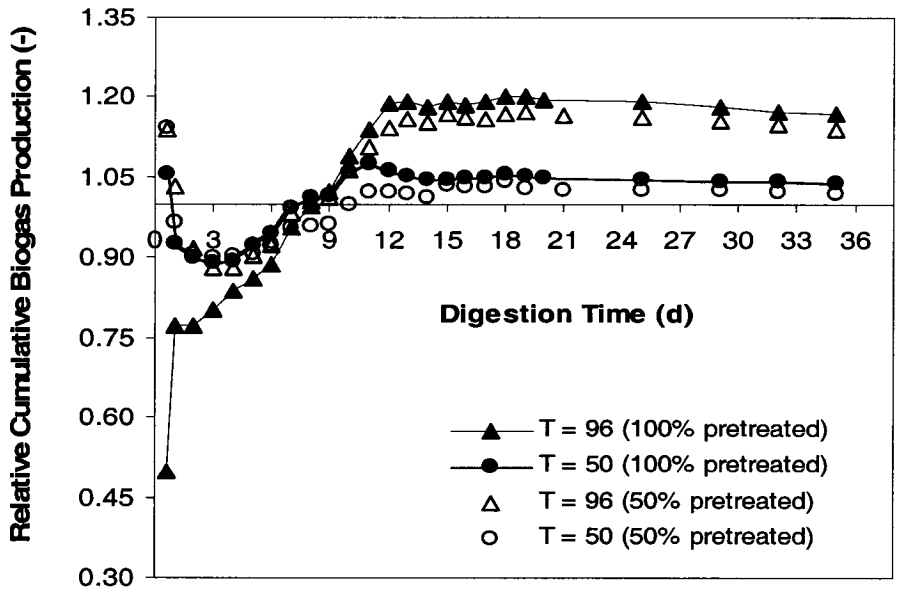
production in the first week of batch digestion. All digesters treating MW pretreated WAS with both low (Figures 3-5a and b) and high (Figures 3-5c and d) sludge concentrations were consistent in their reduced CBP responses indicating that some products were being formed that resulted in a mild acute microbial inhibition compared to the untreated WAS. This mild inhibition may be of some concern for continuous digesters operated at short SRTs but may disappear with further acclimation. However, the mild inhibitory effect was not strong enough to create significant chronic inhibition and therefore CBP rates of control reactors eventually lagged behind CBP from MW pretreated reactors (Figures 3-4a-d). In order to study and evaluate this effect more clearly, continuous flow reactors, with SRT less than 7 d, need to be studied (Eskicioglu *et al.*, 2006).



b)


Relative Cumulative Biogas Production (-)
1.45
1.35
1.25
1.15
1.05
0.95
0.85
0.75
0.65
0
3
6
9
12
15
18
21
24
27
30
33
36
Digestion Time (d)
T = 96 (100% pretreated)
T = 50 (100% pretreated)
T = 96 (50% pretreated)
T = 50 (50% pretreated)

c)


Relative Cumulative Biogas Production (-)
1.35
1.20
1.05
0.90
0.75
0.60
0.45
0.30
0
3
9
12
15
18
21
24
27
30
33
36
Digestion Time (d)
T = 96 (100% pretreated)
T = 50 (100% pretreated)
T = 96 (50% pretreated)
T = 50 (50% pretreated)

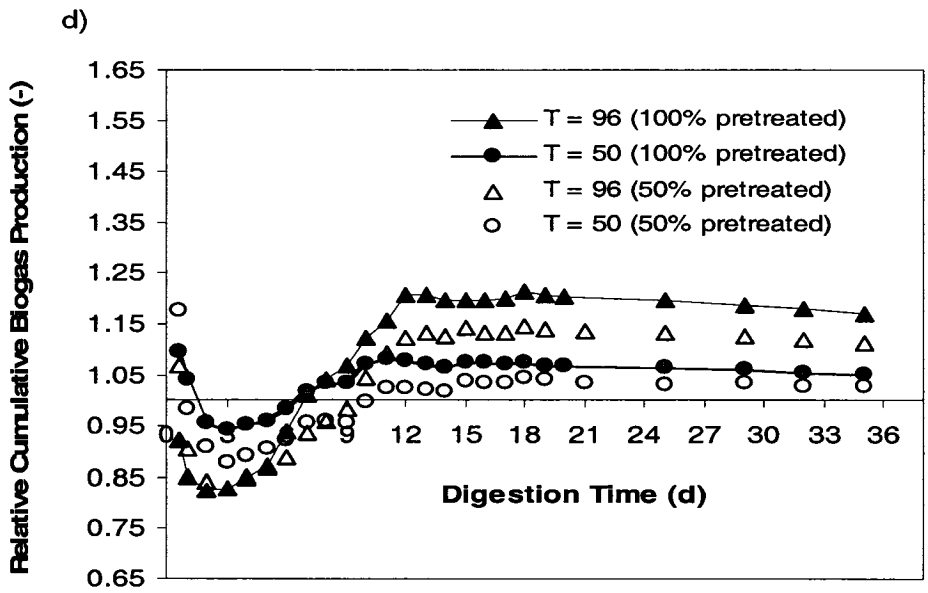


Figure 3-5 Relative (to control) cumulative biogas productions a) WAS with 1.4% TS (Intensity: 100%); b) WAS with 1.4% TS (Intensity: 50%); c) WAS with 3% TS (Intensity: 100%); d) WAS with 3% TS (Intensity: 50%); (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively).

For the low sludge (1.4% TS) concentration, the trends discussed above were the same (Figures 3-4c and d). The WAS, microwaved to 96°C, again produced the highest amount of biogas with 15 ± 0.5 and 14 ± 1.1% increases over the controls at the 100 and 50% MW intensities around the 19th d, respectively. This result suggests that if the concentration of sludge being pretreated is increased, the effect of MW treatment of sludge stabilization and increased CBP also increases, which was also observed in ultrasound pretreatment studies (Barber, 2002).

The effect of MW intensity (slow versus fast cooking) on CBP at high and low sludge concentrations is shown in Figures 3-6a and b, respectively. In general, for both low and high sludge concentrations and for digesters treating both 50 and 100% (v/v)

pretreated WAS, at 50 and 100% MW intensities resulted in very similar CBPs for the same pretreatment temperatures indicating that different MW intensities did not create consistently different CBPs among digesters treating the same concentration of WAS.

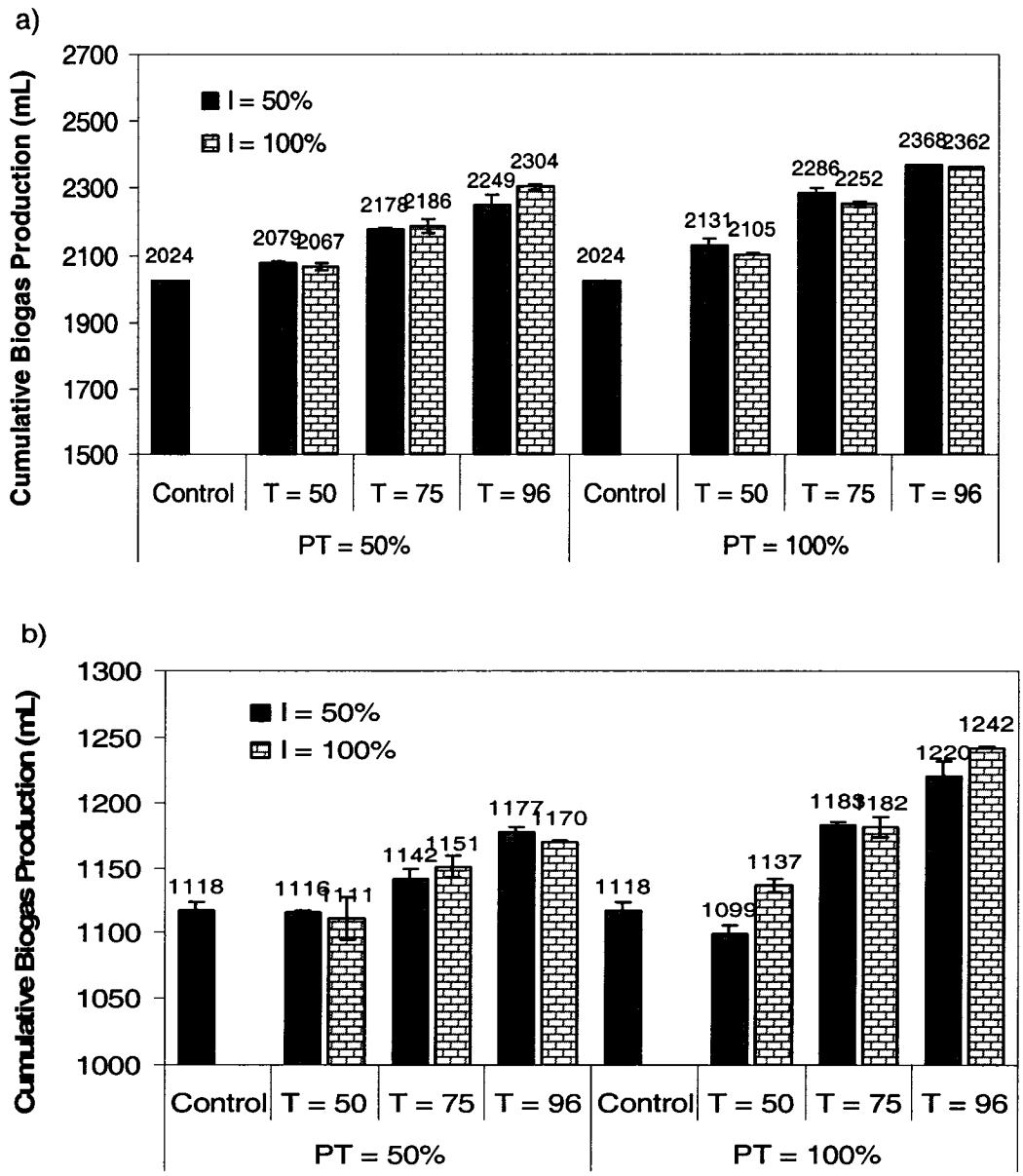


Figure 3-6 Microwave intensity effect on WAS with a) 3% TS; b) 1.4% TS; (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; PT: partial treatment; I: intensity).

The results on partial WAS pretreatment were contradictory to the results from an ultrasound study, which reported that CBP was higher from partially pretreated WAS samples (Barber, 2002). It can be seen from Figures 3-7a and b that except for the initial mild inhibition in the first 7 days, regardless of high or low sludge concentration and for both 50 and 100% MW intensities, reactors receiving 100% pretreated WAS produced higher CBPs than reactors receiving partially pretreated WAS (50% untreated and 50% MW pretreated) for all pretreatment temperatures. If a significant sustained biomass inhibition occurred in 100% WAS pretreated digesters, partially pretreated digesters would produce higher biogas but this was not the case for this study. On the other hand, the increased differences in CPB between totally and partially pretreated WAS reactors were not large. Therefore, the decision on applying partial treatment should be dependent on the energy and cost analysis including dewaterability performance of totally and partially pretreated WAS samples.

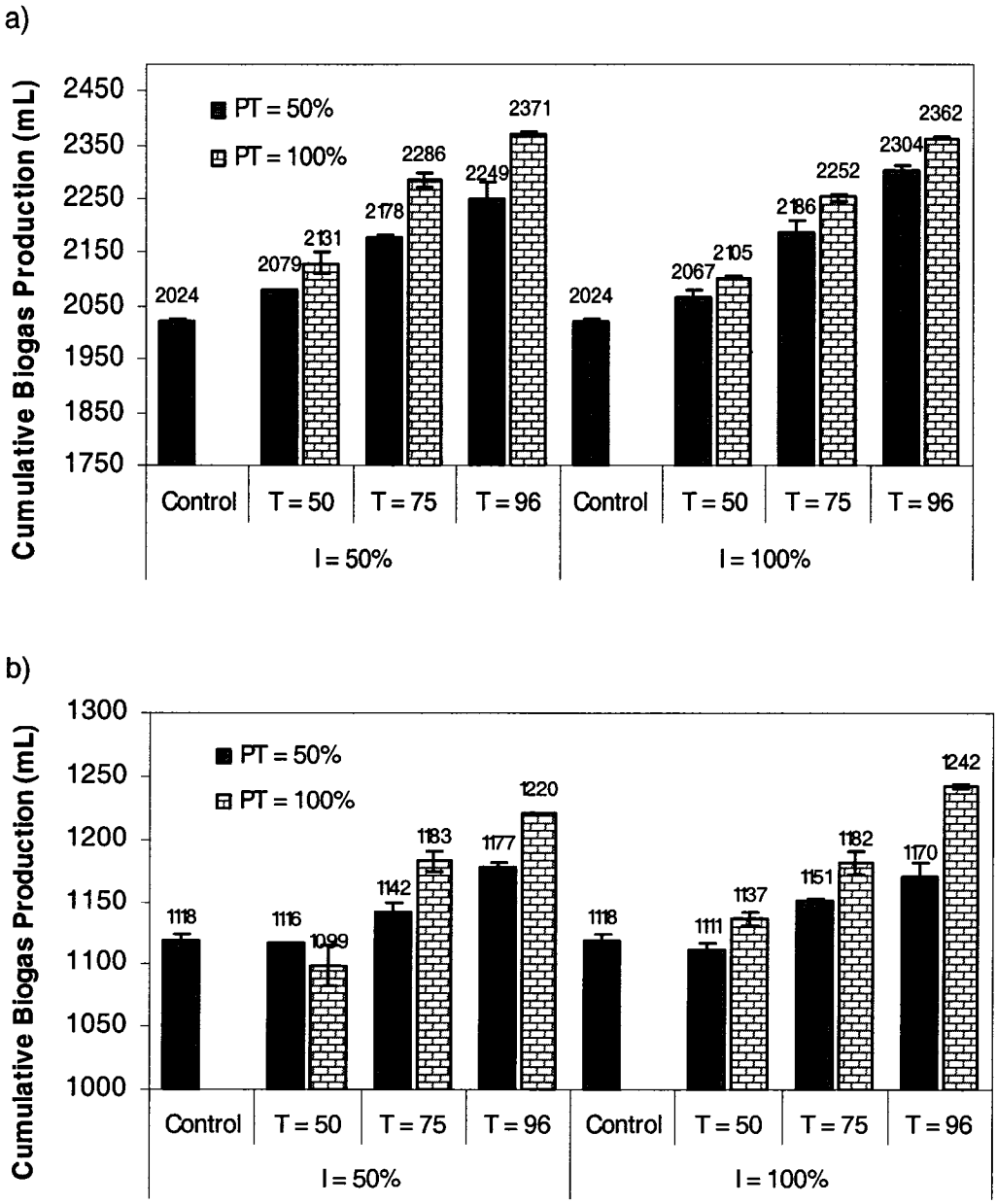


Figure 3-7 Microwave partial treatment effect on WAS with a) 3% TS; b) 1.4% TS; (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; PT: partial treatment; I: intensity).

It was emphasized by previous studies that if pretreatments at high temperature (>175°C), were applied for a longer time than necessary, enzymes can be inactivated, nitrogenous organic materials become less biodegradable and inhibitory concentration of ammonia, can be formed (Stuckey and McCarty, 1984). Although other researchers monitored the ammonia concentration in the anaerobic digesters after pretreatment, no definite ammonia inhibition case was reported. In this study, ammonia concentrations of batch reactors were measured after the BMP test was complete and results are displayed in Figure 3-8 for reactors with 3 and 1.4% TS (w/w). As shown in Figure 3-8, slightly higher ammonia was generated in reactors when pretreatment temperature increased. This was likely due to higher anaerobic degradation efficiency of nitrogenous organic matter in pretreated WAS digesters. MW intensity did not change the ammonia-N concentrations in digesters. As expected, digesters treating 100% MW pretreated WAS contained slightly higher ammonia-N concentrations compared to those treating 50% untreated and 50% MW pretreated WAS. However, the differences between the highest ammonia-N concentrations in the 96°C WAS pretreated reactors and the control were only 15 ± 2% for the reactor with 3% TS (w/w) and 11 ± 2% for the reactor with 1.4% TS (w/w). This agrees with the same relative improvements in CBP and VS stabilization. Since digester pH was controlled by addition of buffer solutions before reactors were sealed, pH values of digesters fluctuated only in a range of 7.3-8.4 during the BMP test (Appendix D, Table D-1) and therefore ammonia was primarily in the less toxic ionized form. Furthermore, inoculum was acclimatized to this level of ammonia in the semi-continuous inoculum reactor (Table 3-4). Therefore all digesters were able to tolerate elevated ammonia concentrations with no longterm decrease in methane production

(Appendix D, Table D-2) or VFA accumulation (Appendix D, Table D-3). Methane content of biogas in digesters fluctuated in a safe range of 81-65% (from high to low with respect to digestion time and pH of the digesters) and there was no significant difference in methane percentages among the control and pretreated digesters. Almost all (98%) of the VFAs were converted to biogas in two weeks. As a disadvantage of applying pretreatment, 100% pretreated digesters had elevated concentrations of SCODs (around 35% higher SCODs compared to controls) in their final effluents (Figure 3-9). Reactors digesting WAS pretreated at 50 and 100% MW intensities produced almost identical SCODs in their effluents as shown in Figure 3-9.

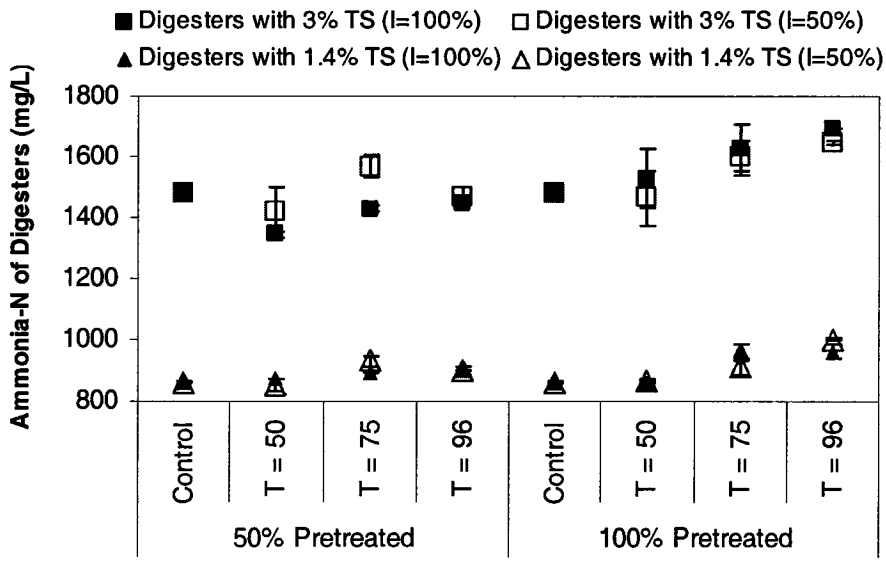


Figure 3-8 Ammonia-N content of batch reactors after BMP test was complete (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; I: intensity).

The biogas or methane yield for an anaerobic digestion process is commonly expressed as a function of the removal of either VS or TCOD. For TWAS with 3-5.4%

TS concentration, sludge digestion experimental TCOD results are less reliable than VS results (sampling error and extreme dilution ratios ~150). Literature values for methane yield may change depending on the characteristics of the sludge digested, but representative values are in the range of 0.49-0.75 L CH_4/g VS removed (Parkin and Oven; 1986; Metcalf and Eddy, 1991). If the sludge is a mixture of PS/WAS, nominally each gram VS removal corresponds to 1 L biogas production (Stephenson et al., 2003). For a reactor treating only WAS, this value is expected to be smaller. In this study, cumulative biogas and methane productions and VS removals from all 54 batch BMP digesters were used and results indicated mean biogas and methane yields of 0.84 ± 0.10 and 0.60 ± 0.07 L per g VS removed respectively at 1 atm. and 25 ± 1°C. Therefore, biogas and methane yields from batch mesophilic digesters were within the range determined by other pretreatment studies.

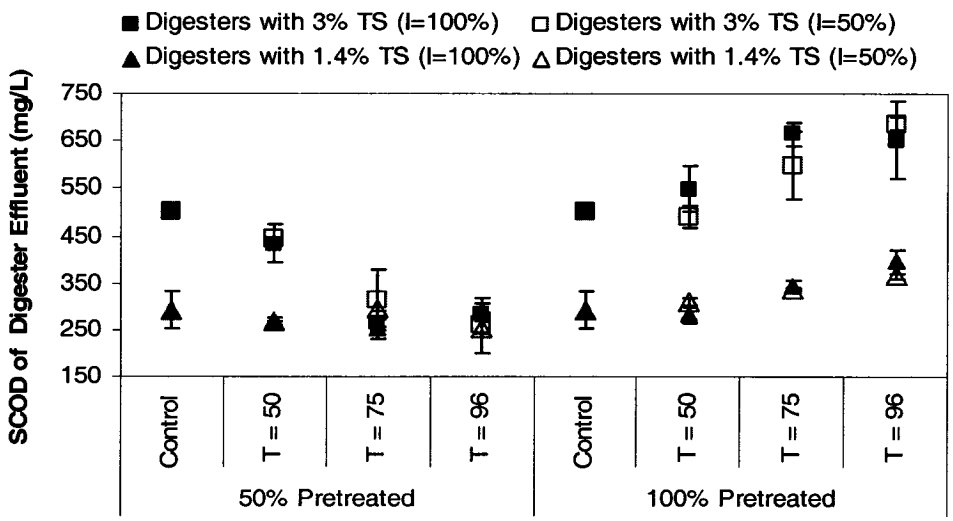


Figure 3-9 SCOD content of digester effluents after BMP test was complete (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; I: intensity).

3.5.3 Statistical Analysis

A multifactor ANOVA [STATGRAPHICS 5.1 software (StatPoint Inc., Virginia, USA] was used to detect significant MW pretreatment factors in the multilevel factorial design shown in Figures 3-1A and B for WAS solubilization (SCOD/TCOD ratios) prior to digestion and then on biogas production from digestion. In this multifactor model, the response (dependent) variables were relative SCOD/TCOD ratio ($SCOD/TCOD_r$ = $SCOD/TCOD_{pretreated}/SCOD/TCOD_{control}$) and relative CBP ($CBP_r$ = $CBP_{pretreated}/CBP_{control}$) in order to eliminate the effects of solubilization and CBP resulting from the controls. Independent MW pretreatment variables were pretreatment temperature (T), intensity (I), and concentration (C) for WAS solubilization and T, I, C and WAS volume percentage treated (PT) for CBP. The ANOVA results shown in Tables 3-5 and 3-6 separate the variability (total sum of squares) of $SCOD/TCOD_r$ and CBP_r into contributions due to other independent factors (sum of squares of each factor) and random error (sum of squares of residuals). Probability (p) values test the statistical significance of each of the factors.

Table 3-5 Results of multifactor ANOVA for $SCOD/TCOD_r$[a].

Source	Sum of squares	Degree of freedom	Mean square	F-ratio*	p-value
Main Effects					
T	2.873	2	1.436	33.18	**0.0000**
I	0.925	1	0.925	21.38	**0.0006**
C	0.840	1	0.840	19.40	**0.0009**
Interactions					
T*I	0.121	2	0.061	1.40	0.2843
T*C	0.810	2	0.405	9.35	**0.0036**
I*C	0.007	1	0.007	0.17	0.6907
T*I*C	0.128	2	0.064	1.48	0.2663
Residuals	0.519	12	0.043		
Total (corrected)	6.224	23			

[a]T = temperature, I= intensity, C = sludge concentration, $SCOD/TCOD_r$ = relative solubilization ratio.
*All F-ratios are based on the residual mean square error.

Table 3-5 indicates that since p-values of 4 variables (T, I, C and interactions of T*C) are less than 0.05, these MW pretreatment factors have a statistically significant effect on WAS solubilization as measured by $SCOD/TCOD_r$ at the 95% confidence level. Similarly, in Table 3-6, the effects of T and C dominate the effects of PT and I; however, p-values of 6 variables (PT, T, C and interactions of T*PT, T*I and PT*I*C) are less than 0.05, indicating that these single, two and three factor interactions have a statistically significant effect on CBP_r at the 95% confidence level. Results from Tables 3-5 and 3-6 imply that higher solubilization ratios achieved at lower MW intensity (50%) during pretreatment did not create statistically significant differences among CBPs of digesters at the end of 35 days of anaerobic digestion; therefore, factor of intensity (I) could be eliminated from the experimental design. However the other factors are important and deserve further study. The ANOVA table is only useful if its assumptions are met:

residuals are independently and identically distributed in a normal distribution. Normal probability plots of residuals displayed in Appendix E, Figures E-3a and b indicated good straight lines, therefore; showing no pattern that might be cause for concern.

Table 3-6 Results of multifactor ANOVA for CBP_r[a].

Source	Sum of squares	Degree of freedom	Mean square	F-ratio*	p-value
Main Effects					
PT	0.014	1	0.014	138.64	**0.0000**
T	0.079	2	0.039	388.15	**0.0000**
I	0.000	1	0.000	1.60	0.2184
C	0.038	1	0.038	373.30	**0.0000**
Interactions					
PT*T	0.003	2	0.001	13.41	**0.0001**
PT*I	0.000	1	0.000	0.01	0.9332
PT*C	0.000	1	0.000	1.36	0.2448
T*I	0.000	2	0.000	1.15	0.3163
T*C	0.002	2	0.001	10.53	**0.0004**
I*C	0.000	1	0.000	2.92	0.0931
PT*T*I	0.000	2	0.000	2.50	0.0932
PT*T*C	0.000	2	0.000	1.87	0.1629
PT*I*C	0.001	1	0.001	11.30	**0.0021**
T*I*C	0.000	2	0.000	2.11	0.1313
PT*T*I*C	0.000	2	0.000	1.62	0.2179
Residuals	0.003	24	0.000		
Total (corrected)	0.142	47			

[a]PT = partial treatment, T = temperature, I= intensity, C = sludge concentration, CBP_r = relative solubilization ratio.
*All F-ratios are based on the residual mean square error.

3.5.4 Dewaterability Analysis on WAS from Batch Digesters

It can be speculated that MW pretreatment of WAS can release of the water originally bound to the EPS and enhance dewaterability by altering the hydration zone since in a WAS sample, water would be the main polar element exposed and affected by MWs. CST method provides a quantitative measure, reported in seconds, of how readily a sludge releases its water. The results can provide information to evaluate sludge conditioning aids and dosages; or, to evaluate coagulation effects on the rate of water release from sludges when used with a jar test and the settleable solids procedure. The method requires a minimum of 5 replicate injections from each sample (APHA, 1995). Due to large sample size in this study, 8 samples including duplicates and controls were randomly selected and dewaterability was tested. Error bars represented the standard deviations among five replicate injections. Despite 10% deterioration in dewaterability of sludge pretreated to 50°C, dewaterability of sludges coming from digesters with pretreatment temperatures of 75 and 96°C was enhanced. Figure 3-10 shows percentage reductions in dewatering time of effluents from pretreated (at 100% intensity) batch digesters relative to controls. As seen in Figure 3-10, relative improvement in dewatering time was significantly increased as WAS pretreatment temperature reached 96°C, indicating 41% better dewaterability compared to controls. Dewaterability results from semi-continuous digesters treating MW-irradiated sludges up to 96°C supported the batch digester results with ~40% better dewaterability compared to controls at SRT of 10 d (Eskicioglu *et al.*, 2006).

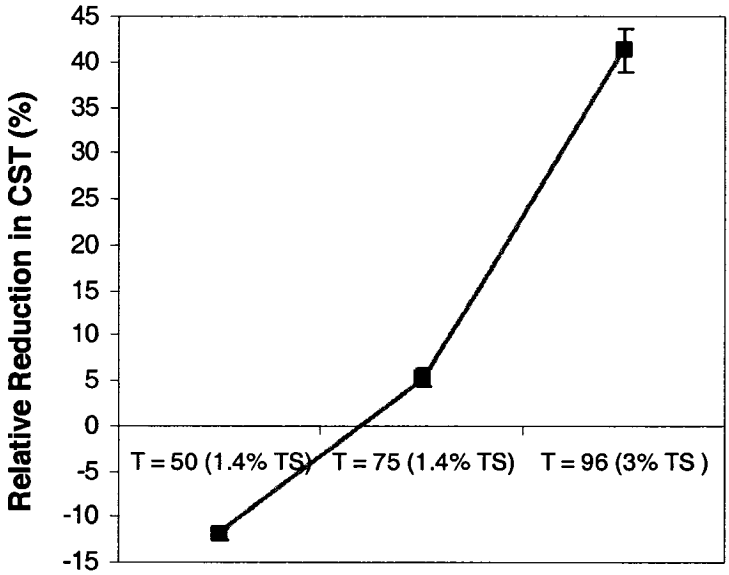


Figure 3-10 Relative (to control) reduction in dewatering time (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; I: intensity).

3.6 Conclusions

Based on the experimental data, the following conclusions are drawn.

(1) Solubilization experiments on microwaved WAS samples resulted in 3.6±0.6 and 3.2±0.1 fold increases in SCOD/TCOD ratios at 5.4% TS and 1.4% TS sludge concentrations, respectively. Solubilization was always slightly higher at 50% than at 100% MW intensities for both sludge concentrations at the same pretreatment temperatures possibly due to longer exposure time to MW field at low MW intensities. A multifactor ANOVA determined pretreatment temperature, MW intensity and WAS concentration as significant factors for WAS solubilization at the 95% confidence level.

(2) Anaerobic digestion of WAS microwaved to 96°C enhanced the ultimate degradability of WAS and produced the highest amount of biogas with 15 ± 0.5,

14 ± 1.1 and 20 ± 0.3 and 21 ± 0.5% increases over the controls after 19 d of digestion at 100 and 50% MW intensities and at 1.4% TS and 5.4% TS sludge concentrations, respectively. A multi-factor ANOVA determined percentage of WAS pretreated, pretreatment temperature, and WAS concentrations as significant factors at the 95% confidence level and MW intensity was eliminated from experimental design.

(3) MW pretreatment did cause some short term inhibition of digestion. But it did not cause any significant longterm chronic inhibition of acclimated biomass during digestion. Pretreated reactors experienced only 15 ± 2% higher ammonia-N concentrations over the controls.

(4) Dewaterability of MW-pretreated sludge to 96°C was significantly enhanced after batch mesophilic digestion.

3.7 Acknowledgments

Funding provided by NSERC, BIOCAP Canada and Environmental Waste International Corporation.

3.8 References

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CHAPTER 4

Empirical Modeling for Effects of Microwave Pretreatment on Secondary Sludge Solubilization and Anaerobic Batch Digestion

Cigdem Eskicioglu, Kevin J. Kennedy, Ronald L. Droste

4.1 Abstract

The effects of microwave (MW) pretreatment on disintegration and hydrolysis of waste activated sludge (WAS) by soluble chemical oxygen demand (SCOD), soluble protein, soluble sugar and nucleic acid leakage detection experiments were investigated. Empirical modeling of the effects of MW temperature (T), intensity (I), WAS concentration (C) and percentage of WAS treated (PT) on solubilization and anaerobic digestion of sludge was studied in two separate multilevel factorial designs containing 24 solubilization runs and 54 batch anaerobic digesters. The results of this study indicated that MW-irradiation has a potential of damaging activated sludge floc structure and cell membranes and releasing extracellular and intracellular compounds (proteins, sugars and nucleic acid) along with the solubilization of particulate COD. The SCOD to total COD (TCOD) ratio of WAS increased from 0.06 ± 0.00 to 0.14 ± 0.01, 0.20 ± 0.02 and 0.18 ± 0.00 at MW temperatures of 50, 75, and 96°C. The MW pretreatment also increased the ultimate bioavailability of sludge components under anaerobic digestion with 17± 0.0% higher cumulative biogas productions compared to controls after 34 days of batch digestion.

KEYWORDS: Microwave, WAS, solubilization, pretreatment, anaerobic digestion, empirical modeling.

4.2 Introduction

Digestion and dewatering of WAS from wastewater treatment processes is a major economical factor for the operation of treatment plants. Researchers have shown that complex activated sludge floc matrix incorporating microbial cells, exocellular polymeric substances (EPS) and divalent cations which improve floc stability creates an additional resistance to hydrolysis, which is known to be the limiting step for the anaerobic digestion of secondary sludges (Higgins and Novak, 1997; Park *et al.*, 2006). EPS cannot only be seen attached to the microbial cell as a *capsule* but also can be excreted into soluble phase as a *slime*. Disintegration of floc structure and extraction of EPS by external forces, such as mechanical, chemical, ultrasound and thermal methods typically yield protein, polysaccharides and smaller amounts of nucleic acid (DNA and RNA) into the surrounding medium (Frølund *et al.*, 1996; Ormeci *et al.*, 2001) which has the potential to enhance the rate as well as the extent of the anaerobic digestion of WAS (Choi *et al.*, 1997; Kim *et al.*, 2003).

The objective of this paper was to test a mild temperature (50-96°C) MW system as an alternative method to disintegrate the floc structure and to enhance the digestion of WAS. The focus was on characterizing the changes that occur in the supernatant phase of WAS after MW pretreatment by conducting SCOD, soluble protein, soluble sugar and nucleic acid leakage tests. Results were then compared to controls to evaluate improvements in anaerobic digestion by monitoring biogas production from batch digesters.

4.3 Materials and Methods

Thickened WAS (TWAS) used in the study was obtained from the thickener centrifuge with total solid (TS) concentration of 5.4% (w/w) at the *Robert O. Pickard Environmental Center (ROPEC)* located in Gloucester, (ON, Canada). ROPEC has preliminary and primary treatment followed by a conventional aerobic activated sludge unit operated at an average sludge retention time (SRT) of 5 d. At ROPEC, TWAS and primary sludge (PS) are blended in a 58:42 v/v ratio and undergo mesophilic anaerobic sludge digestion to produce a stabilized biosolids product for disposal. MW irradiation of TWAS was applied by a 0.045 m^3 capacity household type MW oven [Panasonic NNS53W + inverter, 1250 W, 2450 MHz frequency and 12.24 cm wavelength].

Before anaerobic digestion, the effects of three variables [MW temperature, T, (at three levels: 50, 75, 96°C), MW Intensity, I, (at two levels: 50, 100%), TWAS concentration, C, (at two levels: 1.4 and 5.4% (w/w) TS] on solubilization (SCOD/TCOD) of TWAS. To determine the impact on anaerobic digestion, the effects of four variables [T, I, C, volume percentage of TWAS treated (PT; at two levels: 50 and 100%)] on cumulative biogas production (CBP) from biochemical methane potential (BMP) tests were investigated in two separate experimental multilevel factorial designs. MW intensity of 50% uses only half of the total MW power (1250 W) to reach desired temperature. Therefore at 50% MW intensity, sample heating is done at a slower rate than that of 100% intensity (Eskicioglu *et al.*, 2006). TWAS solubilization experiments contained a total of 24 runs including duplicates (Table 4-1) and BMP tests (Table 4-2) involved 54 mesophilic batch reactors, including controls (no pretreatment) and duplicates. In Table 4-2, digester runs 1 to 12 contain both pretreated WAS (half of the

reactor volume) and untreated WAS (other half). This application called partial treatment (PT) was found more successful than treating the whole stream in ultrasound sludge pretreatment studies (Barber, 2002).

