Innovative Sludge Pretreatment Technologies and Enhanced Anaerobic Digestion

by

WEIGANG YI

B.Eng., Tongji University, China 1999
M.A.Sc., The University of British Columbia, Canada 2003

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Abstract

This dissertation reports the research findings from an investigation into the use of four technologies for municipal wastewater sludge pre-digestion treatment. The technologies explored include microwave, the microwave / hydrogen peroxide process, ultrasound and biological enzyme protease treatment. The general purpose of the sludge pretreatment applications is to improve anaerobic digestion efficiency in terms of biogas production, organic reduction and nutrient recovery.

An examination was first carried out on the effects of these technologies on sludge solubilization (disintegration and hydrolysis), and the various factors influencing treatment efficiencies. Further detailed investigation was undertaken on the sludge macromolecule solubilization, biomass cell destruction and particle size alteration. Finally, an evaluation of the pretreatment impact on both mesophilic and thermophilic anaerobic digestion was done.

This research work found that the degree of sludge solubilization is depending on a numbers of operating factors such as specific energy, temperature, power input, power density, treatment time, and specific oxidant dosage. In general, specific energy is the dominant factor.

Substantial improvements in organic solubilization by the pretreatments were recorded (up to 43% increase in COD, 50% in protein solubilized, at specific energy 5000 kJ/g-DS). Different treatment methods resulted in variation in solubilization effect and digestion performance. Amino acid was found to be the key parameter in correlating to the mesophilic digestion improvements.

Abstract

Pretreatment improves biodegradability in mesophilic digestion (25% total biogas production increase). The mesophilic digestion reaction was found to fit second-order kinetics. Thermophilic digestion was inhibited initially by the large increase in soluble substrates, but recovered at the end of digestion period. The biogas production increase in mesophilic digestion was correlated to the increase in amino acids (R^2=0.9216), not the increase in overall soluble COD. The inhibition in thermophilic digestion was correlated to the sum of increased soluble protein, polysaccharides and amino acids (R^2=0.9822), regardless of the different pretreatment methods used. Overall, ultrasound pretreatment was found to be better energy efficient that other methods tested.

Preface

My supervisors, Dr. Victor K. Lo and Dr. Donald S. Mavinic, have provided great support for this research work. The manuscripts in preparation from this dissertation have been strengthened by input from my supervisory committee, Dr. David Forgie and Dr. Madjid Mohseni, and Research Associate, Dr. Ping H. Liao. Below is a summary of the contributions of co-authors to the work presented in this dissertation.

Chapters 2-6 will be revised into five manuscripts for publication. My contribution to these five manuscripts included all aspects of the research work. This includes identifying the research needs, critically analyzing the relevant literature, designing the research program, finding the appropriate methodologies, constructing the experimental set-ups, conducting the experiments, analyzing the data, organizing and presenting the results and the preparation of these manuscripts.

All co-authors contributed to the completeness of the research scope, to the identification of detailed research needs, to the examination of data, and to the revision of manuscripts.

The following is a list of the manuscripts in preparation that pertain to this dissertation.

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5. YI, W., Lo, K.V., Liao, P.H., Mavinic, D.S., Forgie, D. and Mohseni M. Mesophilic and thermophilic anaerobic digestion of microwave, MW/H_2O_2, ultrasound and protease petreated waste activated sludge. In preparation. (A version of Chapter 6).

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List of Abbreviations

AD	anaerobic digestion
ADM1	anaerobic digestion model No. 1
AOP	advanced oxidation process
BNR	biological nutrient removal
BOD	biochemical oxygen demand
CH/H_2O_2	thermal/hydrogen peroxide treatment
COD	chemical oxygen demand
DD	degree of disintegration
DNA	deoxyribonucleic acids
DS	dried solids
EPS	extracellular polymeric substances
HRT	hydraulic retention time
LCFAs	long chain fatty acids
MW	microwave
MW/H_2O_2	microwave / hydrogen peroxide process
NH_3-N	free ammonia nitrogen
NH_4^+-N	ammonium nitrogen
OH.	hydroxyl radicals
OL	organic loading
OLR	organic loading rate
ORP	oxidation reduction potential
ortho-P	ortho-phosphate
PAHs	polycyclic aromatic hydrocarbons
PAO	polyphosphate-accumulating organisms

PCBs	polychlorinated biphenyls
PO_4-P	ortho-phosphate
poly-P	poly-phosphate
RAS	return activated sludge
PSRP	process to significantly reduce pathogens
SCOD	soluble chemical oxygen demand
SEM	scanning electron microscopy
SRT	solid retention time
TCOD	total chemical oxygen demand
TKN	total kjehldahl nitrogen
TOC	total organic carbon
TP	total phosphorus
TPAD	temperature phased anaerobic digestion
TS	total solids
TWAS	thickened waste activated sludge
US	ultrasound
VFA	volatile fatty acids
VS	volatile solids
WAS	waste activated sludge
WWTP	wastewater treatment plant

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Chapter 1 Introduction

1.1 Background

Sludge treatment is an integral part of the wastewater treatment plant. In practice, the wastewater treatment plant processes are often categorized by liquid treatment stream and sludge treatment stream. Figure 1.1 illustrates a typical wastewater treatment plant process flow diagram, with liquid treatment on the top half and sludge treatment stream at the lower half.

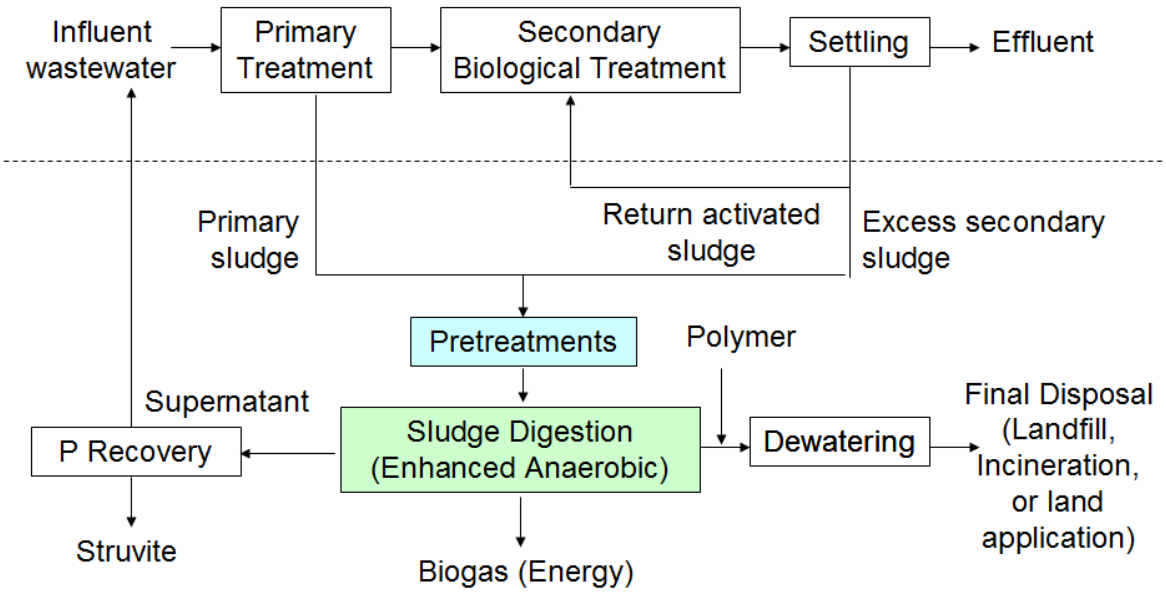

Figure 1.1 Sludge treatment process flow diagram

From a treatment performance point of view, a properly designed and functioning sludge stream is essential to the liquid stream process and the overall treatment plant performance. For example, the activated sludge return and wasting is the key in activated sludge process. From an economic standpoint, the sludge treatment portion could account for 20 to 50 percent of the construction cost, and more than half of annual operating and

maintenance budget.

At the center of sludge treatment stream is the digestion process. The main purpose of sludge digestion is to reduce the sludge organic content so that treated sludge is stabilized for dewatering and final disposal. It also reduces the volume for required dewatering and disposal. In addition, it provides an opportunity for resource (energy and nutrient) recovery.

In recent years, the increasing public attention on energy conservation has put anaerobic digestion into the spotlight of academic research and field practices. Anaerobic digestion is one of the oldest and widely used biological processes. It requires less energy input than aerobic digestion, and produces biogas that could be recovered as alternative energy source. The supernatant from anaerobic digestion is also rich in nutrients (phosphorus and nitrogen), and readily available for recovery processes such as struvite precipitation / crystallization.

However, anaerobic digestion has a number of setbacks that limit its application in medium or small scale treatment plants. The most important one is the slow digestion rate. Due to the slow rate, anaerobic digestion often requires more than 15-30 days of solids retention time to achieve sufficient (38% or more) organic reduction. This long retention time means large digester volume and high construction / maintenance cost are required. Therefore, how to improve the digestion rate is the key to a better sludge treatment / management practice.

Anaerobic digestion is a complex biological process. It involves a number of biological, physical and chemical reactions, in sequence and in parallel. Among them, the disintegration and hydrolysis of particulate organics is considered the rate-limiting step

(Eastman and Ferguson, 1981; Shimizu et al., 1993; Tiehm et al., 2001).

There are two types of sludge generated from the liquid stream treatment process. They are primary and secondary sludge (from primary and secondary settling tanks, respectively). Primary sludge consists of largely settable waste from the influent wastewater. The organic potion of it is considered readily biodegradable. The secondary sludge is the result of biological treatment process that utilizes microorganism metabolism and growth for wastewater biodegradation. As such, the secondary sludge consists of mainly active or dead bio-cells / biomass. Micro-organism cells are protected against cell lysis from the environment stress, by the cell wall and membrane structures (typically peptide and glycan cross-linked bonds). Because of this, secondary biological sludge is particularly resilient to the hydrolysis and anaerobic degradation. In addition, secondary biological sludge also contains inert or slowly biodegradable organics that are the leftovers from the main liquid stream biological process. Some research (Jones et al., 2008; 2009) suggested that these organics requires even longer retention time for anaerobic degradation.

To improve the disintegration and hydrolysis (and consequently the overall digestion process), one could use thermophilic digestion (around 55ºC) instead of conventional mesophilic digestion (around 35ºC). Alternatively, other process configuration could be used, such as temperature phased (thermophilic followed by mesophilic), or acid-gas phased digestion. But these changes would likely require major infrastructure upgrade and expensive operation cost (for heating digestion temperature to 55ºC for example).

Another way for digestion improvement is by having a pre-digestion treatment to accelerate the disintegration and hydrolysis steps. These technologies include thermal

treatment (Hiraoka et al., 1989; Tanaka et al., 1997; Valo et al., 2004; Climent et al., 2007; Bougrier et al., 2007), mechanical treatment (Choi et al., 1997; Baier and Schmidheiny, 1997; Kopp et al., 1997), chemical alkaline treatment (Knezevic et al., 1995; Tanaka et al., 1997; Inagaki et al., 1997; Carballa et al., 2004), ozonation (Weemaes et al., 2000; Battimelli et al., 2003; Goel et a., 2003), ultrasound treatment (Shimizu et al., 1993; Neis et al, 2000), microwave irradiation (Park et al., 2004; Liao et a., 2005a; Eskicioglu et al., 2006, 2007a, 2007b) and advance oxidation processes (Liao et al., 2005b, 2007; Wong et al., 2006a, 2006b, Eskicioglu et al., 2008).

In research or engineering practice, the pre-treatment technologies were often called “disintegration”, “solubilization” or “hydrolysis” processes depending on the specific treatment mechanisms. In this present research work, because there are several fundamentally different treatment methods and mechanisms used (physical disintegration, chemical solublization, and biological hydrolysis), it is considered more appropriate to use the term “pre-digestion treatment”, or “pretreatment”, to reflect they general purpose instead of specific treatment mechanisms.

By improving the disintegration and hydrolysis step, and consequently the overall digestion rate / efficiency, some or all of the following benefits could be achieved.

1. Digester capacity increase - Existing digesters could process more sludge, or the new treatment plants could have smaller digester and/or faster digestion;
2. Biogas production increase (in the case of anaerobic digestion) – More energy recovery;
3. Volatile organic reduction increase - Less sludge volume for dewatering and final disposal;

4. Nutrient recovery increase – Resource recovery as valuable by-products .

Sludge pretreatment research began in the 1990s, and since then a relatively large body of literature has been available reporting the merits and various degree of improvements by different methods. There are also several reports with limited information on cross-comparison of the different treatment methods (Bougrier et al., 2006; Eskicioglu et al., 2008). However, there has not been a successful attempt in correlating the pretreated sludge characteristics (especially from different treatment mechanisms) to the anaerobic digestion performance. The present research was set out to explore this correlation and better understand the linkage between the pretreatment and digestion performance.

In addition, the present research has also taken a practical approach by optimizing the pretreatment technologies at a relatively lower energy cost level (approximately 5,000 kJ/kg-DS). This is particularly important in terms of moving the research findings to potential field application.

1.2 Research Objectives

The objectives of this research program were to investigate the effect of several selected pretreatment methods on secondary biological sludge, and the performance of anaerobic digestion with pretreated feeds. The pretreatments include microwave irradiation, microwave / hydrogen peroxide treatment (MW/H_2O_2), ultrasound treatment, biological enzyme (protease) treatment, and the combined treatment of ultrasound and protease.

More specifically, the research objectives include:

1. Process optimization:
    a. factors influencing microwave, microwave / hydrogen peroxide treatment and ultrasound treatment, at relatively low temperature (40-80°C) or energy level (approximately 5,000 kJ/kg-DS) (reported in Chapter 2, 3 and 4);
    b. non-thermal and/or synergetic effects of MW/H_2O_2 at low temperature conditions (reported in Chapter 3);
    c. MW/H_2O_2 and ultrasound treatment in flow through operations (reported in Chapter 4);
2. Investigation and comparison of sludge bio-chemical and physical characteristics changes due to the different pretreatments mechanisms (reported in Chapter 5);
3. Evaluation and correlation of the pretreatment to mesophilic (35°C) and thermophilic (55°C) anaerobic digestions (at low and high organic loading conditions) (reported in Chapter 6).

1.3 Structure of the Dissertation

This dissertation consists of seven chapters.

Chapter 1 serves as a general guide to research background, objectives, and overall principles in sludge management practice. It also reviews several sludge pretreatment technologies of interest and their underlying mechanisms. Chapters 2 to 6 contain the more relevant literature reviews that led to the specific research topics and experimental designs in each research program.

Chapter 2 reports on an investigation into the process factors in MW/H_2O_2 treatment at a low temperature range (40-80°C). The factors included solids content, temperature, treatment time and hydrogen peroxide dosage. This is the first step in process optimization. Chapter 2 also reports on the use of statistical models (surface response methodology) for factor screening and response prediction.

Chapter 3 reports on an examination of MW/H_2O_2 and thermal/H_2O_2 treatments under identical conditions. The purpose was to identify any non-thermal and synergetic effects of microwave and hydrogen peroxide combined treatment.

Chapter 4 reports on a study of ultrasound treatment in batch and flow through operations, as well as MW/H_2O_2 treatment in flow through operations. This chapter was designed to compare the flow through operation to the batch experiments, so that any benefits derived from flow through operation could be found. Energy aspect of the pretreatment (including specific energy, power input, power density), as well as treatment time, temperature, and hydrogen peroxide dosage rate were examined and discussed. This is to complete the process optimization prior to cross comparison of pretreatment effects at optimized conditions (Chapter 5) and anaerobic digestions (Chapter 6).

Chapter 5 reports on a comparative study of microwave, MW/H_2O_2, ultrasound, protease, and ultrasound/protease treatments. The comparative study was based on these treatments at similar specific energy levels, identified in Chapter 4 as the dominant factor. The results are grouped into two major categories; sludge bio-chemical components solubilization, and sludge physical property changes. The cross comparison allows examination of the contributions from different treatment mechanisms. It also provides the basis for anaerobic digestion study presented in Chapter 6.

Chapter 6 reports on the mesophilic and thermophilic anaerobic digestion performance with various pretreated feeds. Two different organic loading conditions were tested in order to separate the overloading effect from the pretreatment effect. The benefit and impact of pretreated feeds on digestion are reported in terms of biodegradability improvement, reaction rate acceleration and inhibitions. The biogas production (or overall digestion reaction) kinetics was investigated. Correlations of digestion performance and pretreated feed bio-chemical parameters were done to have a better understanding of pretreatment effect on digestion.

Chapter 7 summarizes the research findings and discussions on potential application in pilot-scale study or field engineering practices.

Chapter 8 provides general conclusions and recommendations for future work.

1.4 Literature Review

1.4.1 Sludge characteristics and resource recovery

1.4.1.1 Sludge characteristics

Municipal wastewater treatment sludge is generated from two sources, primary and secondary settling tanks. Primary sludge is mainly the settleable organic material in the raw wastewater influent, and is generally considered readily biodegradable. Secondary sludge is the process sludge that is generated on-site from the secondary treatment processes.

Micro-organisms in this treatment process utilize the organic waste as the energy source for metabolism and growth. By doing so, the organic waste, measured as BOD (biochemical oxygen demand) or COD (chemical oxygen demand), is bio-degraded and reduced to a low concentration to meet discharge requirement. The micro-organism mass in the process / reaction tanks is maintained at certain levels (measured as MLSS, or mixed liquor suspended solids) by removing excess through secondary settling (and return for balancing). Thus, the sludge removed from the bottom of secondary sedimentation tanks consists of mainly micro-organism cell material. Even though it may contain some similar type of pollutants (organic, inorganic, nutrient components, metals, micro-organisms etc.) to that in primary sludge, the characteristics of these two types of sludge are distinctively different.

From a sludge digestion perspective, treatment plants typically blend the thickened primary and secondary sludge for digestion. The primary sludge is more readily available for bio-degradation (relative to the secondary sludge, Jones et al., 2008). It poses no obviously limitation to the digestion rate. The secondary sludge, however, is more resistant

to the bio-degradation, due to the fact that most of the biomass is protected by the cell wall and membrane structures.

Therefore, the pre-digestion treatment usually targets only secondary biological sludge treatment, for practical and economic considerations.

For secondary biological sludge, the variation in treatment processes (activated sludge, trickling filter, biological nutrient removal etc) and / or the operating characteristics (different solids retention times, SRTs, in the activated sludge process) also have a significant impact on the sludge biodegradability. This is due to the changes in sludge compositions (inert or un-biodegradable fraction of the organics, Ekama et al., 2007; Jones et al. 2008). It was suggested that with longer sludge age in the secondary treatment, the inert or non-biodegradable particulates accumulate to a large portion of the secondary sludge that requires longer digestion time (Jones et al. 2008).

Kianmehr et al. (2010) demonstrated that a longer SRT sludge (7 or 15 day secondary treatment SRT versus 1.95 day) is less biodegradable (less SCOD in the sludge feed to the digester, less VFA and methane yield from digester output). Also because of this, the secondary sludge (and long SRT sludge in particular) could see a potentially larger degree of improvement by pretreatments. For example, by applying ozone to the different SRT sludge, Kianmehr et al. (2010) found that a high dose of ozone increased the digestibility significantly on the 15 day SRT sludge, but failed to improve on the 1.95 day SRT sludge.

1.4.1.2 Resource recovery

Organic carbon and nutrient containing (nitrogen, phosphorus) compounds are usually considered resources that could be recovered. The organic carbons can be

bio-degraded to biogas (through anaerobic digestion), which then be used as an alternative energy source. There are other methods for energy recovery, including incineration (Luts et al., 2000), gasification, pyrolysis, or hydrothermal heating at high temperatures (Jaeger and Mayer, 2000; Stolarek and Ledakowicz, 2001). But many of these technologies are still in the development phase.

By converting biodegradable organic carbon into biogas, anaerobic digestion reduces organic content, thus improving the stability of the final sludge. At mesophilic or thermophilic operating temperature, anaerobic digestion can also achieve some degree of pathogen reduction. Therefore, the biosolids (treated sludge with certain degree of organic and pathogen reduction) may be reused in a beneficial way, such as in land application.

A further advantage of anaerobic digestion is that the supernatant contains high levels of nutrients (phosphorus and nitrogen). In conventional treatment plant practice, this supernatant, often called side-stream, is returned to the headwork for further treatment. It adds additional nutrient loading and operational pressure to the treatment plant processes, The additional loading may or may not be accounted for in the initial design. It could result in system failure in some cases. In reality, these nutrients (in the forms of ammonia and phosphate) are often recycled in a closed loop and difficult to remove.

To further complicate the issue, struvite ($MgNH_4PO_4.6H_2O$) formation can naturally occur in the pipes and pumps that deliver the supernatant. Struvite is a white crystalline substance consisting of magnesium, ammonium and phosphate in equal molar concentrations. In some severe case (in biological nutrient removal (BNR) treatment plants mostly), the struvite problem can cripple the side-stream return and results in costly clean-up of the pipes and pumps.

On the other hand, struvite can be used as a valuable fertilizer for many fertilizer markets (Driver et al., 1999). By side-stream phosphorus / struvite recovery, treatment plants could achieve both operational and economic benefits.

1.4.2 Anaerobic digestion

1.4.2.1 Anaerobic digestion principles

The general definition of anaerobic condition is the absence of oxygen or other oxidizing agents. In research, it usually refers to the condition where the oxidation reduction potential below -200 mV. In such conditions, multiple groups of anaerobic microorganisms utilize the biodegradable organics as growth material and as energy. As a result, the large polymer organics are biodegraded into the simple forms of carbon dioxide and methane.

Anaerobic biodegradation follows four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first step in the process is hydrolysis. It is also often referred to as “extracellular solubilization”, and can be further divided into two sub-stages, disintegration and hydrolysis, according to Batstone et al. (2002). The disintegration stage is largely a non-biological process. In this stage, the composite particulate is broken down into particulate protein, carbohydrates (polysaccharides), lipids, and inerts. The second stage is enzymatic hydrolysis of these particulate substrates to amino acids, monosaccharides and long chain fatty acids (Batstone et al., 2002). Due to their close proximity and the variation of digestion model, three terms, “hydrolysis”, “disintegration”, and “solubilization”, are often mixed or confused in the literature. In general, this first step, conventionally termed “hydrolysis” (which include both disintegration and hydrolysis), is

considered a rate limiting step in the anaerobic digestion process (Vavilin et al., 1996; Tiemh et al., 2001; Batstone et al., 2002; Higuchi et al., 2005).

In the second step acidogenesis, the amino acids, monosaccharides, and long chain fatty acids are further degraded to volatile fatty acids (VFAs), carbon dioxide (CO_2) and hydrogen gas (H_2), by acidogenic bacteria. In treatment plants with biological nutrient removal (BNR), the hydrolysis and acidogenesis steps constitute the “fermentation” process. It is to provide readily degradable VFAs for the BNR processes.

In the third step acetogenesis, various forms of volatile fatty acids are broken down to the most basic form of fatty acid, acetic acid (CH_3COOH), along with CO_2 and H_2.

In the last step methanogenesis, acetic acid is split to form methane and CO_2 (Madigan et al., 1997; Batstone et al., 2002).

1.4.2.2 Operation parameters

Like any other biological process, anaerobic digestion requires a careful control of operating parameters such as pH, temperature, solids retention time and mixing.

Anaerobic digestion produces volatile fatty acids, and thus has the tendency to depress pH level. And the same time, carbon dioxide (CO_2) is also generated during the process. The CO_2 presence usually provides sufficient alkalinity buffer capacity to counter the pH drop, and thereby maintains the balance. However, if acid generation is fast enough to exceed the buffer capacity, for example because of any extra pretreatment steps or high organic loading rate, the pH could drop and inhibit the digestion process. The inhibition is particularly severe to the methanogenic bacteria. This is usually referred to as a “sour” or “pickled” reactor in engineering practice. Additional buffer or other operating measures

may be required to maintain the pH condition at near neutral.

Temperature is another major operating factor. Temperature determines the microbial populations, as well as their metabolism and growth rates. It is also likely that it controls the reaction pathway of the digestion process (Batstone et al., 2002). Anaerobic microorganisms can function over a wide range of temperatures. They are generally grouped according to their operating temperature ranges into psychrophilic (4-15°C), mesophilic (20-40°C) and thermophilic (45-70°C). In wastewater engineering, mesophilic digestion is the predominant type with the operating temperature usually controlled at approximately 35°C to 37°C.

Thermophilic digestion, which operates at 55°C, is also used in practice. However, thermophilic digestion requires more heat input for the elevated operating temperature. It could also be more sensitive to the change of sludge characteristics, because of the lower microorganism diversity in thermophilic condition than that in mesophilic condition. More acids and free ammonia may accumulate to levels that inhibit the digestion process due to the increased reaction rates.

Despite all these disadvantages, thermophilic digestion is still appealing to many utilities. It is because when operated properly, thermophilic digestors are capable of producing Class A biosolids, and thus providing beneficial use options for the biosolids final disposal. Other benefits of thermophilic digestion include a faster digestion rate, larger process capacity at a given digester volume, and potentially higher biogas yield.

For both mesophilic and thermophilic digestions, solids retention time (SRT, or hydraulic retention time HRT) and organic loading rate, are just as significant as the pH and temperature. An increase in organic loading rate, or decrease in SRT, will result in the

accumulation of acids and thus a pH drop. Besides the pH impact, the methanogens could also be flushed away if the SRT is too low. Furthermore, lipids might not have sufficient time to break down. As a result of all these factors, the overall volatile reduction could be low. Therefore, a theoretical minimum of 5-10 days SRT is necessary (Tchobanoglous et al., 2003; Turovskiy and Mathai, 2006). In practice, mesophilic digestion is often run with at least 15 days SRT.

1.4.2.3 Inhibition

A number of compounds at elevated concentrations can inhibit the anaerobic digestion process. They include free ammonia (NH_3), volatile fatty acids (VFA), long chain fatty acids (LCFAs), molecular hydrogen, sulphide, various cationic elements and heavy metals. High levels of these inhibitors could be the result of improper digester operation, change of incoming wastewater or sludge characteristics (such as through pretreatments).

During anaerobic digestion, ammonia is produced as the result of amino acid breakdown. In solution, the pH and temperature determines the equilibrium of ammonium (NH_4^+) and free ammonia (NH_3).Free ammonia is toxic to microorganisms (as low as 560–568 mg-NH_3-N/L, Sung and Liu, 2003). An increase in total ammonia, pH, or temperature could all result in higher free ammonia levels. Methanogens are more prone to ammonia inhibition than acidogens (Chen et al., 2008).

It was shown that acclimatization can alleviate the free ammonia toxicity effect (Tchobanoglous et al., 2003; Sung and Liu, 2003). In addition, it is worth bearing in mind that nitrogen is an essential nutrient for microbial growth, and ammonia is the main form of nitrogen source in anaerobic digestion. According to Liu and Sung (2002), a moderate level of ammonia (<200 mg/L) is beneficial to anaerobic digestion.

Volatile fatty acids (VFAs) are the other potential inhibitors in anaerobic digestion. Traditionally, VFAs inhibition is associated with the pH effect. However, research from Siegert and Banks (2005) showed that the VFAs inhibition to cellulose and glucose hydrolysis was independent of pH drops.

Long chain fatty acids (LCFAs) inhibition is less common. The mechanisms involved in LCFAs inhibition have not yet been clearly established. According to Batstone et al. (2002), the inhibition could be irreversible at high levels of LCFAs concentration, mainly due to the death of microbial communities. Therefore, gradual acclimatization is required if anaerobic digesters are fed with lipid-rich waste.

If an industrial wastewater source is part of the treatment plant influent, heavy metal inhibition could be a potential concern. Heavy metals such as zinc, copper, chromium, nickel, cadmium and lead, often end up in both primary and secondary sludge. The inhibitory levels of zinc, nickel, and chromium ions had been reported at 1.0 mg-Zn/L (soluble), 30 mg-Ni/L (total), 2.0 mg-Cr^{3+}/L (soluble) and 3.0 mg-Cr^{6+}/L (soluble) (Turovskiy and Mathai, 2006).

Compared to other inhibitors that affect mostly the methanogens, hydrogen gas may have more impact on the acetogenesis step. Hydrogen partial pressure of 2×10^{-4} bar was found to be 50% inhibitory for butyric and valeric acid degradation, and partial pressure of 7×10^{-5} bar was inhibitory for propionic acid degradation (Batstone et al., 2002).

Inhibition of the anaerobic digestion process has been very well summarized by Batstone et al. (2002), and Appels et al (2008). This aspect is important to sludge pretreatment research, because the sludge characteristics will inevitably be changed during the pretreatments. This change in sludge feed may impose additional stress on the

microorganisms.

1.4.2.4 Biogas production and utilization

Methane and carbon dioxide are the main products of the anaerobic digestion process. In biogas (digester gas), methane constitutes approximately 65%. The biogas also contains nitrogen, hydrogen, hydrogen sulfide, siloxane and water vapor in smaller portions. Methane gas on its own has a heat heating value of 35,800 kJ/m^3, at standard temperature and pressure (20°C and 1 atm). Natural gas (methane, propane and butane mixture) has a higher heating value of 37,300 kJ/m^3. With 65% methane content, biogas's heating value is usually at 22,400 kJ/m^3 (Tchobanoglous et al., 2003).

After treatments to remove water, siloxane and hydrogen sulfide, biogas could be used locally for heat and/or in co-generation for electricity. Currently, these are still the most economical ways to utilize biogas. However, there have also been suggestions that if proven economically feasible in the future, biogas could be used like natural gas (through gas cylinders, or distribution pipelines, Appels et al., 2008).

1.4.3 Sludge pretreatment technologies

As previously discussed in Section 1.1 and 1.4.1.1, secondary biological sludge is more difficult to biodegrade than the primary sludge, because of the secondary biological sludge is mostly biomass (active or dead bio-cells) that is protected by the cell wall and membrane structure. In order to assist cell lysis, a pretreatment step can be used. They include mechanical, electrical, thermal, thermo/chemical, biological and oxidative techniques.

1.4.3.1 Microwave treatment

Thermal or thermo/chemical treatment is one of the more commonly used pretreatment methods (Hiraoka et al., 1989; Tanaka et al., 1997; Penaud et al., 1999; Valo et al., 2004; Climent et al., 2007; Bougrier et al., 2007). Those methods may achieve a significant improvement in organic solubilization, but the processes typically consumed a substantial amount of energy and chemicals (Lin et al., 1997). As an alternative heating mechanism, microwave irradiation provides an efficient source of thermal heating.

Microwaves are located between the 300 MHz and 300 GHz bands in the electromagnetic spectrum. For heating purposes, the dedicated frequencies are 915 ± 25 MHz, 2450 ± 50 MHz, 5800 ± 75 MHz, 22125 ± 125 MHz in North America (Buffler 1993). Household microwave ovens operate at 2450 MHz. For industrial food processing, both 2450 MHz (in Europe) and 915 MHz (in North America) frequencies are commonly seen.

Microwave electric energy is converted to heat by electromagnetically interacting with objects. Two mechanisms involved in microwave heating are generally recognized.

The first mechanism is the acceleration of the movement of ions in the subject material. As the ions oscillate back and forth, they collide with surrounding atoms or molecules, thereby producing heat.

The second mechanism is by the rotation of polar molecules. Water molecules are the main contributor to this dielectric movement. Under the influence of the electromagnetic field, the polar molecules align with the field and energy supplied to them. The electromagnetic field, however, rapidly changes directions and drags the polar molecules along with it. As the polar molecules move and collide with other molecules, energy is transferred in the form of heat.

Thermal inactivation of micro-organisms occurs through the irreversible heat denaturation of proteins, nucleic acids, enzymes or other vital components (Datta & Davidson 2000; Fellows 2000; Heddleson & Doores 1994). Thermal denaturation of proteins and enzymes destroys the metabolic functioning of the cells and causes cell death (Fellows 2000). There are also reports that thermal treatment causes damage to DNA and membranes that leads to cell inactivation (Khalil & Villota, 1988; Heddleson & Doores 1994; Datta & Davidson 2000; Champomier-Verges et al. 2002).

Microwave non-thermal effect, however, has not been well established. Most of the discussion and controversy about microwave non-thermal effect are in microbiology research (Dreyfuss & Chipley 1980; Vasavada, 1986; Kozempel et al. 2000; Datta & Davidson 2000). There are four theories proposed regarding the microwave non-thermal mechanisms on microorganism effect: electroporation, dielectric cell membrane rupture, magnetic field coupling, and selective heating (Kozempel et al. 1998).

The electroporation theory suggests that pore formation in the cell membrane is caused by the stress of the electrical potential applied. This in turn leads to intra-cell material leakage and cell lysis (Kozempel et al. 1998; Datta & Davidson 2000; Brunkhorst et al. 2000). In a similar vein to electroporation theory, dielectric cell membrane rupture theory also focuses on cell membrane destruction caused by electric potential and the sudden changes of voltage (Datta & Davidson 2000; Kozempel et al. 1998; Zimmermann et al. 1974). In magnetic field coupling theory, it is suggested that coupling of electromagnetic energy with protein or DNA causes the cell lysis (Kozempel et al. 1998). Finally, the selective heating theory believes that through microwave heating, the temperature of the microorganisms rises faster than that of the surrounding liquid. While

the temperature of the bulk liquid remains below lethal level, the thermal denaturation has already occurred in the micro-organisms (Kozempel et al. 1998).

Environmental engineering application of microwave irradiation in the laboratory has traditionally been in sample decomposition and sample preparation (Beltra et al., 2003; Perez-Cid et al., 1999, 2001). For waste treatment, microwave was used in applications such as soil remediation (Strack, 1996), processing of scrap tires and plastic wastes (Appleton, 2005), and more recently on waste disinfection and sterilization (Koutchma and Ramaswamy, 2000; Posadas et al., 2001; Hong et al., 2004). It is clear that microwave thermal effect can cause cell lysis and ultimately result in pasteurization and sterilization. Therefore, it is reasonable to suggest microwave heating can also be used for biological sludge treatment.

Park et al. (2004) were among the first to apply microwave heating for sludge pretreatment. Their research program used household microwave ovens in a batch mode for secondary sludge pretreatment. Heating time was the only parameter / control factor. Temperature was measured at the end of microwave heating. An approximately 22% increase in soluble COD was reported. However, the COD removal and methane production for mesophilic anaerobic digestion were recorded at 64% and 79% higher for the pretreated sludge than for the control system, respectively.

A series of tests conducted by Liao et al. (2005a) investigated microwave sludge treatment for enhancing phosphate solubilization from Waste Activated Sludge (WAS). Results showed that microwave treatment was efficient for sludge nutrient solubilization. The advantages stated by the authors include rapid heating, better control, and smaller equipment size than achieved with conventional heating.

Eskicioglu et al., (2006; 2007; 2008) reported the various degrees of sludge solubilization and biogas production increases from microwave treatment on WAS. In Eskicioglu et al., (2007), both acclimated (with 175ºC microwave treated feeds) and non-acclimated inoculums were used in the mesophilic biochemical methane potential (BMP) test. The organic loading in these batch reactors was approximately 30 g-VS/L (or 46-55 g-TCOD/L). Both biogas production improvements and inhibitions were found with all microwave or thermally treated feeds. The authors attributed the biogas increase to the general COD solubilization, and the inhibition or "initial toxicity" to toxic product(s) formed from the pretreatment, or the loss of enzyme activity. However, the extremely high initial organic loading condition could have overshadowed the pretreatments effect.

1.4.3.2 Microwave and hydrogen peroxide treatment

There are suggestions that microwave irradiation may be used to generate hydroxyl radicals (Sanz et al. 2002; Liao et al., 2005b, Wong et al., 2006a, 2007; Eskicioglu et al., 2008). However, there has not been any report that these short-live transient radicals detected and documented by the combination of microwave and hydrogen peroxide process. Therefore, it is prudent to name this treatment as simple as microwave and hydrogen peroxide treatment.

In advanced oxidation process (AOP) research, hydroxyl radical is the key component. Hydroxyl radical is the neutral form of the hydroxide ion, and is a very strong oxidant (oxidation potential: 2.8 V). It can be generated from hydrogen peroxide (H_2O_2) conversion catalyzed by ozone, iron salts and ultraviolet light.

The research that claimed microwave / peroxide advanced oxidation process was loosely based on the findings that the results of the combined treatment was better than

other AOP processes, especially for certainly refractory contaminants, such as phenol for industrial wastewater (Sanz et al, 2002).

Sanz et al. (2002) compared two oxidation processes that use microwave and UV irradiation as catalyzing agents for oxidizing radicals in the treatment of industrial effluents. The results showed that phenol was oxidized completely with H_2O_2/microwave treatment. Operating variables, namely pH, oxidant concentration, and catalyst concentration or microwave irradiation time, were studied. The microwave process was stated to be superior to the Fenton's reagent oxidation. It was also noted that unlike the UV radiation (ionizing radiation) in the AOP process, the microwave radiation is a non-ionizing irradiation, which consumes less energy and induces fewer changes to the material than UV.

Liao et al. (2005b) reported very high phosphate solubilization efficiency from municipal biological sludge using a microwave and peroxide system. More than 84% of the total phosphorous was released at a microwave heating time of 5 min and temperature of 170ºC. The solubilized phosphate was intended for struvite recovery.

Wong et al. (2006a) investigated phosphate and ammonia solubilization with microwave and peroxide system at temperature of 60-120°C. Acid hydrolysis was introduced to break down polyphosphates. It was found that at a reaction time of 5 min, the combination of hydrogen peroxide and acid hydrolysis resulted in up to 61% of total phosphorus and 36% of TKN being released into solution. The nutrients released were in the forms of soluble ortho-phosphate (ortho-P) and ammonia, respectively. To facilitate poly-P break down, hydrogen peroxide was found to be most effective at 80°C and a concentration of 1.5% by wet weight.

Wong et al. (2006b) reported the effects of microwave and peroxide system on sludge COD. They reported a complete COD solubilization at a temperature of 80°C and above. But the peroxide usage was high in these experiments. With regards to struvite recovery from the treated sludge supernatant, ammonia was the limiting factor without H_2O_2, and ortho-P was limiting with H_2O_2 dosage. In addition, microwave and peroxide system was reported as achieving partial sludge pasteurization.

The relationship of COD solubilization, VFA formation and final oxidation with microwave and peroxide system in acid condition was investigated by Liao et al. (2007). It was reported that over 96% of the total COD was solubilized, and up to 25% of this soluble COD was acetic acid. An inorganic acid was used as a stability agent so that the SCOD was retained in solution instead of being oxidized into carbon dioxide. By controlling the amounts of H_2O_2 and acid addition, the microwave peroxide and acid system could either solubilize or reduce the sludge mass (by final oxidation).

Eskicioglu et al. (2008) reported the oxidation and disintegration effect of microwave and peroxide system on thickened WAS (TWAS). It was reported that at a dosage rate of 1 g-H_2O_2/g-TS, the thickened WAS sample lost 11–34% of its TS, total COD and total biopolymers (humic acids, proteins and sugars) via oxidation. With the same acclimated inoculums and similar high organic loading rate (52-64 g-TCOD/L) used in Eskicioglu et al. (2007), the pretreated feed for biochemical methan potential (BMP) test had a lower mesophilic biodegradation rate and ultimate methane production, compared to the untreated control and microwave treated sludge. It was speculated that the soluble organic from microwave and peroxide system was less biodegradable or refractory.

1.4.3.3 Ultrasound

Ultrasound is a mechanical wave. Like a sound wave, it is an oscillation of pressure that passes through solid, liquid or gaseous media. Unlike microwave, it does not interact with the media material on a molecular level, since its wavelength is far larger than the dimensions of the molecule. However, ultrasound has another way of transferring energy to the media, especially liquids. This is done through cavitation.

Cavitation is the formation and collapse of cavities (voids or vacuum bubbles), generated by ultrasound or other high mechanical forces. The cavities are formed when rapidly changing pressure exceeds the tensile strength of the liquid molecules. Solids or gas within the liquid usually create weak spots for cavities to form (Suslick, 1994). Once formed, the cavities grow by absorbing energy from the continuing oscillation pressure. Eventually when they can no longer sustain the growth, the cavities collapse (or implode) violently, and release to the local surroundings all the energy absorbed in a very short period of time (Suslick, 1994).

The sudden release of energy creates extreme conditions in the local (at the micro scale) surroundings. They are called “hot spots”, and are reported to be at temperatures of 5000° C, pressure of 1000 atmospheres, liquid jets of up to 280m/s velocity, heating and cooling rates of 10 billion °C per second (Suslick, 1994). These extreme conditions and the method of their generation would be an advantage in many science and engineering applications. The new term “sonochemistry” was used for chemistry research based on ultrasound cavitation effect.

Cell disintegration by ultrasound has been used for many years in biotechnological laboratories. Recent developments with high power transducers have made ultrasound

treatment of large amounts of municipal waste sludge by means economically feasible. It was reported that ultrasound disintegration enhances anaerobic digestion efficiency and increases bio-degradation and gas production (Chiu et al., 1997; Gronroos et al. 2005; Tiehm et al. 1997, 2001, Baier and Schmidheiny, 1997)

Eder and Gunthert (2002) investigated the mechanical break-up of sludge biomass cells by ultrasound. The treatments were on both wasted activated sludge (WAS) and digested sludge. The authors reported that the treatment of WAS led to a decrease in sludge organic mass of 25%, and consequently an increase in the gas yield of 25%. Further organic biodegradability could also be achieved by the digested sludge disintegration.

Gronroos et al. (2005) reported that ultrasonic disintegration increased the amount of soluble COD of sludge as well as the subsequent methane yield from digestion. It concluded that the significant factors in the disintegration process were the ultrasound power, the dry solid content of the sludge, sludge temperature and ultrasonic treatment time.

Similar results were reported in Tiehm et al. (1997, 2001). Tiehm et al. (2001) studied the impact of different ultrasound frequencies on sludge disintegration. Low frequency ultrasound created large cavitation bubbles, which exert a strong shear force on the liquid and contribute to better sludge disintegration efficiency. Sludge cell lysis, volatile solid reduction and gas production were all improved. The increase in digestion efficiency (volatile solid reduction) was found to be proportional to the degree of sludge disintegration in terms of COD (R^2=0.94).

A study from Neis et al. (2000) showed that with ultrasound treatment, a short solids

retention time of 4 days did not result in a loss in digestion efficiency. Tiehm et al. (1997) also showed stable digestion of disintegrated sludge at a solids retention time of 8 days, with biogas production 2.2 times that of the control.

Martin Kuerth (IWE.tec, Germany, pers. comm.) suggested that a complete cell disruption as described in the literature is not necessary for digestion improvements to occur. Extensive treatment may even have a negative impact on sludge dewatering. Sludge particle disintegration and improved substrate transfer to the micro-organisms were seen as the keys to increased degradation rates. During ultrasound cavitation, substances were cleaved from the cell surface, and the transfer rates were increased.

1.4.3.4 Hydrolytic enzyme treatment

Anaerobic digestion is a complex process which involves a numbers of physical chemical and biological reactions. The fundamental process mechanism is the organic biological degradation by a community of micro-organisms. The sludge fed to the digesters is utilized as substrate for the bio-cell metabolism and growth. Thus the substrate mass transfer, from organic particulate to bulk liquid, and to the intra-cell for anaerobic metabolism, is crucial in this process.

The mass transfer from organics to bulk liquid is often referred to “solubilization”. As previously discussed, this may be further loosely divided into “disintegration” and “hydrolysis”. As Batstone et al. (2002) suggested in their modeling work, “disintegration” could be considered as a physical process, and “hydrolysis” could be considered a chemical or bio-chemical process. This bio-chemical step involves enzyme break down of large organic molecules to intermediate or low molecules, which could then be utilized by micro-organisms for energy and growth.

The molecule weight threshold for diffusing through cell membrane is 800 Dalton (Da, g/mol in biochemistry). The organic polymers, such as protein, polysaccharides and DNAs, are all in the scale of thousands to millions dalton. Therefore, the hydrolysis of these large polymers into smaller molecules (namely amino acids, monosaccharides etc), is particularly important. And hydrolytic enzyme activity is one of the key factors in this process.

The hydrolytic enzymes are mostly extra-cellular type secreted by the active micro-organisms. The enzymes are grouped by their specific functioning target into protease, cellulase, and lipase etc. Depending on where they are located, they can also be classified as ectoenzymes (attached on the cell or solids surface) and exoenzymes (in the bulk liquid).

Another important aspect of this hydrolytic process is the presence of extracellular polymeric substances (EPS). The EPS is a mixture of protein, polysaccharide, lipid, humic substances and other poly-organics. This mixture forms a matrix surrounding the biomass and provides it with a protection barrier.

There are various kinds of reagents and methods for EPS extraction. The reagents include sodium formaldehyde, ethylene- diaminetetraacetic acid (EDTA), sodium tripolyphosphate (STPP), and citric acid (Fang and Jia, 1996; Liu and Fang, 2002; Wawrzynczyk et al., 2008). Methods include cation exchanged resin (CER, Frølund et al., 1995a) and ultrasound cavitation (Yu et al., 2007). In the past, the EPS extraction studies mostly focused on the relationships of EPS with the characteristics of sludge flocs (structure, charge, etc.), sludge settling and dewatering properties. With increasing attention being given to anaerobic digestion and hydrolysis, the effect of EPS and EPS

trapped enzymes on biomass cell lysis has also attracted more research interest (Frølund et al., 1995b; Guellil et al., 2001; Yu et al., 2008).

The knowledge of how EPS interacts with enzyme and hydrolytic activities is not well established. However, there are suggestions that a portion of the exoenzymes is trapped within the EPS (Frølund et al., 1995; Vavilin et al., 1996; Cadoret et al., 2002). Together with the ectoenzymes that are attached closely to the cell and barred by EPS, this makes it difficult for substrate (even in solubilized form) and enzymes to interact. It is therefore reasonable to hypothesize that the extraction or dispersion of EPS from the biomass would have a two-fold benefit for cell hydrolysis: by liberating exoenzymes from EPS, and by exposing biomass (as substrate) for reaction.

Guellil et al. (2001) studied the hydrolysis of enzymes from EPS (by extraction and concentration) on the colloidal faction of the activated sludge. They suggested that protein could be effectively hydrolyzed by enzymes that were originally trapped by EPS. They also disagreed with the general belief that hydrolysis is rate limiting.

Yu et al. (2007; 2008) identified the locations of protease, a-amylase, a-glucosidase, alkaline-phosphatase and acid-phosphatase in activated sludge by several methods of EPS extraction. It concluded that protease is mostly cell surface bound in the same way as portions of the α-amylase and α-glucosidase. Other portions of α-amylase and α-glucosidase are exoenzymes, but immobilized by EPS.

In Yu et al. (2008), ultrasound extraction was used as a pretreatment to sludge aerobic digestion. The study showed improvement in TSS and VSS reduction after pretreatment, without additional enzymes. The authors suggested that ultrasound treatment helped to mobilize the EPS trapped enzymes for better hydrolysis.

Similarly, Wawrzynczyk et al. (2007; 2008) used STPP, citric acid, EDTA and other reagents to “solubilize” sludge. The COD was used as the main measurement of solubilization. The studies also added dosages of various kinds and mixtures of enzymes, namely glycosidic enzymes (Wawrzynczyk et al. 2007), lipase, cellulase, a-amylase, endoxylanase, dextranase and protease (Wawrzynczyk et al. 2008). It was reported that a lower dosage (13.7 mg-enzyme/g-TS) of enzymes with reagents achieved better hydrolysis (50-85%), than a high dosage (68.5 mg-enzyme/g-TS) did without reagents.

Even though enzyme hydrolysis is one of the fundamental subjects in the microbiology field, the use of enzymes in municipal waste sludge treatment is a very rare research topic. There have not been many reports with well-defined conditions. This is probably also due to the complexities of sludge composition and its digestion.

1.5 Summary

The Section 1.4 literature review provides an overview of the sludge pretreatment subject and the background information on several pretreatment technologies. Each of the following research chapters has a subsection for literature review that is more relevant to the specific research topics and experimental designs.

The main objective of sludge digestion pretreatment is to increase the digestion rate. By doing so, it could achieve benefits such as smaller digesters, improved biogas production, improved organic reduction, etc.

The slow anaerobic digestion rate is often due to the slow disintegration / hydrolysis rate on the composite organic particulates (Eastman and Ferguson, 1981; Shimizu et al., 1993; Tiehm et al., 2001). Secondary biological sludge is particularly difficult to digest, when compare to the primary sludge. This is due to fact that the main component of secondary sludge, biomass, is well protected from lysis by the cell wall and membrane structure. It is therefore expected that the pretreatment on secondary biological sludge can achieve greater degree of improvement in anaerobic digestion than on primary sludge.

Anaerobic digestion is one of the most economic processes for sludge handling. It achieves sludge volume reduction, stabilization, and potentially a certain degree of pathogen destruction. It is also an energy-positive process. However, it is also a complex process that involves a series of physic-chemical and biological process. The pretreatment will inevitably alter the sludge feed characteristics, that may have a substantial impact to the anaerobic digestion process.

Most of the pretreatment research did not study the correlation between the pretreatment (feed characteristics) and the digestion. Many of them did not focus on the

energy and cost aspects of the process either. However, these two components are probably the most important parts in technology development and potential field application. Thus the present research will strive to address these two critical issues.

There are many pretreatment methods available. Among them, microwave could replace thermal treatment, and microwave / peroxide system appears to have a higher potential for better results than microwave alone. Ultrasound is another effective and affordable method to yield intense energy impact on sludge. And protease treatment provides an improvement in biological hydrolysis of the bio-polymer, and thus is included in the present research.

Chapter 2 Factors Affecting Microwave And Hydrogen Peroxide Treatment Process *

2.1 Introduction

The sludge management in municipal wastewater treatment plants is a challenging task. At large treatment plants, anaerobic digestion is commonly used to stabilize sludge. The energy recovered from anaerobic digestion (through biogas production) can compensate for part of the heat and/or electricity needs for plant operation. However, one of the drawbacks of anaerobic digestion is the slow reaction rate. It often requires a long solids retention time and large digester volume. Hydrolysis of the sludge particulate organics is the first step in the digestion process. It is considered by many the digestion rate limiting factor (Eastman and Ferguson, 1981; Shimizu et al., 1993; Tiehm et al., 2001).

In order to enhance the efficiency of the hydrolysis stage, pretreatment methods are used to disintegrate or solubilize the particular organics (Odegaard, 2004). A thermo / chemical pretreatment method was reported to have increased bio-degradability by approximately 70%. However, this treatment consumed a substantial amount of energy and chemicals (Lin et al., 1997). An alternative to conventional heating is through microwave irradiation. It has the advantage of being rapid and efficient heating. It may offer potential benefits such as pathogen destruction (through thermal and/or non-thermal

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effect, Khalil and Villota, 1985; 1988; Hong et al., 2004; 2006), sludge mass reduction (Eskicioglu et al., 2007), nutrient extraction (Liao et al., 2005) and enhanced biogas production (Park et al., 2004).

The microwave hydrogen peroxide treatment (MW/H_2O_2) (Sanz et al, 2002; Liao et al., 2005b; 2007; Wong et al., 2006a, 2006b, 2007; Eskicioglu et al., 2008) combines the hydrogen peroxide use with microwave irradiation. It was reported that MW/H_2O_2 achieved up to 100% total chemical oxygen demand (COD) and 84% total phosphorus solubilization (0.35-0.64% solids content secondary biological sludge, Wong, et al., 2006a; 2006b). The soluble form of organic material is usually considered a ready substrate for biodegradation. The increase in soluble COD would benefit the sludge digestion process and could potentially be used in biological nutrient removal (BNR) systems. In addition, the phosphorus and nitrogen released in the sludge solution could be recovered through a crystallization process as potentially valuable by-products, such as struvite (magnesium ammonium phosphate, a slow-release fertilizer).

Most of the previous studies of MW/H_2O_2 sludge treatment (Liao et al., 2005a; 2005b; 2007; Wong, et al., 2006a; 2006b; Chan et al., 2007; Eskicioglu et al., 2008) employed a temperature range of 80-170ºC. The present study investigated the MW/H_2O_2 process at a lower temperature range of 40 to 80 ºC, and with a thickened sludge (from 1% to 3% solids content), in order to reduce the energy cost. The process variables, namely solids content, temperature, treatment time and hydrogen peroxide dosage, were studied for their influence on COD solubilization, volatile fatty acids formation and nutrient release. The relative importance of these factors to the process performance was examined using statistical computing software (Sall et al., 2005).

2.2 Material and Methods

2.2.1 Apparatus

The main experimental apparatus used for MW/H_2O_2 sludge treatment was a closed-vessel microwave digestion system (Ethos TC Digestion Labstation 5000, Milestone Inc., USA). This system provides a maximum power output of 1000 W, at a microwave frequency of 2450 MHz. The process temperature profile is monitored and recorded by a thermocouple inserted in the sample vessel. It also provided real-time temperature control. The closed-vessel can sustain operating temperatures up to 220 °C and pressure up to 30 bars. Twelve 100 mL volume vessels are available for any single run. In this study, the operating sludge volume was 30 mL.

Figure 2.1 Milestone ETHOS TC microwave apparatus

2.2.2 Experiment design

A set of twenty-six experiments, each with three replicates, were conducted for this study. The process variables, or factors, are the sludge solids content, treatment temperature, heating time, and hydrogen peroxide dosage. Experiments were performed at a sludge solids content range of 1%-3%, at heating temperatures from 40-80°C, with a heating time of from 1-9 minutes and with a hydrogen peroxide (30 wt %) dosage from 0.5-2.5 mL in 30 mL sludge (0.5-2.5% in volume, 0.24-1.13 mg-H_2O_2/mg-TCOD, or 0.21-1.05 mg-H_2O_2/mg-DS at 3% solids content).

These variables were input through the Response Surface Design function in statistical analysis software JMP-IN® 5.1. Central Composition Design was used for the experimental design (Sall et al., 2005). Table 2.1 presents the details of these conditions. The experiments are grouped into categories, “A” to “I”, based on microwave temperature and solids concentration.

Response Surface Designs are useful for modeling a curved surface (quadratic) to continuous factors. They are capable of fitting a second order prediction equation for the response. The quadratic terms in these equations model the curvature and find the optimal response within specified ranges of the factors (Sall et al., 2005).

Table 2.1 Summary of experimental conditions

Set	Design Pattern	Solid Content (%)	Heating Temperature (ºC)	Heating Time (minutes)	H_2O_2 dosage (mL)	Group
1	- - - -	1	40	1	0.5	A
2	- - -+	1	40	1	2.5	
3	- -+-	1	40	9	0.5	
4	- -++	1	40	9	2.5	
5	a000	1	60	5	1.5	B
6	-+- -	1	80	1	0.5	C
7	-+-+	1	80	1	2.5	
8	-++-	1	80	9	0.5	
9	-+++	1	80	9	2.5	
10	0a00	2	40	5	1.5	D
11	00a0	2	60	1	1.5	E
12	000a	2	60	5	0.5	
13	0000	2	60	5	1.5	
14	0000	2	60	5	1.5	
15	000A	2	60	5	2.5	
16	00A0	2	60	9	1.5	
17	0A00	2	80	5	1.5	F
18	+- - -	3	40	1	0.5	G
19	+- -+	3	40	1	2.5	
20	+-+-	3	40	9	0.5	
21	+-++	3	40	9	2.5	
22	A000	3	60	5	1.5	H
23	++- -	3	80	1	0.5	I
24	++-+	3	80	1	2.5	
25	+++-	3	80	9	0.5	
26	++++	3	80	9	2.5	

2.2.3 Sludge characteristics, treatment processing and sampling

Secondary aerobic sludge was used for the experiments. It was obtained from the pilot-plant wastewater treatment facilities located at the University of British Columbia (UBC) south campus. Fresh sludge samples were collected daily. They were concentrated with a centrifuge at various rpm to reach the desired solids concentration for the experiments. Table 2.2 defines the characteristics of this secondary biological sludge.

For the treatment processing, hydrogen peroxide was added immediately prior to microwave irradiation. The microwave heating ramp times were set constant at 2 minutes. After reaching the desired experimental temperature, sludge samples were maintained for 1, 5 and 9 minutes of treatment time. Immediately after treatment, samples were taken from the microwave digestion station, and spun in a centrifuge at 15,000 rpm for 10 minutes. The centrates were filtered through Whatman No.4 filters, and analyzed for soluble fraction of the COD, volatile fatty acids, ammonia and phosphate.

Chemical oxygen demand (COD), nitrogen (ammonia, NH_3-N), and phosphorous (orthophosphate, PO_4-P) were determined according to the Standard Methods (APHA, 1995). A Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) was used to measure volatile fatty acids (VFA). Volatile separation was accomplished with an HP FFAP column (0.25 m × 0.31 mm with 0.52 μ film thickness). The injection temperature was set at 175 ºC and the FID detector was at 250 ºC. Helium gas was used as the carrier at a head pressure of 10 psi.

Table 2.2 Thickened secondary sludge characteristics

Parameters	*Units*	*Concentration*		
pH			6.2 - 6.7	
TS	(%)	1.1 ± 0.1	2.0 ± 0.1	2.9 ± 0.1
Total COD	(mg/L)	11,550 ± 1,170	21,540 ± 670	28,410 ± 1,120
Total Phosphorus	(mg-P/L)	330 ± 18	640 ± 15	935 ± 26
TKN	(mg-N/L)	735 ± 24	1,405 ± 63	2,086 ± 30
Initial soluble COD	(mg/L^{-1})		90 ± 33	
Initial soluble PO_4-P	(mg-P/L)		0.54 ± 0.10	
Initial soluble NH_3-N	(mg-N/L)		1.5 ± 0.6	

2.3 Results and Discussion

2.3.1 Solubilization of COD

Chemical oxygen demand (COD) is one of the most commonly used parameters for organics in wastewater engineering. In sludge treatment research, the solubilization of COD is almost always the first important indicator of disintegration results. Since the initial soluble COD (SCOD) in sludge was at low levels (0.2-1% of TCOD), the after-treatment SCOD can be used directly to represent the result of COD solubilization.

2.3.1.1 The effects of solids content and temperature

Figure 2.2 illustrates the significance of operating factors on SCOD, with a prediction profiler and Pareto plot of scaled estimate from the statistic modeling. The models were constructed with the actual experimental data, and represent the general trend of process performance under different conditions.

The prediction profiler displays prediction traces for each variable. A prediction trace is the predicted response (treatment result, in statistical model terminology) as one variable is changed, while the others are held constant. The Pareto plot, a series of bar charts, shows the scaled estimates of variables' influence on the response. It also shows their composition relative to the sum of the scaled estimate value. It is one type of screening tool used to examine the size of effects. In this study, the effect of any quadratic or cross-product term is included and shown in the Pareto plots.

Figure 2.2 shows that solids content and temperature are two of the most significant factors on the SCOD results. The third significant effect is the hydrogen peroxide dosage.

The cross-product of solids content and temperature comes close to the first three, while the effect of heating time was less obvious in the temperature range of 40-80°C.

The increase of SCOD is approximately proportional to the increase in solids content from 1% to 3%, and to the increase in temperature. This is confirmed by the traditional plot of experimental data that is shown in Figure 2.3.

For example, at 80°C, when the solids content increased two and three times, the average SCOD concentrations increased from 2348 mg/L for a 1% solids content, to 3680 mg/L for a 2% and 6061mg/L for a 3% solids content. In the 3% solids content experimental group, when the temperature was raised 20 degrees Celsius (20°C to 40°C), 40 degrees (from 20°C to 60°C), and 60 degrees (from 20°C to 80°C), the average SCOD increases were 2237 mg/L, 4394 mg/L and 5836 mg/L, respectively.

The solids content represents the available biomass subjected to treatment and the temperature represents the kinetic energy of the sludge, which directly related to the extent of microwave treatment. It is therefore reasonable to expect the proportional increase of SCOD resulting from the increases in solids content and temperature.

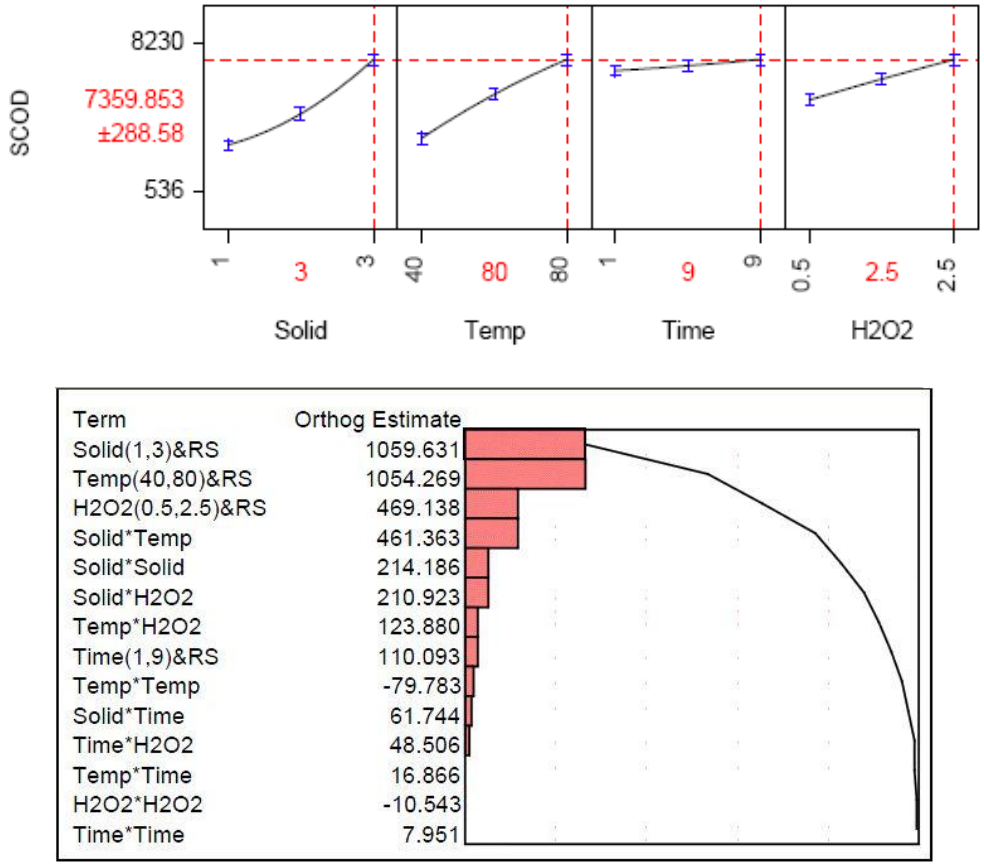


Figure 2.2 Prediction profiler and Pareto plot of scaled estimate for significant factors on SCOD (solids content in %, temperature in °C, time in minutes and H_2O_2 in mL dosage)

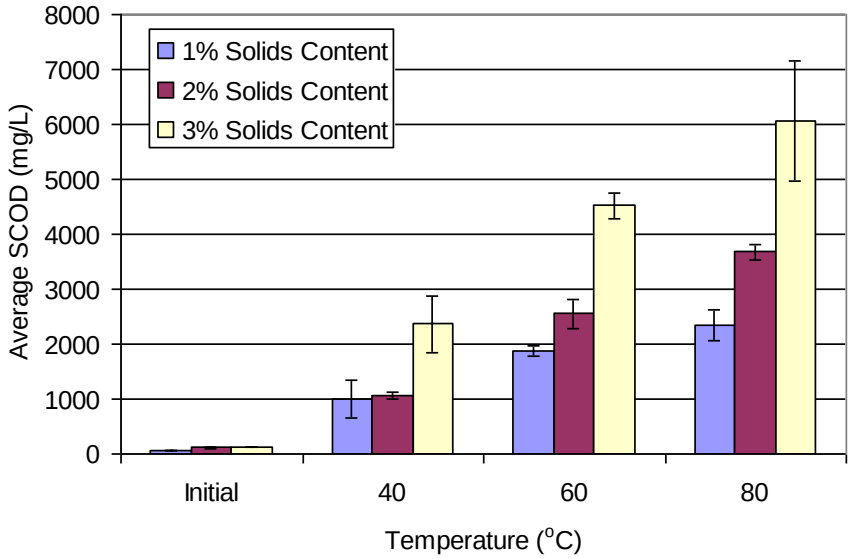


Figure 2.3 Average SCOD concentrations under various treatment conditions

2.3.1.2 The effects of hydrogen peroxide dosage and treatment time

The effects of hydrogen peroxide on SCOD was less obvious than the effects of solids content or temperature. It is clear that a hydrogen peroxide addition enhances COD solubilization. However, it is not conclusive on how much it increases SCOD under different treatment conditions. Figure 2.4 shows the increase of SCOD from hydrogen peroxide additions.

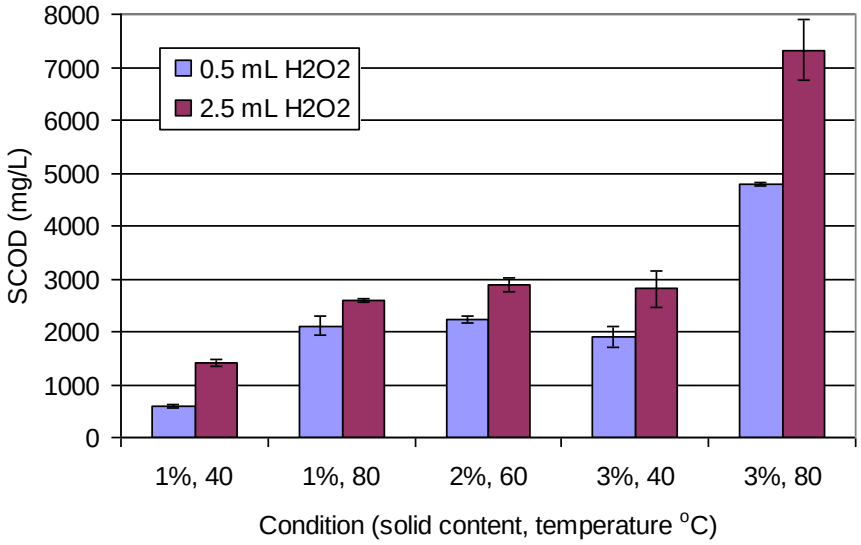


Figure 2.4 The effect of hydrogen peroxide on SCOD increase

In Figure 2.4, for 1% solids content sludge at temperature 80°C, the SCOD level increased 23%, from a 0.5 mL to 2.5 mL hydrogen peroxide dosage. Similar percentage increases were found for conditions of 2% solids content at 60°C (28% of SCOD increase), and 3% solids content at 40°C (34% of SCOD increase).

As for the conditions of 1% solids content at 40°C, and 3% solids content at 80°C, the

percentage SCOD increases were higher than those previously mentioned, at 138% and 53%, respectively (Figure 2.4).

It was also noted that with the same sludge feed conditions (same solids content), the increase in either temperature or hydrogen peroxide could achieve the similar effect. For example, at 3% solids content, similar SCOD results were found at 60°C with a 1.5 mL H_2O_2 dosage, and 80°C with 0.5 mL H_2O_2 (averaged 4518 and 4795 mg/L, respectively).

It is clear that COD solubilzation is the combined result of both physical heat and chemical oxidation treatments. From an operational standpoint, energy input and chemical additions would therefore both be control factors. The process could thus be adjusted accordingly for economic considerations.

In this study, treatment time was defined as the duration of continuous treatment after sludge has reached the desired experimental temperature. Under the current experimental conditions, treatment time showed little effect on SCOD results (Figure 2.2). This is likely due to the enclosed treatment vessel set-up. At the temperature range of 40-80°C, the treatment vessels recorded very little heat loss over a period of 1 to 9 minutes. Because this is a real-time temperature control system, minimum microwave energy input was required to maintain the temperatures. Without microwave power input, the system has little impact on further COD solubilization.

2.3.2 Percentage SCOD to TCOD

In this study, fresh sludge was taken from the UBC wastewater treatment pilot plant for experimental use. The initial soluble COD was at low levels (0.2-1% of TCOD). The after-treatment percentage SCOD to TCOD (SCOD/TCOD%) was therefore used as a simple indicator for treatment efficiency.

The relative influence of various factors on SCOD/TCOD% is shown in Figure 2.5, with the statistical model analysis. The traditional plots of results under various conditions are shown in Figure 2.6 and Figure 2.7.

Figure 2.5 showed that temperature was the dominant factor in SCOD/TCOD%, with hydrogen peroxide the second important factor. This is consistent with a previous study from Liao et al., (2007) which used a lower solids content sludge (0.35-0.45%).

It is reasonable to expect that the variation in solids content should have less impact on SCOD/TCOD%. However, results showed that at an operating temperature of 80°C, higher solids content sludge feeds still favored SCOD/TCOD% (Figure 2.6). For example, at an 80°C treatment temperature and with a 2.5mL hydrogen peroxide addition, a 1% solid content sludge resulted in SCOD/TCOD of 18%, while a 3% solid content sludge yielded 27% SCOD/TCOD. These results suggest that using concentrated sludge is not only beneficial in terms of the energy process (i.e. less microwave energy is consumed), but also for overall treatment efficiency.

Figure 2.7 shows the effect of hydrogen peroxide on SCOD/TCOD%. The hydrogen peroxide dosage had approximately the same effect on the SCOD level as it did on the SCOD/TCOD%. With a 0.5mL peroxide dosage, both the 1% and 3% solids content sludge stabilized at around 15-16% SCOD/TCOD. By increasing the peroxide dosage to 2.5mL,

the 1% solids sludge had only minor increases, resulting in 18% SCOD/TCOD, while the 3% solids sludge reached a 25% SCOD/TCOD.