The anaerobic digesters were started with an inoculum [2.0 ± 0.01% TS (w/w)] acclimatized to MW pretreated WAS (96°C) and had a specific activity of 0.12 ± 0.01 g TCOD/g VSS.d. Refer to Eskicioglu *et al.* (2006) for the MW calibration tests for the pretreatment stage and detailed inoculum acclimation conditions. Inside the anaerobic batch digesters, the highest sludge concentration used was 3% TS (w/w), since the inoculum was acclimatized to this concentration.

Table 4-1 Experimental conditions for evaluation of TWAS solubilization[a].

Run #	T (°C)	I (%)	C (% TS, w/w)	SCOD /TCOD (-)
$1/1_d$	50	50	1.4	0.13 (0.01)†
$2/2_d$	50	50	5.4	0.14 (0.01)
$3/3_d$	50	100	1.4	0.13 (0.00)
$4/4_d$	50	100	5.4	0.12 (0.00)
$5/5_d$	75	50	1.4	0.18 (0.01)
$6/6_d$	75	50	5.4	0.21 (0.02)
$7/7_d$	75	100	1.4	0.16 (0.01)
$8/8_d$	75	100	5.4	0.16 (0.01)
$9/9_d$	96	50	1.4	0.14 (0.00)
$10/10_d$	96	50	5.4	0.18 (0.00)
$11/11_d$	96	100	1.4	0.11 (0.00)
$12/12_d$	96	100	5.4	0.17 (0.01)
$Con/1.4_d$	-	-	1.4	0.06 (0.00)
$Con/5.4_d$	-	-	5.4	0.06 (0.00)

[a]TWAS = thickened waste activated sludge; T, I = microwave temperature and intensity; TS = total solids; TCOD, SCOD = total and soluble chemical oxygen demands; C = TWAS concentration, Con = control
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicates).

Table 4-2 Experimental conditions to determine effect of MW pretreatment on BMP of TWAS[a].

Run #	MW TWAS (mL)	TWAS (mL)	Inoc. (mL)	PT (%, v/v)	T (°C)	I (%)	C (%TS, w/w)	Ultimate CBP (mL)
$1/1_d$	140	140	70	50	50	50	1.4	1116 (1)†
$2/2_d$	140	140	70	50	50	50	3.0	2079 (2)
$3/3_d$	140	140	70	50	50	100	1.4	1111 (7)
$4/4_d$	140	140	70	50	50	100	3.0	2067 (12)
$5/5_d$	140	140	70	50	75	50	1.4	1142 (7)
$6/6_d$	140	140	70	50	75	50	3.0	2178 (3)
$7/7_d$	140	140	70	50	75	100	1.4	1151 (2)
$8/8_d$	140	140	70	50	75	100	3.0	2186 (23)
$9/9_d$	140	140	70	50	96	50	1.4	1177 (4)
$10/10_d$	140	140	70	50	96	50	3.0	2249 (31)
$11/11_d$	140	140	70	50	96	100	1.4	1170 (12)
$12/12_d$	140	140	70	50	96	100	3.0	2304 (10)
$13/13_d$	280	-	70	100	50	50	1.4	1099 (16)
$14/14_d$	280	-	70	100	50	50	3.0	2131 (21)
$15/15_d$	280	-	70	100	50	100	1.4	1137 (5)
$16/16_d$	280	-	70	100	50	100	3.0	2105 (4)
$17/17_d$	280	-	70	100	75	50	1.4	1183 (8)
$18/18_d$	280	-	70	100	75	50	3.0	2286 (14)
$19/19_d$	280	-	70	100	75	100	1.4	1182 (7)
$20/20_d$	280	-	70	100	75	100	3.0	2252 (9)
$21/21_d$	280	-	70	100	96	50	1.4	1220 (1)
$22/22_d$	280	-	70	100	96	50	3.0	2371 (3)
$23/23_d$	280	-	70	100	96	100	1.4	1242 (1)
$24/24_d$	280	-	70	100	96	100	3.0	2362 (6)
$Con/1.4_d$	-	280	70		-	-	1.4	1118 (8)
$Con/3.0_d$	-	280	70		-	-	3.0	2024 (5)
Inoc.	-	-	70		-	-	-	62 (0)

[a]TWAS = thickened waste activated sludge; MW = microwave; PT = percent TWAS treated;
T = temperature; I = intensity; C = TWAS concentration; Con = control (unpretreated); Inoc.= inoculum
TS = total solids; CBP = cumulative biogas production.
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicates).

TS and VS were determined based on Standard Methods procedure 2540G (APHA, 1995). Colorimetric COD measurements were done based on Standard Methods procedure 5250D (APHA, 1995) with a Coleman Perkin-Elmer spectrophotometer Model 295 at 600 nm light absorbance. Before SCOD determination, sludge samples were centrifuged [20 min at 5856 relative centrifugal force (RCF)] and filtered through GN-6 Metricel S-Pack membrane disc filters with 0.45 μm pore size. Total volatile fatty acids (TVFAs; summation of acetic, propionic and butyric acids) were measured by injecting supernatants into a HP 5840A capillary column GC and terminal integrator equipped with HP 7672A autosampler. Biogas production was measured daily by inserting a needle into the reactors attached to a manometer. The concentration of proteins and reducing sugars in the soluble phase (determined by filtration at 0.45 μm) was measured at 595 and 575 nm by a Beckman DU-40 spectrophotometer according to colorimetric methods of Bradford (1976) and Miller (1959), respectively. Bovine serum albumin (BSA) and glucose stock solution were used as the protein and sugar standards. Increase in nucleic acid content of the supernatants after MW irradiation was directly measured at 260 nm using a UV spectrophotometer equipped with 1cm path length quartz cuvettes.

4.4 Results and Discussion

4.4.1 Effect of MW Pretreatment before Anaerobic Digestion

The characteristics of raw TWAS used in the experiments can be summarized as follows: pH: 6.49, TS: 5.4 ± 0.02% (w/w), VS: 3.77 ± 0.01% (w/w), TCOD: 41,667 ± 1190 mg/L, SCOD: 2,357 ± 71 mg/L, TVFA: 913 mg/L, ammonia: 536 ± 8 mg/L. The degree of solubilization was evaluated by SCOD/TCOD ratios (Table 4-1) which resulted in 3.66 ± 0.6 and 3.23 ± 0.1 fold higher SCOD/TCOD ratios compared to controls at high and low TWAS concentrations, respectively (Figure 4-1). This result supports the significant solubilization effects of MWs on WAS previously reported by Hong (2002) and Park *et al.* (2004).

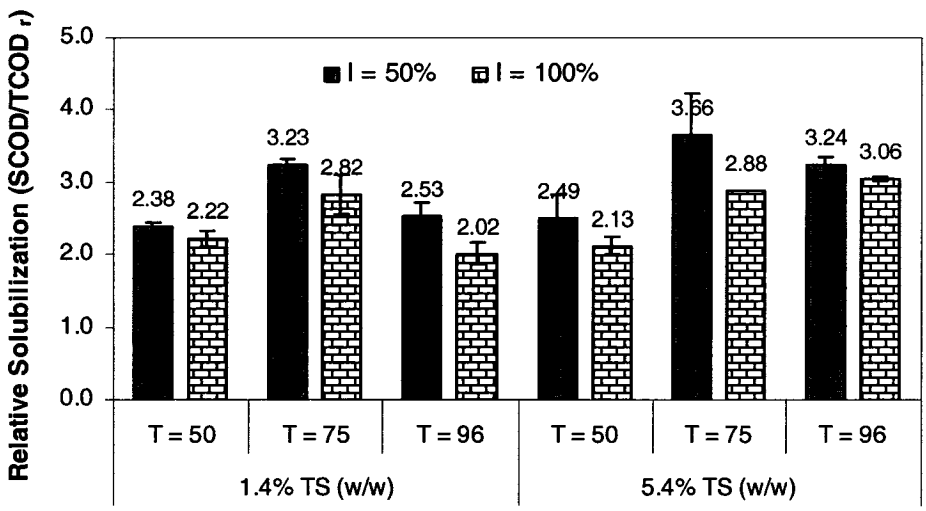

Figure 4-1 Relative (to control) solubilization of TWAS after pretreatment (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; I: microwave intensity; TS = total solids).

Solubilization increased with the TS concentration of WAS. In Figure 4-1, relative SCOD/TCOD values were the highest when samples were irradiated to 75°C at 50% MW intensities for both sludge concentrations indicating that loss of organics by volatilization

could be occurring at 96°C. Solubilization was always slightly higher at 50% than at 100% MW intensities for both sludge concentrations at the same MW temperatures possibly due to longer exposure time to the MW field at low MW intensities.

Solubilization of proteins and sugars was also detected in microwaved TWAS samples (Figure 4-2). MW temperatures of 50, 75 and 96°C resulted in 2.4 ± 0.0, 2.2 ± 0.2 and 4.2 ± 0.3 fold higher soluble protein concentrations compared to the control, respectively. Sugar concentrations in supernatants also increased with temperature, reaching 1.6 and 4.5 fold higher soluble sugars at 50 and 75°C, respectively, and reduced to 3.3 fold at 96°C. The reason for this decrease is unknown but lower sugar concentrations could be resulting from the caramelization or Maillard reactions occurring at high temperatures above 80°C. Millard reactions start between an amino acid and reducing sugar at elevated temperatures and undergo further reaction and polymerization, reducing the solubility of sugars (Labuza and Baisier, 1992). It is possible that at temperatures above 80°C, a portion of reducing sugars in soluble phase (< 0.45 μm) is polymerized and being transformed back to the particulate phase, resulting in a decrease in reducing sugars. There seems to be a disadvantage associated with measuring only reducing sugars, therefore it is recommended to monitor the total sugars as well as the reducing sugars for future thermal pretreatment studies.

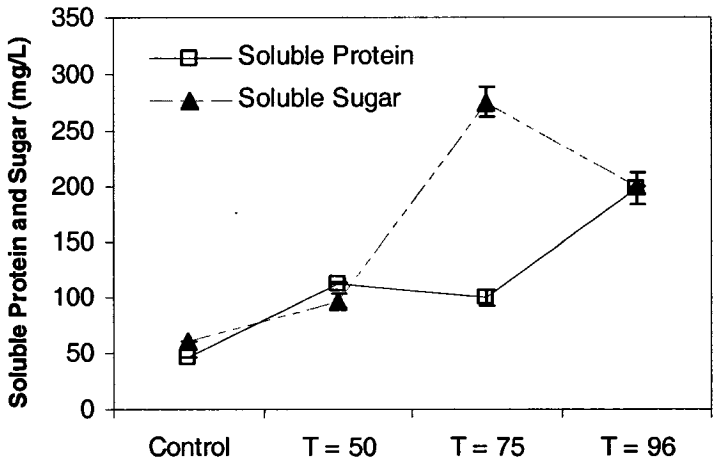

Figure 4-2 Increase in soluble protein and sugar of TWAS with MW temperature (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively, microwave intensity = 100%, TWAS concentration = 5.4% TS w/w).

MW-injured cells are known to release ninhydrin-positive materials such as purines and pyrimidines into suspension which concomitantly absorb UV light at a wavelength of 260 nm (Khalil and Villota 1985; Woo *et al.*, 2000). Figure 4-3 shows the leakage of nucleic acid and its related compounds in units of optical density (OD) at 260 nm as MW temperature increased. In general, the increase in the amounts of these materials along with the release of intracellular proteins indicates damage to bacteria cells at the membrane level especially at elevated MW temperatures. However, some proteins also absorb in these wavelengths due to the presence of the ring structures on the amino acid residues, phenylalanine, tryptophan and tyrosine. Pure nucleic acid absorbs two times more strongly at 260 nm than at 280 nm; therefore pure DNA should have an OD_{260}/OD_{280} ratio of 2:1. In this study OD_{260}/OD_{280} ratios of the supernatants were 1.07, 1.40, 1.35, and 1.85 for the control and at 50, 75 and 96°C, respectively. Although the increase of OD_{260}/OD_{280} ratios towards 2:1 as MW temperature increases would be a strong indication of cell disruption and pure DNA being accumulated in the supernatants

for a pure culture study, the findings of cell lysis products (proteins, DNA) in supernatants of more complex heterogeneous WAS is still somewhat inconclusive with respect to cell lysis. The presence of autolysis products in the EPS matrix complicates a more detailed detection (percentage and characteristic) of intracellular DNA, protein and sugars released to supernatant after MW irradiation (Frølund *et al.*, 1996). However, the results showing dramatic releases of SCOD (Figure 4-1), protein, sugar (Figure 4-2) and DNA (Figure 4-3) into the supernatant phase are compelling evidence of the floc structure of secondary sludge being disintegrated and solubilized as MW temperature increases.

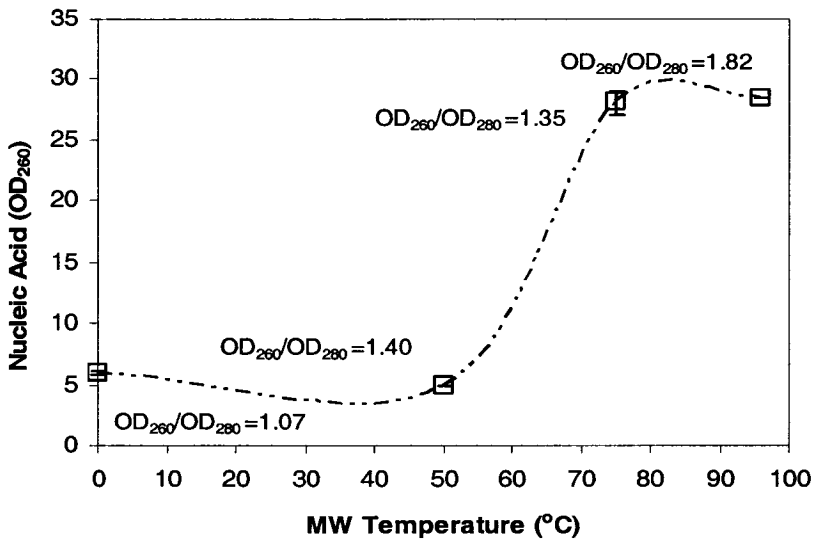

Figure 4-3 Nucleic acid leakage into supernatant of TWAS after MW irradiation (OD_{260}, OD_{280} = optical densities at 260 and 280 nm, respectively; MW: microwave; TWAS concentration = 5.4% TS, w/w; MW intensity = 100%).

4.4.2 Effect of MW Pretreatment on Batch Anaerobic Digesters

The effect of MW irradiation on anaerobic digestion of TWAS was investigated by bench scale mesophilic anaerobic digesters and ultimate (at the end of 35 d of digestion) CBPs are shown for both high and low sludge concentrations in Table 4-2. Figures 4-4a and b indicate relative (to controls) ultimate CBPs from digesters with 3 and 1.4% TS (w/w) WAS concentrations, respectively. For results shown in Figures 4-4a and b, biogas production contributed by inoculum itself (62 ± 0 mL; Table 4-2) was subtracted from the CBP of the other bottles.

In a temperature range of 50-96°C, the severity of the pretreatment temperature, WAS concentration and the volume percentage of MW pretreated WAS present in the reactors influenced the ultimate biogas production. In both Figures 4-4a and b, relative (to control) ultimate CBPs increased with MW temperature, WAS concentration and volume percentage of WAS pretreated. In general, for both low (1.4% TS, w/w) and high (5.4% TS, w/w) sludge concentrations and for digesters with both 50 and 100% (v/v) pretreated WAS, 50 and 100% MW intensities resulted in very similar ultimate CBPs at similar pretreatment temperatures. The results also indicate that within a practical digestion time of 34 days, MW pretreatment enhanced the ultimate degradability of the WAS with an average of 17 and 11% increases over the controls at high (3% TS, w/w) and low (1.4% TS, w/w) sludge concentrations.

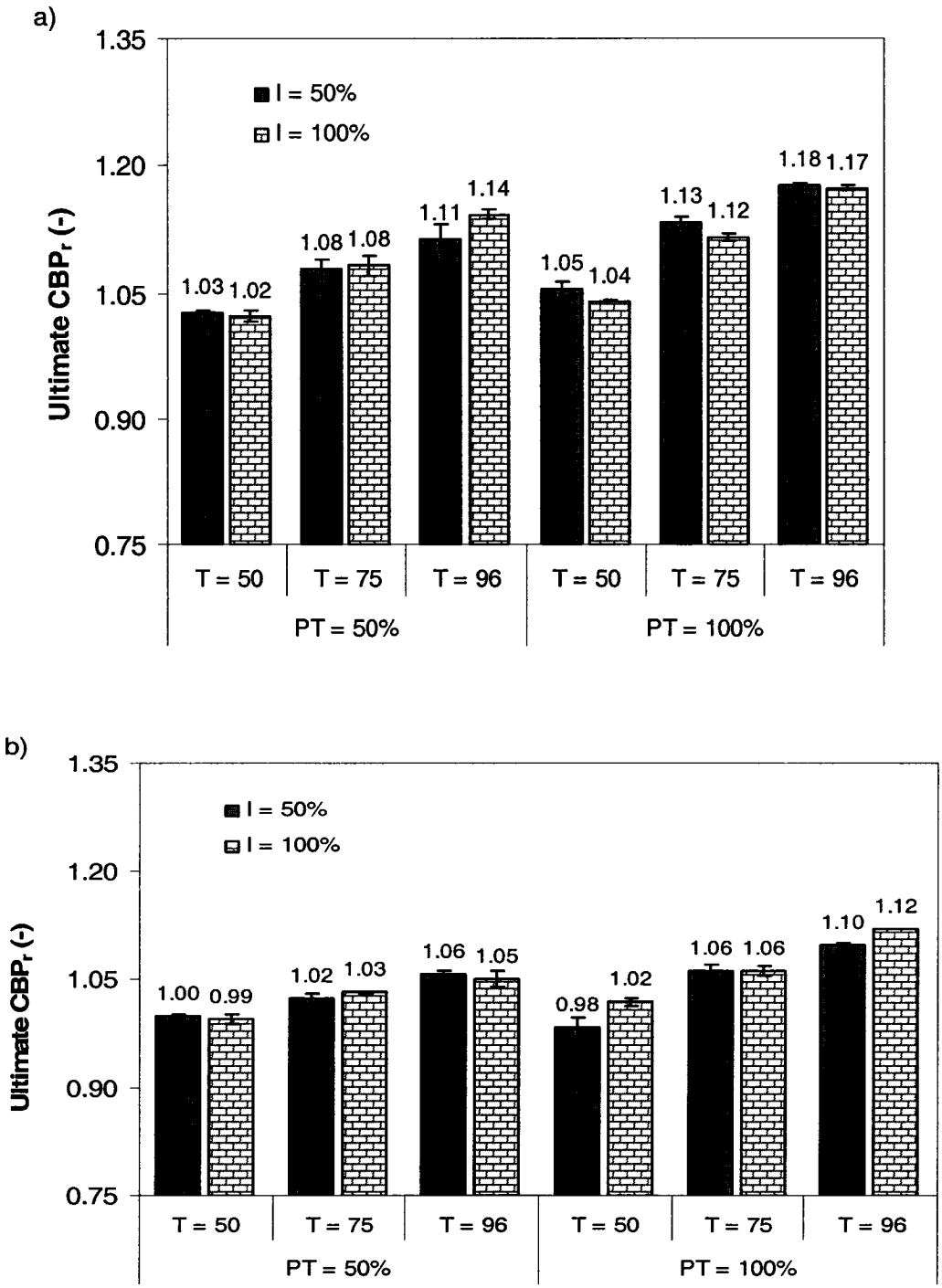

Figure 4-4 Relative (to control) ultimate cumulative biogas production (CBP_r) from pretreated WAS with a) 3% TS (w/w); b) 1.4% TS (w/w); (T = 50, T = 75, T = 96: microwave temperatures at 50, 75, 96°C, respectively; PT: partial treatment or volume percentage of WAS treated in digesters; I: intensity).

4.4.3 Empirical Modeling and Response Surfaces

A software package, *Mathematica 4* (Wolfram Research, Inc., Canada), was used to find the best empirical models for $SCOD/TCOD_r$ $[(SCOD/TCOD_{pretreated})/(SCOD/TCOD_{control})]$ and CBP_r $(CBP_{pretreated}/CBP_{control})$. Models with different structures (from simple to complicated) were tested and R^2 and adjusted R^2 (R^2_a) values were reported in Table 4-3 to evaluate performance of the models. R^2_a gives an adjusted value that can be used to compare different models with different number of experimental data (n) and model parameters (k) to be estimated and is described by equation (4-1):

$$R_a^2 = 1 - \left(\frac{n-1}{n-k}\right)\left(1 - R^2\right) \qquad (4\text{-}1)$$

Table 4-3 Empirical models tested to determine single and multi parameter interactions on solubilization of WAS and enhanced CBP[a].

	Model structure	*k*	R^2	R^2_a	ANOVA table						F ratio	Prob (p)> F
					df		SS		MS			
					Model	Error	Model	Error	Model	Error		
$SCOD/TCOD_r$, X: MW temperature; Y: MW intensity; Z: TWAS concentration, number of experimental data (n) = 24												
1	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z$	4	0.415	0.327	3	20	2.584	3.640	0.861	0.182	4.73	0.012
2	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z+\beta_{12}XY+\beta_{23}YZ+\beta_{13}XZ +\beta_{123}XYZ$	8	0.547	0.348	7	16	3.403	2.821	0.486	0.176	2.76	0.044
3	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z+\beta_4X^2+\beta_5Y^2+\beta_6Z^2$	7	0.745	0.655	6	17	4.638	1.586	0.773	0.093	8.29	0.000
4	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z+\beta_4X^2+\beta_5Y^2+\beta_6Z^2+\beta_{12}XY +\beta_{23}YZ+\beta_{13}XZ+\beta_{123}XYZ$	11	0.877	0.782	10	13	5.457	0.767	0.546	0.059	9.25	0.000
5	**$E(R) = \beta_0+\beta_1Z +\beta_2X^2+\beta_3Y^2+\beta_4YZ+\beta_5XYZ+\beta_6XY^2 +\beta_7YX^2+\beta_8X^3$**	**9**	**0.894**	**0.838**	**8**	**15**	**5.565**	**0.659**	**0.696**	**0.044**	**15.83**	**0.000**
6	$E(R) = \beta_0+\beta_1Z +\beta_2X^2+\beta_3Y^2+\beta_4YZ+\beta_5XYZ+\beta_6XY^2 +\beta_7YX^2+\beta_8X^3+\beta_9Z^4$	10	0.894	0.826	9	14	5.565	0.659	0.618	0.047	13.13	0.000
CBP_r, X: Percentage of TWAS treated; Y: MW temperature; Z: TWAS concentration, number of data (n) = 48												
1	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z$	4	0.920	0.915	3	44	0.130	0.011	0.043	0.000	168.57	0.000
2	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z+\beta_{12}XY+\beta_{23}YZ+\beta_{13}XZ$	7	0.956	0.950	6	41	0.135	0.006	0.023	0.000	149.03	0.000
3	**$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z+\beta_{12}XY+\beta_{23}YZ+\beta_{13}XZ +\beta_{123}XYZ$**	**8**	**0.959**	**0.952**	**7**	**40**	**0.136**	**0.006**	**0.019**	**0.000**	**132.83**	**0.000**
4	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z+\beta_4X^2+\beta_5Y^2+\beta_6Z^2$	7	0.920	0.909	6	41	0.130	0.011	0.022	0.000	79.04	0.000
5	$E(R) = \beta_0+\beta_1X+\beta_2Y+\beta_3Z+\beta_4X^2+\beta_5Y^2+\beta_6Z^2+\beta_{12}XY +\beta_{23}YZ+\beta_{13}XZ+\beta_{123}XYZ$	11	0.959	0.948	10	37	0.136	0.006	0.014	0.000	87.03	0.000

[a]df = degree of freedom; SS = sum of the squares; MS =mean squares; Prob (p) = probability value; CBP_r = relative cumulative biogas production; MW = microwave; E (R) = expected value of the response; k = number of model parameters; R^2 = correlation coefficient; R^2_a= adjusted correlation coefficient; β_0, β_1, …, β_{123} = model parameters (regression coefficients)

In Table 4-3, E(R) represents the expected values of the responses ($SCOD/TCOD_r$ and CBP_r), β_0, β_1, β_2,.., β_{123} are the model parameters or regression coefficients and X, Y, Z indicate parameters T, I, C for $SCOD/TCOD_r$ and PT, T, C for CBP_r, respectively. The parameter microwave intensity (I) was eliminated from CBP_r modeling due to a previous study that showed insignificant effects on biogas production according to multifactor analysis of variance (ANOVA) (Eskicioglu *et al.*, 2006). ANOVA results given in Table 4-3 partition the variability in the response variables ($SCOD/TCOD_r$ and CBP_r) into two parts, one explained by the "Model" and the remaining portion is the uncertainty (Error) that remains after the model is used. The "Model" is considered to be statistically significant if it can account for a large amount of variability in the response. The amount of uncertainty or variability can be measured by the total sum of squares (SS). The SS can be written Σ $(y-ybar)^2$, where ybar is the sample mean. Each SS has corresponding degrees of freedom (df). Total df is one less than the number of observations, n−1. The Model df is the number of independent variables in the model (k) and the Error df is the difference between the Total df (n−1) and the Model df (k), that is, n−k−1. The mean squares (MS) are the SS divided by the corresponding df.

The F value or ratio (Model MS/Error MS) is a statistical test commonly used to judge whether the Model as a whole has statistically significant predictive capability, that is, whether the regression SS is high enough, considering the number of variables necessary to describe it. Under the null hypothesis (Ho) that the Model has no predictive capability--all population regression coefficients are 0 simultaneously--the F statistic follows an F distribution with k numerator degrees of freedom and n−k−1 denominator degrees of freedom. The Ho is rejected if the F ratio is large.

Experimental results for TWAS solubilization ($SCOD/TCOD_r$) was best represented with the model structures incorporating terms for interaction among the parameters (X, Y, Z) and a third-order X (MW temperature) variable (models # 5 and 6; Table 4-3). Among model structures tested in Table 4-3, model # 5 was judged the best with the largest R^2, R^2_a and F ratios. Similarly for biogas production, first-order models (models # 2 and 3), which include terms for interactions among X, Y and Z, were more successful than second-order models (models # 4 and 5). In Table 4-3, model # 3 was selected for CBP_r with the largest R^2, R^2_a values. Addition of second-order parameters to the empirical equations for CBP_r (model # 4 and 5) did not improve the correlation coefficients and they had statistically less significant predictive capacities (smaller F ratios) than models # 1, 2 and 3.

Table 4-4 summarizes the parameter (regression coefficient) estimation, standard error (SE), T statistic tests (TStat) results obtained for each regression coefficient in the selected (best) models. SE can be used for hypothesis testing and constructing confidence intervals. For example; confidence intervals for the TWAS concentration (Z) variable for $SCOD/TCOD_r$ are constructed as (estimated value ± a* SE or $1.20*10^{-1} \pm a* 6.76*10^{-2}$), where a is the appropriate constant depending on the level of confidence desired. For 95% confidence intervals based on large samples, a would be 1.96. The TStat tests the hypothesis that a regression coefficient is zero "when the other predictors are in the model". TStat is the ratio of the sample regression coefficient to its SE. More accurately, it has the form (estimate – hypothesized value)/SE. Since the hypothesized value is zero, the statistic reduces to estimate/SE.

Table 4-4 Parameter estimation results for the models selected to describe solubilization of WAS and enhanced CBP[a].

Regression coefficients	Variables	Estimated value	SE	TStat	Prob (p)> |T|
$SCOD/TCOD_r$, X: MW temperature; Y: MW intensity; Z: TWAS concentration					
β_0	1	$-2.01*10^{-1}$	$6.13*10^{-1}$	−0.329	***0.747***
β_1	Z	$1.20*10^{-1}$	$6.76*10^{-2}$	1.767	***0.098***
β_2	X^2	$1.41*10^{-3}$	$2.04*10^{-4}$	6.909	0.000
β_3	Y^2	$4.39*10^{-4}$	$2.34*10^{-4}$	1.875	***0.080***
β_4	YZ	$-4.87*10^{-3}$	$1.36*10^{-3}$	−3.571	0.003
β_5	XYZ	$6.13*10^{-5}$	$1.44*10^{-5}$	4.263	0.001
β_6	XY^2	$-1.22*10^{-5}$	$6.74*10^{-6}$	−1.815	***0.090***
β_7	YX^2	$1.09*10^{-5}$	$6.92*10^{-6}$	1.568	***0.138***
β_8	X^3	$-1.54*10^{-5}$	$3.12*10^{-6}$	−4.941	0.000
$R^2 = 0.894$, $R^2_a = 0.838$					
CBP_r, X: Percentage of TWAS treated; Y: MW temperature; Z: TWAS concentration					
β_0	1	1.01	$4.40*10^{-2}$	22.965	0.000
β_1	X	$-1.36*10^{-3}$	$5.56*10^{-4}$	−2.445	0.019
β_2	Y	$-4.26*10^{-4}$	$5.77*10^{-4}$	−0.736	***0.466***
β_3	Z	$-1.82*10^{-2}$	$0.01*10^{-2}$	−1.629	***0.111***
β_{12}	XY	$2.62*10^{-5}$	$7.32*10^{-6}$	3.574	0.001
β_{23}	YZ	$4.02*10^{-4}$	$1.47*10^{-4}$	2.739	0.009
β_{13}	XZ	$2.52*10^{-4}$	$1.41*10^{-4}$	1.783	***0.082***
β_{123}	XYZ	$-2.94*10^{-6}$	$1.86*10^{-6}$	−1.587	***0.120***
$R^2 = 0.959$, $R^2_a = 0.952$					

[a]$SCOD/TCOD_r$ = relative solubilization; CBP_r = relative cumulative biogas production; SE = standard error; TStat = T statistics test; Prob (p) = probability; R^2 = correlation coefficient; R^2_a= adjusted correlation coefficient; $\beta_0, \beta_1, \ldots, \beta_{123}$ = model parameters (regression coefficients).

Prob > |T| labels in Table 4-4 are the p values or the observed significance levels for the T statistics. The degrees of freedom used to calculate the p values are given by the Error df from the ANOVA table (Table 4-3). The p values show whether a variable has statistically significant predictive capability in the presence of the other variables, that is, whether it adds something to the equation. In some circumstances, an insignificant p value might be used to determine whether to remove a variable from a model without significantly reducing the model's predictive capability. In Table 4-4, CBP_r model parameters of β_2, β_3, β_{13}, β_{123} could be deemed insignificant at 95% confidence intervals and removed from the model, since they had p values larger than 0.05. The reduced form of the model would have a structure displayed in Table 4-5 with slightly smaller correlation coefficients ($R^2 = 0.954$; $R^2_a = 0.951$), however with much improved F ratio ($F = 304.73$) and p values for each model parameter (<0.05).

However, in some cases, a variable that does not have predictive capability in the presence of the other predictors may have predictive capability when some of those predictors are removed from the model. In Table 4-4, β_0, β_1, β_3, β_6, β_7 parameters of $SCOD/TCOD_r$ were eliminated since they had p values > 0.05 and the predictive capacity of the reduced model ($R^2 = 0.56$; $R^2_a = 0.47$; $F = 6.07$; Table 4-5) was significantly lower than the original model ($R^2 = 0.894$; $R^2_a = 0.838$; $F = 15.83$; Table 4-4). Therefore, while the reduced model (Table 4-5) is used for CBR_r, the original model (Table 4-4) was retained to simulate experimental $SCOD/TCOD_r$ results. Observed and simulated $SCOD/TCOD_r$ and CBP_r values were reported in Tables 4-6 and 4-7, respectively.

Table 4-5 Parameter estimation results of the reduced models to describe solubilization of WAS and enhanced CBP[a].

Model structure	k	R^2	R^2_a	ANOVA table							
				df		SS		MS		F ratio	p> F
				Model	Error	Model	Error	Model	Error		
$SCOD/TCOD_r$, X: MW temperature; Y: MW intensity; Z: TWAS concentration											
$E(R) = \beta_0+\beta_2X^2+ \beta_4YZ+ \beta_5XYZ +\beta_8X^3$	5	0.56	0.47	4	19	3.49	2.73	0.87	0.14	6.07	0.003
Regression coefficients	**Variables**	**Estimate**	**SE**	**TStat**	**(p)> |T|**						
β_0	1	$-8.07*10^{-1}$	$6.34*10^{-1}$	1.272	0.219						
β_2	X^2	$1.21*10^{-3}$	$3.41*10^{-4}$	3.551	0.002						
β_4	YZ	$-2.94*10^{-3}$	$1.74*10^{-3}$	−1.686	0.108						
β_5	XYZ	$4.43*10^{-5}$	$2.29*10^{-5}$	1.933	0.068						
β_8	X^3	$1.08*10^{-5}$	$2.93*10^{-6}$	−3.702	0.002						
CBP_r, X: Percentage of TWAS treated; Y: MW temperature; Z: TWAS concentration											
$E(R) = \beta_0+\beta_1X+\beta_{12}XY+ \beta_{23}YZ$	4	0.954	0.951	3	44	0.135	0.007	0.045	0.000	304.73	0.000
Regression coefficients	**Variables**	**Estimate**	**SE**	**TStat**	**p> |T|**						
β_0	1	$9.70*10^{-1}$	$6.25*10^{-3}$	155.28	0.000						
β_1	X	$-7.61*10^{-4}$	$1.17*10^{-4}$	−6.510	0.000						
β_{12}	XY	$1.96*10^{-5}$	$1.27*10^{-6}$	15.452	0.000						
β_{23}	XYZ	$1.91*10^{-4}$	$1.14*10^{-5}$	16.724	0.000						

[a]df = degree of freedom; SS = sum of the squares; MS =mean squares; Prob (p) = probability value; CBP_r = relative cumulative biogas production; MW = microwave; E (R) = expected value of the response; k = number of model parameters; R^2 = correlation coefficient; R^2_a= adjusted correlation coefficient; β_0, β_1, …, β_{123} = model parameters (regression coefficients)

Table 4-6 Comparison of SCOD/TCOD$_r$ to predicted values[a].

Run #	T (°C)	I (%)	C (% TS, w/w)	SCOD/TCOD$_r$ (-)	
				Observed	**Predicted**
$1/1_d$	50	50	1.4	2.36 (0.10)†	2.36 {0.52}*
$2/2_d$	50	50	5.4	2.47 (0.20)	2.48 {0.52}
$3/3_d$	50	100	1.4	2.22 (0.02)	2.29 {0.54}
$4/4_d$	50	100	5.4	2.12 (0.00)	2.05 {0.54}
$5/5_d$	75	50	1.4	3.23 (0.11)	3.22 {0.52}
$6/6_d$	75	50	5.4	3.63 (0.36)	3.65 {0.52}
$7/7_d$	75	100	1.4	2.81 (0.11)	2.66 {0.52}
$8/8_d$	75	100	5.4	2.88 (0.17)	3.03 {0.52}
$9/9_d$	96	50	1.4	2.53 (0.03)	2.54 {0.52}
$10/10_d$	96	50	5.4	3.24 (0.06)	3.22 {0.52}
$11/11_d$	96	100	1.4	2.01 (0.02)	2.09 {0.53}
$12/12_d$	96	100	5.4	3.06 (0.16)	2.98 {0.53}

[a]T = microwave temperature; I = microwave intensity; C = concentration of TWAS; SCOD/TCOD$_r$ = relative (to control) soluble to total chemical oxygen demand ratios.
†Data represent arithmetic mean of duplicates (± absolute difference between mean and duplicates).
*Data represent predicted values from software {± confidence integrals}.