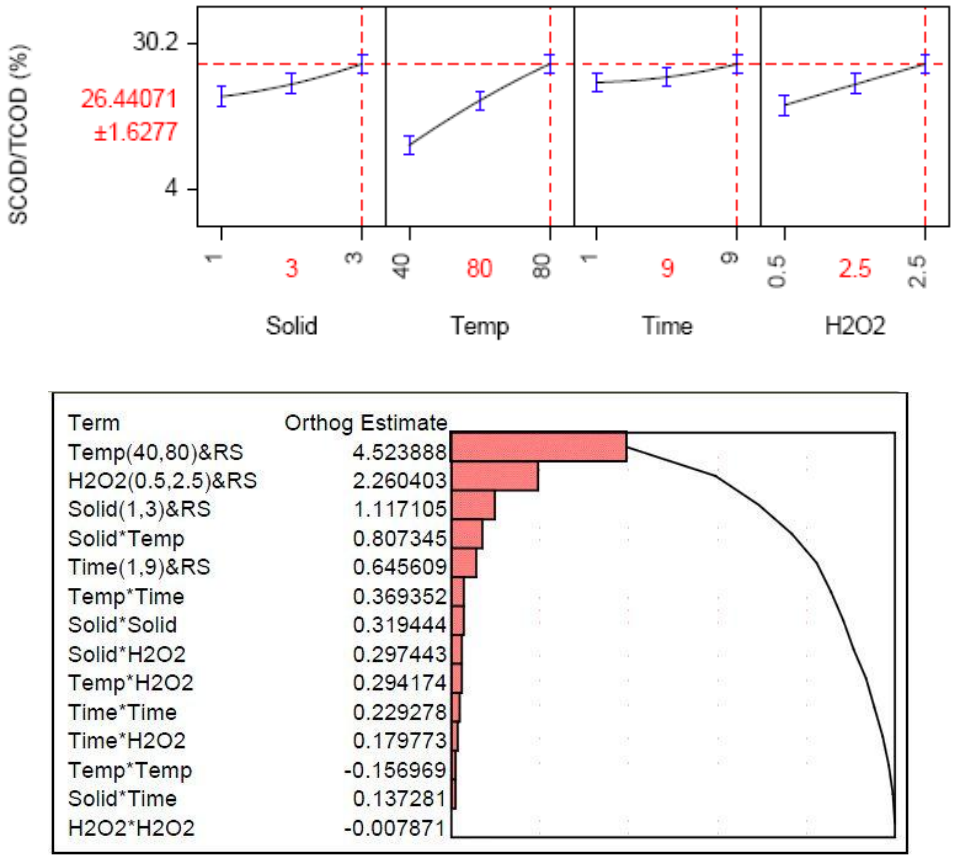


Figure 2.5 Prediction profiler and Pareto plot of scaled estimate for significant factors on SCOD/TCOD% (solids content in %, temperature in °C, time in minutes and H_2O_2 in mL dosage)

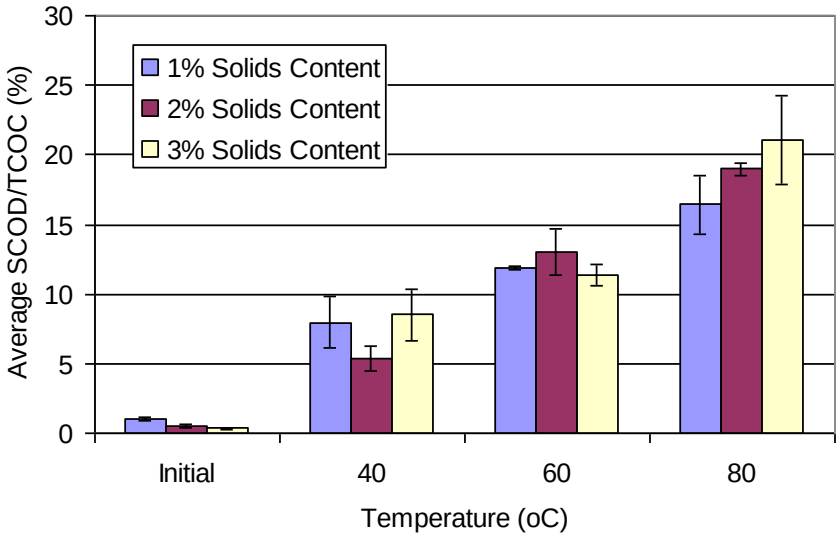


Figure 2.6 Average percentage SCOD/TCOD under various treatment conditions

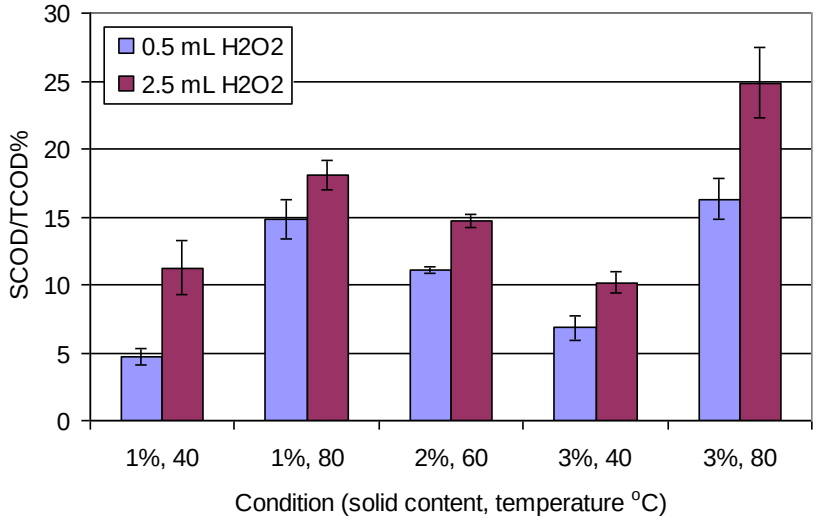


Figure 2.7 The effect of hydrogen peroxide on percentage SCOD/TCOD

2.3.3 Volatile fatty acids formation

Two major reactions, similar to the wet-air oxidation, are assumed to underlie the microwave hydrogen peroxide process (Liao et al., 2007). The first of these reactions was described (by Shanableh, 1999; Shanableh and Jomaa, 2001) as the break-down and solubilization of large particle organic matter, resulting in the accumulation of SCOD. The second reaction is oxidation, which converts the soluble organic substance into oxygenated organic intermediates and eventually into inorganic end products. It is similar to the biological degradation pathway, but involves chemical reactions.

The reactions start by rupturing the R-H bond and forming an organic free radical, $R^{\bullet}$, as depicted in Equation 2-1. The organic free radicals then react with oxygen (Equation 2-2) to yield the intermediate organic peroxy radical, $ROO^{\bullet}$, which in turn reacts with another organic compound, RH. The resulting organic compound, shown in Equation 2-3, represents a simpler organic by-product with fewer carbon atoms and an organic acid link (OOH) (Emanual 1968; Bishop 1968; Shanableh and Jomaa, 2001).

$$RH + HO^{\bullet} \rightarrow R^{\bullet} + H_2O \qquad (2\text{-}1)$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \qquad (2\text{-}2)$$

$$ROO^{\bullet} + RH \rightarrow R^{\bullet} + ROOH \qquad (2\text{-}3)$$

In this study, acetic acid and butyric acid were identified as the main components of VFAs. Acetic acid was more than 89% of the total VFA (TVFA). Figure 2.8 shows the average results of TVFA under various solids content and temperature conditions. The TVFA unit here is milligrams of acetic acid per liter (mg-$C_2H_4O_2$/L).

With 1% and 2% solids content sludge, VFA formation was not obvious under these

experimental conditions. At 40°C, acetic acid was below 5 mg/L, and butyric acid was not detectable. At 60°C, acetic acid was below 8 mg/L, and butyric acid was below 1 mg/L. At 80°C, acetic acid ranged from 5.6 mg/L to 16.4 mg/L, and butyric acid increased slightly to 1.7 mg/L.

With a 3% solids content sludge, both temperature and the hydrogen peroxide addition began to have effects on VFA formation. Figures 2.9 and 2.10 illustrate the differences in VFA formation due to the changes in the addition of hydrogen peroxide and in treatment time, at 40 °C and 80°C.

At a temperature of 40°C (Figure 2.9), acetic acid increased with a longer treatment time and a higher peroxide dosage. Since these changes occurred within a narrow range of a few milligrams per liter, they are not statistically meaningful.

At a temperature of 80°C (Figure 2.10), acetic acid production was significantly affected by both increases in the hydrogen peroxide dosage and in treatment time. It is interesting to note that with a 2.5 mL peroxide addition and with 1 minute of treatment time, the acetic acid level was slightly higher than it was with 9 minutes of treatment time. However, this difference was still within the range of deviation. Butyric acid remained mostly constant at a temperature of 80°C.

According to Shanableh and Jomaa (2001), the amount of oxidant used (hydrogen peroxide in this case) for an oxidation process should provide sufficient but not excessive oxidation power (100% of TCOD). In this study, the amounts of hydrogen peroxide used were between 0.24 and 1.13 times the total COD concentration at 3% solids content sludge. The residual hydrogen peroxide tests were conducted with the Hach test kit (model HYP-1, drop count titration) to examine any peroxide left-over within the samples. The

results showed that more than 96% of hydrogen peroxide was consumed in the treatments.

Under all the experimental conditions, the results for VFAs were below 2% of the SCOD, with most of them less than 0.5%. The after-treatment TCODs were also at approximately the same levels as the initial TCODs (within the standard deviation). There may be an actual lost in TCODs, but the portion would be relative small to the overall TCOD and measurement system errors. Therefore, it is reasonable to suggest that under these experimental conditions, the treatment was mainly on COD solubilization, but not on the secondary oxidation for VFAs production or final oxidation.

In terms of operating variables, the sludge solids content, temperature and hydrogen peroxide dosage are the three important factors for VFA results (Figure 2.11). It is identical to SCOD results (Figure 2.2), and consistent with the previous study by Liao et al. (2007) and Wong et al. (2006a; 2006b).

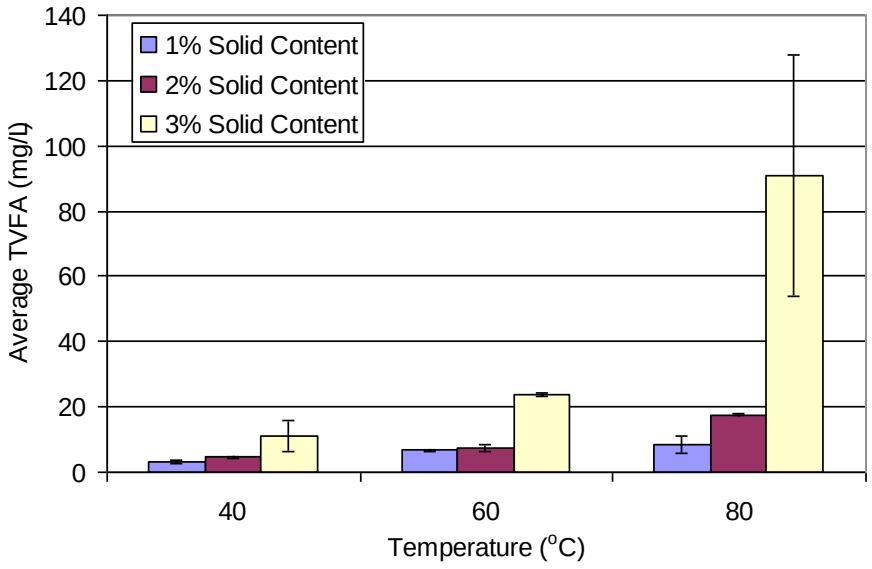


Figure 2.8 Average total volatile fatty acids under various treatment conditions

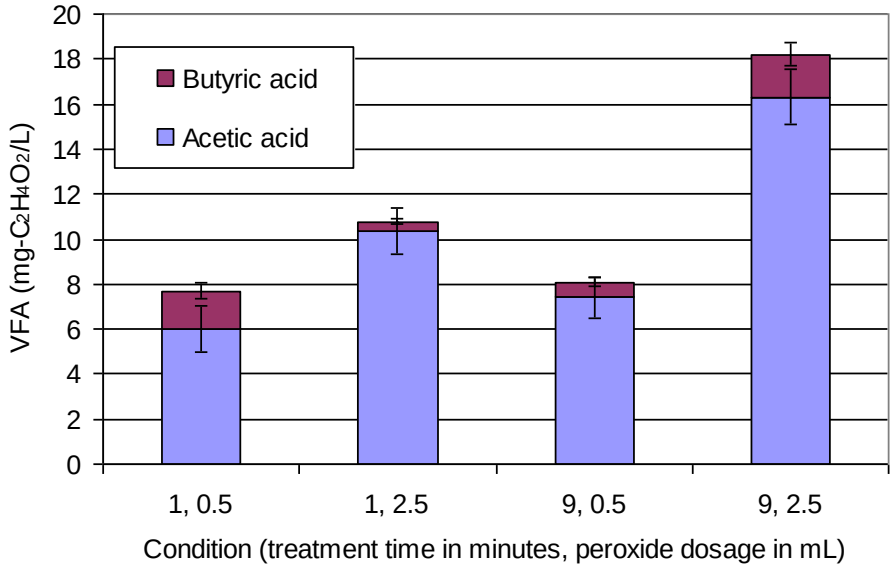


Figure 2.9 Volatile fatty acid production at 3% solids content and temperature 40°C

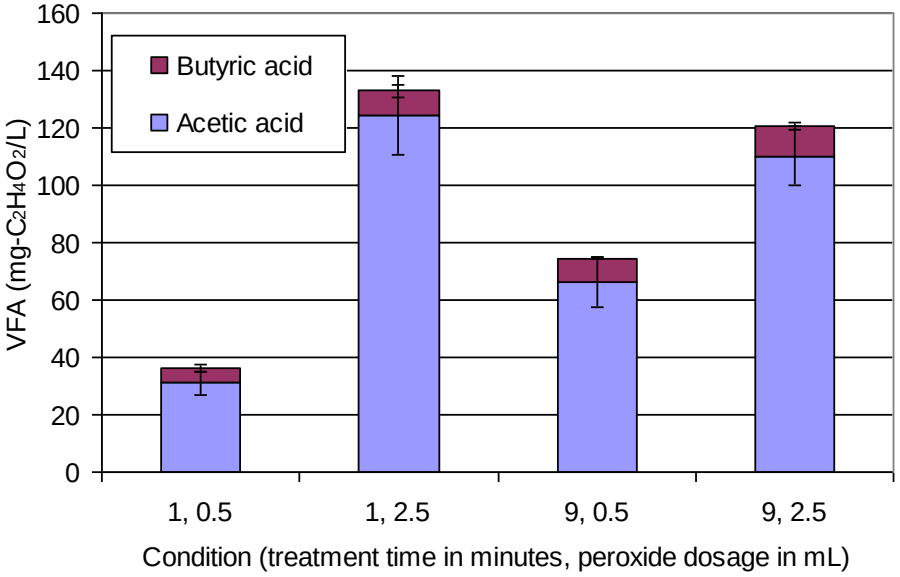
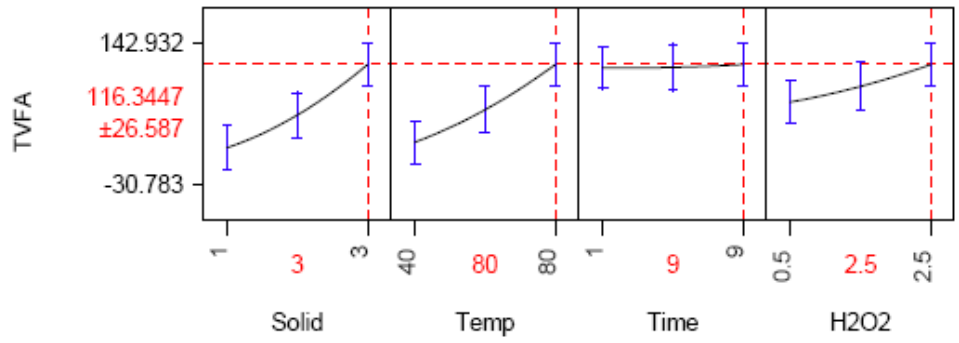


Figure 2.10 Volatile fatty acid production at 3% solids content and temperature 80°C



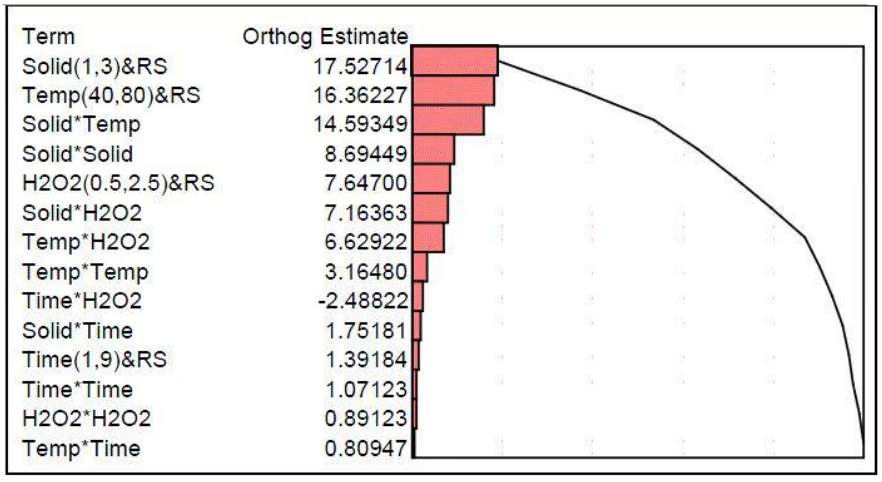


Figure 2.11 Prediction profiler and Pareto plot of scaled estimate for significant factors on TVFA (solids content in %, temperature in °C, time in minutes and H_2O_2 in mL dosage)

2.3.4 Nutrient release

The results for soluble orthophosphate (ortho-P) are shown in Figure 2.12. In this study, the ortho-P released represent 13 to 35% total phosphate (TP) of concentrated sludge. The maximum ortho-P was obtained at approximately 60°C under current experimental conditions (Figure 2.12). These results are consistent with the findings in Liao et al. (2005b), Wong et al. (2006a) and Kuroda et al. (2002). It was suggested (Wong et al., 2006a) that low soluble phosphate levels in the solution at 80°C were due to the presence of intermediate products of polyphosphates. The rate and extent of poly-P release is dependent on the temperature (Kuroda et al. 2002). It has been reported that the initial release of phosphorus from EBPR sludge by heat was entirely poly-P, which then degraded to ortho-P (Kuroda et al. 2002).

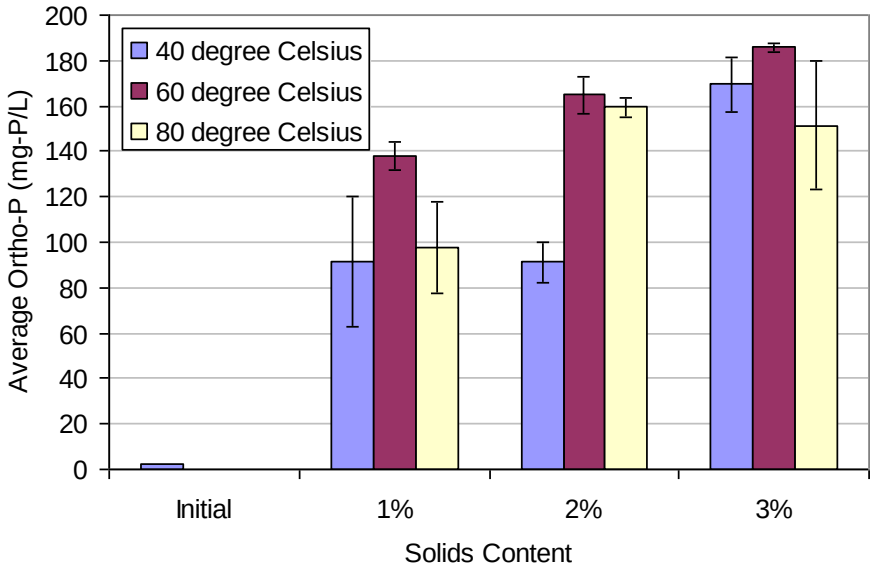


Figure 2.12 Average orthophosphate release under various treatment conditions

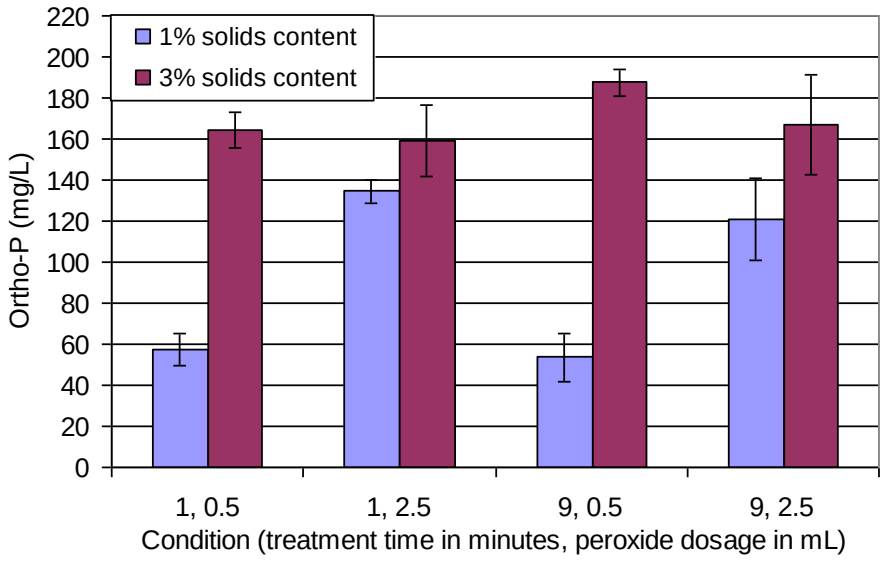
The operating variables of temperature, hydrogen peroxide dosage and solids content all played a role in ortho-P release. Temperature was the most significant factor for ortho-P release. Higher solids content also increased the ortho-P levels in solution. The results and a comparison are shown in Figure 2.13 and Figure 2.14.

Figure 2.13 shows the concentration of ortho-P in solution, and percentage ortho-P to TP, at a temperature of 40°C. For the 1% solids content at 40°C, increasing the hydrogen peroxide dosage appears to have substantially increased the ortho-P concentration in solution. The percentage of ortho-P to TP reached 30-35%, even at this low temperature and with a short treatment time of 1 minute. However, when higher solids content (3%) sludge was used, ortho-P levels seem to have remained relatively constant, regardless of the changes in treatment time or variations in the peroxide addition. This suggests that the mechanism for ortho-P release is likely more than just simple biomass cell destruction.

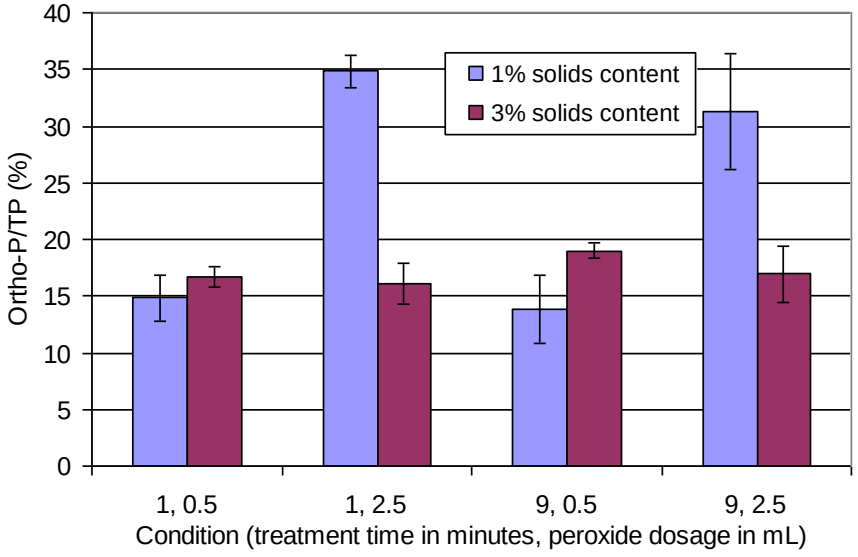
Figure 2.14 shows the ortho-P in solution and the percentage ortho-P to TP at 80°C. For both the 1% and 3% solids content sludge, ortho-P levels seemed to decrease with a longer treatment time and a higher hydrogen peroxide dosage. These results, however, are consistent with Wong et al. (2006a) at lower solids content of 0.35-0.4%. Wong et al. (2006a) explored the possible explanation by having a subsequent acid hydrolysis (Harold, 1960) step to examine the poly-P in solution. It demonstrated the poly-P in solution could be hydrolyzed by acid, and the final mass balance of ortho-P was achieved (Wong et al. 2006a).

The soluble ammonia concentration ranged from 18 to 97.9 mg-N/L (Figure 2.15) and represents between 2 to 7% of TKN in the sludge. It appears that the overall ammonia-N release increased with higher temperatures and greater hydrogen peroxide dosages. The

only exception was with a 3% solids content sludge at a temperature of 80°C. This general pattern is consistent with previous studies (Chan et al. (2007)) at a solids content of 0.35-0.4%. The ammonia to orthophosphate (NH_3/PO_4) molar ratio in 26 sets of experiments ranged from 0.5 to 1.2. If the resulting solution were to be used directly for struvite recovery, ammonia would be the limiting factor.

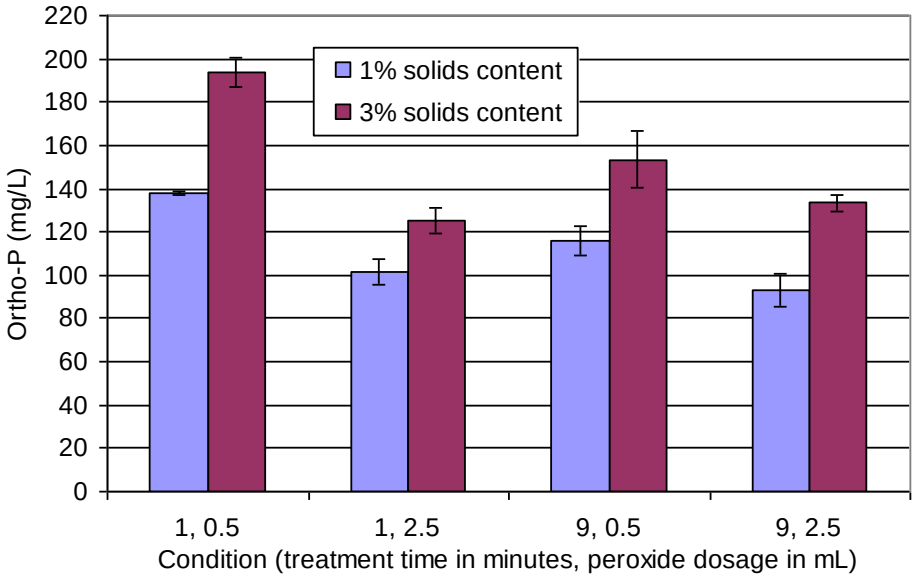


(a)

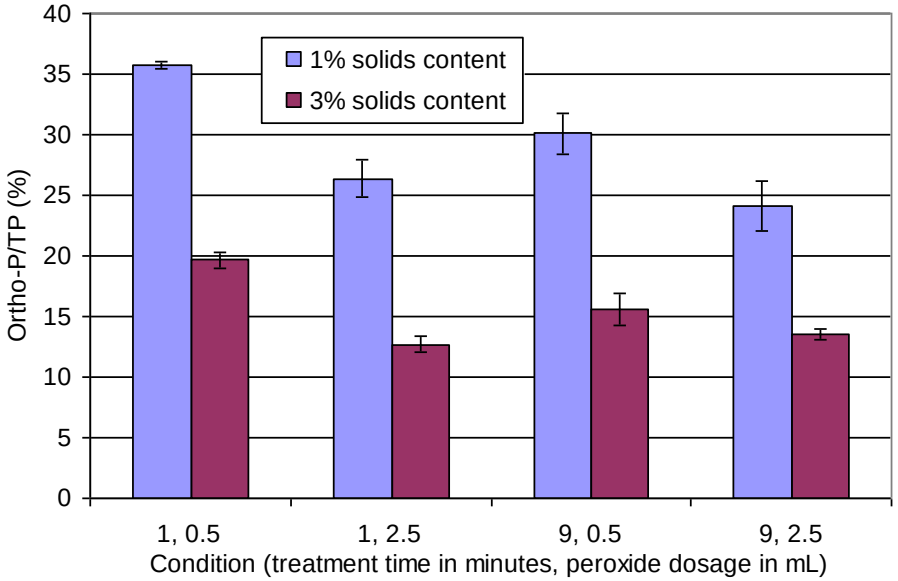


(b)

Figure 2.13 Ortho-P levels (a) and percentage ortho-P/TP (b) at temperature 40°C



(a)



(b)

Figure 2.14 Ortho-P levels (a) and percentage ortho-P/TP (b) at temperature 80°C

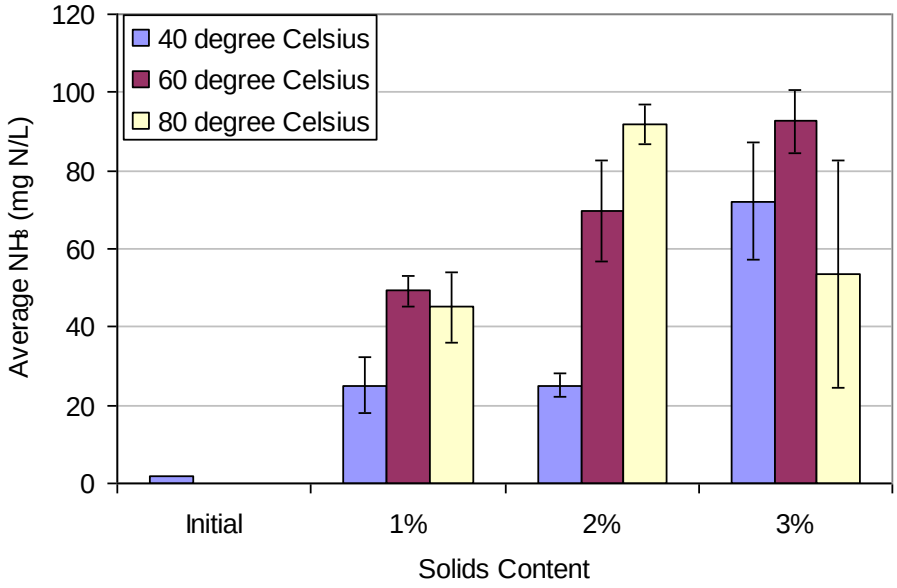


Figure 2.15 Average ammonia release under various treatment conditions

2.3.5 Statistical modeling

The results from the 26 experiments were input to the JMP-IN ® 5.1 statistical modeling program (Sall et al., 2005), where surface response models were constructed to fit the experimental data. The standard least-squares method was used for fitting multiple continuous factors, and all the linear, quadratic and cross product terms were applied (representing two-level interaction factors). These models were presented with a series of simplified prediction formulas, model leverage plots (with the summary of fit) and response surface profilers, in Figure 2.16 (SCOD), Figure 2.17 (percentage SCOD/TCOD), and Figure 2.18 (ortho-phosphate).

In each of these figures, part (a) is the simplified prediction formula. The original prediction formula was simplified by taking off a few quadratic or cross product terms that have minimal impacts. In part (b), the whole model leverage plot (actual by prediction plot) is shown, together with the summary of fit. The R squares for the each of the models are 0.97 for SCOD, 0.92 for percentage SCOD/TCOD, and 0.86 for ortho-P, respectively. They show that these models fit reasonably well with the experimental data and that the general patterns could be confidently identified.

For part (c) and (d), the surface profilers show 3-dimentional plots for the surface responses. Since only response and two factors could be shown in this three dimensional profiler, the other two less significant factors were set to constant. For example, in Figure 2.16 (c), the surface profiler shows response (SCOD) on one axial, and solids content and temperature on the other two. The treatment time and hydrogen peroxide addition were set to constant.

From Figure 2.16 (d) and Figure 2.17 (d), it can be seen that the general trends for

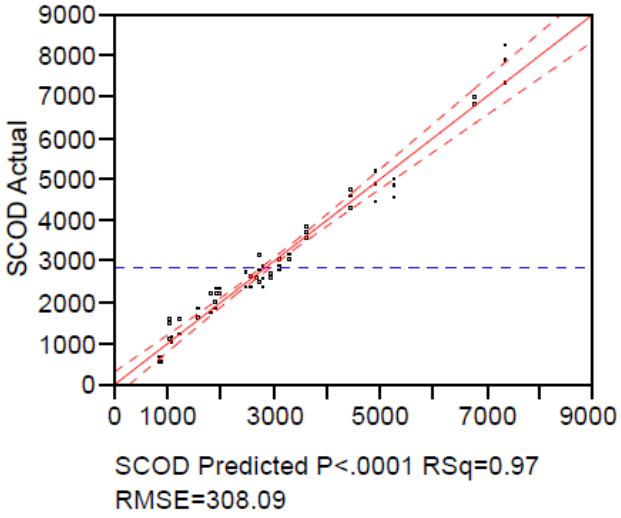
SCOD and SCOD/TCOD%, with regard to temperature and the peroxide addition, were similar, as confirmed in previous findings. The difference between Figure 2.16 (c) and Figure 2.17 (c) shows that SCOD were heavily influenced by the solids content, but not so with SCOD/TCOD%.

One of the main functions of a surface response model is to find the maximum or minimum response and conditions. The prediction models confirmed that the maximum point for SCOD and SCOD/TCOD% would be at 3% solids content, 80°C temperature, 2.5mL hydrogen peroxide addition and 9minutes of treatment time.

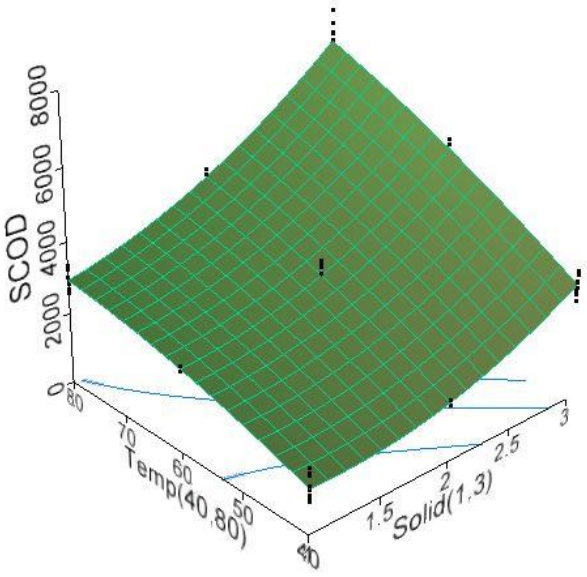
The response surface profiler for ortho-P in Figure 2.18 (c) shows a pattern in which the ortho-P level increased from 40 to 60°C and decreased at 80°C. As discussed in Section 2.3.2, this was probably due to the accumulation of poly-phosphate at around a temperature 80°C. For ortho-P release under these experimental conditions, the optimum point would be at a 3% solids content and a temperature around 60°C. Figure 2.18 (d) shows that with regard to hydrogen peroxide additions at a low solids content of 1%, the slope of the surface was steep. At a high solids content of 3%, the slope turned flat. This suggests that the effect of hydrogen peroxide was not as significant with a 3% solids content sludge as it was with a 1% solids content sludge.

$$SCOD = \begin{bmatrix} 2563 \\ +1273*(Solid-2) \\ +1267*\frac{(Temp-60)}{20} \\ +564*(H_2O_2-1.5) \\ +(Solid-2)*\frac{(Temp-60)}{20}*588 \\ +(Solid-2)*(H_2O_2-1.5)*269 \\ +\frac{(Temp-60)}{20}*(H_2O_2-1.5)*158 \\ +(Solid-2)*(Solid-2)*608 \\ +\frac{(Temp-60)}{20}*\frac{(Temp-60)}{20}*(-225) \end{bmatrix}$$

(a)

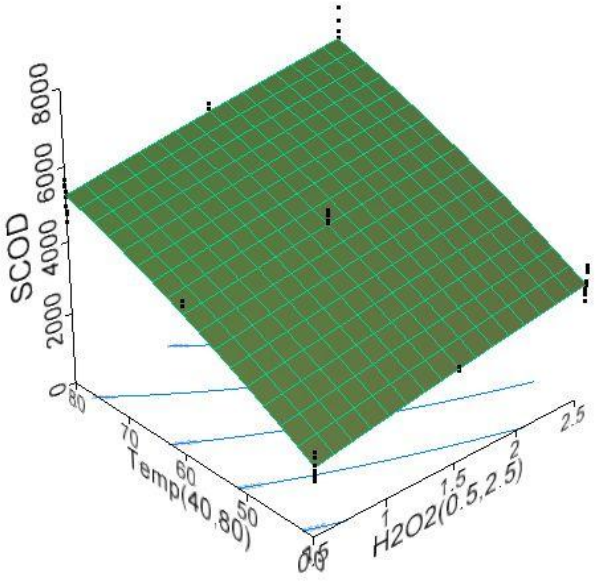


(b)



H_2O_2 2.5mL, Treatment time 9 mins

(c)



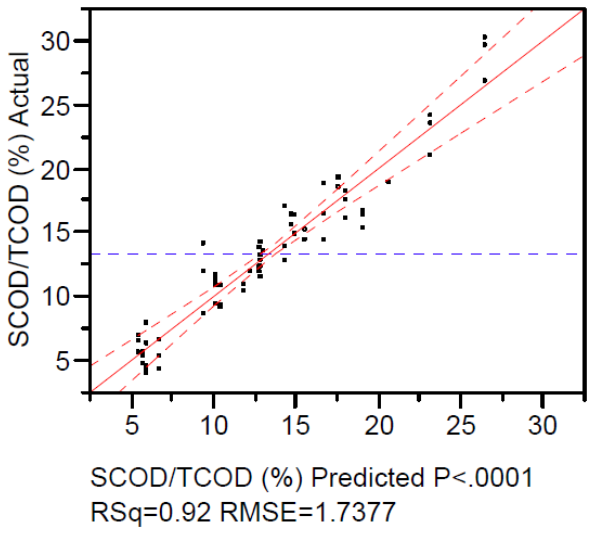
Solids content 3%, Treatment time 9 mins

(d)

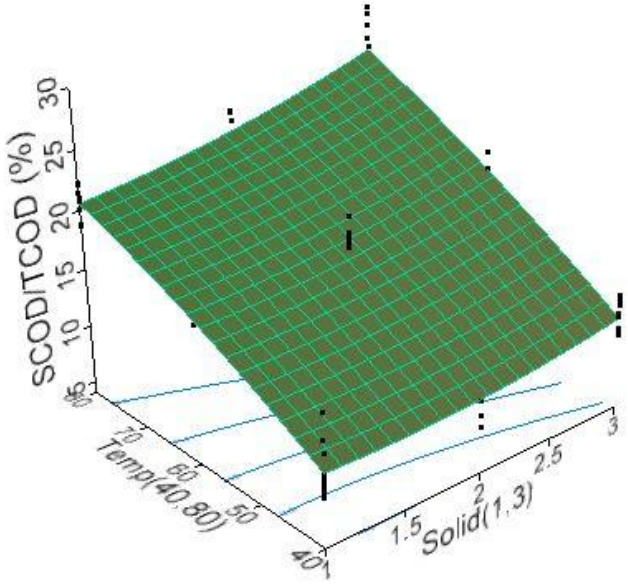
Figure 2.16 Response surface modeling for SCOD (a) simplified prediction formula; (b) model leverage plot; (c) (d) surface profilers

$$\%\left(\frac{SCOD}{TCOD}\right) = \begin{bmatrix} 12.83 \\ +1.34*(Solid-2) \\ +5.44*\dfrac{(Temp-60)}{20} \\ +0.78*\dfrac{(Time-5)}{4} \\ +2.72*(H_2O_2-1.5) \\ +(Solid-2)*\dfrac{(Temp-60)}{20}*1.03 \end{bmatrix}$$

(a)

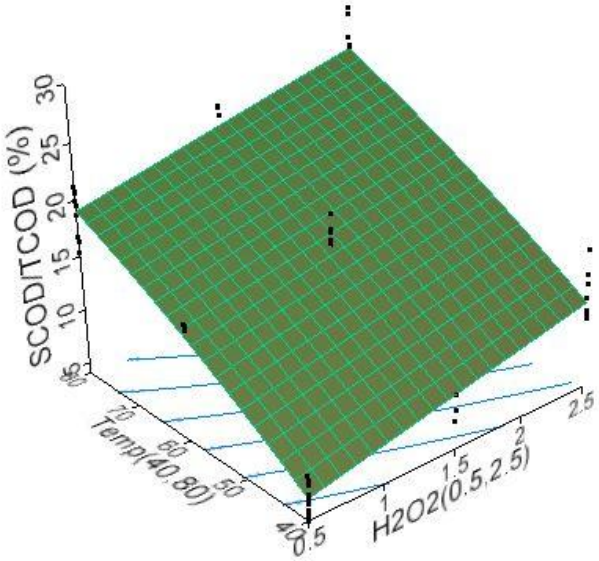


(b)



H_2O_2 2.5mL, Treatment time 9 mins

(c)



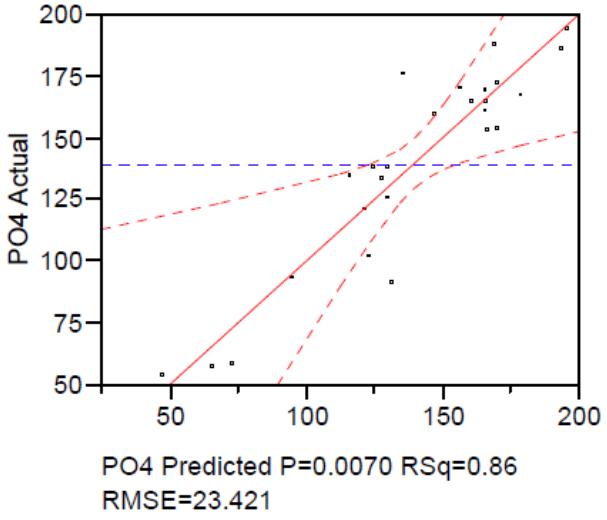
Solids content 3%, Treatment time 9 mins

(d)

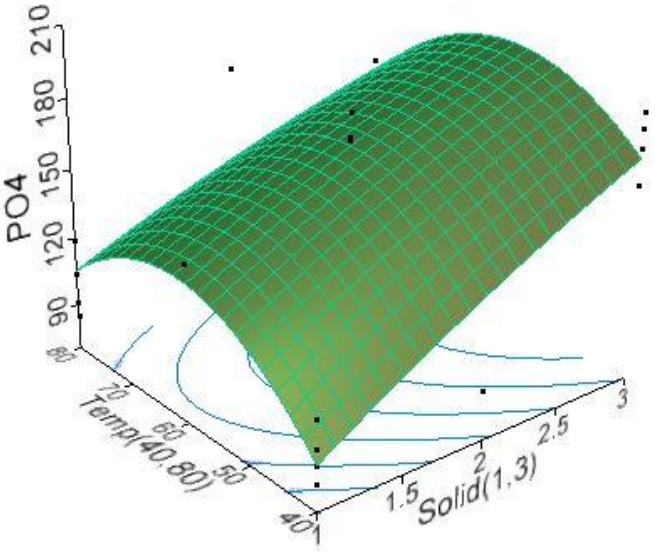
Figure 2.17 Response surface modeling for SCOD/TCOD% (a) simplified prediction formula; (b) model leverage plot; (c) (d) surface profilers

$$PO_4 = \begin{bmatrix} 165.43 \\ +31.98*(Solid-2) \\ +\frac{(Temp-60)}{20}*\frac{(Time-5)}{4}*(-8.42) \\ +(Solid-2)*(H_2O_2-1.5)*(-16.1) \\ +\frac{(Temp-60)}{20}*(H_2O_2-1.5)*(-13.04) \\ +\frac{(Temp-60)}{20}*\frac{(Temp-60)}{20}*(-32.19) \end{bmatrix}$$

(a)

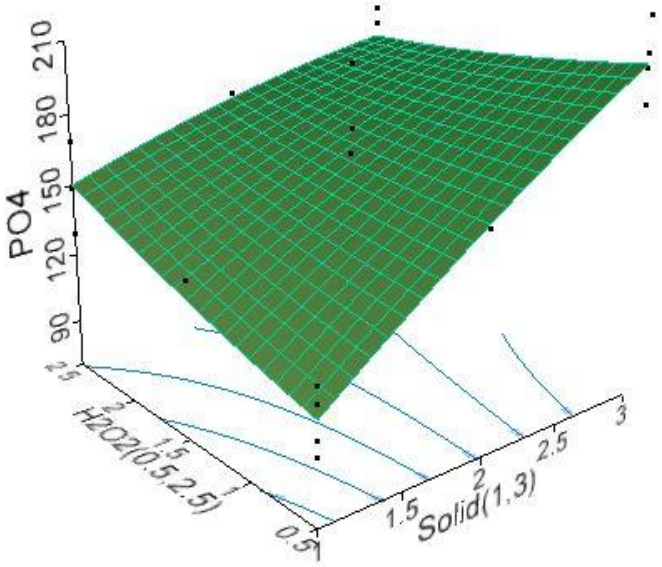


(b)



H_2O_2 1.5mL, Treatment time 5 mins

(c)



Temperature 60°C, Treatment time 5 mins

(d)

Figure 2.18 Response surface modeling for ortho-P (a) simplified prediction formula; (b) model leverage plot; (c) (d) surface profilers

2.4 Conclusions

In this study, the MW/H_2O_2 process was examined for its effect on thickened secondary sludge (1% to 3% solids content) at a low temperature range (40 to 80°C). Hydrogen peroxide dosage and treatment time were also included as the process variables.

The results show that sludge concentration, temperature and hydrogen peroxide dosage all have substantial and interrelated impacts on COD solublization (SCOD level and SCOD/TCOD percentage). The increase of SCOD was found to be proportional to the increase in sludge solids content or temperature. From an operational perspective, the use of thickened sludge is therefore very beneficial in terms of both energy and treatment efficiencies.

The study on volatile fatty acids levels suggests that MW/H_2O_2, at the temperature range of 40 to 80°C, was mainly a disintegration / solubilization process. The main product of VFA formation was acetic acid with more than 89% of total VFA. The overall total VFA was less than 2% of SCOD.

For ortho-P release, temperature and solids content were the two main factors. Hydrogen peroxide was significant for ortho-P release with lower solids content sludge, but less so with a higher solids content sludge. At a temperature of 80°C, ortho-P release was likely affected by poly-phosphorus accumulation.

The statistic models for SCOD, SCOD/TCOD% and ortho-P fitted reasonably well to the data. With linear, quadratic and cross product terms included, these surface response models were very useful for both factor screening and prediction functions.

For potential engineering application of MW/H_2O_2 process, the following discussion was noted.

1. Sludge thickening prior to MW/H_2O_2 treatment is important and necessary in practice.

2. The MW/H_2O_2 treatment at these experimental conditions can not provide sufficient VFA to be used directly for BNR process. For anaerobic digestion, the impact (such as SCOD accumulation) will need to be evaluated in details (reported in Chapter 6).

3. The surface response model approach could be used for pilot-scale or full scale study. It requires less experimental effort than a full factorial design, but provides similar benefits.

Chapter 3 A Comparative Study of The Microwave / Hydrogen Peroxide Process and Thermal / Hydrogen Peroxide Treatment *

3.1 Introduction

Microwave radiation is extensively used in many areas, including food processing, radio communications and in households. It has also been used in environmental applications for sample decomposition and preparation in laboratories (Beltra et al., 2003; Perez-Cid et al., 1999, 2001). More recently, research interest has been on its potential in biological sludge disintegration (or solubilization) by the wastewater engineering industry.

Wastewater sludge presents a significant environmental concern if it is not treated properly. In large-scale treatment plants, anaerobic digestion is the most common process applied to stabilize sludge because of its emphasis on energy conservation and its capacity for sludge volatile solids reduction. However, the speed of anaerobic digestion is limited by the slow rate of hydrolysis. This slow rate results in a long retention time and a large reactor volume. To address this limitation, pretreatment methods have been used break up the biomass cell wall to increase the level of carbon and nutrient solubilization. The various pretreatment methods used include ultrasound treatment (Tiehm et al., 1997, 2001; Eder and Gunthert, 2002; Nickel and Neis 2007), thermo-chemical (Tanaka et al.,

*A version of this chapter will be submitted for publication:

YI, W., Lo, K.V., Liao, P.H., Mavinic, D.S., Forgie, D. and Mohseni M. A comparative study of the microwave/hydrogen peroxide advanced oxidation process and thermal/peroxide treatment.

1997; Penaud et al., 1999; Valo et al., 2004), ozonation (Weemaes et al., 1999; Ahn et al., 2002), and mechanical techniques (Baier and Schmidheiny 1997; Muller et al., 1998). A microwave hydrogen peroxide process (MW/H_2O_2) has also been developed for sludge solubilization (Liao et al., 2005a, 2005b, 2007; Wong et al., 2006a, 2006b).

Liao et al. (2005) and Wong et al. (2006) demonstrated that a large portion of the nutrient (ortho-phosphate and ammonia) could be effectively solubilized by the MW/H_2O_2 process at temperatures above 120°C. A continuing study, reported in Chapter 2 of this dissertation, was conducted to investigate the MW/H_2O_2 process operating at a lower temperature range of 40-80°C and with sludge of various solids contents (1-3%). One of the questions often raised is whether any meaningful non-thermal effects are associated with this microwave heating or MW/H_2O_2 process.

Microwave irradiation's non-thermal effect is itself a myth. Contradictory results have been reported over the past several decades (on microbial inactivation, Khalil and Villota, 1985; Kozempel et al., 1998; Welt et al., 1994; and on microwave-assisted organic synthesis chemistry, Loupy, 2002; Kappe, 2004). In environmental engineering field, Eskicioglu et al. (2007) reported identical results on COD and bio-polymer (protein, carbohydrates etc) solubilization with microwave and conventional heating, but 5-13% of biochemical methane potential enhancement from microwave heating.

In the case of the MW/H_2O_2 process, the strong oxidation capacity and delicate nature of hydrogen peroxide makes the question of non-thermal effects even more intriguing. Hydrogen peroxide is a strong oxidant, with its oxidation potential higher than that of chlorine, chlorine dioxide or potassium permanganate. Through catalysis with iron, ozone or UV-light, it can be converted into hydroxyl radicals (.OH), an even more powerful

and reactive oxidant (2.8V oxidation potential) second only to fluorine. With this catalyzed conversion, some persistent organic pollutants could be treated (Sanz et al., 2002, Cravotto et al., 2005).

It was hypothesized (Sanz et al., 2002; Liao et al., 2005a, 2005b, 2007; Wong et al., 2006a, 2006b; Eskicioglu et al. 2008) that in MW/H_2O_2 process, microwave irradiation acts as the catalysis agent to yield hydroxyl radicals. If that is the case, MW/H_2O_2 process can avoid some other advance oxidation process limitations such as the acidic conditions in Fenton reaction. However, despite all the efforts, there has not been any report to confirm the hydroxyl radical presence, and to what degree the non-thermal or synergistic effect of microwave and hydrogen peroxide contributed to the improvement of sludge disintegration. A direct, side-by-side comparison of MW/H_2O_2 process to thermal/peroxide treatment (CH/H_2O_2) may offer an opportunity to exam these non-thermal or synergistic effect contributions.

As such, the present study was designed to investigate and compare the MW/H_2O_2 process with the thermal/peroxide treatment (CH/H_2O_2) in terms of sludge disintegration over a temperature range of 40 to 80°C and employing different treatment times and peroxide dosages.

3.2 Material and Methods

3.2.1 Apparatus

The main experimental apparatus used for the MW/H_2O_2 sludge treatment was a closed-vessel microwave digestion system (Ethos TC Digestion Labstation 5000, Milestone Inc., USA). The system was described in Section 2.2.1.

Thermal heating of the sludge sample was accomplished by a hot water bath with temperature control. Three individual thermocouples were immersed into the sludge samples. Temperature profiles were recorded by computer.

3.2.2 Experimental design

Two sets of sixteen experiments (Table 3.1), each with duplicate runs and three replicate samples for each run, were carried out to compare the MW/H_2O_2 process and CH/H_2O_2 treatment in terms of sludge disintegration. Process factors such as sludge heating temperature, heating (treatment) time, and hydrogen peroxide dosage were investigated.

Experiments were performed at a sludge solids content of 3%, heating temperatures from 40-80°C, heating time from 0-10 minutes and hydrogen peroxide dosage from 0-1% (wt) in 30 mL sludge. These factors were input to the Response Surface Design function in statistical analysis software JMP-IN ® 5.1 for purposes of screening for effects. Central Composition Design was used for these three continuous variables.

Response Surface Designs are useful for modeling a curved surface (quadratic) to continuous factors. These designs are capable of fitting a second order prediction equation

for the response. The quadratic terms in these equations model the curvature, and find the optimal response within specified ranges of the factors. (Sall et al., 2005).

Table 3.1 Experiment design for both MW/H_2O_2 and CH/H_2O_2 treatment

Set	Design Pattern	Temperature (°C)	Heating Time (minutes)	H_2O_2 Dosage (wt %)
1	- - -	40	0	0
2	- -+	40	0	1
3	a00	40	5	0.5
4	-+-	40	10	0
5	-++	40	10	1
6	0a0	60	0	0.5
7	00a	60	5	0
8	000	60	5	0.5
9	000	60	5	0.5
10	00A	60	5	1
11	0A0	60	10	0.5
12	+- -	80	0	0
13	+-+	80	0	1
14	A00	80	5	0.5
15	++-	80	10	0
16	+++	80	10	1

3.2.3 Treatment processing and sampling

Secondary aerobic sludge was obtained from the pilot-plant wastewater treatment facilities located at the University of British Columbia (UBC) south campus. Fresh sludge samples were collected daily for the experiments. They were concentrated with a centrifuge to reach the desired 3% solids content for the experiments. Table 3.2 defines the characteristics of the secondary aerobic sludge over the course of this study.

Hydrogen peroxide solution is added to the sludge samples and completely mixed. The hydrogen peroxide additions in these two sets of experiment were 0%, 0.5% and 1% to the sample volume in wet weight. With 82% effectiveness (due to natural decomposition), they were calculated at approximately 0, 0.21 and 0.42 mg-H_2O_2/ mg-TCOD (or, 0, 0.2, 0.4 mg-H_2O_2/mg-DS). After rapid dosing and mixing, sludge samples were sent to the microwave station and hot water bath. The microwave heating ramp time was controlled according to the thermal heating ramp time. The sludge samples were maintained at the desired experimental temperature for 0, 5 and 10 minutes of treatment time. After treatment, the mixed liquors were spun in a centrifuge at 15,000 rpm for 10 minutes. The resulting supernatants were filtered through Whatman No.4 filters and analyzed for soluble faction of the COD, volatile fatty acids and phosphate.

Total solids (TS), Volatile solids (VS), Chemical oxygen demand (COD), phosphorus (orthophosphate, PO_4-P), were determined according to the Standard Methods (APHA, 1995). A Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) was used to measure volatile fatty acids (VFA). Volatile separation was accomplished with an HP FFAP column. The injection temperature was set at 175 ºC and the FID detector was at 250 ºC.

Table 3.2 Characteristics of concentrated secondary sludge used in this study

Parameters	*Concentration*
pH	6.2 - 6.7
TS (%)	3.0 ± 0.1
Total COD (mg/L)	29,500 ± 1,600
Total Phosphorus (mg P/L)	910 ± 35
TKN (mg N/L)	2,100 ± 50
Initial soluble COD (mg/L)	120 ± 40
Initial soluble PO_4-P (mg P/L)	2.0 ± 0.5
Initial TVFA (mg-$C_2H_4O_2$/L)	15 ± 8

3.2.4 Degree of disintegration

The traditional method used to quantify the extent of sludge disintegration is by the percentage of soluble COD (SCOD) versus total COD (TCOD). However, this parameter may not accurately indicate the extent of disintegration treatment in cases where initial SCOD was high. Sludge samples also vary between different treatment plants, or from different collection dates. It can also be difficult to compare the results from different batches, even within the same study. Therefore, an expression termed “Degree of Disintegration (DD)”, commonly used in research on ultrasound treatment (Neis et al., 2000; Tiehm et al., 2001; Nickel and Neis 2007) has been adopted for this study. The percentage SCOD to TCOD (SCOD/TCOD%) was also used to illustrate the difference of these two parameters. The “Degree of Disintegration (DD)” is defined as such,

$$DD_{COD} = \frac{(SCOD_{Treated} - SCOD_{initial})}{(SCOD_{NaOH} - SCOD_{initial})}\% \qquad (3\text{-}1)$$

where $SCOD_{Treated}$ is the soluble chemical oxygen demand of the disintegration processed sample (mg/L), $SCOD_{initial}$ is the soluble chemical oxygen demand of the untreated sample (mg/L), and $SCOD_{NaOH}$ is the soluble chemical oxygen demand of a reference sample hydrolysed chemically in a 0.5 molar NaOH solution at 20°C for 22 h (mg/L).

3.3 Results and Discussion

3.3.1 Solubilization of COD

The average SCOD levels in solution are shown in Figure 3.1 (a) for the MW/H_2O_2 process and (b) for CH/H_2O_2 treatment. The highest SCOD for both experimental sets occurred at temperatures of 80°C, heating time of 10 minutes and with a peroxide dosage of 1%. At the maximum point, the SCOD levels were approximately 56 and 49 times the initial SCOD concentration for MW/H_2O_2 and CH/H_2O_2, respectively. Under the same operating condition, SCOD from MW/H_2O_2 was higher than that from CH/H_2O_2.

A student's t-test was performed to statistically compare the means of SCOD data from these two treatments. The results are shown in Figure 3.3. The means diamond (green color diamond shape in Figure 3.3) illustrates a sample mean and 95% confidence interval. The standard error and standard deviation bars are shown in blue color. The student's t-test comparisons are shown in circles that illustrate all possible t-tests. The group means are compared by examining how the comparison circles intersect. The outside angle of intersection indicates whether the group means are significantly different at the 95% confidence interval. Circles that do not intersect or with the intersection angle less than 90 degree suggest that they are significantly different. Circles that intersect with angle more than 90 degree or nest within each other suggest they not significant different. The comparison was separated into 40, 60, 80°C categories, because the temperature is the main factor. At the same temperature MW/H_2O_2 and CH/H_2O_2 can be compared with various hydrogen peroxide conditions.

Without a hydrogen peroxide addition, microwave heating and conventional heating resulted in a similar SCOD. This is consistent with reports from Eskicioglu et al. (2007) on

sludge at a higher solids content of 4.6-5.5%. In Figure 3.1 (b), the hydrogen peroxide addition at an ambient temperature of 20°C, without thermal or microwave heating, yielded average SCOD concentration of 1367 mg/L, and 1884 mg/L for 0.5% and 1% dosage, respectively. With thermal heating at 40°C at the same addition of peroxide, the result was only a slightly increased SCOD to an average of 1810 mg/L and 2097 mg/L. This suggests that low temperature thermal treatment for a short period of time may only impact particulate COD, which could be easily oxidized by hydrogen peroxide. Thermal treatment itself, without a hydrogen peroxide addition and at a temperature of 40°C, yielded approximately 1940 mg/L SCOD.

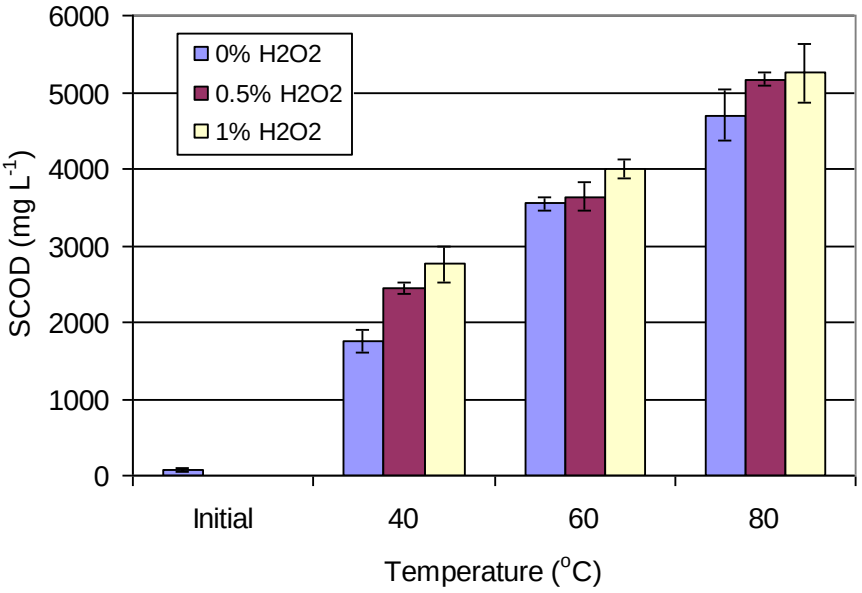
With a hydrogen peroxide addition, MW/H_2O_2 obtained higher COD solubilization, similar to the results reported in Chapter 2. However, with this dosage of hydrogen peroxide (0.21-0.42 mg-H_2O_2/mg-TCOD), the effect from a temperature increase overshadowed the increase due to peroxide. On the other hand, thermal treatment with hydrogen peroxide did not result in significant benefits to SCOD in this temperature range. Figure 3.2 (b) shows the effect of the hydrogen peroxide on thermal treatment from the statistical computing model.

The effect of temperature, heating time and hydrogen peroxide dosage on SCOD level are presented in Figure 3.2, with the prediction profiler and Pareto plot of scale estimates. For both the MW/H_2O_2 process (Figure 3.2 a) and CH/H_2O_2 treatment (Figure 3.2 b), temperature was the dominant factor. With increased temperature, both treatments yielded substantial improvements in SCOD. The hydrogen peroxide addition was the second major factor for MW/H_2O_2. For CH/H_2O_2, the treatment time was the second major factor.

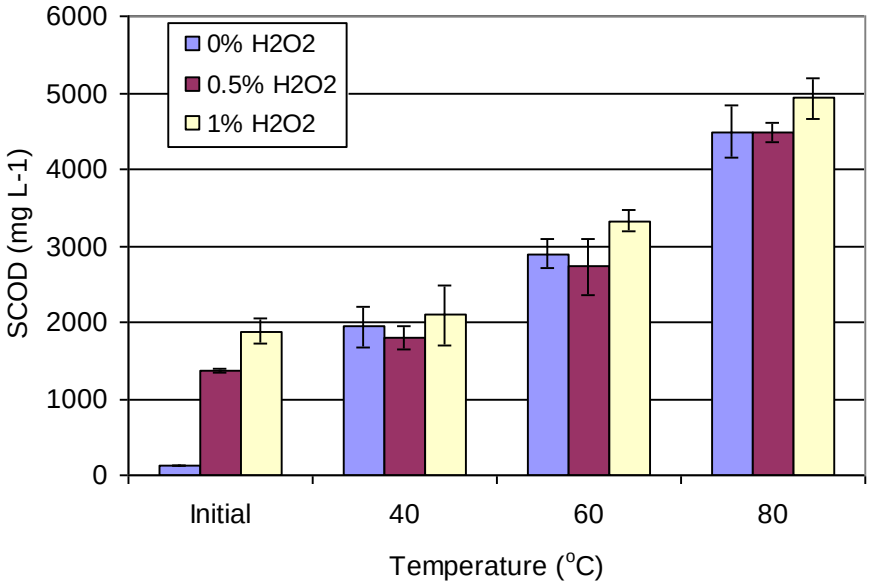
For the MW/H_2O_2 process, treatment time (from 0 to 10 minutes) had less effect on SCOD levels. The probable reason was that little energy was needed for sludge samples to stay at the desired temperature. It was noted that in this concealed vessel setup, heat lost was minimal at these low to moderate temperature levels (40-80°C). For CH/H_2O_2 treatment, time is a factor due to the constant heating from the hot water bath. However, it was observed that at 5 to 10 mins duration, the difference was less obvious.

It is confirmed that without a hydrogen peroxide addition, microwave heating on its own has no statistically-meaningful non-thermal effect on COD solubilization in the experimental conditions (40-80°C). The CH/H_2O_2 treatment did not yield better results with a hydrogen peroxide addition. In contrast, MW/H_2O_2 improves with an increase in hydrogen peroxide.

The student t-test shown on Figure 3.3 suggests that SCOD results from MW/H_2O_2 and CH/H_2O_2 treatment (at same experiment conditions) are different at 95% confidence interval in most of the cases, especially with hydrogen peroxide addition. The MW/H_2O_2 treatment in general has yielded better SCOD result than CH/H_2O_2 treatment. The following section discusses the differences in Degree of Disintegration and percentage SCOD/TCOD.

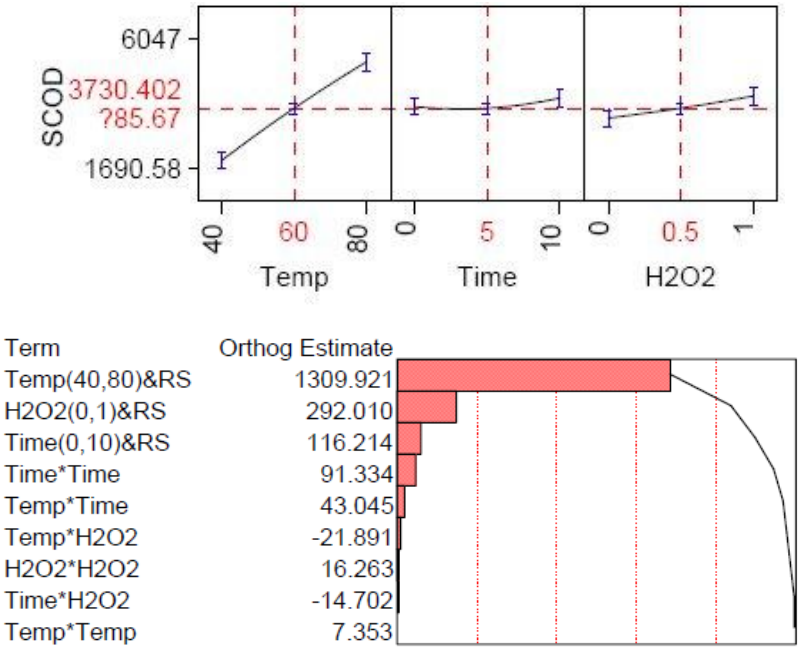


(a) MW/H_2O_2

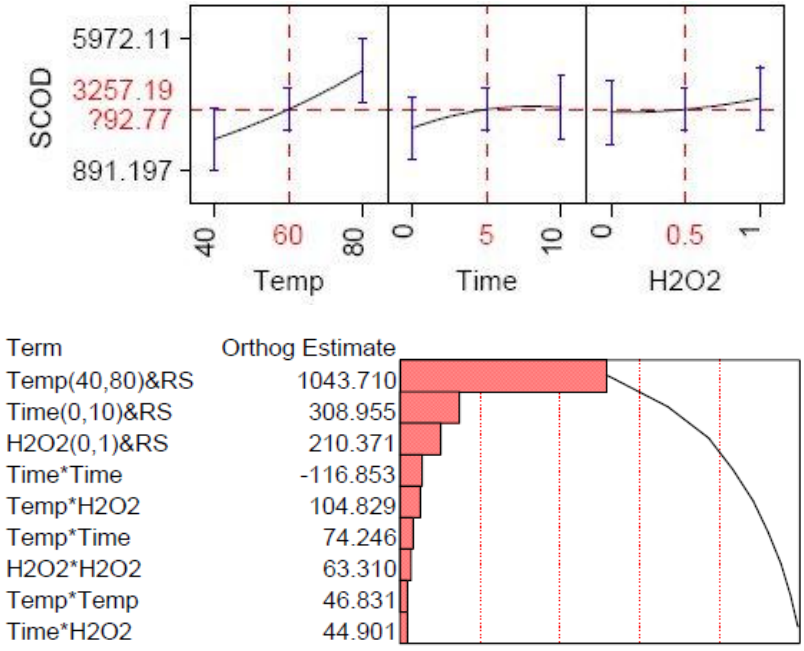


(b) CH/H_2O_2

Figure 3.1 Comparison of average SCOD levels of MW/H_2O_2 (a) and CH/H_2O_2 treatment (b) under the same operating conditions



(a) MW/H_2O_2



(b) CH/H_2O_2

Figure 3.2 Prediction profiler and Pareto plot of scale estimates of significant factors on SCOD levels for MW/H_2O_2 (a) and CH/H_2O_2 treatment (b)

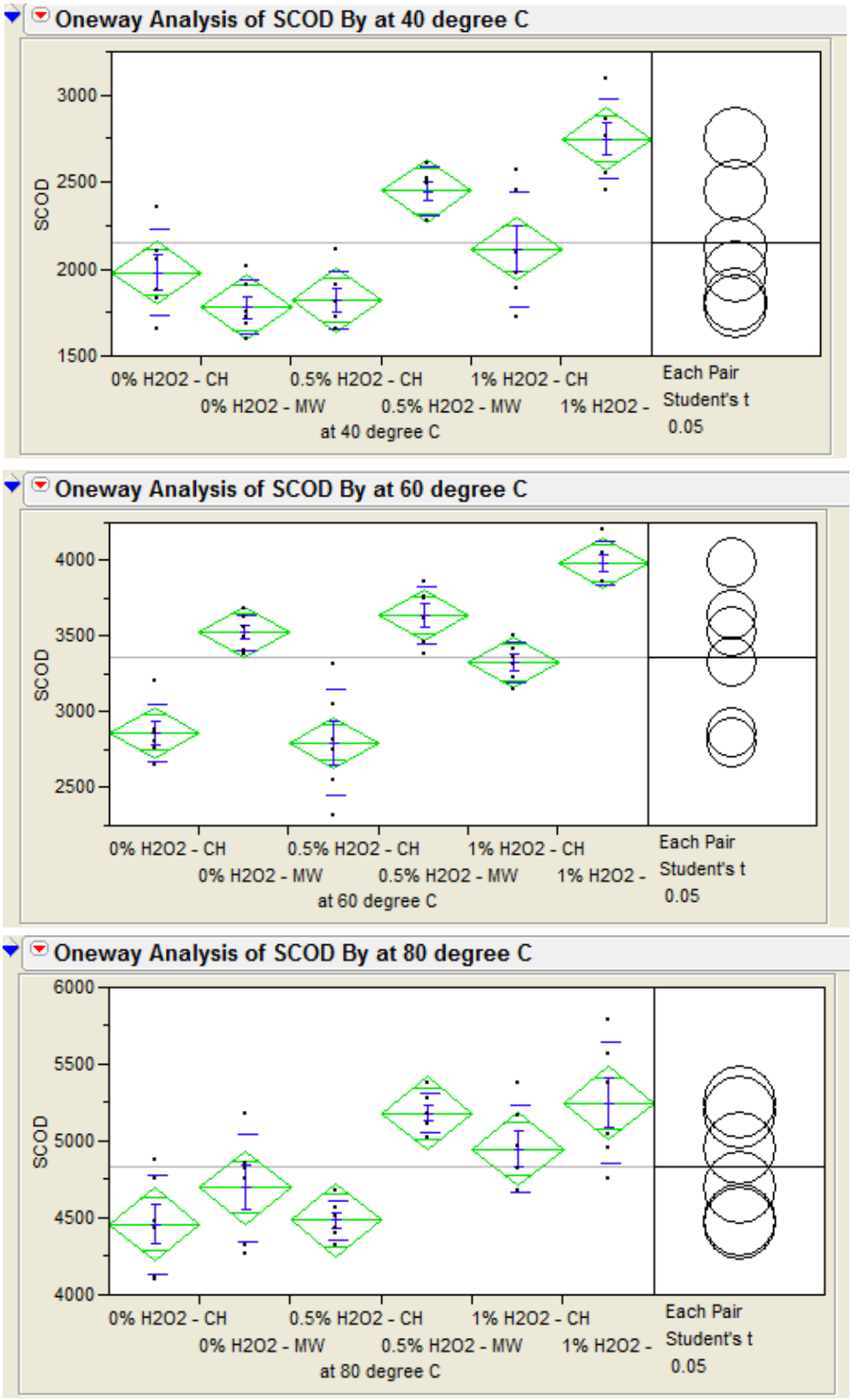


Figure 3.3 Student's t-Test for group comparisons of SCOD result from MW/H_2O_2 and CH/H_2O_2 treatment

3.3.2 Degree of disintegration and percentage SCOD/TCOD

Both the MW/H_2O_2 and CH/H_2O_2 processes yielded between 7 to 24% degree of disintegration, depending on the operating conditions. The comparison of these two processes under the same conditions is shown in Figure 3.4. The overall patterns for both treatments are comparable, with temperature being the dominant factor. The difference is that, with a hydrogen peroxide addition, MW/H_2O_2 consistently yielded approximately 2 degrees of disintegration improvement over CH/H_2O_2, as shown in Figure 3.5.

Assuming results from CH/H_2O_2 treatment represent the effect from thermal heating, hydrogen peroxide oxidation, and any possible combination effect of these two, the additional improvement from MW/H_2O_2 could be attributed to any microwave non-thermal effect and/or synergetic effect with hydrogen peroxide.

For a hydrogen peroxide addition of 0.5%, raising the temperature to 40, 60 and 80°C increased the average degree of disintegration for MW/H_2O_2 to 10.8%, 16.8% and 23.5%, respectively. Under the same conditions, CH/H_2O_2 yielded 7.8% 12.1% and 20.2%. For a 1% hydrogen peroxide dosage, the benefits from MW/H_2O_2 over CH/H_2O_2 were similar to that from a 0.5% dosage. However, it should be noted that results from MW/H_2O_2 at 0.5% hydrogen peroxide were probably overestimated by the relatively low level results from CH/H_2O_2 at 0.5% peroxide (Figure 3.1 b).

When using a traditional parameter of percentage SCOD to TCOD (SCOD/TCOD%), the results from MW/H_2O_2 ranged from 5.7% to 18.5%, while CH/H_2O_2 yielded 5.1% to 16.6%. The difference between the degree of disintegration and SCOD/TCOD% in this case was relatively small, due to the low initial SCOD levels. The average initial SCOD level taken into calculation was 126 mg/L, and the reference SCOD treated by sodium

hydroxide ($SCOD_{NaOH}$) was approximately 72% of total COD. Both of these parameters could be used as indicators of treatment efficiency.

Figure 3.6 showed the models' summaries of fits and response surface profilers for both processes. It shows that the data fitted reasonably well with the models, with R square of 0.95 and 0.93 for MW/H_2O_2 and CH/H_2O_2, respectively. The surface response profilers plotted the degree of disintegration against temperature and hydrogen peroxide additions, while treatment time was kept constant at 5 minutes. It shows that a hydrogen peroxide addition had more influence on MW/H_2O_2 and less on CH/H_2O_2.

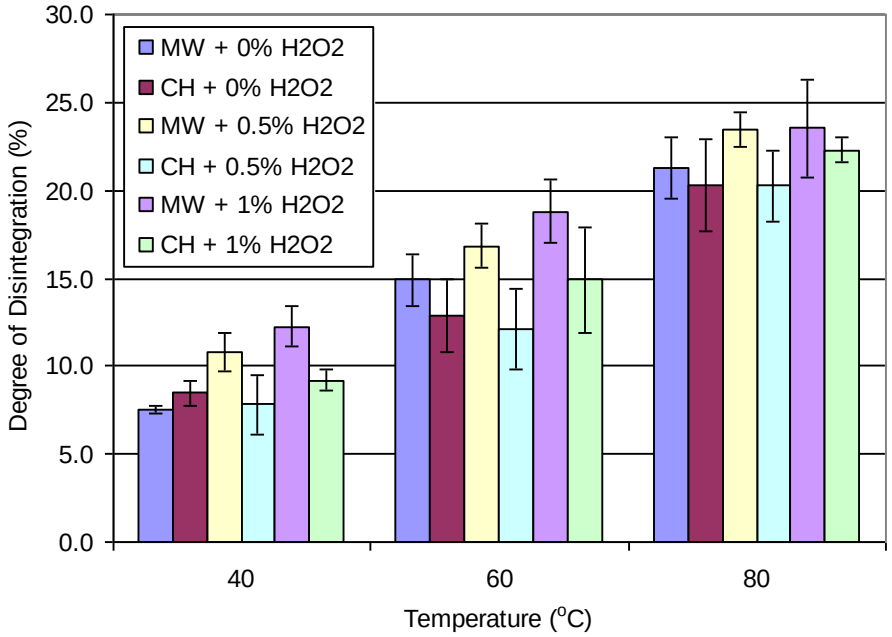


Figure 3.4 Average degree of disintegration for MW/H_2O_2 and CH/H_2O_2

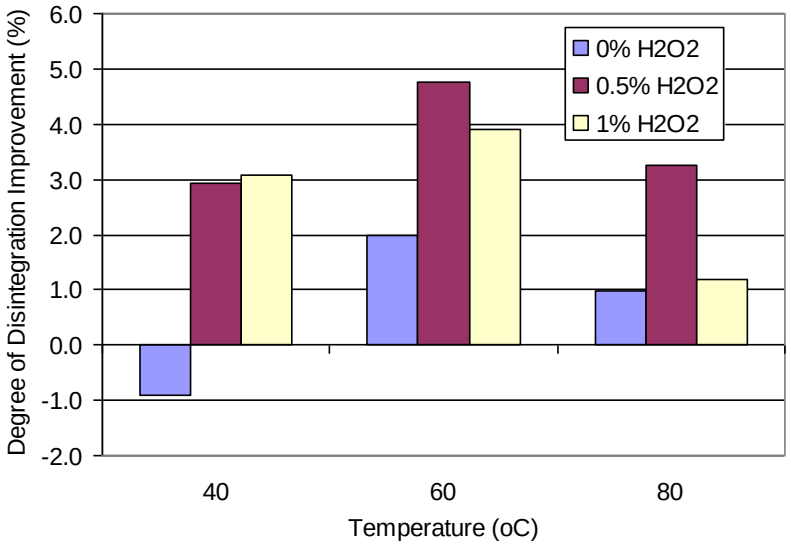
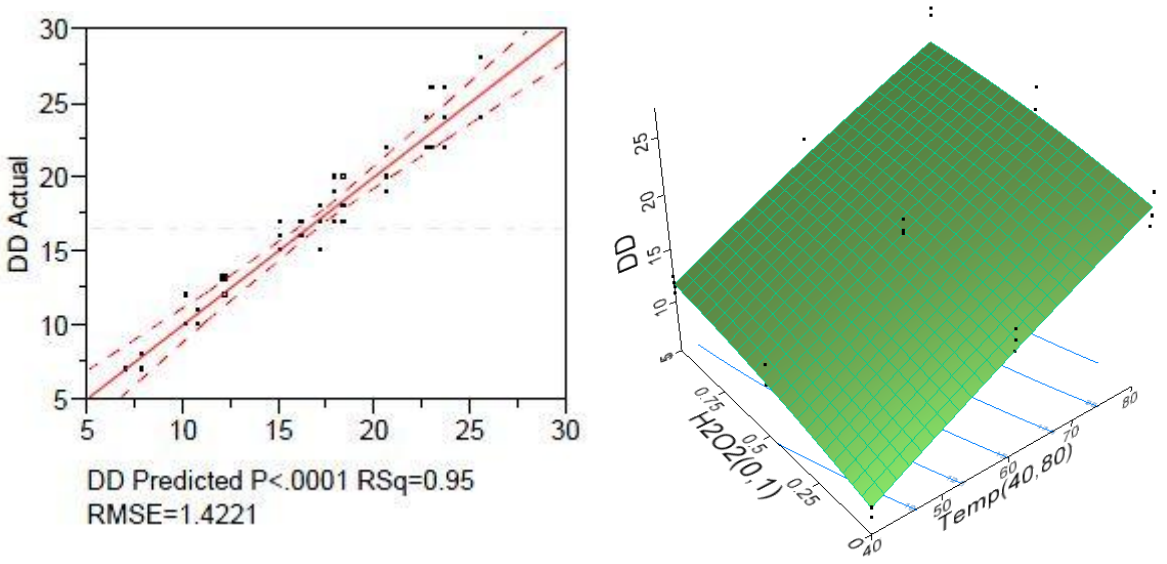
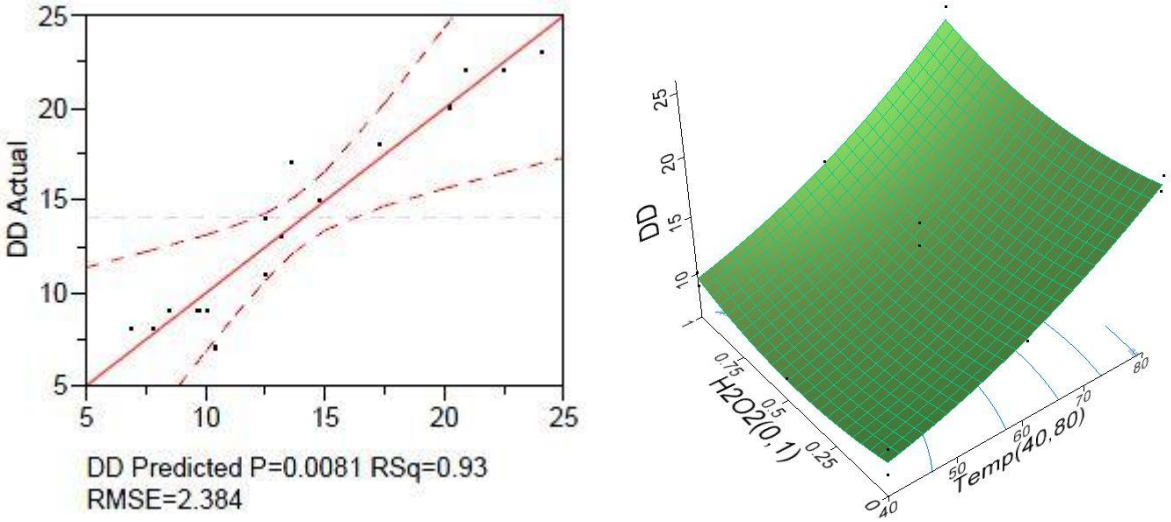


Figure 3.5 Improvements on degree of disintegration by MW/H_2O_2 over CH/H_2O_2



(a) MW/H_2O_2



(b) CH/H_2O_2

Figure 3.6 Summary of fit and response surface profiler of degree of disintegration (DD) for (a) MW/H_2O_2 and (b) CH/H_2O_2

3.3.3 Orthophosphate release

The sludge used in this study was from a biological nutrient removal (BNR) plant. This sludge stores a relatively large amount of phosphate in the form of poly-P. Phosphate release from the microbial cells to the solutions can be another indicator of the extent of sludge disintegration.

The results of soluble orthophosphate (ortho-P) levels for the MW/H_2O_2 process and the CH/H_2O_2 process are shown in Figure 3.6. For the MW/H_2O_2 process, raising the temperature from 40°C to 60°C (without H_2O_2 addition) increased ortho-P from an average of 153 mg/L to 186 mg/L. With an addition of 0.5% H_2O_2 , the increase was from an average 185 mg/L to 193 mg/L. However, this apparent difference may not be statically reliable, since their standard deviations are large enough to cover the ranges of increase. In such case, a pattern could be observed, but conclusions could not be made.

The ortho-P level dropped slightly to 163 mg/L (no H_2O_2) and 153 mg/L (0.5% H_2O_2 addition) when the temperature rose to 80°C. The same pattern was found with the CH/H_2O_2. This is consistent with previous studies reported in Chapter 2, Liao et al. (2006a), Wong et al. (2006a) and Kuroda et al. (2002). Osterberg & Orgel (1972) and Kuroda et al. (2002) commented that trimetaphosphate formation probably occurred in this temperature range, resulting in the relatively low ortho-P. The likely formation of trimetaphosphate does not mean that there is a negative impact from the heating treatment or microwave treatment, since these poly-Ps will gradually be hydrolyzed to ortho-P over time.

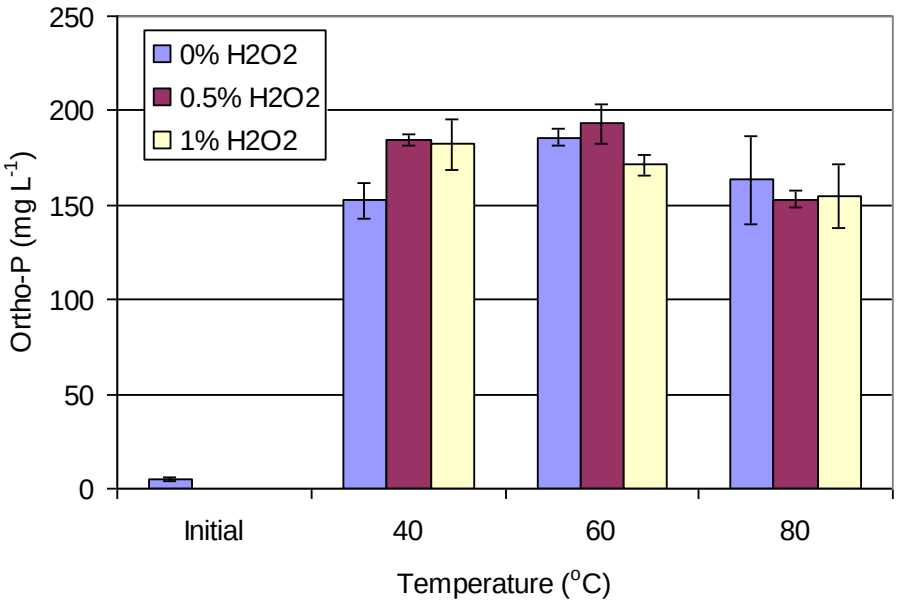
As for the effects resulting from the addition of hydrogen peroxide addition, it was found that ortho-P levels decreased slightly at high H_2O_2 dosage of 1% for both treatment processes. The same pattern was noted in Chapter 2 when hydrogen peroxide increased

from 0.5% to 2.5% in the MW/H_2O_2 process. It is possible that ortho-P release in both processes depends on two effects: first, by partially rupturing cell membranes, as suggested by Kuroda et al. (2002), and secondly, in the low temperature range, by metabolic uptake of the remaining heat resistant microorganisms (Dr. Eric Hall, UBC Civil Engineering, pers. comm.). The final level of ortho-P concentration will likely be the sum of these two effects, plus the negative influence from poly-P formation at 70 to 80°C.

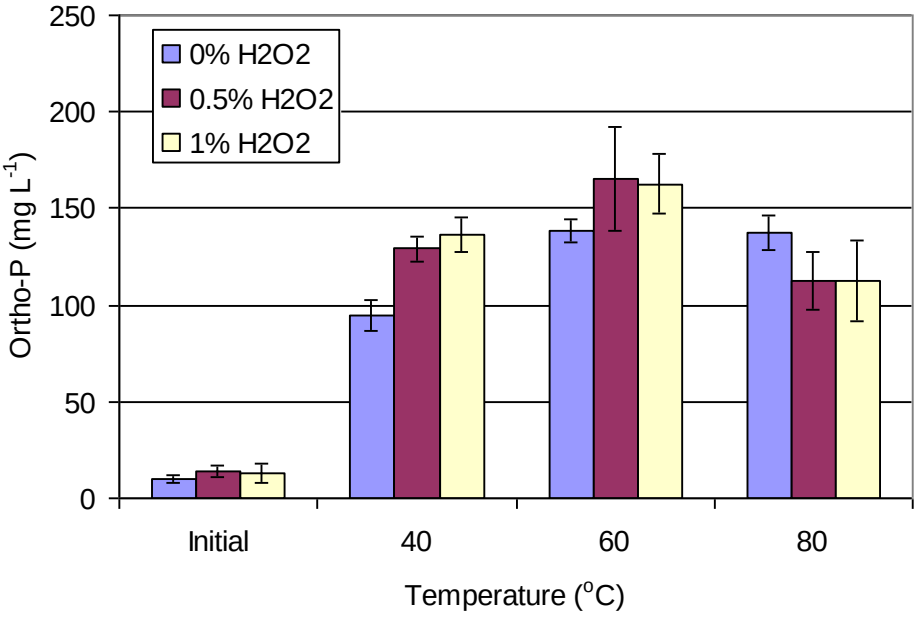
The presence of hydrogen peroxide creates a positive oxidation state. A previous study by Liao et al. (2007) recorded -24mV oxidation-reduction potential (ORP) for microwave heating without hydrogen peroxide, and 252 mV ORP for MW/H_2O_2 with an addition of 1% hydrogen peroxide. This high oxidation state is in fact an aerobic condition, and thus very likely encourages the metabolism and growth of aerobic microorganisms. Under the same conditions, the metabolic rate of aerobic microorganisms is faster than that of its anaerobic counterparts. The ortho-P uptake therefore becomes important. This means that the overall ortho-P in solution would be lower in states of high oxidation. In all previous work (Liao et al. 2006a; Wong et al. 2006a, 2006b) and including Chapter 2 of this work, it can be seen that at temperatures lower than 80°C, hydrogen peroxide limited the ortho-P levels in solution to various extents. When temperature exceeds 100°C, an addition of hydrogen peroxide contributed positively to ortho-P release (Liao et al. 2006a; Wong et al. 2006a, 2006b), since at this point the remaining microorganisms would have been killed or disrupted.

Comparing the two processes under the same conditions, MW/H_2O_2 achieved better overall ortho-P release than did CH/H_2O_2. The same mechanisms that were discussed above can be assumed to be in operation for both MW/H_2O_2 and CH/H_2O_2 treatments.

However, the cell destruction may have been different. Thermal destruction of microorganisms is due to heat denaturation of the membrane, intracellular protein, nucleic acids, enzymes and other vital components (Fellows 2000). Other than the heat effect, microwave irradiation could have achieved bio-destruction through other mechanisms. Food scientists have proposed four theories, with supporting evidence: electroporation, dielectric cell membrane rupture, magnetic field coupling, and selective heating (Kozempel et al. 1998). The results of ortho-P release from MW/H_2O_2 in this study showed that, in addition to the hydrogen peroxide oxidation, some or all of these non-thermal effects could have had an impact on the sludge biomass. At the same time, the results suggest that soluble COD alone is not sufficient to reflect the disintegration efficiency.



(a) MW/H_2O_2



(b) CH/H_2O_2

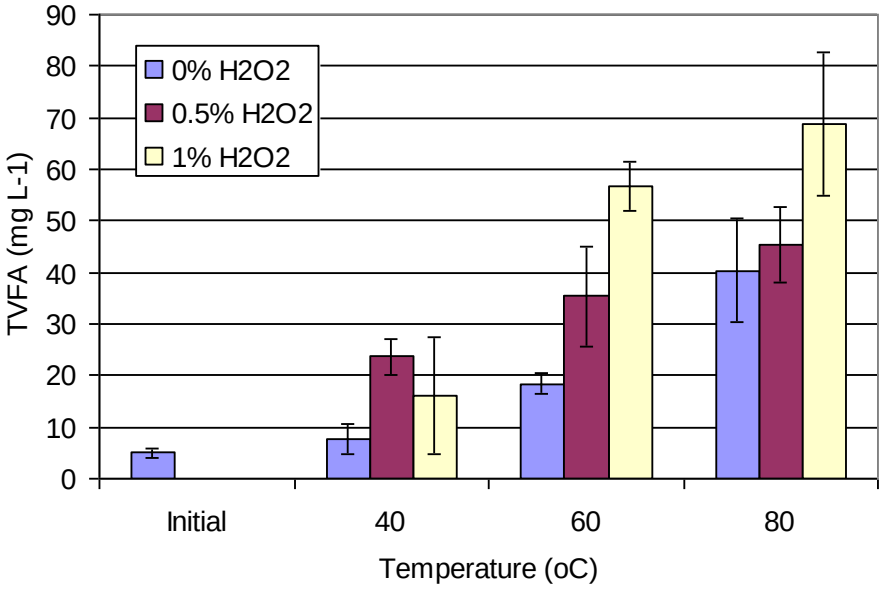
Figure 3.7 Comparison of ortho-P levels of (a) MW/H_2O_2 and (b) CH/H_2O_2

3.3.4 Volatile fatty acids production

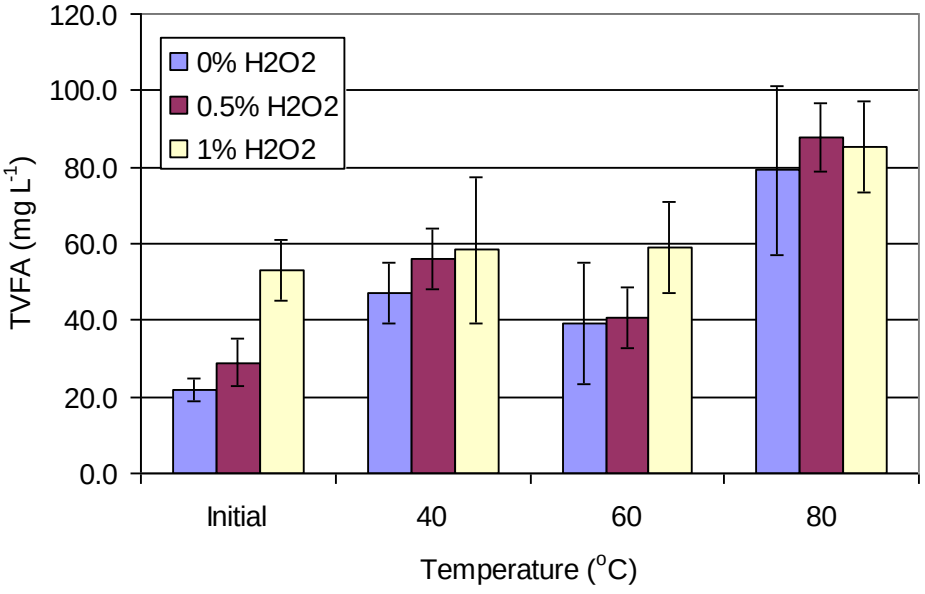
Liao et.al. (2007) reported that MW/H_2O_2 effectively produced VFA (up to 257 mg-$C_2H_4O_2$/L) at a high temperature range 120°C with a peroxide addition of 2.9-5.9 mgH_2O_2/mgTCOD. In Chapter 2, it was reported that VFA production (up to 124 mg-$C_2H_4O_2$/L) was achieved at a lower temperature of 80°C and a lower peroxide addition of 1.13 mgH_2O_2/ mgTCOD, by increasing the solids content (to 3%). In this study, the production of VFAs from the MW/H_2O_2 process was compared to that from the CH/H_2O_2 process. The results are shown in Figure 3.7. The TVFA unit is presented as milligrams of acetic acid per liter (mg-$C_2H_4O_2$/L).

Acetic acid, propionic acid, butyric acid, valeric acid and hexanoic acid were quantified. Acetic acid was more than 85% of TVFA in most cases. It appears that CH/H_2O_2 yield better VFA results than MW/H_2O_2 treatment, but with large deviations. The difference among different conditions will not be statistically reliable due to the large data deviations.

Overall, VFAs from both processes represented less than 2% of SCOD with hydrogen peroxide additions at 0.21 and 0.42 mgH_2O_2/mgTCOD. It was considered that under these conditions, oxidation of soluble COD was a minor side effect of the treatments compared to COD solubilization.



(a) MW/H_2O_2



(b) CH/H_2O_2

Figure 3.8 TVFA productions from (a) MW/H_2O_2 and (b) CH/H_2O_2

3.4 Conclusions

In this chapter, the treatment results and efficiency of two treatment processes, MW/H_2O_2 and CH/H_2O_2, were directly compared under the same experimental conditions. The aim of the research was to identify contributions other than those from thermal heating and hydrogen peroxide oxidation. Effects from process variables, namely temperature, hydrogen peroxide dosage and treatment time were investigated with the assistance of statistical computing software and surface response design.

Results showed that, with an addition of hydrogen peroxide, MW/H_2O_2 and CH/H_2O_2 exhibited slightly different patterns. Similar to the results from a previous study reported in Chapter 2, the MW/H_2O_2 process benefited from a hydrogen peroxide addition, even with the low dosage used in this study (0.2-0.4 mg-H_2O_2/mg-DS). However, the CH/H_2O_2 treatment did not show significant improvement with increased hydrogen peroxide additions over a temperature range of 40-80°C. The treatment time was the second major factor for CH/H_2O_2 rather than the hydrogen peroxide addition. For both treatments, temperature was the most important factor.

The analysis of COD degree of disintegration (and SCOD/TCOD%) showed that MW/H_2O_2 consistently achieved better results than CH/H_2O_2. By isolating the effects from thermal heating and from hydrogen peroxide oxidation, the improvement of MW/H_2O_2 over CH/H_2O_2 could be considered to be contributions from non-thermal effects or from converted hydroxyl radical (.OH).

The differences in Ortho-P release also showed non-thermal effects from MW/H_2O_2 treatment. This could be attributed to different cell membrane rupture mechanisms. However, ortho-P release did not benefit from increases in hydrogen peroxide at

temperatures below 80°C. It was also likely affected by the poly-phosphate formation in temperatures range of 60°C to 80°C. All three effects, from cell membrane rupture, bio metabolism/growth uptake, and poly-phosphate formation, should be considered, in ortho-P release at temperatures below 80°C.

Volatile fatty acid levels remained low (less that 2% of SCOD) throughout the experiments. This suggests that with low amounts of hydrogen peroxide, oxidation of soluble COD is a minor effect compared to COD solubilization or phosphate release.

Chapter 4 Flow Through Operation of Microwave / Hydrogen Peroxide Process and Ultrasound Treatment for Sludge Disintegration *

4.1 Introduction

In the operation of wastewater treatment plants, two types of sludge are produced: primary settling sludge and secondary biological sludge. Both types of sludge can be fed into the anaerobic digestion process for stabilization. During stabilization, volatile organic wastes are reduced and converted to methane biogas for energy recovery. While primary sludge is considered ready for biodegradation, secondary biological sludge is more difficult to treat. Secondary sludge consists mainly of microbial cells or “biomass”. The semi-rigid structure of the microbial cell walls provides the protection against the hydrolysis stress. Hydrolysis is the first step in the anaerobic digestion process, and was identified as the rate-limiting factor (Eastman and Ferguson, 1981; Shimizu et al., 1993; Tiehm et al., 2001). Various pretreatments are therefore been used to improve secondary biological sludge hydrolysis. These include thermal treatments (Hiraoka et al., 1989; Tanaka et al., 1997; Valo et al., 2004; Climent et al., 2007; Bougrier et al., 2007), mechanical treatments (Choi et al., 1997; Baier and Schmidheiny, 1997; Kopp et al., 1997), chemical alkaline treatment (Knezevic et al., 1995; Tanaka et al., 1997; Inagaki et al., 1997; Carballa et al., 2004), ozonation (Weemaes et al., 2000; Battimelli et al., 2003; Goel et a., 2003), ultrasound(Shimizu et al., 1993; Neis et al,

*A version of this chapter will be submitted for publication:

YI, W., Lo, K.V., Liao, P.H., Mavinic, D.S., Forgie, D. and Mohseni M. Flow Through Operation of microwave/ hydrogen peroxide process and ultrasound treatment for sludge disintegration .

2000), microwave irradiation (Park et al., 2004; Liao et a., 2005a; Eskicioglu et al., 2007a, 2007b) and microwave / hydrogen peroxide process (Liao et al., 2005b, 2007; Wong et al., 2006a, 2006b, Eskicioglu et al., 2008).

One hypothesis on microwave hydrogen peroxide process (MW/H_2O_2) is that the microwave irradiation could catalyze the conversion of hydrogen peroxide to hydroxyl radical for further enhancement of sludge solubilization (Liao et al., 2005b, 2007; Wong et al., 2006a, 2006b, Eskicioglu et al., 2008). In research reported in Chapters 2 and 3, as well as in previous studies, hydrogen peroxide has been added and mixed with the sludge before it enters a microwave radiation chamber. Even though the time frame from the peroxide addition to the microwave could be shortened by quick and skillful lab operations, a certain amount of hydrogen peroxide will be consumed by reaction with the sludge substrate and through decomposition by catalase. If the process was carried out in a flow through mode and the hydrogen peroxide injected immediately prior to the entry of the sludge into the microwave chamber, there may provide more hydrogen peroxide available for the catalyze conversion. In addition, the use of a flow through operation would also be both convenient and indicative for the scale-up application of this technology. The research reported here was therefore carried out with a newly designed and constructed flow-through MW/H_2O_2 system. The reason that it was tested under the batch flow-through operation (instead of a single pass continuous flow) was have a better control on the experimental conditions (temperature etc), so that the result could be compared to the previous studies.

In order to provide a reference point, another pretreatment method with ultrasound cavitation, was also investigated and compared with the MW/H_2O_2 process. Both ultrasound and microwave are energy waves. Ultrasound is a mechanical wave that propagates through

media and microwave is electromagnetic radiation capable of travelling through a vacuum. Ultrasound is characterized as cyclic sound pressure with a frequency greater than the upper limits of human hearing, approximately 20 kilohertz (kHz). It has a vast number of applications, including sonography, non-destructive testing, etc. With high power ultrasound, cavitations are produced. The formation and violent collapse of micro-bubbles result in extreme local conditions of value for chemical and biological science processing.

In environmental applications of ultrasound cavitation, three primary mechanisms are at work: the shear force occurring at the solid-liquid interface, localized heating, and free radicals (OH., H., $HO_{2.}$) and hydrogen peroxide formation. It has also been suggested (Tiehm et al., 2001; Wang et al., 2005) that, at low frequency power (20-41 kHz), an ultrasound system performs better in terms of sludge disintegration when a hydrodynamic shear force is the dominant contributor to cell disruption and intercellular substance release. In this study, both batch and flow through ultrasound disintegration were examined. Hydrogen peroxide was also added in the flow through operation.

The addition of an oxidant in ultrasound pretreatment has a double-sided impact. On the one hand, oxidation contributes to COD solubilization. The gas molecules from oxidation would also create a weak-spot in the liquid, thereby reducing the cavitation threshold. The result would be greater cavitation. On the other hand, gas bubbles could also serve as “cushions” when cavitation bubbles collapse. This would reduce the shear force necessary for cell disruption. As in the MW/H_2O_2 pretreatment process, the disintegration product of soluble organic material in ultrasound pretreatment could also be further degraded or oxidized to intermediates such as fatty acids and inorganic end-products. This is a complex process, and only one publication by Gronroos et al. (2005) has reported SCOD results with

an oxidant addition. They recorded no increase in SCOD from a hydrogen peroxide addition used with ultrasound for disintegration. In the present study, a more thorough investigation was conducted, including COD solubilization, phosphate release and volatile fatty acids formation-degradation.

4.2 Material and Methods

4.2.1 Apparatus

An ultrasonic flow cell set (UIP1000 ultrasonic processor, Figure 4.1) from Hielscher Ultrasonics GmbH, Berlin, Germany, was used for both batch testing and flow through operation. It includes a 1,000 watts ultrasonic processor (transducer and generator), two sonotrodes, five boosters, and a stainless steel flow cell. The ultrasonic processor UIP1000 has a frequency of 20 kHz (auto-scan), amplitude 25 micron (adjust. 50-100%), and can be operated under continuous or batch conditions. The amplitude is controlled electronically and mechanically to remain constant under various load conditions. This provides reproducible conditions and continuous operation. The booster set could be used for mechanical increase (or decrease) of the amplitude at the sonotrode.

(a) Batch Testing (b) Continuous flow

Figure 4.1 UIP1000 ultrasonic processor (Hielscher Ultrasonics GmbH, Germany)

The microwave flow through system consists of a modified household 2.2 cu ft countertop microwave oven (Panasonic NN-P994), a silicon flow-through cell in the microwave chamber, a control box with an irradiation leakage detection probe, and a Master-Flex peristaltic pump. Figure 4.2 shows the front view of the flow-through cell in the microwave chamber and the control box.

This microwave oven has a heating power of 1200W. The cavity dimension of the microwave chamber is 18.5×18.5×11 inches. Two connectors were built at the back of the microwave oven. These connect the peristaltic pump to the flow-through cell. The coiled flow-through cell is made of 12 feet of silicon tubing with an inner diameter of ½ inch. For safety considerations in case of irradiation leakage or pressure build-up in the flow through cell, two automatic shut-off mechanisms were applied. In this study, both the microwave and

ultrasound system operated in a “multiple passes” mode to reach the desired treatment conditions. Figure 4.3 illustrates the microwave system and its main components.

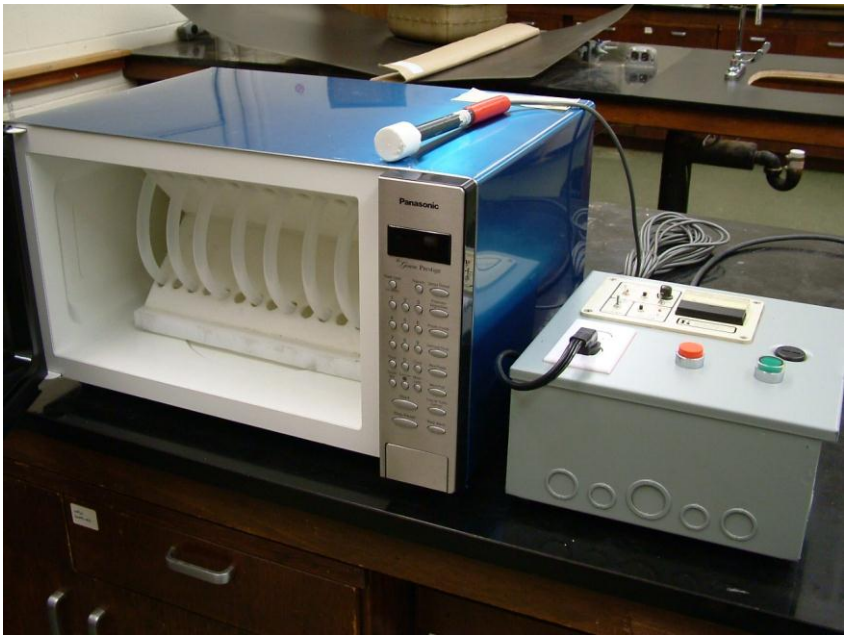


Figure 4.2 Front view of the microwave flow through cell and control box

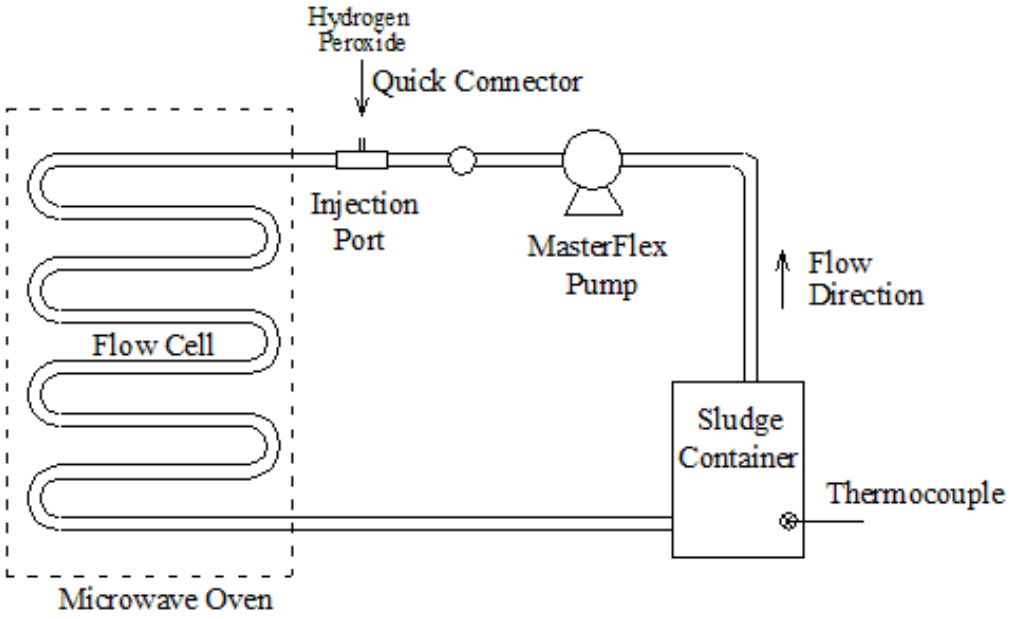


Figure 4.3 Simplified illustration of continuous-flow microwave system

4.2.2 Experimental design

A total of three sets of experiments were conducted for preliminary batch ultrasound treatment, flow through ultrasound with a hydrogen peroxide treatment, and flow through MW/H_2O_2 treatment. The experimental designs are shown in Table 4.1, 4.2, and 4.3, respectively. The Central Composition Design (statistical software JMP-IN ® 5.1, Sall et al., 2005) was used for the experimental designs. Using three distinct values (data points) for each factor, the results were fit to a surface response model in order to find the maximum or minimum response inside the factor region. This also provided a screening function for the process factors.

The factors for the flow through MW/H_2O_2 treatment were input power level, temperature and hydrogen peroxide dosage. In order to compare with the previous batch operation studies (Chapters 2 and 3), the temperature factors for the experiments were set to 40, 60 and 80°C.

The microwave power input was 372W, 678W and 920W, at 40%, 70% and 100% power level settings, respectively. The microwave power determination was done by International Microwave Power Institute (IMPI) 2 Liter-Test (Buffler, 1993).

The hydrogen peroxide dosages for both flow through MW/H_2O_2 treatment and ultrasound/peroxide treatment were 0%, 0.5% and 1% in wet weight, or approximately 0, 0.15 and 0.3 mg-H_2O_2/mg-TCOD.

For ultrasound treatment, the factors were power level, treatment time and hydrogen peroxide dosage. The power inputs were set to 40%, 70% and 100% amplitudes of the sonotrode installed. For batch operation with open beakers (Figure 4.1a), they were recorded at average 179W, 254W and 329W (Table 4.1) with the PC control module. For the flow

througt operation, with a stainless steel flow-through cell (Figure 4.1b), the power levels recorded were 234W, 325W and 416W (Table 4.2).

The treatment times were set to 2, 4 and 6 minutes for batch ultrasound treatment on a 600 mL sludge sample for each experiment. They were 5, 10 and 15 minutes for flow through ultrasound/peroxide treatment on a 2 L sludge sample in order to maintain similar specific energy levels.

Table 4.1 Experiment design for preliminary batch ultrasound treatment

Set	Design Pattern	Power Input (W)	Treatment Time (minutes)
1	- -	179	2
2	a0	179	4
3	- +	179	6
4	0a	254	2
5	00	254	4
6	00	254	4
7	0A	254	6
8	+ -	329	2
9	A0	329	4
10	++	329	6

Table 4.2 Experiment design for flow through ultrasound/peroxide treatment

Run	Design Pattern	Power Input (W)	Treatment Time (minutes)	Hydrogen Peroxide dosage (wt%)
1	- - -	234	5	0
2	- -+	234	5	1
3	a00	234	10	0.5
4	- + -	234	15	0
5	-++	234	15	1
6	0a0	325	5	0.5
7	00a	325	10	0
8	000	325	10	0.5
9	000	325	10	0.5
10	00A	325	10	1
11	0A0	325	15	0.5
12	+ - -	416	5	0
13	+ - +	416	5	1
14	A00	416	10	0.5
15	++ -	416	15	0
16	+++	416	15	1

Table 4.3 Experiment design for flow through microwave/peroxide advance oxidation process

Run	Design Pattern	Power Input (W)	Temperature (°C)	Hydrogen Peroxide dosage (wt%)
1	- - -	372	40	0
2	- -+	372	40	1
3	a00	372	60	0.5
4	- + -	372	80	0
5	-++	372	80	1
6	0a0	678	40	0.5
7	00a	678	60	0
8	000	678	60	0.5
9	000	678	60	0.5
10	00A	678	60	1
11	0A0	678	80	0.5
12	+ - -	920	40	0
13	+ - +	920	40	1
14	A00	920	60	0.5
15	++ -	920	80	0
16	+++	920	80	1

4.2.3 Sludge treatment processing and sampling

Secondary biological sludge was obtained from Metro Vancouver's Lulu Island Wastewater Treatment Plant, located at the south end of the City of Richmond, BC. Sludge samples were collected weekly and stored at 4 ºC in a refrigerator. Table 4.4 defines the characteristics of this secondary sludge (thickened waste activated sludge, or TWAS).

For preliminary batch ultrasound treatment, a sludge sample of 600 mL was sonicated directly with the sonotrode submerged 2-3 cm below the sludge liquid line. Power input, energy consumed and temperature profiles were recorded with the PC control module.

For both flow through ultrasound/peroxide treatment and MW/H_2O_2 treatment, sludge samples of 2L from the holding tank were pumped into the flow-through cell and re-circulated with multiple passes in order to reach the treatment time in the case of the ultrasound/peroxide treatment, and treatment temperature for the MW/H_2O_2 process. Hydrogen peroxide was injected into the sludge line before it entered the flow-through cell. The injection was flow-paced by a peristaltic pump. Sludge in the holding tank was constantly mixed to maintain uniform conditions. The temperature profile in the holding tank was recorded with a thermocouple connected to the PC control. In all experiments, duplicate runs and three replicate samples were taken.

After each experiment, treated sludge samples were spun in a high-speed centrifuge at 15,000 rpm for 10 minutes. The resulting supernatants were filtered through Whatman No.4 filters and analyzed for soluble fraction of the COD, volatile fatty acids and phosphate.

Total solids (TS), Volatile solids (VS), Chemical oxygen demand (COD) and phosphorus (orthophosphate, PO_4-P) were determined according to the Standard Methods (APHA, 1995). A Hewlett Packard 5890 Series II gas chromatograph, equipped with a flame

ionization detector (FID), was used to measure volatile fatty acids (VFA). Volatile separation was accomplished with an HP FFAP column (0.25 m × 0.31 mm with 0.52 μ film thickness). The injection temperature was set at 175 ºC and the FID detector was at 250 ºC.

Table 4.4 Characteristics of thickened secondary sludge used in this study

Parameters	*Concentration*
pH	6.5 ± 0.2
TS (%)	4.2 ± 0.1
Total COD (mg/L)	41,200 ± 1670
Total Phosphorus (mg P/L)	1050 ± 90
Initial soluble COD (mg/L)	3090 ± 125
Initial soluble PO_4 (mg P/L)	43 ± 9
Initial TVFA (mg-$C_2H_4O_2$/L)	450 ± 30

4.3 Results and Discussion

4.3.1 Preliminary batch ultrasound treatment

4.3.1.1 SCOD, degree of disintegration and temperature effect

The SCOD results are shown in Figure 4.4 (a), plotted against specific energy (kJ/kg-DS, dried solids). Despite three levels of power input, it showed that the main function for SCOD or COD solubilizaiton was the energy consumption. In Figure 4.4 (b) for Degree of Disintegration (DD_{COD}, described in Chapter 3, Section 3.2.4) and SCOD/TCOD%, all data points were assembled in a single series and the 2 level polynomial trendline from this series showed a reasonable fit (R^2=0.96, 0.95) with minor variations. The variations were mostly from different power inputs.

At the same specific energy level, a higher power input with short treatment time appeared to produce slightly better results than with lower power input and long treatment time (Figure 4.4a). It is consistent with, but less obvious than the report from Eder and Gunthert (2002), or Gronroos et al. (2005). A possible reason could be that the power input (or density, W/L) increments in the present study was less than that from the above mentioned research. In ultrasound assisted chemistry (sonochemistry), the rates of methyl ethanoate hydrolysis (Couppis and Klinzing, 1974) or iodine yield from sonolysis of aqueous KI (Henglein, 1993) were also found to be directly proportional to the power intensity of ultrasound input, until a limiting value was reached. Beyond this value, the cavitation energy was severely dampened by the excess bubbles' "cushioning" effect. Different systems with various ultrasound equipment and subject media, had different limiting values on power input (Henglein, 1993).

These variations in equipments and subject media would have also resulted in the

differences on Degree of Disintegration (DD_{COD}) between different researches. In the present study, the DD_{COD} was found to be between 10 to 38%, when the specific energy ranged from approximately 1000 to 5000 kJ/kg-DS (Figure 4.4 b). Similar DD_{COD} were reported in Neis et al. (2000) and Tiehm et al., (2001), with higher specific energy consumed. The SCOD/TCOD% in the present study is also reported in Figure 4.3 (b), alongside DD_{COD} for a reference. It ranged from 14% to 32%.

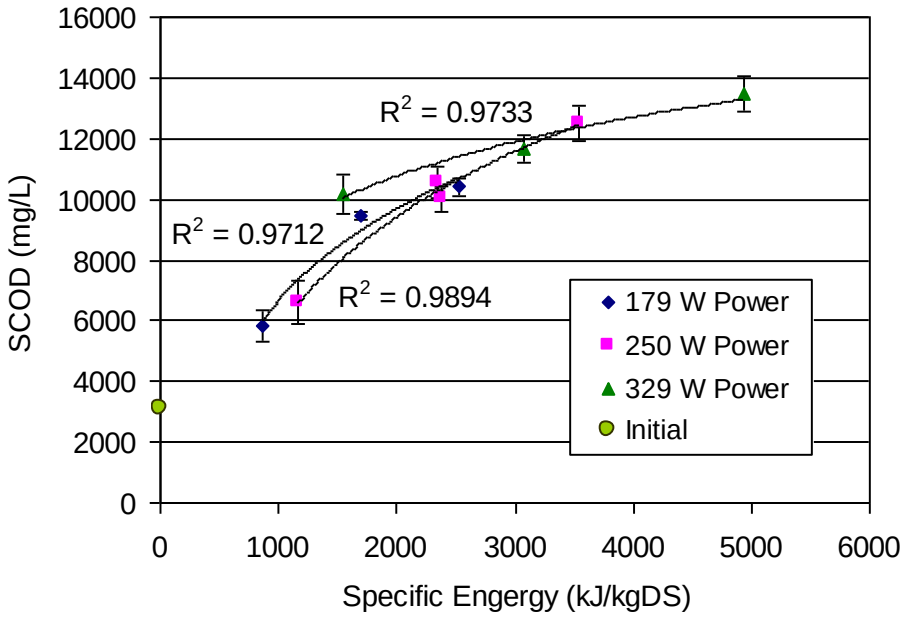
In this study, it appears that the increase of SCOD or DD_{COD} slowed down at a certain level, where any further increase in specific energy over 4000 kJ/kg-DS could no longer facilitate substantial COD solublization. Two likely sources might have contributed to this limitation. For one, the temperature could have played a role. At a specific energy of 5000 kJ/kg-DS, the temperature was raised to 55°C (Figure 4.5 a). At higher bulk temperatures, ultrasound cavitation was thought to have less drastic effect (lower local temperature and pressure when bubbles collapse), due to the decrease in liquid surface tension and viscosity (Mason and Lorimer, 2002). The other possible explanation, as Khanal et al. (2007) put it, was the exhaustion of readily disintegrable substrates, or the exhaustion of dissolved gas that aids cavitation bubble formation.

The temperature profile versus specific energy is shown in Figure 4.5 (a) and the comparison of SCOD increase at the same thermal condition is shown in Figure 4.5 (b). The temperature profile indicated that the bulk temperature raise was a strict function of specific energy, regardless of the differences in power levels. The thermal effect of ultrasound cavitation was the cumulative result of the local heating through the collapse of cavitation bubbles. At higher temperature, heat loss started to become important, while, at the same time, the cavitation effect was reduced. Therefore, it is advantageous to operate ultrasound

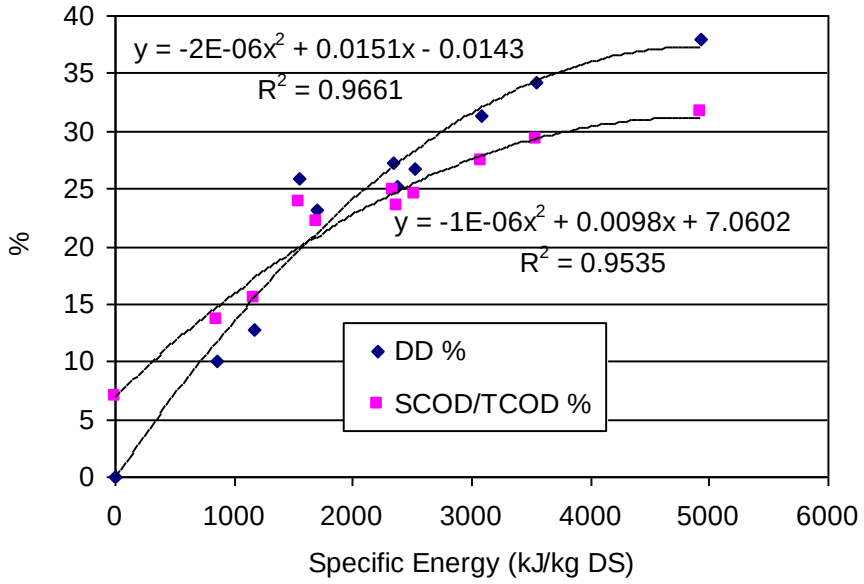
treatment at a relatively low temperature range (below 55 ºC in this study).

However, to consider using ultrasound-pretreated sludge for anaerobic digestion purposes, the thermal effect is beneficial for supplementing a portion of the heating requirement for sludge to reach 35 ºC for mesophilic or 55 ºC for thermophilic digestion. In addition, thermal denaturing of biomass is also part of sludge treatment. The contribution of thermal effect on SCOD increase was approximately 69 and 53% in this study at 40 ºC and 55 ºC, respectively (Figure 4.5 (b)). The rest of the SCOD increase could be attributed to the non-thermal effect of ultrasonication. Therefore, it may be logical to control temperature at low levels for academic research in cavitation. But it will be more practical in field applications to allow the temperature to rise to 55 ºC. Further increase in temperatures would, however, have an adverse effect on cavitation, hence affecting the energy costs.

The effects screening of operating variables, power input and treatment time, on SCOD are shown in Figure 4.6 (a) (b) from the statistic program analysis (JMP-IN ® 5.1). It suggested that both power and treatment time had significant influence on SCOD, with treatment time ranked first. In Figure 4.6 (c), the summary of the prediction model fit indicated that the standard least squares model fits well with the actual data (R^2=0.96, significant probability P=0.0035). The Surface profiler in Figure 4.6 (d) showed a 3-dimentional plot of surface response of SCOD versus power inputs and treatment time.

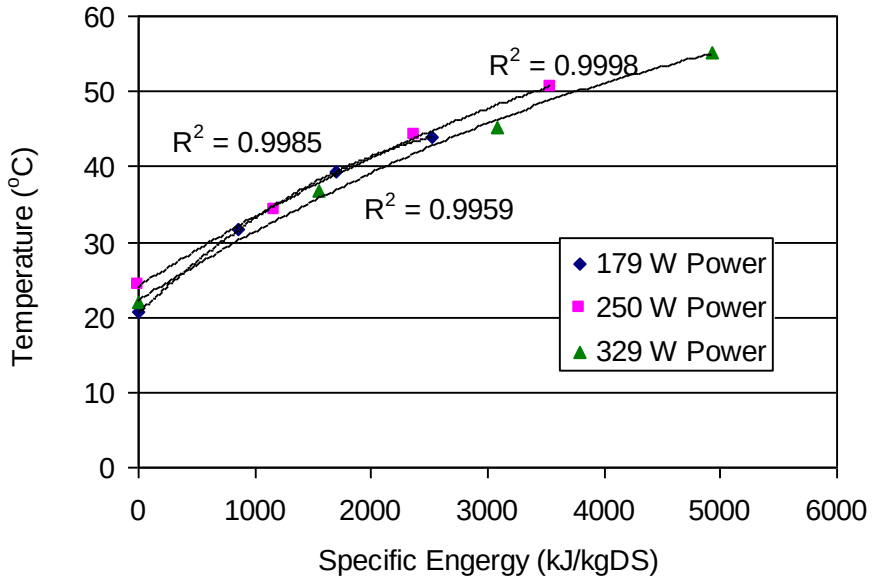


(a)

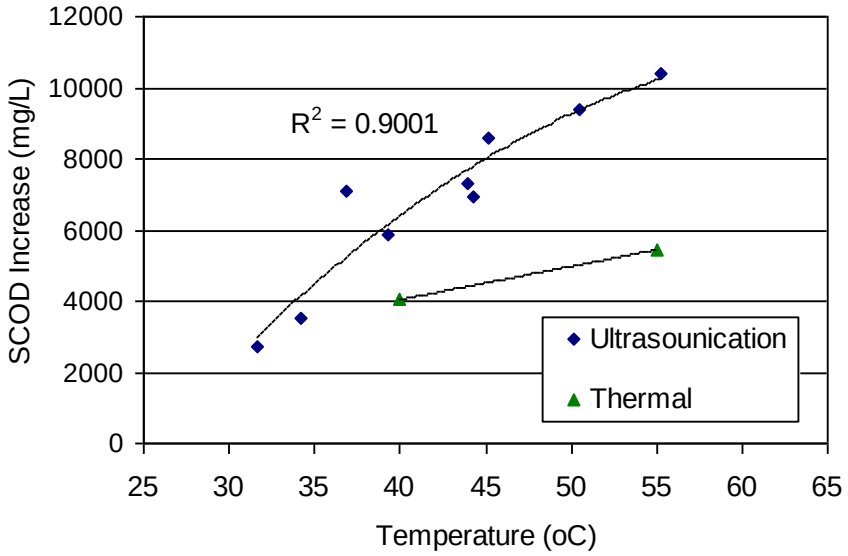


(b)

Figure 4.4 Batch ultrasound treatment (a) SCOD levels and (b) degree of disintegration

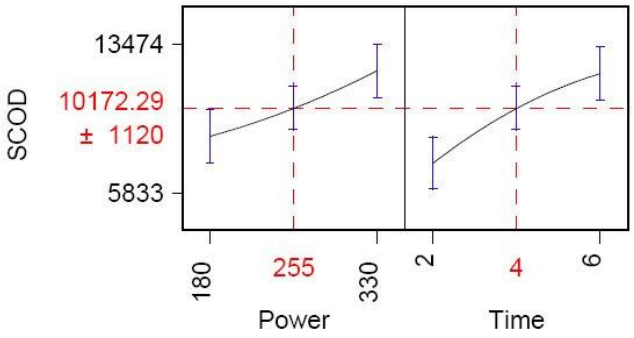


(a)

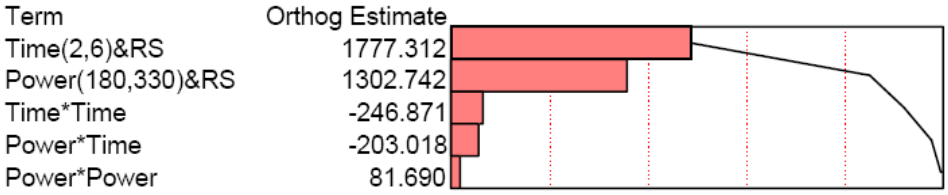


(b)

Figure 4.5 Batch ultrasound treatment (a) temperature profile and (b) SCOD increase at the same thermal condition



(a)



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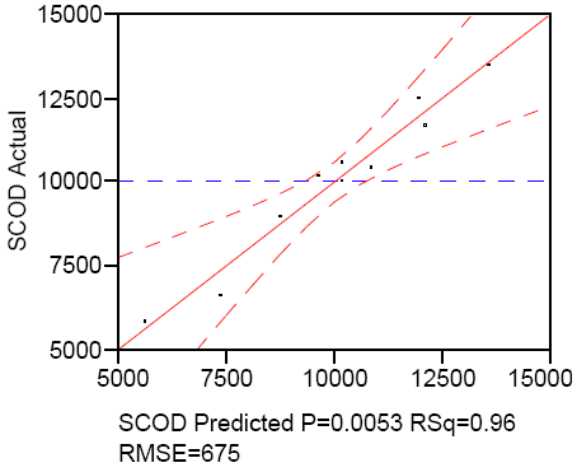
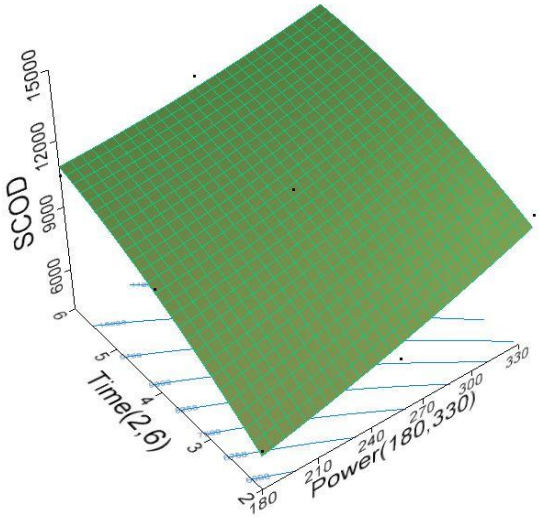


(c)



(d)

Figure 4.6 Prediction profiler (a), Pareto plot of scale estimates (b), prediction model summary of fit (c) and surface profiler (d) on SCOD levels for batch ultrasound treatment

4.3.1.2 Orthophosphate

By far the most commonly applied parameter in ultrasound sludge disintegration research has been Soluble COD. However, as demonstrated in Chapter 3, SCOD alone may not be sufficient to reflect overall disintegration efficiency. Phosphate release is another parameter that could be used to supplement the SCOD data.

Poly-phosphate stored within the biomass could be efficiently released and subsequently hydrolyzed to orthophosphate by thermal treatment (Chapter 3; Kuroda et al., 2002), microwave (Liao et al., 2005) or MW/H_2O_2 (Chapter 2; Chapter 3; Liao et al. 2006a; Wong et al. 2006a, 2006b). Even though numerous publications have reported ultrasound pretreatment studies, none have adopted phosphate release as an indicator. The potential of phosphorus recovery adds to the importance of examining the phosphate release aspect of sludge pretreatment methods.

The results for soluble orthophosphate (ortho-P) versus specific energy are shown in Figure 4.7. The ortho-P increased from an initial 43 mg-P/L to 173 mg-P/L at approximately 5000 kJ/kg-DS. It is interesting to note that, at the same specific energy, less ortho-P was obtained with higher ultrasound power. Figure 4.7 shows that ortho-P data and trendline of higher power are below those from lower power. This is contradictory to the SCOD results, which demonstrated a high power input with a short treatment time for better COD solubilization. In ortho-P release, longer treatment time appeared to be more advantageous. This could be due to the time needed for poly-phosphate hydrolysis. It is believed that the ultrasound cavitation effect also facilitated poly-phosphate hydrolysis after their release from biomass intracellular storage. In sonochemistry research, hydrodynamic shear of cavitation bubble implosion has generally been used for polymer degradation, such as polystyrene in

benzene or toluene, aqueous polyacrylic acid, etc (Mason and Lorimer, 2002).

These results suggest that treatment time was probably the determining factor in ortho-P release, once a sufficient level of energy (2000-4000 kJ/kg-DS in this system) was provided for cell rupturing. The statistical software analysis (shown in Figure 4.8) confirmed that treatment time was the main factor. In comparison, power input was a less influential factor in ortho-P release.

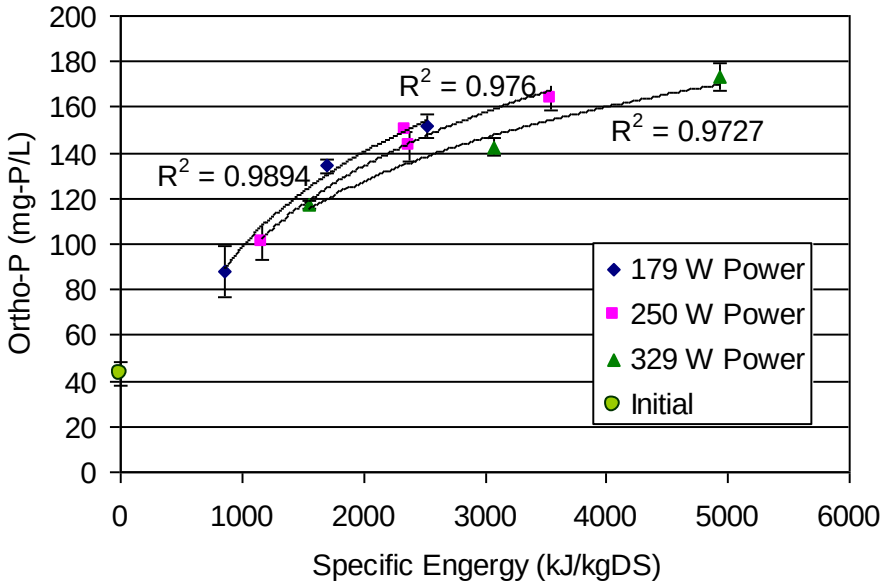
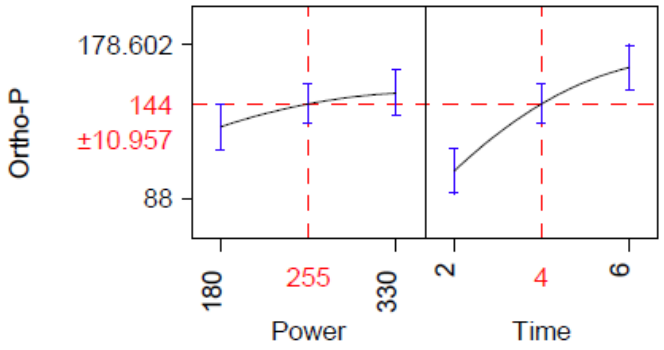
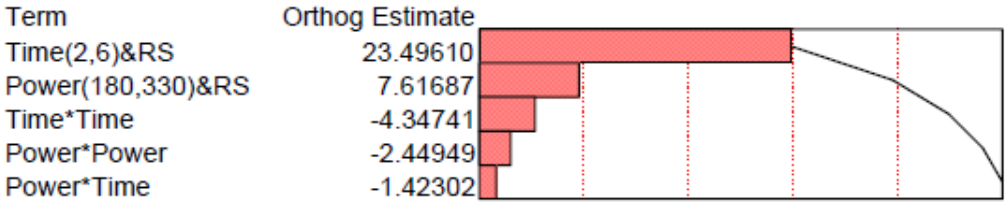


Figure 4.7 Orthophosphate release from batch ultrasound treatment



(a)



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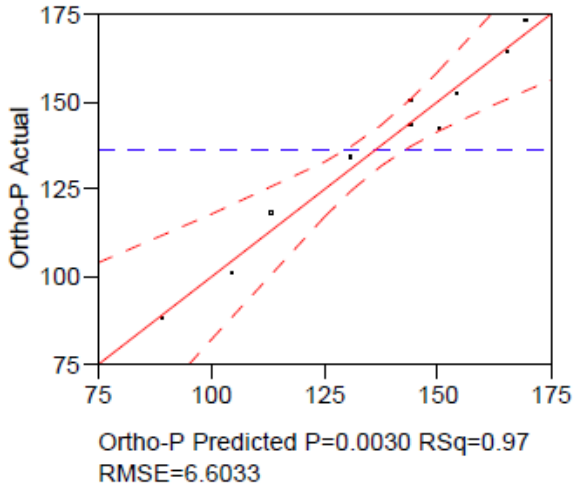

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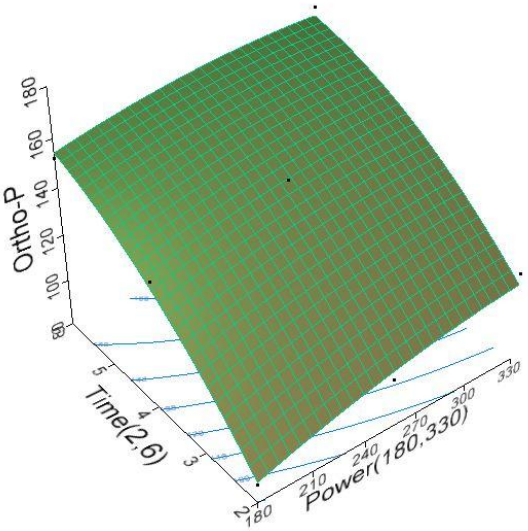


(d)

Figure 4.8 Prediction profiler (a), Pareto plot of scale estimates (b), prediction model summary of fit (c) and surface profiler (d) on ortho-P release for batch ultrasound treatment

4.3.1.3 Volatile fatty acids

A number of volatile fatty acids were quantified in this study. They were acetic acid (CH_3COOH, or $C_2H_4O_2$), propionic acid (CH_3CH_2COOH), n-butyric acid ($CH_3(CH_2)_2COOH$), i-butyric acid ($(CH_3)_2CHCOOH$), n-valeric acid ($CH_3(CH_2)_3COOH$), i-valeric acid ($(CH_3)_2CHCH_2COOH$), and hexanoic acid ($CH_3(CH_2)_4COOH$). Figure 4.9 (a) shows the total VFAs (TVFA) expressed in milligrams of acetic acid per liter (mg-$C_2H_4O_2$/L) plotted against specific energy (kJ/kg-DS). The percentages of TVFA versus SCOD are shown in Figure 4.9 (b). The individual VFA numbers from all batch ultrasound treatments are shown in the bar chart in Figure 4.10.

The untreated sludge samples collected from the Lulu Island treatment plant were high in VFAs, with average of 740 mg-$C_2H_4O_2$/L at approximately 24% of the initial SCOD (3029 mg/L). After ultrasound treatment, most samples showed mild increases in total VFAs, mainly from the increase in acetic acid (Figure 4.10). However, when compared to the increase in SCOD, the VFA yield was minor. The percentage TVFA/SCOD dropped from 24% to a range of 5 to 15% (Figure 4.9 (b)), mainly due to the large increase in SCOD, from 3029 mg/L to as much as 13474 mg/L.

With respect to each individual volatile fatty acid, it was found that acetic acid increased with ultrasound treatment, while butyric acid (n- and i-butyric) and valeric acid (n- and i-valeric) decreased (Figure 4.10). The increase in acetic acid could be attributed to the degradation of butyric and valeric acids as well as from other medium or long chain fatty acids in SCOD. These degradations of butyric, valeric and propionic acid have been reported with ultrasound irradiation time at 200 kHz frequency (Yoo et al., 1997). The order of degradation rate was as follows: propionic acid < n-butyric acid < n-valeric acid.

In Yoo et al. (1997), the VFAs used were pure chemical acids and there was only one level of power input at 200 kHz frequency. The results from this present study confirmed a similar finding, but employed actual sludge samples and various power input (or power density) at 20 kHz frequency.

Abundant in the untreated sludge, all VFAs (including acetic and propionic acid) were at first reduced with a high power input (329W and 2 minutes irradiation, Figure 4.9a, Figure 4.10). With continuing ultrasound treatment (329W and 4-6 minutes), the acetic and propionic acid levels started to recover and eventually increased to more than their initial levels. Butyric and valeric acid recovered slightly and remained at approximately 30% and 50% of their initial levels, respectively.

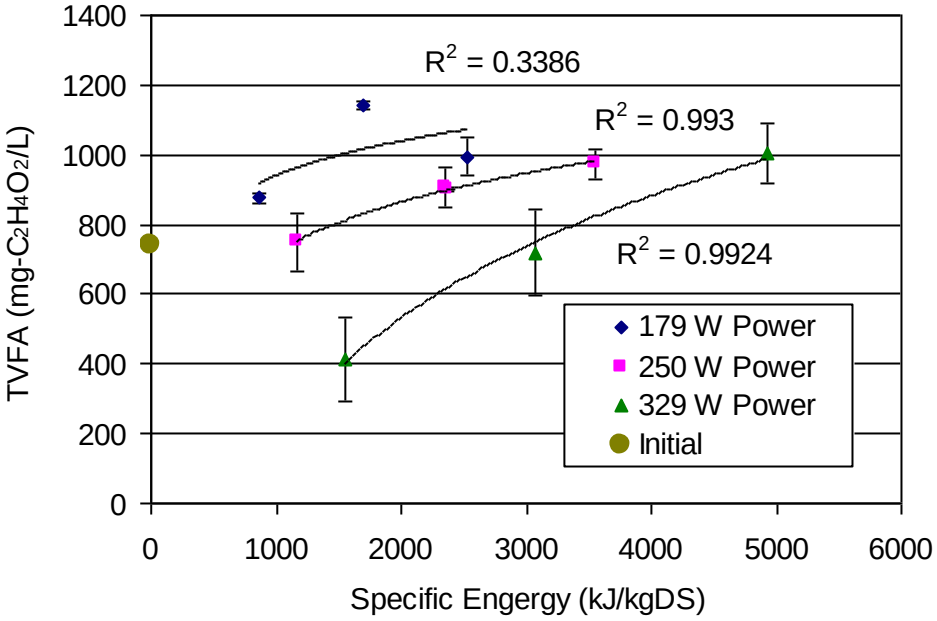
Because of the changes in acetic, propionic acids, and the relatively stable increase in SCOD (Figure 4.4(a)), the percentage TVFA to SCOD plot showed an increasing trend for the high power experiment set (Figure 4.9(b)).