Table 4-7 Comparison of CBP_r to predicted values[a].

Run #	PT (%, v/v)	T (°C)	I (%)	C (%, w/w)	CBP_r (-)	
					Observed	Predicted
$1/1_d$	50	50	50	1.4	1.00 (0.00)†	0.99 {0.03}*
$2/2_d$	50	50	50	3.0	1.03 (0.00)	1.03 {0.03}
$3/3_d$	50	50	100	1.4	0.99 (0.01)	0.99 {0.03}
$4/4_d$	50	50	100	3.0	1.02 (0.01)	1.03 {0.03}
$5/5_d$	50	75	50	1.4	1.02 (0.01)	1.03 {0.03}
$6/6_d$	50	75	50	3.0	1.08 (0.00)	1.08 {0.03}
$7/7_d$	50	75	100	1.4	1.03 (0.00)	1.03 {0.03}
$8/8_d$	50	75	100	3.0	1.08 (0.01)	1.08 {0.03}
$9/9_d$	50	96	50	1.4	1.06 (0.00)	1.05 {0.03}
$10/10_d$	50	96	50	3.0	1.11 (0.02)	1.13 {0.03}
$11/11_d$	50	96	100	1.4	1.05 (0.01)	1.05 {0.03}
$12/12_d$	50	96	100	3.0	1.14 (0.01)	1.13 {0.03}
$13/13_d$	100	50	50	1.4	0.98 (0.01)	1.01 {0.03}
$14/14_d$	100	50	50	3.0	1.05 (0.01)	1.04 {0.03}
$15/15_d$	100	50	100	1.4	1.02 (0.01)	1.01 {0.03}
$16/16_d$	100	50	100	3.0	1.04 (0.01)	1.04 {0.03}
$17/17_d$	100	75	50	1.4	1.06 (0.01)	1.06 {0.03}
$18/18_d$	100	75	50	3.0	1.13 (0.01)	1.12 {0.03}
$19/19_d$	100	75	100	1.4	1.06 (0.01)	1.06 {0.03}
$20/20_d$	100	75	100	3.0	1.12 (0.00)	1.12 {0.03}
$21/21_d$	100	96	50	1.4	1.10 (0.00)	1.11 {0.03}
$22/22_d$	100	96	50	3.0	1.18 (0.00)	1.18 {0.03}
$23/23_d$	100	96	100	1.4	1.12 (0.00)	1.11 {0.03}
$24/24_d$	100	96	100	3.0	1.17 (0.00)	1.18 {0.03}

[a]PT = percentage of TWAS pretreated; T = microwave temperature; I = microwave intensity; C = concentration of TWAS; CBP_r = relative (to control) cumulative biogas production.
†Data represent arithmetic mean of duplicates (± absolute difference between mean and duplicates).
*Data represent predicted values from software {± confidence integrals}.

Figures 4-5 and 4-6 display the predicted three-dimensional response surfaces generated by the models selected. In Figure 4-5, TWAS solubilization was the highest at 75°C and at 50% MW intensity for both 1.4 and 5.4% TS TWAS concentrations and observed SCOD/$TCOD_r$ values increased as % TS concentration of pretreated TWAS increased. Figure 4-6 shows similar trends to Figure 4-5 but clearly indicates the advantage of lower MW intensity (50%) and lower temperatures with lower TS concentrations on improved solubilization. At the lower sludge concentrations and lower MW intensities, the additional water in the system and longer exposure for heating may increase the thermal solubilization response since polar molecules of water are one of the simple MW absorbers. These results are in agreement with the experimental results. It is important to emphasize that TWAS solubilization results obtained in this study were also a function of pretreatment equipment used. In this study, due to problems related to monitoring temperature profiles inside a kitchen type MW oven, such as interaction with the MW field causing local heating within the sample (Lorenz 1999; Loupy, 2002), temperature measurements were done outside the MW oven as soon as microwaving was terminated. It is likely that some of the organics were being lost at high pretreatment temperatures (96°C) after the container was removed from MW and while the sample was vigorously stirred and the maximum temperature was recorded within 10 s with thermocouple probes. As a result of this, SCOD/TCOD values first increased from 0.06 ± 0.00 to 0.20 ± 0.02 in a MW temperature range of 50-75°C and decreased to 0.18 ± 0.00 at 96°C. If a more sophisticated MW system which incorporates the proper temperature probes inside the heating vessel was used, the SCOD/TCOD profile would indicate a linear relation. Further experiments are required to prove this hypothesis.

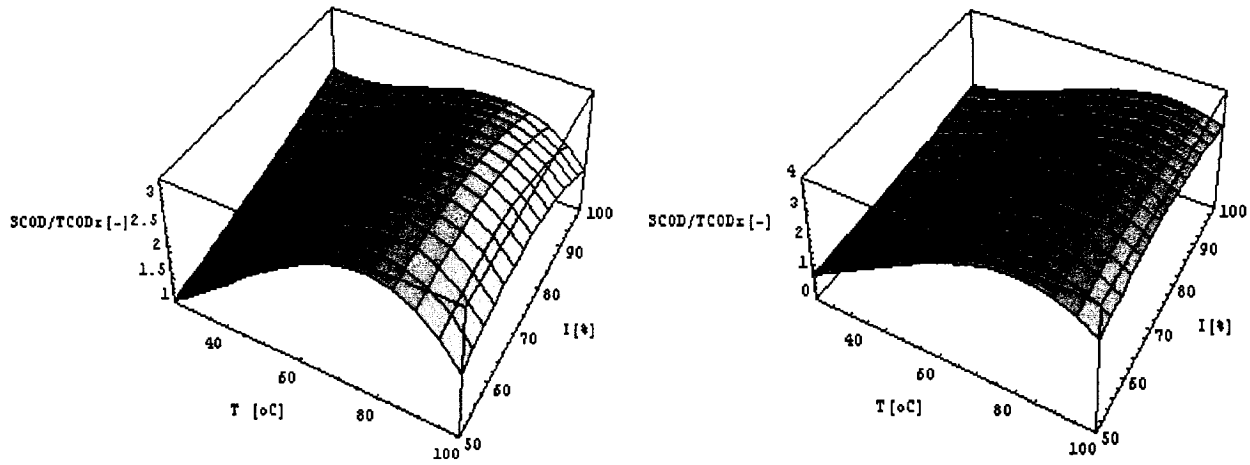

Figure 4-5 Predicted relative (to control) solubilization ($SCOD/TCOD_r$) for TWAS with 1.4 (on the left) and 5.4 (on the right) % TS (w/w) as a function of T and I [T = microwave temperature (°C), I: microwave intensity (%)].

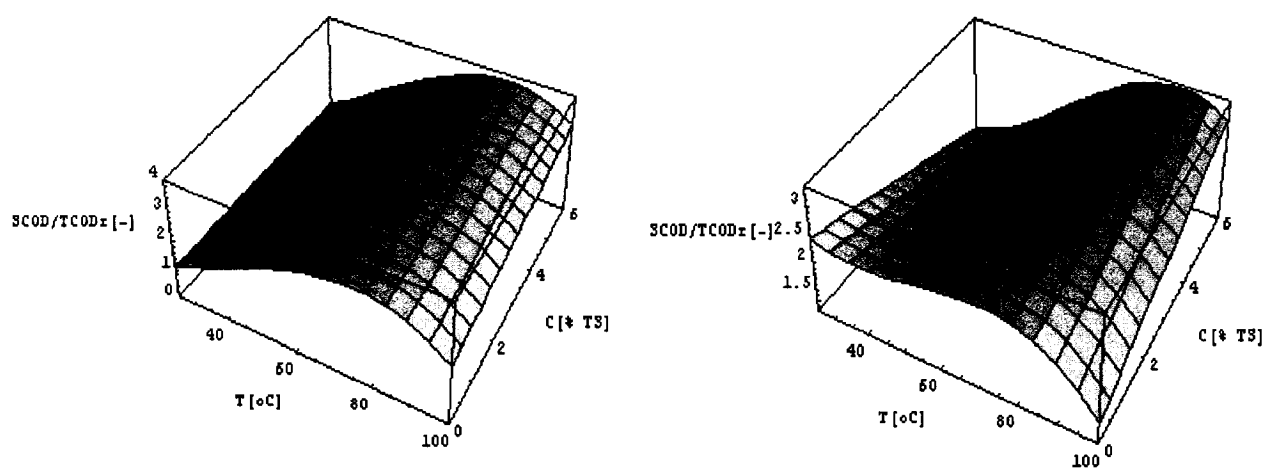

Figure 4-6 Predicted relative (to control) solubilization ($SCOD/TCOD_r$) for TWAS pretreated at 100% (on the left) and 50% (on the right) microwave intensities as a function of T and C [T = microwave temperature (°C), C: TWAS concentration (%TS, w/w)].

The reduced model [equation (4-2)] used for CBP_r simulation shows that low T (around 40-50°C) MW pretreatment could only yield CBP_r value of 1 (no or insignificant improvement over control) even when 100% (v/v) of sludge in batch digesters was pretreated (Figure 4-7, on the left). This result is in agreement with the experimental observations. Similar to solubilization results, MW temperature and % TS of TWAS pretreated (Figure 4-8) created a significant effect on biogas production from digesters with a CBP_r value of 1.17 (17% higher biogas production compared to controls) at higher temperatures and TS concentrations (96°C and 5.4% TS concentration, respectively, Figure 4-7, on the right). Based on the shape of the responses for CBP, it may be speculated that further work at higher temperature and higher concentrations may be warranted especially if a MW, which has ability to minimize loss of volatile components released at high temperature, is used.

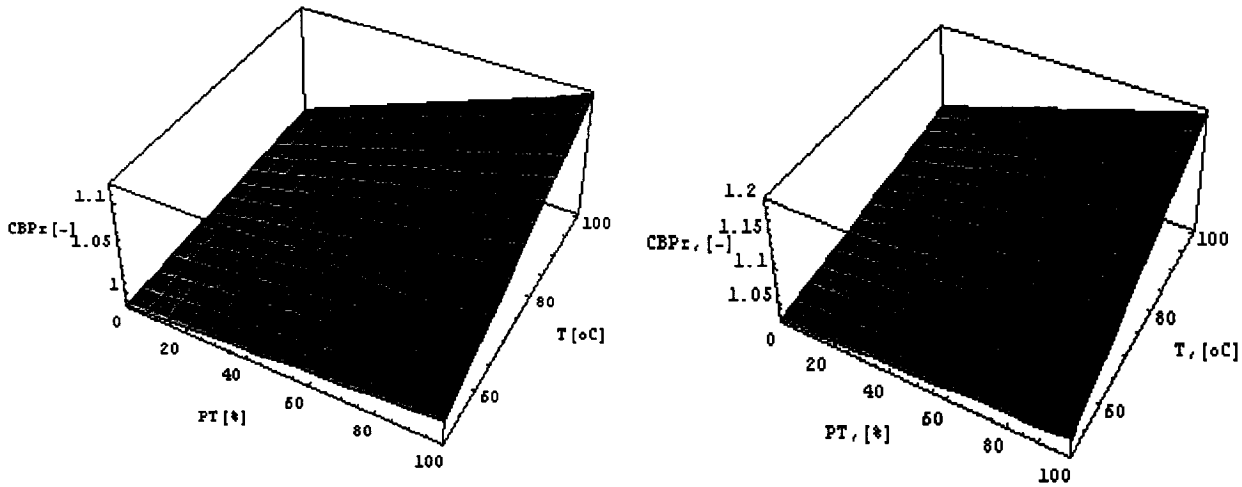

Figure 4-7 Predicted relative (to control) cumulative biogas production (CBP_r) from digesters with TWAS pretreated at 1.4 (on the left) and 5.4 (on the right) % TS (w/w) as a function of PT and T [PT = percentage of TWAS pretreated (v/v); T = microwave temperature (°C)].

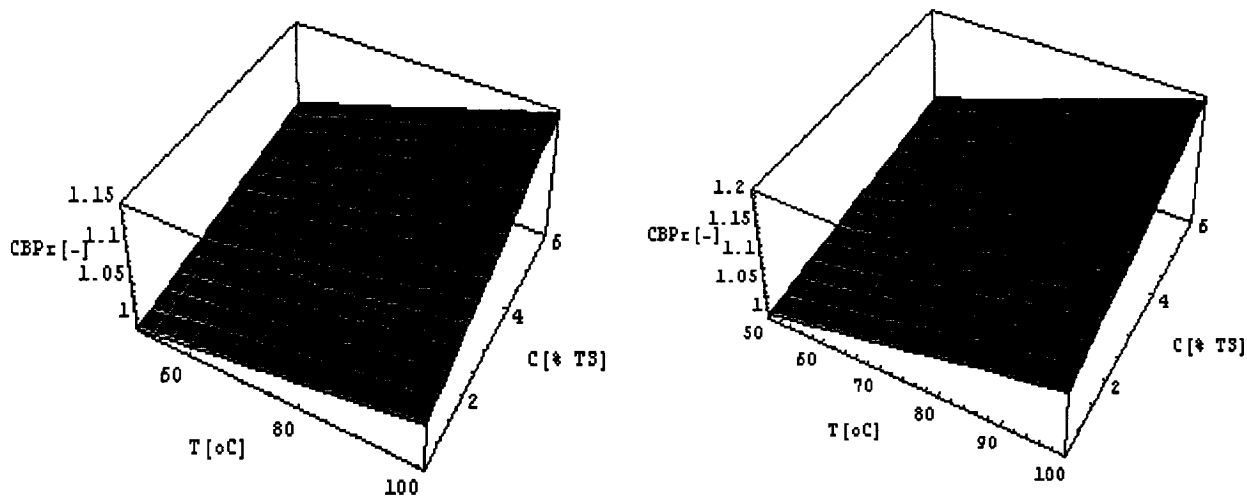

Figure 4-8 Predicted relative (to control) cumulative biogas production (CBP_r) from digesters with 50 (on the left) and 100% (on the right) (v/v) pretreated TWAS as a function of T and C [T = microwave temperature (°C), C: TWAS concentration at pretreatment stage (%TS, w/w)].

Distribution of residuals can indicate whether assumptions made here (residuals are independently and normally distributed) are reasonable and our choice of model is appropriate. The three most common plots to examine the residuals are histograms, normal distribution and dot plots. Since most of the experimental designs including this study have limited treatment options, sample sizes of residuals are generally small (<50) so a histogram is not the best choice for judging the distribution of residuals. In this study, a more sensitive graph, the normal probability plot was used for residual analysis. Normal probability plots for $SCOD/TCOD_r$ and CBP_r (Figures 4-9 and 4-10, respectively) imply that it is reasonable to assume that the random errors for these predictions are drawn from approximately normal distributions. In each case there is a clear linear relationship ($R^2 = 0.92$ in Figure 4-9 and $R^2 = 0.89$ in Figure 4-10) between the residuals and the theoretical values from the standard normal distribution. Of course, the residuals plots do indicate that the relationship is not perfectly deterministic (and it will not be in most cases), but the linear relationship is still clear and the choice of models is appropriate.

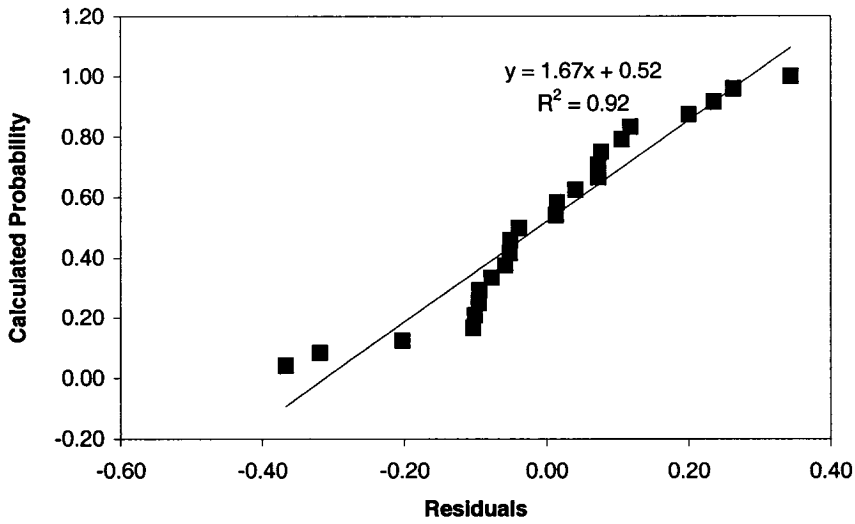

Figure 4-9 Normal probability plot of residuals from SCOD/TCOD$_r$ modeling.

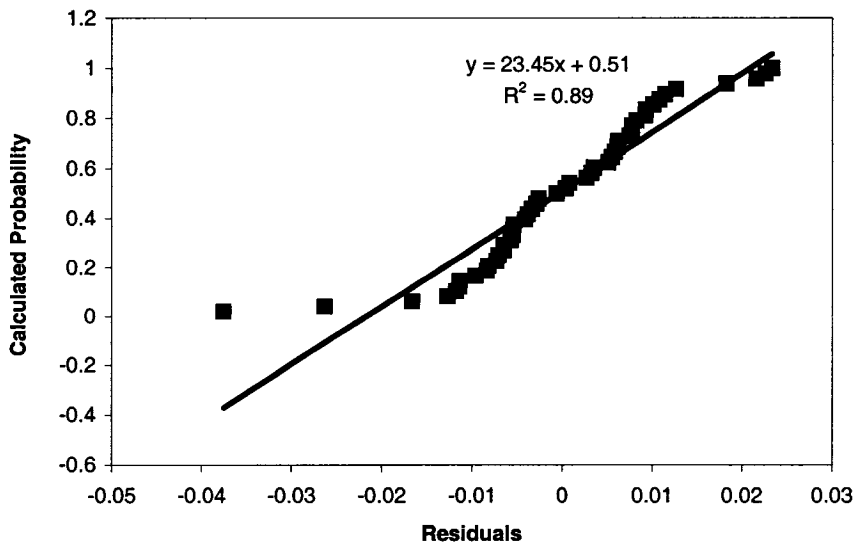

Figure 4-10 Normal probability plot of residuals from CBP$_r$ modeling.

4.5 Conclusions

The results of this study indicated that MW-irradiation has a potential of damaging activated sludge floc structure and cell membranes and releasing extracellular and possibly intracellular compounds (proteins, sugars and nucleic acid) along with the

solubilization of particulate COD. The SCOD/TCOD ratio of TWAS increased from 0.06 ± 0.00 to 0.14 ± 0.01, 0.20 ± 0.02 and 0.18 ± 0.00 at MW temperatures of 50, 75 and 96°C. MW pretreatment also increased the bioavailability of sludge components under anaerobic digestion with 17 ± 0.00% higher ultimate cumulative biogas productions compared to controls after 34 days of batch mesophilic digestion.

4.6 References

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CHAPTER 5

Enhancement of Continuous Flow Waste Activated Sludge Digestion by Microwave Pretreatment

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5.1 Abstract

Effects of microwave (MW) pretreatment on thickened waste activated sludge (TWAS) in mesophilic semi-continuous (SC) anaerobic digestion with acclimatized inoculum at sludge retention times (SRTs) of 5, 10 and 20 d are presented. Two pretreatment temperatures (50 and 96°C) were tested in a total of 10 SC digesters including duplicates and controls. Digesters using conventionally heated (CH) TWAS were also run to investigate *thermal* and *athermal* effects of MW pretreatment. Soluble chemical oxygen demand (SCOD) to total COD (TCOD) ratios of 0.07 for untreated TWAS increased to 0.15-0.2 for MW and CH pretreated TWAS samples. Solubilization was always higher after CH compared to MW heating at similar temperatures due to the extended exposure of CH to achieve a given temperature compared to MW irradiation. In general, incremental increases in total solid (TS), volatile solids (VS) and TCOD removal efficiency of pretreated digesters compared to controls dramatically increased as SRT was gradually shortened from 20 to 10 to 5 d. TWAS pretreated to 96°C by MW and CH achieved 29 and 32% higher TS and 23 and 26% higher VS removal efficiencies compared to controls at SRT of 5 d, while similar reactors at SRT of 20 d had only 16% higher TS and 11 and 12% higher VS removals than those of controls, respectively. SCOD and ammonia concentrations increased in digester effluents as the pretreatment temperature was increased and as SRT was shortened. Control samples were the most difficult to dewater for all SRTs. Results indicated that MW pretreatment is a good alternative to CH to enhance the biodegradability and dewaterability of the TWAS before anaerobic digestion.

KEYWORDS: Microwave, conventional heating, TWAS, continuous flow, SRT, pretreatment, anaerobic.

5.2 Preliminary Studies on MW Pretreatment of WAS

Recently, attempts involving thermal (Jolis *et al.*, 2004; Skiadas *et al.*, 2004), chemical (Chu *et al.*, 2002; Stephenson *et al.*, 2003), ultrasonic or mechanical pretreatments (Muller *et al.*, 2003; Cartmell *et al.*, 2004) have been made to disintegrate the floc structure of activated sludge and to extract both intracellular (within the microbial cell) and extracellular materials (within the polymeric network) before it is sent to anaerobic digesters. In most of the studies, pretreatments solubilized the waste activated sludge (WAS), which subsequently improved anaerobic digestion. Among the pretreatment methods studied to date, thermal disintegration methods with high temperatures (160-170°C) and pressures (600-800 kPa) appear to be superior with a surplus energy gained due to higher biogas production and better pathogen removal efficiency compared to controls (Barnard *et al.*, 2002; Abraham and Kepp, 2003).

MW heating is a new alternative pretreatment method, which supports sustainable development since it consumes less energy than CH due to lower thermal losses in transferring energy. Initial MW pretreatment studies have been conducted at temperatures less than 100°C and focused on understanding basic phenomena occurring during MW irradiation of municipal sludge, such as MW penetration depths, temperature depth profiles and death of coliforms in order to produce Class A sludge. Using MW irradiation, fecal coliforms were not detectable in primary sludge (PS) and WAS pretreated to 65 and 85°C, respectively (Hong, 2002). Solubilization of WAS due to MW irradiation have also been reported and SCOD/TCOD ratios of WAS increased from 0.08 (control) to 0.18 after MW to 70°C (Hong, 2002) and from 0.06 (control) to 0.18 after MW to 96°C (Eskicioglu *et al.*, 2006). SCOD/TCOD ratios of 0.19 and 0.21 were also

reported for WAS MW-irradiated to 91°C and boiling temperatures, respectively (Park *et al.*, 2004). MW-irradiation was also successful in damaging bacteria cell membranes and releasing intracellular and extracellular components such as; proteins, sugars and DNA from the floc polymeric network to supernatant phase along with the solubilization of particulate COD (Chapter 4, Section 4.4.1). Park *et al.* (2004) studied SC anaerobic digesters using pretreated WAS microwaved to 91°C at 8, 10, 12 and 15 d SRTs and reported 64 and 31% higher COD removal and methane production, respectively, compared to controls at SRT of 15 d. Hong (2002) also studied performance of SC digesters at SRTs of 20, 15, 10 and 5 d after microwaving sludge; however, results related to biogas production, VS/TS, and TCOD treatment efficiencies were not reported since their study focused on fecal coliform destruction in continuous flow digesters (Hong *et al.*, 2004).

Except for Park *et al.* (2004), MW sludge pretreatment has focused on batch rather than continuous flow anaerobic digesters, which are used in full-scale applications. Zheng (2005) studied the effects of MW irradiation [temperature and sludge concentration ranges of 35-90°C and 1-4% TS (w/w), respectively] on PS digestion and observed the highest degree of improvement (45% higher biogas production compared to control) when sludge concentration of batch digesters was increased to 4% TS (w/w) at a MW pretreatment temperature of 90°C. This research emphasized the importance of the "*sludge concentration*" factor in pretreatment studies, which was also corroborated by other researchers (Barber, 2002; Eskicioglu *et al.*, 2006). However, results from a MW pretreatment batch study using combined (primary + secondary) waste sludge from a sequencing batch reactor (SBR) operated at an SRT of 20 d found the opposite result.

Using a statistical approach, Thibault (2005) first analyzed the effects of MW temperature (in a range of 45-85°C), MW intensity (60-100%) and sludge concentration [1.5-4% TS (w/w)] by simply looking at the SCOD/TCOD ratios of the pretreated mixed PS/WAS SBR sludge and concluded that MW intensity and sludge concentration did not have an effect on solubilization. Based on this conclusion, sludge pretreatment concentration was kept constant [~2% TS (w/w)] and batch mesophilic digesters exhibited 16% higher biogas production than the control after MW pretreatment at 85°C. These results may be due to the type of sludge pretreated in Thibault's study [extended aeration mixed (PS+WAS) SBR sludge], since the highest solubilization ratio (SCOD/TCOD) achieved was only 0.07 at a MW temperature of 85°C which was much lower than ratios observed at similar temperatures in other MW studies (Hong, 2002; Park *et al.*, 2004). In order to further increase solubilization, Thibault (2005) tried to weaken cell membranes with sludge exposure to 2 g NaOH/L overnight followed by MW pretreatment (at 85°C). Although chemical addition followed by MW heating elevated the SCOD/TCOD ratio to ~0.18, these samples did not produce more biogas than MW-irradiated sludge alone indicating that the increased soluble material was most likely recalcitrant in nature. Dewaterability of digester sludge effluent from digesters treating MW-irradiated sludges deteriorated which does not agree with other MW and thermal pretreatment studies (Barnard *et al.*, 2002; Dereix *et al.*, 2005; Eskicioglu *et al.*, 2006). It is possible that initial sludge characteristics may influence final pretreatment outcomes so that general statements of performance cannot always be made.

Eskicioglu *et al.* (2006) used a *multilevel factorial* statistical design to differentiate the effects of WAS concentration exposed to MW pretreatment, MW temperature,

percentage mixtures of MW pretreated and un-pretreated WAS and MW intensity on mesophilic anaerobic batch digestion using MW acclimated inoculum. The first three factors were found to be the most important parameters governing biogas production while MW intensity during pretreatment had no statistically significant effect on biogas production at the 95% confidence level which supports solubilization results reported by Thibault (2005). MW pretreatment temperatures of 50-96°C were evaluated using mesophilic batch digesters. TWAS microwaved to 96°C resulted in the highest improvement in the amount of biogas produced with 15 ± 0.5% and 20 ± 0.3% increases over controls after 19 d of digestion at low [1.4% TS (w/w)] and high [5.4% TS (w/w)] sludge concentrations, respectively. MW pretreated TWAS reactors also experienced 15 ± 2% higher ammonia concentrations after digestion and dewaterability of digested sludge was significantly enhanced over the controls (Eskicioglu *et al.*, 2006). The primary objective of this paper was to verify the effects of MW pretreatment of TWAS on mesophilic SC anaerobic digesters at SRTs of 5, 10 and 20 d.

Despite a limited number of studies on MW pretreatment to enhance sludge digestion, the effectiveness of MW on microbial viability (sterilization) has been investigated. Interestingly, the mechanism of MW sterilization is still not fully understood. The death of organisms is considered to be due to the *thermal* effect of MW exposure (Stiles, 1963; Vela *et al.*, 1979; Fung *et al.*, 1980) and there was no evidence of a *non-thermal* effect or it was indistinguishable from the *thermal* effect (Vela *et al.*, 1979; Jeng *et al.*, 1987; Welt *et al.*, 1994). However, other MW researchers have argued that death of cells at temperatures lower than their thermal death point has also been observed (Cunningham, 1978; Dreyfuss *et al.*, 1980; Khalil and Villota, 1985). MW-irradiated

microbial cells showed greater damage than CH cells to similar temperatures (Kakita, 1995; Kozempel *et al.*, 1998; Hong *et al.*, 2004). These studies have premised an additional effect called *non-thermal (athermal* or *microwave)* effect caused by polarized parts of macromolecules lining up with the poles of the electromagnetic field (orientation or pearl chain) resulting in possible breakage of hydrogen bonds leading to denaturation and death (Kingston and Jassie, 1988; Loupy, 2002).

MW pretreatment studies of WAS have not yet addressed the thermal versus athermal issue. If a significant athermal effect indeed existed, MW-irradiated WAS pretreated to lower temperatures would show better primary treatibility performance (increased rates or overall biogas production, COD or VS destruction) or secondary performance (improved solubility/denaturation or dewaterability) along with the higher pathogen removal efficiency reported by Hong *et al.* (2004), than CH sludges. The secondary objective of this paper was to ascertain whether thermal and athermal effects of MWs were distinguishable by simply comparing the performances of mesophilic anaerobic digesters treating MW and CH TWAS samples.

5.3 Materials and Methods

5.3.1 Experimental Design

This paper evaluates the effects of MW pretreatment on TWAS using acclimated inoculum in SC anaerobic digesters operating at SRTs of 5, 10, and 20 d. Two pretreatment temperatures (low and high) were tested in a total of 10 SC reactors including duplicates and controls (Figure 5-1).

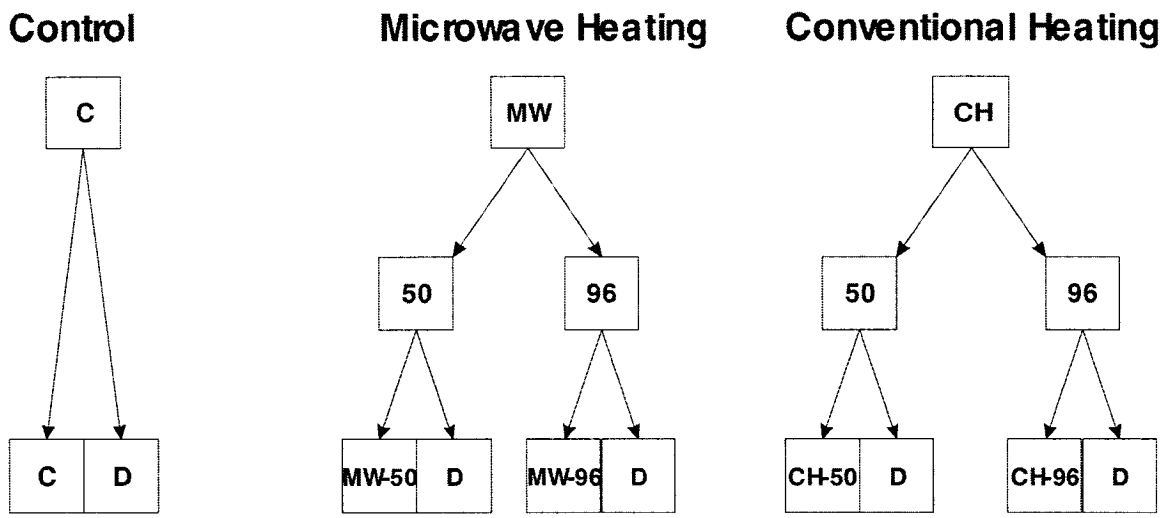

Figure 5-1 Experimental design for semi-continuous flow digesters (C: control; MW: microwave; CH: conventional heating; D: duplicate).

MW pretreatment of 96°C was selected as the "high" temperature since improvement in cumulative biogas production from batch reactors (previously studied) was highest at this temperature (Eskicioglu *et al.*, 2006). Low temperature (50°C) TWAS pretreated reactors were also run to investigate whether athermal effects of MWs were distinguishable by simply comparing the performances of MW and CH pretreatment at low temperatures. If an athermal effect indeed existed, MW-irradiated digesters would show better treatibility than CH digesters at pretreatment temperatures lower than the thermal destruction point of bacteria cells, such as 50°C.

Side-armed erlenmeyer flasks with a volume of 1 L [Pyrexplus (29410-993) plastic-coated graduated flasks, VWR, Montreal, QC, Canada] were used as SC flow reactors. Erlenmeyer flasks were sealed with rubber stoppers [two-hole black rubber stoppers (59582-326), VWR, Montreal, QC, Canada] and side arms were used to feed the reactors (Appendix A.4, Figure A-6) sampling ports (first port to withdraw the sludge from the digester) were bored into the rubber stoppers. The second sampling port was

connected to a 1 L tedlar bag for biogas collection. The tedlar bags [Tedlar gas sampling bags (TDP070710), Chromatographic Specialties Inc., ON, Canada] were equipped with on/off valves and a septum fitting that was used for gas composition sampling. Volume of daily biogas collected was measured by a manometer.

The SC digesters were started with an inoculum [2.0 ± 0.01% TS (w/w)] acclimatized to MW pretreated WAS (96°C) and had a specific activity of 0.12 ± 0.01 g TCOD/ g VSS. d. Total operating volume of SC digesters was 700 mL. SRTs were maintained by first removing and then adding a constant mixed liquor volume from the reactors by a modified wide mouth 50 mL glass syringe. TWAS used in the study was obtained from the thickener centrifuge at the *Robert O. Pickard Environmental Center (ROPEC)* wastewater treatment plant located in Gloucester, ON, Canada. ROPEC has primary treatment followed by a conventional aerobic activated sludge (SRT of 5 d) process with ferric chloride P removal and anaerobic sludge digesters for biosolid handling. TWAS was obtained from ROPEC on three separate occasions for the three different SRT runs. TS concentrations of TWAS changed in a range of 4.8-5.8% (w/w). MW and CH were applied at these high TS concentrations since the efficiency of pretreatment increases with increased concentration of sludge (Barber, 2002; Eskicioglu *et al.*, 2006). After pretreatment, TWAS was diluted to 3% TS (w/w) and fed to digesters since the inoculum was acclimatized at this sludge concentration. Each SRT run was started with freshly prepared feed which was stored at 4°C. Feed was brought to room temperature in a water bath immediately prior to feeding. Inoculum and feed characteristics (in duplicate) of SC digesters are given in Tables 5-1 and 2, respectively. MW irradiation was applied to 500 g TWAS samples (in a 1L plastic MW resistant

container) by a household MW oven [Panasonic NNS53W + inverter, 0.045 m^3 capacity, 1250 W, 2450 MHz frequency and 12.24 cm wavelength, 100% MW intensity] and CH was applied (500 mL glass volumetric flask) in a 0.023 m^3 concentric ring water bath [1650 W, Boekel Scientific, PA, USA].

Table 5-1 Inoculum characteristics for semi-continuous digesters.

Parameter	Acclimatized Inoculum
pH [-]	7.70
TS [%, (w/w)]	2.0 ± 0.01†
VS [%, (w/w)]	1.0 ± 0.00
TCOD [mg/L]	17,714 ± 286
SCOD [mg/L]	607 ± 107
NH_3-N [mg/L]	1383 ± 34
[a]TVFA [mg/L]	0 ± 0.0
[2]Alkalinity	4239 ± 9.9
Specific Activity [g TCOD/g VSS.d]	0.12 ± 0.01

†Data represent arithmetic mean of duplicates ± absolute difference between mean and duplicates.
[a]TVFA = summation of acetic, propionic and butyric acids.
[2] Bicarbonate alkalinity in units of mg/L as calcium carbonate ($CaCO_3$).

Table 5-2 TWAS feed characteristics for semi-continuous digesters[a].