At a lower power input of 179W, the degradation of acetic and propionic acids appears to be overshadowed by the degradation of other longer chain fatty acids (butyric, valeric acid, etc.). This resulted in VFA increase from the beginning. The results for the degradation of butyric and valeric acid were found to be similar to those at a high power input.

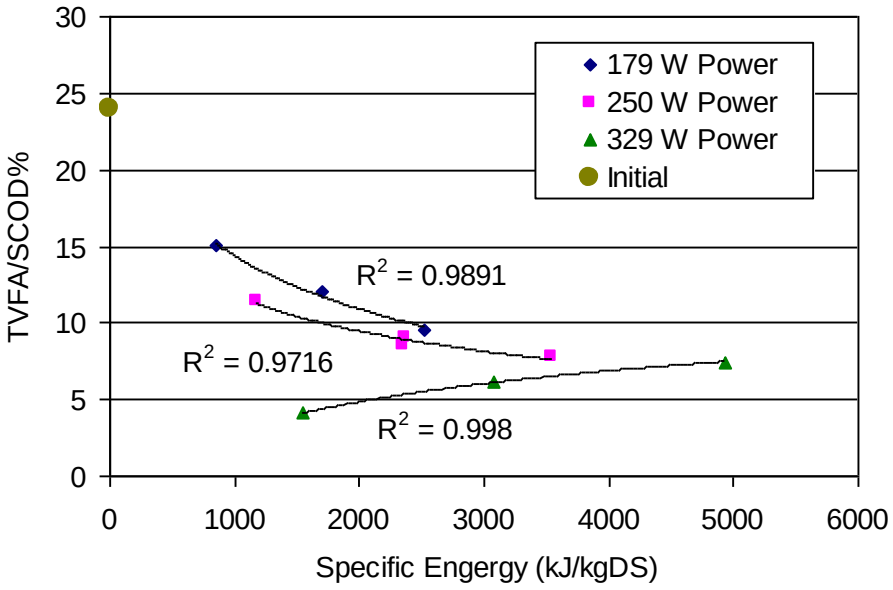
For both 179 W and 250 W power experiment sets, the percentage TVFA to SCOD trends appear to be gradually decreasing (Figure 4.9(b)). In fact, both TVFA and SCOD are increasing, only that the rates of their increase are different. The rates of SCOD increase are higher than that of the TVFA, thus a decreasing trend in percentage TVFA to SCOD.

Hexanoic acid, with a initial level of 0.8 mg/L, increased to a range of 13 to 18 mg/L, with slightly higher concentrations at high power inputs.

The VFA results indicate that ultrasound cavitation impacted the whole spectrum of sludge organic compounds, from large particulates to the short chain VFAs (even acetic acid). The changes in each individual VFA were determined by the degradation of its own and from longer chain VFAs. The rate of change was significantly affected by the power input (or power density). Longer chain fatty acids appeared to be more susceptible to ultrasound cavitation, degrading towards short chain ones. The difference was obvious with low power input. Here cavitation formation might not have been sufficient for degradation of the short chain fatty acids. On the other hand, when high power input was provided, the short chain VFAs, such as acetic and propionic acid, also underwent a different degree of degradation. With a longer treatment duration, all VFAs, but especially the shortest chain acetic and propionic acids, started to accumulate.



(a)



(b)

Figure 4.9 Total volatile fatty acids from batch ultrasound treatment

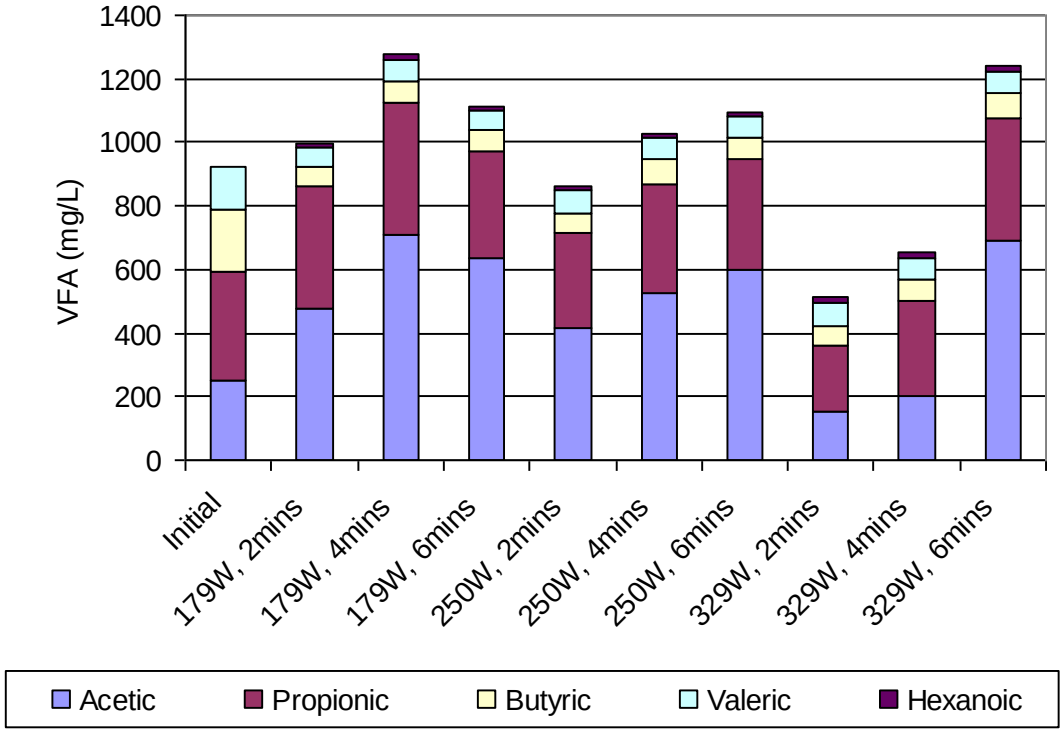


Figure 4.10 Individual volatile fatty acids from batch ultrasound treatment

4.3.2 Flow through ultrasound/peroxide treatment

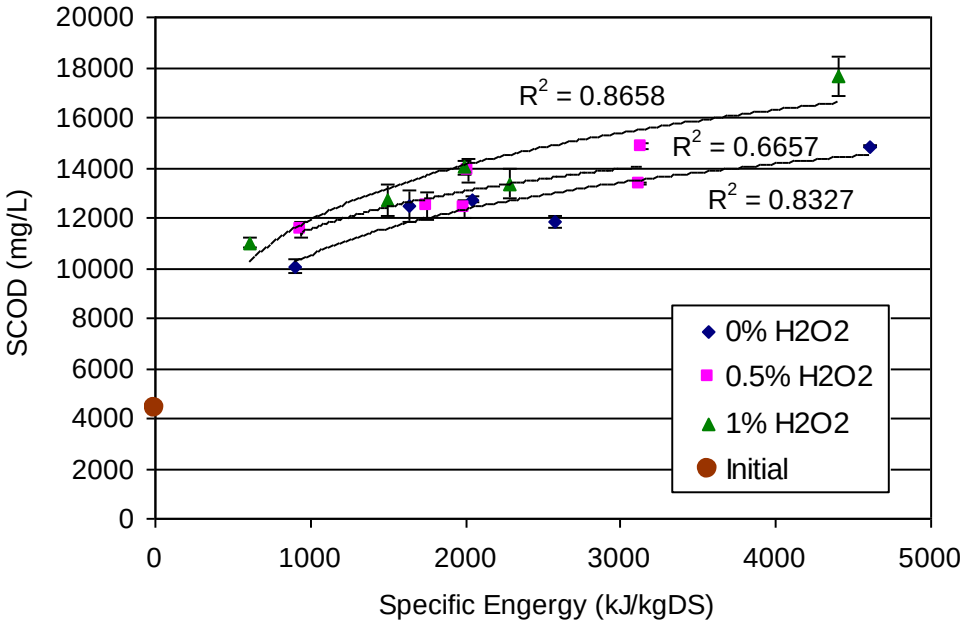
4.3.2.1 SCOD, degree of disintegration

Results for SCOD of the flow through ultrasound/peroxide treatment and the comparison of DD_{COD} to the previous batch ultrasound experiments are shown in Figure 4.11 (a) and (b), respectively. In Figure 4.11 (a), the results showed that the addition of 0.5 and 1% hydrogen peroxide (0.15 and 0.3 mg-H_2O_2/mg-TCOD) increased SCOD levels. At the same specific energy input, the SCOD data and trendline with a hydrogen peroxide addition were approximately 3-27% higher than those without the hydrogen peroxide. This indicated that the oxidation benefit of hydrogen peroxide out-weighed the negative "cushioning" effect from oxidation bubbles. The difference between this finding and the findings of Gronroos et al. (2005) in which no increase was observed was likely due to the amount of hydrogen peroxide added ("25-70 kg/t-DS" in their study). It was also noted in Gronroos et al. (2005) that "perhaps the wrong oxidizing agent dosage might be the reason for a minor effect on oxidizing results". The sufficient amount of hydrogen peroxide introduced through the flow through operation accounted for both the positive contribution of oxidation on COD solubilization, and the negative "cushioning" effect on weakening hydrodynamic shear forces. The results suggest that sludge disintegration, in terms of COD solubilization, improved with the addition of hydrogen peroxide.

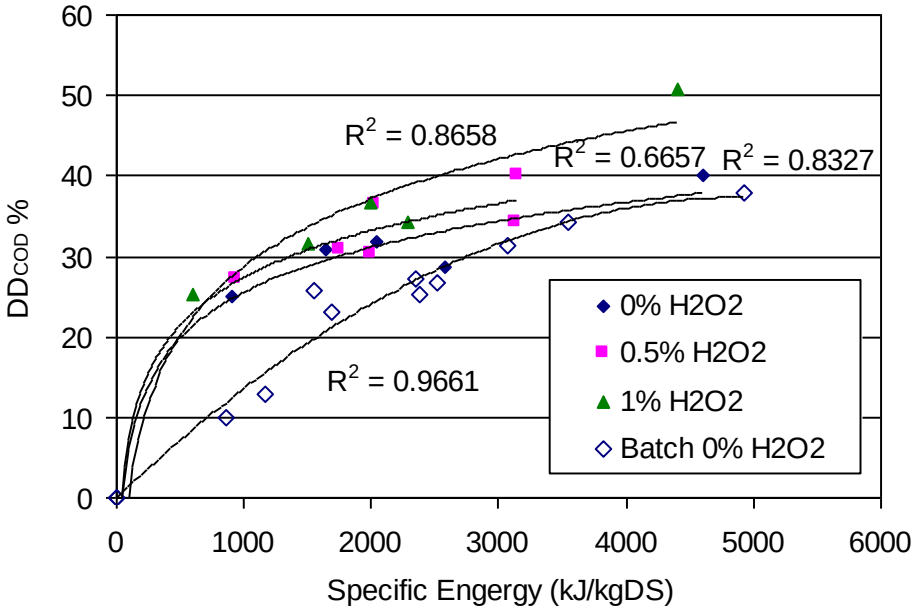
It is interesting to observe the difference in DD_{COD} when comparing the flow through ultrasound/peroxide treatment to the pervious batch ultrasound experiment. With no addition of hydrogen peroxide, the flow through treatment achieved better DD_{COD} under lower specific energy conditions. When specific energy increased to over 4000 kJ/kg-DS, the results from both sets were at the same level.

The difference under low specific energy conditions demonstrates that power density (W/L) also has a significant impact on sludge disintegration. With a flow through operation, the sludge was pumped upward through the stainless steel flow cell (Figure 4.1 b) which had a volume of approximately 250 mL. This means that at any point in time during the flow through treatment, the actual power density (W/L) was 2.4 times that from the batch experiments (with 600 mL of working sludge sample in an open beaker). Even though the power input (W) or overall specific energy (kJ/kg-DS) stayed the same, the power density is higher for the flow through operation. The sludge subjected to higher power density ultrasound treatment yielded better disintegration results until a certain maximum condition was reached, which in this case was 4000 kJ/kg-DS. The economic implication of this is that by using an ultrasound probe system (high power intensity W/cm^2) and a smaller flow cell (high power density W/L), the ultrasound sludge disintegration can be optimized in terms of specific energy costs.

The statistical analysis and prediction model for this set of flow through ultrasound/peroxide treatments are shown in Figure 4.12. The model (R^2=0.94, P=0.0057) confirmed that all three factors, power input, time and H_2O_2 addition, contributed positively to COD solubilization, where time and power input are the two most significant factors.

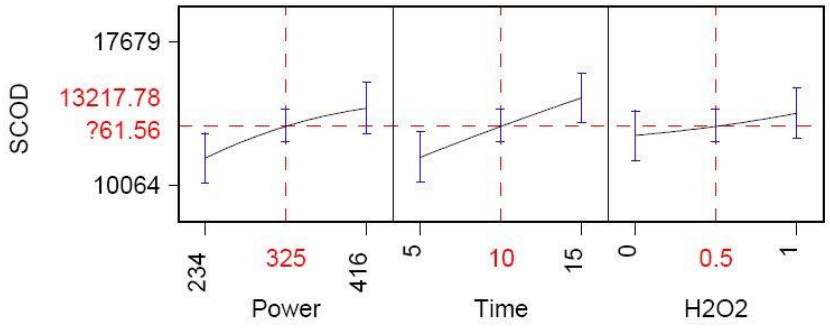


(a)

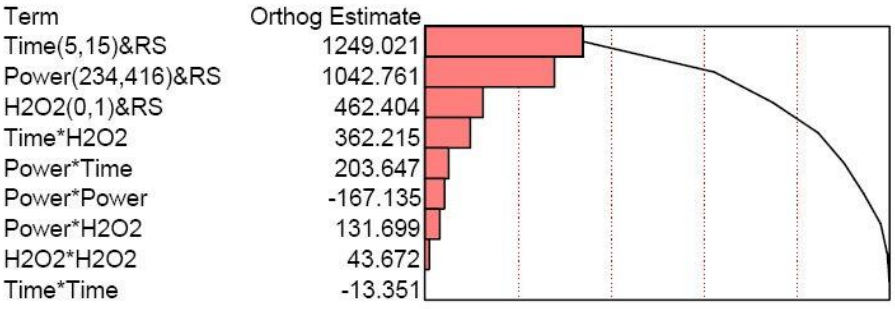


(b)

Figure 4.11 Flow through ultrasound/peroxide treatment (a) SCOD levels and (b) degree of disintegration



(a)



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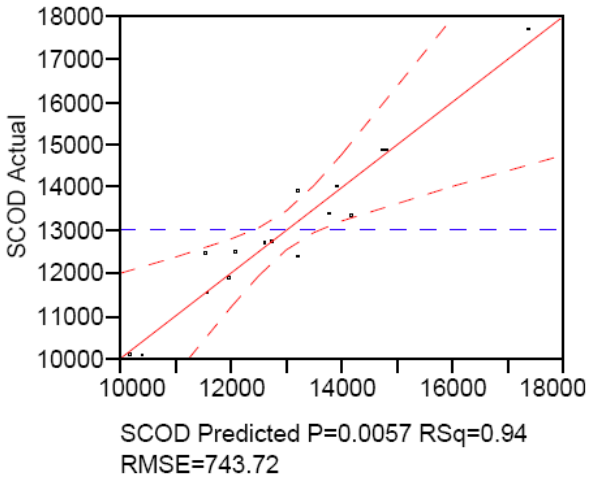

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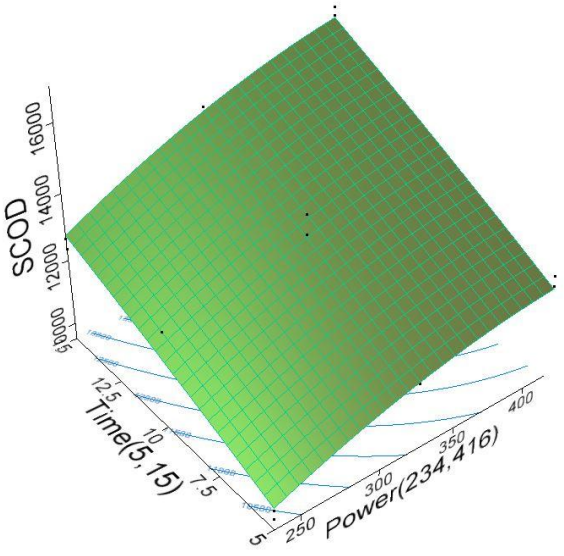


(d)

Figure 4.12 Prediction profiler (a), Pareto plot of scale estimates (b), prediction model summary of fit (c) and surface profiler (d) on SCOD levels for flow through ultrasound/peroxide treatment

4.3.2.2 Orthophosphate

The orthophosphate release results for the flow through ultrasound/peroxide treatments are shown in Figure 4.13. At each level of H_2O_2 dosage, the ortho-P concentration increased with higher specific energy. However, an increase of the H_2O_2 dosage from zero to 1% appeared to have lowered the ortho-P results. The ortho-P concentration ranged from 140 to 220 mg/L without any H_2O_2 dosage, and from 100 to 160 mg/L with a 1% H_2O_2 dosage. The oxidation-reduction potential (ORP) recorded average was -62 mV for a zero H_2O_2 addition and 280 mV for the 1% H_2O_2 addition experiment. The bulk sludge temperatures for all experiments were below 50°C. It is likely that the obstacle to ortho-P release in this ultrasound/peroxide system was the high oxidation state created by hydrogen peroxide. At a relatively low temperature range, cell metabolism and growth is likely still active. As previously suggested (Chapter 3 and section 4.3.1.2), cell membrane rupture, poly-P hydrolysis, and the metabolic uptake of ortho-P (under low temperature conditions),can all have an impact on the overall ortho-P in solution.

All factors, power input, specific energy, treatment time and the addition of hydrogen peroxide, played an part in ortho-P release. Table 4.5 shows a direct comparison of the ortho-P results of these factors. Figure 4.14 presents the statistical analysis and prediction model for ortho-P release in this system. Overall, treatment time was the most important factor. With all other factors held constant, increasing the treatment time will expose the sludge to a longer period of poly-P hydrolysis. The addition of hydrogen peroxide was found to be the second factor, with negative contributions (discussed above). Power input was the third most important factor. Higher power input (W), also expressed as power intensity (W/cm^2) or power density (W/L), benefits ortho-P release by increasing cavitation intensity.

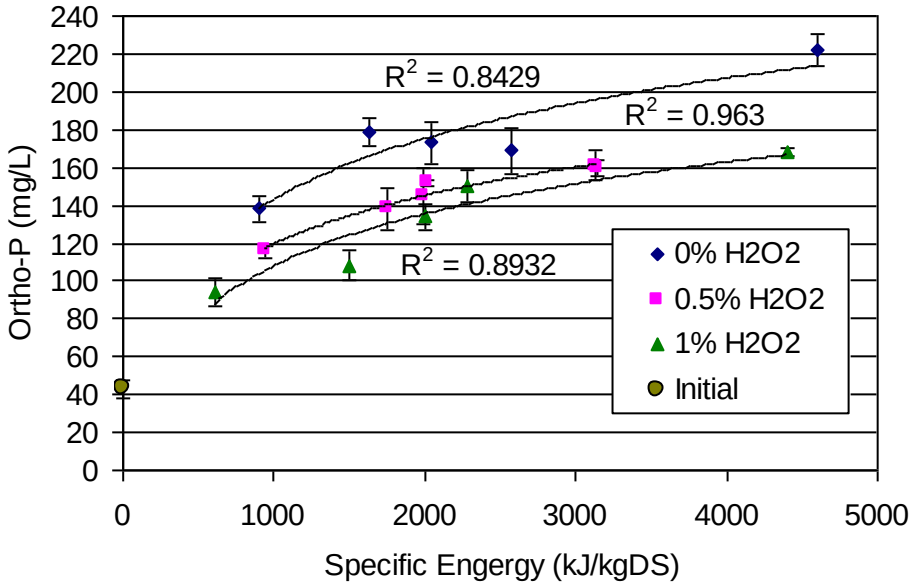
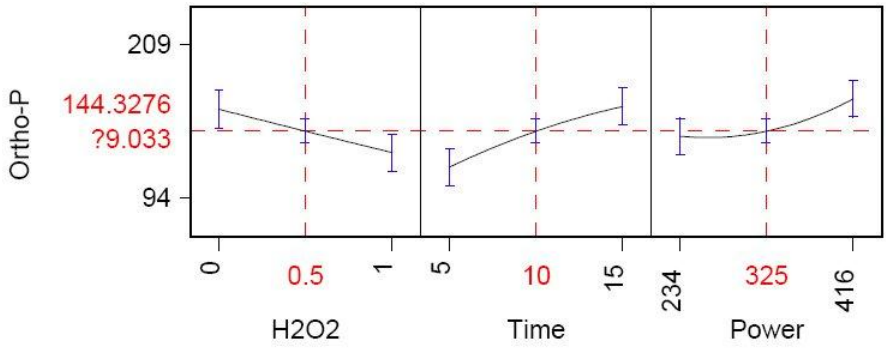


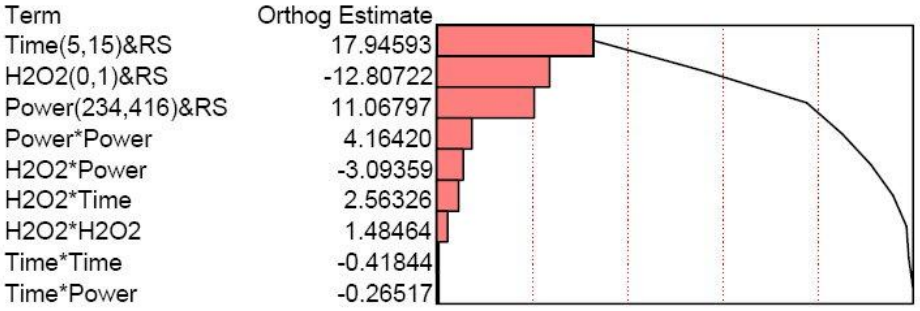
Figure 4.13 Orthophosphate release from flow through ultrasound/peroxide treatment

Table 4.5 Comparison of ortho-P release under multiple factor conditions

Design Pattern	H_2O_2 (%)	Treatment Time (minutes)	Designated Power (W)	Actual Power (W)	Energy Consumed (kJ)	Specific Energy (kJ/kg-DS)	P Average (mg/L)	Standard deviation (mg/L)
- - -	0	5	234	246	76.75	901	138	7
- - +	0	5	416	437	139.5	1637	179	7
- + -	0	15	234	232	219.55	2577	169	12
- + +	0	15	416	401	392	4601	222	8
+ - -	1	5	234	231	51.5	604	94	8
+ - +	1	5	416	418	127.8	1500	108	8
+ + -	1	15	234	233	194.9	2288	150	4
+ + +	1	15	416	399	375	4401	168	2



(a)



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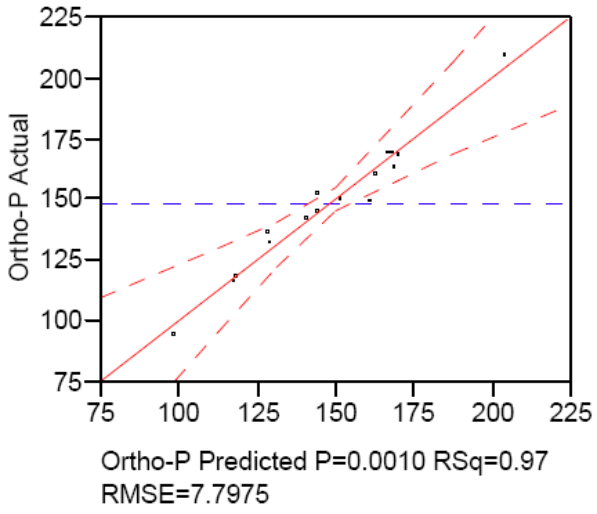

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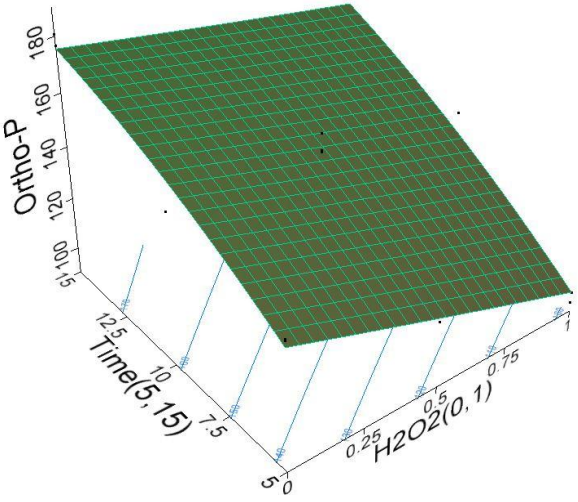


(d)

Figure 4.14 Prediction profiler (a), Pareto plot of scale estimates (b), prediction model summary of fit (c) and surface profiler (d) on ortho-P release for flow through ultrasound/peroxide treatment

4.3.2.3 Volatile fatty acids

Results for volatile fatty acids obtained from the flow through ultrasound/peroxide treatments are presented in Figure 4.15. With higher ultrasound power and/or longer treatment time, most VFAs (except i-valeric acids) increased. A hydrogen peroxide addition converted part of the propionic, butyric and valeric acids to acetic acid. The total VFA increased with increased power, treatment time and hydrogen peroxide additions. However, the overall percentage TVFA to SCOD decreased from 20% to approximately 8-12%, due to the large increase in SCOD. This is consistent with previous batch ultrasound treatment without hydrogen peroxide, discussed in Section 4.3.1.3.

The role that power and hydrogen peroxide played in the transformation of these VFAs is an intricate one. At a power input of 234 W, acetic acid levels decreased for 5 minutes of treatment, while propionic and butyric acid increased. With the addition of hydrogen peroxide, a portion of the propionic and butyric acids were degraded to acetic acids, resulting in drops in propionic and butyric levels, but increases in acetic acid levels. With longer treatment time (15 minutes), or increased power input (416 W), all VFAs increased, but the same pattern remained. The addition of hydrogen peroxide increased levels of acetic acid and decreased levels of propionic and butyric acid compared to those without hydrogen peroxide.

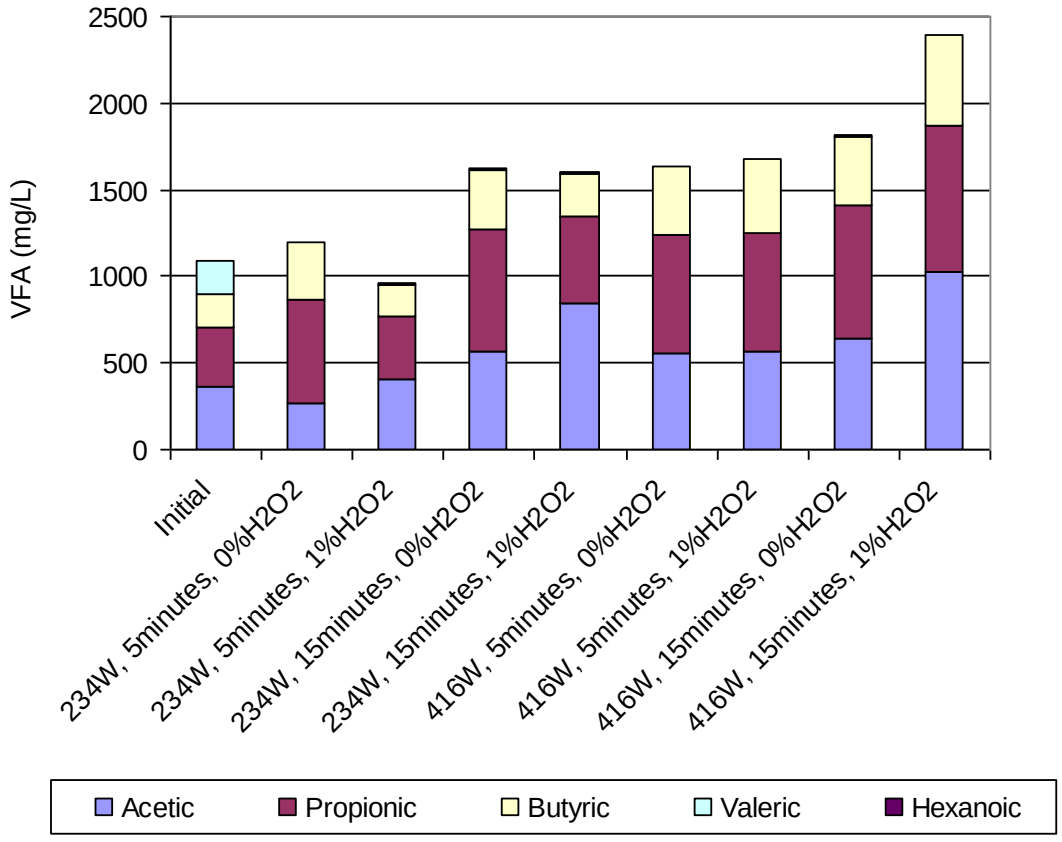


Figure 4.15 Individual volatile fatty acids from continuous-flow ultrasound/peroxide treatment

4.3.3 Flow through operation microwave / hydrogen peroxide process

4.3.3.1 SCOD, degree of disintegration

The SCOD results for the flow through MW/H_2O_2 process are shown in Figure 4.16, plotted against the specific energy. The energy numbers were calculated by multiplying the calibrated power inputs (372W, 678W and 920W) to the treatment time (required to reach desired temperatures of 40, 60 and 80°C). The SCOD data, grouped according to the hydrogen peroxide dosage (0, 0.5% and 1%), were consistent with specific energy (R^2=0.9536, 0.9139, 0.9416, respectively), despite the three levels of power deployed. This suggests that the microwave/peroxide process has a stronger correlation with specific energy than with power. This is also confirmed by the statistical analysis and prediction model illustrated in Figure 4.17.

In Figure 4.17 (a) model prediction profiler, and (b) Pareto plot of scale estimates, the results show that temperature and hydrogen peroxide dosage were the two main factors for the flow through MW/H_2O_2 system. Temperature (increase), as a representation of energy consumed or absorbed by the sludge sample during the process, was clearly the most significant factor in COD solubilization. The addition of hydrogen peroxide also contributed to the positive increase in SCOD. The power level parameter turned out to be a minor and negative contributor in this flow through MW/H_2O_2 system. This is likely due to the increasing heat lost and longer treatment duration when the system was operated at a low power level. In order to reach the same desired temperature, runs with a lower power setting required a longer treatment time. The effect of this was more heat lost through sludge pumping and also in the holding tank, especially when a higher temperature (80°C) was desired. The longer treatment duration (for low power input sets) resulted in more

COD solubilization but at the cost of additional energy input.

Table 4.6 shows a comparison of SCOD and the degree of disintegration from the flow through MW/H_2O_2 system and the batch testing at EOS microwave station at 80°C. In the batch testing, the degree of disintegration increased from an average of 25% with no H_2O_2 addition, to 28% with a 1% H_2O_2 addition. In the flow through system, this increment was from approximately 30% to 36%. The flow through appears to have better synergetic effect of microwave and hydrogen peroxide treatments. However, this would require further confirmation with better controlled microwave flow through system. At the same desired temperature of 80°C, the current flow through system has significantly higher power and energy cost than the batch treatment (Table 4.6), due to the heavy heat loss. This was the first attempt at devising a flow through MW/H_2O_2 treatment system. It points to the need for further research and equipment development that addresses the energy cost aspects of this type of system.

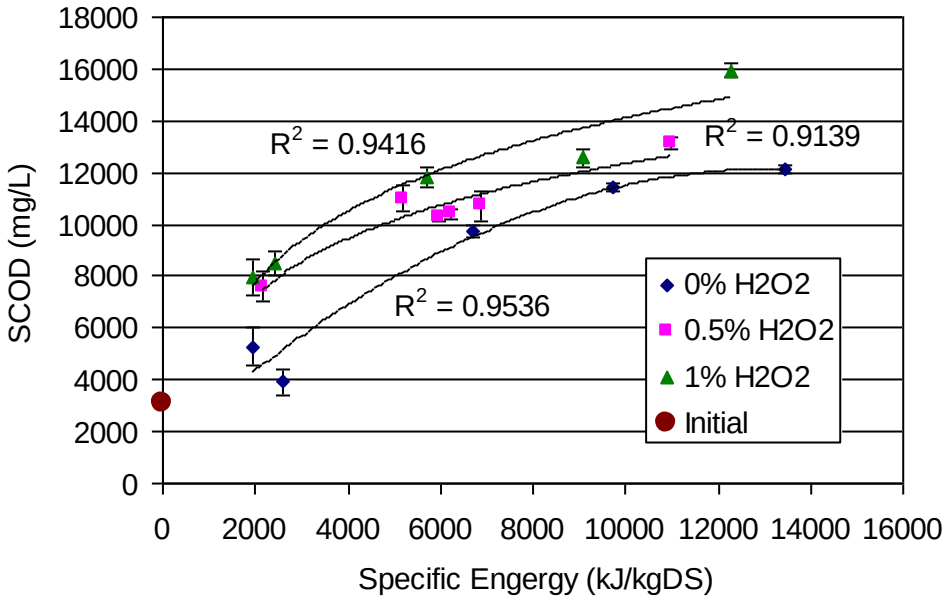
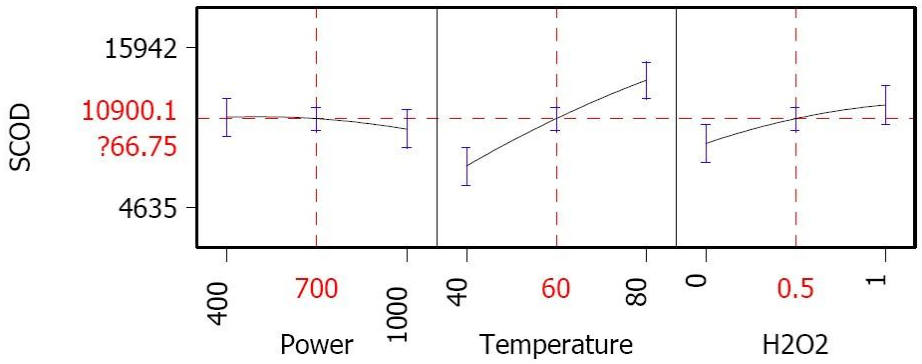
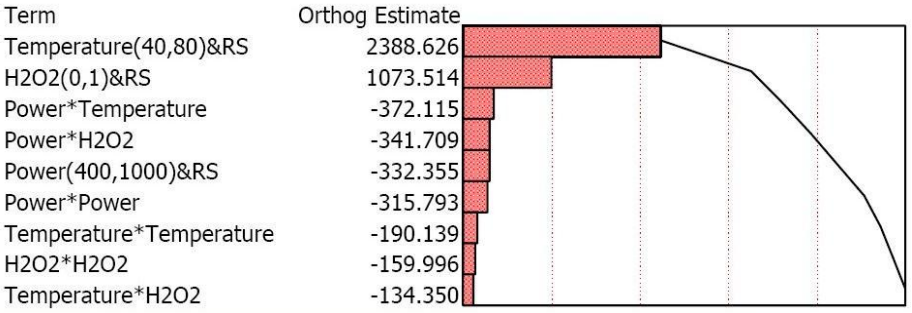


Figure 4.16 Flow through microwave / hydrogen peroxide process SCOD levels



(a)



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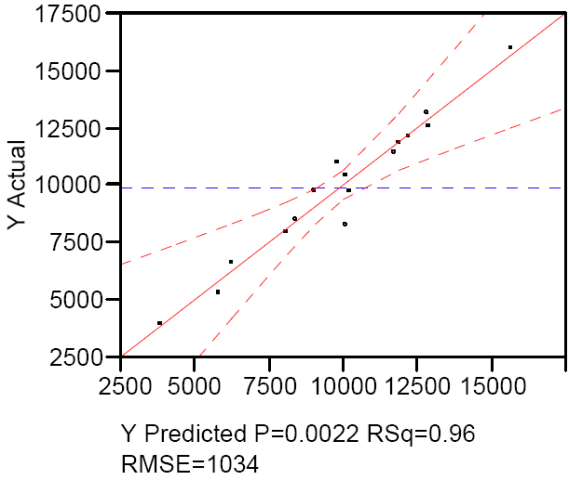

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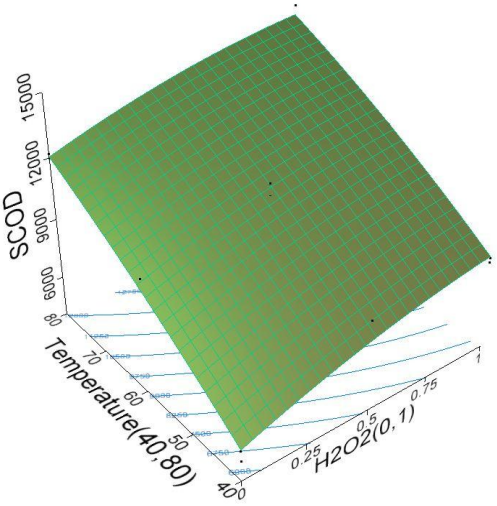


(d)

Figure 4.17 Prediction profiler (a), Pareto plot of scale estimates (b), prediction model summary of fit (c) and surface profiler (d) on SCOD levels for flow through microwave / hydrogen peroxide

Table 4.6 Comparison of SCOD result from the flow through and batch runs

MW/H_2O_2 process

Power	Temperature	H_2O_2	Specific Energy	SCOD Average	Standard deviation	Degree of Disintegration	DD Standard deviation
(W)	(°C)	(%)	(KJ/KgDS)	(mg/L)	(mg/L)	(%)	(%)
Flow through MW/ H_2O_2							
920	80	0	9718	11405	715	30	2.6
920	80	1	9070	12858	345	36	1.3
Batch run MW/ H_2O_2							
505	80	0	6159	9772	443	25	1.2
519	80	1	6329	10603	297	28	0.8

4.3.3.2 Orthophosphate

Results for soluble orthophosphate versus specific energy and its statistical analysis are shown in Figure 4.18 and Figure 4.19, respectively. The results show that as in the ultrasound/peroxide treatment, more ortho-P was released into the soluble form with an increased energy input. A hydrogen peroxide addition was found to have a negative effect for ortho-P solubilization in this treatment range below 80°C. Furthermore, the lowest levels were not at a 1% peroxide addition, but at 0.5% (Figure 4.18) or at a saddle point between 0.5% to 1%, as the statistical analysis suggested (Figure 4.19a).

The initial soluble ortho-P level was 43 mg/L. By injecting hydrogen peroxide and creating high oxidation (aerobic) states, the aerobic phosphate uptake became important in the process. A low temperature such as 40°C encourages such growth and uptake. A drop in soluble ortho-P to below the initial levels was recorded for 40°C treatment conditions (approximately 2000 kJ/kg-DS). With further treatment to 60°C and 80°C and/or a larger addition of hydrogen peroxide, soluble ortho-P increased to surpass the initial levels. This could be indicating more cell rupture occurs than microbial activity.

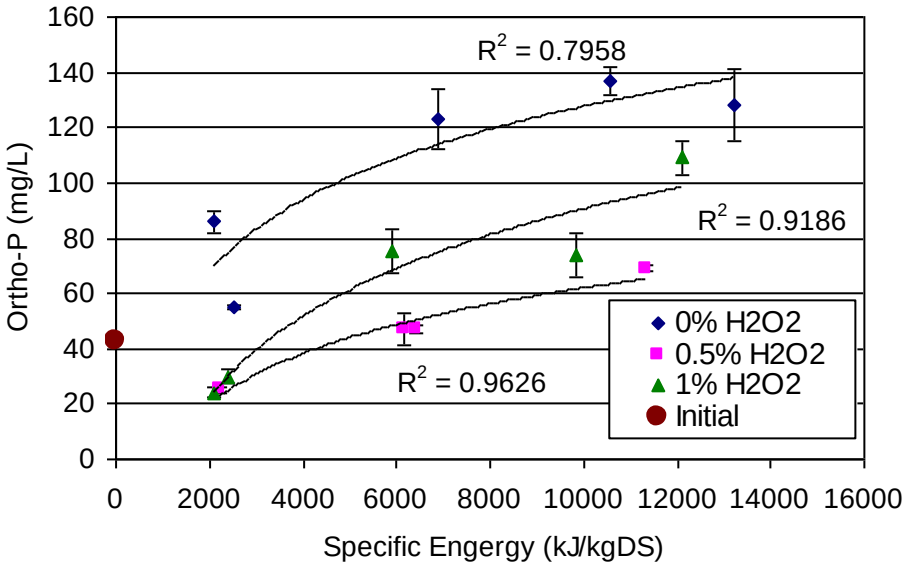
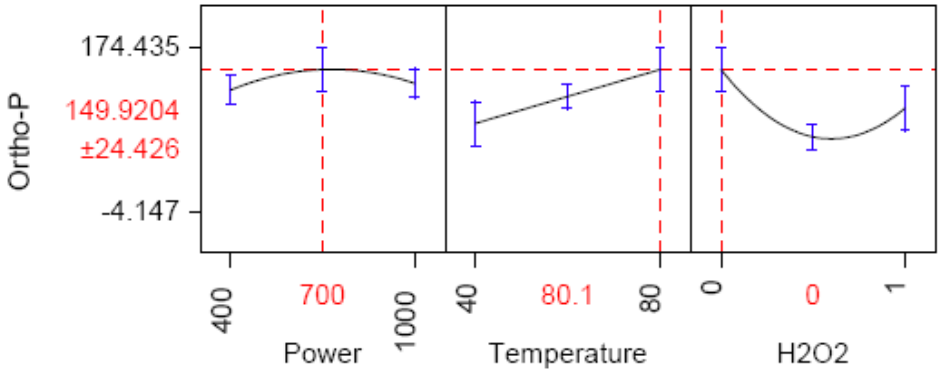


Figure 4.18 Orthophosphate levels from flow through MW/H_2O_2



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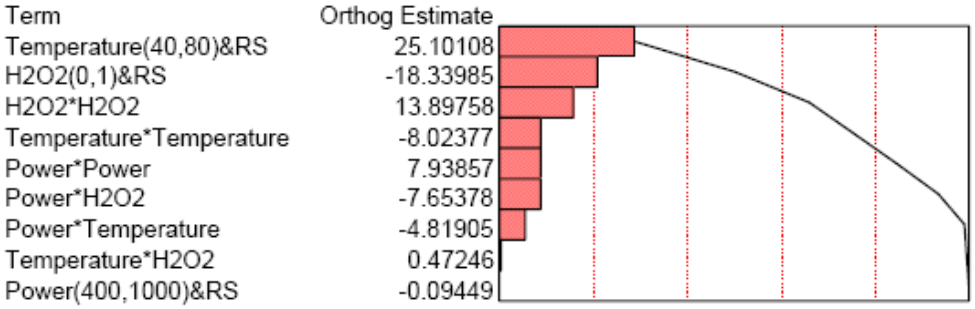

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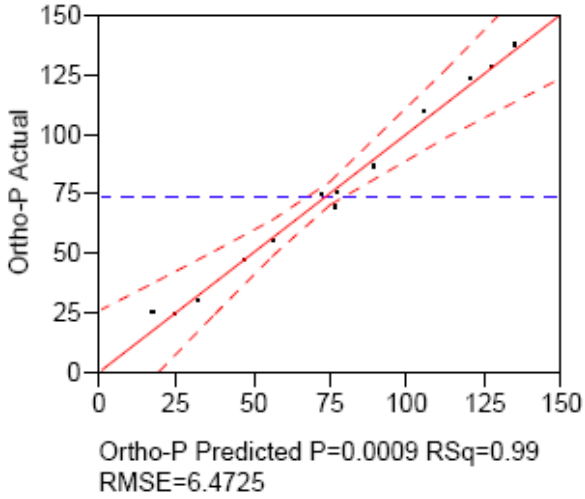

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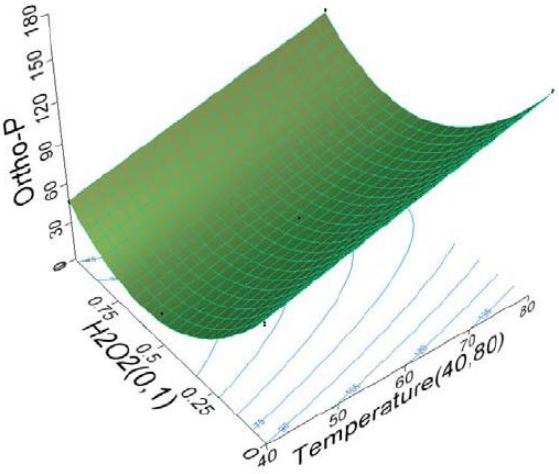


(d)

Figure 4.19 Prediction profiler (a), Pareto plot of scale estimates (b), prediction model summary of fit (c) and surface profiler (d) on ortho-P for flow through MW/H_2O_2 process

4.3.3.3 Volatile fatty acids

The volatile fatty acid results for the flow through MW/H_2O_2 process are presented in Figure 4.20. The initial sludge sample was high in all VFAs, except hexanoic acid. With microwave treatment alone, the individual VFAs were at lower levels, including propionic, i- and n-butyric, i- and n-valeric. On the other hand, acetic acid increased. It is likely that the other acids were broken down to acetic acid. The overall total VFA, expressed in milligrams of acetic acid per liter, increased modestly.

With an addition of hydrogen peroxide, large molecule soluble organics are oxidized into the small molecule fatty acids. All short chain VFAs, from acetic to hexanoic acids significantly increased compared to both the initial measurements and to those taken for the microwave treatment alone. The most noticeable ones were those that decreased with microwave treatment alone, for example hexanoic acid. Hexanoic acid increased from less than 1 mg/L to 77 mg/L.

Acetic acid was more than double the initial level. Propionic acid increased 33% from the initial value. The i- and n-butyric, i- and n-valeric acids were similar to the initial level, and significantly higher than those with microwave treatment alone.

With a 1% hydrogen peroxide addition, the total VFA increased 25-66%, adding to the already high initial value. However, when compared to the even larger increase in SCOD, the TVFA/SCOD% dropped from an initial 17% to approximately 7-13%.

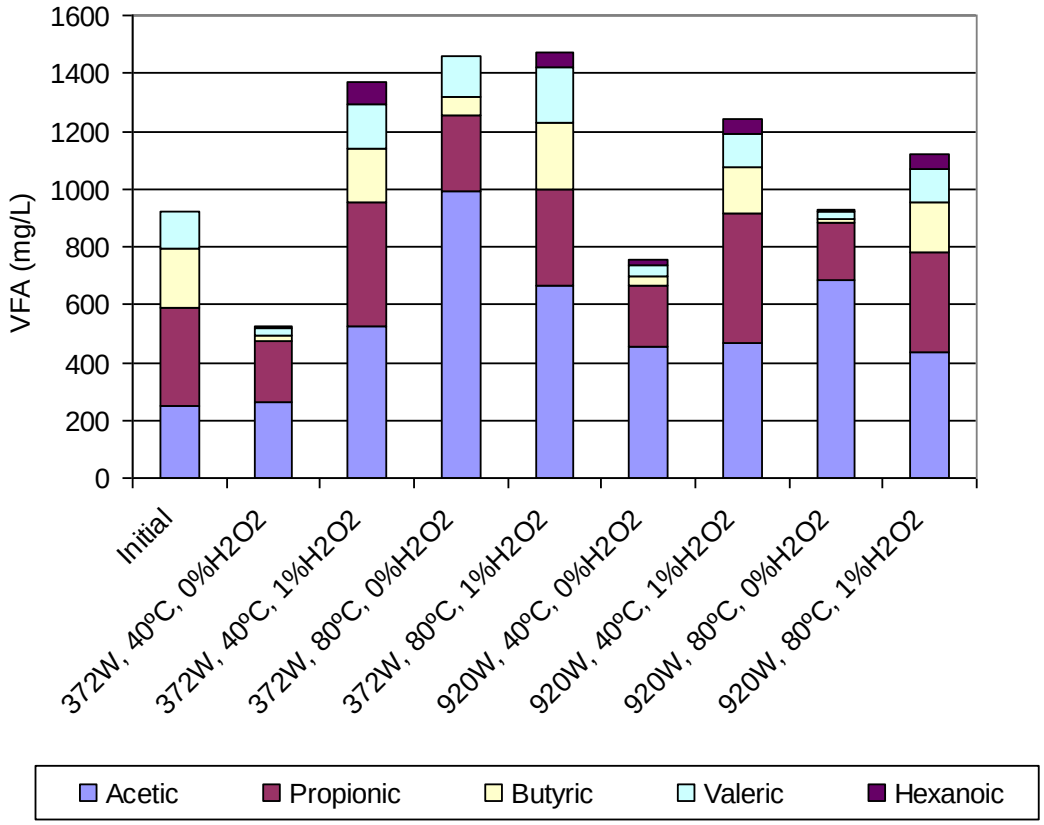


Figure 4.20 Individual volatile fatty acids from flow through MW/H_2O_2 process

4.4 Conclusions

In this study, batch ultrasound treatment, flow through ultrasound/peroxide treatment and flow through MW/H_2O_2 processes were investigated in terms of COD solubilization, orthophosphate release and VFA transformation. Process variables, namely power input, specific energy level, treatment time, temperature, and hydrogen peroxide additions were thoroughly examined.

With batch ultrasonication, it was found that:

- The most influential function for COD solubilizaiton was specific energy;
- At the same specific energy level, a higher power input with a short treatment time gave slightly better SCOD results than did a lower power input and a longer treatment time;
- It is beneficial to allow the bulk temperature to rise to 55°C, thereby taking advantage of the thermal denaturing of biomass and conserving energy;
- In ortho-P release, treatment time was the determining factor;
- Ultrasound cavitation impacted the whole spectrum of sludge organic compounds, from large particulates to the short chain VFAs.
- Changes in each individual VFA was determined by self-degradation and from the longer chain VFAs. The rate of this change was significantly affected by the power input (or power density).

With flow through ultrasound/peroxide treatment, it was found that:

- A hydrogen peroxide addition at 0.5% and at 1% increased COD solubilization;
- With no hydrogen peroxide addition, the flow through treatment achieved better DD_{COD} at lower specific energy conditions than did than batch ultrasonication;

- Power density (W/L) also had a significant impact on sludge disintegration;
- A hydrogen peroxide addition had a negative effect on ortho-P release. Treatment time was the most important factor here;
- The total VFA increased with power, treatment time and a hydrogen peroxide addition. However, the overall percentage of TVFA to SCOD decreased, due to the greater increase in SCOD;
- The addition of hydrogen peroxide increased acetic acid levels, but decreased propionic and butyric acid levels compared to those without hydrogen peroxide.

With a flow through MW/H_2O_2 process, it was found that:

- The flow through MW/H_2O_2 treatment had a stronger correlation with specific energy than with power input;
- Temperature and the addition of hydrogen peroxide were two main factors in COD solubilization;
- The flow through system appears to have better synergistic effect of microwave and hydrogen peroxide by injecting the peroxide immediately before the microwave irradiation. However, the current system operated at a higher power and specific energy cost due to the heat loss and low microwave power efficiency;
- Microwave treatment alone decreased level of propionic, i- and n-butyric, i- and n-valeric acids, but increased the acetic acid level;
- The MW/H_2O_2 process increased all VFA levels, but decreased the percentage of TVFA to SCOD, due to the large increase in SCOD.

Chapter 5 Effect of Microwave, Microwave/H_2O_2, Ultrasound, and Protease Treatment on Thickened Waste Activated Sludge Solubilization and Physical Properties *

5.1 Introduction

In wastewater treatment, chemical oxygen demand (COD) is a convenient term that is used to estimate the sum of the overall organic compounds in wastewater or sludge. It measures the oxygen equivalent of the wastewater material that can be oxidized chemically by dichromate in an acid solution. Even though there are limitation about the COD measurement (such as inorganic substance and non-biodegradable organics interference), it has become more popular in research and engineering application due to its many advantages. The main advantages of COD are the rapid response and the lower instrumentation requirement than other parameters such as biochemical oxygen demand (BOD) or total organic carbon (TOC). The solubilization of COD (SCOD, SCOD/TCOD% or Degree of Disintegration) is also commonly used as the key indicator in sludge pretreatment. It is sufficient as a general parameter in evaluating the extent of sludge disintegration and hydrolysis step. However, it does not provide sufficient detail to link the pretreatment to the acidogenesis, acetogenesis and methanogenesis steps in the anaerobic digestion of sludge.

*A version of this chapter will be submitted for publication:

YI, W., Lo, K.V., Liao, P.H., Mavinic, D.S., Forgie, D. and Mohseni M. Effect of microwave, microwave/H_2O_2, ultrasound and protease treatment on thickened wasted activated sludge solubilization and physical properties.

Anaerobic digestion is the most commonly used sludge stabilization process. Its biodegradation mechanism has been described in three theoretical stages: hydrolysis, fermentation (acidogenesis and acetogenesis), and methanogenesis (Tchobanoglous et al., 2003, McCarty and Smith, 1991). In IWA Anaerobic Digestion Model No. 1 (Batstone et al., 2002), the first extracellular solubilization stage was further differentiated into two steps. The first step was the disintegration of composite particulate material. The second step was the enzymatic hydrolysis of these disintegration products, namely, proteins, carbohydrates (polysaccharides) and lipids, into amino acids, monosaccharides, and long chain fatty acids.

Secondary biological sludge, including wasted activated sludge (WAS), consists mainly of biomass cell material. Protein is the dominant component of biomass. The typical composition of a bacterial cell is 55% protein in dry weight (Madigan et al., 1997), and in wasted activated sludge the protein ranges from 32 to 41% of the total solids (Tchobanoglous et al., 2003,). Bougrier et al. (2008) reported in their thermal pretreatment research that protein appears to be more resistant than carbohydrates during solubilization. It is therefore postulated that the disintegration and hydrolysis of protein might hold the key to this rate-limiting step in the anaerobic digestion of waste activated sludge.

The present study examined the effects of pretreatment on the macro molecule components of WAS, including protein, carbohydrates (polysaccharides), humic substances and COD. The subsequent products of disintegration / hydrolysis were also investigated. They included amino acids, ammonia, orthophosphate and volatile fatty acids. The release or leakage of deoxyribonucleic acids (DNA) was used as an indicator for evaluating the degree of biomass cell destruction. For a more thorough examination, the effects of pretreatments on the physical properties of the physical properties of sludge were also investigated using

particle size distribution analysis, floc microscopic imaging, and scanning electron microscopy (SEM) imaging.

The pre-treatment methods subjected to comparison were microwave irradiation, the microwave / hydrogen peroxide process (MW/H_2O_2), ultrasound, protease enzymatic treatment, and a combination of ultrasound and protease treatment. The addition of Protease enzyme and its combination with ultrasound specifically targeted protein hydrolysis. Ultrasound treatment has been reported to be capable of dispersing or extracting the extracellular polymeric substances (EPS) (Matin-Cereceda et al., 2001; Yu et al., 2007; 2008). The EPS has been determined to be a gel-like matrix that protects the biomass cell against environmental stresses (Li and Ganzarczyk, 1990; Morgan et al., 1990). By exposing the biomass cell to protease after ultrasound treatment, it was expected that protein hydrolysis would be enhanced.

The study reported in Chapter 4 showed that specific energy is the regulating factor in both microwave irradiation and ultrasound cavitation. In engineering practice, energy cost is also a very important consideration. It would therefore be valuable to compare various treatment methods at similar specific energy levels. In this study, all specific energy levels were controlled within a range of from 4933 to 6671 kJ/kg-DS. For the microwave and MW/H_2O_2 treatments, the final temperature of the treated sludge reached 80°C. This was considered an effective treatment level (Chapter 2, 3 and 4). With ultrasonication, this specific energy range had also provided sufficient disintegration results (Chapter 4).

5.2 Material and Methods

5.2.1 Sludge characteristics

Secondary sludge was obtained from Metro Vancouver's Lulu Island Wastewater Treatment Plant, located at the south end of the City of Richmond, BC. Sludge samples were collected weekly and stored at 4 ºC for the duration of the experiments. Table 5.1 defines the characteristics of this secondary sludge (thickened waste activated sludge, or TWAS).

5.2.2 Microwave apparatus and treatment processing

A closed-vessel microwave digestion system (Ethos TC Digestion Labstation 5000, Milestone Inc., U.S.A.) was used in this study. The system was described in Section 2.2.1 and Figure 2.1. For both microwave treatment and the MW/H_2O_2 treatment, the temperature was set to 80°C. Energy consumption and power input were recorded by the Ethos control module as presented in Table 5.2. The hydrogen peroxide addition for the MW/H_2O_2 process was 1% in wet weight, approximately 0.29 mg-H_2O_2/mg-TCOD (or 0.38 mg-H_2O_2/mg-DS).

5.2.3 Ultrasound apparatus and treatment processing

An ultrasonic flow cell set (UIP1000 ultrasonic processor) from Hielscher Ultrasonics GmbH, Berlin, Germany, was used for ultrasound treatment testing in this study. The system was described in Section 4.2.1 and Figure 4.1. The ultrasound operation frequency was at 20 kHz. Energy consumption and power input were recorded by the PC-control. They are also presented in Table 5.2.

5.2.4 Protease treatment

A non-specific protease, P5147 (SIGMA product), type XIV bacterial protease from *Streptomyces griseus*, were used in this study. The protease was supplied as a dry powder. Prior to use, it was prepared as a stock solution of 1000 mg/L. The sludge samples, with and without ultrasound pre-treatment, received a protease dosage of 100 mg/L (specific dosage rate 0.002 g-Protease/g-COD or 0.0026 g-Protease/g-DS).

5.2.5 Chemical analysis

5.2.5.1 Sample processing for soluble faction

After each experiment, treated sludge samples were spun in a high-speed centrifuge at 15,000 rpm for 10 minutes. The resulting supernatants were filtered through Whatman No.4 filters and analyzed for soluble faction of the COD, protein, polysaccharides, humic acids, amino acids, ammonia, ortho-phosphate, volatile fatty acids and DNA.

5.2.5.2 Total solids, volatile solids, chemical oxygen demand

Total Solids (TS), Volatile Solids (VS), and Chemical Oxygen Demand (COD) were determined according to the Standard Methods (APHA, 1995).

5.2.5.3 Protein and humic acids

The modified Lowry assay (Lowry et al., 1951) was used to quantify proteins and humic-like compounds, in which bovine serum albumin (BSA) and humic acids were used as the standards, respectively (Frolund et al., 1996). The Lowry assay is a colorimetric method. A HACH DR/2800 spectrophotometer was used for the measurement of absorbance at 750 nm wavelength. The procedures of the modified Lowry assay were adopted from Keleti and

Lederer (1974).

In the Lowry procedure, proteins and humic compounds interfere with each other during the analysis (Frolund et al., 1996). When the mixture is prepared without the addition of copper ion, the color development is due to humic-like compounds and chromogenic amino acids. In that case, the color developed by BSA decreased to 20%, as $CuSO_4$ was absent, but no decrease was observed for humic acids. The absorbance values for proteins and humic-like compounds were therefore calculated using the following equations (Pattanayak, 2007).

$$A_{total} = A_{proteins} + A_{humic\text{-}like}$$

$$A_{blind} = 0.2A_{proteins} + A_{humic\text{-}like}$$

$$A_{proteins} = 1.25\ (A_{total} - A_{blind})$$

$$A_{humic\text{-}like} = A_{blind} - 0.2A_{proteins}$$

Where A_{total} is the total absorbance value of the mixture with the addition of CuSO4, A_{blind} is the total absorbance value of the mixture without an addition of CuSO4, $A_{humic\text{-}like}$ is the absorbance value due to humic-like compounds, and $A_{proteins}$ is the absorbance value due to proteins. The concentrations of proteins and humic-like substances in the EPS extract were calculated by fitting the values of $A_{proteins}$ and $A_{humic\text{-}like}$ into the standard curves of BSA and humic acids, respectively.

5.2.5.4 Polysaccharides

The Dubois Assay (Dubois et al., 1956) was used for the measurement of polysaccharides, with glucose as the standard. The Dubois assay is a colorimetric method, based on the reaction of phenol-sulphuric acid with carbohydrates. The HACH DR2800

spectrophotometer was used for the measurement of absorbance at a 490 nm wavelength. The procedures of the Dubois assay were adopted from Keleti and Lederer (1974).

5.2.5.4 Amino acids

The modified Ninhydrin assay (Moore and Stein, 1948; 1954) was used for the measurement of amino acids, with leucine used as the standard. The Ninhydrin assay is a colorimetric method. The HACH DR2800 spectrophotometer was used for the measurement of absorbance at a 570 nm wavelength. The procedures of the Dubois assay were adopted from Keleti and Lederer (1974). Ammonia is a positive interference in this chemistry. The contribution of ammonia to the absorbance was subtracted by measuring the sample ammonia concentrations using the Lachat QuickChem method (Section 5.2.5.5). A standard absorbance curve of the known ammonia concentrations was then prepared.

5.2.5.5 Ammonia and orthophosphate

Ammonia and orthophosphate (ortho-P) were determined by flow injection analysis using a Lachat QuikChem 8000 Automated Ion Analyzer. QuickChem Method No. 10-107-06-1-D (phenolate method) and No. 10-115-01-1-Z (ascorbic acid method) were used for ammonia and ortho-P measurements, respectively.

5.2.5.6 Volatile fatty acids

A Hewlett Packard 5890 Series II gas chromatograph, equipped with a flame ionization detector (FID), was used to measure volatile fatty acids (VFA). Volatile separation was accomplished with an HP FFAP column (0.25 m × 0.31 mm with 0.52 μ film thickness). The injection temperature was set at 175 ºC and the FID detector was at 250 ºC.

5.2.5.7 Deoxyribonucleic acids (DNA)

Deoxyribonucleic acids (DNA) were determined by the DNA-DAPI (4,6-diamidino -2-phenylindole·2HCl) fluorimetric method (Kapuscinski and Skoczylas, 1977; Brunk et al., 1978). A DNA marker (Invitrogen, 100 bp DNA ladder) was used as standard. The buffer solution consisted of 0.1M NaCl, 0.01M EDTA, 0.01M Tris, pH = 7.0. The DAPI solution contained 100 ng/L DAPI in the buffer solution. In sample preparation, 1 mL of sample was transferred into the 1.5 mL mini-centrifuge tube, heated at 100°C for 10 min and immediately chilled on ice to denature the DNA. The sample was then expelled with a syringe several times to degrade crude DNA. The amount of 10 µL of sample/standard was added into 5 mL DAPI solution and measured with a fluorometer (Turner Designs, Model 10-AU-005-CE).

5.2.6 Physical properties examination

5.2.6.1 Particle size analysis

Particle size measurement was conducted through a Malvern Instrument Mastersizer 2000 analyzer with a Hydro S automated sample dispenser unit. It used the laser diffraction technique, based around the principle that particles passing through a laser beam will scatter light at an angle that is directly related to their size. The material sizes measured in the Mastersizer 2000 ranged from 0.2 µm to 2000 µm. A volume distribution, showing the volume percentage of particles that have a given size, is also reported.

5.2.6.2 Floc microscopic imaging

The microscopic images of sludge flocs were obtained with a phase contrast microscope (ECLIPSE E, Nikon Instrument).

5.2.6.3 Scanning electron microscopy (SEM) imaging

Scanning electron microscopy (SEM) was used to scan the surface of the specimens. Using a 2-3 nm spot of electrons, SEM generates secondary electrons that are then detected by a sensor so as to produce an image of the surface that gives the impression of three dimensions. SEM work included fixation (fixing, drying, applying conductive coating) and imaging of the samples. The SEM in this study was conducted at the UBC BioImaging Facility, using the HITACHI S-4700 Field Emission Scanning Electron Microscope (FESEM).

Table 5.1 Characteristics of thickened secondary sludge used in this study

Parameters	*Concentration*
pH	6.5 ± 0.2
TS (%)	4.1 ± 0.1
VS (%)	87.0 ± 0.1
Total COD (g/L)	50.9 ± 2
Soluble COD (g/L)	2.4 ± 0.6
Total protein (g/L)	13.7 ± 2.1
Soluble protein (g/L)	0.19 ± 0.05
Total polysaccharides (g/L)	3.2 ± 0.2
Soluble polysaccharides (g/L)	0.14 ± 0.07
Total humic acids (g/L)	5.4 ± 0.3
Soluble humic acids (g/L)	0.45 ± 0.19

Table 5.2 Power and specific energy levels for the various treatment methods

(specific energy from hydrogen peroxide dosage, in bracket)

		Power W	Specific Energy kJ/kg-DS
25/03/2009	MW	505	6159
Set 1	MW/H_2O_2	519	6329 (987)
	US	362	5545
05/05/2009	MW	473	5768
Set 2	MW/H_2O_2	547	6671 (987)
	US	344	5251
	US+Protease	336	4933

5.3 Results and Discussion

5.3.1 Total solids, volatile solids, total COD and COD solubilizaiton

The total solids and volatile solids results from three separate batches of experiments are reported in Table 5.3. The student's t-test (described in Section 3.3.1) were performed at 95% confidence interval, and reported in Figure 5.1 and Figure 5.2 for total solids and total CODs. The data showed relatively constant values in total solids, organic solids and total COD after microwave, MW/H_2O_2 and ultrasound treatments. Two exceptions were from the protease treatment and one microwave treatment set. These results were compared to the findings in a report from Eskicioglu et al. (2008), in which hydrogen peroxide was dosed at a rate of 1 g-H_2O_2/g-TS. In the present study, the hydrogen peroxide dosage was at 0.38 g-H_2O_2/g-TS or 0.29 mg-H_2O_2/mg-TCOD.

In Eskicioglu et al. (2008), the TS, VS and TCOD from microwave only treatment remained relatively unchanged, or had only minor increases. This was probably due to the water evaporation in heating. This is consistent with the present study. A reduction in TS and TCOD was found in the peroxide only and MW/H_2O_2 treatments from temperatures of 60°C to 120°C in Eskicioglu et al. (2008). The likely cause here is a higher peroxide dosage rate. Liao et al. (2007) have also shown TCOD reduction at peroxide dosage rates of 2.9 to 5.9 g-H_2O_2/g-TCOD in the same temperature range. In the present study, it was found that under low dosage hydrogen peroxide conditions, most of the organic solids remained in either particulate or solubilized forms. If any final oxidation occurred, it would have been small and within measurement error. Ultrasound treatment at this energy level did not result in significant organic reduction either. Overall, solubilization, or disintegration, appeared to be the main process in these various pretreatments.

Soluble COD results from these treatments are shown in Figure 5.3. The SCOD levels increased from an initial average of 2243 mg/L to 12917 mg/L, 14052 mg/L, 18776 mg/L, 7014 mg/L and 19639 mg/L for microwave, MW/H_2O_2, ultrasound, protease and ultrasound/protease treatments, respectively. They corresponded to approximately 27%, 30%, 41%, 12% and 43% in Degree of Disintegration (DD_{COD}) as defined in Section 3.2.4. Given that energy was directly introduced into the sludge by submerging an ultrasound probe 2-3 cm below the sludge line, ultrasound treatment appeared to be more energy efficient at COD solubilization with 41% DD_{COD}. COD solubilization at 12% for the Protease only treatment was relatively small compared to the microwave, MW/H_2O_2 or ultrasound treatments. However, little energy was consumed in protease treatment other than for vortex mixing. Neither did a Protease addition after ultrasound treatment significantly increase COD solubilization. The effect of protease hydrolysis was probably overshadowed by the substantial SCOD increase which occurred with ultrasound only treatment.

The student's t-test for SCOD results (Figure 5.4) showed that the results from various treatments are mostly different, except the comparison between microwave and MW/H_2O_2 treatments in set 1.

Table 5.3 Total solids, volatile solids and total COD results from the various batch treatments (standard deviation shown in bracket)

		Total solids g/L	Volatile solids g/L	VS/TS %	Total COD g/L
25/03/2009	WAS	38.5 (0.1)	33.4 (0.1)	86.8	50.9 (1.7)
(Set 1)	MW	40.5 (0.2)	35.4 (0.3)	87.6	49.3 (1.9)
	MW/H_2O_2	39.4 (0.0)	34.1 (0.3)	86.6	49.5 (2.6)
	US	39.5 (0.2)	33.6 (0.4)	85.1	50.5 (1.9)
	Protease	35.2 (0.1)	30.1 (0.2)	85.6	51.2 (2.7)
05/05/2009	WAS	41.1 (0.0)	34.8 (0.2)	84.8	57.4 (2.5)
(Set 2)	MW	42.6 (0.0)	36.1 (0.3)	85.4	61.3 (2.0)
	MW/H_2O_2	40.1 (0.1)	33.9 (0.5)	84.6	54.0 (2.1)
	US	39.9 (0.1)	34.1 (0.1)	85.5	55.8 (0.7)
	Protease	38.3 (0.0)	32.2 (0.4)	84.2	56.1 (3.0)
	US+Protease	39.9 (0.0)	33.7 (0.1)	84.5	55.8 (0.9)

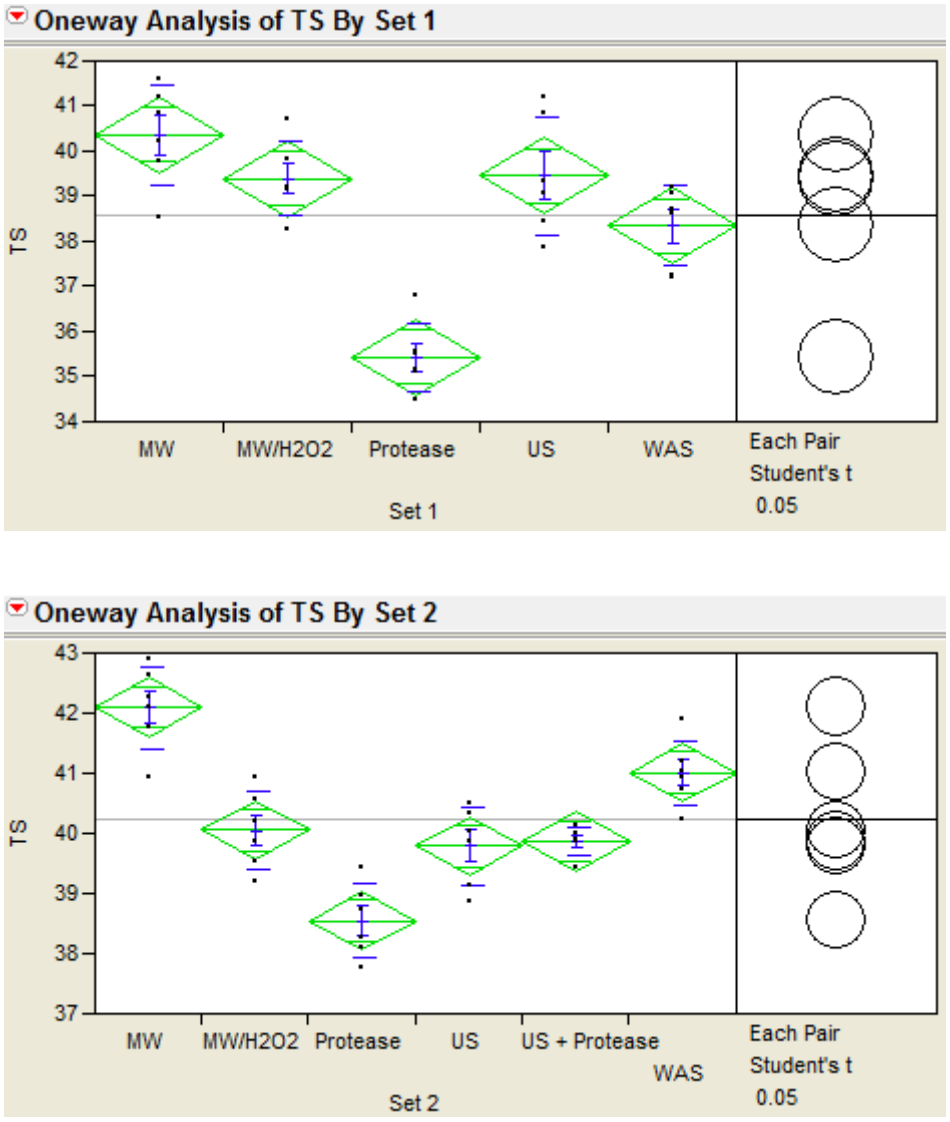


Figure 5.1 Student's t-Test for group comparisons of total solids (TS) result from various treatments

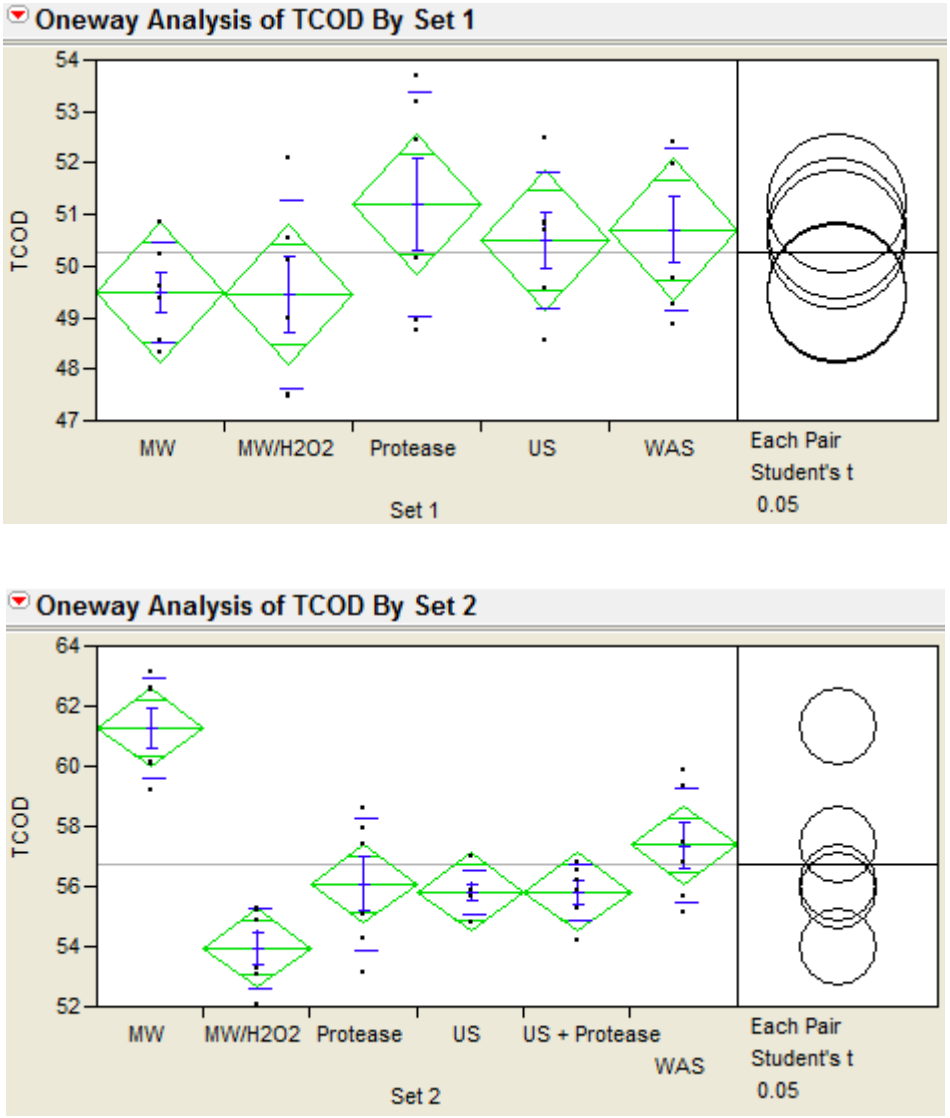


Figure 5.2 Student's t-Test for group comparisons of TCOD result from various treatments

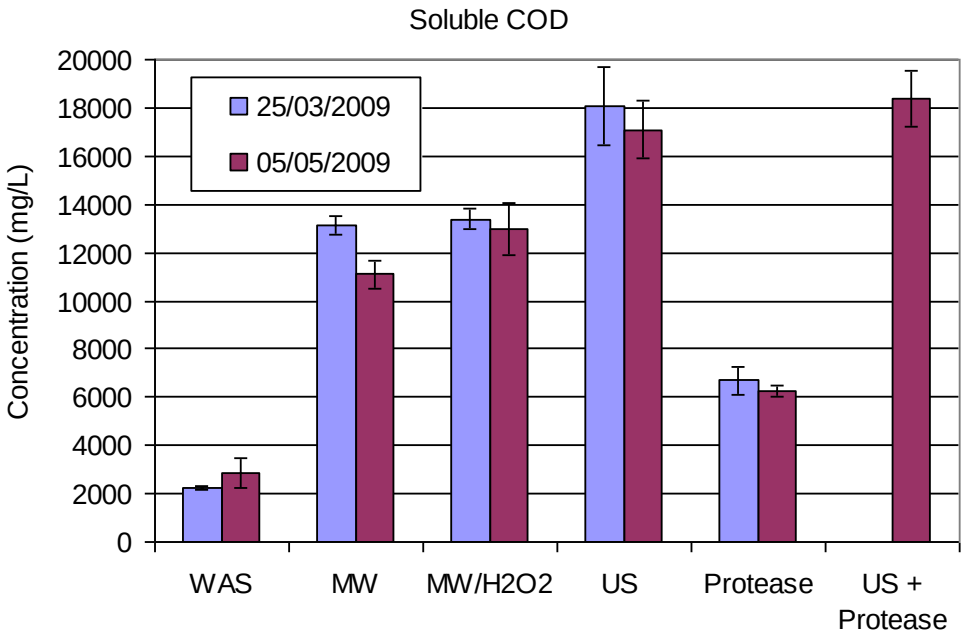


Figure 5.3 SCOD results from various batch treatments

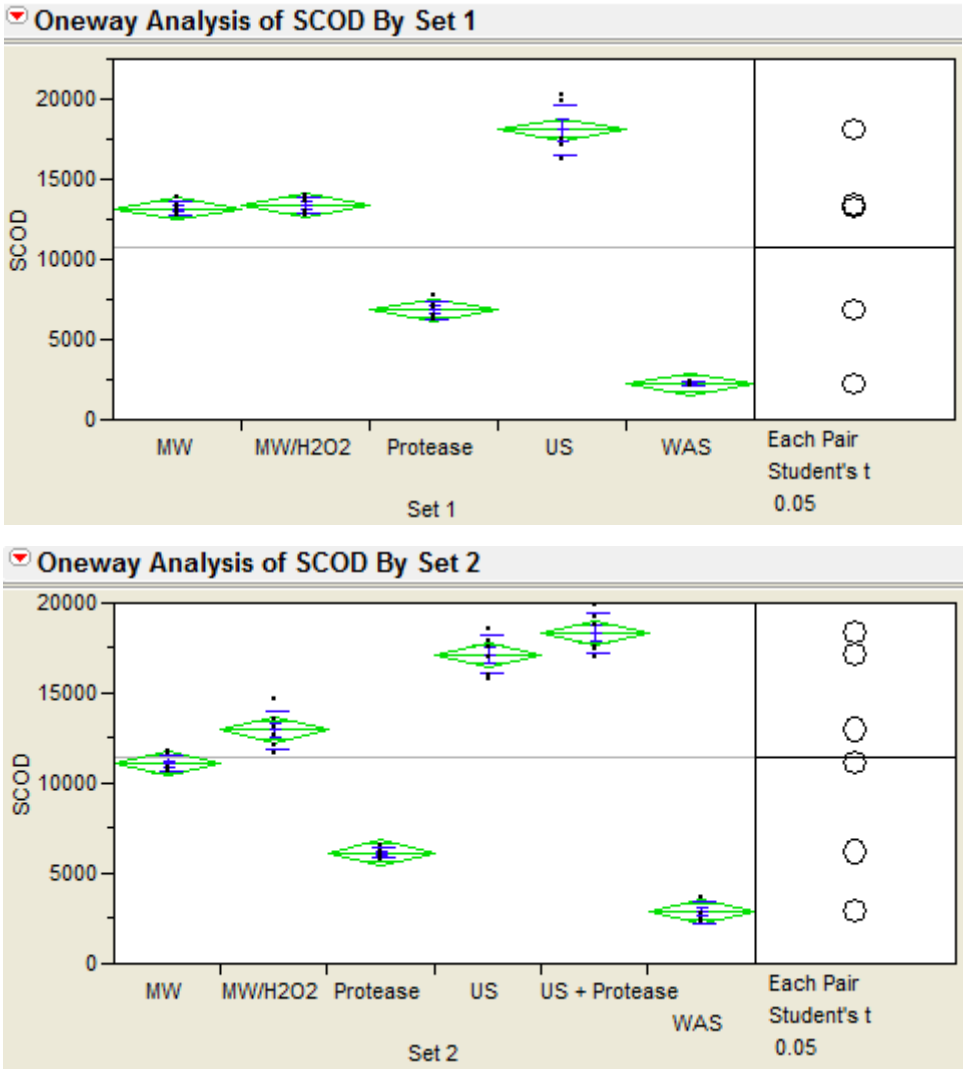


Figure 5.4 Student's t-Test for group comparisons of SCOD result from various treatments

5.3.2 Protein, amino acids and ammonia

The soluble protein and amino acids results are reported in Figure 5.5 and Figure 5.6, respectively. Student's t-test comparisons are shown in Figure 5.7 and Figure 5.8 for soluble protein and amino acids, respectively. Microwave only treatment yielded approximately 15% of soluble protein to total protein. With an addition of hydrogen peroxide, MW/H_2O_2 treatment achieved 20% of protein solubilization. Ultrasound and protease treatment resulted in 9% and 4% protein solubilization, respectively.

However, ultrasound treatment achieved better amino acids results compared to microwave, MW/H_2O_2 or protease. It indicates that ultrasound has a better effect on protein hydrolysis. With a protease dosage after ultrasound treatment, the amino acids level reached an average 8111 mg/L, while the initial WAS amino acids level had been at 343 mg/L level. The student t-tests confirmed that these results are substantially different.

In order to better understand the changes in terms of protein which occurred subsequent to these treatments, the mass balance and distribution of particulate protein, soluble protein and amino acids were plotted in Figure 5.9. The measurements were done on total protein, soluble protein, and amino acids. Particulate protein shown on Figure 5.9 was obtained by subtracting soluble protein from total protein in the same sample. The student t-tests for protein mass balance are shown in Figure 5.10.

The Figure 5.9 and Figure 5.10 show the sum of total protein (particulate and soluble) and amino acids remained at relatively constant levels before and after the treatments, despite the substaintial changes between the three groupings. For the microwave treatment, approximately 4451 mg/L of protein was solubilized, half of which was hydrolyzed into amino acids. For the MW/H_2O_2 treatment, a large amount of 5033 mg/L of particulate protein was

solubilized, and approximately the same amount (as with microwave treatment) 2247 mg/L was converted to amino acids.

With the ultrasound treatment, a slightly higher protein solubilization from particulate (average 6235 mg/L) was found compared to microwave and MW/H_2O_2 treatments at approximately the same specific energy level. The major difference, however, was that about 80% of these soluble proteins were hydrolyzed to amino acids. The protease only treatment did not solubilize a significant amount of particulate protein, but most of the products were in amino acids form. When protease was added after ultrasound treatment, the hydrolysis effect was enhanced. Not only did it hydrolyze the soluble protein remaining after ultrasound treatment, the protease had access to the remaining particulate protein. This is likely due to the ultrasound cavitation forces that acted on the protective EPS and exposed the biomass. The end result was that almost 60% of initial particulate protein was disintegrated and hydrolyzed. Approximately 86% of this was into amino acid form.

The ammonia results (ammonium nitrogen, NH_4^+-N) are presented in Figure 5.11. The increases in ammonium levels from microwave treatment and MW/H_2O_2 treatment were moderate, from an initial 112 mg/L in the WAS to an average 163 mg/L and 126 mg/L, respectively. Protease treatment also raised ammonium levels to an average 178 mg/L. The larger increase was seen with ultrasound treatment, where ammonium levels reached an average of 341mg/L. However, even at this level, ammonium only represented less than 2% of the initial total protein. This indicates that, at these energy and peroxide dosage levels, disintegration and hydrolysis were the main pathways. Mineralization of organic to inorganic nitrogen from these treatments was at a minimum.

Ammonia nitrogen, as an essential nutrient, is important for microorganism growth and

metabolism. However, elevated levels of free ammonia (NH_3-N) are toxic for anaerobic digestion (Chen et al., 2008; Sung and Liu, 2003). It is therefore not only important to monitor ammonia levels as an indicator of pretreatment progression, but also to ensure that there is no negative impact on the subsequent digestion process. The free ammonia level is subject to three factors: the total ammonia concentration, temperature and pH. Hence, thermophilic digestion is more susceptible to the impact of free ammonia toxicity (Hansen et al., 1998; Sung and Liu, 2003). Sung and Liu (2003) reported that 560-568 mg/L of free ammonia can cause a 50% inhibition of methanogenesis at pH 7.6 under thermophilic conditions.

In this study, the sludge samples from the microwave and MW/H_2O_2 treatments reached 80°C, while the ultrasound treatment raised the temperature to 55°C. The treated sludge was cooled to room temperature before introducing it to the digestion process (Chapter 6). In practice, pretreated or heated sludge would likely be subjected to heat exchange for energy conservation. This makes it important to estimate the free ammonia levels in pretreated sludge at mesophilic (35°C) and thermophilic (55°C) temperatures if the ammonium levels from pretreatments are high. Table 5.4 presents the results for the potential free ammonia levels in the treated sludge obtained through equilibrium calculations for hypothetical digestion scenarios at pH 7.0 and 7.5. The results show that in most circumstances all pretreated sludge should pose no immediate threat to digestion from free ammonia toxicity. In fact, according to Liu and Sung (2002) these ammonium levels might be beneficial for microorganism growth.

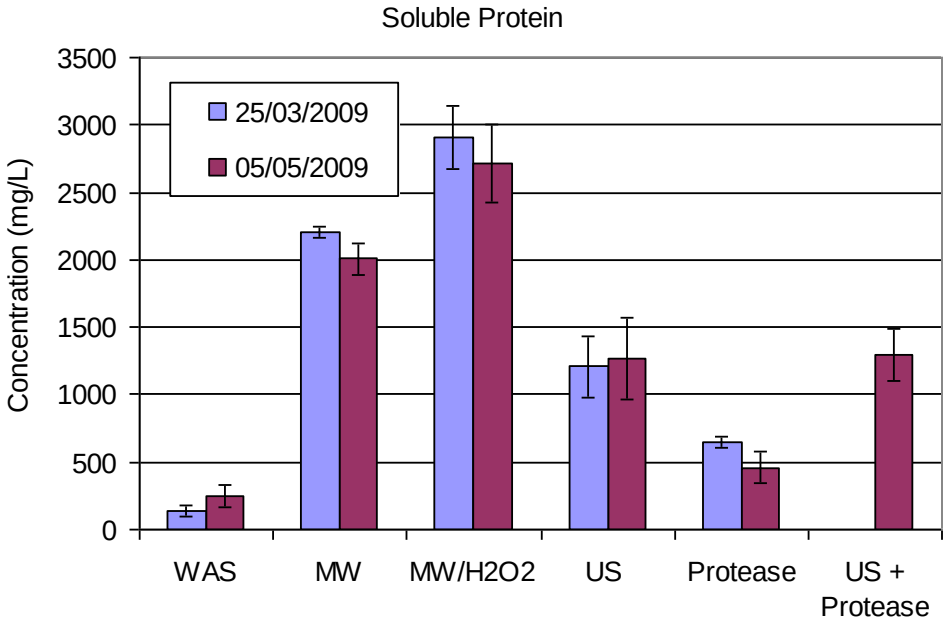


Figure 5.5 Soluble protein from various batch treatments

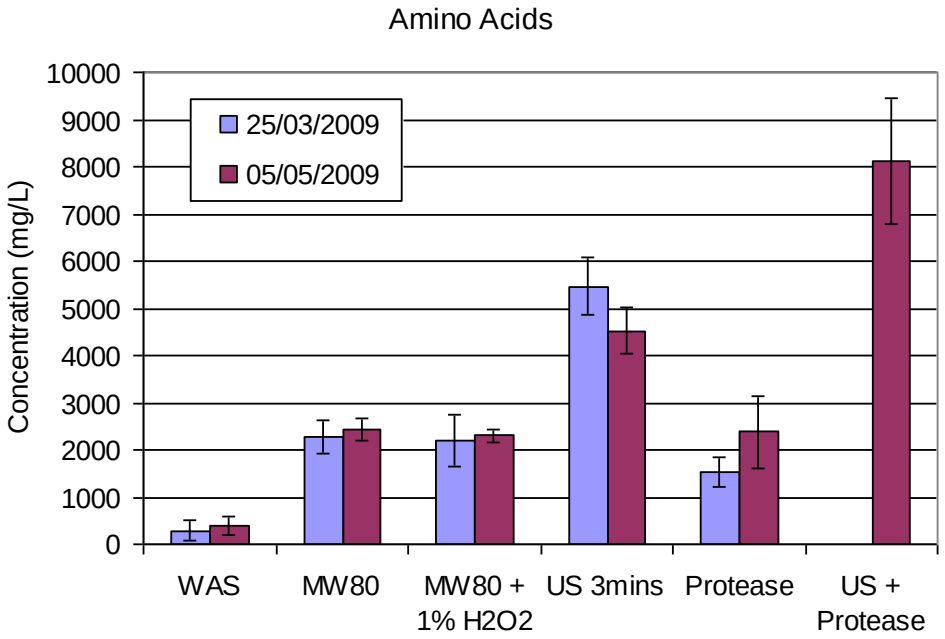


Figure 5.6 Soluble amino acids from various batch treatments

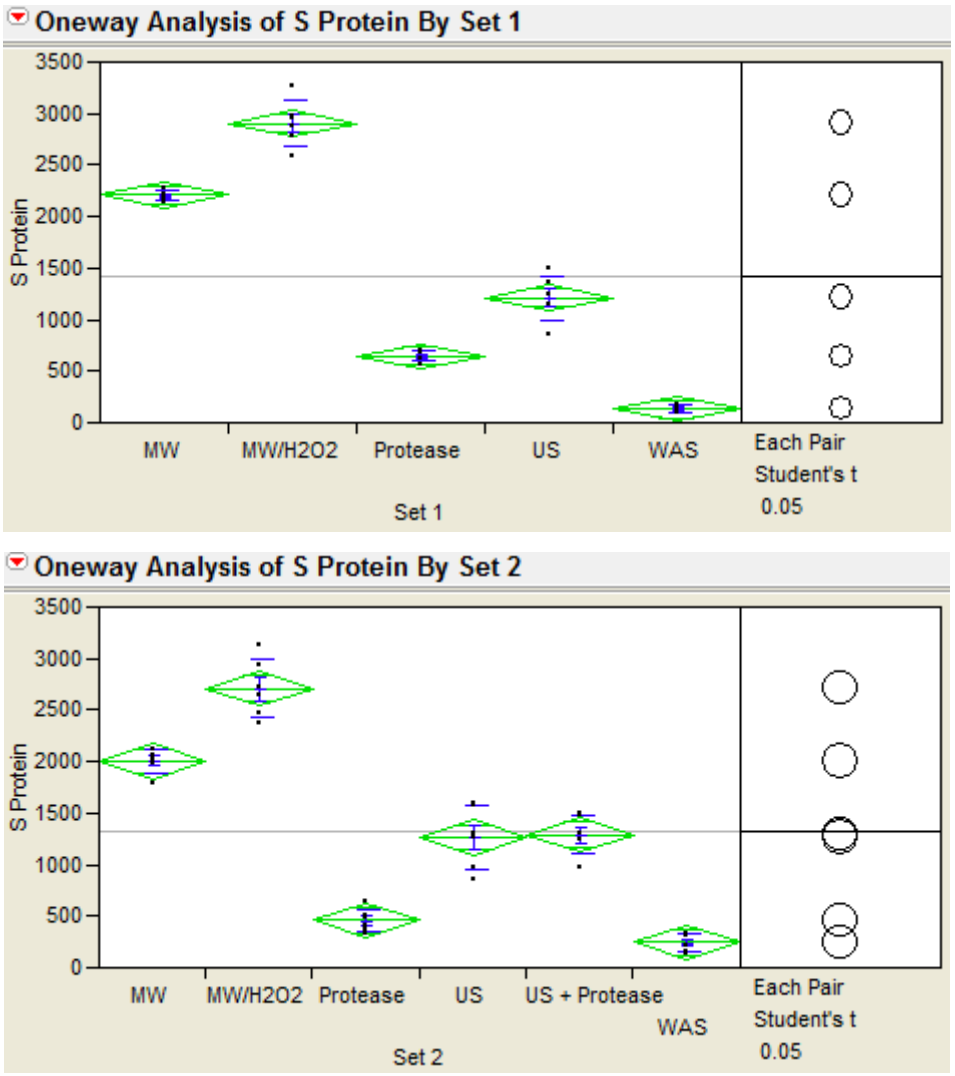


Figure 5.7 Student's t-Test for group comparisons of soluble protein result from various treatments

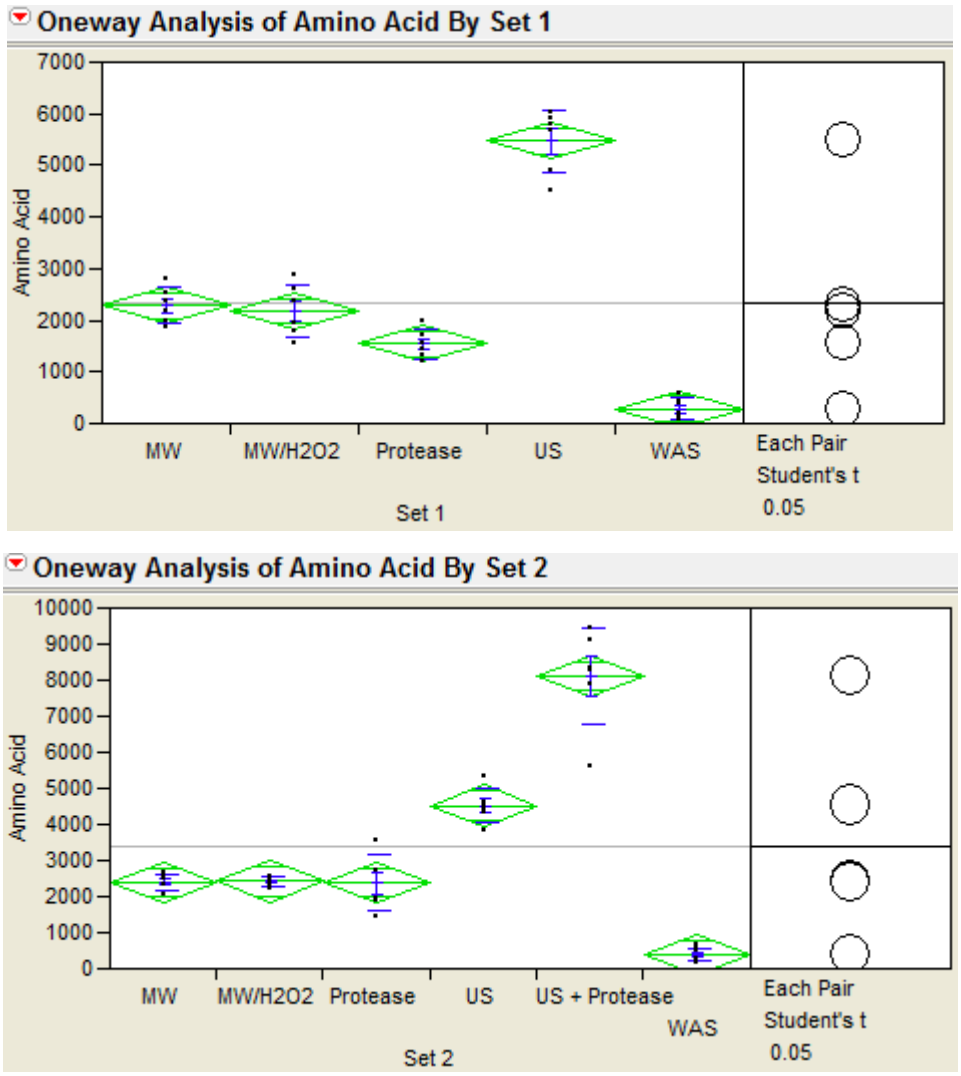


Figure 5.8 Student's t-Test for group comparisons of amino acids result from various treatments

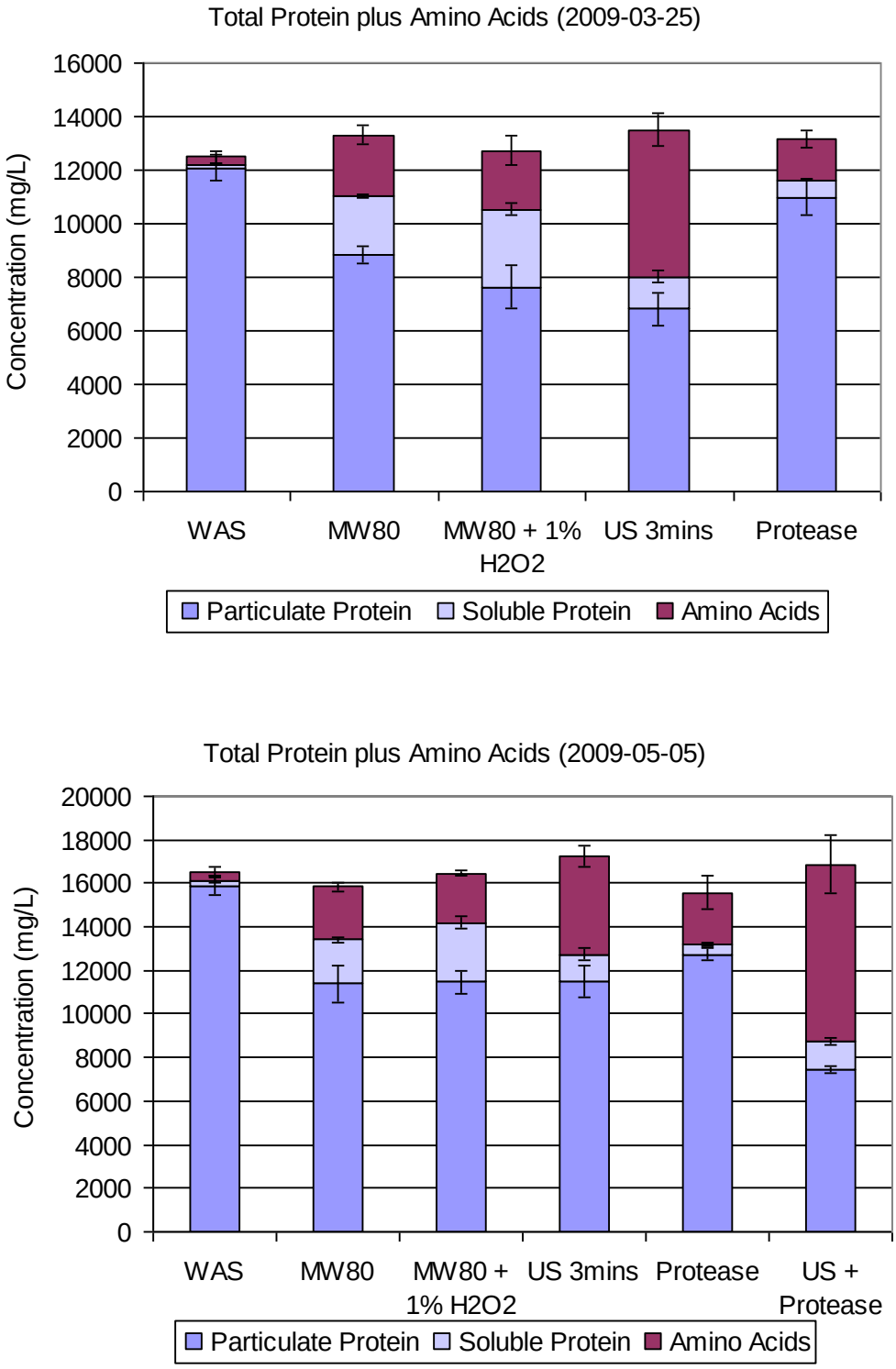


Figure 5.9 Total protein plus soluble amino acids from various batch treatments

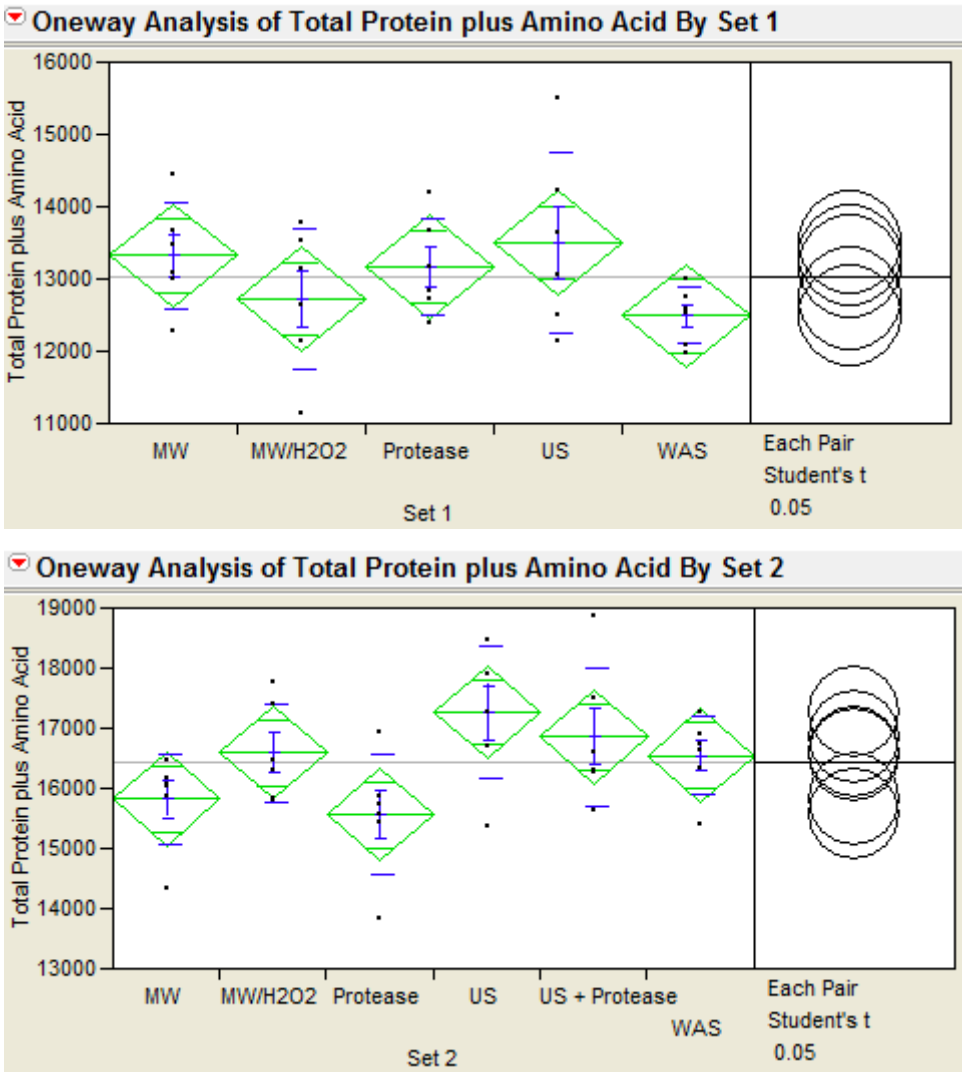


Figure 5.10 Student's t-Test for group comparisons of protein mass balance from various treatments

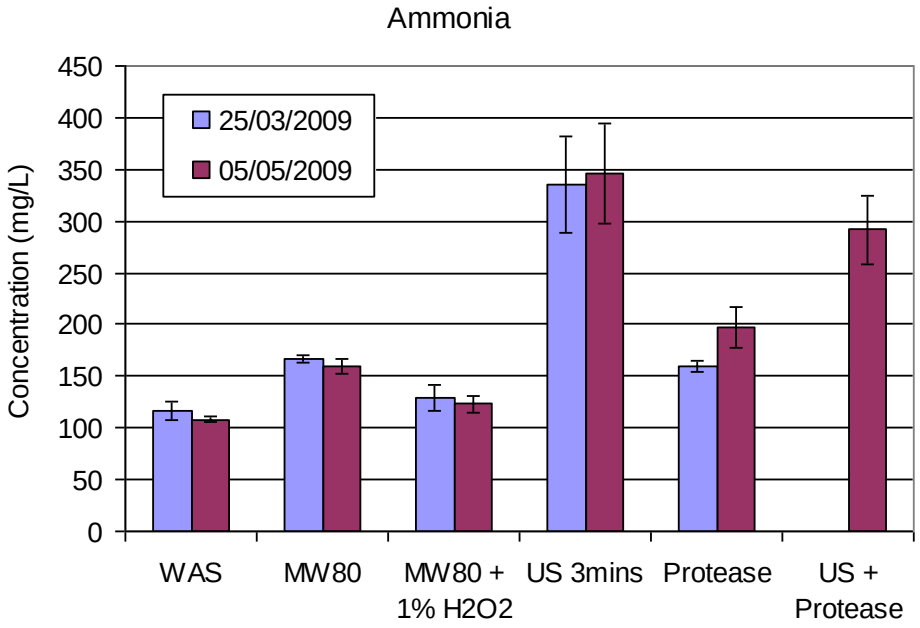


Figure 5.11 Soluble ammonia from various batch treatments

Table 5.4 Ammonium and potential free ammonia under various conditions

		Ammonium	Potential free ammonia			
			pH=7.0		pH=7.5	
			35°C	55°C	35°C	55°C
		mg/L	mg/L	mg/L	mg/L	mg/L
25/03/2009	WAS	117 ± 10	1.3	4.5	4.1	14.4
	MW	167 ± 3	1.9	6.4	5.9	20.6
	MW/H2O2	129 ± 12	1.4	5.0	4.5	15.9
	US	336 ± 37	3.7	12.9	11.8	41.5
	Protease	160 ± 5	1.8	6.1	5.6	19.7
05/05/2009	WAS	108 ± 3	1.2	4.2	3.8	13.4
	MW	159 ± 7	1.8	6.1	5.6	19.7
	MW/H2O2	123 ± 8	1.4	4.7	4.3	15.2
	US	347 ± 29	3.9	13.3	12.2	42.8
	Protease	197 ± 20	2.2	7.6	6.9	24.4
	US+Protease	292 ± 33	3.2	11.2	10.3	36.1

5.3.3 Polysaccharides and humic acids

The results for soluble polysaccharides are reported in Figure 5.12. Microwave, MW/H_2O_2 and ultrasound treatments all showed increased polysaccharide solubilization from an initial 140 mg/L in WAS, to an average 1396 mg/L, 1650 mg/L and 1789 mg/L, respectively. They represented approximately 41%, 50% and 53% of total polysaccharide, respectively. Protease treatment did not increase polysaccharide solubilization. The minor increase was probably due to the vortex mixing. A Protease dosage after ultrasound treatment yielded similar results to those for treatments with the ultrasound only.

The soluble humic acids results are shown in Figure 5.13. Humic acids are the product of the biodegradation of dead organic matter, such as lignin. The term refers to a complex mixture of many different acids containing the carboxyl and phenolate groups. By nature, these humic acids are resistant to further biodegradation (Stevenson, 1994). Also, the complexes formed by humic acids with ions are considered one of the main sources of EPS that protects the biomass cell from stress (Peter and Wuhrman, 1970; Riffaldi et al., 1982; Frolund et al., 1995). The levels of humic acids solubilization from sludge floc or EPS should be regarded as another aspect of pretreatment disintegration.

Both the microwave and MW/H_2O_2 treatments solubilized approximately 20-30% of humic acids. These results are consistent with the report from Eskicioglu et al. (2008). Interestingly, protease treatment also increased humic acid solubilization to an average of 16%. This might be attributed to the EPS structural changes resulting from extracellular protein hydrolysis. Ultrasound treatment, with its violent cavitation shear force, yielded the most humic acids solubilization at an average of 48%.

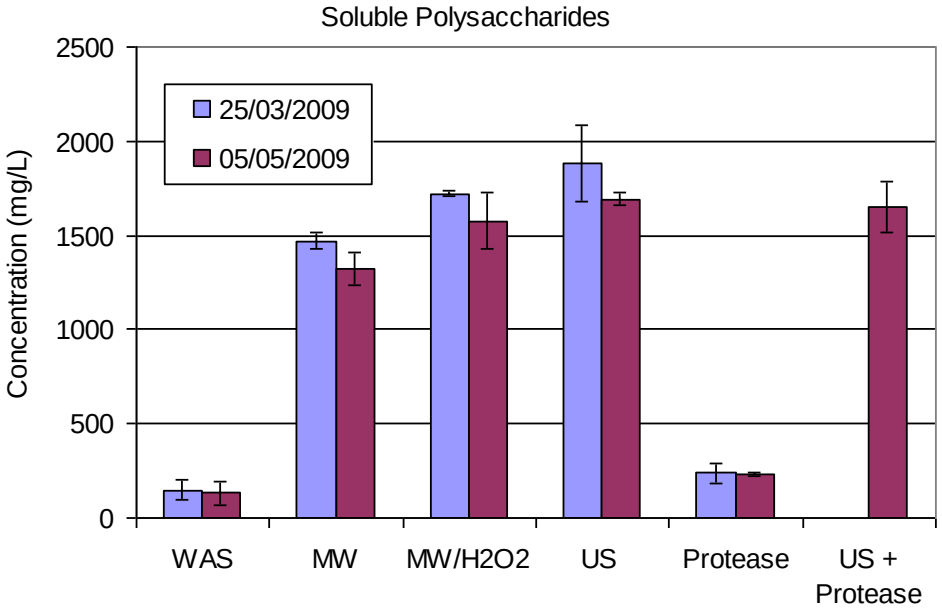


Figure 5.12 Soluble polysaccharides from various batch treatments

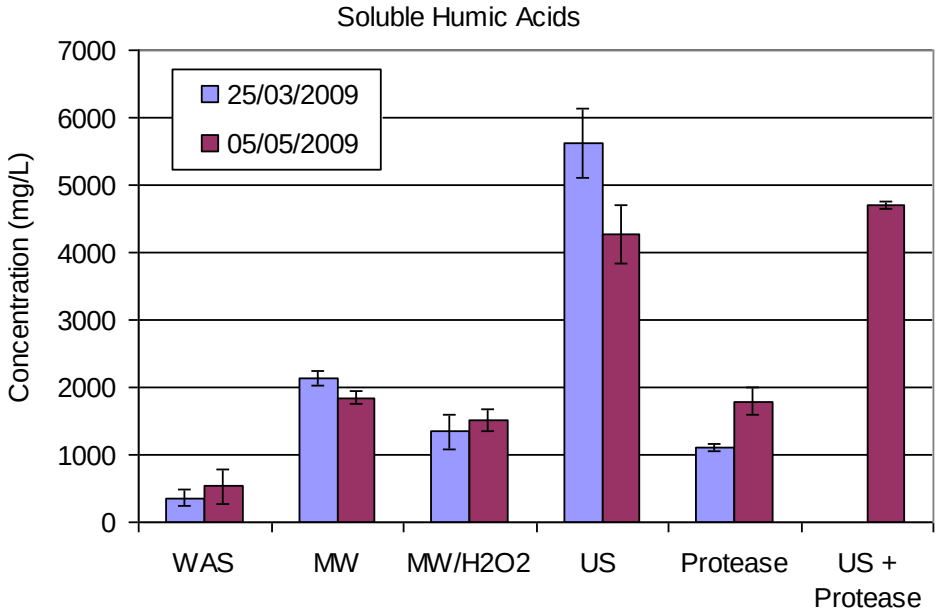


Figure 5.13 Soluble humic acids from various batch treatments

5.3.4 Orthophosphate

The soluble ortho-P levels are reported in Figure 5.14. The treatments which only used microwave yielded an average 180 mg/L ortho-P release, a substantial increase from the 33 mg/L in the initial WAS. With an addition of hydrogen peroxide, MW/H_2O_2 results showed less soluble ortho-P at the same treatment level (temperature of 80°C). These results were consistent with previous work (Chapter 3, 4; Liao et al. 2006a; Wong et al. 2006a, 2006b). The likely cause, as already discussed, was probably the high oxidation state created by the addition of hydrogen peroxide. Ultrasound treatment raised the ortho-P levels even further, to an average of 259 mg/L, while the Protease treatment was found to have a relatively minor increase on ortho-P solubilization, at 60 mg/L. A Protease dosage subsequent to ultrasound treatment did not increase ortho-P levels from treatment using only ultrasound.

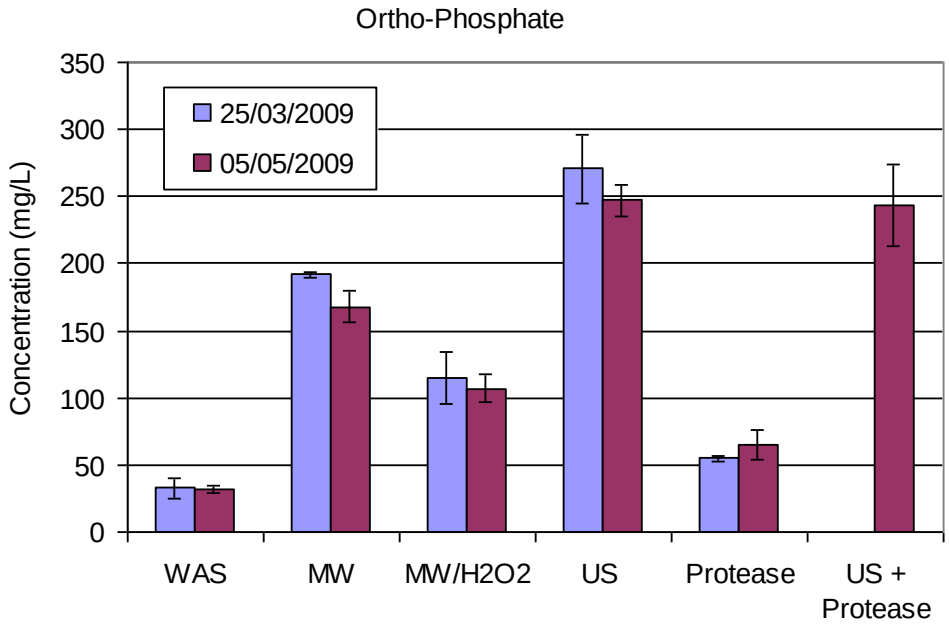
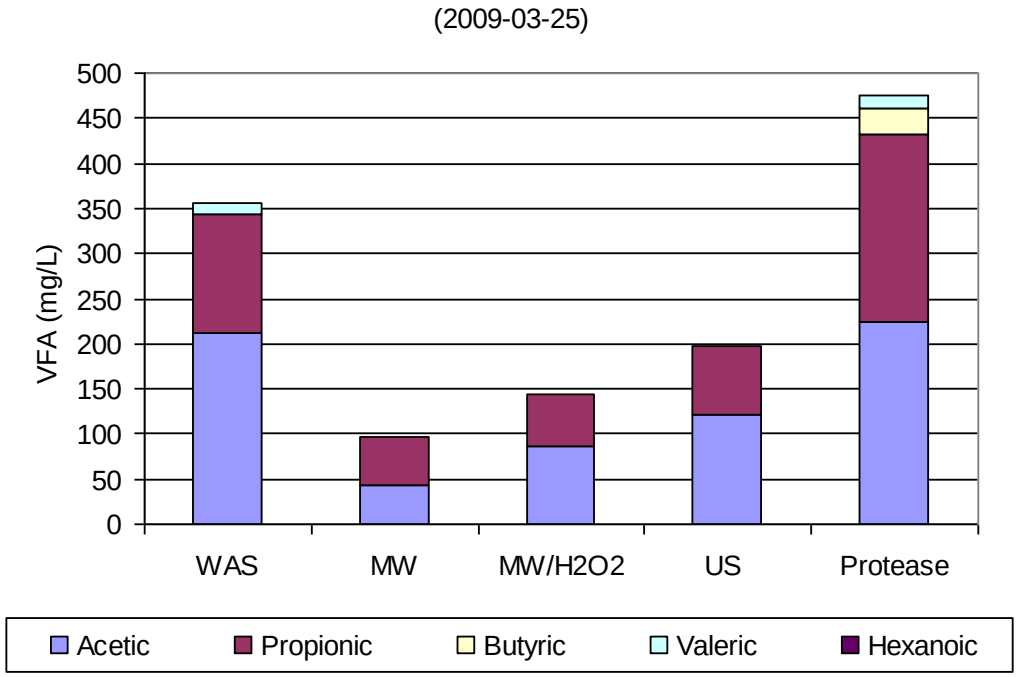


Figure 5.14 Orthophosphate release from various batch treatments

5.3.5 Volatile fatty acids

The volatile fatty acids results are reported in Figure 5.15. The initial VFA levels were high, with acetic and propionic acids being the main components. The total VFA (TVFA), expressed in milligrams of acetic acids per liter, was approximately 14-16% of the initial SCOD. These high values were partly due to the relatively low initial SCOD levels. With pretreatments, except for the protease treatment, all VFAs appeared to be in decline. As discussed in Chapter 4, VFA levels went through different phases, from an early decline to a later increase, depending on the treatment energy and oxidant levels. For microwave, MW/H_2O_2 and ultrasound treatments at the current level, VFAs declined. The TVFA values dropped to various degrees, and the TVFA to SCOD percentage was only at 0.7-1.9%.

In contrast, with the Protease treatment, all VFA levels, from acetic acid to hexanoic acid, increased. It was very likely that these increases were due to the larger amount of amino acids available for bio-degradation. The addition of Protease not only hydrolyzed protein to amino acids, but also facilitated bioactivity by providing more available substrates.



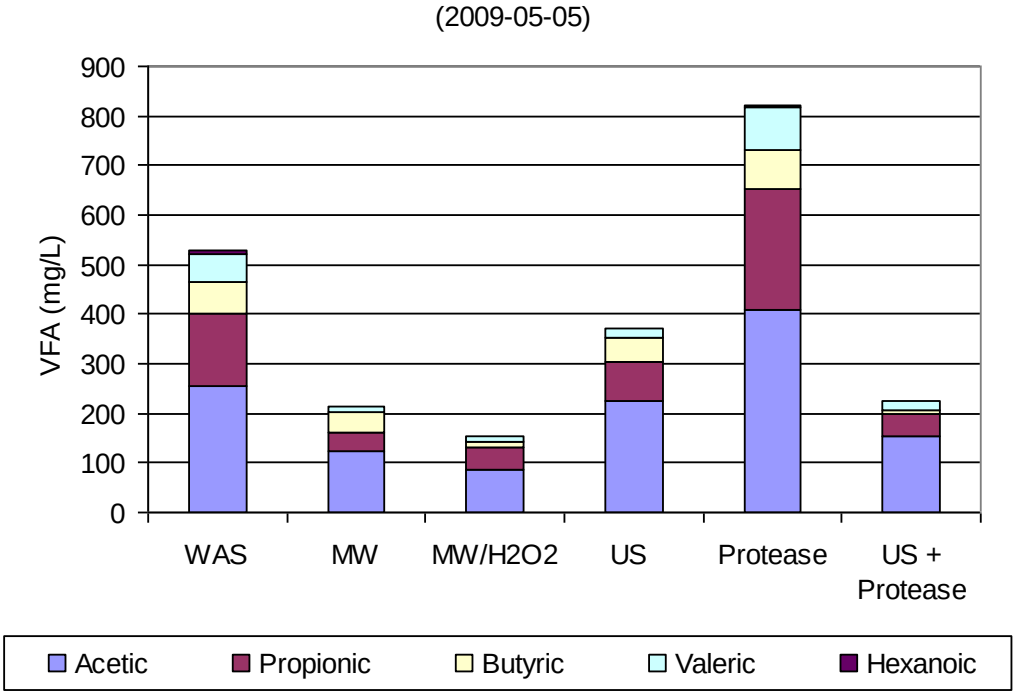


Figure 5.15 Individual volatile fatty acids from various treatments

5.3.6 DNA

The DNA release from the pretreatments is reported in Figure 5.16. In general DNA material is well hidden behind the biomass cell wall. The initial DNA level in the WAS was low, at an average of 24 mg/L. Therefore, any leakage in DNA could be considered as the direct consequence of cell destruction resulting from the pretreatments. Both microwave and MW/H_2O_2 treatments showed a substantial increase in DNA levels in the supernatant. Microwave treatment yielded an average of 133 mg/L, and MW/H_2O_2 reached 153 mg/L. These results were compared to Yu et al. (2010), where a diluted sludge (0.4% TS) was used. The higher DNA leakage recorded in the present study (at the same temperature and H_2O_2 specific dosage), show that sludge thickening has beneficial effects. The ultrasound treatment results recorded were 110 mg/L, lower than both microwave and MW/H_2O_2 treatment results at a similar energy level. Protease treatment did not result in major cell destruction, with a recorded DNA leakage of only 35 mg/L.

Microwave energy poses a direct stress on the cell membrane of a microorganism. It does so through both thermal and non-thermal effects. The thermal effect is mainly the denaturation of cell materials (Fellows, 2000). The non-thermal effects include electroporation, dielectric cell membrane rupture, magnetic field coupling, and selective heating (Kozempel et al. 1998). Ultrasound, on the other hand, works by its cavitation effect. In this case, at a low frequency (20 kHz), hydrodynamic shear force was considered the main source of cell destruction (Tiehm et al., 2001; Wang et al., 2005). At similar energy levels, these pretreatments yielded different degrees of cell destruction, with MW/H_2O_2 achieving the highest level of DNA leakage.

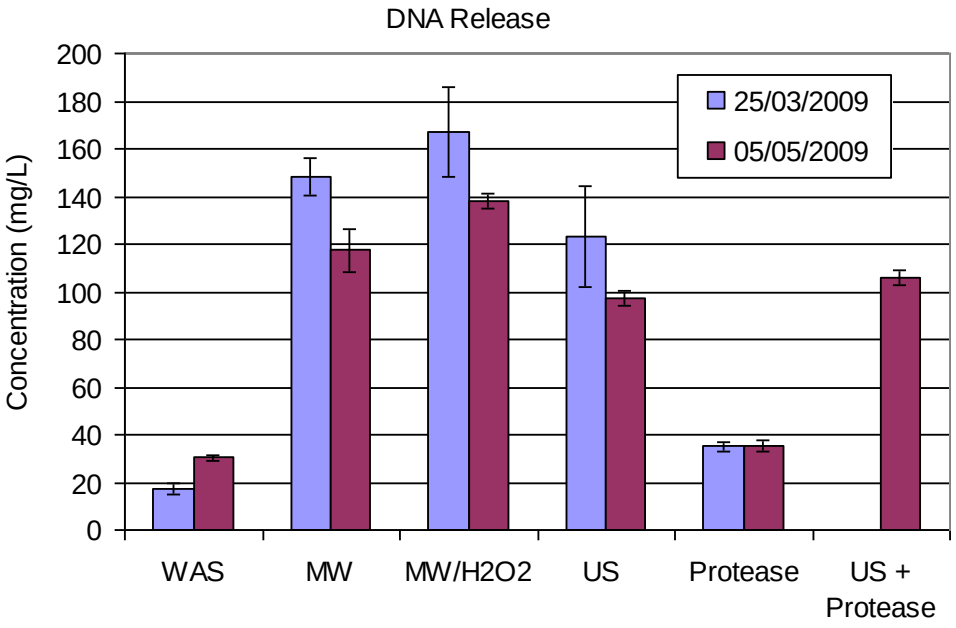
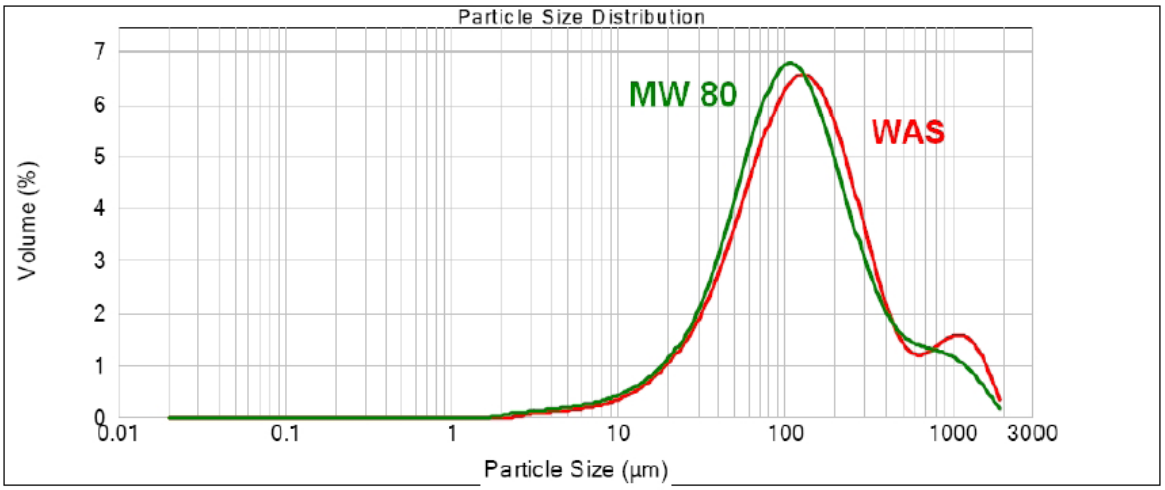
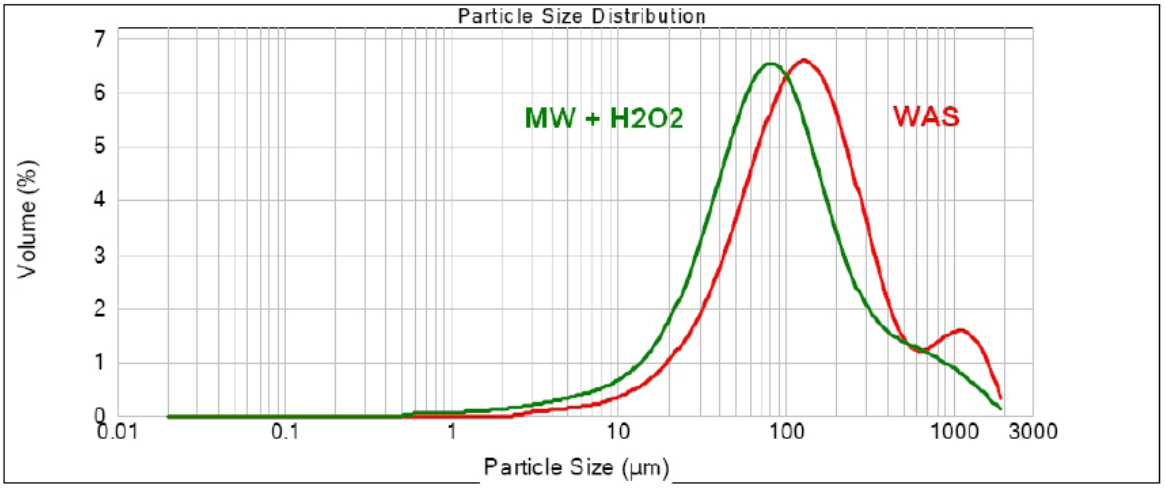


Figure 5.16 DNA release from various batch treatments

5.3.7 Particle size distribution

The direct contrast of particle size distribution, before and after pretreatment, is shown in Figure 5.11. The volume distributed diameter, peak size range and peak volume are reported in Table 5.5. The d(0.1), d(0.5) and d(0.9) each represented 10%, 50% and 90% of volume distributed below these diameter values. For the initial WAS, the particle size volume medium diameter [d(0.5)] was at 128 µm, with the peak ranging between 120 and 138 µm. With microwave treatment, the volume medium diameter and peak shifted slightly toward the smaller sizes. However, the distributions were still similar. The MW/H_2O_2 treatment further shifted the distribution toward the smaller sizes, with d(0.5) at 89 µm. The peak volumes remained the same. For both of them, the second peak in WAS, which was at the larger size range, appeared to be decreasing. This could indicate that the microwave energy or thermal effect could have fractured some of the bulky sludge flocs. With ultrasound cavitation, the particle size distribution turned more heterogeneous, with d(0.5) at 80 µm and peak volume much lower at 4.5%. The d(0.1) was drastically reduced from 35 µm to 8 µm. It showed that particles from size 1 to 10 µm had largely increased. Protease treatment also reduced the overall particle sizes, but the distribution pattern remained the same.





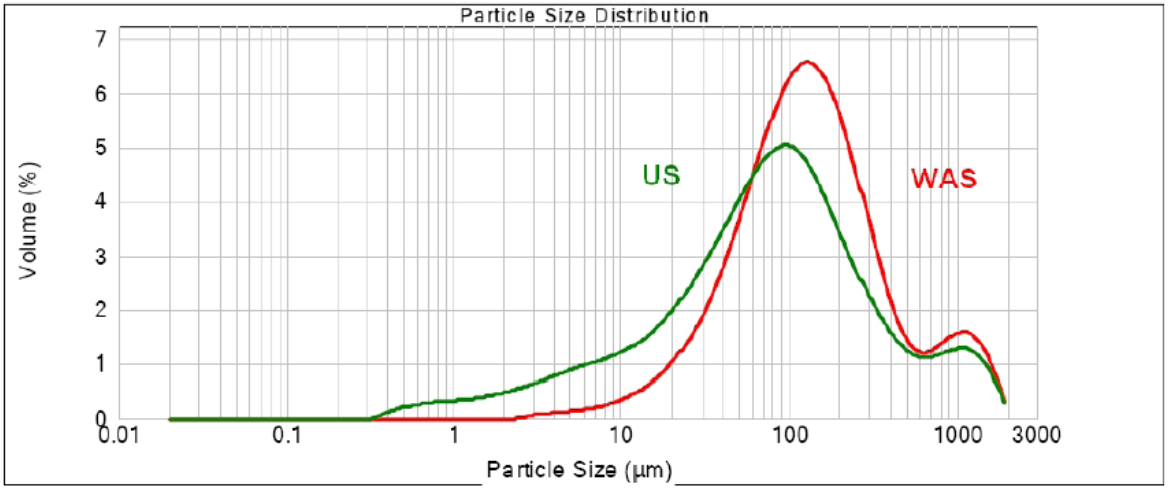


Figure 5.17 Particle size distributions before and after treatments

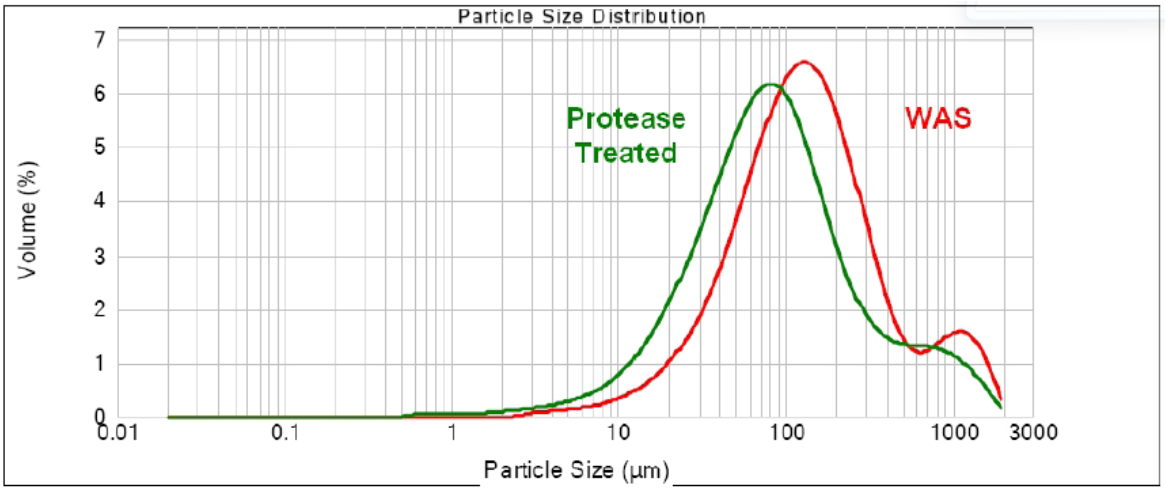


Figure 5.17 Particle size distributions before and after treatments (cont)

Table 5.5 Volume distributed diameters and peak volumes of particles

	d (0.1)	d (0.5)	d (0.9)	peak	peak volume
	µm	µm	µm	µm	%
WAS	35	128	581	120-138	5.93
MW	32	112	468	104-120	6.11
MW/H_2O_2	22	89	551	79-91	5.61
US	8	80	476	91-104	4.51
Protease	20	81	441	79-91	5.55

5.3.8 Floc microscopic imaging

Figure 5.12 shows the comparison of sludge floc before and after the treatments in visual images, while Figure 5.13 shows the comparison in microscopic images. Visual observation revealed that the WAS floc structure had gone through various degrees of destruction. Microwave treatment loosened up some parts of the tightly bonded sludge floc. However, no significant changes were observed. With the addition of hydrogen peroxide and its associated bubbling, the sludge floc structure was further disrupted after the MW/H_2O_2 treatment and more floc material was mobilized. Ultrasound treatment showed a greater capability for disrupting the floc structure. This is largely due to the fact that cavitation is essentially a physical phenomenon. The hydrodynamic shear force it generated was likely the dominant source of this disruption (Tiehm et al., 2001; Wang et al., 2005). Protease treatment did break loose some of the floc structure. The enzymatic effect on EPS protein breakdown might have contributed to this change, but it is also likely that it was partly due to the vortex mixing.

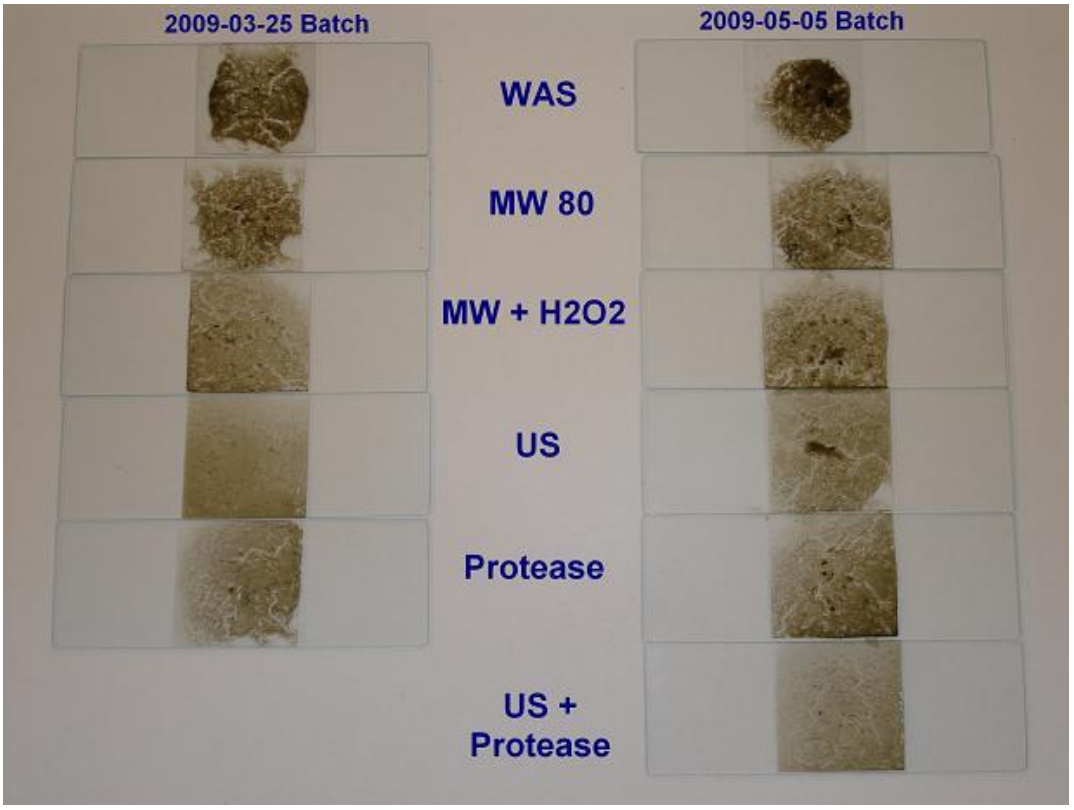
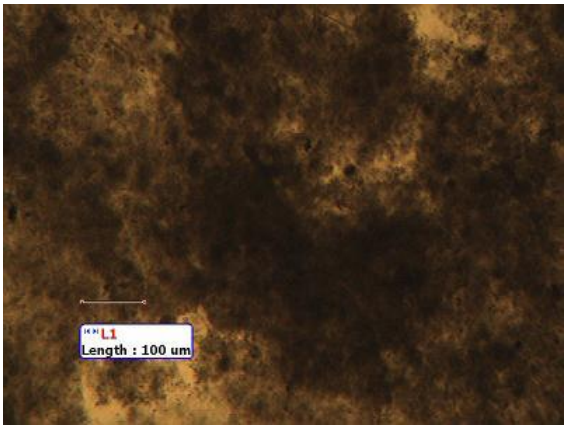
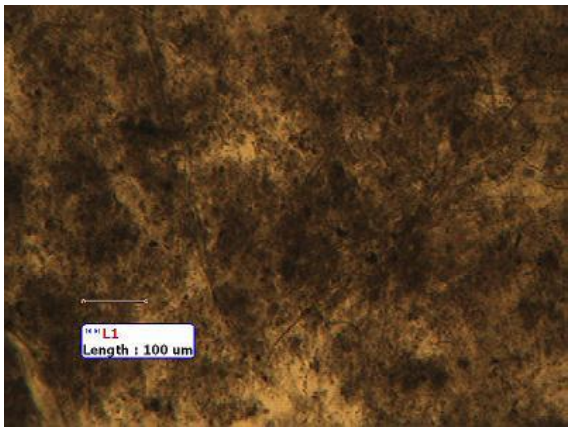


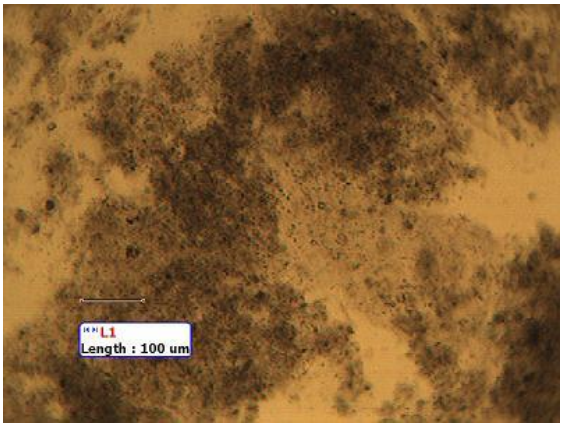
Figure 5.18 Visual comparison of sludge floc changes from various batch treatments



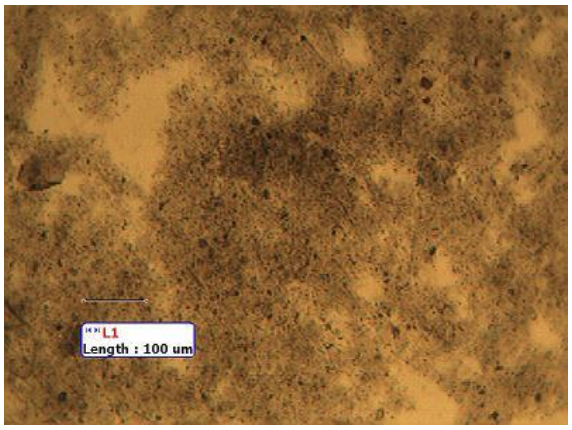
WAS



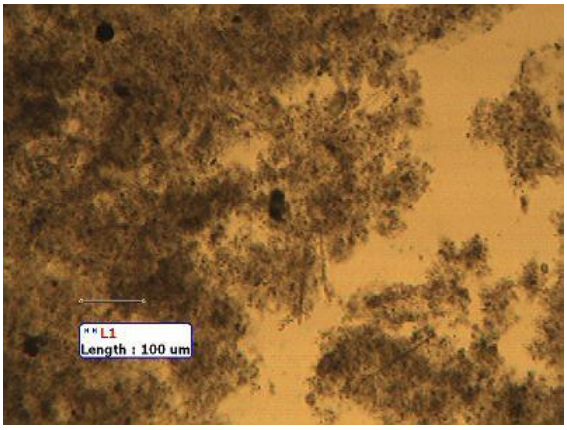
Microwave



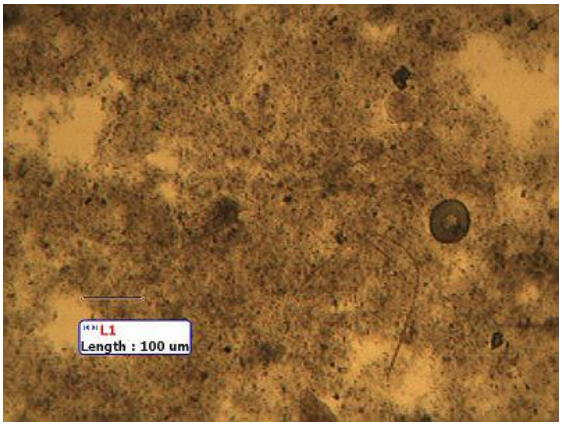
MW/H_2O_2



Ultrasound



Protease



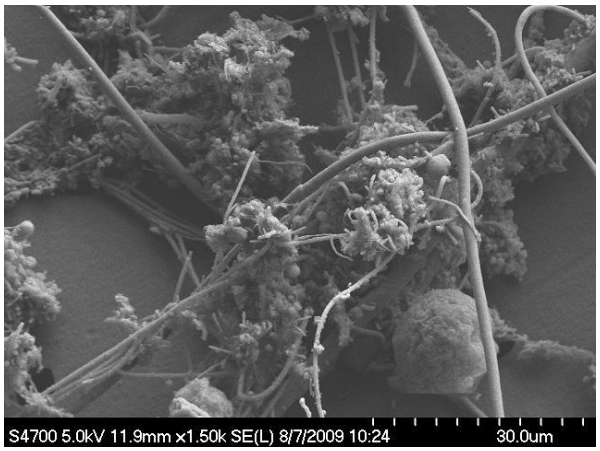
US+Protease

Figure 5.19 Microscopic comparison of sludge floc changes from various batch treatments (2009-05-05)

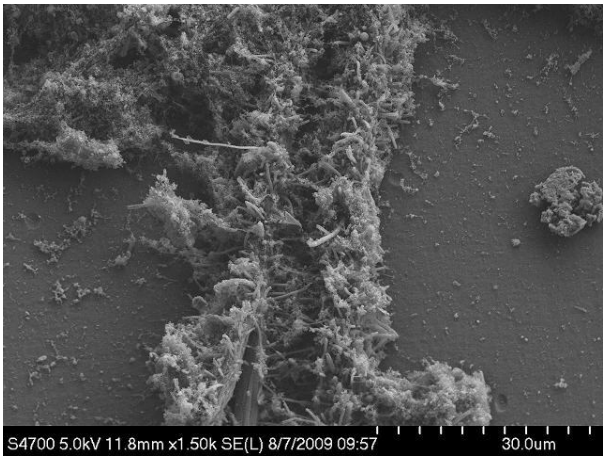
5.3.9 Scanning electron microscopic (SEM) imaging

The scanning electron microscopic imaging not only provided a more detailed view of the changes in the sludge floc, but also allowed observations on the actual effects of the treatments on biomass cell destruction. Two groups of SEM images are presented in Figure 5.14 for sludge floc changes, and in Figure 5.15 for cell destruction.