	SRT = 20 d					SRT = 10 d					SRTs = 7 and 5 d				
	Control	**MW-50**	**CH-50**	**MW-96**	**CH-96**	**Control**	**MW-50**	**CH-50**	**MW-96**	**CH-96**	**Control**	**MW-50**	**CH-50**	**MW-96**	**CH-96**
pH [-]	6.79	6.69	6.51	6.74	6.52	7.58	7.58	7.60	7.54	7.50	7.12	6.85	6.56	7.17	6.65
TS [%w/w]	2.8 (±0.04)†	3.0 (±0.00)	2.8 (±0.12)	3.0 (±0.00)	2.9 (±0.00)	3.0 (±0.01)	2.9 (±0.02)	3.0 (±0.01)	3.0 (±0.00)	2.9 (±0.00)	3.0 (±0.02)	3.0 (±0.00)	3.0 (±0.00)	3.1 (±0.01)	3.2 (±0.00)
VS [%w/w]	2.0 (±0.04)	2.1 (±0.00)	2.0 (±0.10)	2.1 (±0.00)	2.1 (±0.00)	1.9 (±0.00)	1.9 (±0.00)	1.9 (±0.02)	2.0 (±0.00)	2.0 (±0.00)	2.1 (±0.02)	2.1 (±0.00)	2.1 (±0.01)	2.1 (±0.00)	2.2 (±0.00)
TCOD [mg/L]	41,333 (±2476)	45,143 (±2286)	40,524 (±143)	39,143 (±381)	39,619 (±286)	36,840 (±2040)	38,040 (±240)	38,280 (±0)	38,220 (±420)	35,520 (±480)	34,400 (±114)	37,714 (±124)	33,771 (±400)	34,914 (±629)	34,857 (±571)
SCOD [mg/L]	3029 (±7)	6293 (±143)	6729 (±157)	5850 (±7)	7886 (±29)	2881 (±24)	4650 (±221)	6071 (±0)	5471 (±114)	6179 (±179)	3162 (±67)	5371 (±186)	6171 (±100)	5393 (±107)	6252 (±29)
[2]Alk.	939 (±4)	1027 (±385)	760 (±3.4)	298 (±30)	310 (±46)	1491 (±6.6)	1832 (±6.6)	932 (±13.1)	788 (±26.3)	791 (±23)	1261 (±9.9)	1443 (±4.9)	670 (±9.9)	552 (±0.0)	296 (±0.0)
NH_3-N [mg/L]	264 (±1)	276 (±7)	280 (±26)	99 (±1)	98 (±0)	278 (±3)	272 (±7)	297 (±39)	101 (±1)	98 (±0)	282 (±5)	283 (±7)	299 (±41)	102 (±1)	105 (±0)
Volatile fatty acids															
Acetic acid [mg/L]	506 (±44)	389 (±63)	458 (±25)	295 (±19)	187 (±17)	820 (±75)	1013 (±41)	721 (±7)	519 (±13)	72 (±53)	887 (±10)	1189 (±38)	696 (±6)	658 (±4)	278 (±10)
Propionic acid [mg/L]	190 (±15)	179 (±13)	77 (±0)	127 (±5)	69 (±5)	421 (±38)	449 (±24)	137 (±4)	295 (±5)	98 (±2)	457 (±6)	617 (±12)	0 (±06)	381 (±6)	0 (±0)
Butyric acid [mg/L]	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	210 (±8)	237 (±16)	73 (±73)	0 (±0)	0 (±0)

[a]MW-50, MW-96 = pretreated with microwave to 50 and 96°C, respectively; CH-50, CH-96 = pretreated with conventional heating to 50 and 96°C, respectively.

†Data represent arithmetic mean of duplicates (± absolute difference between mean and duplicate measurements).

[2] Bicarbonate alkalinity in units of mg/L as calcium carbonate ($CaCO_3$).

SC reactors were kept in a temperature controlled shaker at 35°C [PhycroTherm, New Brunswick Scientific Co. Inc., NB, Canada]. Exposure times to reach the desired temperatures were 1.5 and 5 min for MW and 23 and 80 min for CH at pretreatment temperatures of 50 ± 1 and 96 ± 1°C, respectively (Figure 5-2). During CH, water bath temperatures fluctuated around 60 ± 2 and 98 ± 2°C to increase the temperature of a TWAS sample from 20 ± 1 to 50 ± 1 and from 20 ± 1 to 96 ± 1°C, respectively. Duration of exposure once the desired temperature was reached was zero minutes for both MW and CH.

SC reactor operation was first started at a typical SRT of 20 d used in anaerobic digestion. The reactors were operated until reactors reached steady-state (SS) conditions and then maintained in this state over a period of three SRTs (Ekama *et al.*, 1986). Upon completion of this run, the SRT was reduced to 10 d. Again SS operation over almost three SRTs was maintained. Although the lowest SRT of interest was 5 d, digesters were first operated at SRT of 7 d over a week and then reduced to 5 d SRT to achieve a smooth transition, since the digesters were expected to be less stable at the higher volumetric OLR. Figure 5-3 shows the operational pattern of one set of digesters (MW-50) to clearly illustrate the experimental approach. When daily biogas production values from digesters were observed to be stable, pH, TCOD, SCOD, TS, VS, VFA, ammonia-N, biogas composition, alkalinity and dewaterability analyses were done biweekly during SS conditions. Standard Methods (APHA, 1995) and techniques explained in Eskicioglu *et al.* (2006) were used for all analyses.

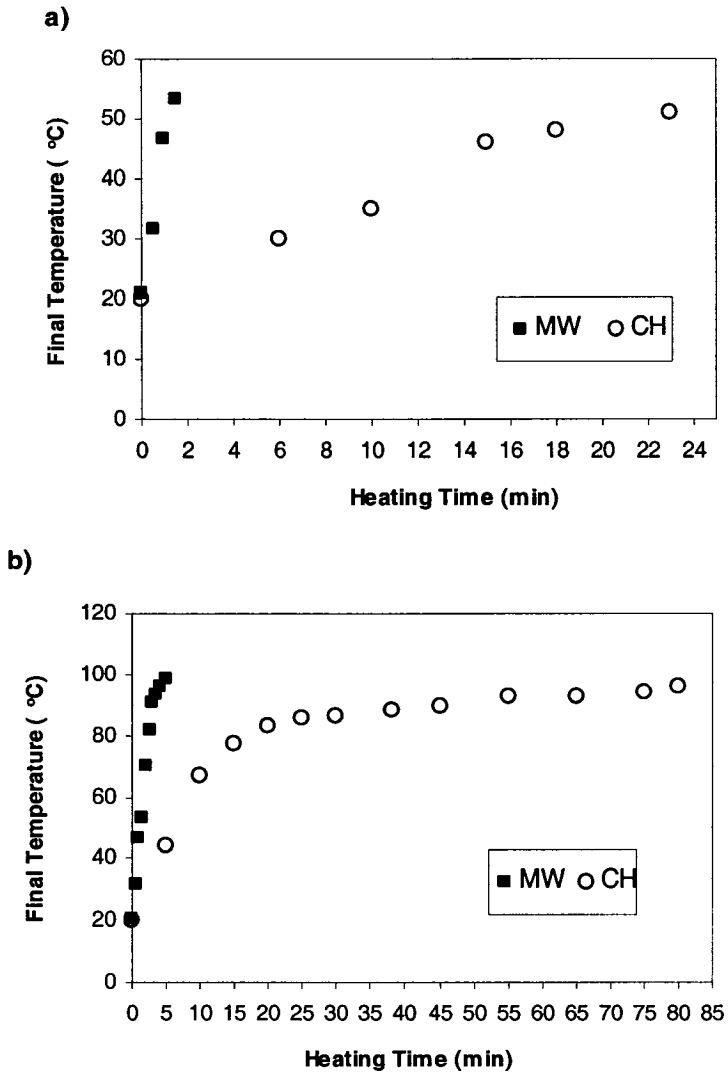

Figure 5-2 High (a) and low (b) temperature profiles of TWAS samples (MW: microwave; CH: conventional heating).

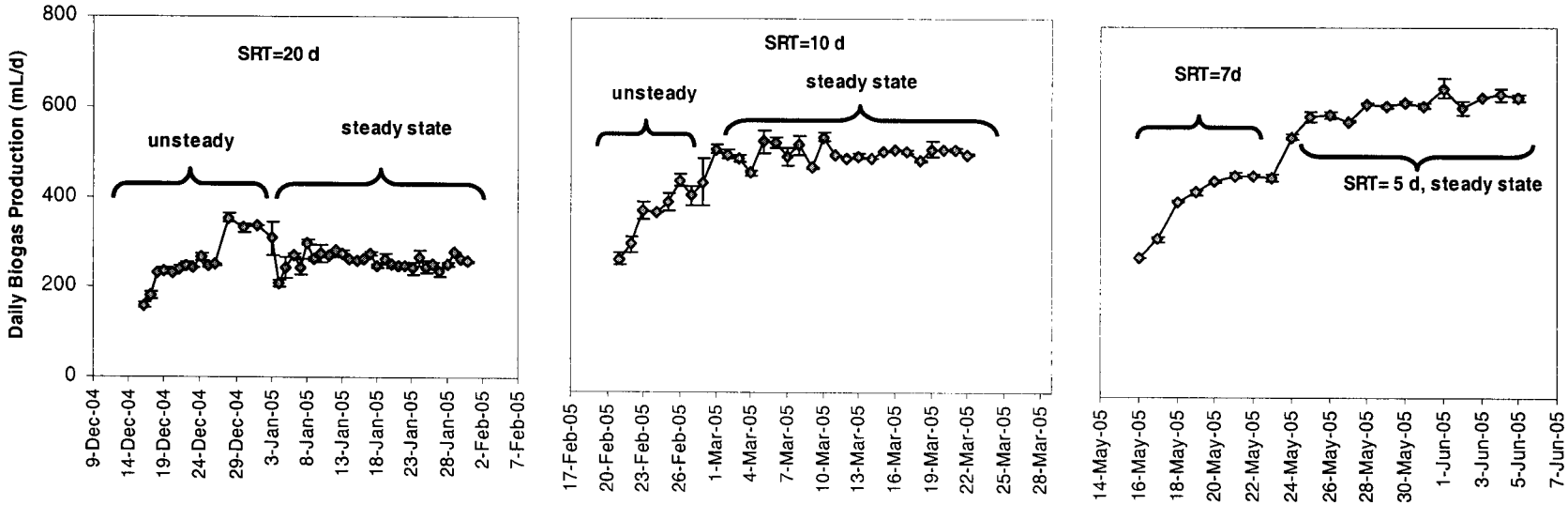

Figure 5-3 Operational pattern for MW-50 digesters samples (SRT: sludge retention time).

Dewaterability of WAS digested in control and pretreated reactors was determined using a capillary suction timer [Model 440, Fann Instrument Company, TX, USA] without polymer addition based on Standard Methods procedure 2710G (APHA, 1995). The method consists of injecting a sludge sample onto a small cylinder placed on a sheet of chromatography paper. While the paper extracts liquid from the sludge by capillary suction, water released from sludge travels between two contact points on the chromatography paper and the travel time or capillary suction time (CST in seconds) is recorded by a digital timer. In this project, sludge temperature and volume were kept constant (22 ± 1°C and 5 mL, respectively) since variations in temperature and sample volume can affect CST results. At the end of experiments, CST values indicated by the timer were divided by TS concentration of sludge samples in order to prevent bias among samples with different solids concentrations.

5.4 Results and Discussions

5.4.1 Solubilization of Pretreated TWAS

It was anticipated that both CH and MW would rupture microbial cell membranes, solubilize a portion of particulates and denature proteins at the higher temperature. Despite small variations in SCOD/TCOD ratios at similar temperatures, solubilization trends for TWAS feeds for the three different SRTs to be evaluated were similar and reproducible (Figure 5-4, Table 5-2). Variations in solubilization ratios at similar temperatures for different SRT feeds possibly originated from variations in solid concentrations of raw TWAS which were 5.54, 4.75, 5.76% TS (w/w) for feeds used at SRTs of 20, 10 and 5 d, respectively. The SCOD/TCOD ratio of the control TWAS feeds used at each SRT was also different indicating that TWAS characteristics of ROPEC

sludge were not consistent throughout the year (not unexpected). The degree of TWAS solubilization always increased with temperature for both conventional and MW heating. Interestingly, SCOD/TCOD ratios were always higher after CH than after MW heating at similar temperatures. It is postulated that the extended exposure of CH to achieve a given temperature compared to MW exposure (Figure 5-2) has an effect on pretreatment. Increasing MW dwell times should be explored to obtain a better CH vs. MW comparison (not possible with present equipment). From a simple pretreatment solubilization point of view it would seem that MW pretreatment does not exhibit any significant athermal effects that can easily be substantiated. In fact as mentioned above, any possible MW athermal effects are smaller than thermal effects caused by longer CH exposure time.

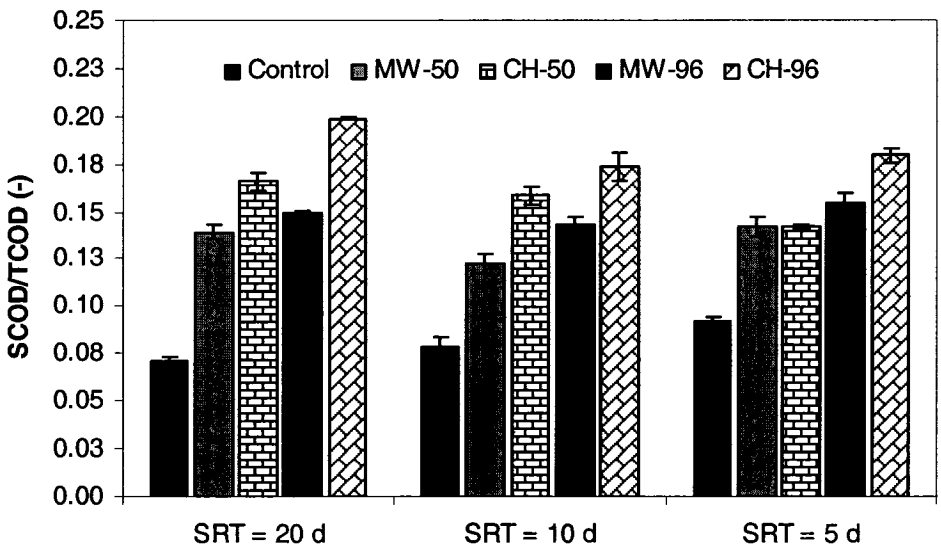

Figure 5-4 Solubilization effects of pretreatments on feed sludge (MW-50, MW-96: microwave at 50, 96°C; CH-50, 96: conventional heating at 50, 96°C; SRT: sludge retention time).

5.4.2 Effect of Pretreatment on Continuous Flow Digestion of TWAS

Table 5-3 summarizes the SS data for reactors fed with MW and CH TWAS. Values in Table 5-3 are the arithmetic means of measurements taken at SS; standard

deviations and number of data points are also given in parentheses, respectively. Despite higher fluctuations in effluent qualities of the control at SRT of 5 d (higher standard deviations for removal percentages, Table 5-3) compared to that of longer SRTs, all reactors were able to tolerate high OLRs (4.12-4.29 gVS/L.d) and short SRT (5 d) with no dramatic decrease in methane production. VFA concentrations in all reactors were negligible at 20 and 10 d SRTs and at 5 d SRT they increased up to ~190 mg/L but stayed well with in the safe range (less than 250 mg/L, Metcalf and Eddy, 1991) indicating that the majority of VFA were converted to biogas even at SRT of 5 d. It is believed that the main reason for process stability was the use of inoculum with good bioactivity acclimatized to MW-irradiated feed resulting in concomitant smooth transition from long to short SRTs.

Table 5-3 Steady state results for semi-continuous digesters at 5, 10 and 20 d SRTs[a].

	SRT = 20 d					SRT = 10 d					SRT = 5 d				
Parameters	**Control**	**MW-50**	**CH-50**	**MW-96**	**CH-96**	**Control**	**MW-50**	**CH-50**	**MW-96**	**CH-96**	**Control**	**MW-50**	**CH-50**	**MW-96**	**CH-96**
Loading conditions for duplicate reactors															
[1]OLR	0.98†	1.03	0.99	1.05	1.03	1.91	1.85	1.95	2.04	1.99	4.12	4.15	4.12	4.29	4.40
[g VS/[n]L/d]	(0.02, 2)	(0.00, 2)	(0.05, 2)	(0.00, 2)	(0.00, 2)	(0.00, 2)	(0.02, 2)	(0.02, 2)	(0.00, 2)	(0.00, 2)	(0.04, 2)	(0.01, 2)	(0.02, 2)	(0.00, 2)	(0.00, 2)
OLR	2.07	2.26	2.03	1.96	1.98	3.68	3.80	3.83	3.82	3.55	6.88	7.54	6.75	6.98	6.97
[g TCOD/[n]L/d]	(0.12, 2)	(0.22, 2)	(0.35, 2)	(0.04, 2)	(0.00, 2)	(0.20, 2)	(0.02, 2)	(0.00, 2)	(0.04, 2)	(0.05, 2)	(0.02, 2)	(0.02, 2)	(0.08, 2)	(0.13, 2)	(0.11, 2)
Removal efficiencies															
VS	46.5	49.4	46.9	51.8	51.9	45.5	44.1	46.7	50.4	50.5	32.9	37.6	40.1	40.5	41.7
[%]	(1.6, 14)	(1.8, 14)	(1.7, 14)	(1.2, 14)	(1.5, 14)	(1.4, 14)	(1.6, 14)	(1.4, 14)	(0.8, 14)	(1.3, 14)	(7.4, 10)	(3.8, 10)	(3.2, 10)	(3.5, 10)	(2.9, 10)
TS	31.5	34.6	31.4	36.6	36.6	29.8	27.8	28.9	33.8	33.6	22.6	26.3	29.3	29.3	29.9
[%]	(1.7, 14)	(2.4, 14)	(2.3, 14)	(1.5, 14)	(2.1, 14)	(4.3, 14)	(2.8, 14)	(3.2, 14)	(2.8, 14)	(1.6, 14)	(5.6, 10)	(3.4, 10)	(2.4, 10)	(2.9, 10)	(2.8, 10)
TCOD	52.7	55.3	50.9	49.8	51.2	44.9	46.4	47.7	50.3	48.2	32.6	39.6	38.4	38.1	37.9
[%]	(2.2, 14)	(1.1, 14)	(2.5, 14)	(2.1, 14)	(1.5, 14)	(2.4, 14)	(2.0, 14)	(1.0, 14)	(2.7, 14)	(1.7, 14)	(3.7, 10)	(3.6, 10)	(2.7, 10)	(2.9, 10)	(5.8, 10)
[2]Biogas	0.26	0.26	0.27	0.27	0.26	0.37	0.38	0.39	0.39	0.38	0.56	0.65	0.69	0.72	0.76
[L/d]	(0.02, 48)	(0.02, 48)	(0.01, 48)	(0.02, 48)	(0.01, 48)	(0.02, 42)	(0.02, 42)	(0.02, 42)	(0.01, 42)	(0.02, 42)	(0.13, 24)	(0.06, 24)	(0.05, 24)	(0.06, 24)	(0.05, 24)
CH_4	72	75	72	75	74	65	65	65	65	65	63	65	64	64	63
[%]	(7, 6)	(2, 6)	(9, 4)	(1, 5)	(6, 7)	(0.38, 7)	(1.99, 7)	(0.68, 7)	(1.87, 7)	(0.88, 7)	(0.39, 3)	(0.23, 4)	(0.06, 2)	(0.75, 3)	(0.99, 3)
pH in reactors	7.59	7.60	7.63	7.61	7.59	7.56	7.59	7.60	7.59	7.56	7.40	7.48	7.41	7.46	7.41
	(0.07, 6)	(0.02, 6)	(0.05, 6)	(0.05, 6)	(0.02, 6)	(0.03, 8)	(0.04, 8)	(0.05, 8)	(0.04, 8)	(0.05, 8)	(0.07, 6)	(0.08, 6)	(0.09, 6)	(0.18, 6)	(0.03, 6)
Effluent supernatant characteristics															
[3]TVFA	24.10	0.35	3.11	0.64	1.11	0.00	2.08	0.00	0.00	0.00	29.86	15.38	187.46	91.50	168.89
[mg/L]	(33.3, 10)	(1.1, 10)	(4.6, 10)	(1.2, 10)	(2.6, 10)	(0.0, 8)	(5.9, 8)	(0.0, 8)	(0.0, 8)	(0.0, 8)	(37.4, 4)	(30.8, 4)	(186.9, 4)	(13.0, 4)	(101, 4)
SCOD	396.8	390.3	381.4	415.4	445.5	431.2	500.4	470.2	557.4	450.8	612.9	659.3	920.4	1103.0	1203.9
[mg/L]	(26.8, 12)	(18.5, 12)	(30.7, 12)	(34.9, 12)	(25.9, 12)	(18.0, 14)	(78.2, 14)	(80.5, 14)	(43.2, 14)	(10.9, 14)	(64.2, 8)	(45.4, 8)	(80.2, 8)	(118.7, 8)	(56.9, 8)
NH_3-N	1130.2	1175.9	1181.7	1370.8	1360.1	1219.3	1221.7	1298.2	1340.1	1298.5	735.4	883.7	884.2	1620.6	1145.9
[mg/L]	(272.8, 6)	(122.6, 6)	(77.6, 6)	(60.7, 6)	(60.8, 6)	(193.6, 6)	(152.8, 6)	(352.4, 6)	(221.7, 6)	(116.5, 6)	(5.36, 4)	(38.7, 4)	(42.08, 4)	(376, 4)	(1.50, 4)

[a]MW-50, MW-96 = pretreated with microwave to 50 and 96°C, respectively; CH-50, CH-96 = pretreated with conventional heating to 50 and 96°C, respectively.

†Data represent arithmetic mean of measurements (standard deviation, number of data points); [n]liter of reactor.

[1]OLR = Organic loading rate; [2]Biogas production data at SRT of 5 d are calculated from biogas yield coefficient of 0.83 L/ g VS removed.

[3]TVFA = Total Volatile Fatty Acids (summation of acetic, propionic and butyric acids).

Organic removal efficiency of digestion is generally judged by the VS and TCOD removals. Table 5-3 shows TS, VS and TCOD removal efficiencies of control and pretreated digesters at different SRTs. Although TS and VS removal performances of all digesters decreased when SRT was shortened, control digesters performed less efficiently compared to CH or MW pretreated SC reactors. Figures 5-5 and 5-6 show improvements (relative to control) in TS and VS removal efficiencies of CH or MW pretreated digesters, respectively. It is very clear from both figures that relative improvements in solids removal efficiencies of pretreated digesters were significantly increased as SRT was shortened and as pretreatment temperature increased. The highest relative improvement in TS removals from both MW and CH digesters was observed at SRT of 5 d and pretreatment at 96°C, which were 29 and 32% higher than controls, respectively.

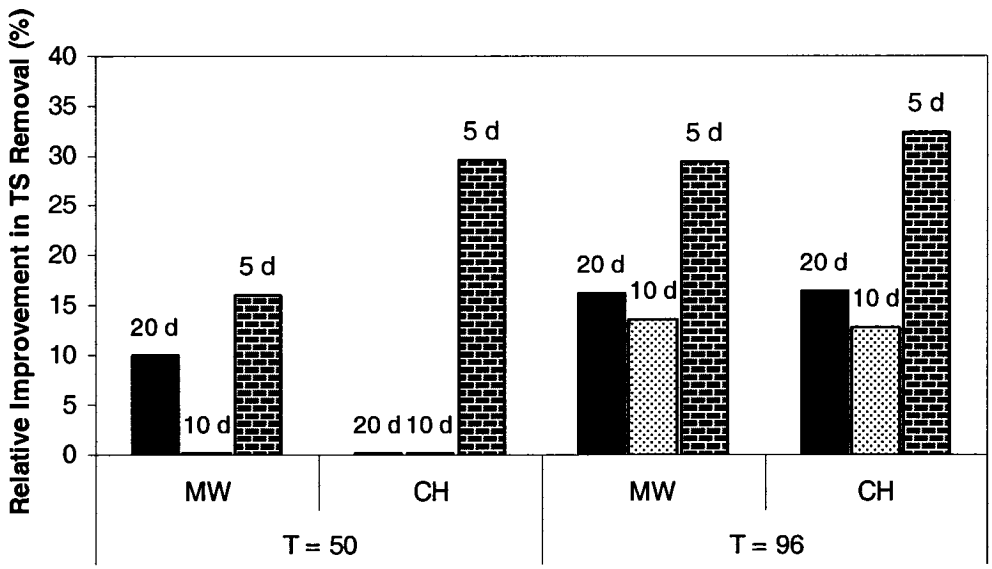

Figure 5-5 Relative improvements in TS removal efficiencies (T = 50, T = 96: temperature at 50, 96°C; MW: microwave; CH: conventional heating; TS: total solid; 5, 10 and 20 d: sludge retention times).

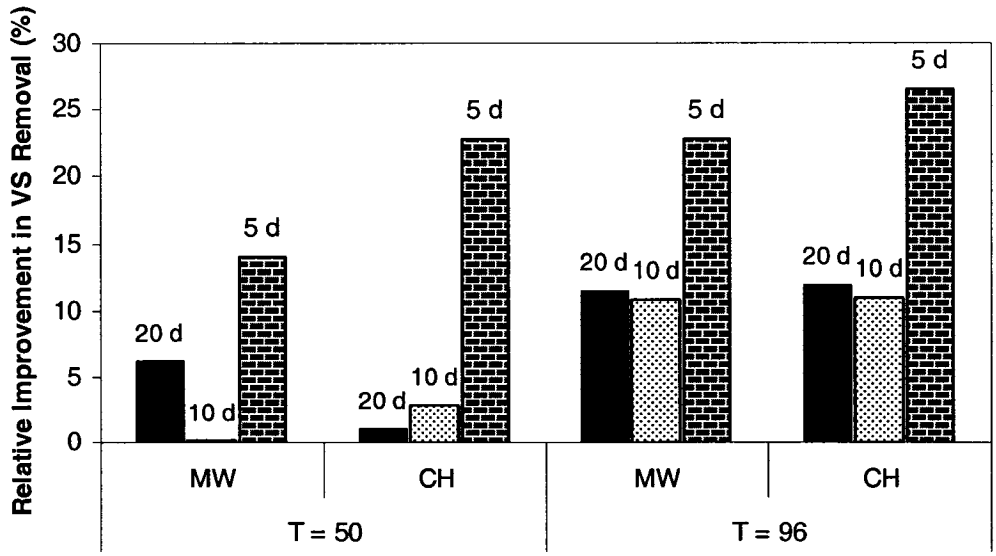

Figure 5-6 Relative improvements in VS removal efficiencies (T = 50, T = 96: temperature at 50, 96°C; MW: microwave; CH: conventional heating; TS: total solid; 5, 10 and 20 d: sludge retention times).

Relative improvements in VS removals for pretreated SC digesters followed a similar pattern to TS removals and resulted in 23 and 26% better VS removals than controls for MW-96 and CH-96, respectively. Improvements in TCOD removal efficiencies from SC digesters treating pretreated TWAS relative to controls were also plotted in Figure 5-7. In general, improvements in TCOD removal efficiencies of pretreated SC digesters with respect to controls increased by 16-22% at the shortest SRT (5 d), an indication that controls were challenged by the organic characteristics of untreated TWAS at high OLRs. At a 10 d SRT a similar but less dramatic 3-11% increase in TCOD removal efficiencies occurred. On the other hand, at longer SRTs (20 d), controls were performing nearly as well as pretreated digesters with pretreated digester only producing relative TCOD removal improvements less than 5%. These results are logical since pretreatments change the characteristics of sludge and are known to

accelerate the hydrolysis step but in general do not change the amount of organic material in the TWAS sample. Therefore TS/VS and TCOD results were in agreement with the general hypothesis that incremental increase of pretreatment effects compared to controls would be higher for high temperature MW and CH TWAS pretreatments when the SRT of the system was reduced.

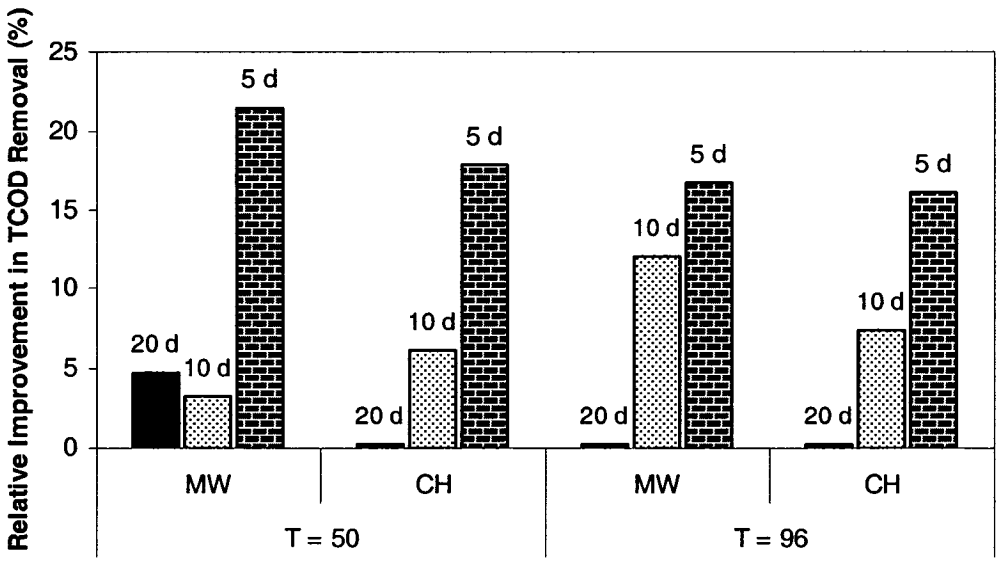

Figure 5-7 Relative improvements in TCOD removal efficiencies (T = 50, T = 96: temperature at 50, 96°C; MW: microwave; CH: conventional heating; TCOD: total chemical oxygen demand; 5, 10 and 20 d: sludge retention times).

Results on TS/VS removal efficiencies from SC digesters treating TWAS pretreated to low temperature (MW-50 and CH-50) were further analyzed to investigate whether solids removal performances might indicate enhancing MW athermal effects. Figure 5-6 shows that CH-50 digesters had relative improvements of 4 and 23% in VS removal efficiencies which were higher than the <1 and 15% improvements for MW-50 at shorter 10 and 5 d SRTs respectively. Better CH-50 improvements are likely due to a higher concentration of soluble organics (SCOD) available (Figure 5-4) for

biodegradation in CH-50 than in MW-50. Even if the athermal effect was present, it is possible that it was masked by the extended duration of CH compared to MW exposure to achieve a given temperature. CH duration most likely had a dominant effect, creating higher SCOD/TCOD ratios in both CH-50 and CH-96 SC digesters which eventually resulted in better solids removal efficiencies compared to MW-50 and MW-96 SC reactors.

The biogas or methane yield for an anaerobic digestion process is commonly expressed as a function of the removal of either VS or TCOD. For a TWAS digestion study, TCOD measurements can be less reliable than VS, measurements due to extreme dilution ratios (~150) required for TWAS samples with 5.4% TS (w/w) concentration. Literature values for methane production may change depending on the type of the sludge digested, but representative values are usually in the range of 0.49-0.75 L CH_4/g VS removed (Parkin and Oven, 1986; Metcalf and Eddy, 1991). If the sludge is a mixture of PS/WAS, nominally each gram VS removal corresponds to 1 L biogas production (Stephenson *et al.*, 2003). For a reactor treating only WAS, this value is expected to be lower. In this study, sludge digesters encountered gas leakage problems especially when OLR was increased to ~4 g VS/L/d at SRT of 5 d. Therefore, daily biogas productions at 5 d SRT were calculated as a function of VS removals of digesters with a biogas yield coefficient of 0.83 L biogas/g VS removed generated by preliminary batch digester studies on similar ROPEC TWAS (Eskicioglu *et al.*, 2006) and reported in Table 5-3 along with the observed biogas values at SRTs of 5 and 10 d. Selecting another value of biogas yield would only change the daily biogas productions from a digester but does not change improvements in biogas productions relative to another digester or relative to the

control. Figure 5-8 shows improvements in biogas productions relative to control digesters as SRT was shortened. As expected from VS and TCOD removal efficiencies of digesters, improvements in biogas production from all digesters dramatically increased (30 and 35% higher biogas production over controls for MW-96 and CH-96, respectively) as SRT was reduced to 5 d.

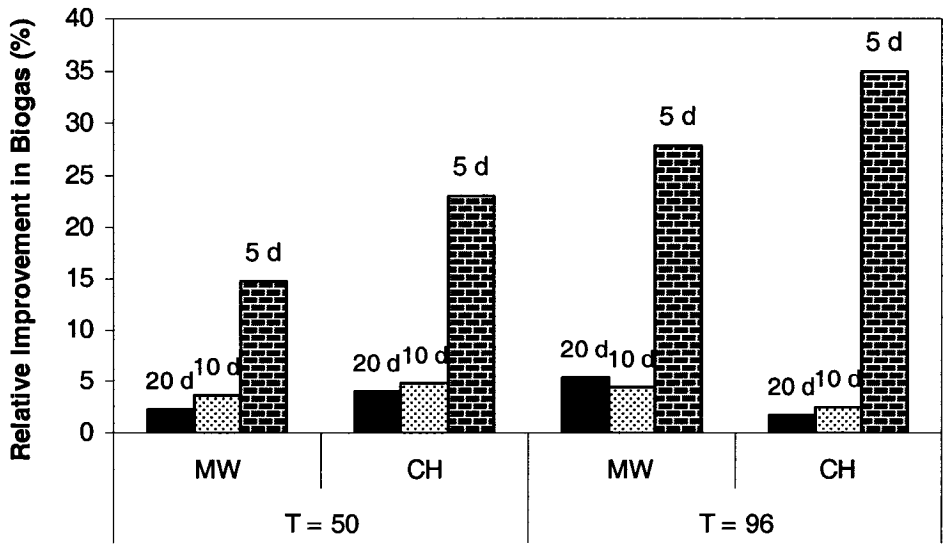

Figure 5-8 Relative improvements in biogas production (T = 50, T = 96: temperature at 50, 96°C; MW: microwave; CH: conventional heating; 5, 10 and 20 d: sludge retention times).

Results (MW of 91°C) generated by Park *et al.* (2004) and results from this study (MW of 96°C) predict different performances for continuous flow digesters. Although both studies were digesting secondary sludge, at a similar SRT of 10 d and OLR of ~1.9 g VS/L/d, TCOD and VS treatment efficiencies of control SC digesters were 13.8 ± 0.5 and 23.2 ± 1.2%, respectively (Park *et al.*, 2004) while TCOD and VS removals from controls in this study were 44.9 ± 2.4 and 45.5 ± 1.4%, respectively. Obviously, there was a large difference between the performances of the controls in the two studies which

could explain the much higher (64 and 43% higher at SRTs of 15 and 10 d) COD removals observed by Park *et al.* (2004) compared to the control for MW-pretreated (to 91°C) digesters. Different digestion performances could arise from different initial TWAS characteristics. The type of activated sludge plant (conventional, nitrification, etc.) or SRT of the activated sludge unit [not reported in Park *et al.* (2004)] as well as other plant operating procedures that could effect extracellular polymeric substances (EPS) and divalent cation compositions which determine the amount of biodegradable materials, the floc structure and strength of activated sludges, respectively (Higgins and Novak, 1997; Novak *et al.*, 2003), all may have some impact on pretreatment and final digester performance.

Results related to performances of the SC digesters (displayed in Table 5-3 and Figures 5-5 to 5-8) imply that MW pretreatment at temperatures under the boiling point (100°C at 1 atm) have a significant potential to increase biodegradability of TWAS in full-scale continuous flow sludge digesters.