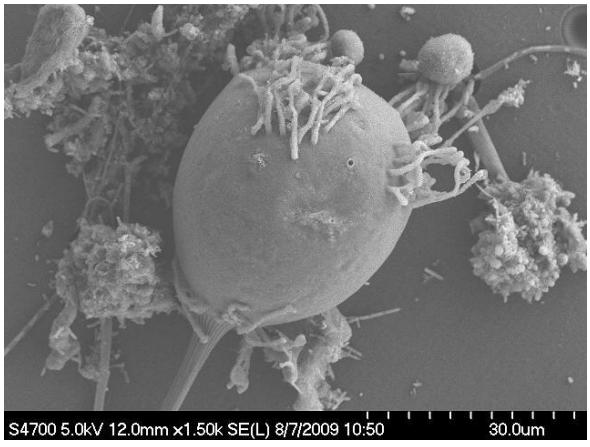
In the first row of Figure 5.14, the comparison of WAS with the ultrasound/protease treatment showed that the sludge floc structure had gone from intact to complete destruction. The destruction rendered by the microwave, MW/H_2O_2, protease and ultrasound only treatments was of different degrees along this continuum. Despite a high level of DNA leakage, a significant portion of cell organisms remained intact after both microwave and MW/H_2O_2 treatments. In Figure 5.15, the images show that in all but the ultrasound/protease treatment, the bacterial cells were not destroyed. This suggests it was unlikely that the DNA leakage came from a complete destruction of the cell wall. Electroporation and dielectric cell membrane rupture (Kozempel et al. 1998; 2000; Datta and Davidson 2000; Brunkhorst et al. 2000) could have contributed to the DNA leakage. Surprisingly, ultrasound/protease treatment yielded an almost complete destruction of the cell wall (Figure 5.15).



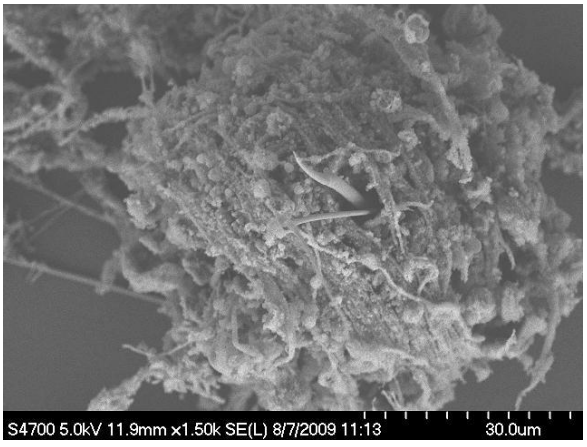
WAS



US+Protease

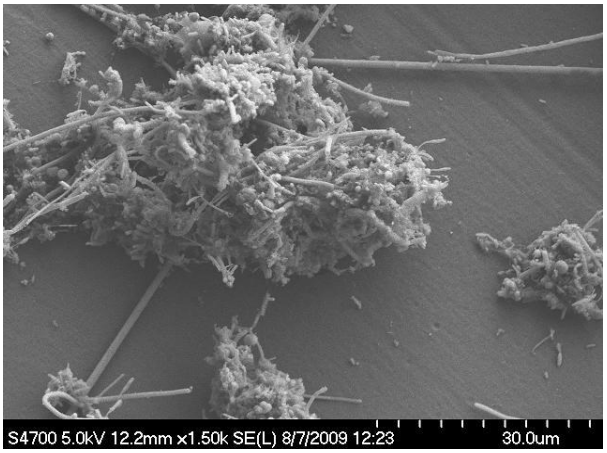


MW



MW/H_2O_2

US



Protease

Figure 5.20 SEM images for sludge floc changes

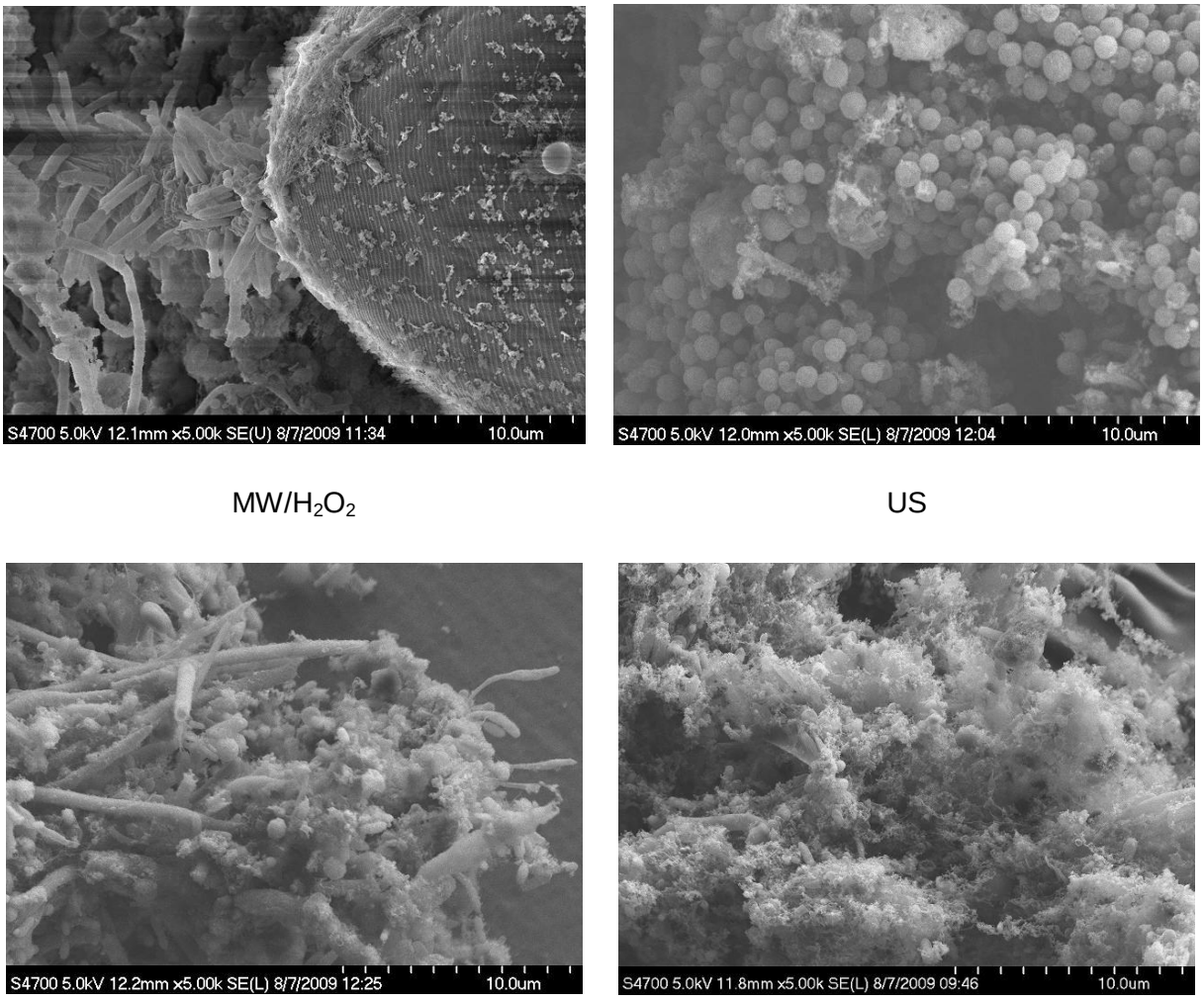


Figure 5.21 SEM images for cell destruction

5.4 Conclusions

In this chapter, the effect of microwave, MW/H_2O_2, ultrasound, protease and ultrasound/protease treatments on WAS solubilization and physical properties was investigated. The comparison was based on similar specific energy levels in the range of 4933 to 6667 kJ/kg-DS. The study examined WAS solubilization from macromolecule organics (VS, COD, protein, polysaccharides, humic acids, DNA) to micromolecules organic (amino acids, VFAs) and inorganic nutrients (NH_4^+/NH_3-N, PO_4-P). The physical property changes were investigated in terms of particle size distribution, floc imaging and SEM imaging. The conclusions from this study are listed as follows:

- TS, VS and TCOD from all treatments remained relatively constant, indicating that the treatments at these levels were mostly from the disintegration/hydrolysis stage;
- Ultrasound treatment appeared to be slightly more energy efficient in terms of COD solubilization;
- Microwave and MW/H_2O_2 treatments resulted in a higher soluble protein in the supernatant. However, when considering all amino acids levels, it was found that ultrasound treatment yielded better protein disintegration and hydrolysis;
- A dosage of protease subsequent to ultrasound treatment further enhanced protein degradation;
- Low levels of ammonium after all treatments indicate that protein degradation largely proceeded only to amino acids;
- Microwave, MW/H_2O_2 and ultrasound treatments all yielded a high degree of polysaccharide solubilizaton. Protease had little or no effect on polysaccharides;
- Ultrasound treatment resulted in a substantial increase in soluble humic acids;

- All treatments except protease achieved significant levels of ortho-P solubilization;
- All treatments except protease decreased total and individual VFAs. Protease, on the other hand, facilitated more VFA production by providing more available amino acids substrates;
- Protease treatment yielded good results in amino acids and VFAs, but not in SCOD, polysaccharides and ortho-P.
- Microwave and MW/H_2O_2 pretreatments had higher levels of DNA leakage than ultrasound and protease. This was likely due to the non-thermal effects of electroporation on cell membranes;
- The particle sizes were reduced by all treatments;
- The particle size distribution pattern remained similar for the microwave, MW/H_2O_2 and protease treatments, before and after treatment. Ultrasound pretreatment altered this pattern to a further non-uniform distribution;
- SEM imaging revealed that cell wall destruction was not completed by microwave, MW/H_2O_2, ultrasound only or protease treatments. Ultrasound pretreatment with protease appeared to have a better result in terms of cell wall destruction.

Chapter 6 Mesophilic and Thermophilic Anaerobic Digestion of Microwave, Microwave/H_2O_2, Ultrasound, and Protease Pretreated Waste Activated Sludge *

6.1 Introduction

Chapters 2 through 5 have shown that (microwave treatment and the microwave/ hydrogen peroxide process (MW/H_2O_2) can achieve substantial organic solubilization and floc/cell destruction in waste activated sludge (WAS). Ultrasound treatment was showen to be more energy efficient due to its physio-chemical cavitation effect. The protease treatment improved the amino acid formation, and the process does not required additional energy input other than mixing. All these pretreatments proved to be effective to some degree in the first step of WAS digestion. However, any pretreatment must be connected to anaerobic digestion in order to prove its net worth.

Anaerobic digestion is a critical step in a wastewater treatment plant. It provides benefits, such as putrescible organic stabilization, pathogen reduction and energy recovery. Even though anaerobic digestion (under mesophilic conditions) seems robust, a successful ongoing operation requires the maintenance of a healthy microorganism community and the delicate balancing of operating conditions such as pH, temperature and organic loading. Thermophilic digestion is often more susceptible to changes in conditions than mesophilic

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digestion. Pretreatments inevitably alter the chemical and physical characteristics of the WAS which serves as feed to the process. A careful examination of the benefits and potential impact on mesophilic and thermophilic digestion of the various pretreatments is therefore necessary.

The impact of both the MW/H_2O_2 and protease treatments on anaerobic digestion is largely unknown. Eskicioglu et al. (2008) reported that MW/H_2O_2 treated WAS had lower mesophilic biodegradation rates and ultimately, lower methane production, when compared to WAS control and to microwave-treated sludge. These authors have suggested that the soluble organics from MW/H_2O_2 were slow to biodegrade or were refractory. However, the inoculums used in the study were acclimated to microwave treated (at 175°C) WAS and the COD loading (52-64 g/L) was in excess of the level recommended (2 g/L) by Owen et al. (1979). The high initial loading and the very specific acclimation could have masked the effects of altered biodegradability and of any possible toxicity for digestion.

Ultrasound treated sludge used for mesophilic anaerobic digestion has been well studied (Shimizu et al., 1993; Neis et al, 2000; Tiem et al., 2001; Gronroos et al., 2005; El-Hadj et al., 2007). Many researchers have reported positive volatile solids reduction results and increased biogas production (Wang et al., 1999; Bougrier et al., 2004; Hogan et al., 2004; Bragulia et al., 2008). Some research results, however, have been contradictive (Tiem et al., 1997; Latitte-Trouque and Forster 2002). These inconclusive results were likely the result of variations in equipment, energy levels, initial sludge quality, inoculums, loadings, operating conditions, etc. Other than for soluble COD, there is also little information regarding the correlation of digestion efficiency or biogas production to the changes resulting from pretreatments. As was said in earlier chapters, COD is a convenient

parameter. On its own, however, it may not accurately reflect disintegration (and hydrolysis) or the impact of pretreatment on the subsequent complex steps of acidogenesis, acetogenesis and methanogesis.

In this chapter, the details for both mesophilic and thermophilic digestion of microwave, MW/H_2O_2, ultrasound and protease pre-treated WAS are presented. Two levels of organic loading, 1.7 and 6.8 g-VS/L (or TCOD loading 2.5 and 10.1 g-COD/L) were studied in terms of changes in biodegradability and for possible toxicity (or inhibition) resulting from the pretreatments. The correlation between biogas production and various pre-treatment parameters (reported in Chapter 5) was also investigated.

6.2 Material and Methods

6.2.1 Sludge characteristics

Thickened secondary waste activated sludge (TWAS) was obtained from the Lulu Island Wastewater Treatment Plant (WWTP), located at the south end of the City of Richmond, BC. This plant uses Trickling Filter - Solids Contact (TF-SC) processes to provide secondary sewage treatment for a population of approximately 197,000 (year 2010 estimate by City of Richmond). Secondary waste activated sludge taken from sludge contact tanks was thickened by dissolved air floatation and then fed to the mesophilic digesters.

Fresh sludge samples for this study were collected for the same day treatment and digestion. The typical characteristics of this sludge are reported in Table 5.1.

6.2.2 Inoculums

The inoculum for mesophilic digestion was obtained from the Lulu Island Wastewater Treatment Plant digesters. These operate at 37°C and 33 days of solids retention time (SRT). A mixture of primary and secondary sludge is digested. The organic loading rate of the digesters is about 1 kg-VS/m^3/day. The average volatile solids reduction is approximately 65%.

The inoculum for thermophilic digestion was obtained from the Annacis Island Wastewater Treatment Plant digesters. These operate at 55°C and 28 days of total SRT. The Annacis Island WWTP is also a secondary treatment plant. It uses a Trickling Filter - Solid Contact (TF-SC) as the main wastewater treatment processes. Located at Annacis

Island, between the cities of Richmond, Surrey and New Westminster, BC, it serves approximately a million people. The Annacis Island WWTP sludge digestion system consists of four large thermophilic digesters operated in parallel. In order to kill pathogens, three Flow Through Vessels (FTV) are operated in series at thermophilic temperatures. The organic loading rate is approximately 1.5 kg-VS/m^3/day and the average volatile solids reduction from this extended thermophilic digestion process is 63%.

6.2.3 Microwave, ultrasound apparatus and treatment processing

A closed-vessel microwave digestion system (Ethos TC Digestion Labstation 5000, Milestone Inc., U.S.A.) and an ultrasonic set (UIP1000 ultrasonic processor from Hielscher Ultrasonics GmbH, Berlin, Germany) were used in this study. The microwave system was described in Section 2.2.1 and Figure 2.1. The ultrasound system was described in Section 4.2.1 and Figure 4.1. The treatment processing details were reported in Section 5.2.2 for microwave treatment and MW/H_2O_2 treatment, in Section 5.2.3 for ultrasound treatment, and in Section 5.2.4 for the protease treatment. In summary, specific energy was controlled at 4933-6671 kJ/kg-DS. The hydrogen peroxide addition for MW/H_2O_2 treatment was 1% in wet weight, averaged 0.29 mg-H_2O_2/mg-TCOD (or 0.38 mg-H_2O_2/mg-DS). The protease (P5147) specific dosage was at 0.002 g-Protease/g-COD (or 0.0026 g-Protease/g-DS). The chemical analysis of the WAS and treated samples was previously described in Section 5.2.5.

6.2.4 Mesophilic and thermophilic anaerobic digestion

The biodegradability and potential impact of pretreated WAS on both mesophilic and thermophilic anaerobic digestion were conducted in biochemical methane potential assay tests (Owen et al. 1979). Serum bottles (total volume of 150 mL) with butyl rubber stoppers were used in this evaluation. The pretreated WAS samples, 5 mL and 20 mL, were added to inoculums of 95 mL and 80 mL, respectively, to make up a total volume of 100 mL in each bottle. Accordingly, the organic loadings of these feeds were approximately 1.7 and 6.8 g-VS/L (or TCOD loading 2.5 and 10.1 g-COD/L). The measured alkalinities in both inoculums were in the range of 3500 to 4000 mg/L. No additional alkalinity was therefore added. Nitrogen sparging was applied to each batch reactor bottle at the beginning of digestion. An INNOVA 4230 Incubator/Shaker (New Brunswick Scientific, NJ, USA) was used to provide a dark, temperature-controlled (35±1°C and 55±1°C) incubation environment. The shaker moved at 100 rpm in order to provide uniform mixing conditions. Each condition was run with five replicates. Biogas production was measured twice daily for the first seven days and once daily thereafter. Measurement was done by inserting a needle attached to a manometer (Fish Scientific, USA). Biogas composition was determined early in the process at day 5 or day 7, and again at the end of digestion. A Fisher-Hamilton Gas Partitioner was used.

6.3 Results and Discussion

6.3.1 Mesophilic anaerobic digestion

6.3.1.1 Microwave-pretreated WAS

The mesophilic biogas production from untreated WAS (feed control), microwave-pretreated sludge and the inoculums (seed control) are shown and compared in Figure 6.1. The standard deviation of the biogas production in these batch digestion tests were found to be generally less than 3% of the group average, thus the deviation bars were omitted from these figures for better clarity to the figures. However, the standard deviations are shown on the student's t-test in Figure 6.2, for the statistical comparison of the daily biogas production between the treated feed and untreated WAS feed. The comparison was made at 95% confidence interval.

The first row of Figure 6.1 records the total accumulated biogas volume over a period of 40 days, for both loading conditions (1.7 g-VS/L on the left, 6.8 g-VS/L on the right column). The second row of Figure 6.1 shows the daily biogas production, and the third row illustrates the accumulated biogas production from the feed. This was obtained by subtracting the contribution by inoculums from the total accumulated biogas.

Results for the seed-control biogas production shown in Figure 6.1 establish that the inoculums were healthy and active. It was critical to first ascertain that there was a stable and working microorganism community in order to separate the effects of the pretreatments from other interferences from any operating issues. As long as close and accurate monitoring of the seed control is carried out in these types of experiments, the biogas production from the feed and that from the inoculums can be separated.

At both loadings of 1.7 and 6.8 g-VS/L, the microwave-pretreated sludge results showed no inhibition of the digestion process when compared to the results for untreated WAS. With an organic loading of 1.7 g-VS/L, the biogas production was at its maximum for digestion on day 1 and decreased thereafter. The amount of microorganism in the inoculums was sufficient to take on any additional available (soluble) substrates. The biogas production from the feed (both pretreated and untreated) decreased to a minimum at day 11. This indicates that the biodegradation of feed had virtually been completed.

At an organic loading of 6.8 g-VS/L, both untreated WAS and microwave-pretreated sludge appeared to be slightly overloaded for the amount of seeding. In both cases, the maximum daily biogas production occurred on the second day of digestion. This one day of lag time was apparently the result of the overloading and not due to the pretreatment, since the control with untreated WAS feed showed exactly the same pattern. By digestion day 14, the biodegradation of feed had been completed. The rest of the reaction could be considered the respiratory decay of the microorganisms themselves.

The student's t-test shown on Figure 6.2 confirmed that the biogas production from untreated WAS and microwave treated sludge are statistically different at 95% confidence interval (except the first day with 1.7 g-VS/L).

Figure 6.3 (a) shows the increase in biogas production from the microwave-pretreatment feed as a percentage increase over the untreated WAS feed control for the total accumulated biogas. Figure 6.3 (b) shows this for the daily biogas. The total biogas increase represents the biodegradability improvement, and the daily biogas increase represents the reaction speed (rate) acceleration.

By the end of digestion, the overall biodegradability improvement from microwave

pretreatment was approximately 12-15% for the treatment levels of temperature 80°C (specific energy 6159 kJ/kg-DS). The maximum total biogas increase was at day 5 and day 6. This was very likely due to the time needed for resistant particulates in untreated WAS to be broken down and hydrolyzed. Once this hydrolysis was completed, the untreated WAS regained some of the loss relative to the pretreated sludge.

A daily biogas increase was found from digestion day 1 to day 7 for loading condition of 1.7 g-VS/L. For the 6.8 g-VS/L loading conditions, this occurred from day 1 to day 14. This daily biogas increase, or reaction rate acceleration, was likely the result of the increases in soluble substrate resulting from pretreatment. The maximum daily biogas increase was approximately 25% for organic loading at 1.7 g-VS/L, and 70% for the 6.8 g-VS/L loading conditions. The higher percentage increase at the higher loading rate (6.8 g-VS/L) was likely due to the availability of 4 times more substrate, as shown in the reaction kinetic study.

The specific biogas production from feed (1.7 g-VS/L condition) were 0.83 and 0.84 L/g-VS-destroyed, for untreated WAS and microwave treatment feed, respectively. They were 0.84 and 0.90 L/g-VS-destroyed, for the high loadings conditions (6.8 g-VS/L). These numbers are summarized in Section 6.3.1.5, and compared to other pretreated feeds.

It was found that the biogas production was a second order reaction (Figure 6.3). This finding contradicted to a previous hypothesis of first order kinetics (Eskicioglu et al., 2008). The Integration Method (Tchobanoglous et al., 2003) was used to determine the reaction rate by plotting $\ln(V_m/(V_m-V))$ versus digestion time t, for the first-order reaction (Figure 6.4 a), and $1/(V_m-V)$ versus t, for the second-order reaction (Figure 6.4 b). The abbreviation of V stands for cumulative biogas (or methane) volume, and Vm stands for the maximum

cumulative biogas (or methane) volume at the end of digestion period.

The reaction rate laws (equation), and integrated rate laws, in terms of substrate (biodegradable organic material remaining concentration, C, and initial concentration C_o) degradation could typically be expressed as following:

$$r_c = -\frac{dC}{dt} = kC \qquad \text{(first order rate law)}$$

$$r_c = -\frac{dC}{dt} = kC^2 \qquad \text{(second order rate law)}$$

and

$$\ln\frac{C}{C_o} = -kt \quad \text{or} \quad C = C_o e^{-kt} \qquad \text{(first order integrated rate law)}$$

$$\frac{1}{C} - \frac{1}{C_o} = kt \quad \text{or} \quad C = \frac{C_o}{ktC_o + 1} \qquad \text{(second order integrated rate law)}$$

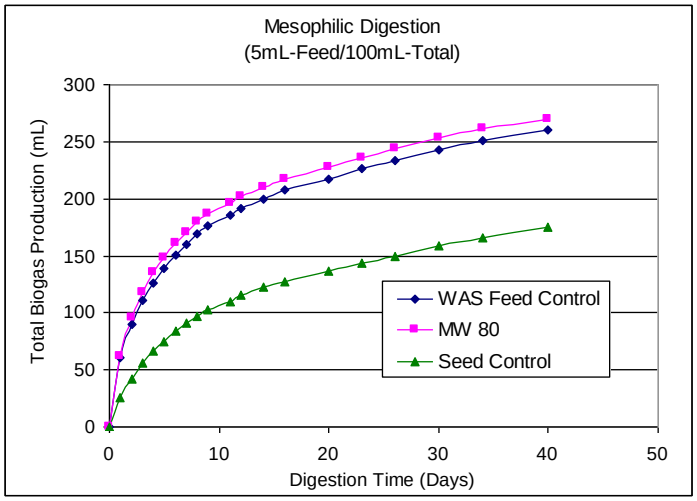
The expressions in terms of biogas production could be as modified by substitute (V_m-V) for C and V_m for C_o.

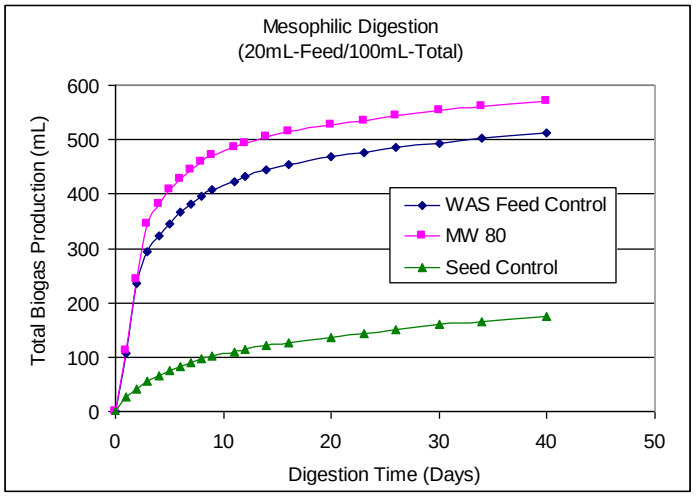
$$\ln\frac{(V_m - V)}{V_m} = -kt \quad \text{or} \quad V = V_m(1 - e^{-kt}) \qquad \text{(first order integrated rate law)}$$

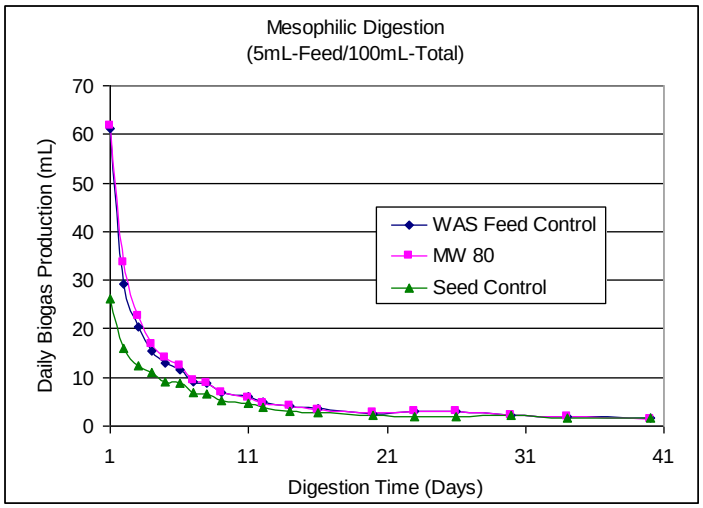
$$\frac{1}{(V_m - V)} - \frac{1}{V_m} = kt \quad \text{or} \quad V = V_m\left(1 - \frac{1}{ktV_m + 1}\right) \qquad \text{(second order integrated rate law)}$$

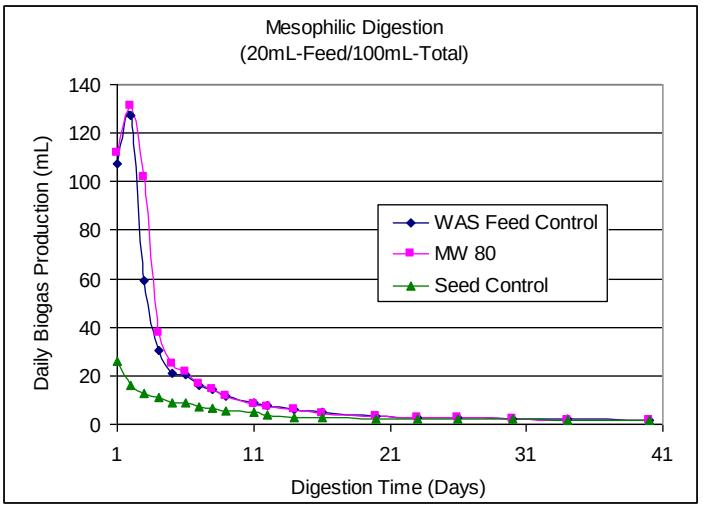
The second order reaction rate coefficient (k) and kinetic fits (R^2) are shown in Figure 6.1 (third row graphs) and in Figure 6.4 (b). Figure 6.4 (a) shows the first order kinetic fits. It demonstrates that the second order kinetic was a better fit to the actual biogas production. This indicates that, in a well-controlled environment with a sufficiently healthy microorganism community, the biogas production or volatile solid reduction reaction rate is a function of squared substrate concentration ($r_C = -kC^2$).

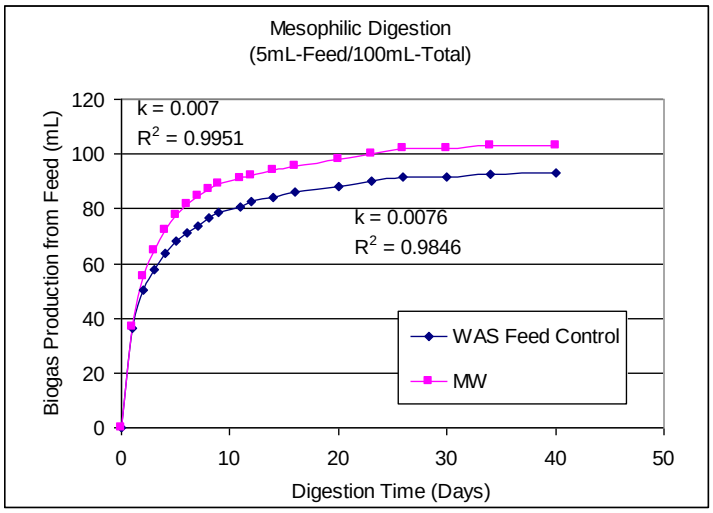
The biogas composition from microwave-pretreated feed at digestion days 5 and 33 is shown in Figure 6.5. As expected, the biogas consisted mostly of methane (CH_4) and carbon dioxide (CO_2). The ratios of methane to carbon dioxide were in the range of 1.6 to 1.88. This is the normal range in batch operations (Tchobanoglous et al., 2003). The initial nitrogen gas in the headspace was diluted by the volume of biogas produced.











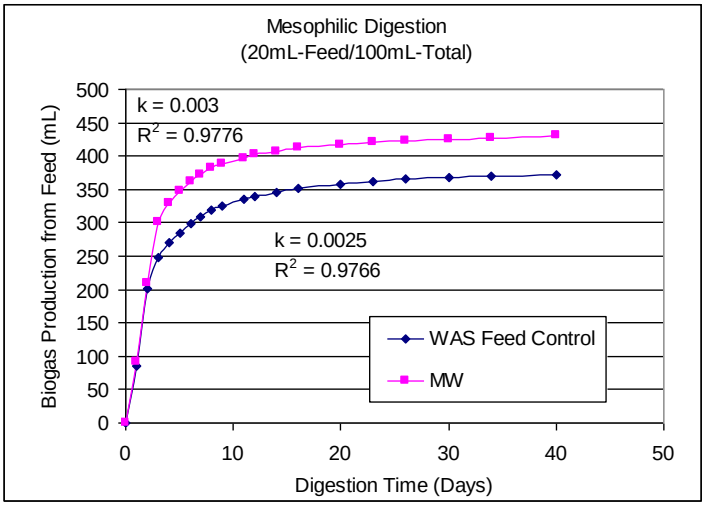


Figure 6.1 Mesophilic digestion of microwave treated sludge at two organic loading conditions (1.7 g-VS/L on the left column, 6.8 g-VS/L on the right column)

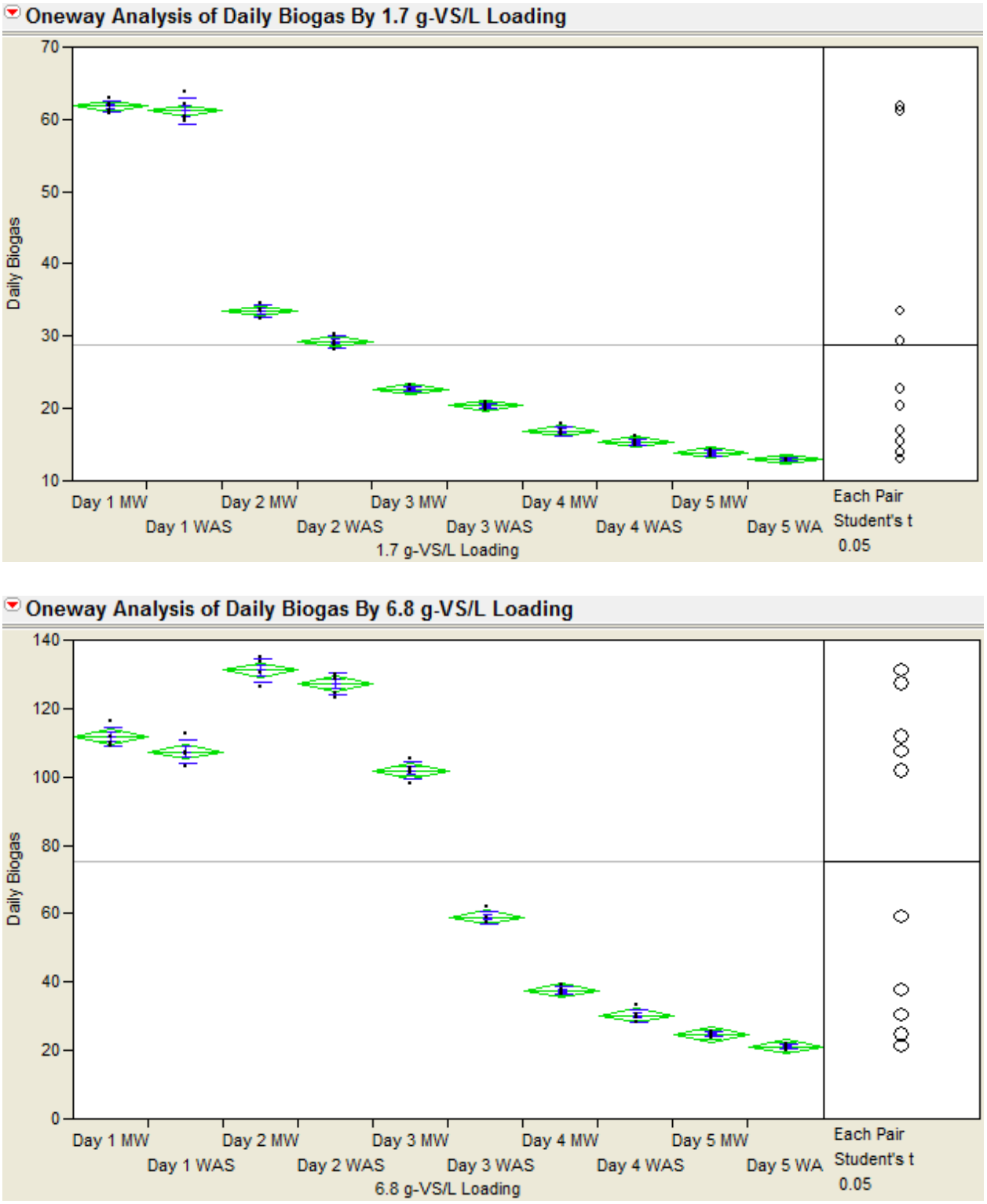
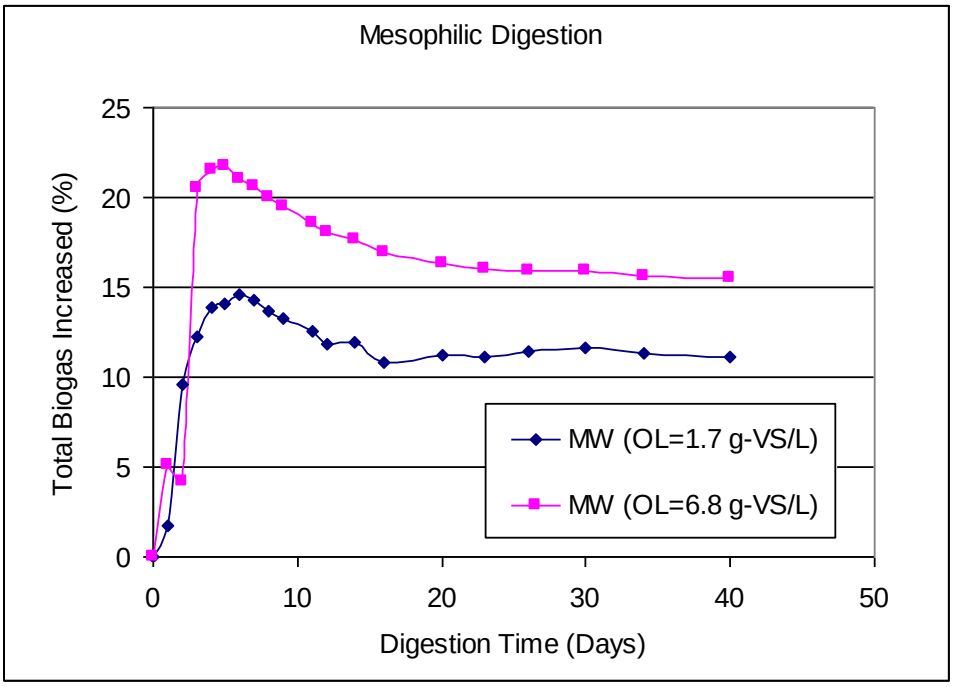
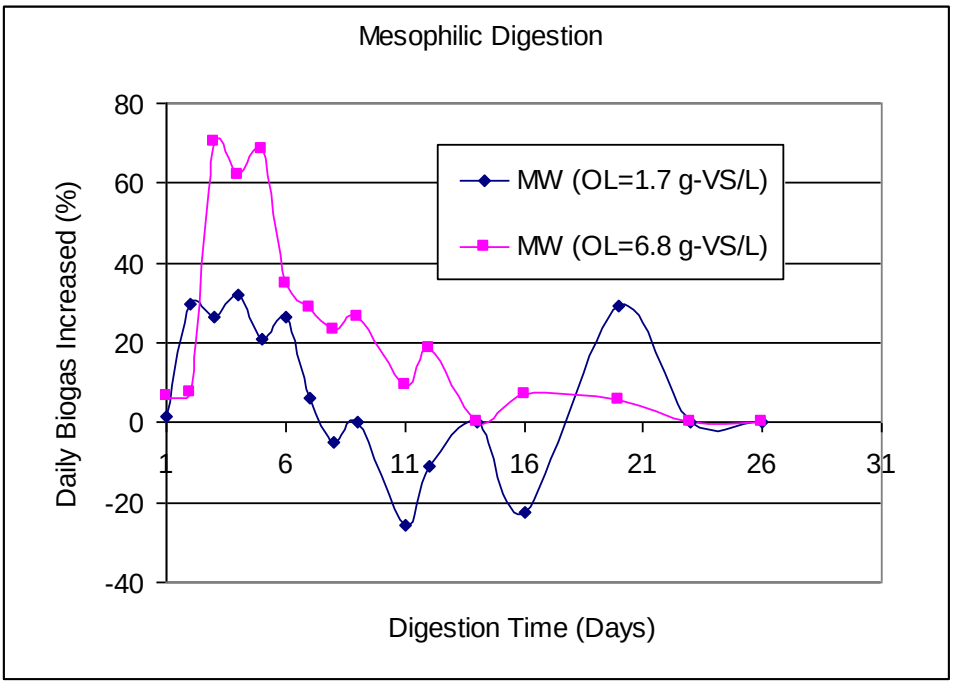


Figure 6.2 Student's t-Test for comparisons of mesophilic daily biogas production from untreated WAS and microwave treated sludge

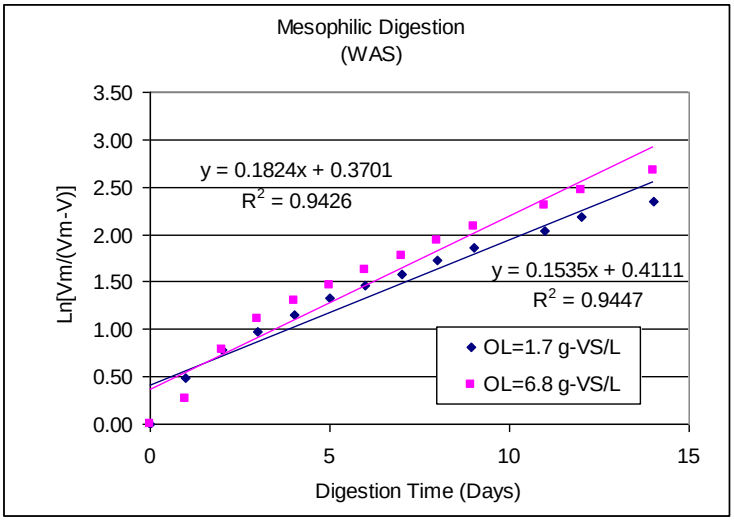


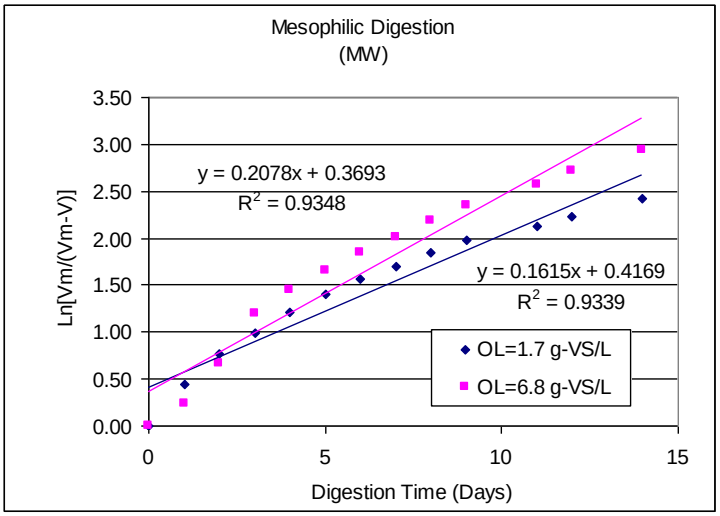
(a)



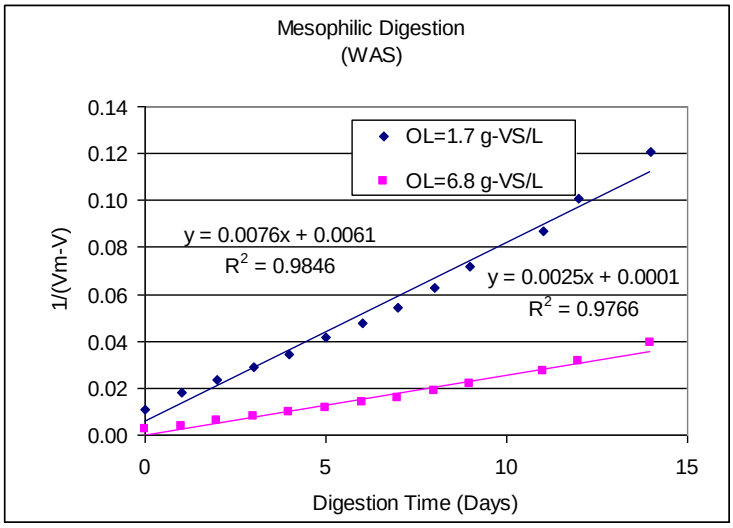
(b)

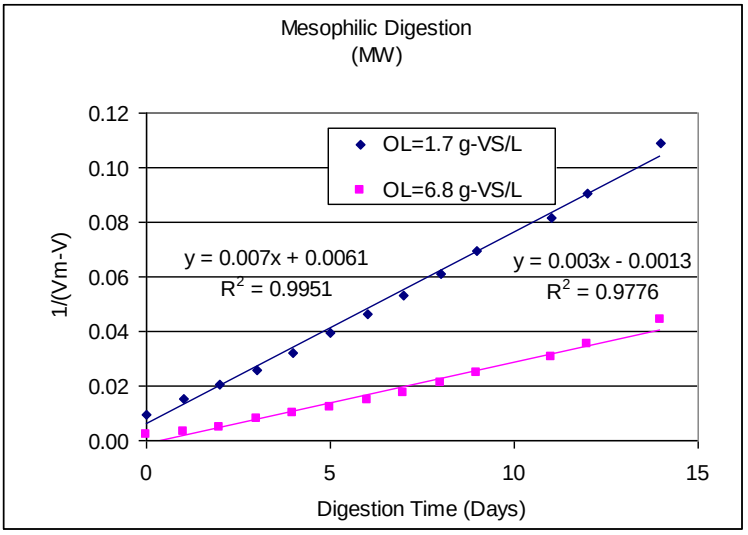
Figure 6.3 Mesophilic total (a) and daily biogas (b) increase from microwave treated sludge in comparison to WAS feed control





(a)





(b)

Figure 6.4 Reaction kinetics for mesophilic biogas production from untreated WAS and microwave treated feed (first order plots (a) and second order plots (b))

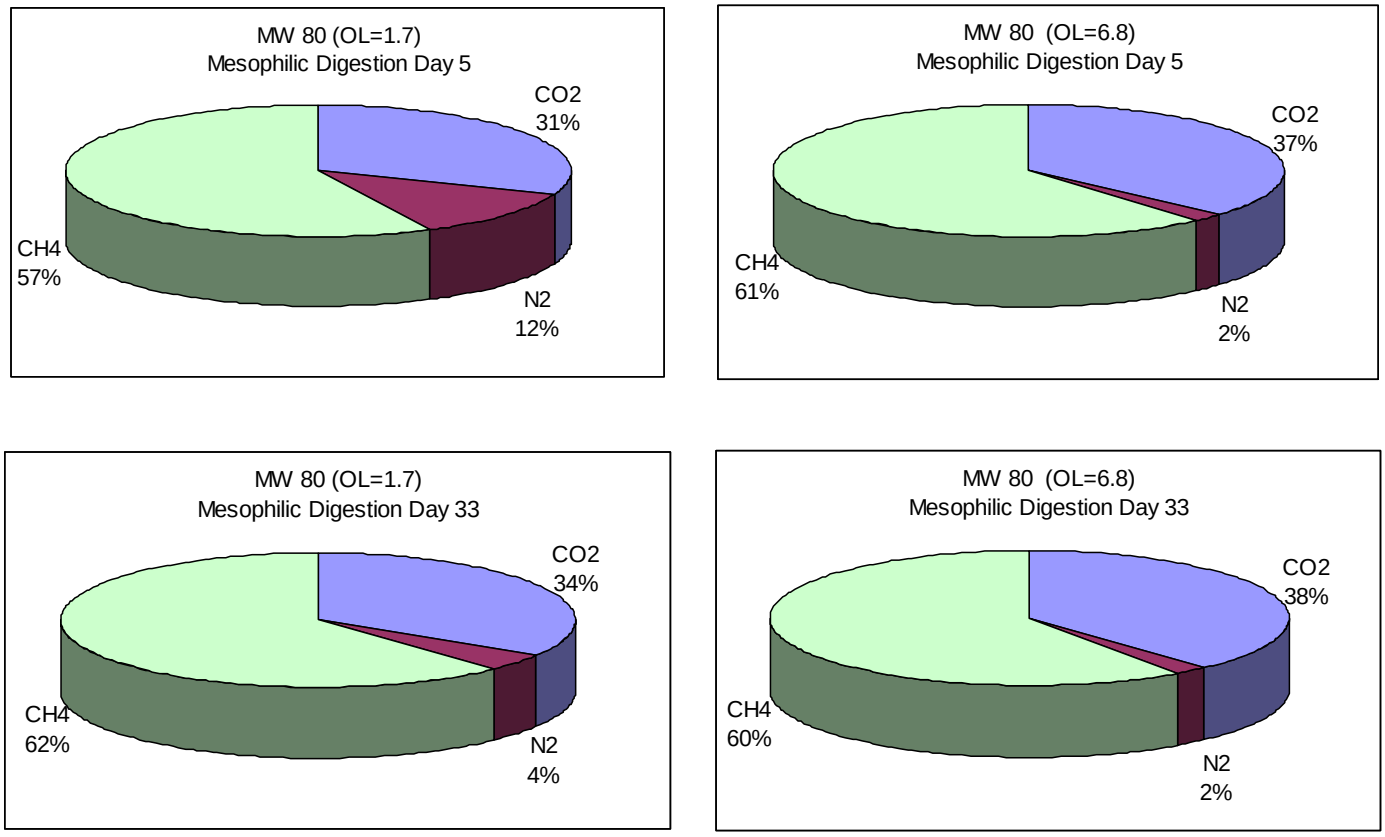


Figure 6.5 Biogas composition from microwave treated sludge at mesophilic digestion day 5 and day 33

6.3.1.2 MW/H_2O_2-pretreated WAS

The mesophilic biogas production from untreated WAS (feed control), MW/H_2O_2 pretreated sludge and the inoculums (seed control) are shown and compared in Figure 6.6. This figure shows the results for two organic loading conditions of 1.7 and 6.8 g-VS/L in the left and right columns, respectively. Under both loading conditions, no inhibition of biogas production resulted from MW/H_2O_2 pretreatment. The student's t-test shown on Figure 6.7 confirms that the biogas production from untreated WAS and MW/H_2O_2 pretreated sludge are statistically different. The biodegradability improvement (accumulated biogas increase) and the reaction rate acceleration (daily biogas production) are both shown in Figure 6.8.

Results for biogas production from the MW/H_2O_2-pretreated feed (Figure 6.6) showed a similar pattern to those for microwave-pretreated feed (Figure 6.1). However, Figure 6.8 shows some of the difference, especially for the first six days of digestion. Under low loading conditions (1.7 g-VS/L), the total biogas from the MW/H_2O_2-pretreated feed for digestion day 1 showed a 10% increase compared to that for untreated WAS. In contrast, the microwave treated feed showed only a 2% increase. The maximum total biogas increase was 18% at digestion day 6 from the MW/H_2O_2-pretreated feed, and 15% from the microwave-pretreated feed. This indicates that the MW/H_2O_2-pretreated feed provided more immediate biodegradable substrate for digestion than did the microwave-pretreated sludge. For digestion day 1, microorganisms in the seed had more than sufficient capacity to utilize the immediately available substrates under low loading conditions. Whereas microwave-pretreated sludge showed similar biogas production to the untreated WAS, the MW/H_2O_2-pretreated feed yielded an almost five times greater increase than either untreated WAS or microwave-pretreated sludge.

Under the high loading conditions (6.8 g-VS/L), this initial (day 1) difference was marginalized (5% and 7% increase for microwave and MW/H_2O_2 feed, respectively). This was likely due to the high overall organic loading that required the inoculums to acclimate. By digestion day 3, the daily biogas increases over untreated WAS were 70% and 87% for microwave and MW/H_2O_2-pretreated feed, respectively. This acclimation is not due to pretreatments, since the untreated WAS needed the same amount of time for acclimation. Instead, it was the effect of high overall organic loading. The final total biogas increase (biodegradability improvement) settled at 12% (1.7 g-VS/L loading condition) and 16% (6.8 g-VS/L), for the MW/H_2O_2-pretreated sludge.

With regard to the reaction kinetic, the MW/H_2O_2-pretreated feed showed a closer fit to the second order reaction (Figure 6.9 (b), R^2=0.9953 and 0.9809 for loading condition 1.7 g-VS/L and 6.8 g-VS/L, respectively) than to the first order kinetic (Figure 6.9 (a), R^2=0.9213 and 0.9293). These results demonstrate that with greater immediate availability of substrate for sufficient healthy inoculums, the overall digestion reaction moved towards a second order kinetic reaction.

Figure 6.10 shows biogas composition results for MW/H_2O_2-pretreated feed. The ratio of methane to carbon dioxide was similar to untreated WAS and microwave-pretreated feed, at 1.65 to 1.87.

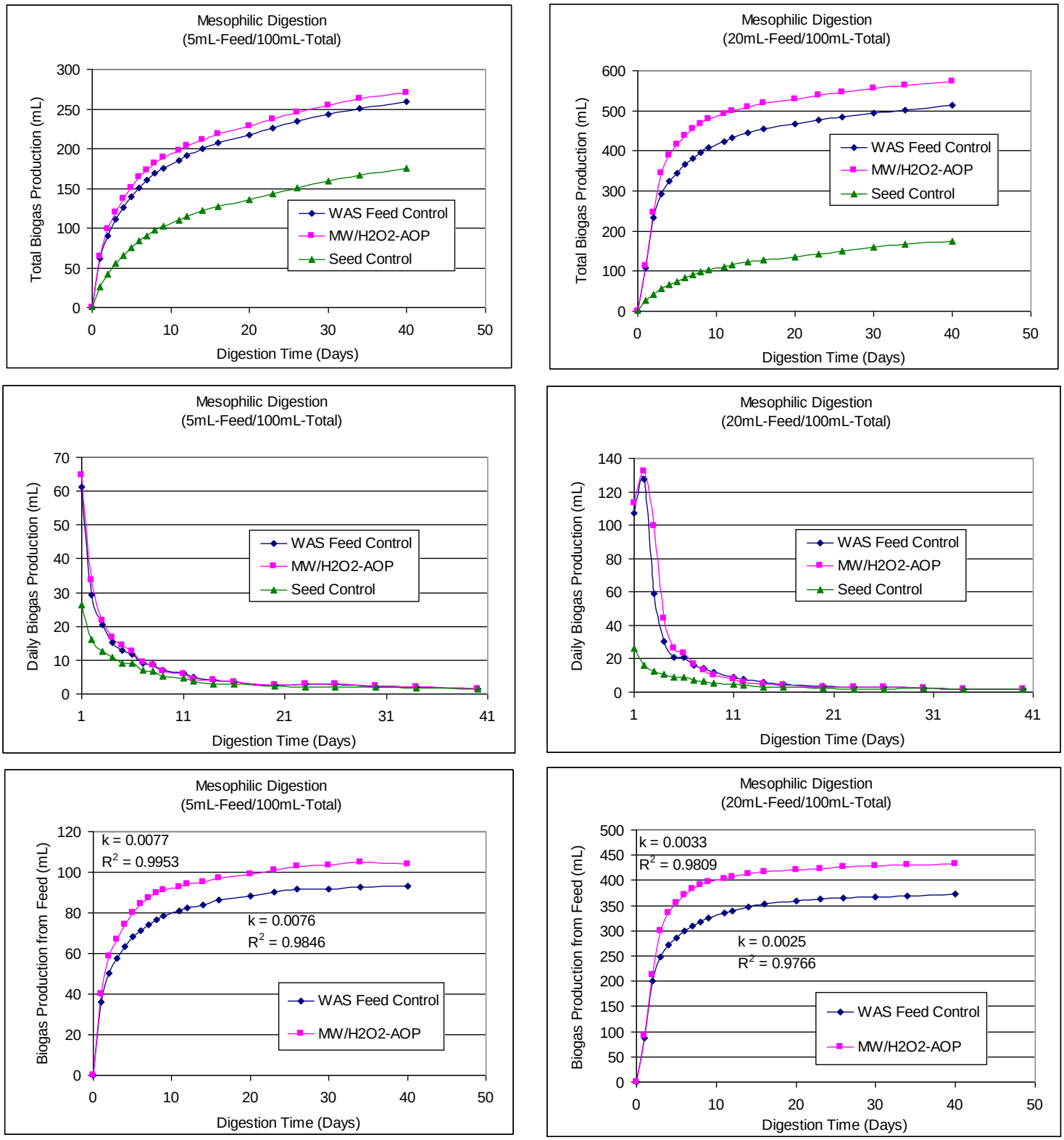


Figure 6.6 Mesophilic digestion of MW/H_2O_2 treated sludge at two organic loading conditions (1.7 g-VS/L on the left column, 6.8 g-VS/L on the right column)

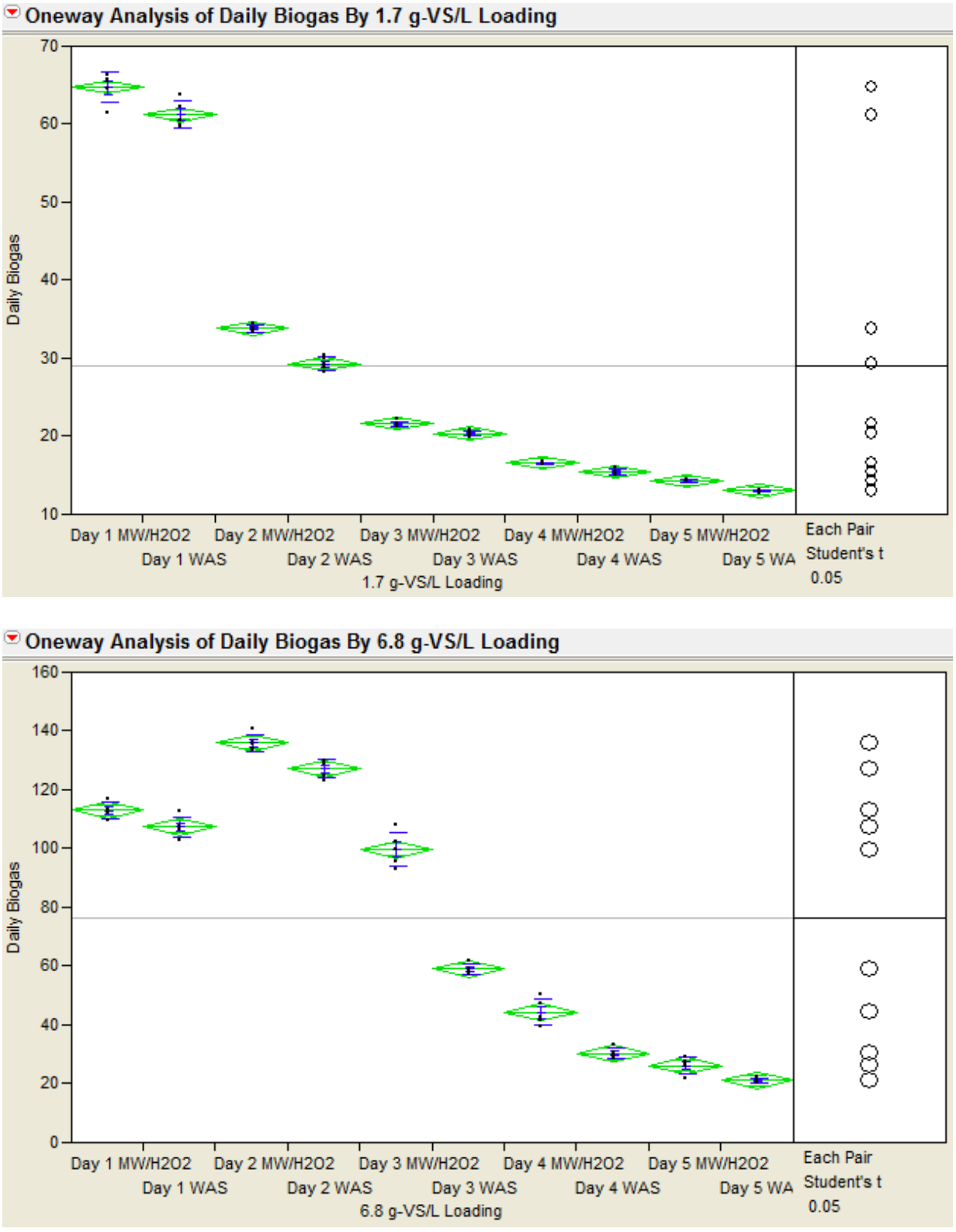
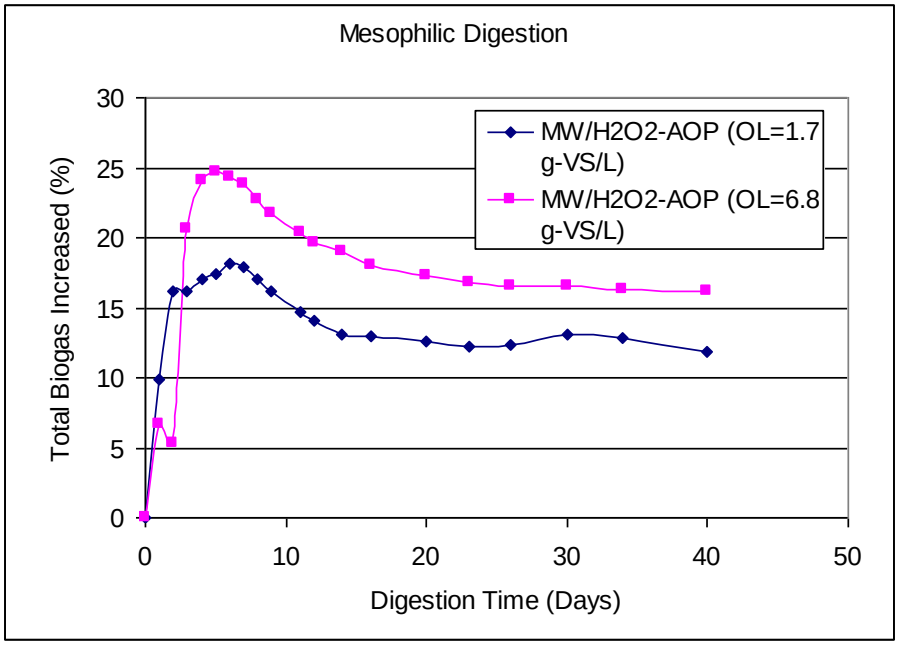
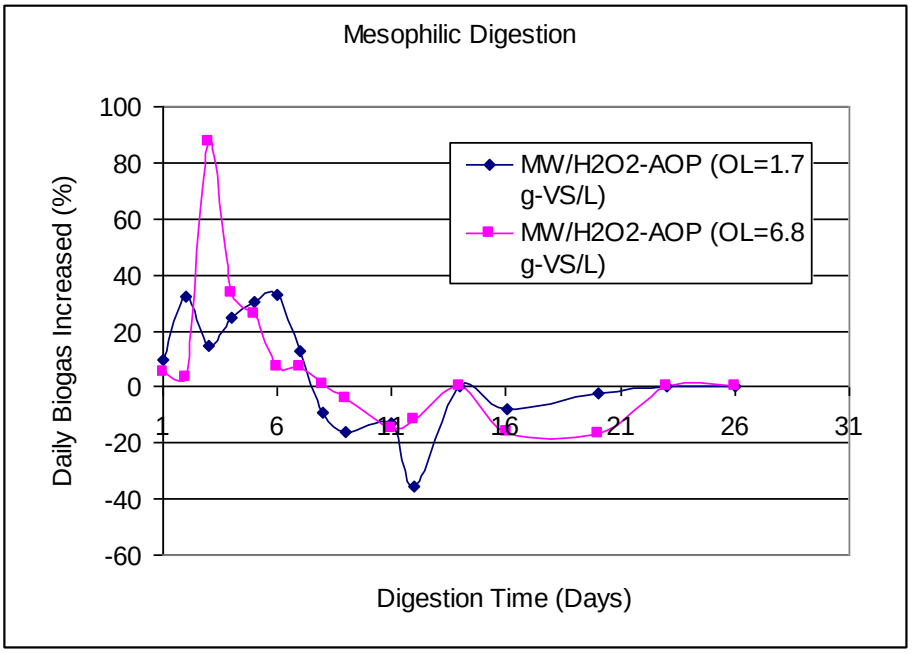


Figure 6.7 Student's t-Test for comparisons of mesophilic daily biogas production from untreated WAS and MW/H_2O_2 treated sludge

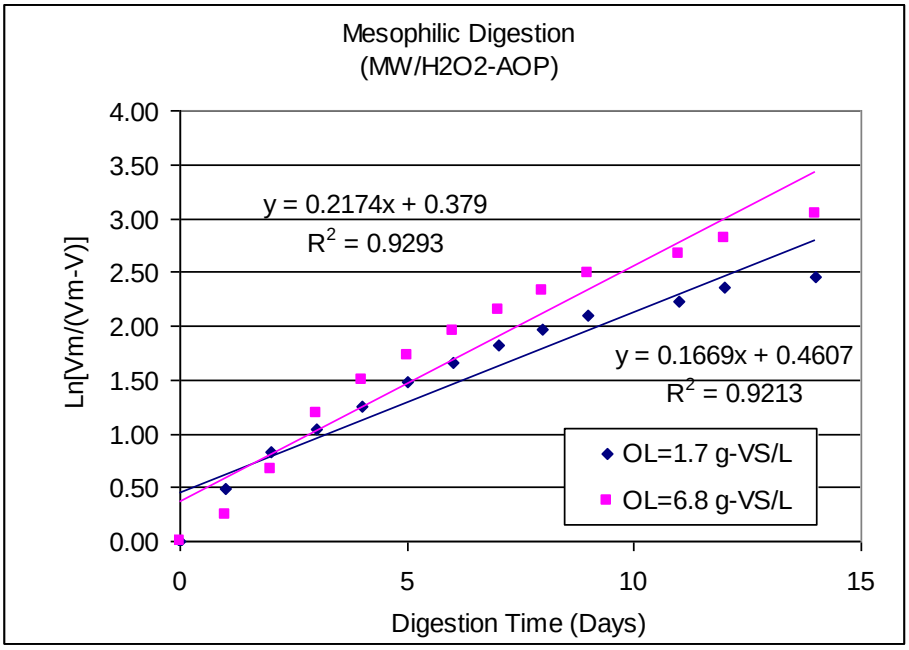


(a)

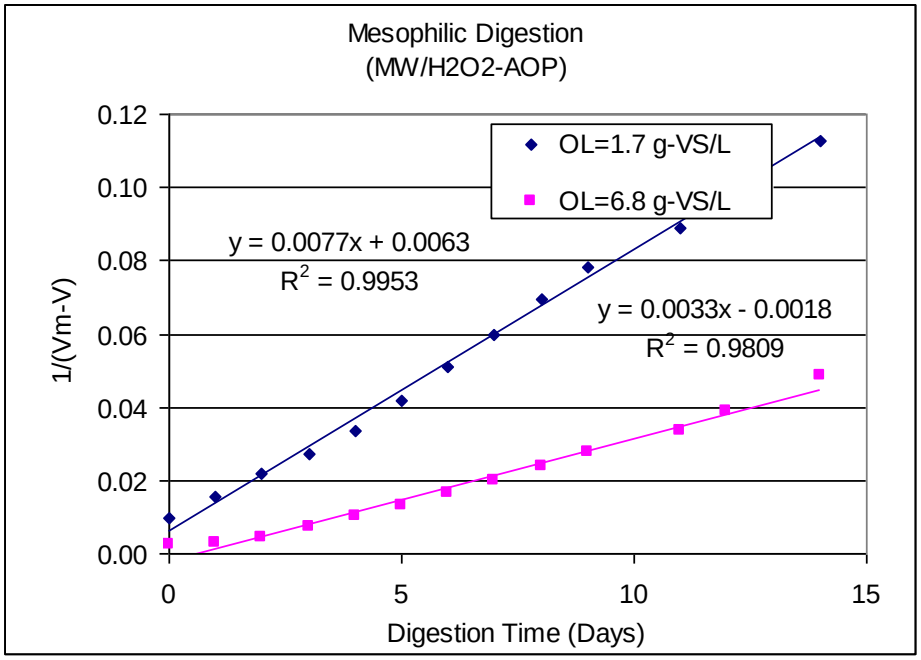


(b)

Figure 6.8 Mesophilic total (a) and daily biogas (b) increase from MW/H_2O_2 treated sludge in comparison to WAS feed control



(a)



(b)

Figure 6.9 Reaction kinetics for mesophilic biogas production from MW/H_2O_2 treated feed (first order plots (a) and second order plots (b))

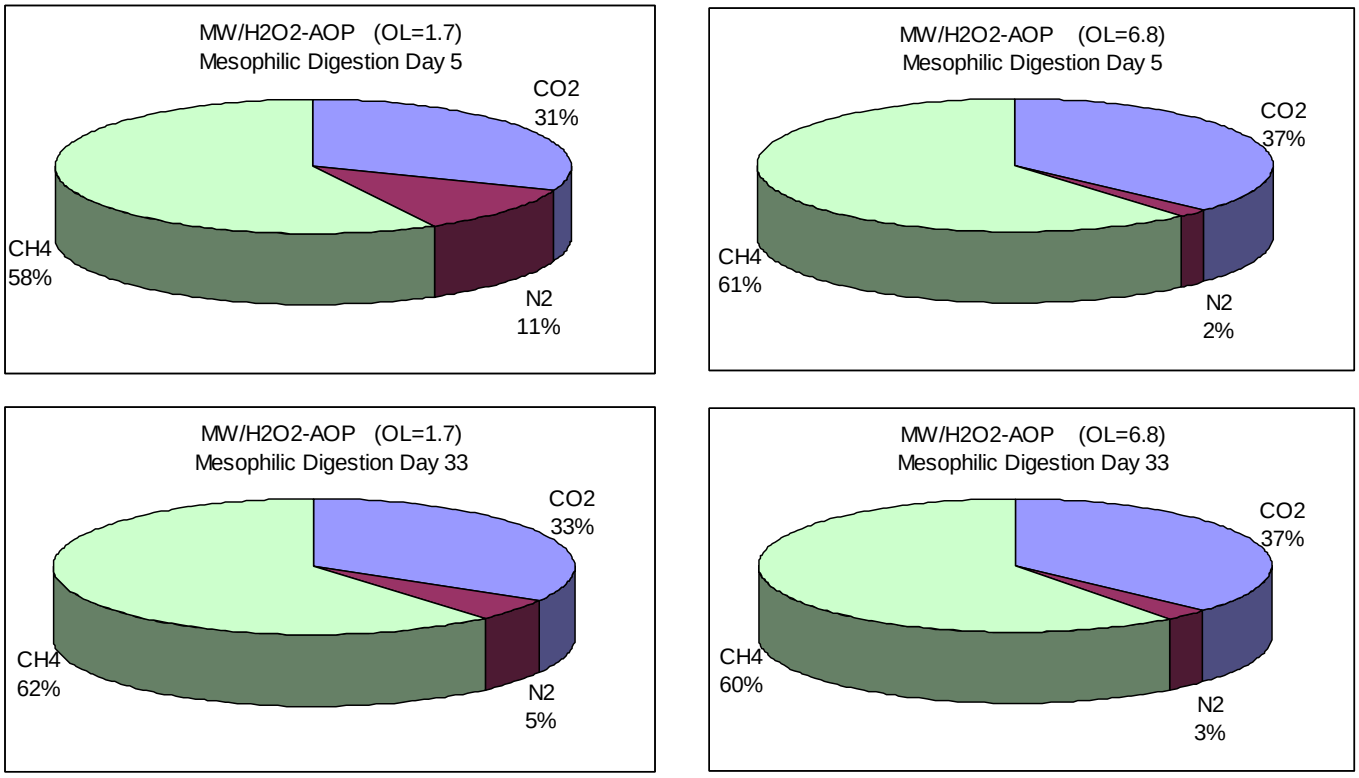


Figure 6.10 Biogas composition from MW/H_2O_2 treated sludge at mesophilic digestion day 5 and day 33

6.3.1.3 Ultrasound-pretreated WAS

The mesophilic biogas production from ultrasound-pretreated sludge, untreated WAS (feed control) and the inoculums (seed control) are shown in Figure 6.11. The two organic loading conditions, 1.7 and 6.8 g-VS/L, are shown in the left and right columns in Figure 6.11, respectively. Similar to microwave and MW/H_2O_2-pretreated sludge, there was no obvious inhibition on mesophilic biogas production with ultrasound pretreated sludge, under both organic loading conditions. The student t-test shown on Figure 6.12 confirmed that the observe difference between untreated WAS and ultrasound treated sludge are reliable at 95% confidence interval.