5.4.3 Effect of Pretreatment on Digester Supernatant Characteristics

Applying pretreatment before anaerobic digestion had two main disadvantages; elevated levels of SCOD and ammonia concentrations in the digested supernatant. Effluent supernatant SCOD values of control and pretreated digesters are reported in Table 5-3. Relative (to control) increase in SCOD concentrations of the effluents are plotted in Figure 5-9. MW and CH digesters at SRTs of 10 and 20 d contained relatively lower SCOD values at similar pretreatment temperatures. Relative increases in SCOD of supernatants were 5 and 29% for MW-96 and 12 and 5% for CH-96 at SRTs of 20 and 10 d, respectively. However, SCOD of both MW and CH digesters at SRT of 5 d were

extremely high with 80 and 96% higher SCODs over controls for MW-96 and CH-96, respectively.

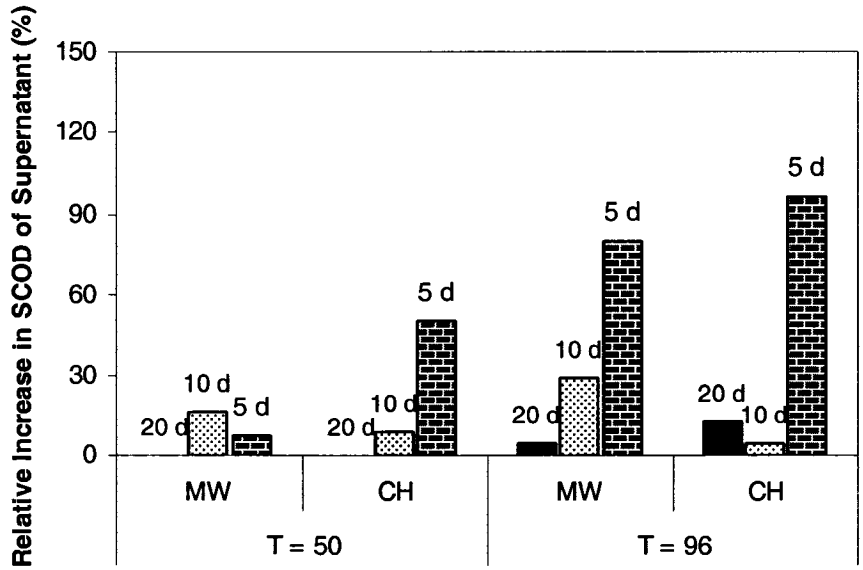

Figure 5-9 Relative increase in SCOD of supernatants (T = 50, T = 96: temperature at 50, 96°C; MW: microwave; CH: conventional heating; SCOD: soluble chemical oxygen demand 5, 10 and 20 d: sludge retention times).

There are contradictory reports regarding the toxicity of ammonia in anaerobic digestion. Although many researchers monitored ammonia concentration in digesters after pretreatment, no definite ammonia inhibition case was reported. Kroeker (1979) observed that ammonia concentrations of 7000 mg/L were not inhibitory if sufficient acclimatization was achieved. In another treatment plant, digesters were operated at 2650 mg/L of ammonia without any adverse effect (Barnard *et al.*, 2002). In this study, ammonia-N concentrations of SC reactors were measured (Table 5-3). More ammonia-N was generated in reactors when MW temperature was increased, due to higher anaerobic degradation efficiency of nitrogenous organic matter in pretreated digesters. The relative increases between the SC reactor with the highest ammonia-N concentration and the

control were only 21 and 10% for MW-96 at SRTs of 20 and 10 d, respectively. However, when reactors were being operated at 5 d SRT, the difference in effluent ammonia between control and MW-96 increased to 120%. Since pH values of digesters fluctuated only in a range of 7.40-7.63 during the digestion, the ammonia was primarily in the less toxic ionized form. Furthermore, inoculum was acclimatized to this level of ammonia (Table 5-1). Therefore all digesters were able to tolerate elevated ammonia concentrations with no noticeable decrease in methane production and composition or VFA accumulation (Table 5-3).

5.4.4 Dewaterability Analysis on WAS from Continuous Flow Digesters

Figure 5-10 presents dewaterability results from SC reactors with SRTs of 20, 10 and 5 d. As observed from Figure 5-10, in general, control digesters had the highest CST values indicating the worst dewaterability characteristics. Dewaterability results of CH digester sludges were always better than MW-pretreated results at similar pretreatment temperatures for all three SRTs. In terms of the greatest improvement in dewaterability compared to control, 39 and 29% better dewaterability was observed for MW-96 and CH-96 at SRT of 10 d.

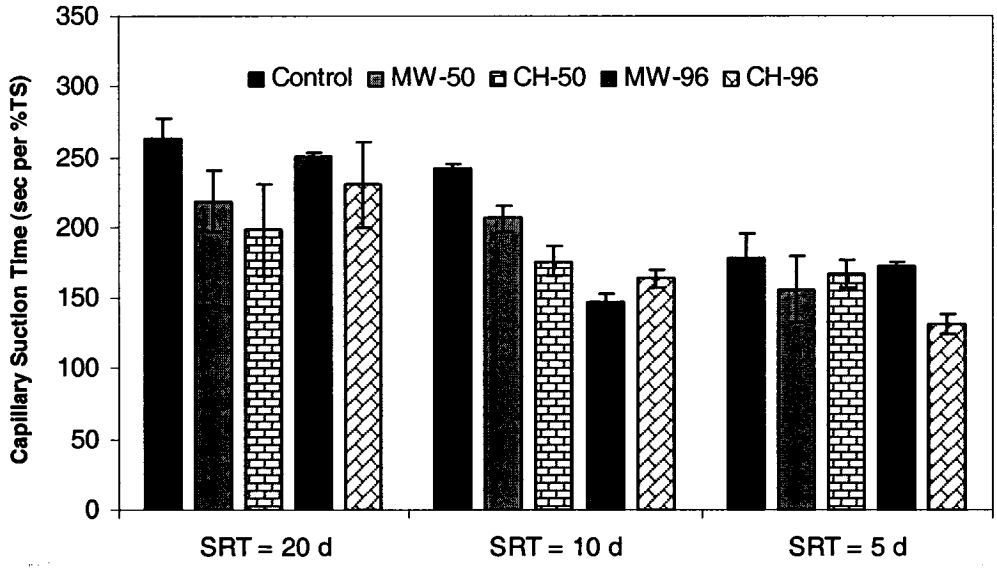

Figure 5-10 Dewaterability results from control and pretreated digesters (MW-50, MW-96: microwave at 50, 96°C; CH-50, 96: conventional heating at 50, 96°C; SRT: sludge retention time; TS: total solids).

5.5 Conclusions

Based on the experimental data, the following conclusions are drawn.

(1) After pretreatment, SCOD/TCOD ratios of both MW and CH TWAS samples increased 2-3 fold from the control value of 0.07 to between 0.15-0.2. Solubilization was always higher after CH compared to MW heating to similar temperatures, likely due to the extended duration of CH exposure to MW exposure to achieve a given temperature. There was no pattern of MW digesters showing better performances due to *athermal* effects at low MW temperatures (50°C). In fact, any possible MW *athermal* effects were smaller than *thermal* effects caused by longer CH exposure time.

(2) Incremental increases in TS/VS and TCOD removal efficiency of pretreated digesters compared to controls dramatically increased as SRT was gradually shortened from 20 to 10 to 5 d and as pretreatment temperature was increased from 50 to 96°C. TWAS pretreated to 96°C by MW and CH achieved 29 and 32% higher TS and 23 and 26% higher VS removal efficiencies compared to controls at SRT of 5 d, while similar reactors had only 16% higher TS and 11 and 12% higher VS removals than those of controls at SRT of 20 d, respectively.

(3) SCOD and ammonia-N concentrations were dramatically increased in digester effluent as pretreatment temperature was increased and SRT of the digester was decreased. MW-96 and CH-96 digesters had 80 and 96% higher SCOD and 120 and 56% higher effluent ammonia-N compared to controls, respectively, when OLR was increased corresponding to an SRT of 5 d.

(4) Control samples were the most difficult to dewater for all SRTs. MW-96 and CH-96 digesters achieved 39 and 46% better dewaterability compared to the control, respectively, at SRT of 10 d. This result again indicated that thermal pretreatment methods have potential to enhance dewaterability after anaerobic digestion.

5.6 Acknowledgments

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CHAPTER 6

Characterization of Soluble Organic Matter of Waste Activated Sludge Before and After Thermal Pretreatment

Cigdem Eskicioglu, Kevin J. Kennedy, Ronald L. Droste

6.1 Abstract

Microwave (MW) irradiation and conventional heating (CH) at 96°C was successful in disrupting the complex waste activated sludge (WAS) floc structure and releasing extracellular and intracellular biopolymers, such as protein and sugars from activated sludge flocs into soluble phase along with the solubilization of particulate chemical oxygen demand (COD). Soluble CODs of CH and MW irradiated WAS were 361 ± 45 and 143 ± 34% higher and resulted in 475 ± 3 and 211 ± 2% higher cumulative biogas productions relative to the control at the end of 23 days of mesophilic anaerobic digestion, respectively. Ultrafiltration (UF) was used to characterize the soluble molecular weight (Mw) distributions of control (unpretreated), CH and MW irradiated WAS. Depending on the Mw fraction, the range of substrate volumetric utilization rate increases from anaerobic digesters was between 94-184% for the CH and 26-113% for the MW compared to the control for the first 9 days of the digestion. Digesters treating high Mw materials (Mw >300 kDa) resulted in smaller biodegradation rate constants, *k*, indicating that microorganisms require a longer time to utilize high Mw fractions which are most likely the cell wall fragments and exopolymers.

Keywords—Microwave, conventional heating, WAS, molecular weight distribution, biodegradability, pretreatment, solubilization, ultrafiltration.

6.2 Introduction

Rapid and complete stabilization of WAS via anaerobic digestion has not been achievable due to the rate limiting hydrolysis step of large organic molecules associated with microbial cells. Recent studies have indicated that activated sludge has a more complex floc structure than first realized. It is comprised of different groups of microorganisms, organic and inorganic matter agglomerated together in a polymeric network formed by microbial extracellular polymeric substances (EPS) and cations (Li and Ganzarczyk, 1990; Frølund *et al.*, 1996). It is believed that hydrolysis of EPS and/or microbial biomass together within the activated floc limits the rate and extent of degradation (Higgins and Novak, 1997). EPS does not only originate from the metabolism and cell autolysis associated with activated sludge bacterial cells but also originates in part from the raw influent wastewater coming into the treatment plant (Urbain *et al.*, 1993; Frølund *et al.*, 1994). According to the most recent WAS-floc agglomeration concept, EPS and divalent cations may be the most important parameters governing WAS hydrolysis. These two parameters rather than microbial cells represent the major organic fraction determining the floc structure, integrity and strength (Higgins and Novak, 1997; Novak *et al.*, 2003). Disruption of the EPS and divalent cation network followed by subsequent enhanced stabilization of microbial biomass should result in enhancing the rate and extent of WAS biodegradability (Park *et al.*, 2003) and increase dewaterability (Ormeci and Vesilind, 2000) during and after anaerobic digestion.

Recent WAS pretreatment studies have focused on thermal (Jolis *et al.*, 2004; Skiadas *et al.*, 2004), chemical (Lin *et al.*, 1999; Stephenson *et al.*, 2003), ultrasonic or mechanical pretreatment (Muller *et al.*, 2003; Cartmell *et al.*, 2004) methods to

disintegrate the floc structure of sludge and to extract both intracellular (within the microbial cell) and extracellular (within the polymeric network) materials before WAS is sent to the anaerobic digesters. In most of the studies, pretreatments solubilized the WAS, which subsequently improved anaerobic digestion of sludge.

As an alternative to conventional heating (CH), it is hypothesized in this study that interactions which bind the biopolymers together can be further disrupted by the *dipole rotation* or *orientation* effects of MW-irradiation. The MW orientation effect is mainly caused by polarized parts of macromolecules lining up with the poles of the electromagnetic field resulting in the possible breakage of hydrogen bonds (Kingston and Jassie, 1988; Loupy, 2002). It is believed that orientation (athermic) and subsequent heating (thermic) effects break apart the polymeric network ending with release of mainly extracellular and possibly intercellular materials (depending on the intensity of MW-irradiation) such as; polysaccharide, protein and smaller amounts of DNA and RNA into the soluble phase.

Little effort has gone into evaluation of the specific characteristics of different wastes (landfill leachate and effluents from different biological treatment processes) such as size distribution or nature of the soluble compounds as they undergo waste treatment (Gourdon *et al.*, 1989; Barker *et al.*, 1999; Abdessemed *et al.*, 2002). Pretreatment studies mostly focused on the particle size distribution of WAS. Significant size reductions of sludge solids were reported after pretreatments (Muller *et al.*, 1998; Tiehm *et al.*, 2001; Kim *et al.*, 2003). However, no studies could be found relating the size and impact of soluble products produced by WAS pretreatment to anaerobic stabilization of WAS in an attempt to obtain a better understanding of the advantages or disadvantages of various

pretreatment strategies (i.e., why more pretreatment may or may not result in decreased rates or extent of WAS degradation).

In this paper, batch anaerobic digestion of supernatants (soluble fraction) from both untreated (raw) and pretreated thickened WAS (TWAS) was examined. BMP results from the soluble fraction of TWAS pretreated to 96°C by both MW and CH were compared. Additionally, using ultrafiltration, the distribution of soluble COD fractions from raw and pretreated sludge and the overall anaerobic biodegradability and biodegradation rate of soluble fractions with different molecular weight cutoffs (MwCOs) were addressed.

6.3 Materials and Methods

6.3.1 Sample Preparation and Pretreatments

TWAS was obtained from the thickener centrifuge at the *Robert O. Pickard Environmental Center (ROPEC)* located in Gloucester, (ON, Canada). ROPEC has preliminary and primary treatment followed by a conventional aerobic activated sludge unit operated at an average sludge retention time (SRT) of 5 d. Ferric chloride is added to WAS for P removal prior to WAS thickening. TWAS and primary sludge (PS) are blended in a 58:42 v/v ratio and undergo mesophilic anaerobic sludge digestion to produce a stabilized biosolids product for disposal. For feed characterization, raw TWAS was sampled from ROPEC at different times of the year and general characteristics are displayed in Table 6-1. TWAS was characterized as young sludge based on the 5 d SRT as supported by the relatively high VS/TS ratio of about 0.70. Pretreatment and ultrafiltration (UF) experiments were done on a TWAS sample obtained from ROPEC on

31/08/2005 and total solid (TS) concentration of TWAS was 5.9 ± 0.07% TS (w/w). MW and CH were applied at these high TS concentrations since efficiency of pretreatment increases with concentration of sludge (Barber, 2002; Eskicioglu *et al.*, 2006).

Table 6-1 General characteristics of raw TWAS from ROPEC[a].

	Date of sampling				
Parameters	**17/03/2004**	**9/12/2004**	**17/02/2005**	**03/05/2005**	**31/08/2005**
pH [-]	7.5	6.8	7.6	7.1	6.5
TS [% (w/w)]	5.4 (0.01)†	5.5 (0.18)	4.7 (0.00)	5.8 (0.00)	5.9 (0.07)
VS [% (w/w)]	3.7 (0.02)	4.0 (0.01)	3.2 (0.01)	4.0 (0.01)	4.0 (0.06)
VS/TS [-]	0.69 (0.00)	0.70 (0.00)	0.67 (0.00)	0.70 (0.00)	0.69 (0.00)
TCOD [mg/L]	67,301 (5873)	81,450 (2470)	59,019 (2040)	65,831 (114)	60,189 (315)
SCOD [mg/L]	3957 (29)	5968 (7)	4615 (24)	6051 (61)	3621 (396)
SCOD/TCOD [-]	0.06 (0.00)	0.07 (0.04)	0.08 (0.00)	0.09 (0.00)	0.06 (0.01)

[a]TS, VS: total and volatile solids; TCOD, SCOD: total and soluble chemical oxygen demand, respectively.
†Data represent arithmetic mean of duplicates (absolute difference between mean and dup. measurements).

MW irradiation was applied to 500 g TWAS samples (in a 1 L, 19 x 12 x 4.4 cm, plastic MW resistant container) by a household type MW oven with a capacity of 0.045 m^3 [Panasonic NNS53W + inverter, 1250 W, 2450 MHz frequency and 12.24 cm wavelength, 100% MW intensity]. CH was applied to the same amount of TWAS sample in a glass volumetric flask placed in concentric ring water bath (0.023 m^3 water capacity, 110 V, 1650 W, Boekel Scientific, PA, USA] set at 97 ± 2°C to increase the temperature of a TWAS sample from 25 ± 1 to 96 ± 1°C. Due to lower energy consumption at low MW temperatures, this study focused on a temperature of 96°C, which was achievable with a kitchen type MW oven and plastic MW transparent container designed for food applications. If it was desired to exceed the boiling point, an industrial type of MW and a properly sealed vessel designed for high temperatures and pressures would be required. Exposure time to raise the temperature of the TWAS from room temperature to the

desired temperature (96 ± 1°C) was 5 min for MW and 80 min for CH, respectively (Figure 6-1). Duration of exposure at the desired temperature was zero for both MW and CH. Temperature readings were taken with thermo-couple probes [Cole-Parmer (P-08506-75), T-type, fine-gauge Teflon PTFE insulated probe with a response time of 0.5 s, Labcor Technical Sales Inc., ON, Canada] connected to a module for analog-to-digital conversion and recorded by a laboratory computer system [LabVIEW Software Version 6, National Instruments Co., Austin, TX, USA].

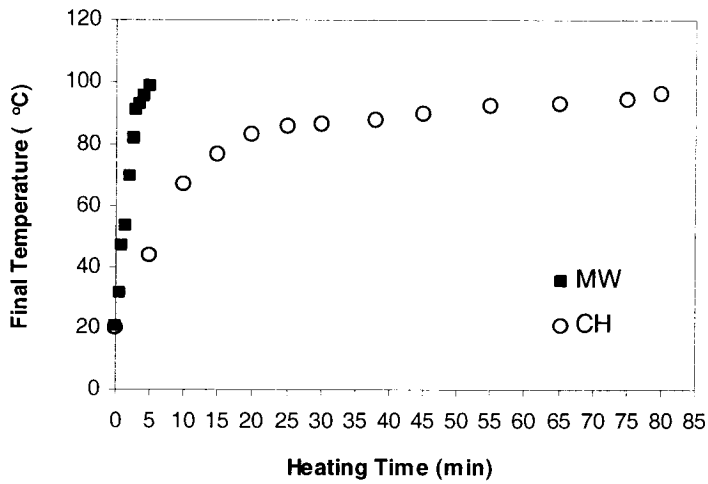


Figure 6-1 Pretreatment temperature profiles of raw TWAS (MW: microwave; CH: conventional heating).

After pretreatment, both raw and pretreated TWAS samples were centrifuged at 6300g [International Refrigerated Centrifuge, Model B-20, International Equipment Co., USA] for 40 min. The centrifuged supernatants underwent a primary filtration through a membrane disc filter with 0.45 μm pore size [GN-6 Metricel S-Pack] to separate any residual biomass. In order to prevent immediate clogging of UF membranes, preliminarily filtered supernatants were diluted and then used for Mw fractionation. The dilution ratio was 3.7:1 for control and CH or MW pretreated primary filtered

supernatants. Before UF fractionation, sample supernatants were characterized for COD, protein, sugar and TVFA concentrations (Table 6-2).

Table 6-2 Soluble phase characteristics of raw TWAS before and after pretreatment[a].

Parameter	Raw TWAS	MW-96	CH-96
pH [-]	7.5	6.9	7.0
SCOD [mg/L]	3,621 (396)†	8,669 (264)	16,518 (185)
Soluble sugar [mg/L]	80 (1)	91 (1)	325 (4)
Soluble protein [mg/L]	88 (8)	224 (3)	124 (8)
Soluble TVFA[2] [mg/L]	281 (12)	1346 (32)	936 (54)
Acetic acid [mg/L]	0 (0)	944 (30)	778 (104)
Propionic acid [mg/L]	281 (12)	402 (2)	158 (158)
Butyric acid [mg/L]	0 (0)	0 (0)	0 (0)

[a]MW-96 = pretreated with microwave to 96°C; CH-96 = pretreated with conventional heating to 96°C.
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicates).
[2]TVFA = total volatile fatty acids (summation of acetic, propionic and butyric acids).

6.3.2 Apparent Molecular Weight Distribution (AMwD) by Ultrafiltration (UF)

The AMwD of COD in supernatants before and after CH and MW pretreatments were determined by UF membranes in a 400 mL Amicon model 8400 stirred cell [Amicon Corp., MA, USA (Appendix A.4, Figure A-8)]. Flat (7.6 cm dia.), high recovery hydrophilic membranes [Millipore, MA, USA], which assured the highest possible retention with the lowest possible adsorption of DNA and other macromolecules during filtration were used. Membranes with molecular weight cutoffs (MwCOs) of 1 (YM1), 10 (YM10), 100 (YM100) and 300 kDa (PES300) were set-up in a cascading series method to reduce the risk of clogging for membranes with smaller MwCOs as shown in Figure 6-2.

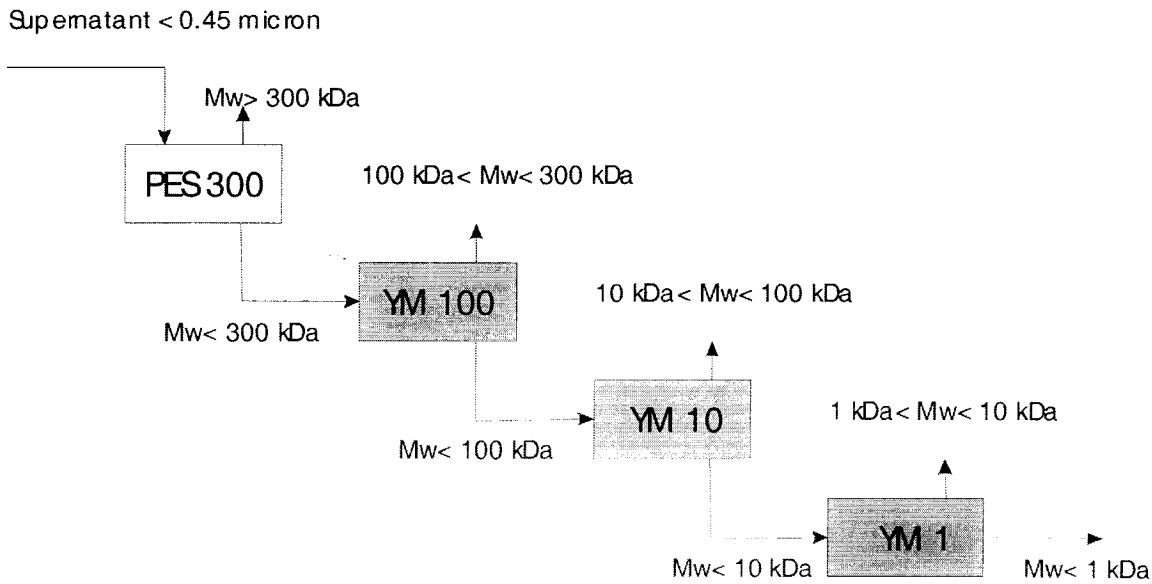


Figure 6-2 Schematic diagram of series UF for determination of AMwD (PES300: Polyethersulfone membrane with 300 kDa molecular weight cut off (MwCO); YM100, 10, 1: UF membranes with 100, 10, 1 kDa MWCOs, respectively; Mw: molecular weight).

The membranes were first rinsed with Milli-Q water in a beaker (by floating it glossy side down) for at least one hour, changing water three times. The rinsed membranes were placed into the Amicon model 8400 cell pressured with nitrogen and further rinsed with Milli-Q water for a minimum of 15 minutes. Nitrogen gas pressures were a maximum 10 psi for PES300 and YM100 membranes and 50 psi for YM10 and YM1 membranes as recommended by the manufacturer. After rinsing was completed, the stirred cell was loaded with 300 mL of the primary filtered (0.45μm) and diluted (3.7:1) supernatants from untreated, MW-irradiated and CH sludge samples. After 270 mL (90% of sample by volume) of the effluent had passed through the membrane, the pressure source was stopped and the cell was depressurized. The cell was stirred for another 15 min. to improve recovery from the membrane surface. A retentate volume of 30 mL was to be collected. The procedure was repeated until sufficient volume of retentate and

permeate was collected from each membrane with different MwCOs. After filtration was completed, membranes were rinsed with a 0.1 M NaOH solution and kept in a 10% (v/v) ethanol–water solution at 4°C as recommended by the manufacturer. Samples of permeates and retentates were analyzed for COD, TVFA, protein and sugars. Results for the control, MW-96 and CH-96 were expressed in terms of %w/w of the SCOD, soluble VFA, soluble protein and soluble sugars in the 300 mL preliminary filtered and diluted sample.

6.3.3 Determination of Anaerobic Biodegradability

The anaerobic degradability of the retentates and permeates from UF analysis was determined by batch biochemical methane potential (BMP) tests in 125 mL serum bottles [Wheaton (223748) borosilicate glass bottles, VWR, Montreal, QC, Canada (Appendix A.1, Figure A-7)] sealed with butyl rubber stoppers [Wheaton (224100-181) gray butyl snap-on stoppers for serum bottles with 13 x 20 mm mouth I.D. and O.D., VWR, Montreal, QC, Canada] and crimped with aluminum caps [Wheaton (224223-01) aluminum seals, VWR, Montreal, QC, Canada]. The BMP test performed in this study included 50 mesophilic (33 ± 1°C) batch reactors including controls and duplicates and the procedure described by Owen *et al.* (1979) was used. Experimental conditions for the BMP test are given in Table 6-3.

Table 6-3 Experimental conditions for the BMP test.

Run #	Reactor codes	Acclimatized inoculum (mL)	Supernatants of TWAS (mL)	Pretreatment temperature (°C)	Heating method
1	[a]$R_{MW1}/R_{MW1\text{-}d}$	15	70	96	MW
2	$R_{MW10}/R_{MW10\text{-}d}$	15	70	96	MW
3	$R_{MW100}/R_{MW100\text{-}d}$	15	70	96	MW
4	$R_{MW300}/R_{MW300\text{-}d}$	15	70	96	MW
5	$P_{MW1}/P_{MW1\text{-}d}$	15	70	96	MW
6	$P_{MW10}/P_{MW10\text{-}d}$	15	70	96	MW
7	$P_{MW100}/P_{MW100\text{-}d}$	15	70	96	MW
8	$P_{MW300}/P_{MW300\text{-}d}$	15	70	96	MW
9	$R_{CH1}/R_{CH1\text{-}d}$	15	70	96	CH
10	$R_{CH10}/R_{CH10\text{-}d}$	15	70	96	CH
11	$R_{CH100}/R_{CH100\text{-}d}$	15	70	96	CH
12	$R_{CH300}/R_{CH300\text{-}d}$	15	70	96	CH
13	$P_{CH1}/P_{CH1\text{-}d}$	15	70	96	CH
14	[1]$P_{CH10}/P_{CH10\text{-}d}$	15	70	96	CH
15	$P_{CH100}/P_{CH100\text{-}d}$	15	70	96	CH
16	$P_{CH300}/P_{CH300\text{-}d}$	15	70	96	CH
17	$R_{Cont\text{-}1}/R_{Cont\text{-}1\text{-}d}$	15	70	-	-
18	[2]$R_{Cont\text{-}10}/R_{Cont\text{-}10\text{-}d}$	15	70	-	-
19	$R_{Cont\text{-}100}/R_{Cont\text{-}100\text{-}d}$	15	70	-	-
20	$R_{Cont\text{-}300}/R_{Cont\text{-}300\text{-}d}$	15	70	-	-
21	$P_{Cont\text{-}1}/P_{Cont\text{-}1\text{-}d}$	15	70	-	-
22	$P_{Cont\text{-}10}/P_{Cont\text{-}10\text{-}d}$	15	70	-	-
23	$P_{Cont\text{-}100}/P_{Cont\text{-}100\text{-}d}$	15	70	-	-
24	$P_{Cont\text{-}300}/P_{Cont\text{-}300\text{-}d}$	15	70	-	-
Inoc.	I/I_d	15	-	-	-

[a]$R_{MW1}/R_{MW1\text{-}d}$, R = Retentate, MW = Microwave, 1 = UF membrane with MwCO of 1kDa, d = duplicate.
[1]$P_{CH10}/P_{CH10\text{-}d}$, P = Permeate, CH = Conventional heating, 10 = UF membrane with MwCO of 10 kDa.
[2]$R_{Cont\text{-}10}/R_{Cont\text{-}10\text{-}d}$, R = Retentate, Cont = Control (no pretreatment), 10 = UF memb. with MwCO of 10 kDa.

Inoculum for the BMP test was taken from the effluent line of the anaerobic sludge digesters treating a mixture of TWAS and PS [58:42 (v: v)] at ROPEC. In order to eliminate gross underestimation of the BMP of pretreated sludge due to possible lag-phase and/or inhibition at the early stages of digestion, inoculum was acclimatized to TWAS MW pretreated to 96°C. MW toxicity effect on secondary sludge digestion was expected to increase with MW temperature (Hong, 2002); therefore 96°C was used to pretreat feed sludge coming to the acclimation reactor. For acclimation, one 5 L anaerobic semi-continuous reactor, fed with MW-irradiated sludge, was run at approximately 20 d SRT by gradually increasing the influent flowrate over a period of three SRTs (Ekama *et al.*, 1986). Organic loading rate (OLR) of the acclimation reactor was 2.0 ± 0.04 g TCOD/ L. d. Acclimated inoculum had a specific activity of 0.12 ± 0.01 g TCOD/ g VSS. d.

For BMP set-up, acclimated inoculums (15 mL) were inoculated into serum bottles and then supernatant samples (70 mL) were added according to Table 6-3. Nitrogen sparging was applied to batch reactors when supernatants and inoculum were mixed to prevent exposure to air and reactors were sealed after addition of an equal mixture of $NaHCO_3$ and $KHCO_3$ to achieve an alkalinity of 4000 mg/L (as $CaCO_3$). Serum bottles were kept in a temperature controlled incubator shaker [PhycroTherm, New Brunswick Scientific Co. Inc., NB, Canada] at 33 ± 1°C and at 90 rpm until they stopped producing biogas. Daily biogas produced was measured by inserting a needle attached to a manometer. Biodegradation rates of permeates and retentates with different Mw fractions were calculated from experimental results and compared.

6.3.4 Analysis

TS and volatile solid (VS) of raw TWAS samples were determined based on Standard Methods procedure 2540G (APHA, 1995). Colorimetric TCOD and SCOD measurements were done based on Standard Methods procedure 5250D (APHA, 1995) using a Coleman Perkin-Elmer spectrophotometer Model 295 at 600 nm light absorbance. TVFAs (acetic, propionic and butyric acids) were measured by injecting supernatants to a HP 5840A capillary column GC and terminal integrator equipped with HP 7672A autosampler. Biogas composition (nitrogen, methane and carbon dioxide) was determined with a HP GC Model 5710 equipped with a thermal conductivity detector using helium as the carrier gas. The concentration of proteins and sugars in the soluble phase was measured at 595 and 575 nm by a Beckman DU-40 spectrophotometer according to colorimetric methods of Bradford (1976) and Miller (1959), respectively. Bovine serum albumin (BSA) and glucose stock solution were used as the protein and sugar standards.

6.4 Results and Discussion

6.4.1 Disintegration and Solubilization Effect of Pretreatments

It was hypothesized that MW-irradiation would disrupt the complex activated sludge floc structure as CH does and release extracellular (i.e., EPS) and intracellular biopolymers, such as protein and sugars from the activated sludge floc structure into the soluble phase as well as enhance the solubilization of particulate (microbial) COD. Table 6-2 shows the soluble phase characteristics of raw TWAS before and after pretreatments.

As expected, COD, protein, sugar and TVFA concentrations of soluble phases increased after MW and CH. However, although TWAS was pretreated to a similar

temperature (96 ± 2°C) by both MW and CH, the level of release of COD and biopolymers was different for MW and CH (Tables 6-1 and 6-2). Comparison of component concentrations before and after UF (Table 6-4) displays recoveries that range between 95-105% indicating negligible sorption of compounds to the membrane. Figure 6-3 shows the relative (to control) increases in SCOD, soluble biopolymers (protein and sugars) and soluble TVFA during CH and MW pretreatment. SCOD and soluble sugar concentration increased 361 ± 45 and 308 ± 2% respectively compared to the control for CH sludge and were always higher than those of MW-irradiated TWAS which were 143 ± 34% higher for SCOD and 15 ± 0% higher for soluble sugar compared to the controls.

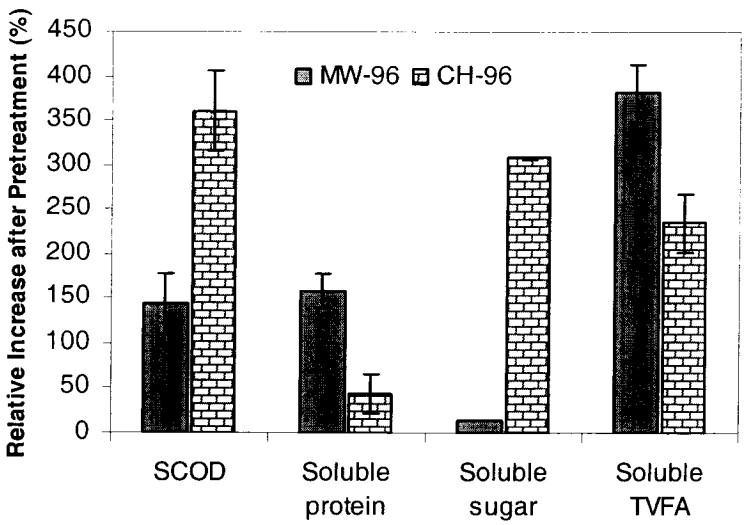


Figure 6-3 Solubilization effects of pretreatments on supernatants of raw TWAS (MW-96: microwave to 96°C; CH-96: conventional heating to 96°C; TVFA: total volatile fatty acids).

Table 6-4 Apparent molecular weight distribution using ultrafiltration (UF) for control and pretreated samples[a].

	Before UF [mg/L]	After UF* [mg/L]	Mw <1 [% w/w]	1< Mw <10 [% w/w]	10< Mw <100 [% w/w]	100< Mw <300 [% w/w]	Mw >300 [% w/w]
SCOD	{mass in mg}						
Control	3621 {3229}	3562 {3177}	53.0 (0.2)†	12.5 (0.3)	8.6 (0.6)	9.0 (0.0)	16.9 (0.7)
MW-96	8669 {7731}	8283 {7387}	33.7 (0.3)	15.6 (0.3)	12 (0.6)	9.2 (0.2)	29.5 (0.4)
CH-96	16518 {14732}	15878 {14161}	34.4 (0.1)	22.1 (0.3)	9.7 (0.1)	9.1 (0.1)	24.7 (0.3)
Soluble sugars							
Control	80 {71}	96 {86}	39.4 (0.4)	7.0 (0.0)	9.8 (0.0)	7.8 (0.2)	36.1 (0.2)
MW-96	91 {81}	140 {125}	11.5 (1.5)	11.5 (1.8)	13 (0.3)	7.6 (0.8)	56.4 (4.5)
CH-96	325 {290}	314 {280}	19.3 (0.4)	29.4 (0.6)	7.4 (0.0)	10.9 (0.0)	33.0 (0.2)
Soluble proteins							
Control	88 {78}	127 {114}	16.1 (0.1)	3.0 (0.8)	17.4 (0.7)	8.8 (0.0)	54.6 (1.6)
MW-96	224{200}	150 {134}	8.6 (0.4)	19.1 (0.2)	16.6 (0.2)	20.2 (0.4)	35.6 (0.1)
CH-96	124 {114}	131 {117}	4.8 (0.1)	34.8 (0.0)	3.2 (0.2)	6.6 (0.2)	50.6 (0.1)
Soluble TVFA[2]							
Control	281 {250}	n/a	n/a	n/a	n/a	n/a	n/a
MW-96	1346 {1201}	1474 {1315}	53.6 (0.2)	9.4 (0.3)	7.0 (0.4)	8.8 (0.1)	21.3 (0.4)
CH-96	936 {835}	956 {852}	40.5 (2.5)	16.5 (0.2)	8.9 (0.7)	15.8 (5.3)	18.4 (1.9)
Calibration of similar UF membranes by polyethylene glycol (PEG) (Barker et al., 1999)							
Prepared PEG standard			75.0	5.0	20.0	0.0	0.0
Measured PEG standard			89.3	2.3	8.4	0.0	0.0

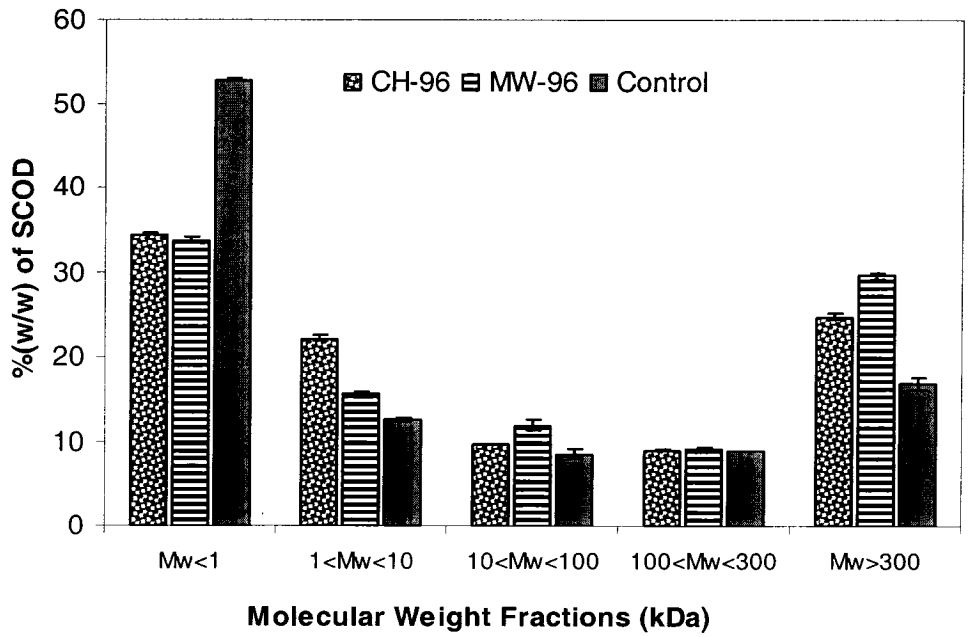
[a]MW-96 = pretreated with microwave to 96°C; CH-96 = pretreated with conventional heating to 96°C; Mw = molecular weight in kDa; n/a = information not available; [2]TVFA = summation of acetic, propionic and butyric acids.
*Calculated from the total amount of COD (or protein, sugar, TVFA) obtained in the 5 molecular weight fractions after UF.
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements).

Comparison of the SCOD/TCOD ratio gives a general indication of the extent of hydrolysis. The SCOD/TCOD ratio increased from 0.06 (control) to 0.15 and 0.27 for MW and CH pretreatments, respectively. TWAS pretreated with MW resulted in higher soluble protein (157 ± 20% higher compared to control) and higher soluble TVFA (381 ± 32% higher compared to control) concentrations in the UF supernatants compared to CH (43 ± 22% increase in soluble protein and 235 ± 34% higher soluble TVFA compared to controls). It is possible that the extended duration of exposure of CH to achieve a given temperature compared to MW exposure (Figure 6-1) has an effect on pretreatment. Higher duration of heat exposure to CH not only resulted in higher increase in SCOD and soluble sugar concentrations, but also caused higher level of denaturation of soluble proteins to ammonia and sugars and loss of TVFA at extended elevated temperatures in the water bath. Application of MW dwell times and heating rates should be explored to obtain a better CH vs. MW comparison (not possible with present equipment). While a direct comparison of CH and MW heating is problematic due to the difference in heating rates caused by equipment limitations, from a simple pretreatment solubilization point of view it would seem that MW pretreatment does not exhibit any significant athermal effects that can easily be substantiated. In fact as mentioned above, any possible MW athermal effects are smaller than thermal effects caused by longer CH exposure time. Additionally, the above changes in soluble concentrations have shown differences at the macro-scale (general soluble characterization) based on the type of pretreatment. It is highly likely that changes at the micro-scale (component fractionation based on Mw) have occurred that could affect the extent or rate of WAS stabilization.

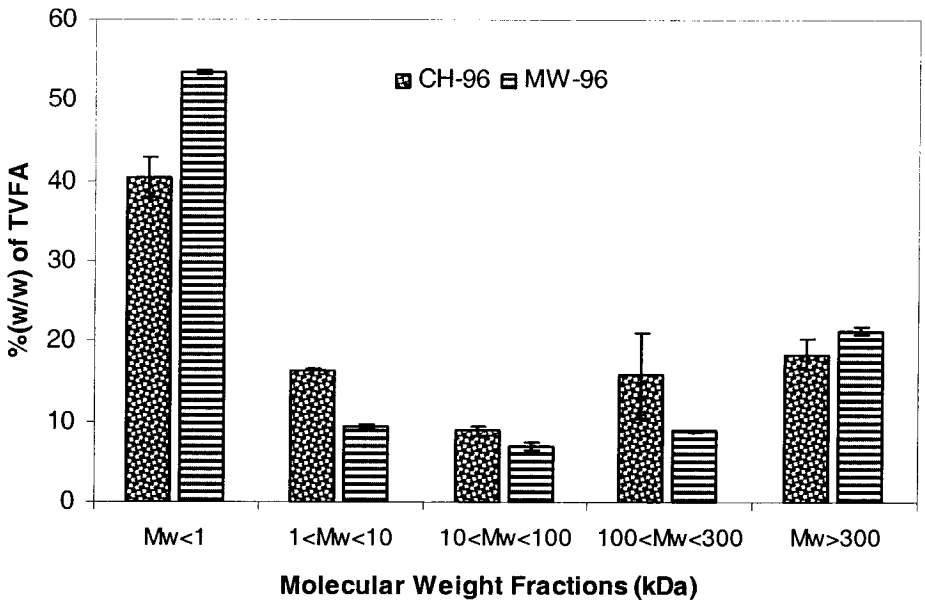
6.4.2 Apparent Molecular Weight Distribution (AMwD) by Ultrafiltration (UF)

The results from UF analysis are shown in Table 6-4 and Figures 6-4a-d. It can be seen from Figure 6-4a that for all three samples (control, MW-96 and CH-96), the bulk of the SCOD appeared to be in the Mw range <1 kDa and was expected to contain the VFAs (Barker *et al.*, 1999). Results presented in Figure 6-4b support this and indicate that 53.6 ± 0.2 and 40.5 ± 2.5% (w/w) of soluble TVFA in MW-96 and CH-96 pretreated samples respectively were present in the Mw fraction <1 kDa. The AMwD of TVFA in the control sample could not be analyzed because this sample contained very low TVFA concentration (76 mg/L; Table 6-4) compared to those in pretreated samples. The error range of the GC used for VFA analysis was ± 25 mg/L, therefore; it was impossible to determine accurate soluble TVFA concentrations for the control in most of the permeate fractions. A portion of the remainder of the SCOD fraction with Mw <1 kDa was most likely biopolymers (protein and sugars) as shown in Figures 6-4c-d.

a)


60
50
40
30
20
10
0
%(w/w) of SCOD
CH-96
MW-96
Control
Mw<1
1<Mw<10
10<Mw<100
100<Mw<300
Mw>300
Molecular Weight Fractions (kDa)

b)


60
50
40
30
20
10
0
%(w/w) of TVFA
CH-96
MW-96
Mw<1
1<Mw<10
10<Mw<100
100<Mw<300
Mw>300
Molecular Weight Fractions (kDa)

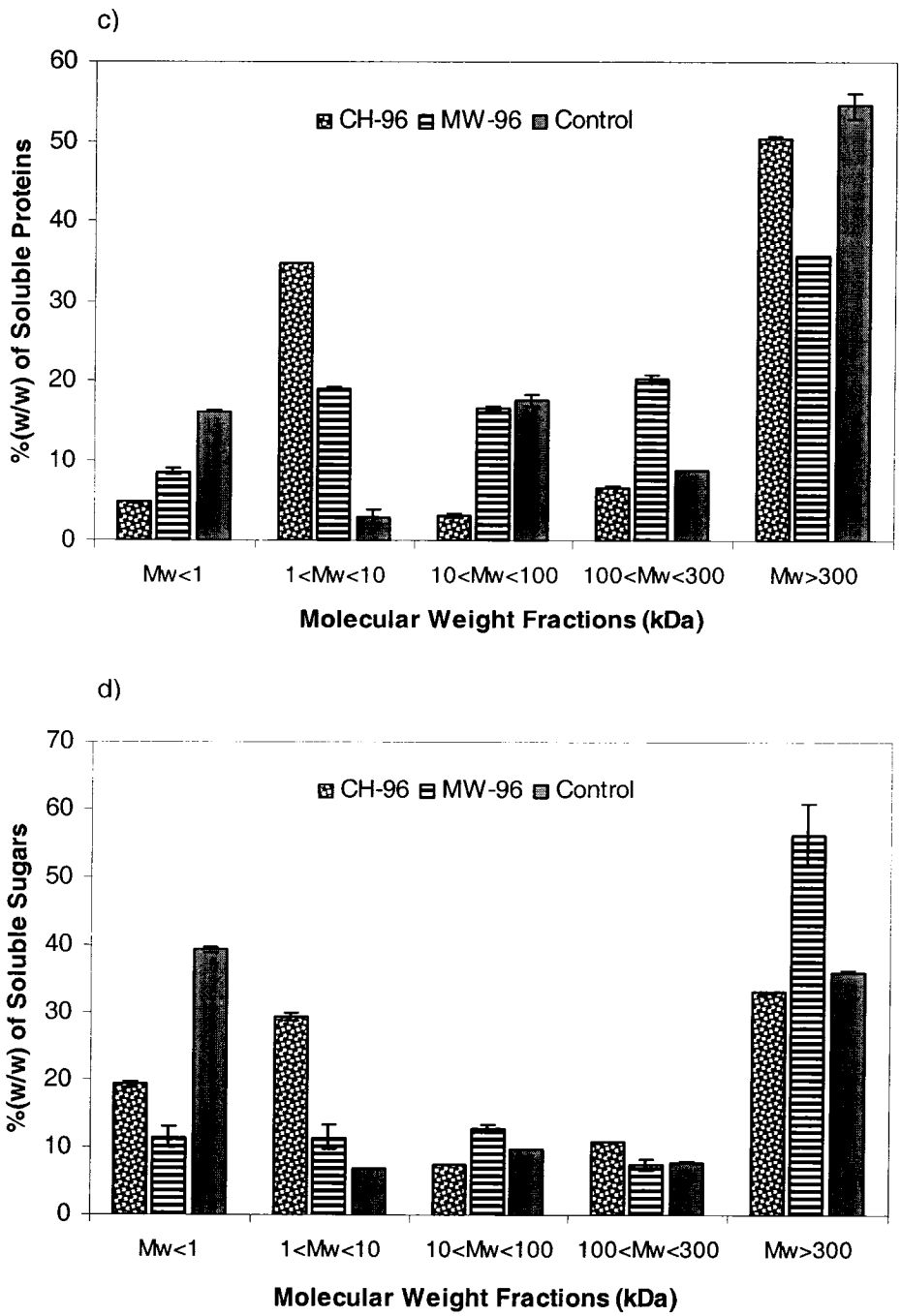


Figure 6-4 Apparent molecular weight distribution of a) SCOD; b) Soluble TVFA; c) Soluble proteins; d) Soluble sugars in control and pretreated samples (MW-96: microwave to 96°C; CH-96: conventional heating to 96°C; Mw: molecular weight).

The control contained a significant SCOD fraction of high Mw material (Mw >300 kDa, Figure 6-4a) and this portion was most likely cell wall fragments, exopolymers such as; exopolysaccharides, proteins, humic acids and nucleic acids (Manka and Rebhun 1982; Namkung and Rittman, 1986). The fact that the mass as well as proportion of high Mw material (Mw >300 kDa) increased in both pretreated samples (Figure 6-4a) was a strong indication that pretreated samples had higher portion of cell wall fragments and intra- and extra-cellular components released from activated sludge flocs compared to that of the control. Concomitantly, the largest mass and portion of soluble proteins in both MW-96 [35.6 ± 0.1% (w/w)] and CH-96 [50.6 ± 0.1% (w/w)] samples should be and was in the range above Mw >300 kDa (Figure 6-4c). Similarly the largest mass and portion of soluble sugars, 56.4 ± 4.5% (w/w) of soluble sugars in MW-96 and 33.0 ± 0.2% (w/w) of soluble sugars in CH-96 samples were high Mw >300 kDa compounds (Figure 6-4d). Interestingly, all three samples contained comparatively little SCOD material in the 10-300 kDa Mw range (Figure 6-4a). Similarly, soluble sugar Mw fractionation results for all three samples (Figure 6-4d) were similar with 13 ± 0.3% (w/w) or less in the 10-300 kDa range. However, MW-96 and CH-96 pretreatment resulted in different protein Mw distributions. Figure 6-4c indicates that, MW-96 pretreatment resulted in an approximately equal distribution of soluble protein in each Mw size range. However, for CH-96 samples the overall mass and proportion of the soluble protein fraction was bimodal with over 85% of the protein material found either in >300 kDa or between 1-10 kDa [50.6 ± 0.01% (w/w) with Mw >300 kDa and 34.8 ± 0.0% (w/w) with Mw between 1-10 kDa]. This might be the result of extended temperature exposure during CH that results in a greater proportion of solubilization of

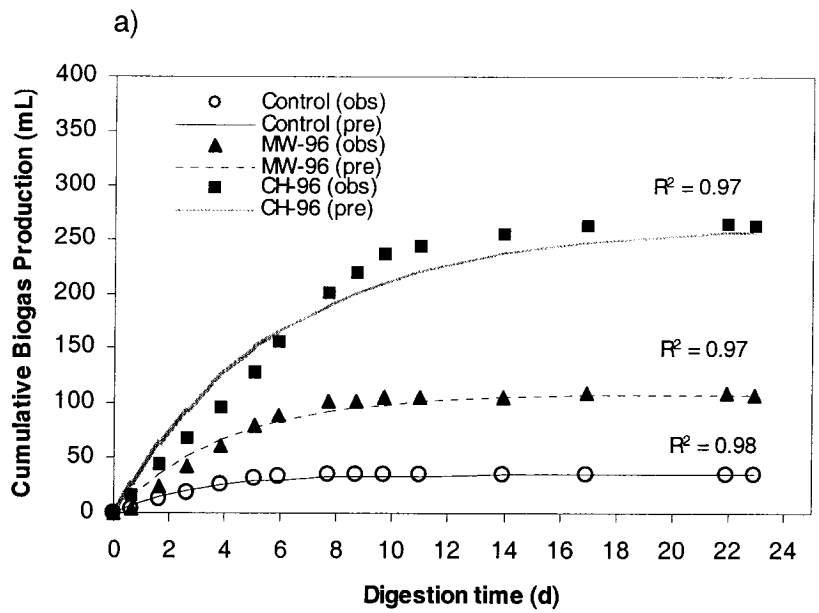
very large particulate proteins and subsequently a higher proportion hydrolyzed to smaller Mw.

It is worth emphasizing that at this time the pretreatment results reported above and further discussed below are most likely site specific and may be used for guidance but not for design of a specific pretreatment strategy. It is highly likely that macro-characteristics as well as the micro characteristics such as Mw distribution of organics found in the control and pretreated samples are likely influenced by the operating strategies of a particular wastewater treatment plant. Types of WAS produced based on carbonaceous biochemical oxygen demand (cBOD) removal, or biological nutrient removal processes, SRT of activated sludge (i.e., conventional vs. extended aeration) as well as chemical agents used for nutrient removal or dewatering can impact pretreatment. In the future it may be better to characterize pretreatment based on sludge types. In this project, the activated sludge SRT was ~5 d with minimal nitrification and might be characterized as a young cBOD removal based municipal WAS. Raw TWAS, used in this study, contained high amounts of biodegradable organics trapped in activated sludge flocs which were eventually disintegrated and released from the polymeric network as SCOD and soluble biopolymers during pretreatments. If TWAS was taken from an extended aeration activated sludge unit with a much longer SRT, control samples would likely contain more recalcitrant material with a lower percentage of high Mw materials (Mw >300 kDa) and a higher fraction of smaller material below <1 kDa. Kuo and Parkin (1996) used UF membranes in-series for fractionation of soluble microbial products (SMPs) in effluents from anaerobic treatment and observed that SMPs in the Mw < 1kDa varied in a range of 16-48% (w/w) depending on the SRT of the treatment units.

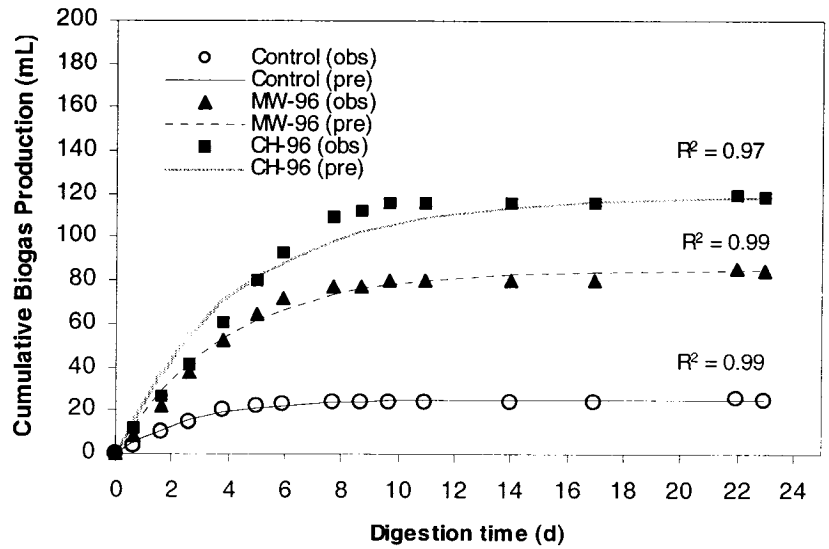
Despite the extensive usage of UF for Mw fractionation, readers should be aware that this method has a number of limitations. Factors; such as temperature, pH, ionic strength and trans-membrane pressure that can affect the advective and diffusive transport of organics through membranes as well as variable molecule size and shape all can impact on fractionation results for different membrane materials (Logan and Jiang, 1990). Table 6-4 indicated the measured concentrations of organics in the supernatants before and after UF fractionation to evaluate the mass recovery percentage of membranes. Results from this study and previous studies with similar membranes indicate that there is a high level of confidence in the data presented above. According to Table 6-4, recovery percentages for SCOD concentrations in all three samples were very high with error values of 1.6, 4.4 and 3.9% for the control, MW-96 and CH-96, respectively. Similarly, error percentages for soluble TVFA fractions were 2.1 and 9.5% for CH-96 and MW-96 samples, respectively. However, when the concentrations of soluble proteins in the solutions were measured, error percentages became larger. While MW-96 sample indicated only 67% recovery of total proteins, control samples resulted in 145% protein recovery after UF. These results are not only due to adsorption of some proteins by membranes during UF, but also due to different error percentages of the Bradford method (1976) for different protein concentrations in retentate and permeate of a particular sample. As a result of limitations emphasized above, polyethylene glycol (PEG) calibration tests conducted by Barker *et al.* (1999, Table 6-4) on similar membranes revealed that AMwD by UF technique can slightly overestimate lower Mw fractions in solutions which supported the results by Amy *et al.* (1987).

6.4.3 BMP Test Results

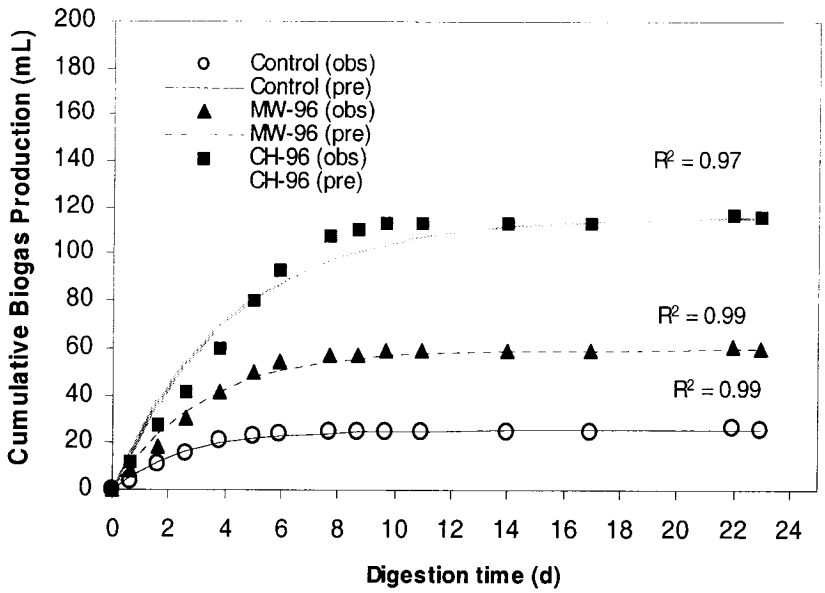
Figures 6-5a-d present observed cumulative biogas productions from reactors digesting various Mw retentates; while ultimate biogas productions of all reactors are tabulated in Table 6-5. In general, the BMP test results indicated that for the worst case only refractory non-biodegradable COD remained at the end of 23 days of mesophilic digestion at optimal conditions. In fact in many cases as early as the 8th day, the biodegradation could be considered to be completed; two additional weeks did not display any significant changes in COD although digesters continued producing small amounts of biogas. As biogas data indicated (Table 6-5), the CH-96 sample produced the highest amount of biogas (475 ± 3% higher from the overall sample relative to control) followed by the MW-96 sample (211 ± 2% higher from the overall sample relative to control). These results were not unexpected since supernatants of CH-96 and MW-96 contained 361 ± 45 and 145 ± 34% higher SCODs than the control (Figure 6-3).



b)


Cumulative Biogas Production (mL)
Digestion time (d)
Control (obs)
Control (pre)
MW-96 (obs)
MW-96 (pre)
CH-96 (obs)
CH-96 (pre)
R² = 0.97
R² = 0.99
R² = 0.99

c)


Cumulative Biogas Production (mL)
Digestion time (d)
Control (obs)
Control (pre)
MW-96 (obs)
MW-96 (pre)
CH-96 (obs)
CH-96 (pre)
R² = 0.97
R² = 0.99
R² = 0.99

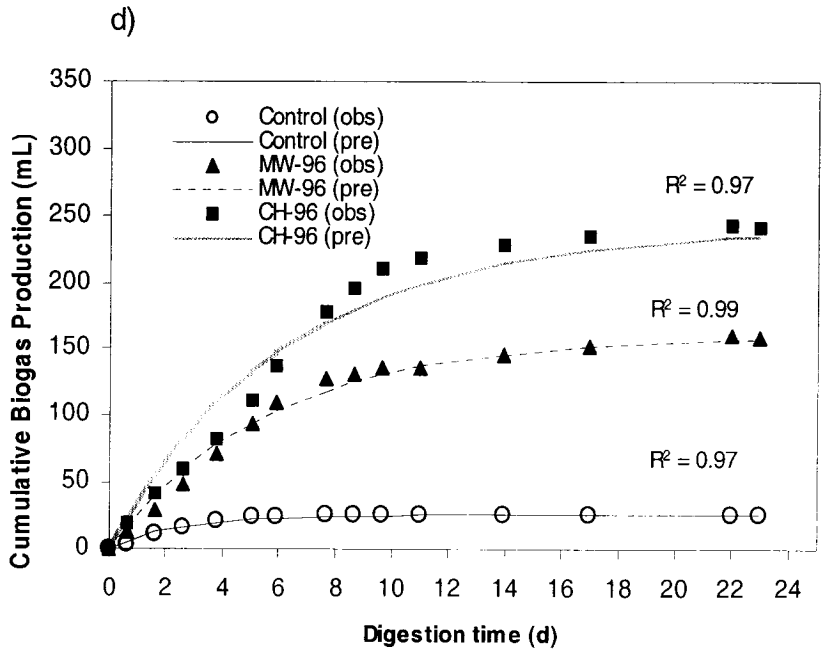


Figure 6-5 Observed and predicted cumulative biogas productions from molecular weight fractions of a) 1kDa<Mw<10kDa; b) 10kDa<Mw<100kDa; c) 100kDa<Mw<300kDa; d) Mw>300kDa (MW-96: microwave to 96°C; CH-96: conventional heating to 96°C; Mw: molecular weight; obs: observed; pre: predicted).

Table 6-5 Anaerobic biodegradability of molecular weight (Mw) fractions[a].

	Cumulative Biogas Produced [mL][n]			μ = Ultimate COD removal [% (w/w)]*			Reaction rate constant, k [d^{-1}]		
Mw (kDa)	**Control**	**MW-96**	**CH-96**	**Control**	**MW-96**	**CH-96**	**Control**	**MW-96**	**CH-96**
Retentates				{μ, predicted by first order model}				[R^2]	
1<Mw<10	35.0 (0.0)†	108.8 (0.6)	262.9 (0.3)	94.4 (1.0){94.4}	97.0 (0.4) {96.6}	93.4 (0.4) {91.5}	0.35 [0.98]	0.25 [0.97]	0.17 [0.97]
10<Mw<100	25.1 (0.9)	84.7 (0.7)	119.2 (0.6)	89.1 (1.7) {89.2}	91.7 (0.0) {91.4}	95.9 (0.2) {95.4}	0.37 [0.99]	0.26 [0.99]	0.23 [0.97]
100<Mw<300	25.4 (0.6)	59.5 (0.3)	116.5 (0.5)	88.0 (5.6) {88.0}	97.0 (0.3) {96.9}	94.2 (0.3) {93.8}	0.40 [0.99]	0.32 [0.99]	0.24 [0.97]
Mw>300	35.0 (0.0)	159.1 (0.3)	241.9 (3.5)	92.1 (0.7) {92.1}	95.1 (3.5){93.6}	92.7 (0.1) {90.2}	0.33 [0.97]	0.18 [0.99]	0.16 [0.97]
Permeates									
Mw<1	23.5 (0.3)	35.4 (0.8)	86.8 (1.0)	94.9 (2.3) {94.8}	99.0 (1.0) {98.9}	98.2 (0.4) {98.0}	0.39 [0.98]	0.33 [0.99]	0.26 [0.98]
Mw<10	27.3 (0.3)	50.5 (0.3)	127.0 (6.4)	96.7 (0.7) {96.7}	98.4 (0.4) {98.3}	96.4 (0.2) {95.8}	0.35 [0.99]	0.30 [0.99]	0.23 [0.98]
Mw<100	27.0 (0.2)	56.1 (0.1)	117.1 (0.1)	95.5 (0.0) {95.5}	96.5 (0.6) {96.3}	97.0 (0.2) {96.5}	0.39 [0.99]	0.29 [0.98]	0.24 [0.98]
Mw<300	28.7 (0.1)	59.2 (0.0)	118.4 (0.4)	94.7 (0.9) {94.7}	96.3 (0.1) {96.2}	97.4 (0.2) {96.8}	0.34 [0.99]	0.29 [0.99]	0.23 [0.98]
Overall sample[z]	**144.0 (1.8)**	**447.5 (2.7)**	**827.3 (5.9)**	**91.7**	**95.9**	**94.9**	**0.37**	**0.27**	**0.21**

[a]MW-96 = pretreated with microwave to 96°C; CH-96 = pretreated with conventional heating to 96°C; Mw = molecular weight in kDa;
[n]In calculating biogas amount and yield of the sample, the biogas produced in the inoculum bottle was subtracted from biogas produced in all other reactors.
*In calculating COD removal efficiency of the sample, the final COD in the inoculum bottle was subtracted from final COD concentrations in all other reactors.
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements).
[z]Overall sample = summation of fractions from all retentates + fraction of Mw<1 kDa.

The anaerobic biodegradability of both control and pretreated supernatants was judged by the ultimate COD removal percentages [μ = % (w/w)] given in Table 6-5. Appendix F, Table F-1 displays the initial and final COD concentrations of soluble fractions in the batch bottles. All of the retentates and permeates were highly biodegradable with μ values $\geq$ 88%. In general, anaerobic biodegradability of permeates were slightly higher than those of retentates. The methane (or biogas) yield for an anaerobic digestion is commonly expressed as a function of reduction in COD. In this project, the accuracy in determination of methane percentage of biogas in the head space of the digesters was low due to the small volume of biogas production (~35 mL from control digesters) relative to the volume of head space (40 mL) in BMP bottles. Therefore, biogas production and COD removal from 50 batch digesters in the BMP test were used and results indicated a biogas yield of 0.46 L per g COD removed at 1 atm. and 25 $\pm$1^{o}C as shown in Figure 6-6. The methane yields were in a range of 0.32 to 0.35 L/g COD removed based on a methane content 70 to 75% as measured by GC for BMP tests for both soluble and particulate fractions. These values are close to the theoretical methane production of 0.35 L/g COD removed and provide confidence in the results based on a COD balance.

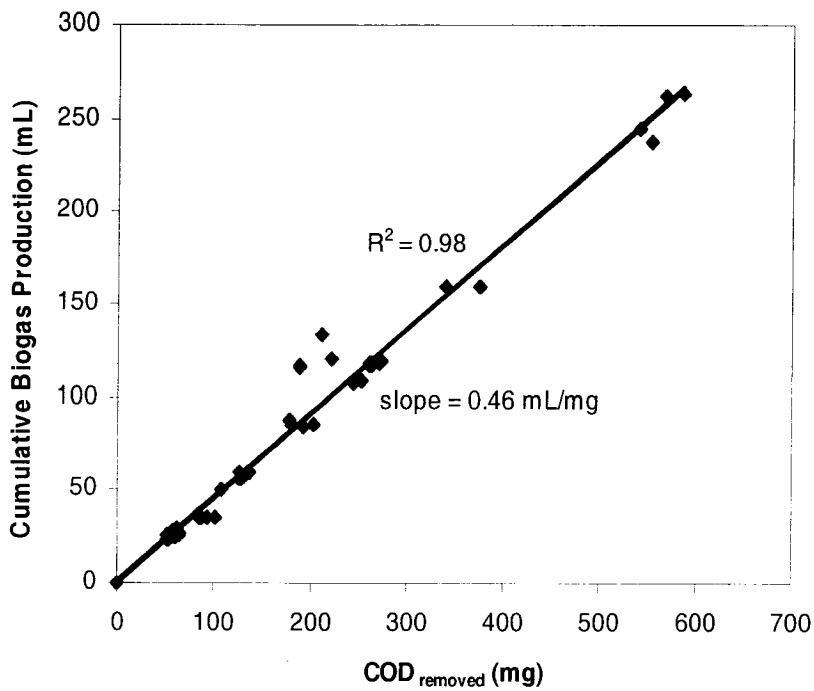


Figure 6-6 Relationship between biogas production versus COD removed (number of the data points = 50).

6.4.4 Kinetics for Anaerobic Degradation

Results from the BMP test were used to evaluate the anaerobic digestion rates of supernatant fractions with different Mw sizes. Anaerobic digestion is commonly expressed as a first-order reaction. The substrate utilization rate, r_{su} [mg/L.d], of anaerobic digesters in the BMP test can be represented by first-order reaction kinetics [eqn (6-1)];

$$r_{su} = dC/dt = -kC \tag{6-1}$$

in which C is the amount of organics (mg COD/L) and k is the anaerobic degradation rate constant (d^{-1}). Integration and rearrangement of eqn (6-1) yields;

$$Y_t = L_u \left(1 - e^{-kt}\right) \tag{6-2}$$

in which Y_t is the amount of organics removed at time t (mg COD/L) and L_u is the ultimate biodegradable organics (mg COD/L) in the sample and t is the digestion time (d). Therefore ultimate biodegradability of organics (μ) can be calculated as L_u/initial COD of the sample.

For determination of the kinetic coefficient based on Mw fractions, the amount of COD removed with respect to time, Y_t, was calculated from the biogas yield (biogas production per gram of COD) of the digesters (Figure 6-6) and cumulative biogas productions given in Table 6-5. The anaerobic degradation constant, k, was then determined by minimizing the sum of the squares of deviations of the observed COD removal values from predicted COD removals by eqn (6-2). The coefficient of determination, R^2 [(eqn (6-3)] was used as the primary discriminator to evaluate the adequacy of fit along with visual judgment.

$$R^2 = \left(1 - \frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2} \right) \tag{6-3}$$

in which $\bar{y}$ is the mean of observed values; y is the observed value; $\hat{y}_i$ represents the predicted value and n is the number of data points.

Using the Microsoft Excel Solver tool which has a nonlinear optimization code, k, μ values for control and pretreated digesters were estimated and reported in Table 6-5 along with R^2 values. The squared correlation coefficient, R^2, was generally close to unity, being greater than 0.97 for all cases. For visual observation, predicted cumulative biogas productions for retentates were also plotted along with the observed biogas values

(Figures 6-5a-d). Thus, the kinetics of COD removal and gas production was satisfactorily predicted with the first-order model. Interestingly, the retentate fractions from both control and pretreated samples yielded the largest anaerobic rate constant, *k*, values in adjacent mid-range Mw size ranges of 100 kDa <Mw> 300 kDa and 10 kDa <Mw> 100 kDa (Table 6-5). BMP digesters with COD fraction containing high Mw materials (Mw> 300 kDa) resulted in the smallest *k* values indicating that microorganisms took a longer time to utilize high Mw fractions which are most likely the more complex cell wall fragments and exopolymers such as; exopolysaccharides, proteins, humic acids and nucleic acids.

In general, most WAS pretreatment methods are known to increase the overall extent and anaerobic biodegradation rate constant (*k*) of whole pretreated WAS due to higher SCOD/TCOD ratios in pretreated digesters compared to controls (Lin *et al.*, 1999). However, rate constants from digesters utilizing various Mw SCOD fractions showed a different result (Figure 6-7). Comparison of the control and pretreated samples which were digesting SCODs with identical Mw fractions, *k* values were the highest for controls followed by MW-96 and CH-96. All permeate and retentate fractions were consistent in responses indicating that in a particular Mw fraction, denatured organics generated by thermal pretreatment methods were being digested at a slower rate than those in the control.