Figure 6.13 shows the biodegradability improvement and digestion rate acceleration resulting from ultrasound pretreatment in total and daily biogas increases over untreated WAS. On digestion day 1 at low loading (1.7 g-VS/L) the total and daily biogas increase was approximately 64%. This large initial improvement indicated a substantial increase in the immediate availability of substrate for digestion as a result of the ultrasound pretreatment. The daily biogas increase dropped quickly after day 1. This was different than it was in the case of both microwave and MW/H_2O_2-pretreated feed, where the increase was sustained for 6 days.

Under high loading conditions (6.8 g-VS/L), the inoculums required one day of acclimation, the same as for the untreated, microwave and MW/H_2O_2 pretreated feeds. The maximum daily biogas increase reached 83%, close to, but less than the 87% from the MW/H_2O_2 -pretreated feed. However, this daily increase was sustained longer, for another 3 days. Thus, the final total biogas increase over the untreated WAS (biodegradability improvement) was recorded at 25%.

As with the untreated, microwave and MW/H_2O_2-pretreated feed studies, the digestion reaction for ultrasound treated feed was found to be a second order reaction. The kinetic coefficients and the comparison to first order fits are shown in Figure 6.14. The comparison of kinetic fit, from second order reaction at low organic loading (R^2=0.9974) to the first order kinetic fit (R^2=0.8722) further supported the earlier finding that with large amounts of available substrate and sufficient inoculums, the digestion reaction was a function of a squared substrate concentration (second order reaction).

The biogas composition is shown in Figure 6.15. The methane fraction was found to be no different than it was with the other pretreated feed or with untreated WAS feed.

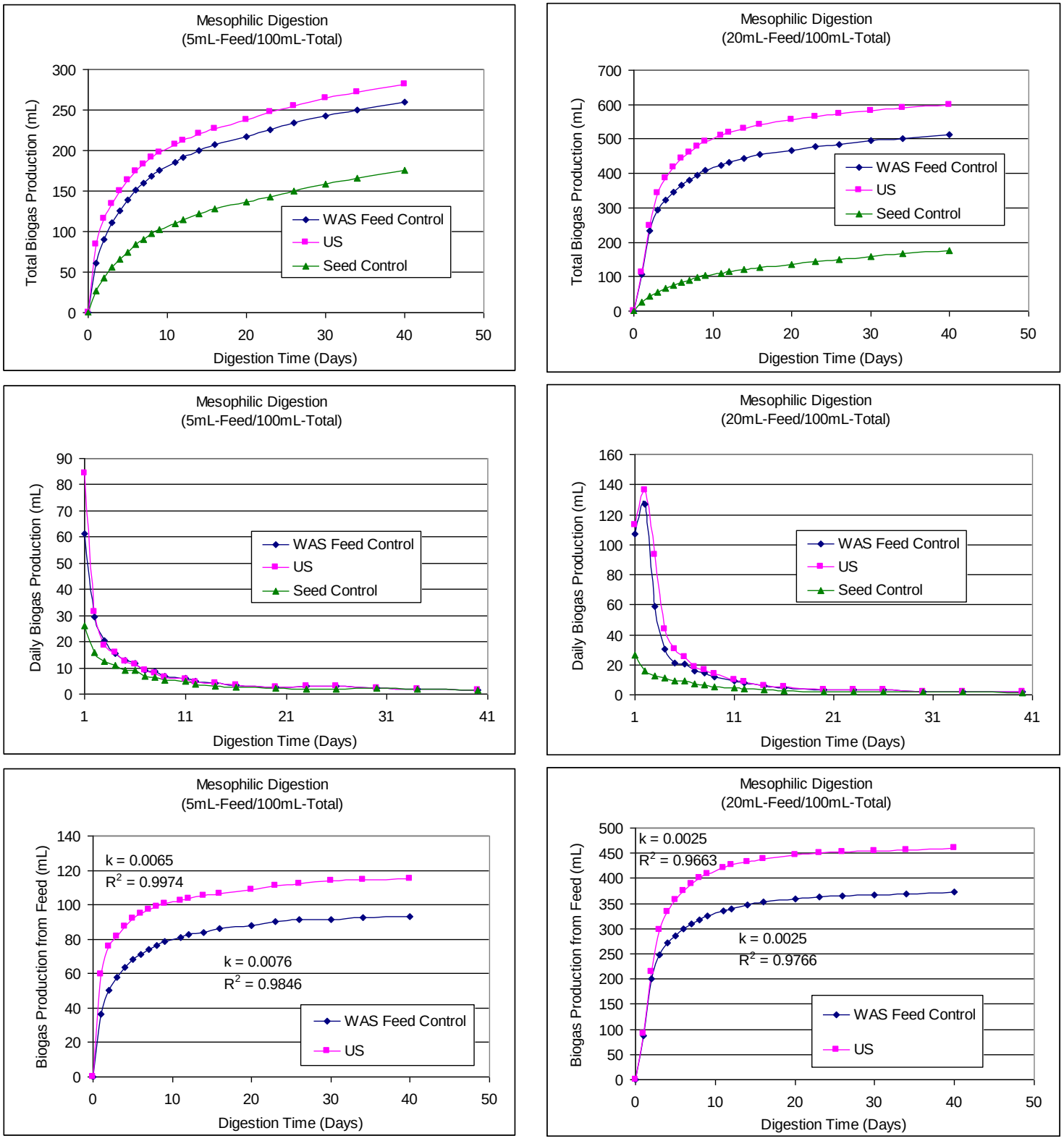


Figure 6.11 Mesophilic digestion of ultrasound treated sludge at two organic loading conditions (1.7 g-VS/L on the left column, 6.8 g-VS/L on the right column)

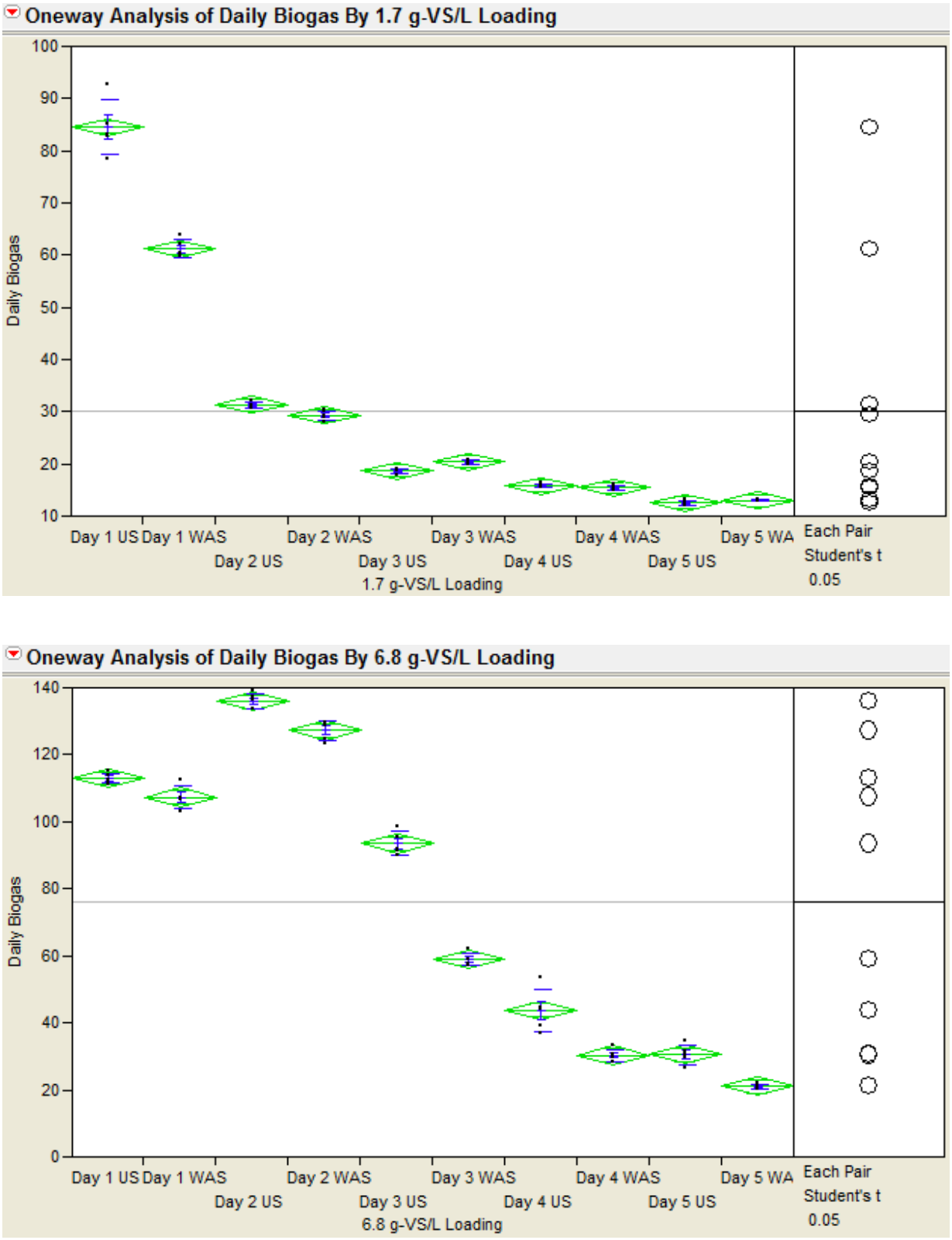
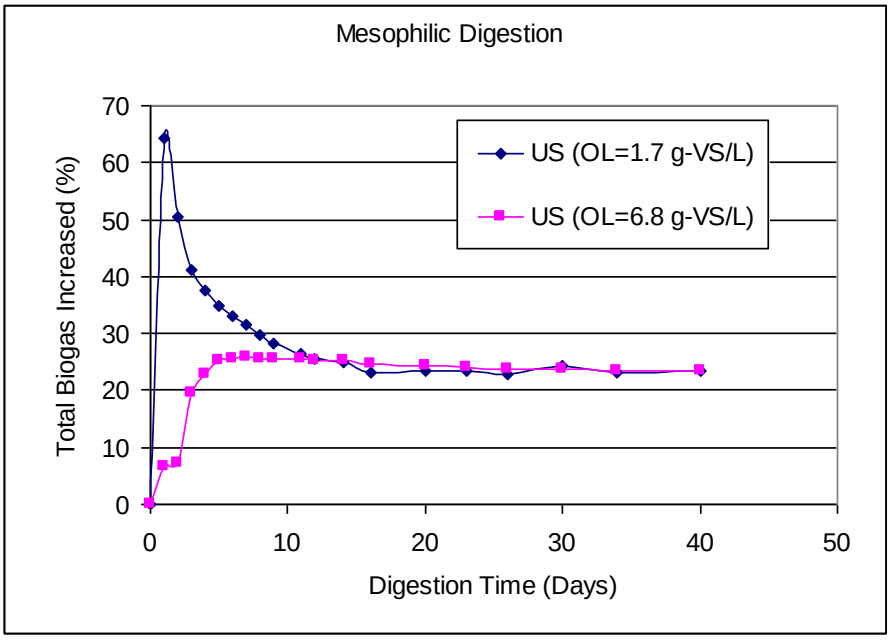
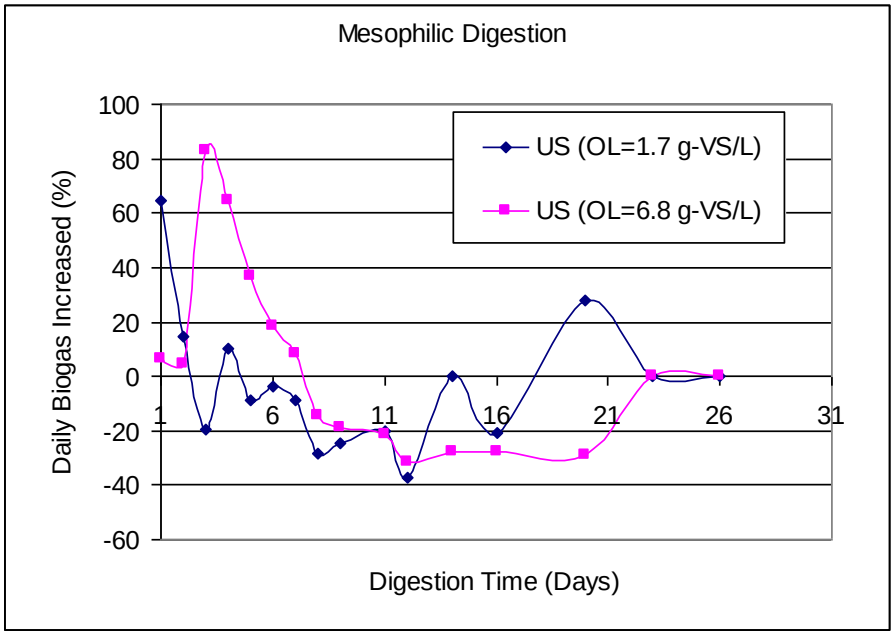


Figure 6.12 Student's t-Test for comparisons of mesophilic daily biogas production from untreated WAS and ultrasound treated sludge

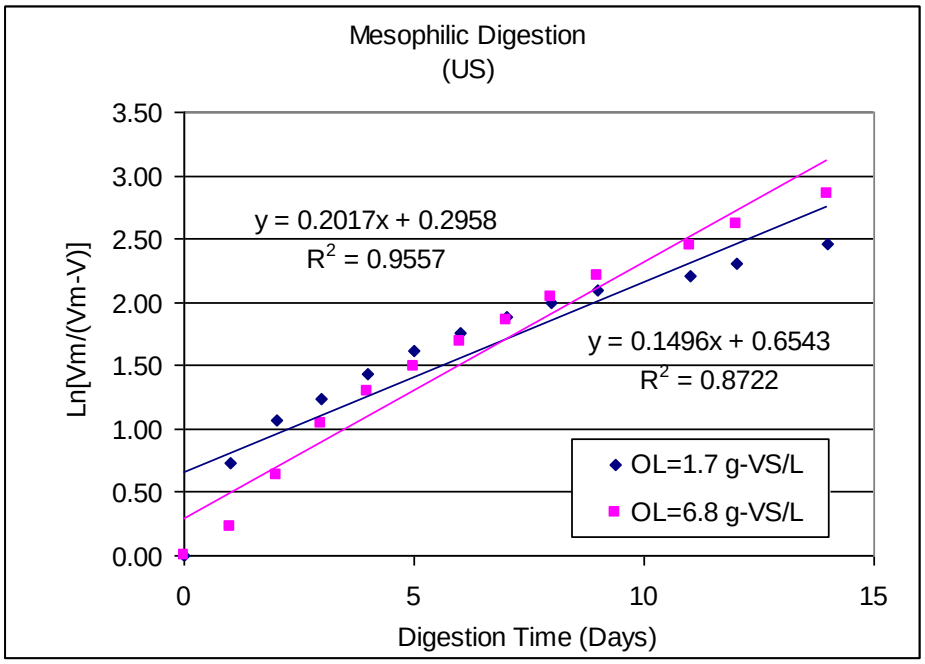


(a)

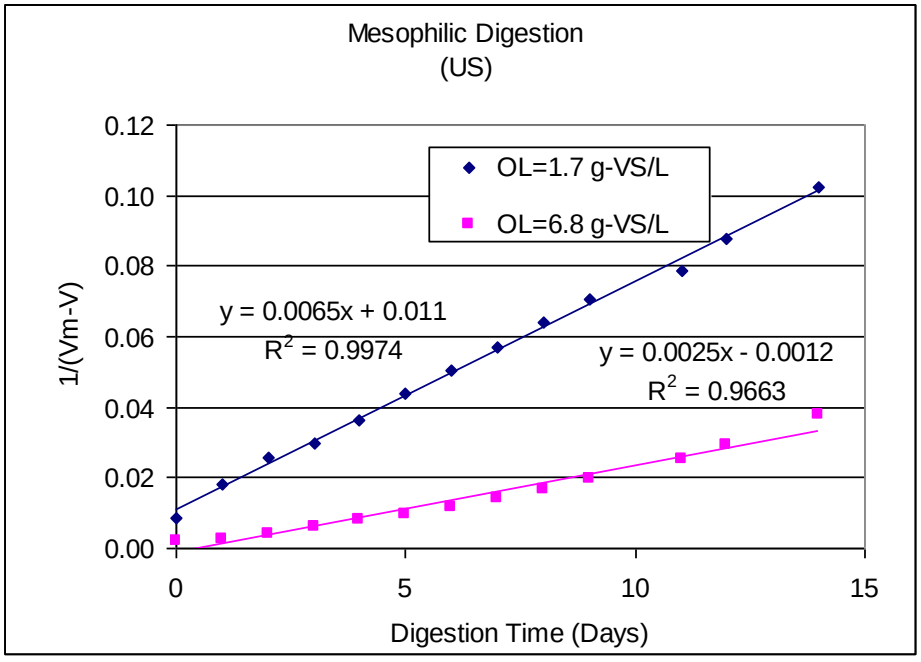


(b)

Figure 6.13 Mesophilic total (a) and daily biogas (b) increase from ultrasound treated sludge in comparison to WAS feed control



(a)



(b)

Figure 6.14 Reaction kinetics for mesophilic biogas production from ultrasound treated feed (first order plots (a) and second order plots (b))

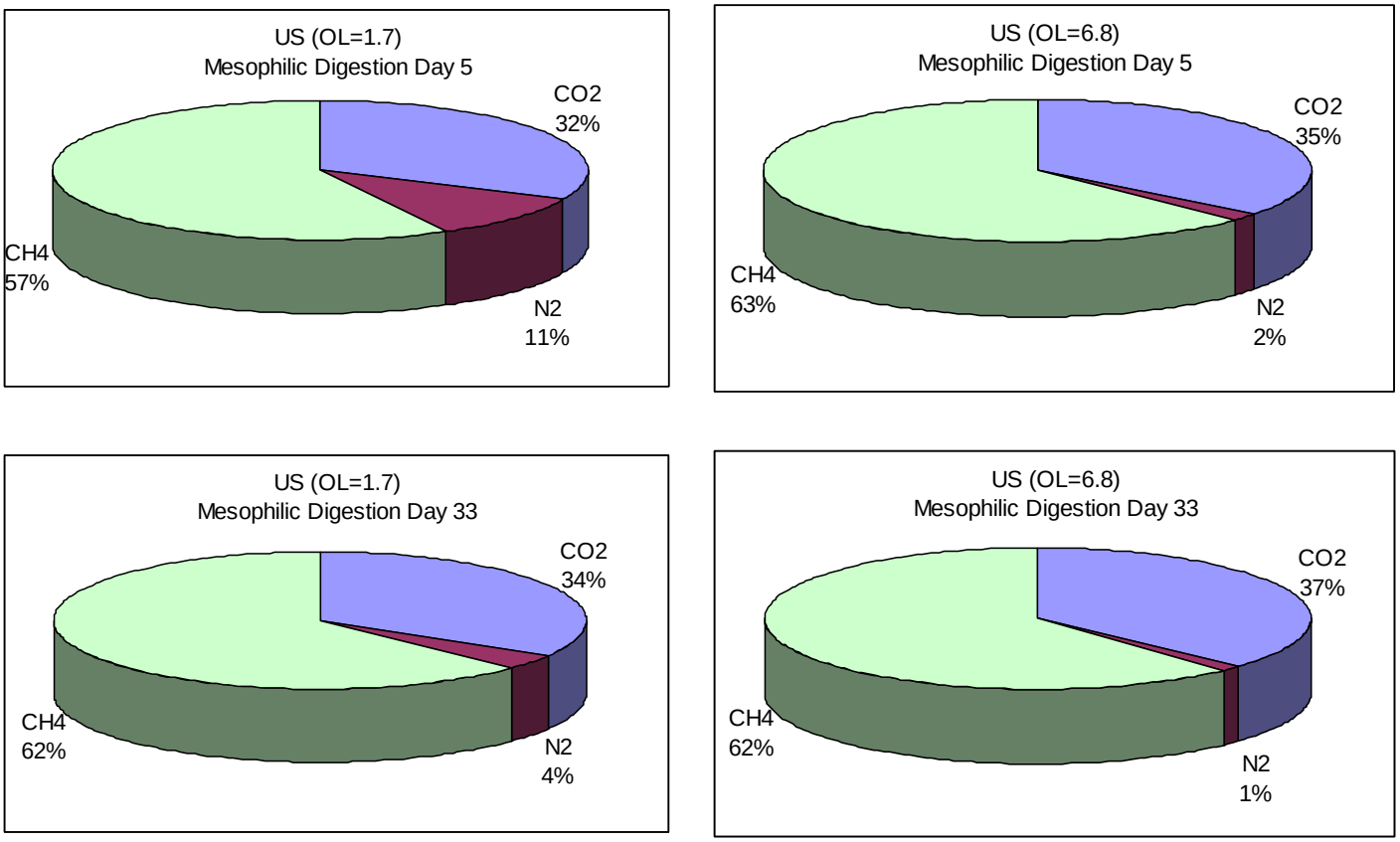


Figure 6.15 Biogas composition from ultrasound treated sludge at mesophilic digestion day 5 and day 33

6.3.1.4 Protease-pretreated WAS

Figure 6.13 reports the mesophilic biogas production from protease-pretreated sludge, together with biogas production from untreated WAS (feed control) and the inoculums (seed control). Unlike previous microwave, MW/H_2O_2 or ultrasound-pretreated feed, protease-pretreated feed exhibited a minor inhibition in biogas production for the first two days of digestion at an organic loading of 6.8 g-VS/L. This inhibition is better illustrated in Figure 6.14, where it is contrasted with untreated WAS feed in terms of total and daily biogas production.

The total accumulated biogas production for these first two days of digestion were 6% and 4% less than that from the untreated feed. The daily biogas numbers were 6% and 2% less. The possible source of this inhibition was the elevated volatile fatty acid levels in protease treated feed (421 mg-TVFA/L, expressed in acetic acid, Chapter 5). It was likely that the protease dosage tipped the balance of the inoculums' microorganism component in favor of acidogens. The result was further VFA accumulation. Batstone et al. (2000) suggested that a VFA concentration at 6.7-9.0 mol/m^3 would have a toxic effect on methanogens. The initial available VFA in protease treated feed was 7 mmol/L. In such a case, methanogens were not able to remove the hydrogen and VFA fast enough and required more time for acclimation.

By digestion day 3, the large biogas production increase indicates that a stable working methanogen community had been restored. This acclimation time was similar to all the other feeds. This could suggest that high organic loading was also partly responsible. The difference was that while the other pretreated feeds had minor positive increases, protease-pretreated feed showed a decrease over the untreated WAS control. At the end of

digestion, the total biogas increase (biodegradability improvement) from protease treated sludge was at 11%.

The kinetic study (Figure 6.15) showed that, despite the first two days of inhibition (two slightly deviated data points), biogas production (overall digestion reaction) was still a second order reaction. The biogas composition as shown in Figure 6.16 confirms that biogas makeup from the protease treated feed was the same as for the other treatments.

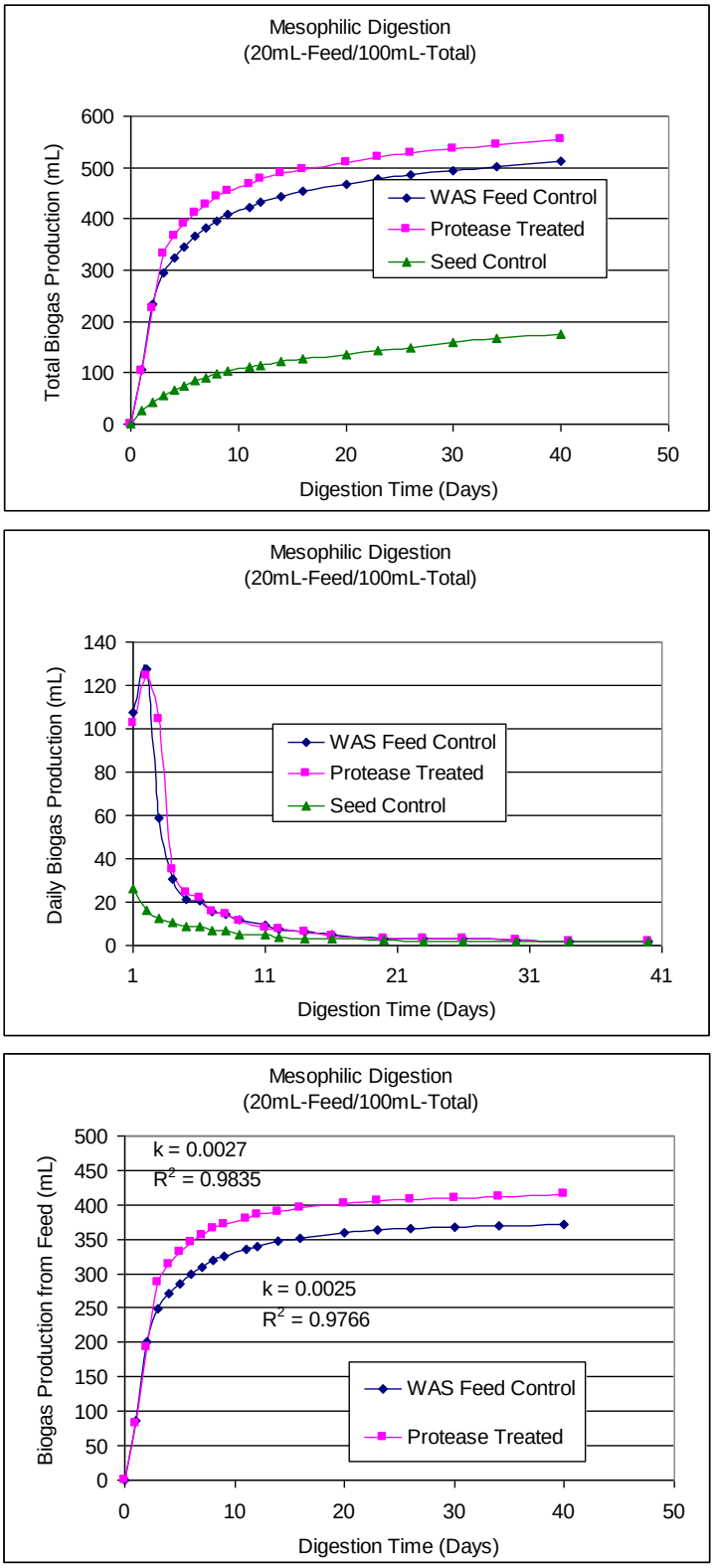


Figure 6.16 Mesophilic digestion of protease treated sludge at organic loading of 6.8 g-VS/L

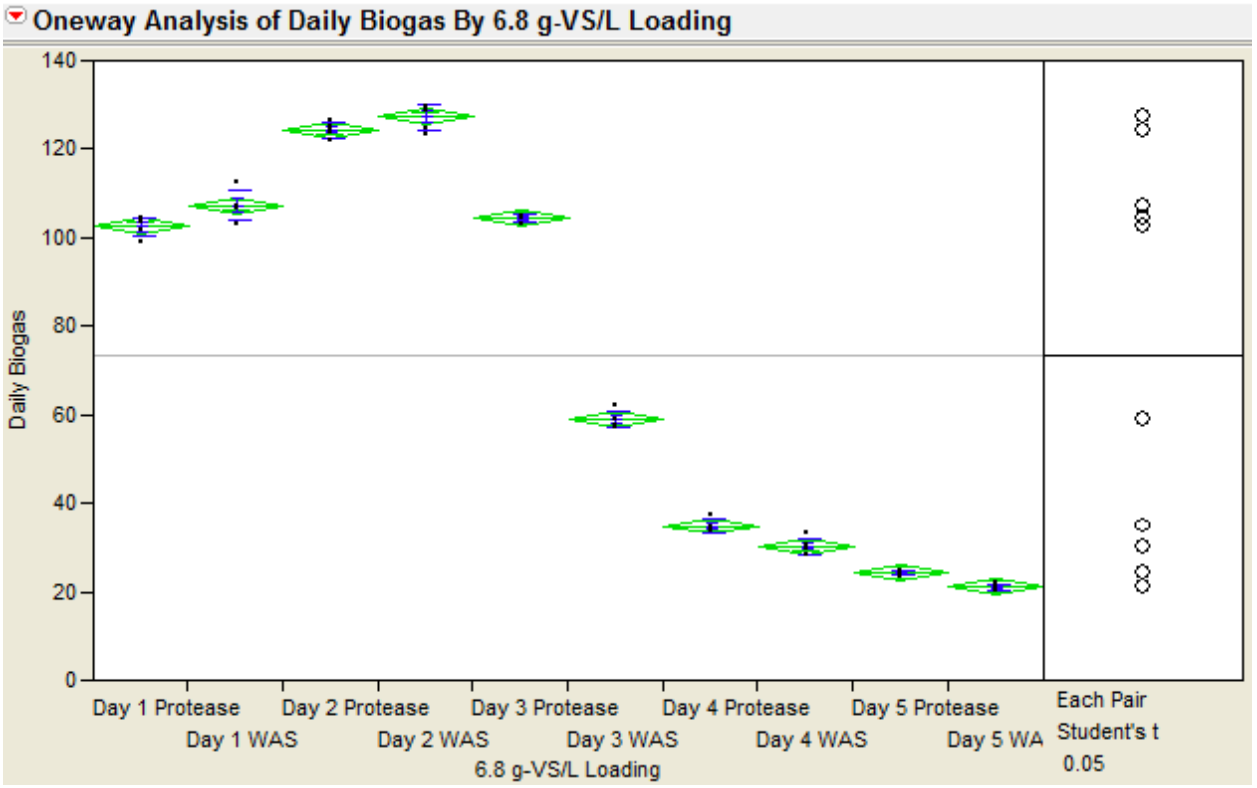
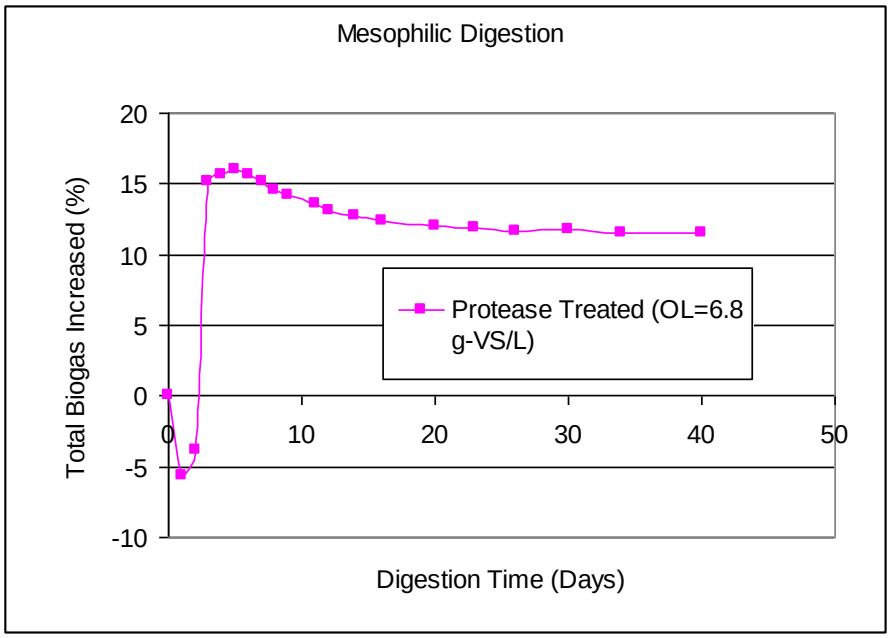
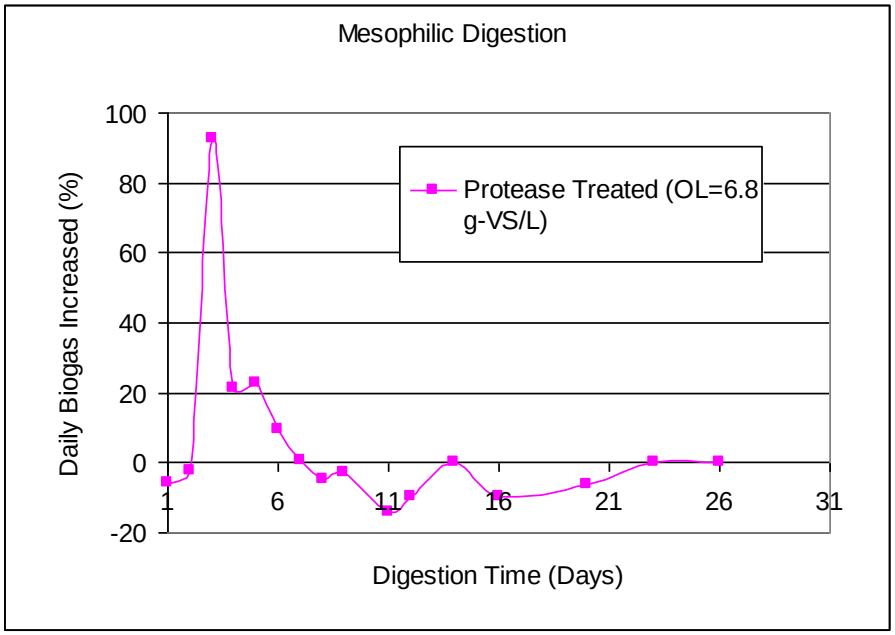


Figure 6.17 Student's t-Test for comparisons of mesophilic daily biogas production from untreated WAS and protease treated sludge

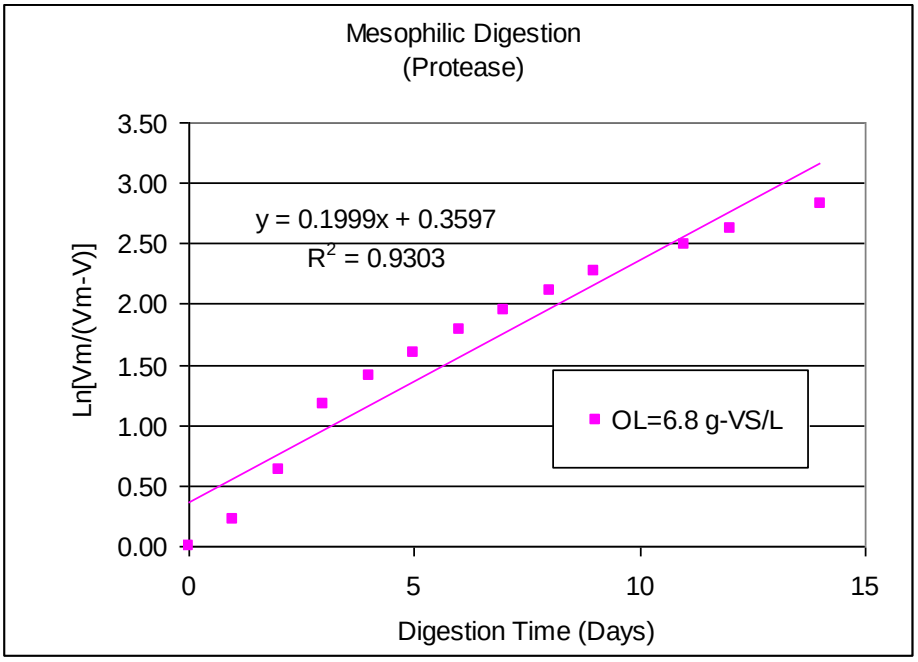


(a)

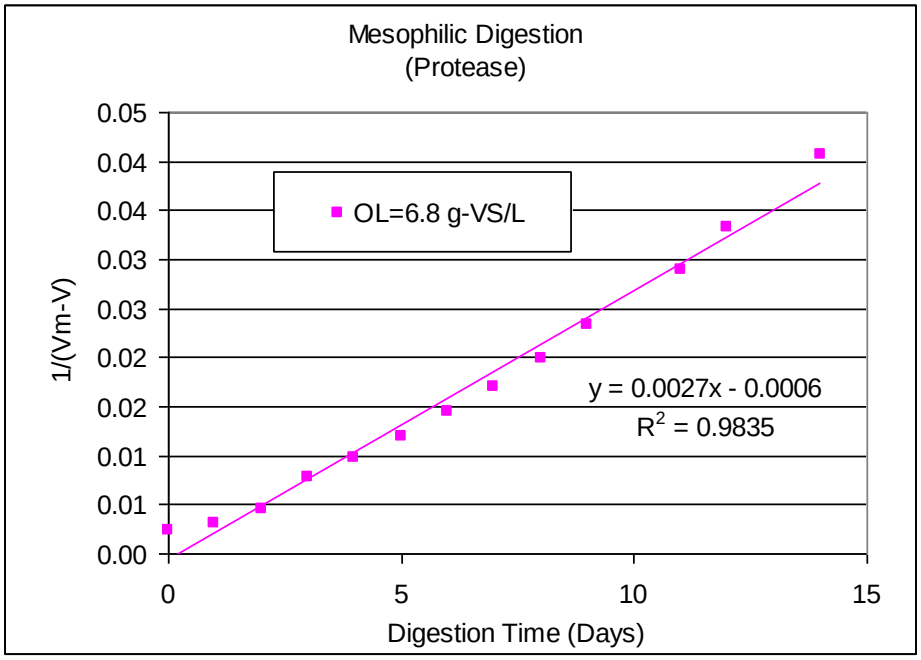


(b)

Figure 6.18 Mesophilic total (a) and daily biogas (b) increase from protease treated sludge in comparison to WAS feed control



(a)



(b)

Figure 6.19 Reaction kinetics for mesophilic biogas productions from protease treated feed (first order plots (a) and second order plots (b))

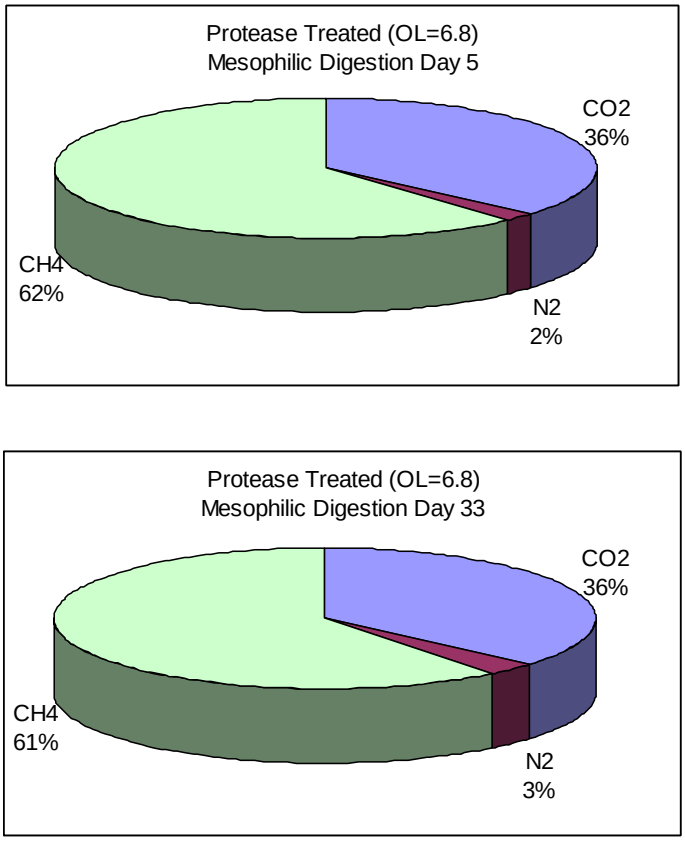


Figure 6.20 Biogas composition from protease treated sludge at mesophilic digestion day 5 and day 33

6.3.1.5 Summary

Table 6.1 reports the volatile solids reduction, COD degradable fraction and specific biogas production rate from mesophilic digestion of untreated WAS and pretreated feeds. The VS reductions are in the range of 64.2% to 69.5%. The COD substrate biodegradable fraction was calculated, based on a theoretical conversion (McCarty, 1964), from the total biogas (methane) produced at the end of digestion. Microwave pretreatment increased the COD biodegradable fraction from 69% in untreated WAS to an average 80.7% (under two organic loading conditions). The MW/H_2O_2, ultrasound and protease pretreatments increase to 81.1%, 85.9% and 76.5%, respectively. These results suggest that pretreatments improved acceleration of the digestion rate as well as the conversion of a portion of the inert organics to mesophilic digestables. The specific biogas production rates also increased with pretreatment applications. Microwave, MW/H_2O_2, ultrasound and protease treatments each recorded an average 0.87, 0.93, 1.04, and 1.07 L-biogas /g-VS-destroyed, respectively. This compares to 0.83 L/g-VS-destroyed for untreated WAS.

In short, treatments prior to mesophilic digestion did not result in inhibition under the two tested loading conditions (1.7 and 6.8 g-VS/L), except for a minor negative impact from protease treated feed at the beginning of digestion. However, the inoculums required one day of acclimation under high organic loading conditions. The acclimation was a result of overloading and not a result of the pretreatments. The total biogas increase from all pretreatments was between 12 to 25%. The biogas production (overall digestion) kinetics was found to closely fit a second order reaction. Biogas composition had consistent methane content in the range of 61 to 65%.

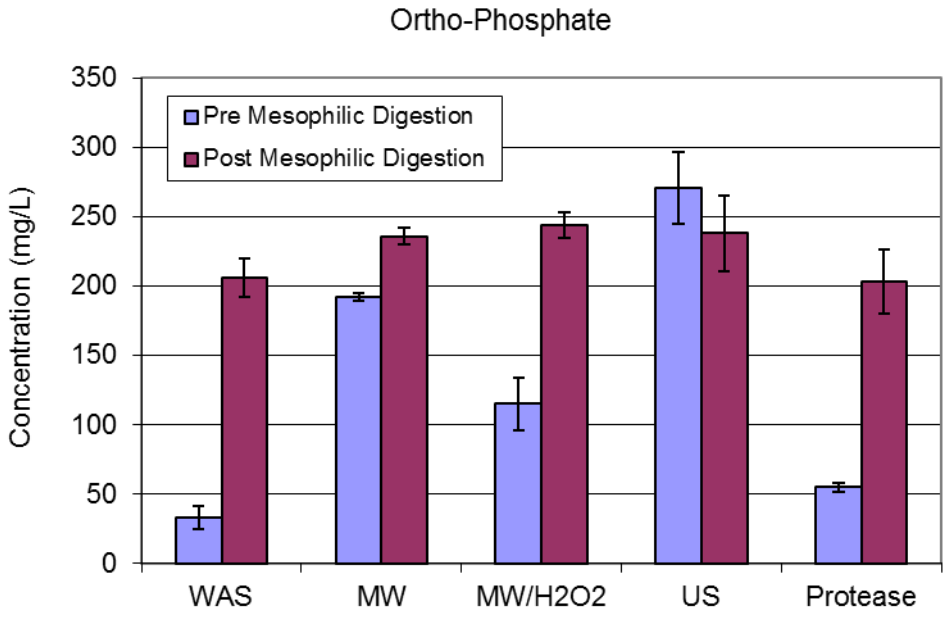
The ortho-phosphate levels in post digestion supernatant were shown on Figure 6.21

(a), along with pre-digestion levels for comparison (previous Chapter 5 Section 5.3.4). The Figure 6.21 (b) shows the student's t-test (95% confidence interval) for group comparison of post digestion ortho-phosphate results. It appears that microwave, MW/H_2O_2 and ultrasound pretreatment increase the ortho-phosphate release into the supernatant after mesophilic anaerobic release (digestion), by approximately 20% when compared to untreated WAS feed. Protease treatment did not increase the ortho-phosphate release, both before and after the digestion.

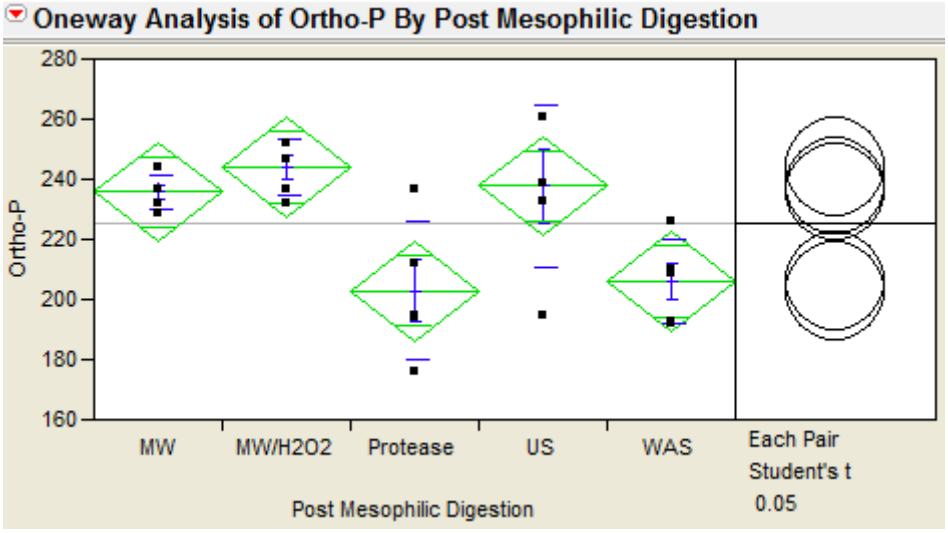
Table 6.1 Volatile solids reduction, biodegradable COD faction and specific biogas production from mesophilic digestion

	Initial volatile solids	Volatile solids after digestion	Volatile solids reduction	Initial measured total COD	Total biogas production from feed	Theoretical COD substrate for biogas *	Bio-degradable COD fraction	Specific biogas from feed
	g/L	g/L	%	g/L	mL	g/L	%	L/g-VS-destroyed
OL=1.7 g-VS/L								
WAS	33.4	10.9	67.4	50.9	93.0	35.1	69.0	0.83
MW	35.4	10.8	69.5	49.3	103.0	39.0	79.1	0.84
MW/H_2O_2	34.1	11.0	67.8	49.5	104.0	39.5	79.8	0.90
US	33.6	11.0	67.3	50.5	115.0	43.3	85.8	1.02
OL=6.8 g-VS/L								
WAS	33.4	11.2	66.4	50.9	372	35.1	69.0	0.84
MW	35.4	11.5	67.6	49.3	430	40.6	82.3	0.90
MW/H_2O_2	34.1	11.5	66.1	49.5	433	40.8	82.4	0.95
US	33.6	11.6	65.5	50.5	460	43.4	85.9	1.05
Protease	30.1	10.8	64.2	51.2	415	39.1	76.5	1.07

* Theoretically 0.350 m^3 of methane produced from every 1 kg COD converted (McCarty, 1964)



(a)



(b)

Figure 6.21 Ortho-phosphate results before and post mesophilic digestion (a) and Student's t-Test for group comparisons of post digestion (b)

6.3.2 Thermophilic anaerobic digestion

6.3.2.1 Microwave-pretreated WAS

The thermophilic biogas production from untreated WAS (feed control), microwave-pretreated sludge and the inoculums (seed control), over 30 days of digestion, are shown in Figure 6.22. Figure 6.23 shows the students' t-test on daily biogas production for the first 7 (1.7 g-VS/L loading) and 8 days (6.8 g-VS/L loading), at 95% confidence interval. For untreated WAS feed, there was no inhibition with the increased organic loading from 1.7 g-VS/L (on the left column) to 6.8 g-VS/L (on the right column). Under both loading conditions, daily biogas production peaked on digestion day 1 then gradually decreased. No lag time or acclimation was needed. The biogas production (overall digestion) followed the saturation curve described by the second order kinetics (Figure 6.25). This suggests that the thermophilic inoculums were sufficient and healthy for untreated WAS feed under both loading conditions. This set the base line for confidently studying the impacts from the pretreatments. With untreated WAS feed, the digestion process was completed at digestion day 9 for both loading conditions. Daily biogas production was at minimum after that, and close to that from the seed control.

Figure 6.24 shows the total and daily biogas production increases from microwave treated feed relative to untreated WAS. Under low loading (1.7 g-VS/L) conditions, microwave-pretreated feed results showed similar biogas production to those from the untreated WAS feed. However, inhibition was found during the first two days of digestion, when daily biogas production was lower than it was from untreated WAS (24% and 8% less for day 1 and 2 respectively, Figure 6.24 (b)). By digestion day 4, daily biogas production had recovered and increased compared to that from the feed control. The total

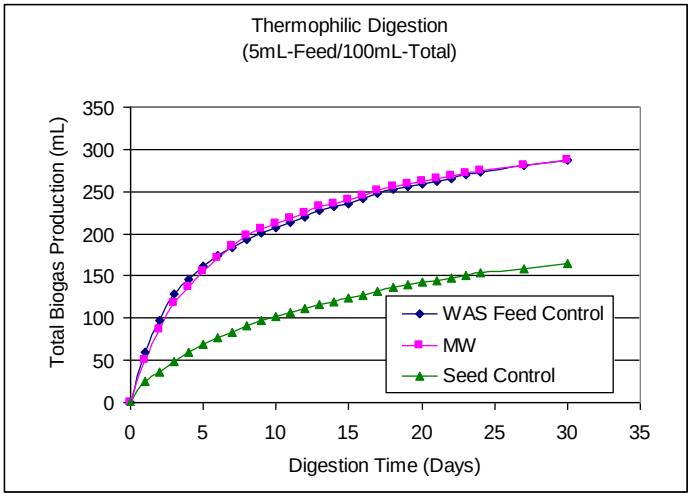
accumulated biogas crossed over the equal point and became positively higher at digestion day 7. By day 10, slightly behind the feed control, digestion was largely completed.

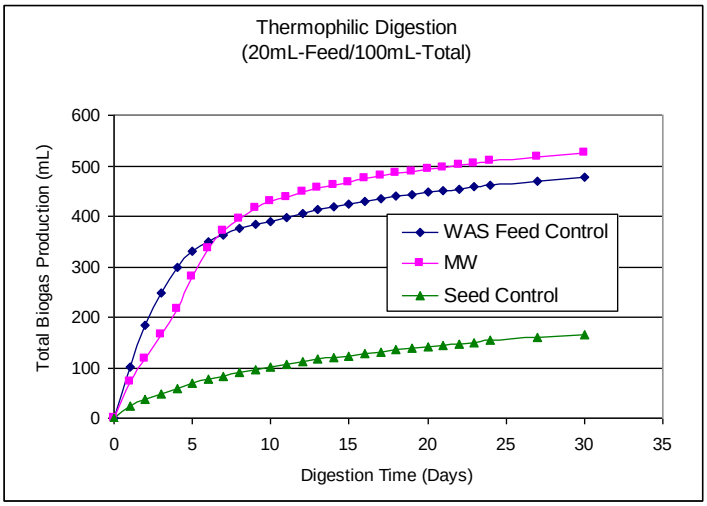
At the higher loading rate (6.8 g-VS/L), the inhibition from the microwave-pretreated feed was more severe. For three days, the daily biogas production was 32-53% less than that from untreated WAS. By digestion day 4, the daily biogas production from microwave-pretreated reached the equal point. It remained higher than that from untreated WAS for another 12 days. The total biogas increase (biodegradability improvement) recovered from the early setback and eventually settled at 14% by the end of the digestion period. The digestion was completed at day 16, approximately 7 days behind the untreated WAS run.

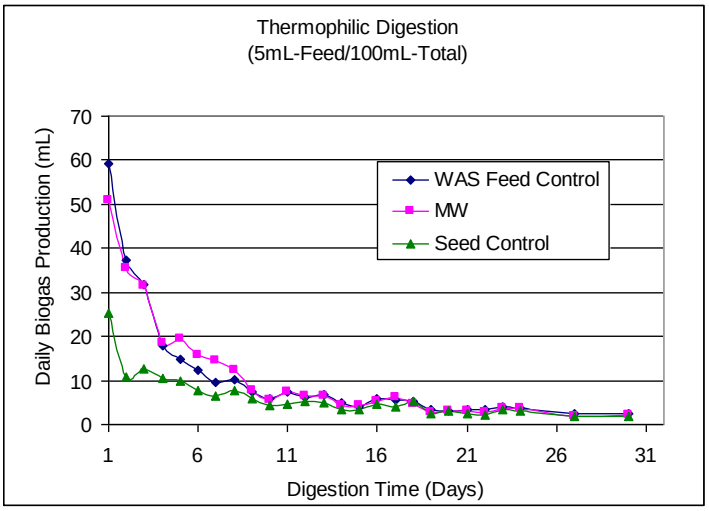
The inhibition for microwave-pretreated feed was not solely attributable to the overall organic loading, although increasing loading magnified the impact. The initial VFA level in the microwave-pretreated feed was lower than it was for untreated WAS (188 and 454 mg/L of TVFA, respectively, Chapter 5). However, microwave-pretreatment did provide more immediate substrate in other forms (soluble COD, protein, polysaccharide, amino acids, etc, Chapter 5). This increase in available substrates accelerated mesophilic digestion (Section 6.3.1.1), but inhibited thermophilic digestion during the early days. At thermophilic temperatures, the hydrolysis rate is higher than at mesophilic temperatures. Thermophilic temperatures allow the soluble macromolecules to be degraded to VFAs at a much faster pace if there is an active acidogens community. Even though the initial VFA in the feed was at a safe level, VFA accumulation can still happen and result in inhibition of the thermophilic digestion process.

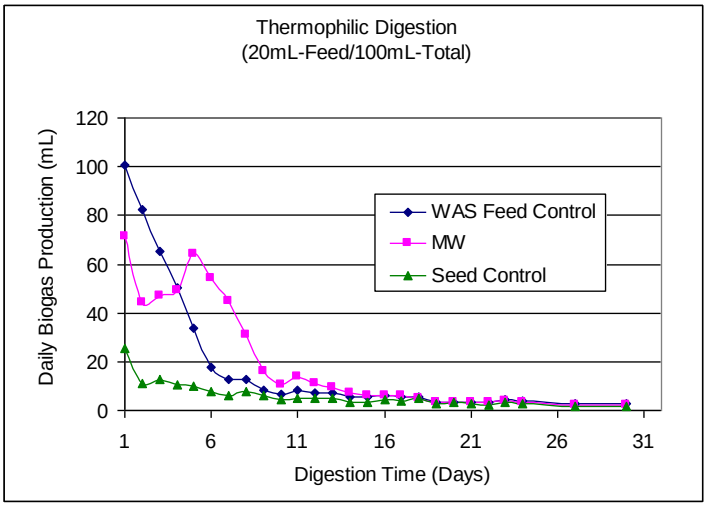
By comparing the first order and second order reaction kinetics (Figure 6.25), it was found that the first order reaction was a better fit for thermophilic digestion of microwave-pretreated feed under both organic loading conditions (R^2=0.9826 and 0.9835 for loading 1.7 and 6.8 g-VS/L conditions, respectively). In contrast, the second order reaction still applied to the untreated WAS feed (R^2=0.9867 and 0.9906 for 1.7 and 6.8 g-VS/L conditions, respectively). This indicates that the microwave pretreatment reduced the biogas production (overall digestion) rate because of the acclimation time needed.

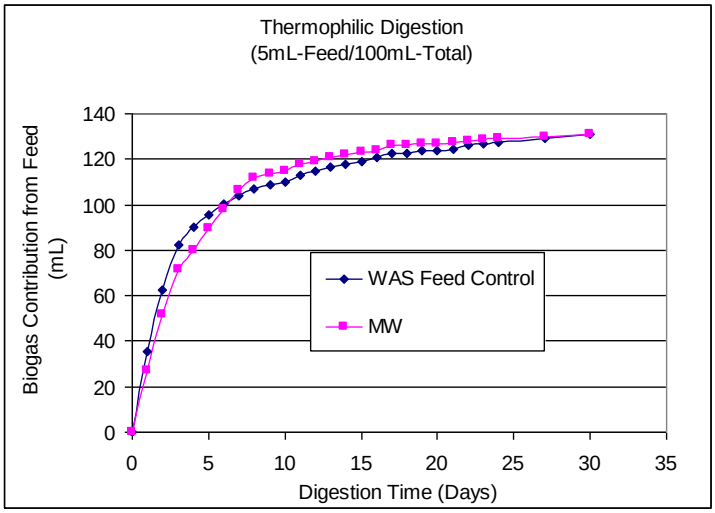
Figure 6.26 reports the biogas composition from microwave-pretreated feed on digestion day 7 and day 29. The methane content from thermophilic digestion was similar to that from mesophilic digestion at 60-66%. Nitrogen content followed the same dilution pattern, in accordance with the biogas production.