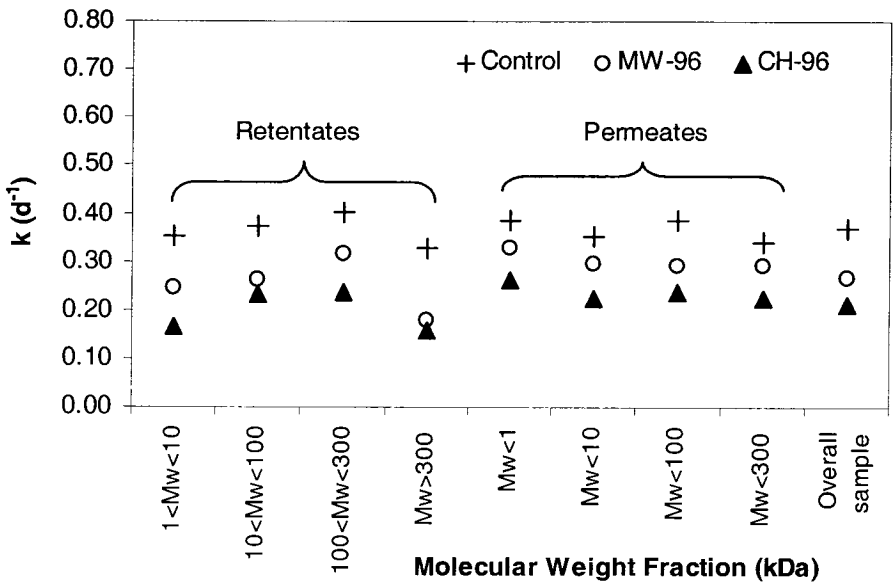


Figure 6-7 Degradation rate constant of digesters (MW-96: microwave to 96°C; CH-96: conventional heating to 96°C; Mw: molecular weight).

Additionally CH-96 samples had lower reaction rate constants than the similar MW-96 samples for all retentate and permeate fractions (Table 6-5; Figure 6-7). Overall k values based on all retentate fractions and Mw fraction <1 kDa were 0.37, 0.27 and 0.22 d^{-1} for control, MW-96 and CH-96, respectively. It is possible that lower k values for MW or CH pretreated samples could be the result of heat inactivation or denaturation of in situ WAS enzymes and biopolymers that contribute to degradation. Stuckey and McCarty (1984) reported a considerable amount of reduction in bioconvertibility and increase in toxicity of thermochemically (CH at 200°C for 1 h with NaOH) pretreated nitrogen compounds and mixtures present in WAS from a BMP test. If this is the case, the effects were more apparent for the CH-96 samples possibly due to the longer exposure to heat pretreatment (Figure 6-1). However it is necessary to emphasize that while the overall reaction rate constant, k, values were slightly smaller, substrate utilization rates (r_{su}) of both MW-96 and CH-96 digesters for the first 9 days of the

anaerobic digestion [from eqn (6-1)] were still much higher than those of controls for all retentate and permeate fractions because of the higher SCOD concentrations resulting from pretreatment (Figure 6-8). In all cases for each comparable Mw fraction, substrate removal rates r_{su} were greatest for CH-96 followed by MW-96 and the control. Depending on the Mw fraction, the range of r_{su} increases was between 94-184% for the CH-96 and 26-113% for the MW-96 compared to the control. Overall average substrate volumetric removal rates increased 136 and 75% for CH-96 and MW-96 pretreatments, respectively.

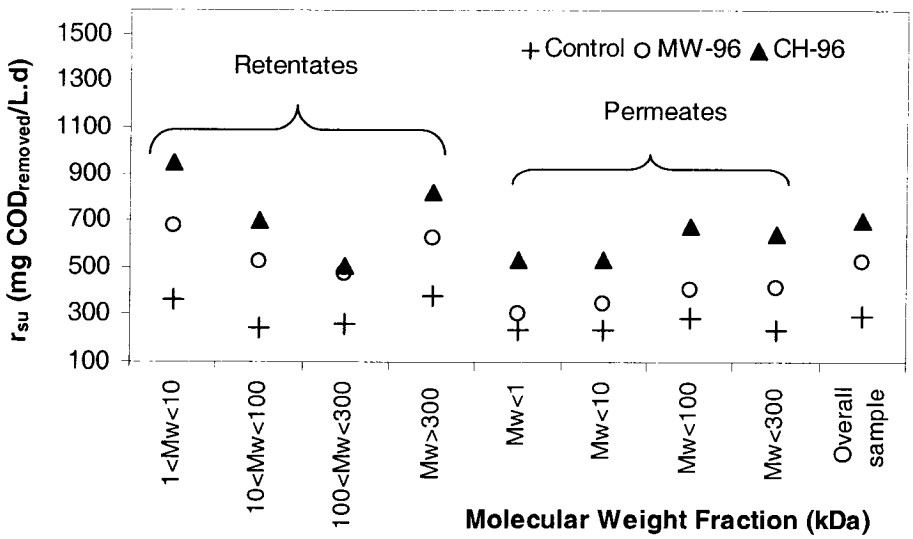


Figure 6-8 Substrate utilization rate of reactors for the first 9 days of digestion (MW-96: microwave to 96°C; CH-96: conventional heating to 96°C; Mw: molecular weight).

Biogas production and anaerobic biodegradability results from this study suggested that there is an advantage provided to anaerobic digestion by thermally pretreated supernatants of WAS since pretreated supernatants resulted in higher substrate stabilization per volume of reactor per unit time, hence more efficient digestion. It might be speculated that if no further reduction in k values results at higher temperatures or

exposures than found in this study, efficiency of digesters treating pretreated WAS may be further increased if a more sophisticated MW, capable of controlling and achieving higher temperatures and pressures resulting in complete or higher particulate COD solubilization, is used. Depending on the level of MW WAS solubilization, anaerobic digestion of only pretreated WAS supernatants might be possible and preferred resulting in higher organic loading rates and smaller reactor volumes compared to the digestion of total pretreated WAS containing both particulate and soluble fractions. At the elevated pretreatment temperatures and pressures, disposal of the particulate portion of WAS without digestion would be safer since MW technology has been shown to be capable of enhancing the pathogen destruction and dewaterability of WAS (Hong *et al.*, 2002; Toreci *et al.*, 2006). On the other hand, if k values are inversely proportional to increased temperatures and temperature exposures greater than 96°C any advantages of increased solubilization may be lost.

Assuming that no MW athermal affects are occurring, it might be argued that the MW pretreatment is the effect of temperature change on k while the CH pretreatment is a measure of the impact of temperature exposure to the same temperature on k. In only the crudest of comparisons, the change in k due to temperature change (compared to the control) was a 28% decrease or 5.5% decrease per minute exposure to 96°C. On the other hand, comparison of k values of MW-96 and CH-96 indicates that a 75 minute exposure to 96°C resulted in only a further 21% decrease in k (compared to MW-96) or 0.3% decrease per minute of exposure time (Figure 6-9). Overall the combination of exposure temperature and exposure time on k determined by comparing the control to the CH-96 was a 43% reduction. Based on this crude estimation, at a temperature close to the boiling

point, it would seem that temperature effects may have a greater impact on k than exposure effects and be indicative of an optimal pretreatment condition that has to take into account temperature, exposure, solubilization and biodegradation rate, k, effects.

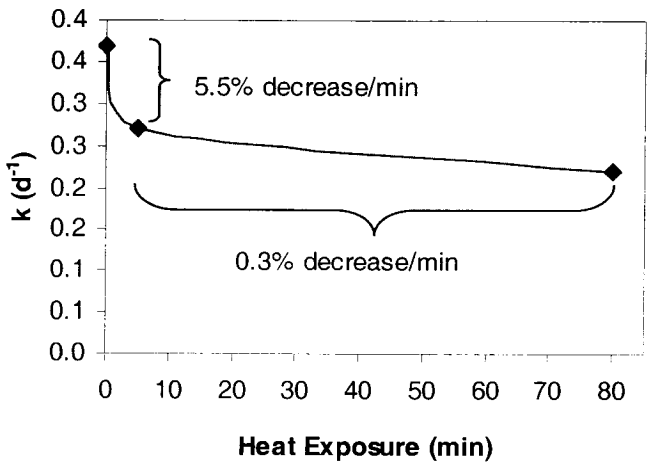


Figure 6-9 Relationship between heat exposure and degradation rate constant, k.

6.5 Conclusions

Based on the experimental data and analysis the following conclusions are drawn.

(1) Both MW-irradiation and CH at 96°C was successful in disrupting the complex activated sludge floc structure and releasing extra- and intra-cellular biopolymers, such as protein and sugars from activated sludge flocs into soluble phase along with the solubilization of particulate COD. However, the level of increase in SCOD, soluble sugar, soluble protein and soluble TVFA was different for MW-96 and CH-96 samples. Greater WAS solubilization with the CH-96 pretreatment was most likely due to extended duration of exposure of CH to achieve a given temperature compared to MW-irradiation.

(2) The majority of the SCOD for the control, MW-96 and CH-96 pretreatments was in <1 kDa Mw range and all samples contained little SCOD material in the 10-300 kDa Mw range.

(3) The BMP test indicated that retentate and permeate of all three samples were highly biodegradable with ultimate biodegradability values ≥ 88% (w/w). In general, anaerobic biodegradability of permeates were slightly higher than those of retentates. CH-96 samples produced the highest amount of biogas followed by the MW-96 samples which were 475 ± 3 and 211 ± 2% higher relative to control, respectively.

(4) The kinetics of COD removal and biogas production were satisfactorily predicted by a first-order reaction. Digesters with high Mw materials (Mw > 300 kDa) resulted in lower k values indicating that microorganisms required a longer time to utilize high Mw fractions which are most likely composed of cell wall fragments and exopolymers. Pretreatment resulted in slightly lower rate constants but overall high reaction rates based on first-order kinetics.

(5) Higher overall reaction rates based on biogas production and anaerobic biodegradability suggested that depending on the level of WAS solubilization, reduced digester volume and increased waste stabilization may be achievable via anaerobic digestion of pretreated supernatants only over the digestion of pretreated WAS containing both particulate and soluble fractions.

6.6 Acknowledgments

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CHAPTER 7

OVERALL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Based on the results of this research, the following general conclusions can be made:

(1) MW-irradiation in a temperature range of 50-96°C at 2450 MHz frequency had a potential of disintegrating activated sludge floc structure and releasing extracellular and intracellular compounds (proteins, sugars and nucleic acid) along with the solubilization of particulate COD.

(2) Solubilization experiments on microwaved WAS in a temperature range of 50-96°C resulted in 3.6 ± 0.6 and 3.2 ± 0.1 fold increases in SCOD/TCOD ratios at 5.4% TS (w/w) and 1.4% TS (w/w) concentrations compared to controls (unpretreated), respectively. Solubilization was always slightly higher at 50% than at 100% MW intensities for both sludge concentrations at the same MW temperatures possibly due to longer exposure time to MW field at low MW intensities. A multifactor ANOVA determined MW temperature, MW intensity and WAS concentration as significant factors for WAS solubilization at the 95% confidence level.

(3) MW pretreatment increased the bioavailability of sludge components under batch mesophilic anaerobic digestion. MW pretreatment did cause some short term inhibition of digestion but it did not cause any significant longterm chronic inhibition of acclimated biomass during batch mesophilic digestion. Mesophilic digestion of WAS (5 d SRT) microwaved to 96°C enhanced the ultimate

degradability of WAS and produced 15 ± 0.5, 14 ± 1.1 and 20 ± 0.3 and 21 ± 0.5% increases in biogas production over controls after 19 d of digestion at 100 and 50% MW intensities and at 1.4% TS (w/w) and 5.4% TS, respectively. A multifactor ANOVA determined volume percentage of MW pretreated WAS in mesophilic digesters, MW temperature, and concentration of WAS pretreated as significant factors for enhanced biogas production (at the 95% confidence level) and MW intensity was eliminated from experimental design.

(4) WAS solubilization and continuous mesophilic digester performance was always better for CH pretreatment compared to MW heating at similar temperatures and was due to the extended duration of exposure of CH to achieve a given temperature compared to MW irradiation. Semi-continuous flow mesophilic digesters compared the performance of MW and CH WAS (at 50 and 96°C) with acclimatized inoculum at SRTs of 5, 10 and 20 d. In general, incremental increases in TS, VS and TCOD removal efficiencies of pretreated digesters compared to controls dramatically increased as SRT was gradually shortened from 20 to 10 to 5 d. TWAS pretreated to 96°C by MW and CH achieved 29 and 32% higher TS and 23 and 26% higher VS removal efficiencies compared to controls at SRT of 5 d, while similar reactors at SRT of 20 d had only 16% higher TS and 11 and 12% higher VS removals than those of controls, respectively.

(5) Control samples were the most difficult to dewater for all SRTs. Sludge from reactors digesting MW and CH WAS at 96°C achieved 39 and 29% better dewaterability compared to the control, respectively, at SRT of 10 d. This result

again indicated that thermal pretreatment methods have potential to enhance dewaterability after anaerobic digestion.

(6) SCOD and ammonia-N concentrations were dramatically increased in digester effluent as pretreatment temperature was increased and SRT of the digester was decreased.

(7) Batch mesophilic anaerobic digestion of supernatants (soluble fraction) from both untreated (raw) and pretreated WAS with MW and CH at 96°C was also examined. SCODs of CH and MW irradiated WAS were 361 ± 45 and 143 ± 34% higher and resulted in 475 ± 3 and 211 ± 2% higher cumulative biogas productions relative to the control at the end of 23 days digestion, respectively.

(8) UF was used to characterize the soluble organic matter of WAS by their molecular weight. The BMP test indicated that retentate and permeates of both control and pretreated samples were highly biodegradable with ultimate biodegradability values ≥ 88% (w/w). The kinetics of COD removal and biogas production were satisfactorily predicted by a first-order reaction.

(9) Lower biodegradation rate constant, *k*, values for supernatants of CH and MW pretreated samples were observed compared to those of controls with similar molecular weight distribution possibly as a result of heat inactivation or denaturation of in situ WAS enzymes and biopolymers that contribute to degradation. Therefore for an optimal pretreatment condition, temperature, exposure, solubilization and biodegradation rate, *k*, effects should be taken account.

(10) Results from this research imply that MW pretreatment at temperatures under the boiling point (100°C at 1 atm) has a significant potential to disintegrate the secondary sludge floc structure and to enhance the hydrolysis and biodegradation of WAS in full-scale mesophilic sludge reactors.

7.2 Recommendations

In order to improve the understanding of all the phenomena involved in MW pretreatment studies, the following recommendations are suggested:

(1) Study the effects of MW pretreatment on secondary sludges taken from different activated sludge units (conventional, nitrification, etc.) with a wide range of SRT and operating conditions (chemical agents used for nutrient removal or dewatering).

(2) Increase the energy efficiency of the MW pretreatment step by increasing the solid percentage of WAS samples to feasible concentrations, that has to be determined for each sludge sample, before pretreatment.

(3) Explore higher MW temperatures as well as dwell times with a more sophisticated MW capable of reaching and holding certain temperatures at variety of MW intensities and durations to obtain greater solubilization and better CH vs. MW comparison.

(4) Explore the role of extracellular polymeric substances and cations that hold the polymeric network together in the general concept of MW pretreatment studies.

(5) Apply all of the above recommendations to thermophilic anaerobic sludge digestion.

APPENDIX A

A.1 MICROWAVE OVEN and PC SYTEM USED

Microwave (MW) irradiation was applied by a 0.045 m^3 capacity household type MW oven (displayed in Figure A-1; Panasonic NNS53W + inverter, 1250 W, 2450 MHz frequency and 12.24 cm wavelengths). Due to lower energy consumption at low MW temperatures, this study focused on a temperature range of 50-96°C, which was achievable with a kitchen type MW oven and a plastic MW transparent container designed for food applications. Temperature readings were taken with fast response insulated thermo-couple probes [Figure A-1; Cole-Parmer (P-08506-75), T-type, fine-gauge Teflon PTFE response time of 0.5 s, Labcor Technical Sales Inc., ON, Canada] connected to a module for analog-to-digital conversion and recorded by a laboratory computer system, LabVIEW Software Version 6, National Instruments Co., Austin, TX, USA (Figure A-2).

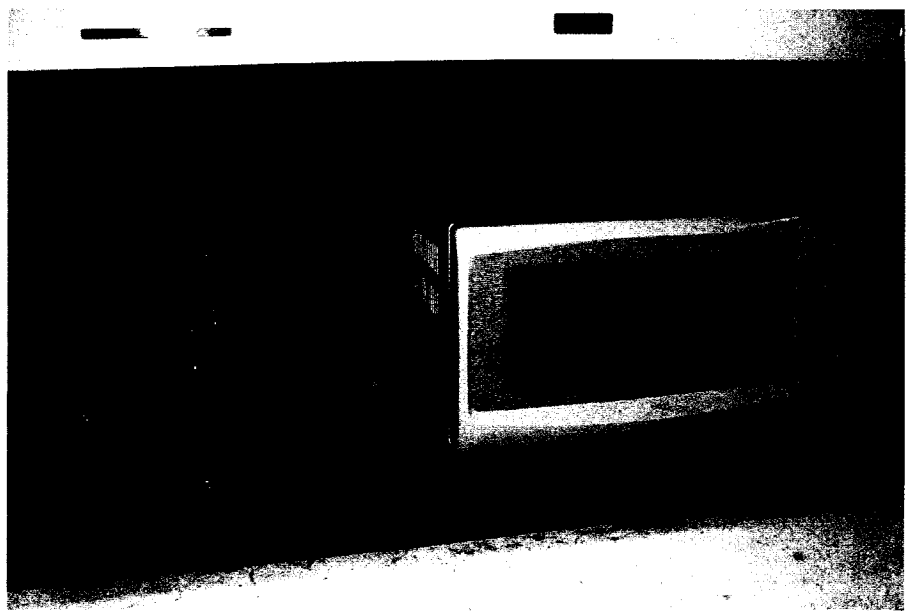


Figure A-1 MW oven and thermocouple probes used for temperature readings.

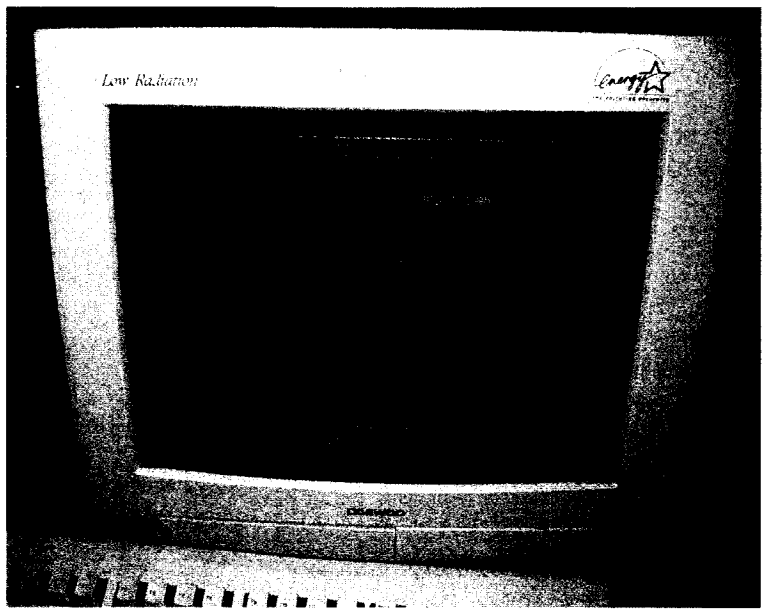


Figure A-2 LabVIEW software used for temperature readings display and recording.

A.2 BATCH ANAEROBIC SLUDGE DIGESTERS

The statistical design explained in Chapter 3 contained in 54, 500 mL glass bottles [shown in Figure A-3; Wheaton (219919) bottles with polypropylene caps (240746), screw cap size of 45 mm, VWR, Montreal, QC, Canada] with butyl rubber stoppers [see Figure A-3; Wheaton (22400-503) lyophilization stopper, screw cap size of 43 mm, VWR, Montreal, QC, Canada].

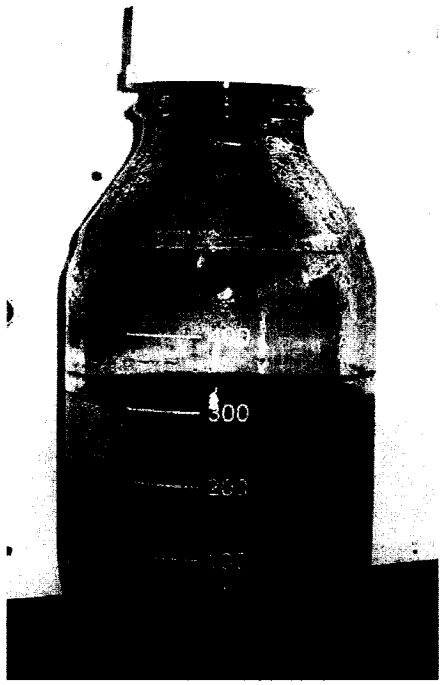


Figure A-3 Batch glass bottle used for the multilevel factorial design.

Batch reactors were kept in a temperature controlled incubator shaker [Figure A-4; PhycroTherm, New Brunswick Scientific Co. Inc., NB, Canada] at 33 ± 1°C and mixed at 90 rpm to keep the bacteria - sludge mixture in suspension.

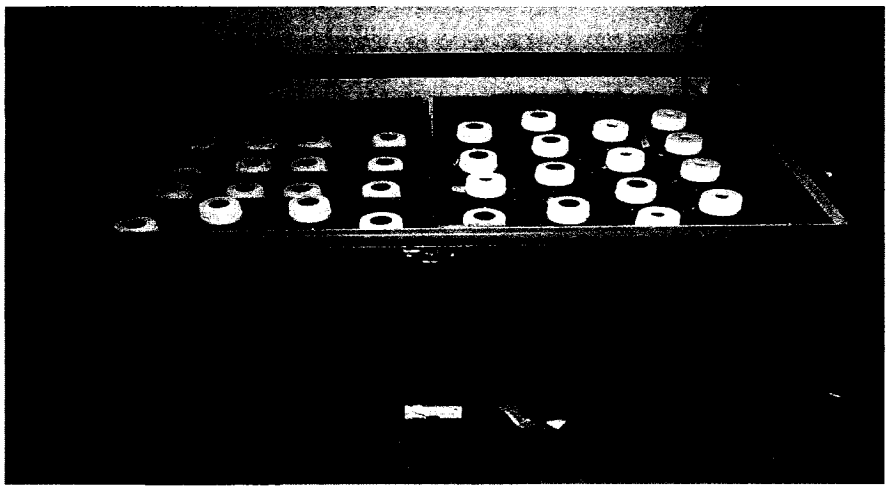


Figure A-4 Batch reactors and temperature controlled incubator.

A.3 INOCULUM ACCLIMATION BEFORE ANAEROBIC DIGESTION

Inoculum for the biochemical methane potential (BMP) test, explained in Chapter 3, was taken from the effluent line of the anaerobic sludge digesters treating a mixture of primary sludge (PS) and TWAS [58:42 (v: v)] at municipal wastewater treatment plant (ROPEC). For acclimation, one 5 L anaerobic semi-continuous (SC) reactor (Figure A-5), fed with MW-irradiated sludge (96°C), was run at approximately 20 d sludge retention time (SRT) by gradually increasing the influent flowrate over a period of approximately three SRTs. Organic loading rate (OLR) of the acclimation reactor was 2.0 ± 0.04 g TCOD/L.d.

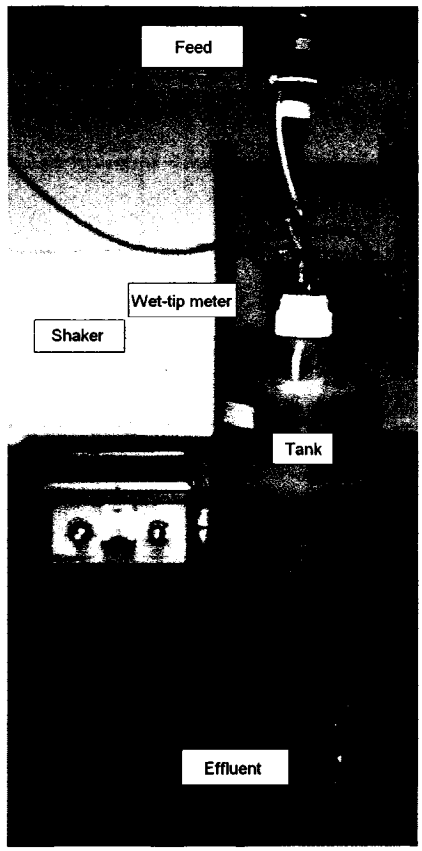


Figure A-5 Semi-continuous reactor used for acclimation of inoculum to MW pretreated TWAS.

A.4 SEMI-CONTINOUS ANAEROBIC SLUDGE DIGESTERS

SC anaerobic sludge digestion was discussed in Chapter 5. Side-armed erlenmeyer flasks with a volume of 1 L [see Figure A-6; Pyrexplus (29410-993) plastic-coated graduated flasks, VWR, Montreal, QC, Canada] were used as SC flow reactors. The total number of SC digesters was 10 including controls and duplicates. Erlenmeyer flasks were sealed with rubber stoppers [two-hole black rubber stoppers (59582-326), VWR, Montreal, QC, Canada] and side arms were used to feed the reactors. Two sampling ports (first port to withdraw the sludge from the digester) were bored into the rubber stoppers. The second sampling port was connected to a 1 L tedlar bag for biogas collection. The tedlar bags [Tedlar gas sampling bags (TDP070710), Chromatographic Specialties Inc., ON, Canada] were equipped with on/off valves and a septum fitting that was used for gas composition sampling. Volume of daily biogas collected was measured by a manometer.

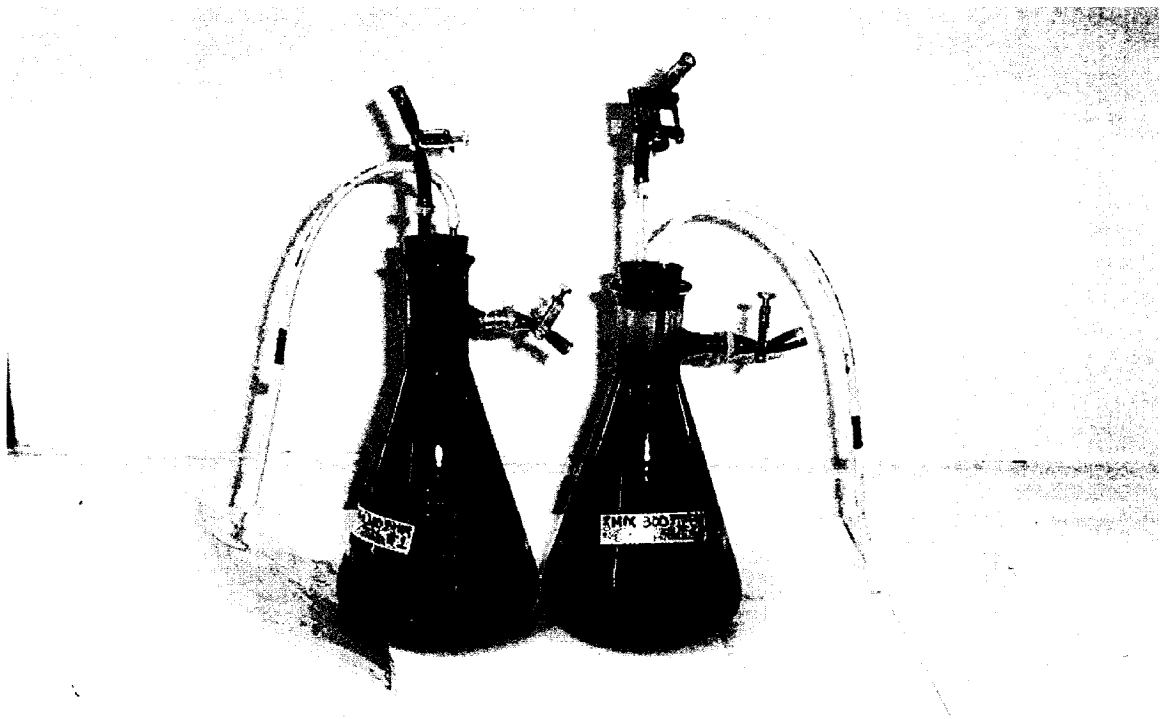


Figure A-6 Semi-continuous anaerobic sludge digesters.

A.5 BATCH DIGESTERS FOR CHARACTERISATION OF SOLUBLE FRACTION OF TWAS

In Chapter 6, the anaerobic degradability of soluble fraction of TWAS was determined by 125 mL serum bottles [see Figure A-7; Wheaton (223748) borosilicate glass bottles, VWR, Montreal, QC, Canada] sealed with butyl rubber stoppers [Wheaton (224100-181) gray butyl snap-on stoppers for serum bottles with 13 x 20 mm mouth I.D. and O.D., VWR, Montreal, QC, Canada] and crimped with aluminum caps [Wheaton (224223-01) aluminum seals, VWR, Montreal, QC, Canada]. The BMP test performed in Chapter 6 included 50 mesophilic (33 ± 1°C) batch reactors including controls and duplicates.

Figure A-7 Serum bottle used for characterization of soluble fraction of TWAS.

A.6 STIRRED ULTRAFILTRATION (UF) CELL

The apparent molecular weight distribution (AMwD) of chemical oxygen demand (COD) in supernatants before and after conventional heating (CH) and MW pretreatments were determined by UF membranes in a 400 mL Amicon model 8400 stirred cell [see Figure A-8; Amicon Corp., MA, USA]. Flat (7.6 cm dia.), high recovery hydrophilic membranes [Millipore, MA, USA], which assured the highest possible retention with the lowest

possible adsorption of DNA and other macromolecules during filtration were used. Membranes with molecular weight cutoffs (MwCOs) of 1 (YM1), 10 (YM10), 100 (YM100) and 300 kDa (PES300) were set-up in a cascading series method to reduce the risk of clogging for membranes with smaller MwCOs.

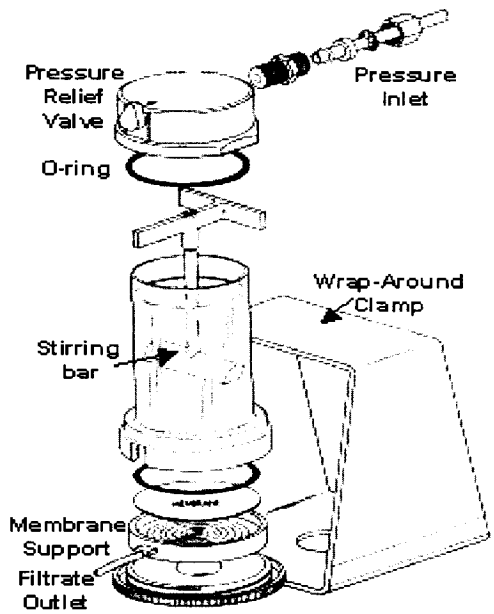
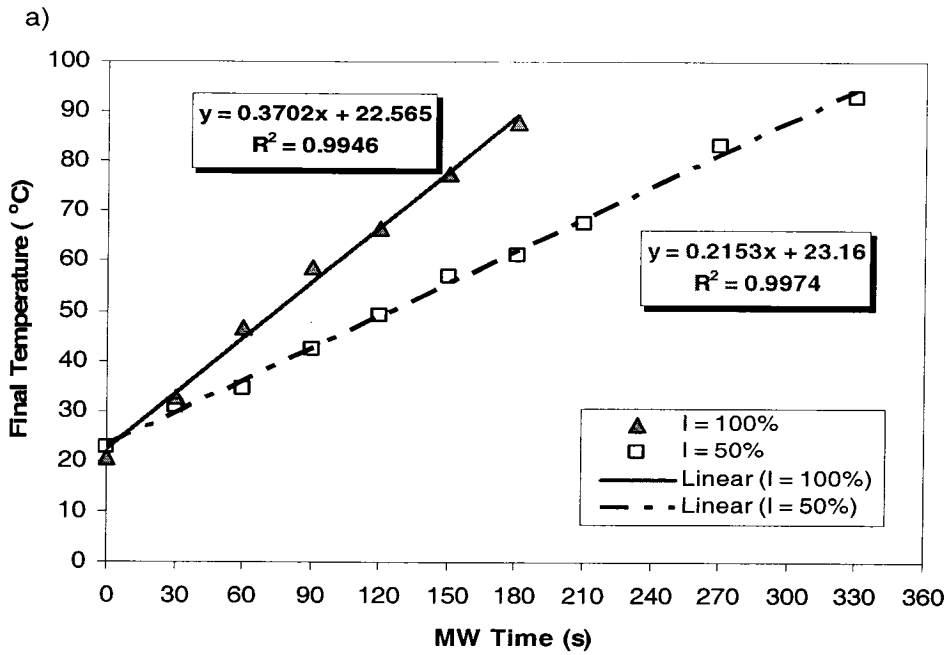


Figure A-8 Stirred UF cell.

APPENDIX B

MICROWAVE CALIBRATION CURVE FOR TWAS (1.4% TS)

Microwave (MW) calibration curves were prepared for TWAS with 1.4% TS (w/w) at two different MW intensities (50 and 100%) to reach desired MW temperatures (50, 75 and 96°C). For each pretreatment, 500 g of TWAS sample at room temperature ($T_{initial}$ = 20 ± 1°C) was transferred to a 1 L (19 x 12 x 4.4 cm) plastic MW resistant container and subsequently exposed to a MW field for different durations of times while using the turning table in the MW oven. The container was covered with a plastic lid to prevent evaporation during the irradiation. After the container was removed, the sample was vigorously stirred and the maximum temperature was recorded within 10 s. Experimentally prepared calibration curves (Figures B-1a, b) were used to estimate microwaving times of TWAS samples for each pretreatment step.



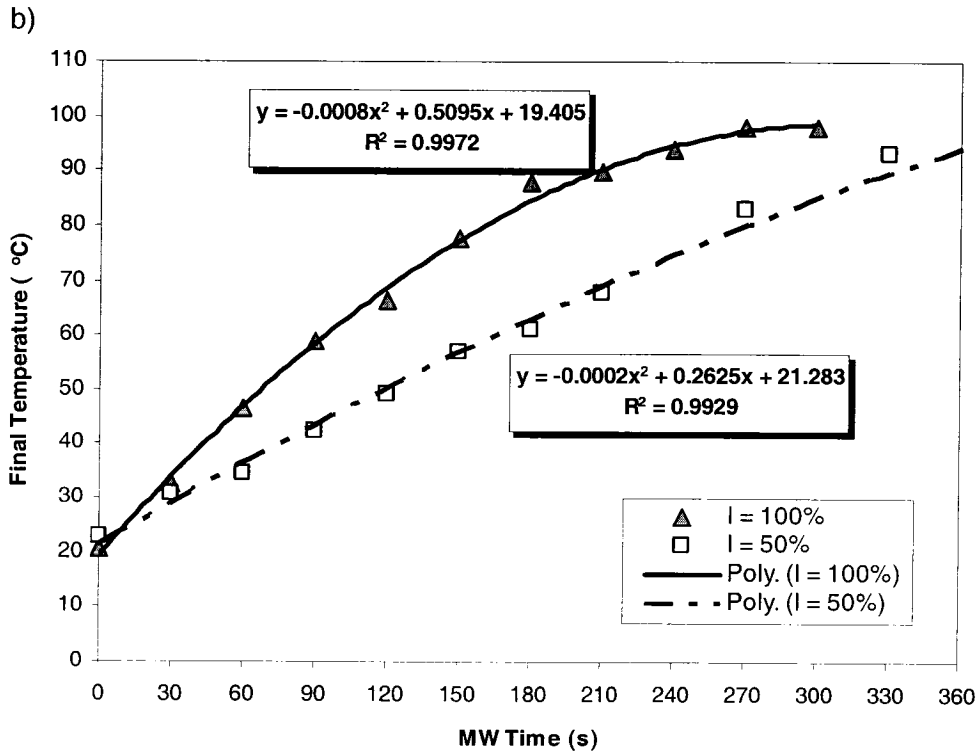


Figure B-1 Microwave calibration curves for TWAS [1.4% TS (w/w)] a) under boiling point; b) at the boiling point (MW: microwave; I: intensity).

In Figure B-1a, only temperature measurements up to 90°C were considered. In this range, it was clear that there was a linear relationship between MW time and temperature increase of WAS at both MW intensities. Due to the lower amount of power applied at low intensities, 50% MW intensity resulted in a longer exposure time for the same sludge sample to reach the same temperature as a sample microwaved at a 100% MW intensity. On the other hand, when a larger range (up to 100°C) was covered, the calibration curve was transformed to a second-order function (Figure B-1b). The logical explanation for this result is that as temperature values closer to the boiling point were reached, some of the heat energy added to the system was used to change state (from liquid to gas) instead of raising the temperature of the system.

APPENDIX C

CHARATERIZATION OF TWAS AND ANAEROBIC INOCULUM USED FOR BATCH ANAEROBIC DIGESTION

Thickened waste activated sludge (TWAS) used in Chapters 3 and 4 was obtained from the thickener centrifuge with total solid (TS) concentration of 5.4% (w/w) at the *Robert O. Pickard Environmental Center (ROPEC)* located in Gloucester, (ON, Canada). Anaerobic inoculum used for the BMP test (Chapters 3 and 4) was originally taken from the effluent line of the anaerobic sludge digesters treating a mixture of primary sludge and TWAS [58:42 (v: v)] at ROPEC and acclimatized to microwave pretreated TWAS (96°C). Results from the analysis on characterization of raw ROPEC TWAS are presented in Table C-1.

Table C-1 Characteristics of raw TWAS used in batch digestion.

Parameter	Raw TWAS	Inoculum (BA*)	Inoculum (AA*)
pH [-]	6.49	7.65	8.08
TS [%, (w/w)]	5.40 ± 0.02†	2.13 ± 0.01	1.96 ± 0.00
VS [%, (w/w)]	3.77 ± 0.01	1.21 ± 0.01	1.02 ± 0.00
TSS [%, (w/w)]	5.13 ± 0.03	1.99 ± 0.01	1.79 ± 0.02
VSS [%, (w/w)]	3.66 ± 0.04	1.10 ± 0.00	0.90 ± 0.02
TCOD [mg/L]	41,667 ± 1190	21,429 ± 857	18,514 ± 343
SCOD [mg/L]	2,357 ± 71	383 ± 56	557 ± 14
NH_3-N [mg/L]	536 ± 8	937 ± 0	1280 ± 0
[a]TVFA [mg/L]	913	0	0
[2]Alkalinity		4239 ± 10	5824 ± 5

†Data represent arithmetic mean of duplicates ± absolute difference between mean and duplicates.
[a]TVFA = summation of acetic, propionic and butyric acids; *BA, AA: before and after acclimation, respectively; [2] Bicarbonate alkalinity in units of mg/L as calcium carbonate ($CaCO_3$).

APPENDIX D

BIOGAS COMPOSITION, VFA AND pH VALUES OF BATCH DIGESTERS

Reactor pH (Table D-1), biogas composition (nitrogen, methane and carbon dioxide percentage; Table D-2) and total volatile fatty acids (TVFAs; summation of acetic, propionic and butyric acids; Table D-3) were monitored weekly during the anaerobic digestion of thickened waste activated sludges (TWAS) in multilevel statistical design explained in Chapter 3. Fisher Accumet Model 925 pH/ion meter was used for pH measurements and TVFAs were measured by injecting supernatants to a HP 5840A capillary column GC and terminal integrator equipped with HP 7672A autosampler. Biogas composition was determined with a HP GC Model 5710 equipped with a thermal conductivity detector using helium as the carrier gas.

Table D-1 Reactor pH levels during batch anaerobic digestion[a].

		Digestion time (d)				
Run #	**T/I/C/PT**	**0***	**8**	**15**	**26**	**37**
$1/1_d$	50/50/1.4/50	6.92 ± 0.00†	7.76 ± 0.02	8.25 ± 0.01	7.78 ± 0.09	7.34 ± 0.02
$2/2_d$	50/50/3/50	6.99 ± 0.00	7.87 ± 0.16	8.67 ± 0.05	8.06 ± 0.00	7.52 ± 0.01
$3/3_d$	50/100/1.4/50	6.91 ± 0.00	7.77 ± 0.02	8.43 ± 0.08	7.29 ± 0.05	7.37 ± 0.00
$4/4_d$	50/50/3/50	6.97 ± 0.00	8.22 ± 0.01	8.57 ± 0.01	7.98 ± 0.07	7.50 ± 0.01
$5/5_d$	75/50/1.4/50	6.78 ± 0.00	7.93 ± 0.01	8.27 ± 0.03	7.58 ± 0.00	7.45 ± 0.00
$6/6_d$	75/50/3/50	6.96 ± 0.00	7.98 ± 0.03	8.63 ± 0.05	7.80 ± 0.16	7.55 ± 0.05
$7/7_d$	75/50/1.4/50	6.98 ± 0.00	7.94 ± 0.20	8.34 ± 0.00	7.66 ± 0.05	7.42 ± 0.01
$8/8_d$	75/100/3/50	6.98 ± 0.00	7.83 ± 0.02	8.63 ± 0.02	7.64 ± 0.14	7.51 ± 0.00
$9/9_d$	96/50/1.4/50	6.86 ± 0.00	7.97 ± 0.00	8.28 ± 0.05	7.27 ± 0.02	7.38 ± 0.03
$10/10_d$	96/50/3/50	7.22 ± 0.00	8.10 ± 0.12	8.58 ± 0.02	7.95 ± 0.05	7.55 ± 0.00
$11/11_d$	96/100/1.4/50	6.87 ± 0.00	7.86 ± 0.06	8.10 ± 0.03	7.68 ± 0.07	7.39 ± 0.03
$12/12_d$	96/100/3/50	7.07 ± 0.00	7.70 ± 0.03	8.30 ± 0.03	8.29 ± 0.02	7.52 ± 0.03
$13/13_d$	50/50/1.4/100	7.04 ± 0.00	7.79 ± 0.03	7.78 ± 0.06	7.77 ± 0.02	7.35 ± 0.02
$14/14_d$	50/50/3/100	7.18 ± 0.00	7.73 ± 0.20	8.21 ± 0.09	7.56 ± 0.08	7.52 ± 0.01
$15/15_d$	50/100/1.4/100	7.01 ± 0.00	7.82 ± 0.02	8.26 ± 0.12	8.05 ± 0.23	7.39 ± 0.00
$16/16_d$	50/100/3/100	7.13 ± 0.00	7.48 ± 0.03	8.09 ± 0.04	7.66 ± 0.04	7.54 ± 0.00
$17/17_d$	75/50/1.4/100	6.74 ± 0.00	7.83 ± 0.03	8.10 ± 0.28	7.47 ± 0.01	7.39 ± 0.02
$18/18_d$	75/50/3/100	7.12 ± 0.00	7.80 ± 0.08	8.28 ± 0.05	7.62 ± 0.06	7.60 ± 0.05
$19/19_d$	75/100/1.4/100	7.14 ± 0.00	8.12 ± 0.07	8.42 ± 0.05	7.65 ± 0.04	7.49 ± 0.07
$20/20_d$	75/100/3/100	7.14 ± 0.00	7.76 ± 0.03	8.26 ± 0.06	7.54 ± 0.01	7.57 ± 0.02
$21/21_d$	96/50/1.4/100	6.90 ± 0.00	7.80 ± 0.01	8.42 ± 0.16	7.90 ± 0.00	7.41 ± 0.02
$22/22_d$	96/50/3/100	7.35 ± 0.00	7.66 ± 0.12	8.33 ± 0.08	7.50 ± 0.15	7.60 ± 0.01
$23/23_d$	96/100/1.4/100	6.94 ± 0.00	8.00 ± 0.01	8.01 ± 0.32	8.11 ±0.13	7.48 ± 0.00
$24/24_d$	96/100/3/100	7.34 ± 0.00	7.65 ± 0.01	8.30 ± 0.00	7.60 ±0.08	7.55 ± 0.01
$C/C1.4_d$	Control/1.4	6.81 ± 0.00	7.32 ± 0.11	8.06 ± 0.06	7.49 ±0.12	7.38 ± 0.01
$C/C3_d$	Control/3	6.99 ± 0.00	7.37 ± 0.08	8.25 ± 0.04	7.56 ±0.05	7.52 ± 0.00
Inoc.	Blank	7.22 ± 0.00	-	-	-	-

[a]T = temperature (°C); I = intensity (%); C = concentration (%TS, w/w); PT = percent treated (%, v/v).
*0 indicates the initial pH of the bottles after and before addition of inoculum and buffers, respectively.
†Data represent arithmetic mean of duplicates ± absolute difference between mean and duplicates.

Table D-2 Reactor biogas composition during batch anaerobic digestion[a].

Digestion time (d)		8			15			26		
Run #	T/I/C/PT	N_2	CH_4	CO_2	N_2	CH_4	CO_2	N_2	CH_4	CO_2
$1/1_d$	50/50/1.4/50	4 (0) †	76 (1)	20 (0)	1 (0)	68 (0)	30 (0)	1 (0)	67 (0)	32 (0)
$2/2_d$	50/50/3/50	0 (0)	78 (1)	22 (1)	0 (0)	68 (0)	32 (0)	0 (0)	66 (0)	34 (0)
$3/3_d$	50/100/1.4/50	4 (0)	76 (0)	20 (1)	1 (0)	68 (0)	31 (0)	1 (0)	68 (0)	32 (0)
$4/4_d$	50/50/3/50	0 (0)	77 (0)	23 (0)	0 (0)	68 (0)	32 (0)	0 (0)	66 (0)	34 (0)
$5/5_d$	75/50/1.4/50	4 (0)	77 (1)	20 (1)	1 (0)	70 (0)	30 (0)	0 (0)	68 (0)	32 (0)
$6/6_d$	75/50/3/50	0 (0)	80 (0)	20 (0)	0 (0)	70 (1)	30 (1)	0 (0)	67 (1)	33 (1)
$7/7_d$	75/50/1.4/50	6 (5)	75 (3)	18 (2)	0 (1)	69 (1)	30 (1)	0 (0)	68 (0)	32 (0)
$8/8_d$	75/100/3/50	0 (0)	80 (1)	20 (1)	0 (0)	70 (1)	30 (1)	0 (0)	68 (0)	32 (0)
$9/9_d$	96/50/1.4/50	3 (0)	77 (1)	19 (0)	1 (0)	69 (0)	30 (0)	1 (0)	68 (0)	31 (0)
$10/10_d$	96/50/3/50	0 (0)	79 (1)	21 (1)	0 (0)	71 (1)	29 (1)	0 (0)	67 (0)	33 (0)
$11/11_d$	96/100/1.4/50	3 (0)	78 (0)	19 (0)	1 (0)	70 (0)	30 (0)	0 (0)	68 (0)	32 (0)
$12/12_d$	96/100/3/50	0 (0)	80 (0)	20 (0)	0 (0)	71 (0)	29 (1)	0 (0)	67 (0)	33 (0)
$13/13_d$	50/50/1.4/100	2 (0)	78 (1)	20 (1)	0 (0)	70 (1)	29 (0)	0 (0)	68 (0)	32 (0)
$14/14_d$	50/50/3/100	0 (0)	80 (1)	20 (0)	0 (0)	66 (0)	33 (0)	0 (0)	66 (0)	34 (0)
$15/15_d$	50/100/1.4/100	2 (0)	77 (0)	20 (0)	1 (0)	69 (1)	30 (1)	1(0)	68 (0)	32 (0)
$16/16_d$	50/100/3/100	0 (0)	80 (1)	20 (0)	0 (0)	66 (0)	34 (0)	0 (0)	66 (0)	34 (0)
$17/17_d$	75/50/1.4/100	2 (0)	78 (1)	20 (1)	0 (0)	71 (1)	29 (1)	0 (0)	69 (0)	31 (0)
$18/18_d$	75/50/3/100	0 (0)	80 (1)	20 (1)	0 (0)	70 (1)	30 (1)	0 (0)	67 (0)	33 (0)
$19/19_d$	75/100/1.4/100	2 (0)	79 (0)	19 (0)	0 (0)	71 (0)	29 (0)	0 (0)	69 (0)	31 (0)
$20/20_d$	75/100/3/100	0 (0)	81 (0)	19 (0)	0 (0)	69 (0)	31 (0)	0 (0)	67 (0)	33 (0)
$21/21_d$	96/50/1.4/100	2	79	20	0 (0)	70 (0)	30 (0)	0 (0)	68 (0)	32 (0)
$22/22_d$	96/50/3/100	0 (0)	81 (0)	19 (0)	0 (0)	69 (1)	32 (0)	0 (0)	66 (0)	34 (0)
$23/23_d$	96/100/1.4/100	2 (0)	79 (0)	19 (0)	0 (0)	71 (1)	29 (1)	0 (0)	68 (0)	32 (0)
$24/24_d$	96/100/3/100	0 (0)	80 (0)	20 (0)	0 (0)	68 (0)	32 (0)	0 (0)	66 (0)	34 (0)
$C/C1.4_d$	Control/1.4	3 (0)	76 (0)	22 (0)	1 (0)	68 (0)	32 (0)	1 (0)	67 (0)	33 (0)
$C/C3_d$	Control/3	0 (0)	77 (0)	23 (0)	0 (0)	66 (0)	34 (0)	0 (0)	66 (0)	34 (0)
Inoc.	Blank	-	-	-	-	-	-	-	-	-

[a]T = temperature (°C); I = intensity (%); C = concentration (%TS, w/w); PT = percent treated (%, v/v).
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements) in percentages (%).

Table D-3 Reactor volatile fatty acid (VFA) values during batch anaerobic digestion[a].

Digestion time (d)		0*			8			15			26		
Run #	T/I/C/PT	AA	PA	BA	AA	PA	BA	AA	PA	BA	AA	PA	BA
$1/1_d$	50/50/1.4/50	265 (0)†	100 (0)	0 (0)	12 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$2/2_d$	50/50/3/50	642 (0)	233 (0)	12 (0)	29 (6)	315 (19)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$3/3_d$	50/100/1.4/50	488 (0)	163 (0)	6 (0)	11 (3)	2 (2)	0 (0)	69 (60)	5 (5)	0 (0)	0 (0)	0 (0)	0 (0)
$4/4_d$	50/50/3/50	629 (0)	220 (0)	12 (0)	39 (7)	329 (49)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$5/5_d$	75/50/1.4/50	335 (0)	121 (0)	0 (0)	41 (1)	11 (4)	0 (0)	12 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$6/6_d$	75/50/3/50	505 (0)	173 (0)	9 (0)	119 (15)	658 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$7/7_d$	75/50/1.4/50	383 (0)	134 (0)	0 (0)	30 (3)	4 (0)	0 (0)	6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$8/8_d$	75/100/3/50	622 (0)	197 (0)	8 (0)	107 (20)	529 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$9/9_d$	96/50/1.4/50	265 (0)	100 (0)	0 (0)	38 (3)	11 (1)	0 (0)	12 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$10/10_d$	96/50/3/50	444 (0)	173 (0)	8 (0)	145 (42)	475 (89)	0 (0)	7 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$11/11_d$	96/100/1.4/50	306 (0)	121 (0)	0 (0)	49 (0)	24 (2)	0 (0)	7 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$12/12_d$	96/100/3/50	485 (0)	187 (0)	6 (0)	137 (1)	726 (83)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$13/13_d$	50/50/1.4/100	0 (0)	0 (0)	0 (0)	9 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$14/14_d$	50/50/3/100	755 (0)	266 (0)	755 (0)	189 (53)	531 (47)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$15/15_d$	50/100/1.4/100	446 (0)	126 (0)	12 (0)	8 (2)	0 (0)	0 (0)	73 (63)	6 (6)	0 (0)	0 (0)	0 (0)	0 (0)
$16/16_d$	50/100/3/100	728 (0)	239 (0)	728 (0)	90 (18)	605 (55)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$17/17_d$	75/50/1.4/100	140 (0)	42 (0)	0 (0)	51 (0)	122 (18)	0 (0)	9 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$18/18_d$	75/50/3/100	481 (0)	145 (0)	18 (0)	279 (74)	682 (111)	0 (0)	8 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$19/19_d$	75/100/1.4/100	236 (0)	67 (0)	0 (0)	103 (40)	136 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$20/20_d$	75/100/3/100	713 (0)	194 (0)	15 (0)	281 (24)	684 (12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

$21/21_d$	96/50/1.4/100	0 (0)	0 (0)	0 (0)	50 (2)	157 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$22/22_d$	96/50/3/100	358 (0)	146 (0)	15 (0)	282 (36)	587 (58)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$23/23_d$	96/100/1.4/100	82 (0)	42 (0)	0 (0)	75 (8)	197 (21)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$24/24_d$	96/100/3/100	440 (0)	175 (0)	12 (0)	283 (5)	692 (102)	0 (0)	7 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$C/C1.4_d$	Control/1.4	137 (0)	52 (0)	137 (0)	17 (3)	6 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$C/C3_d$	Control/3	295 (0)	111 (0)	295 (0)	39 (2)	239 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Inoc.	Blank	(0)	(0)	0 (0)	-	-	-	-	-	-	-	-	-

[a]T = temperature (°C); I = intensity (%); C = concentration (%TS, w/w); PT = percent treated (%, v/v); AA, PA, BA = acetic, propionic, butyric acids in mg/L.
*0 indicates the initial volatile fatty acid concentration of reactors after and before addition of inoculum and buffers, respectively.
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements).

APPENDIX E

MULTILEVEL FACTORIAL DESIGNS

In Chapter 3, two different multilevel factorial designs were used for thickened waste activated sludge (TWAS) solubilization during microwave (MW) pretreatment and cumulative biogas production (CBP) from batch anaerobic digesters. Multifactor analysis of variance (ANOVA) [STATGRAPHICS 5.1 software (StatPoint Inc., Virginia, USA] was used to detect significant factors in the multilevel factorial designs shown in Tables E-1 and E-2 for TWAS solubilization and CBP, respectively. Soluble chemical oxygen demand (SCOD) to total COD (TCOD) ratio is generally used for an indication of TWAS solubilization for pretreatment studies. In Table E-1, the response (dependent) variable was relative SCOD/TCOD ratio ($SCOD/TCOD_r = SCOD/TCOD_{pretreated}/SCOD/TCOD_{control}$) and independent MW pretreatment variables were temperature (T; analyzed at three levels; 50, 75, 96°C), intensity (I; at two levels: 50, 100%), and TWAS concentration (C; at two levels: 1.4, 5.4% TS). Similarly in Table E-2, the effects of 4 variables; partial treatment (PT), T, I and C were analyzed on relative CBP ($CBP_r = CBP_{pretreated}/CBP_{control}$) in a 3x2x2x2 factorial design.

Table E-1 Variables and levels in the 3x2x2 factorial design for $SCOD/TCOD_r$[a].

Variables	T (°C)	I (%)	C [% TS (w/w)]
Levels			
1	50	[n]50	1.4
2	75	100	5.4
3	96	n/a	n/a

[a]T = temperature, I = intensity, C = sludge concentration, n/a = not available.
[n]50% of maximum MW power (1250 W) was used.

Table E-2 Variables and levels in the 3x2x2x2 factorial design for CBP_r[a].

Variables	PT (%)	T (°C)	I (%)	C [% TS (w/w)]
Levels				
1	[l]50	50	[n]50	1.4
2	100	75	100	5.4
3	n/a	96	n/a	n/a

[a]PT = partial treatment, T = temperature, I = intensity, C = sludge concentration, n/a = not available.
[l]50% of total volume of sludge was being microwaved and the other 50% was non-pretreated.
[n]50% of maximum MW power (1250 W) was used.

The 3x2x2 factorial experimental design shown in Table E-1 was run with duplicates equaling 24 experimental units. The linear equation for this experiment is as follows:

$$Y_{ijkl} = \mu + L_i + M_j + A_k + LM_{ij} + LA_{ik} + MA_{jk} + MLA_{ijk} + \varepsilon_{(ijk)l} \qquad \text{(E-1)}$$

where

i, j, k = levels of the first, second and third variables (i = 1, 2, 3; j = 1, 2; k = 1, 2)

l = number of the replicates (1, 2)

Y_{ijkl} = the $SCOD/TCOD_r$ ratio of the l^{th} replicated TWAS with k^{th} TS concentration pretreated at j^{th} microwave intensity and at i^{th} microwave temperature

μ = the overall mean

L_i = the fixed effect of the i^{th} microwave temperature

M_j = the fixed effect of the j^{th} microwave intensity

A_k = the fixed effect of the k^{th} TWAS concentration

LM_{ij} = the interaction effect of the i^{th} microwave temperature and j^{th} microwave intensity

LA_{ik} = the interaction effect of the i^{th} microwave temperature and the k^{th} TWAS concentration

MA_{jk} = the interaction effect of the j^{th} microwave intensity and the k^{th} TWAS concentration

MLA_{ijk} = the interaction effect of the i^{th} microwave temperature, j^{th} microwave intensity and the k^{th} TWAS concentration

$\varepsilon_{(ijk)l}$ = the random effect of the l^{th} $SCOD/TCOD_r$ ratio within the ijk^{th} treatment combination. The $\varepsilon_{(ijk)l}$ are assumed to be independently and identically distributed in a normal distribution with mean zero and variance of σ^2.

ANOVA can be obtained for all factors and factor interactions. They can be easily tested against the error $\varepsilon_{(ijk)l}$ with the following seven null hypotheses [symbolically H_o; the effect of any particular independent variable (in terms of error created by each variable, Φ) on each of the response variables does not exist]: H_o:Φ(LMA) = 0, H_o:Φ(MA) = 0, H_o:Φ(LA) = 0, H_o:Φ(LM) = 0, H_o:Φ(A) = 0, H_o:Φ(M) = 0, H_o:Φ(L) = 0. The ANOVA table (Table 3-4, Chapter 3) presents the sum of the squares, degree of freedom, mean squares (sum of the squares/degree of freedom), F- ratio (mean square of the variable/mean square of residuals) and probability (p) value of each variable. Variables are considered significant if they have a probability of 0.05. Table 3-4 indicates that since p-values of 4 variables (T, I, C and interactions of T*C) are less than 0.05, these MW pretreatment factors have a statistically significant effect on WAS solubilization as measured by $SCOD/TCOD_r$ at the 95% confidence level.

The information given above is also true for ANOVA of 3x2x2x2 factorial design (Table 3-5, Chapter 3) for CBPr with an additional main effect and interaction terms coming from the PT variable. Similarly, in Table 3-5, the effects of T and C dominate the effects of PT and I; however, p-values of 6 variables (PT, T, C and interactions of T*PT, T*I and PT*I*C) are less than 0.05, indicating that these single and two and three factor interactions have a statistically significant effect on CBP_r at the 95% confidence level.

Figures E-1a-c display the scatter plots for $SCOD/TCOD_r$ by the level codes of the parameters; temperature, intensity and concentration, respectively. In Figures E-1c and d, TWAS solubilization decreased with MW intensity and increased with TWAS concentration. According to Figure E-1a, $SCOD/TCOD_r$ was the highest at 75°C.

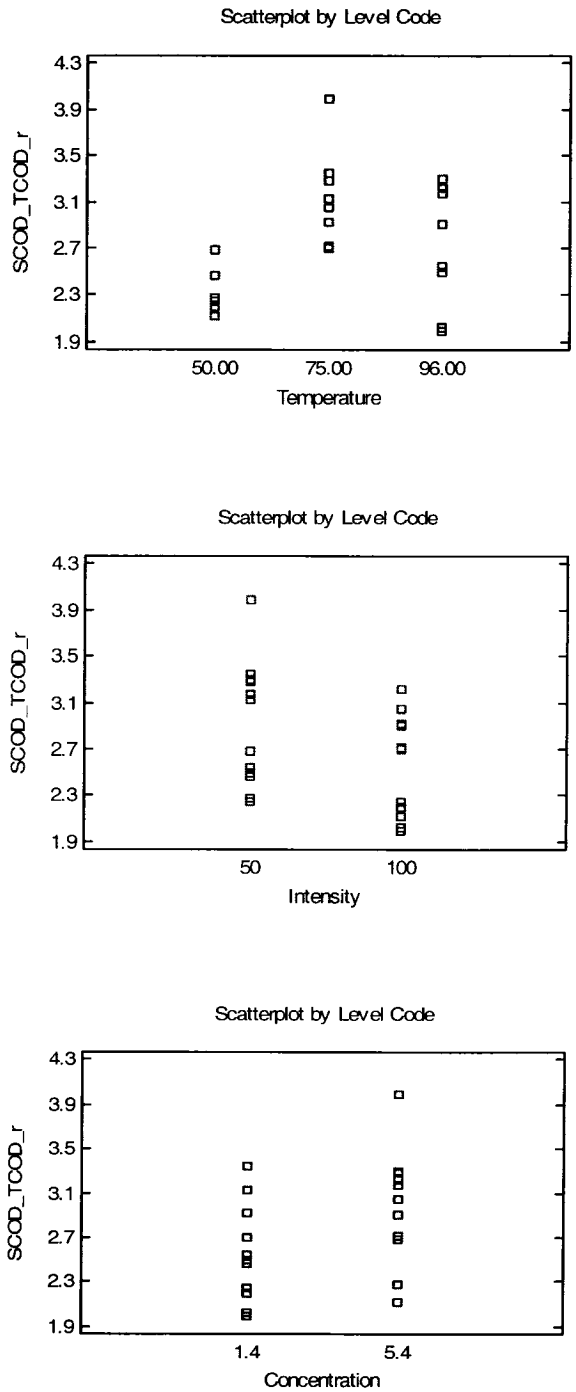
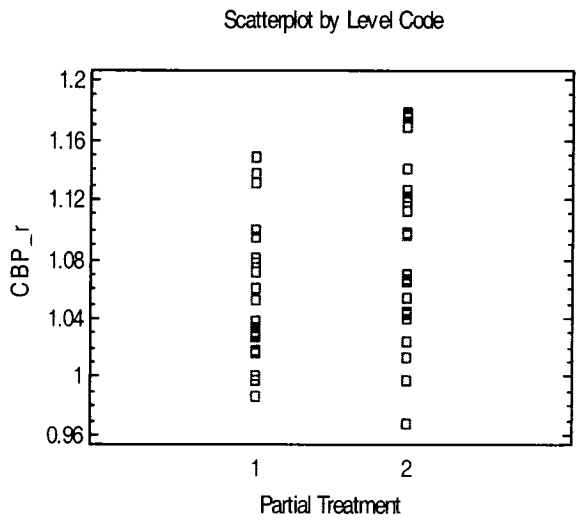
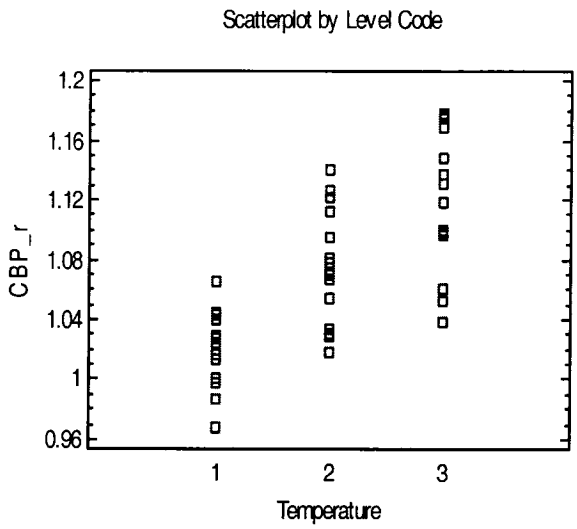


Figure E-1 Scatter plots for SCOD/TCOD$_r$ by level code of parameters of a) MW temperature; b) MW intensity and c) TWAS concentration.

Similarly, Figures E-2a-d display the scatter plots for CBP$_r$ by the level codes of the parameters; partial treatment, temperature, intensity and concentration, respectively.

In Figures E-2a, b and d, CBP_r increased with percentage of TWAS treated, MW temperature and TWAS concentration. As it can be observed from Figure E-2c, MW intensities at both levels (1 and 2) yielded very similar CBP_r.





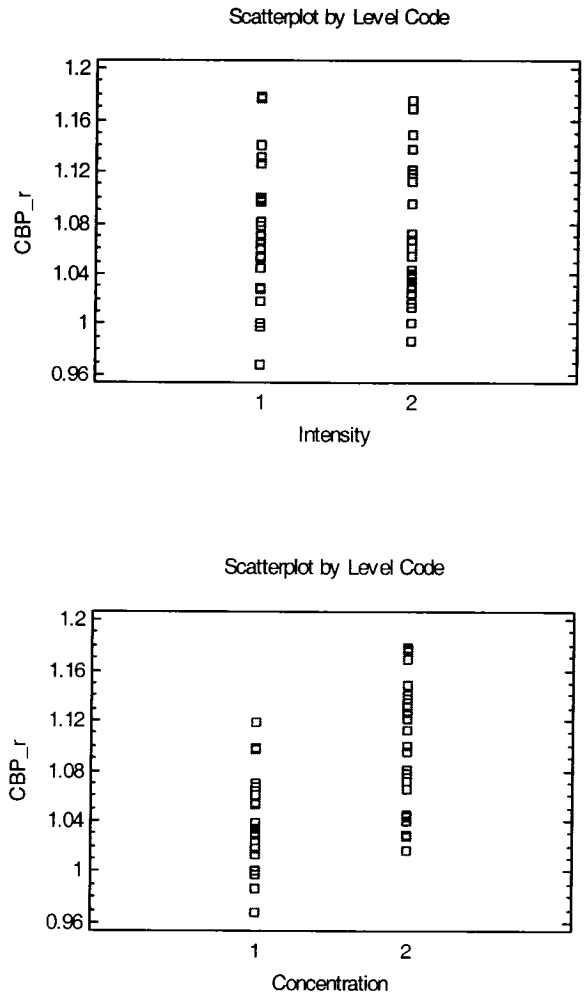
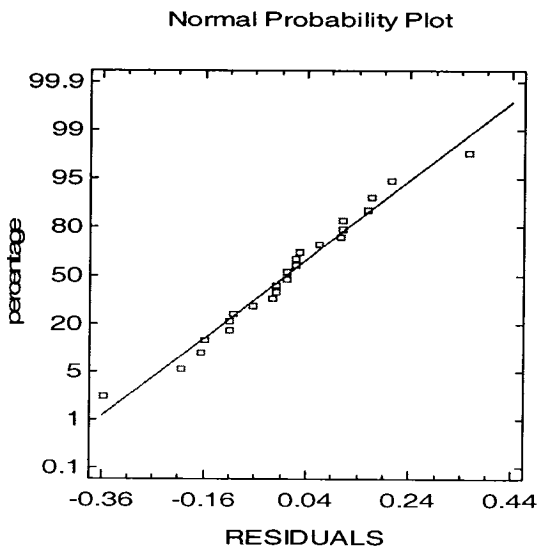


Figure E-2 Scatter plots for CBP_r by level code of parameters of a) partial treatment; b) MW temperature c) MW intensity and d) TWAS concentration.

The ANOVA table is only useful if its assumptions are met: residuals are independently and identically distributed in a normal distribution with a mean zero and variance sigma squared (σ^2). Figures E-3a and b display the normal probability plots of residuals from ANOVA for both $SCOD/TCOD_r$ and CBP_r, respectively. In both Figures E-3a and b, normal probability plots are showing good straight lines, therefore; showing no pattern that might be cause for concern. Accordingly, ANOVAs for both $SCOD/TCOD_r$ and CBP_r should be fully valid.



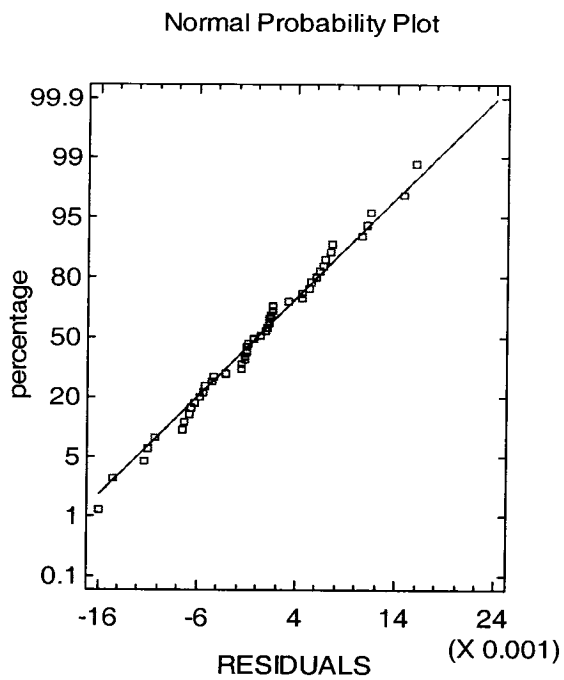


Figure E-3 Normal probability plots of residuals from ANOVAs for a) SCOD/TCOD$_r$ b) CBP$_r$.

APPENDIX F

COD CONCENTRATION OF SUPERNATANTS OF TWAS

Table F-1 Initial and final chemical oxygen demand (COD) concentrations of supernatants in batch digesters[a].

Mw (kDa)	Control		MW-96		CH-96	
Retentates	COD_i (mg/L)	*COD_f (mg/L)	COD_i (mg/L)	*COD_f (mg/L)	COD_i (mg/L)	*COD_f (mg/L)
1<Mw<10	1082 (24)†	61 (12)	3012 (59)	91 (14)	7259 (82)	476 (21)
10<Mw<100	776 (47)	84 (8)	2394 (88)	199 (6)	3341 (12)	137 (7)
100<Mw<300	755 (2)	91 (42)	1576 (47)	47 (3)	2359 (6)	137 (7)
Mw>300	1241 (53)	97 (4)	4412 (59)	214 (150)	6941 (94)	506 (16)
Permeates						
Mw<1	667 (4)	34 (16)	988 (0)	10 (10)	2279 (132)	41 (8)
Mw<10	732 (26)	24 (6)	1297 (3)	21 (5)	2647 (59)	96 (3)
Mw<100	776 (14)	35 (1)	1553 (12)	55 (9)	3176 (12)	96 (6)
Mw<300	767 (14)	41 (8)	1600 (59)	59 (1)	3165 (0)	84 (5)

[a]MW-96 = pretreated with microwave to 96°C; CH-96 = pretreated with conventional heating to 96°C; Mw = molecular weight in kDa;
COD_i, COD_f = initial and final chemical oxygen demand (COD) of samples in the batch digesters.
*In calculating final COD of the sample, the final COD in the inoculum (blank) bottle was subtracted from final COD concentrations in all other reactors.
†Data represent arithmetic mean of duplicates (absolute difference between mean and duplicate measurements).