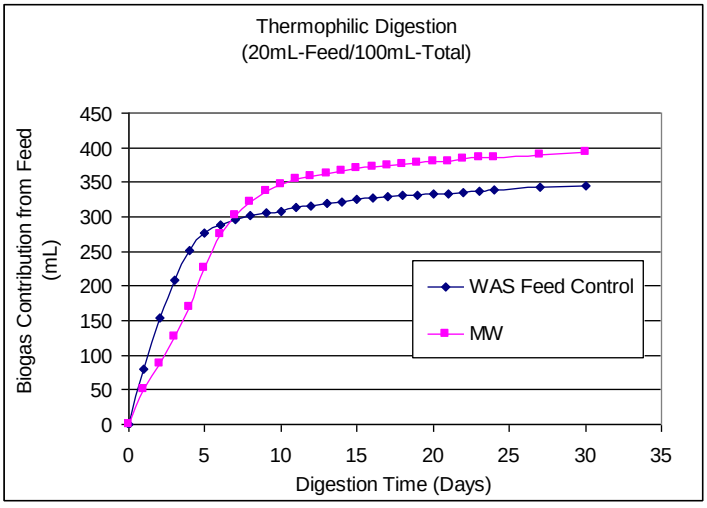


Figure 6.22 Thermophilic digestion of microwave treated sludge at two organic loading conditions (1.7 g-VS/L on the left column, 6.8 g-VS/L on the right column)

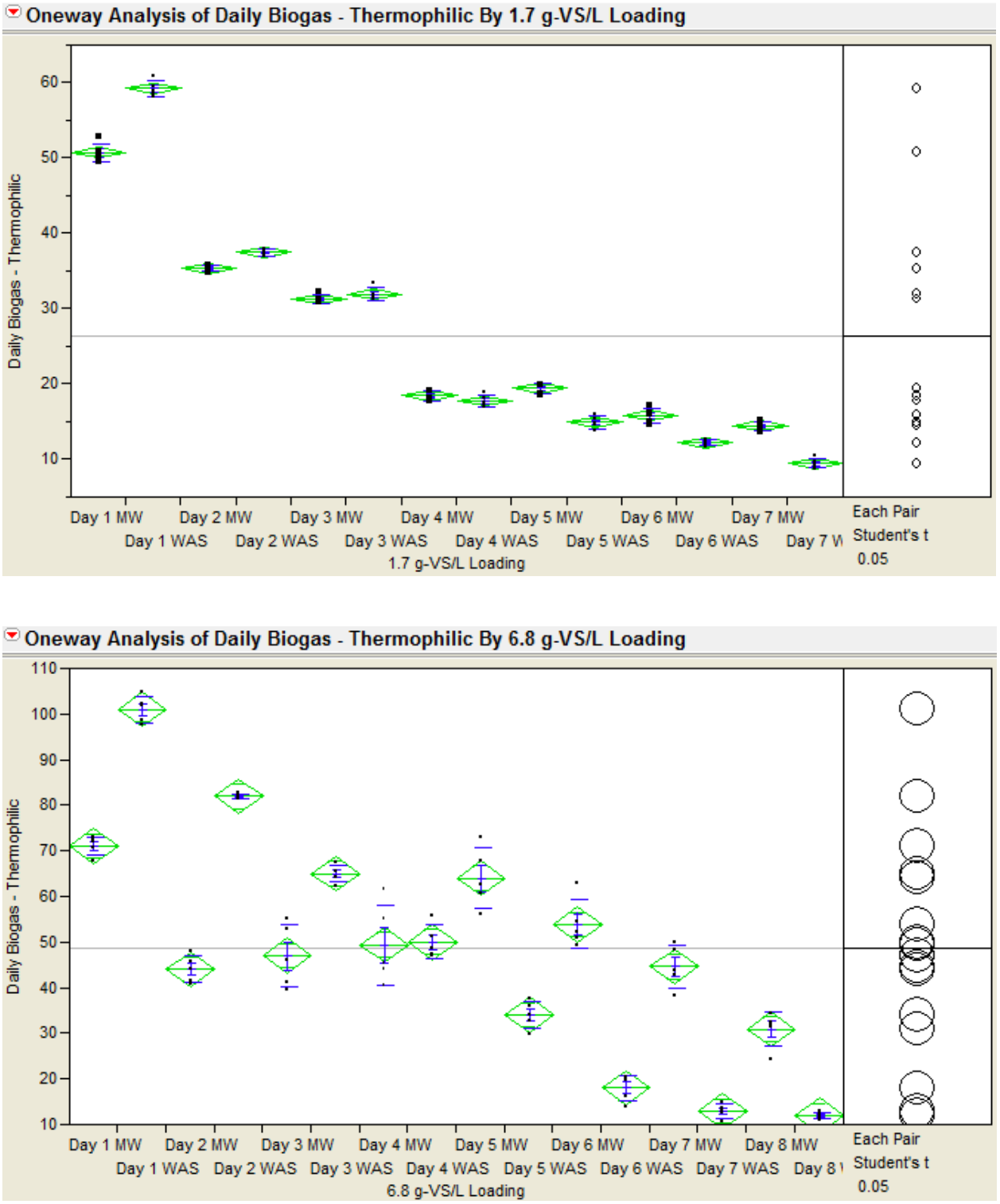
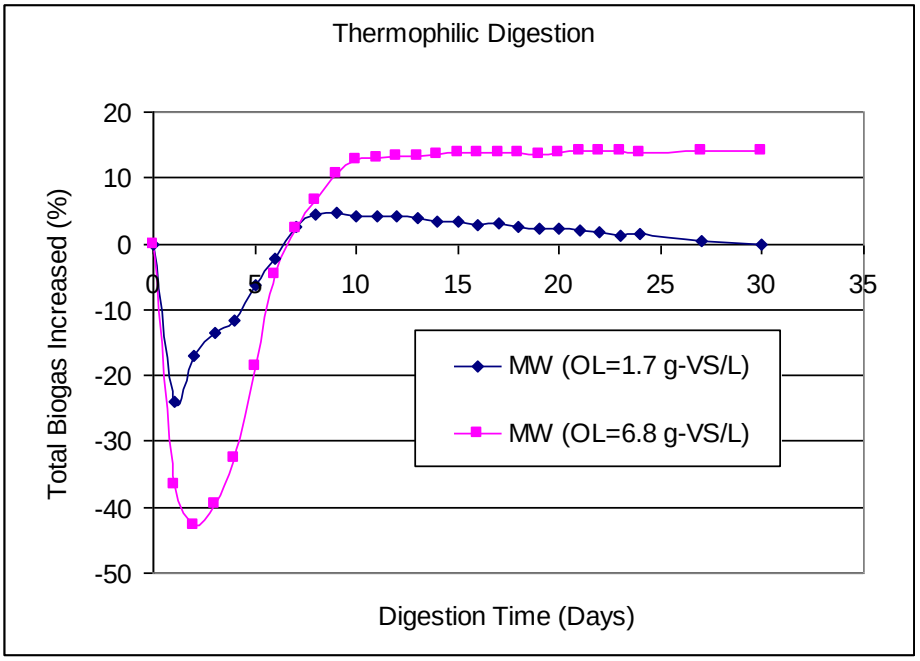
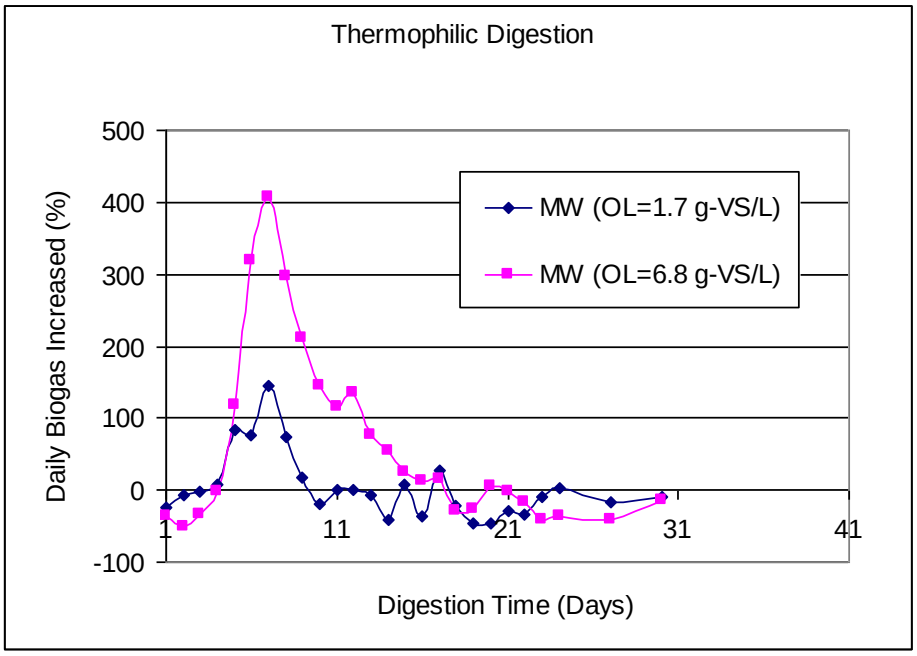


Figure 6.23 Student's t-Test for comparisons of thermophilic daily biogas production from untreated WAS and microwave treated sludge

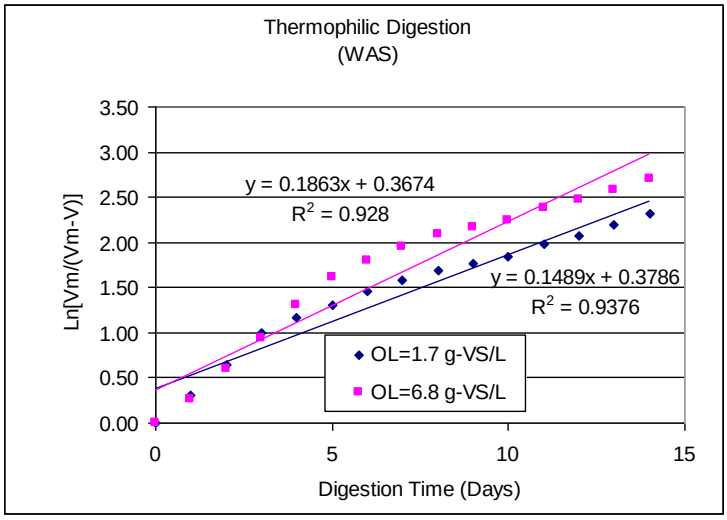


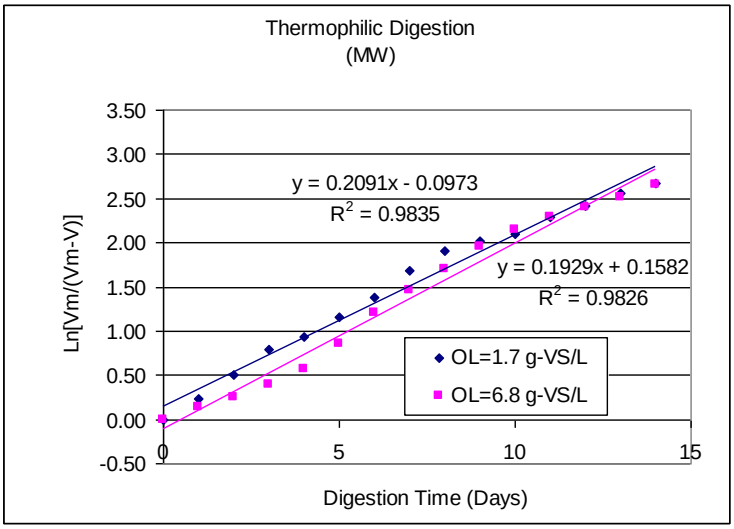
(a)



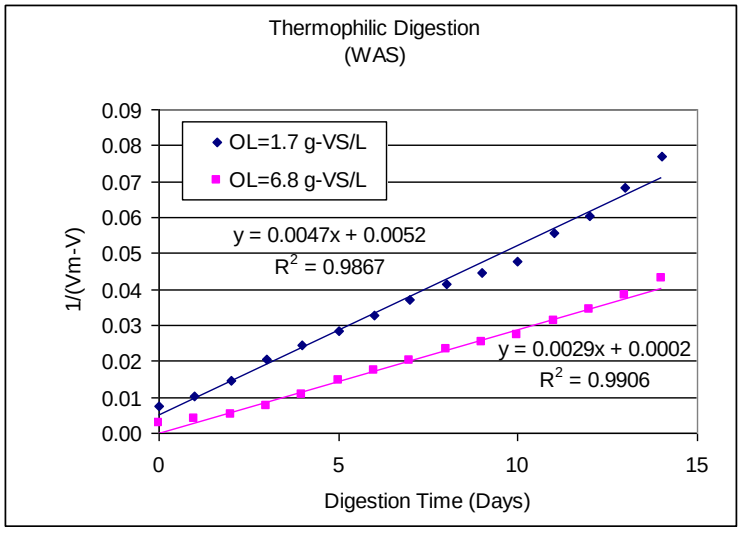
(b)

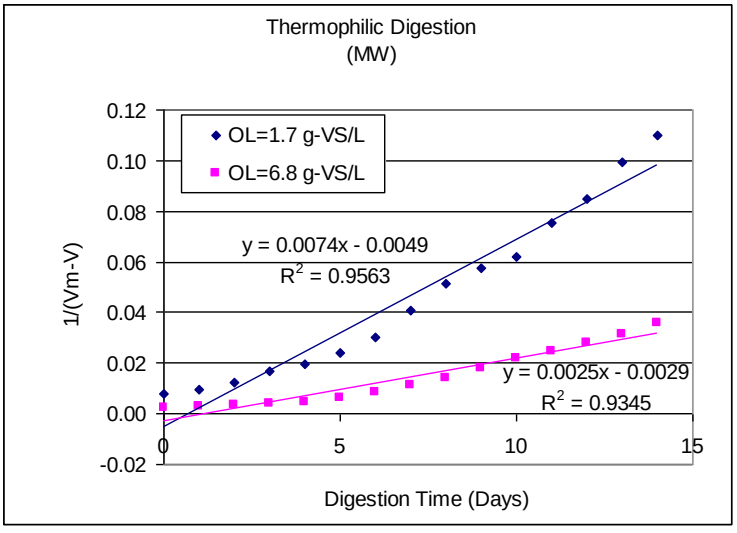
Figure 6.24 Thermophilic total (a) and daily biogas (b) increase from microwave treated sludge in comparison to WAS feed control





(a)





(b)

Figure 6.25 Reaction kinetics for thermophilic biogas productions from untreated WAS and microwave treated feed (first order plots (a) and second order plots (b))

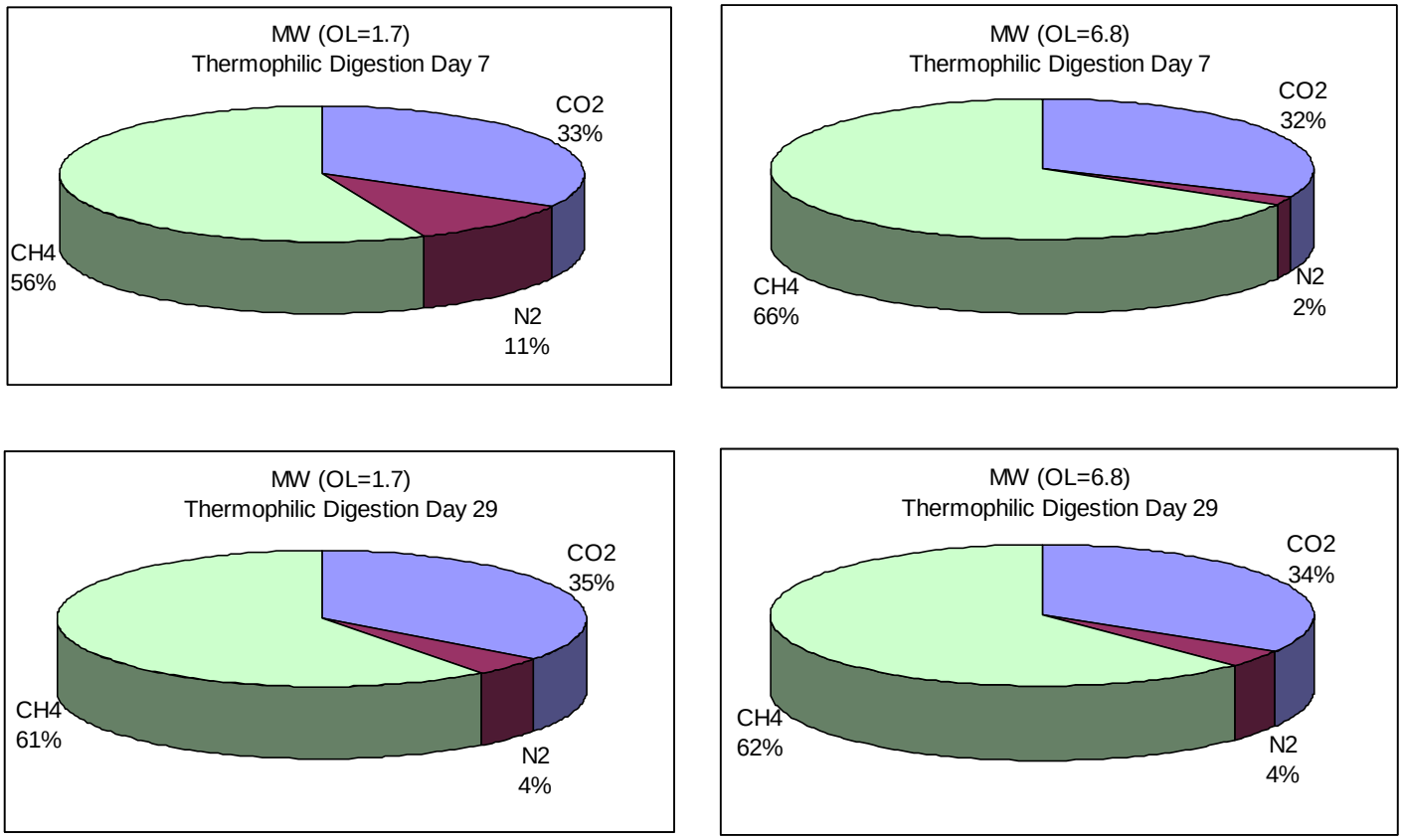


Figure 6.26 Biogas composition from microwave treated sludge at thermophilic digestion day 7 and day 29

6.3.2.2 MW/H_2O_2-pretreated WAS

Figure 6.27 reports results for thermophilic biogas production from MW/H_2O_2-pretreated feed, the untreated WAS (feed control) and inoculums (seed control). The students' t-test at 95% confidence interval is shown in Figure 6.28. As in the case with microwave treated feed, biogas production was inhibited for the first two days at organic loading of 1.7 g-VS/L and for the first three days for the 6.8 g-VS/L loading.

Under the lower loading conditions, the total biogas increase (biodegradability improvement) versus WAS feed control (Figure 6.29 (a)) turned positive at day 5, two days earlier than in the microwave treated case. It eventually settled at 14% by the end of digestion. Under the higher loading conditions, this final improvement was 15%, consistent with the low loading runs. It was at approximately the same level as it was in the mesophilic digestions results (12-16%). This indicates that the conversion of part of the inert organics to biodegradables by MW/H_2O_2 treatment could not be achieved by thermophilic (55°C) hydrolysis itself.

The overall reaction rate for MW/H_2O_2-pretreated feed, however, was slower due to inhibition. The first order kinetics was found to be a better fit (R^2=0.9796 and 0.9878 for 1.7 and 6.8 g-VS/L conditions, respectively, Figure 6.30) than for the second order ones. The consistent biogas composition (Figure 6.31) confirmed that there was no fundamental change in overall thermophilic digestion with pretreated feeds.

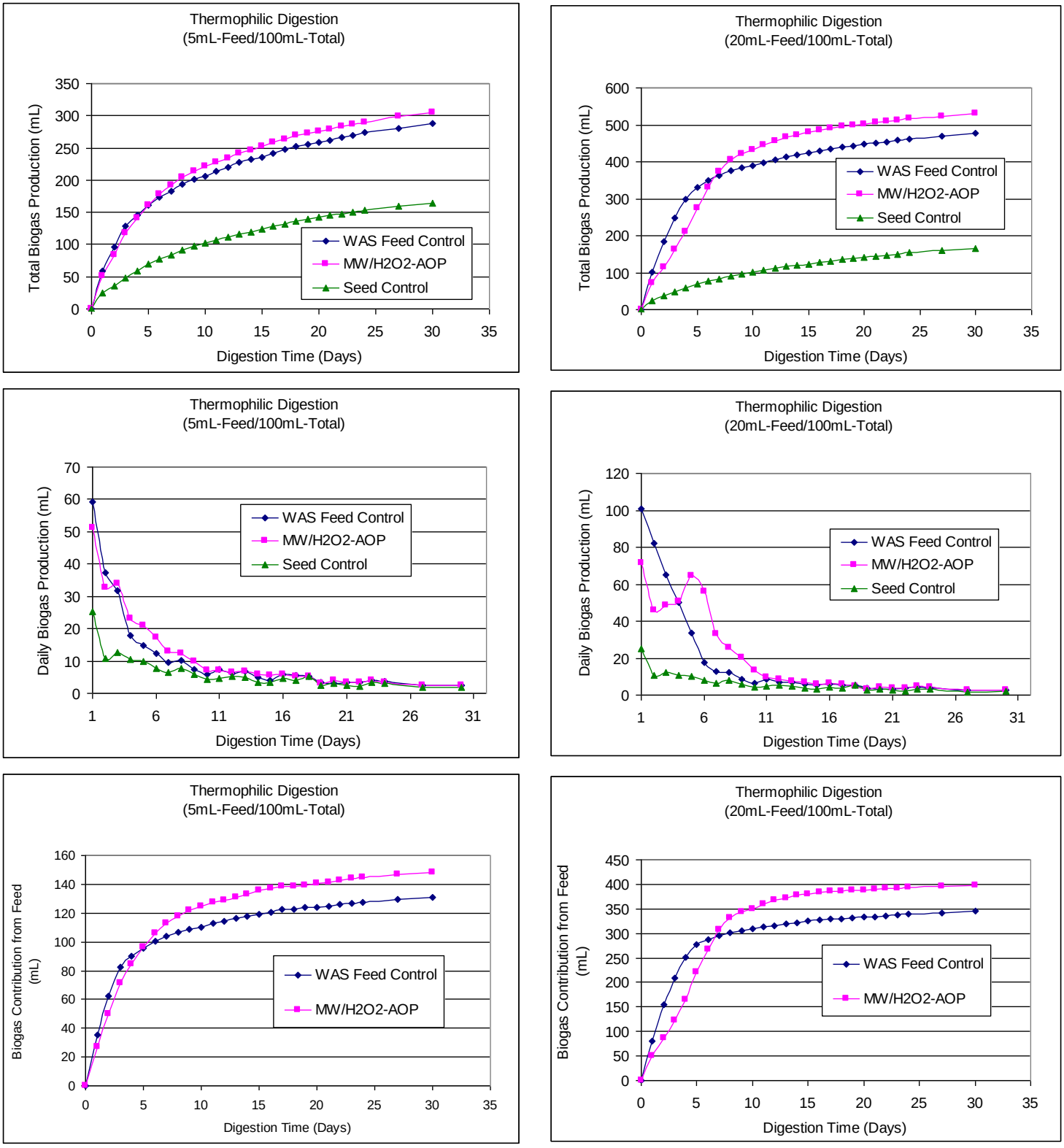


Figure 6.27 Thermophilic digestion of MW/H_2O_2 treated sludge at two organic loading conditions (1.7 g-VS/L on the left column, 6.8 g-VS/L on the right column)

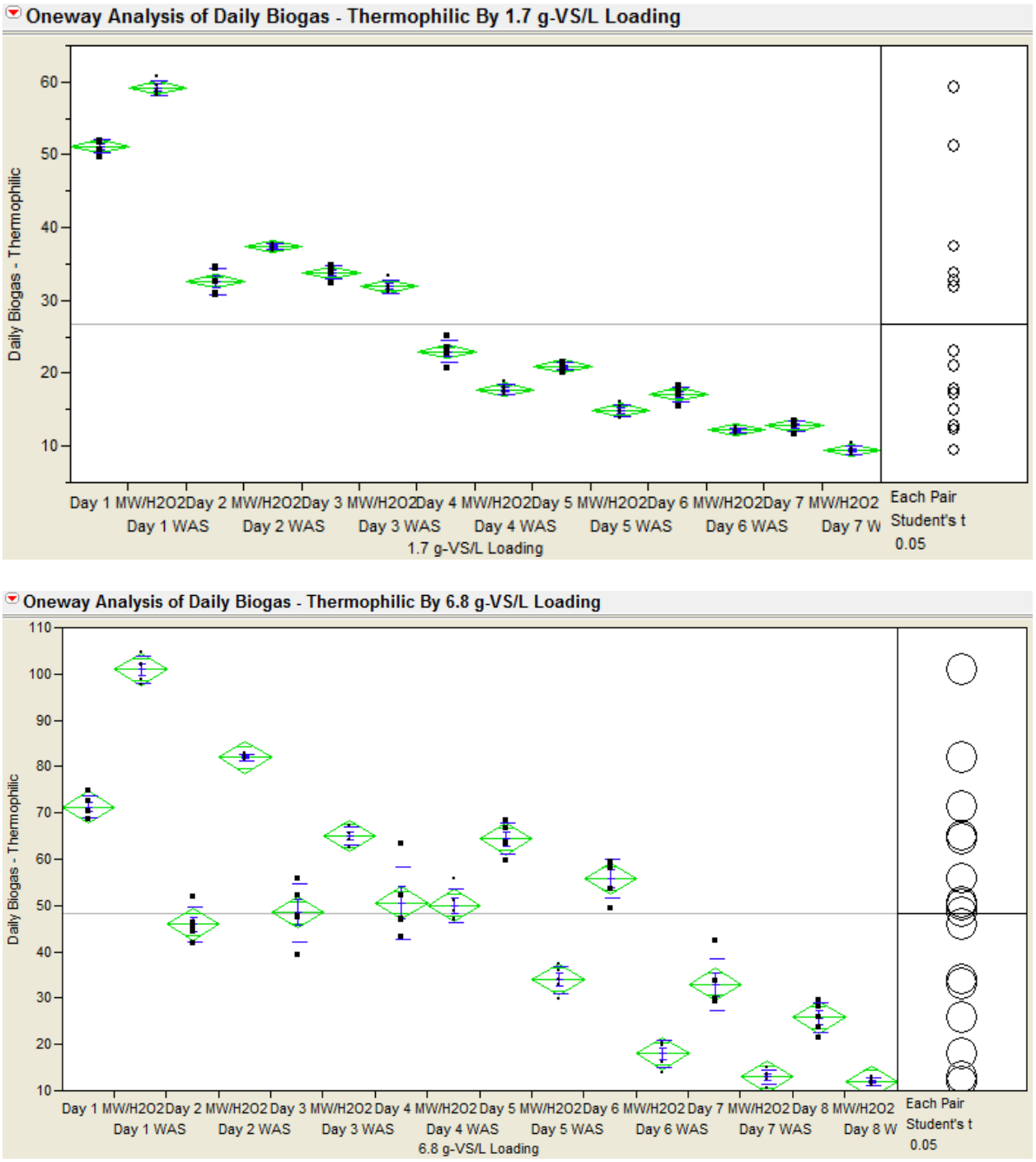
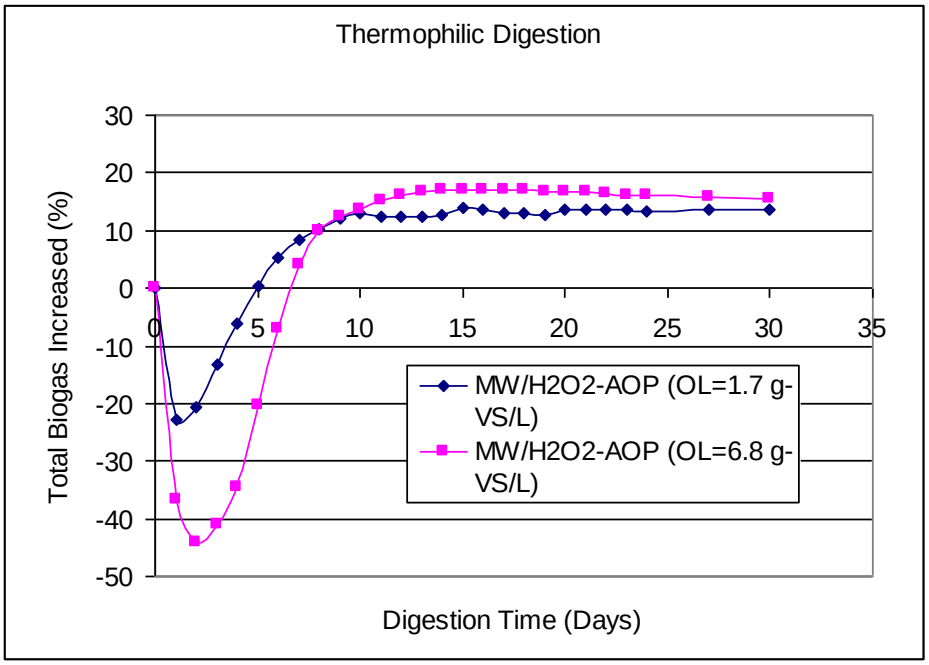
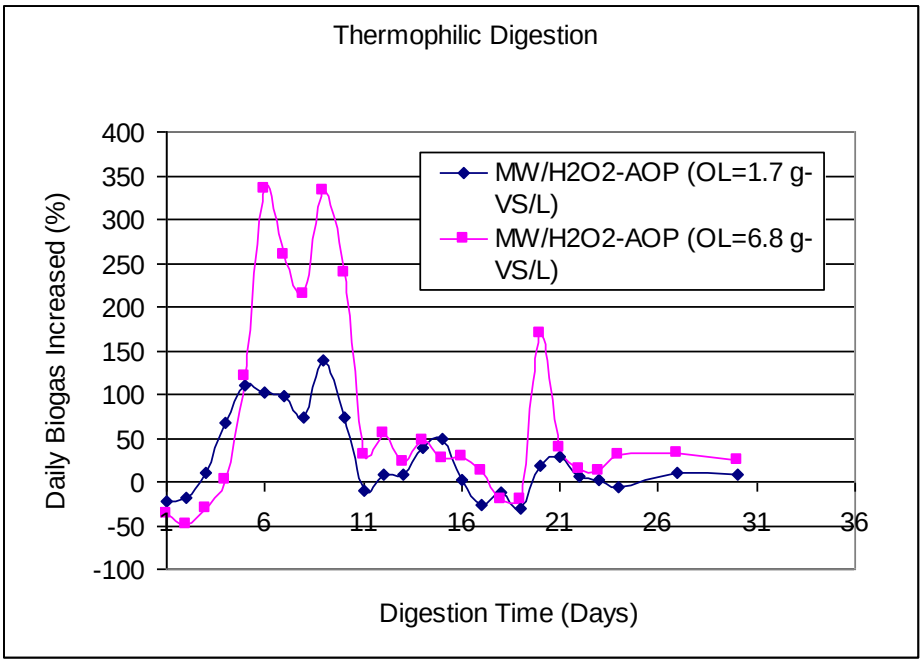


Figure 6.28 Student's t-Test for comparisons of thermophilic daily biogas production from untreated WAS and MW/H_2O_2 treated sludge

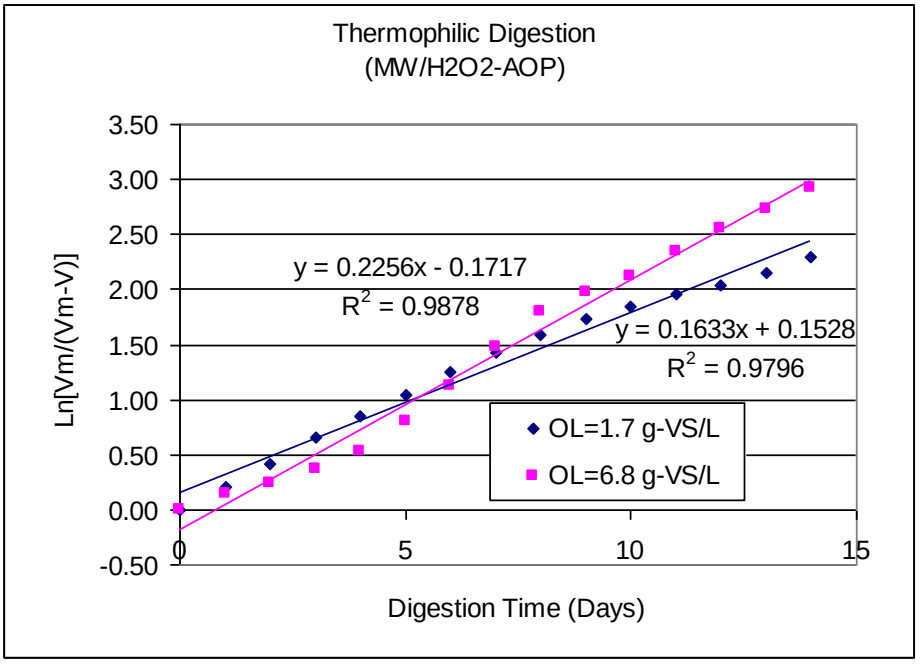


(a)

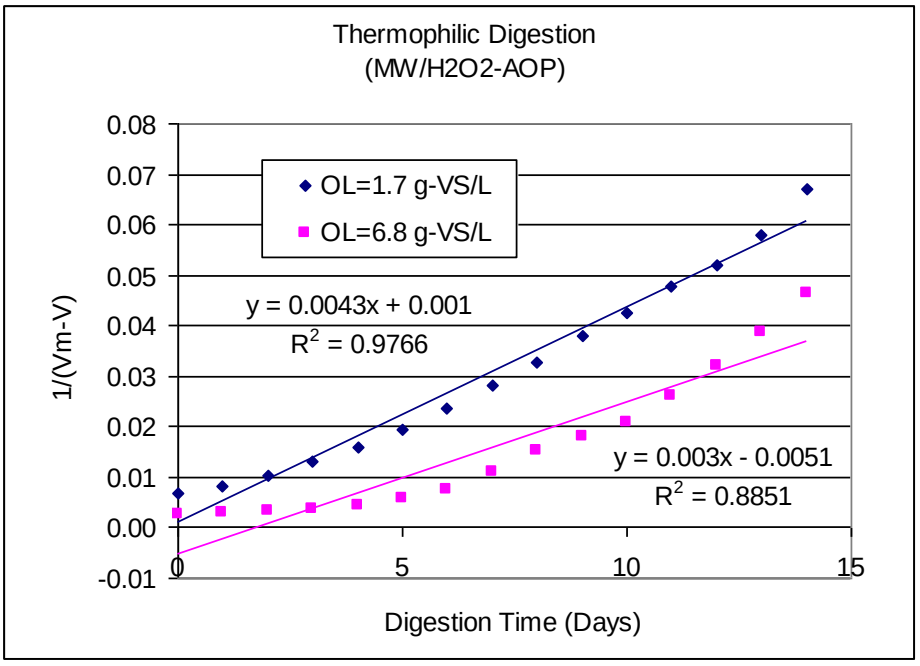


(b)

Figure 6.29 Thermophilic total (a) and daily biogas (b) increase from MW/H_2O_2 treated sludge in compare to WAS feed control



(a)



(b)

Figure 6.30 Reaction kinetics for thermophilic biogas productions from MW/H_2O_2 treated feed (first order plots (a) and second order plots (b))

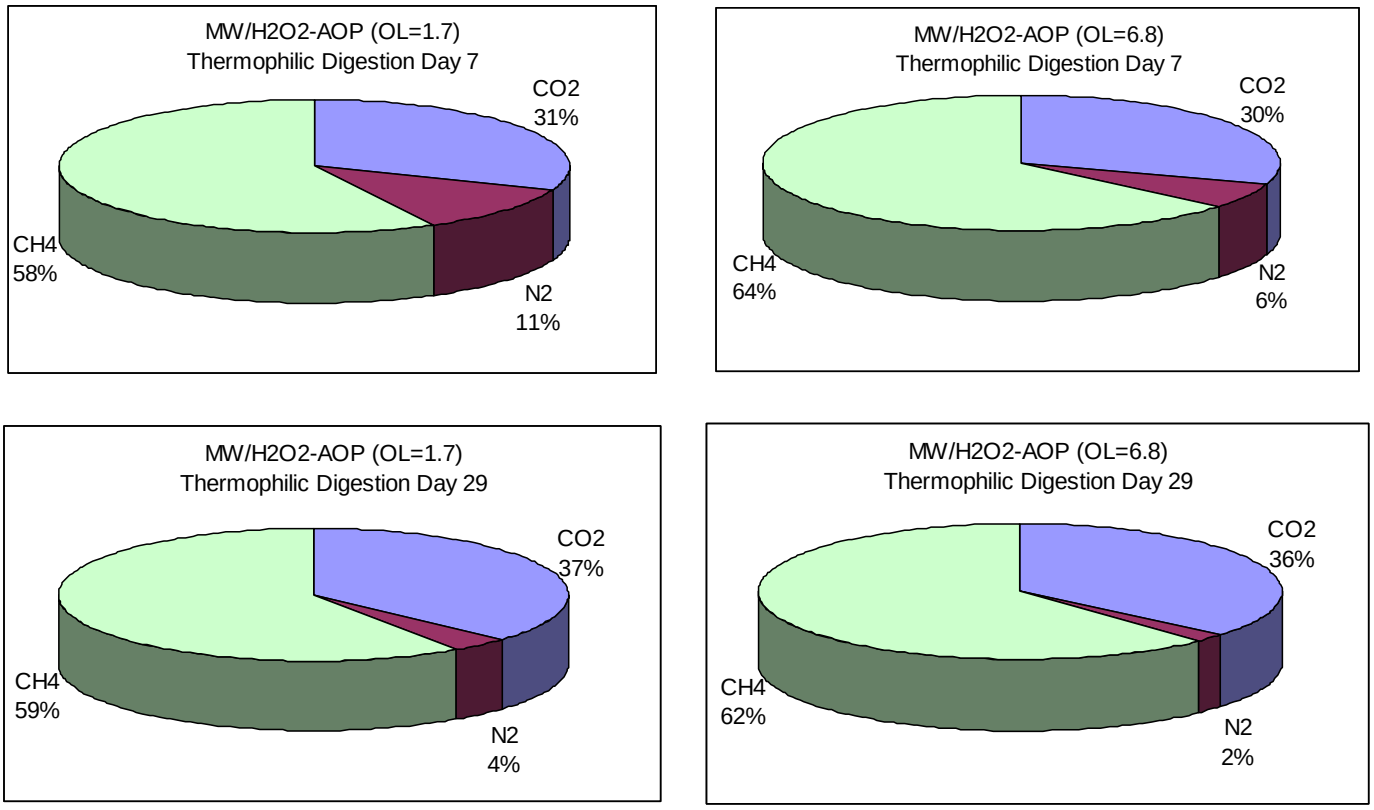


Figure 6.31 Biogas compositions from MW/H_2O_2 treated sludge at thermophilic digestion day 7 and day 29

6.3.2.3 Ultrasound-pretreated WAS

The thermophilic biogas production from ultrasound-pretreated feed also showed inhibition under both organic loading conditions (Figure 6.32). The students' t-test at 95% confidence interval is shown in Figure 6.33. The pace of its recovery was slower as well compared to the microwave and MW/H_2O_2-pretreated feeds, especially at the higher organic loading. At an loading of 6.8 g-VS/L, it took 10 days for the total accumulated biogas to surpass that from the untreated WAS feed. The figure for both microwave and MW/H_2O_2-pretreated feeds was 7 days. The time to complete the overall digestion was similar, at 16 days.

The total biogas increases over untreated WAS feed (biodegradability improvement) for both organic loadings were at 11% (Figure 6.34(a)), significantly lower than in mesophilic digestion (25%, Section 6.3.1.3). This suggests that part of the biodegradability improvement made by ultrasound pretreatment could actually be accomplished by thermophilic hydrolysis. The 11% improvement was similar, but lower, than the 15% for MW/H_2O_2-pretreated feed.

The reaction kinetics was fitted to the first order reaction (R^2=0.9959 and 0.9752 for 1.7 and 6.8 g-VS/L conditions, respectively, Figure 6.35). It is slower than the second order reaction in mesophilic digestion. But with severe initial inhibition, the thermophilic microorganism community is still resilient and recovered by the end of digestion period. The biogas composition at digestion day 7 and day 29 is shown in Figure 6.36. There were no noticeable changes compared to the other feeds, including untreated WAS.

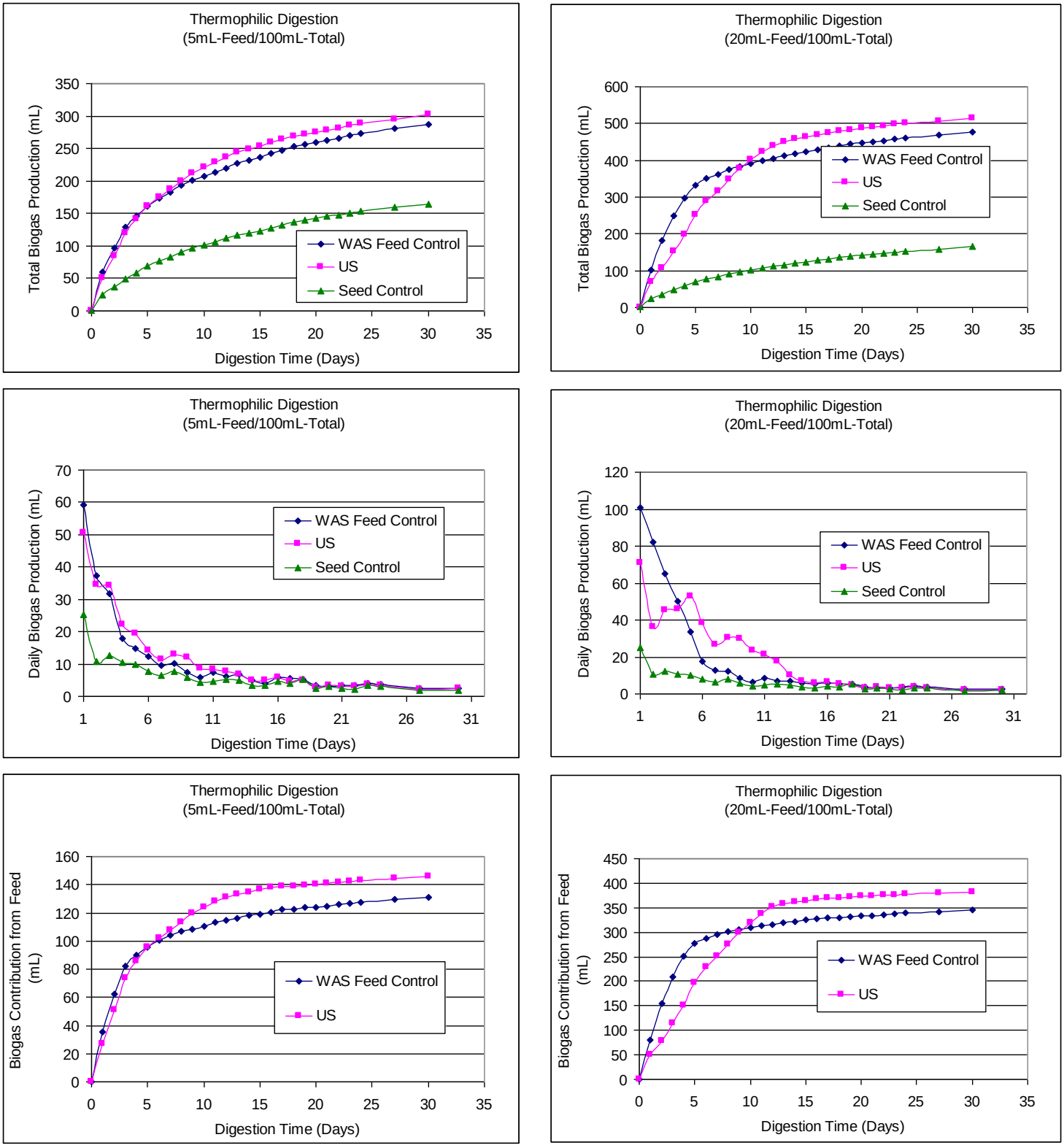


Figure 6.32 Thermophilic digestion of ultrasound treated sludge at two organic loading conditions (1.7 g-VS/L on the left column, 6.8 g-VS/L on the right column)

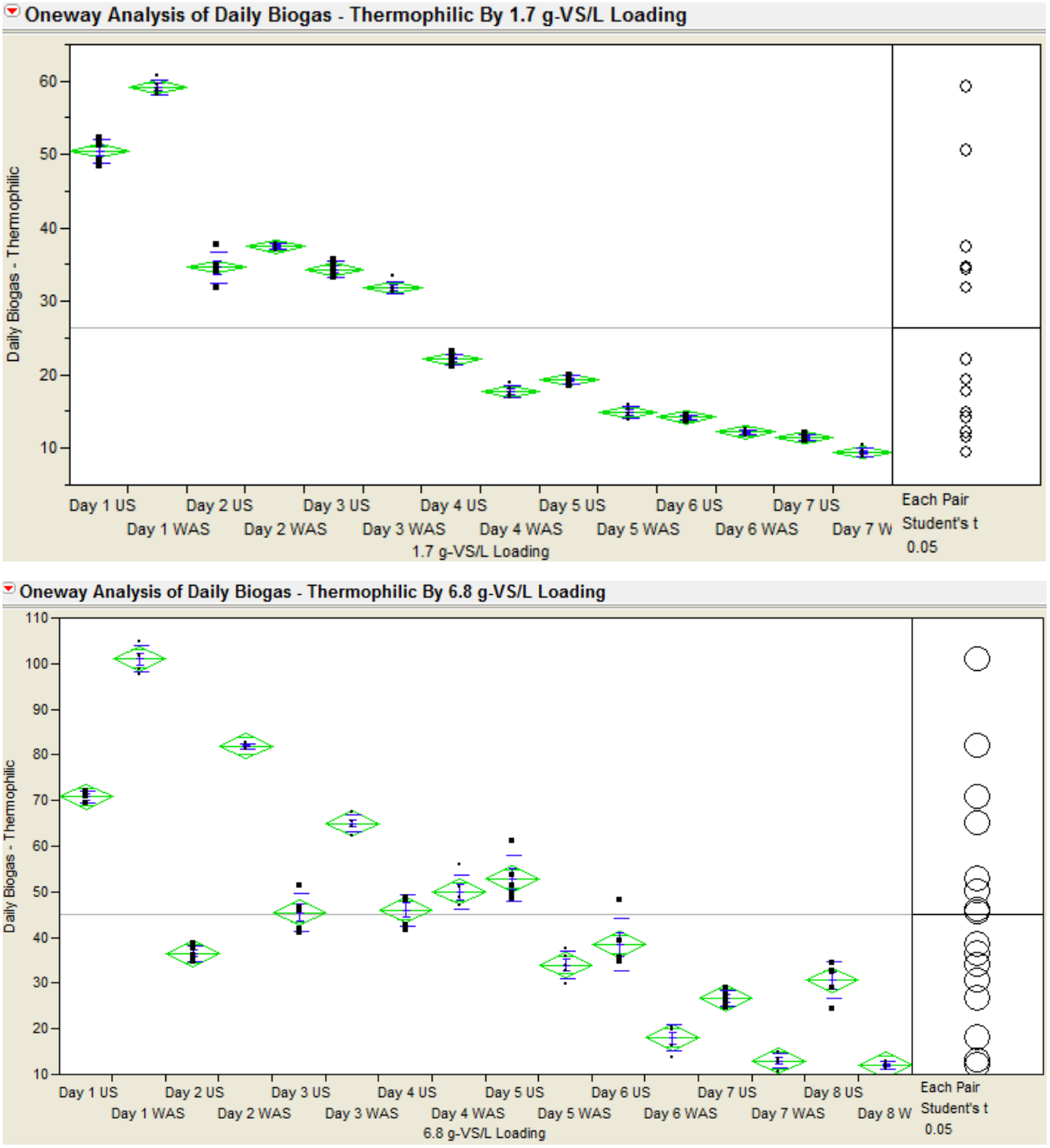
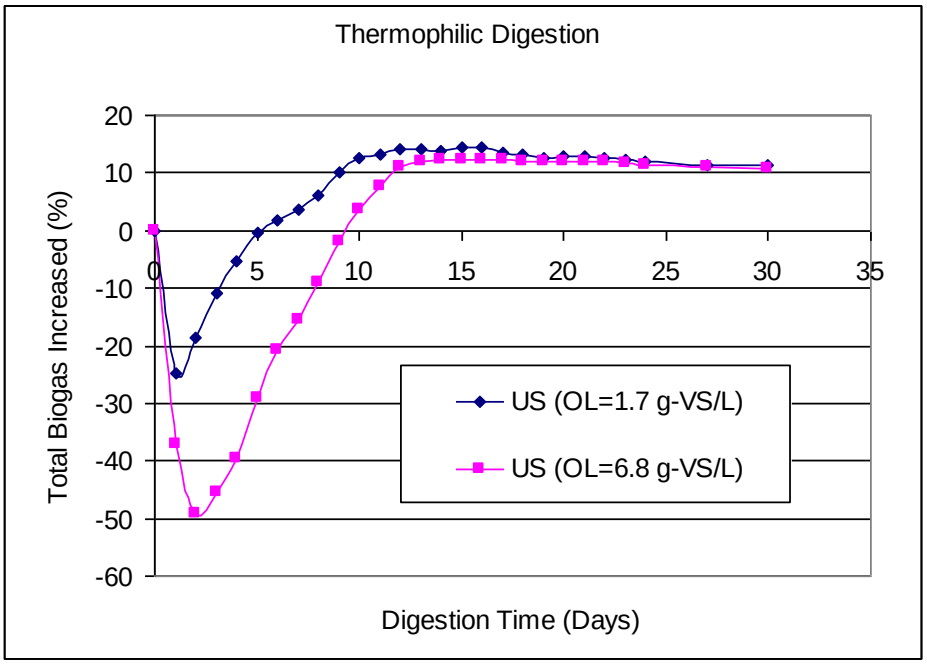
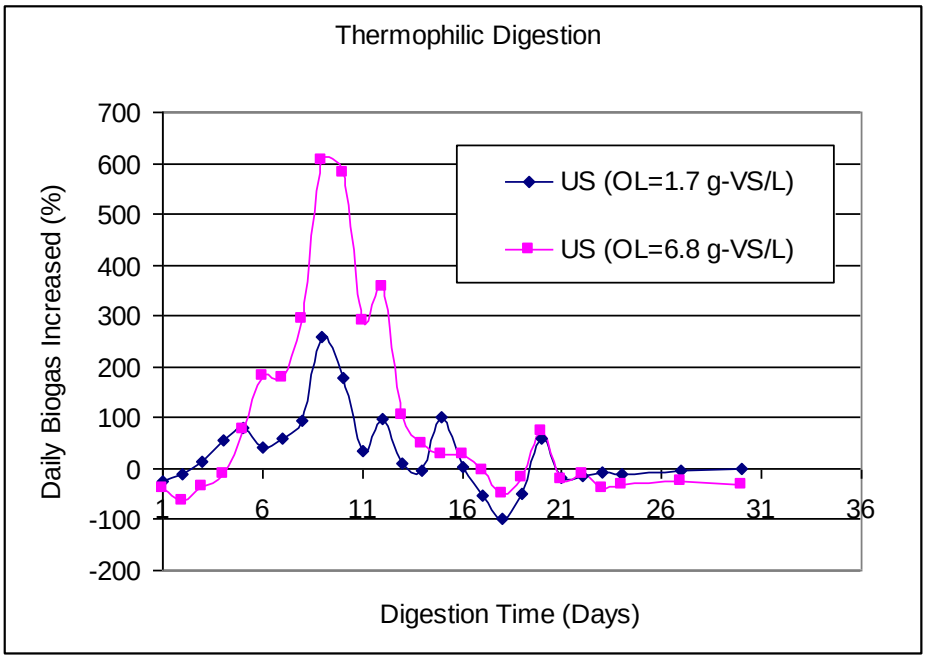


Figure 6.33 Student's t-Test for comparisons of thermophilic daily biogas production from untreated WAS and ultrasound treated sludge

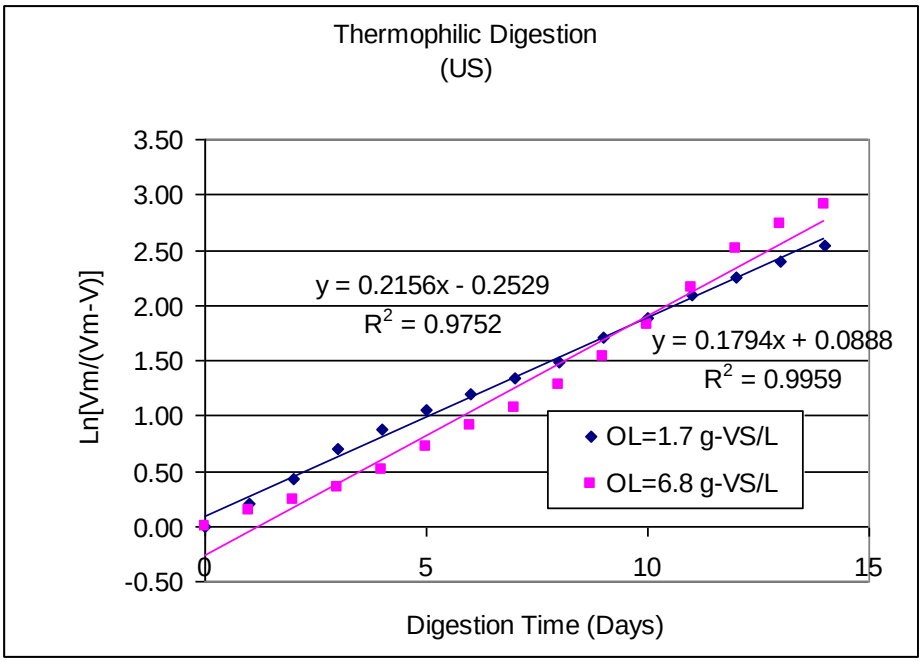


(a)

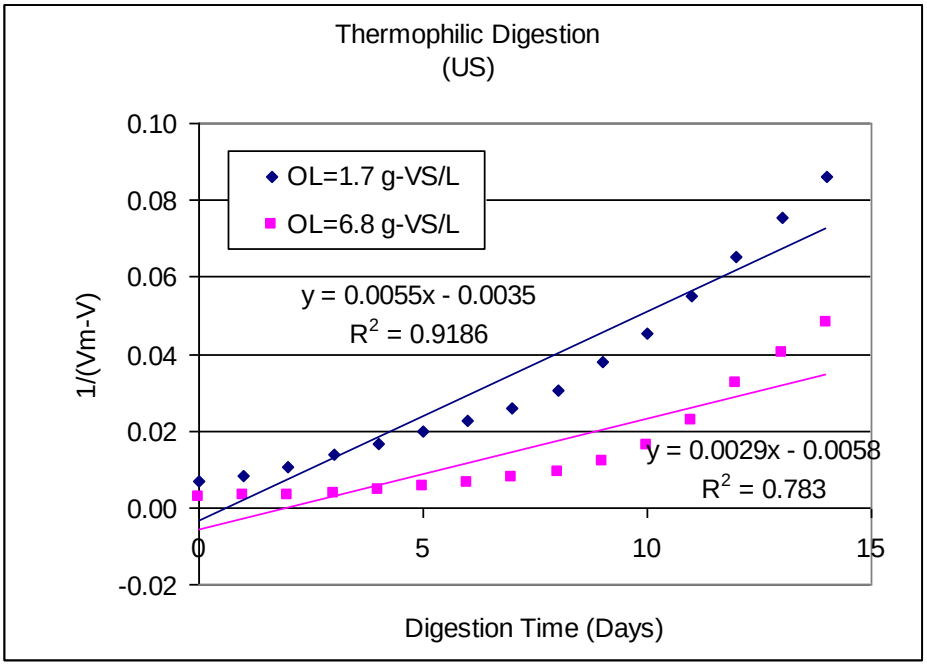


(b)

Figure 6.34 Thermophilic total (a) and daily biogas (b) increase from ultrasound treated sludge in compare to WAS feed control



(a)



(b)

Figure 6.35 Reaction kinetics for thermophilic biogas productions from ultrasound treated feed (first order plots (a) and second order plots (b))

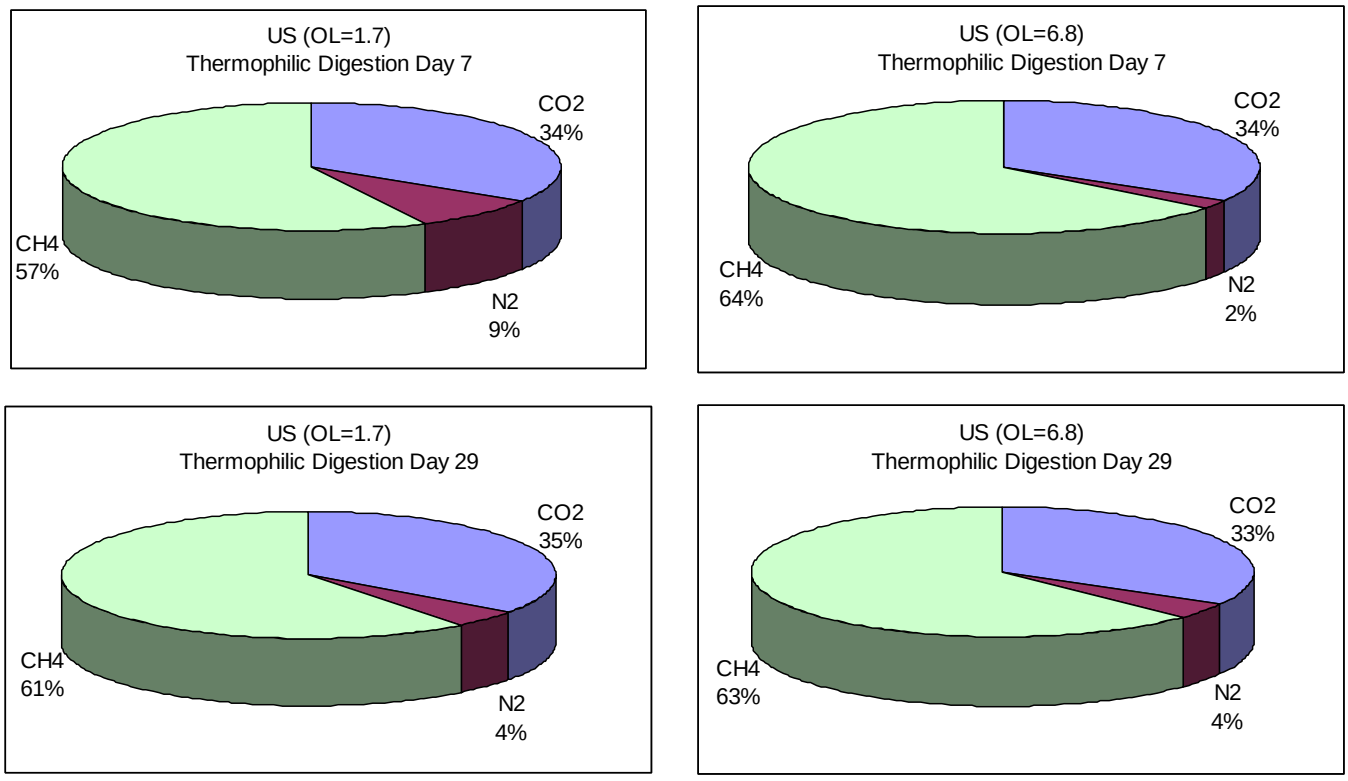


Figure 6.36 Biogas compositions from ultrasound treated sludge at thermophilic digestion day 7 and day 29

6.3.2.4 Protease and ultrasound/protease-pretreated WAS

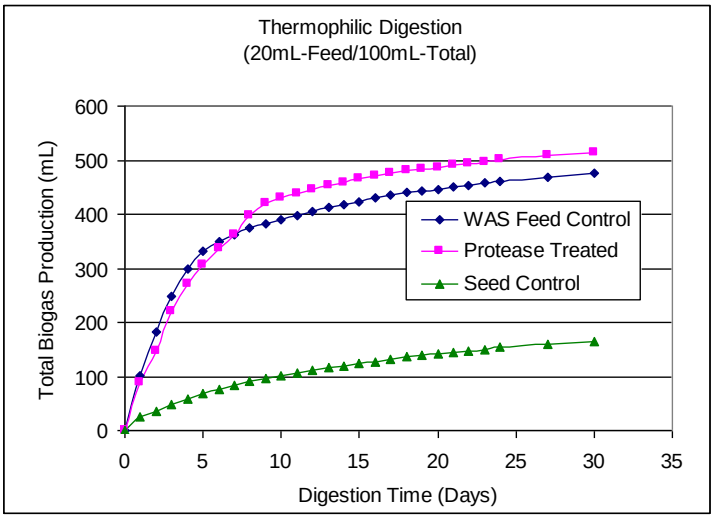
Figure 6.37 reports the thermophilic biogas production from protease and ultrasound/protease-pretreated sludge, together with biogas from untreated WAS (feed control) and the inoculums (seed control). The students' t-test at 95% confidence interval is shown in Figure 6.38. At an loading of 6.8 g-VS/L, protease-pretreated feed disgestion experienced a relatively minor inhibition compared to microwave, MW/H_2O_2, and ultrasound-pretreated feeds. On the other hand, the ultrasound/ protease-pretreated feed had the most severe inhibition of all the pretreated feeds. It took 15 days for the total accumulated biogas to surpass that from untreated WAS, and 25 days to complete the overall digestion process.

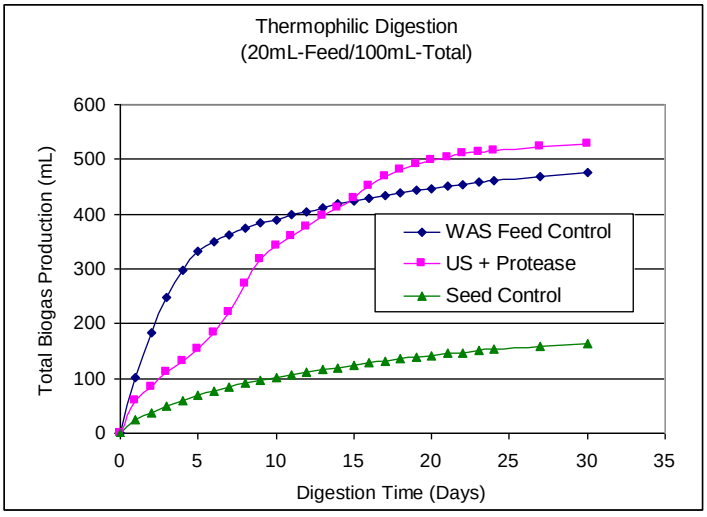
For protease treated feed, the acclimation time was the same as for the other pretreated feeds. Figure 6.39(a) shows that at digestion day 7, the total biogas increase (biodegradability improvement) turned positive. The initial decrease during the first two days (-23%) was significantly less than it was for microwave (-43%), MW/H_2O_2 (-44%), and ultrasound (-49%) pretreated feeds. This indicates that the addition of protease and the resulting soluble substrates, were relatively better adapted by the thermophilic inoculums. The soluble substrates from protease treatment were higher in VFA, approximately the same in amino acids, and substantially lower in soluble COD, protein, and polysaccharides (Chapter 5), compared to the other pretreatments.

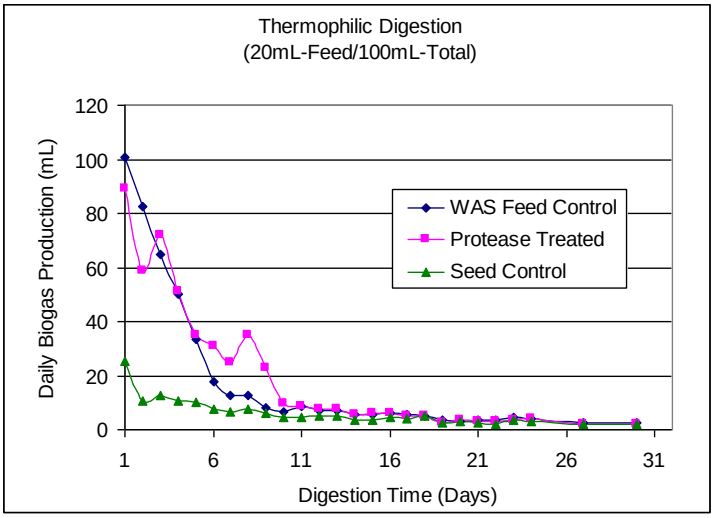
In the case of ultrasound/protease-pretreated feed, the impact of pretreatment resulted in a 67% decrease in total biogas at digestion day 4, compared to the base line from untreated WAS feed. The ultrasound/protease treated feed had low VFA and low soluble protein, but high amino acids compared to the other pretreatments. Thus, the initial

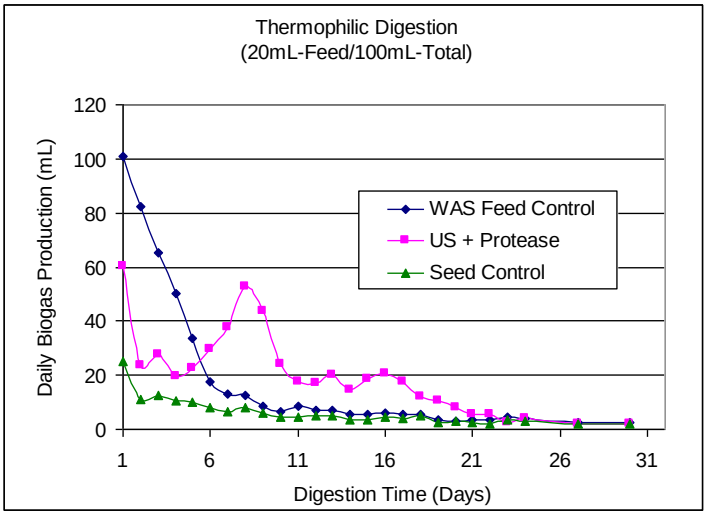
VFA in these feeds was not the major factor influencing inhibition. Amino acids, together with other immediately available substrates such as soluble protein and polysaccharides, were the likely contributors to the negative impact. The correlation of these various parameters to the inhibition (impact) on biogas production was made and is discussed in Section 6.3.3.

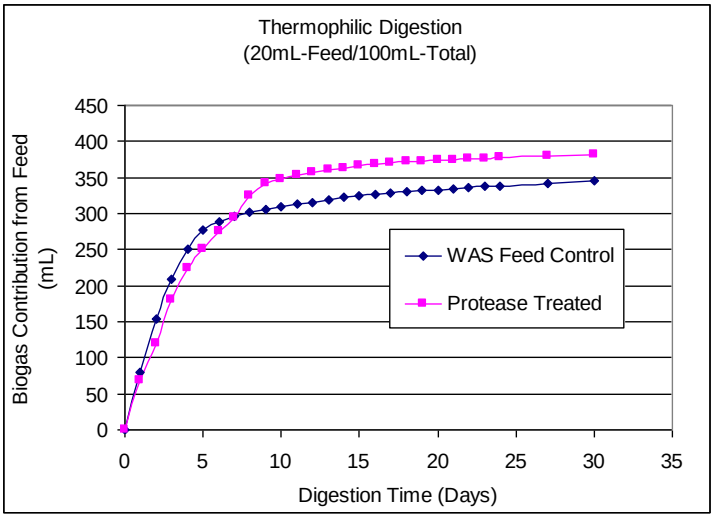
First order reaction kinetics could be used to describe digestion for protease and ultrasound/protease-pretreated feeds (R^2=0.9899 and 0.9583, respectively, Figure 6.40). Second order kinetics was far from accurate in these two cases. Biogas composition at digestion day 7 and day 29 are reported in Figure 6.41. Methane, at between 62-65%, was the dominant component at the end of digestion.











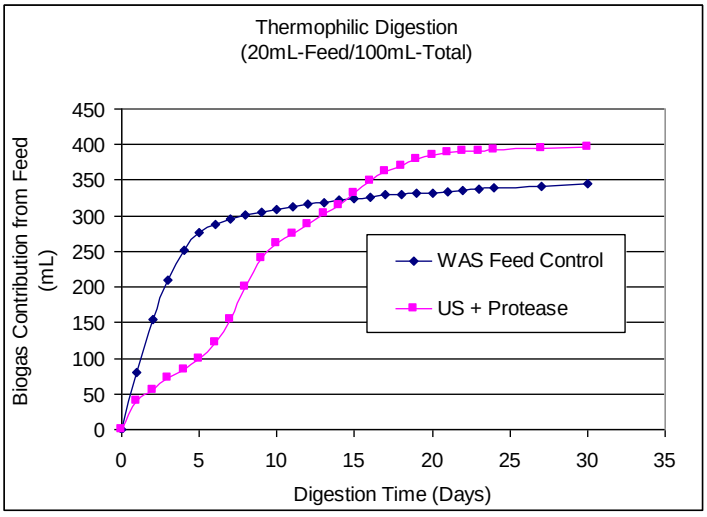


Figure 6.37 Thermophilic digestion of protease and ultrasound/protease treated sludge at organic loading of 6.8 g-VS/L

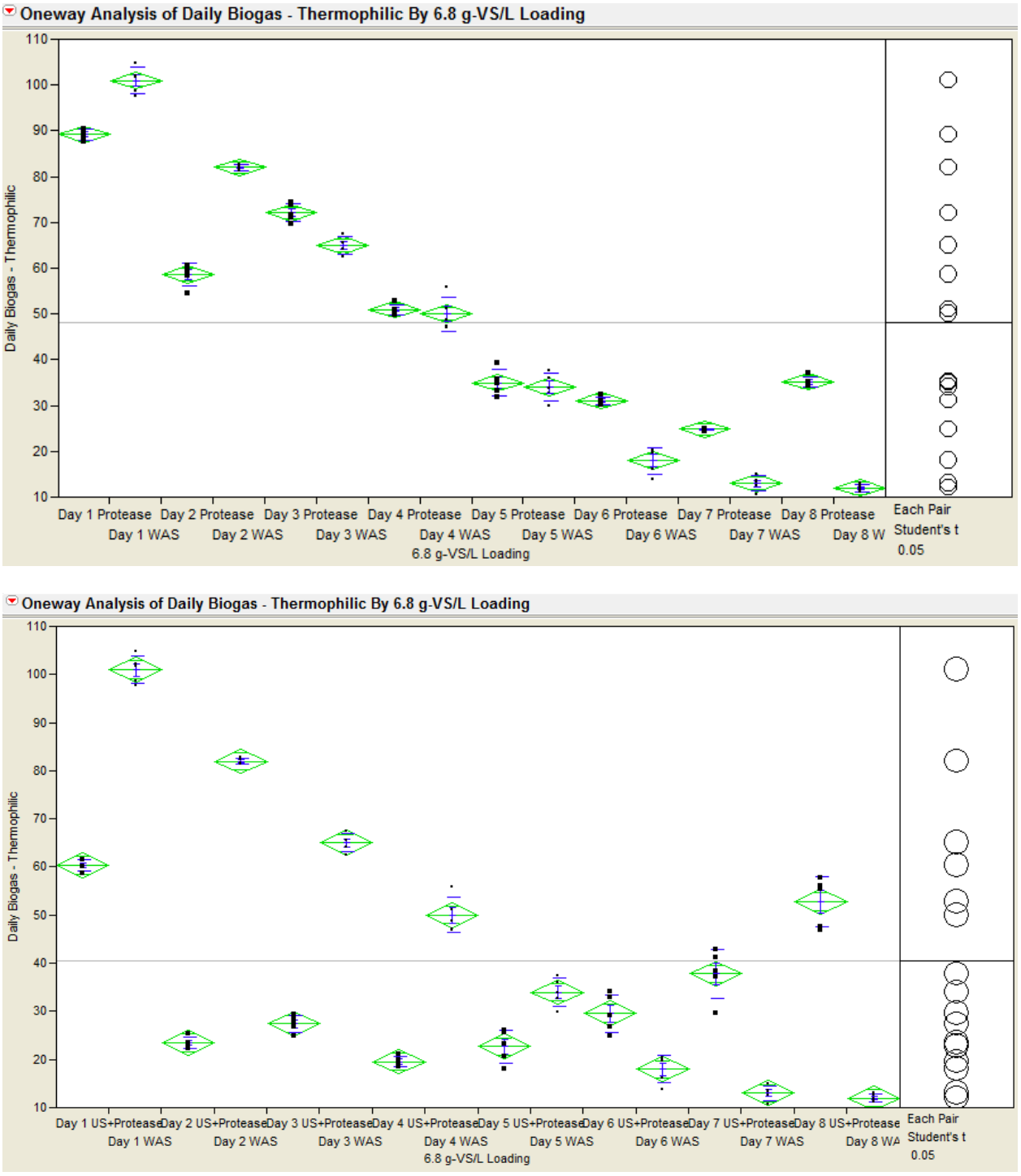
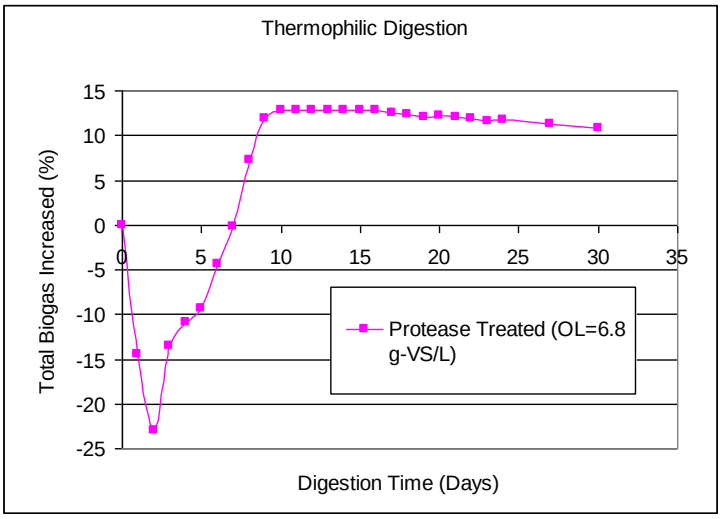
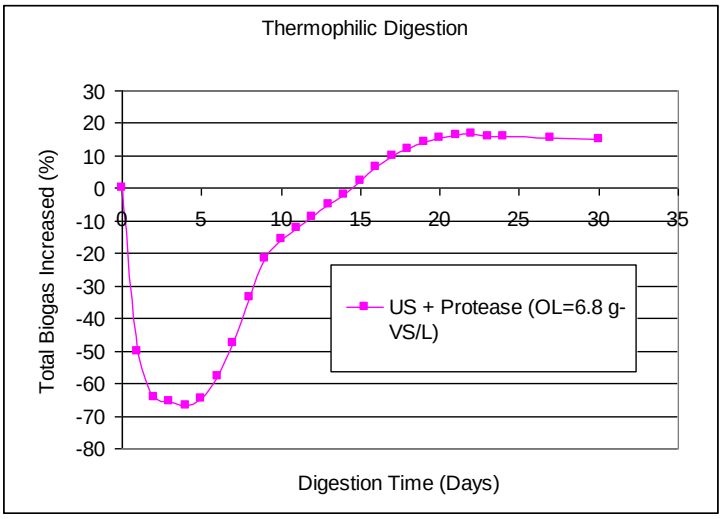
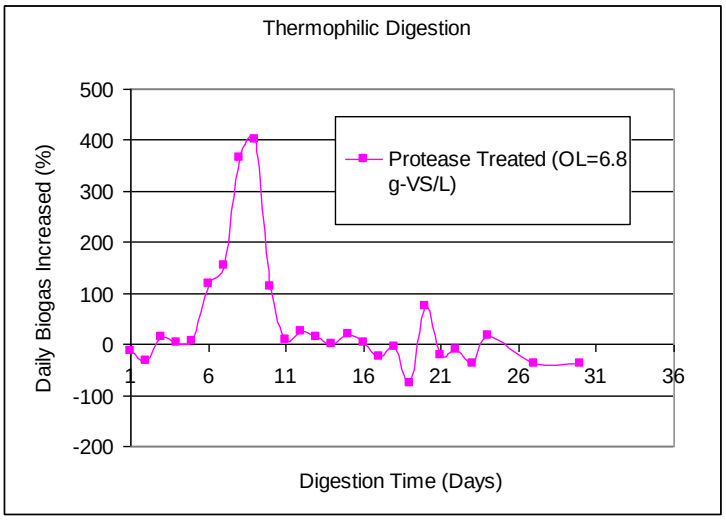


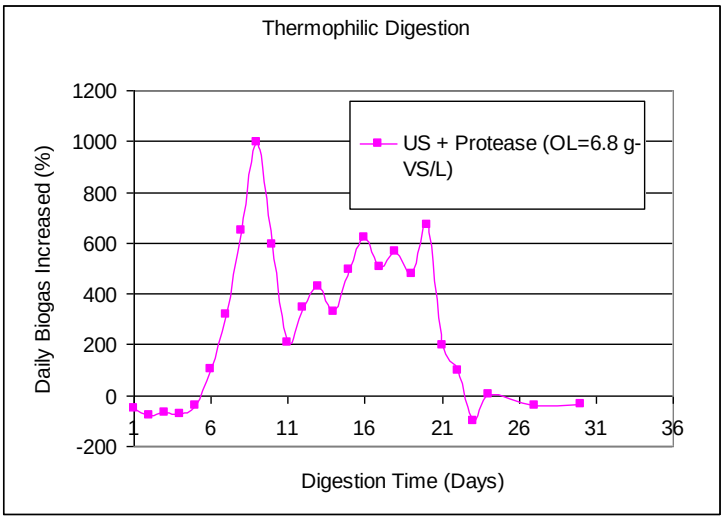
Figure 6.38 Student's t-Test for comparisons of thermophilic daily biogas production from untreated WAS, protease and ultrasound/protease treated sludge





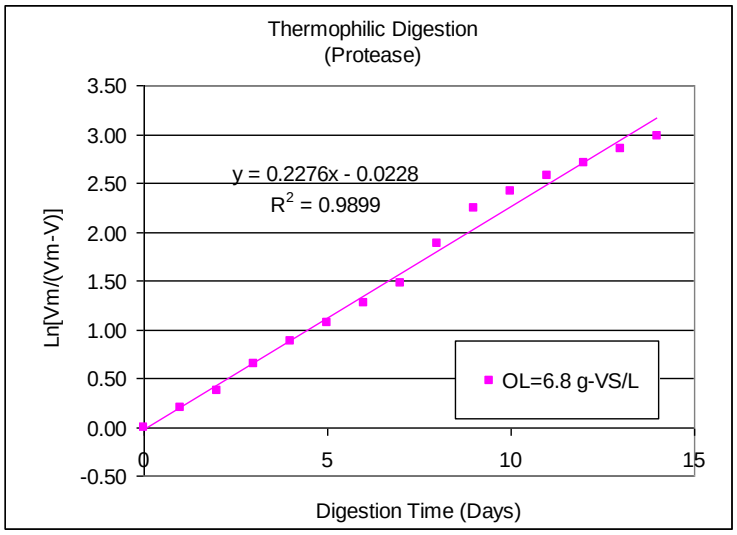
(a)

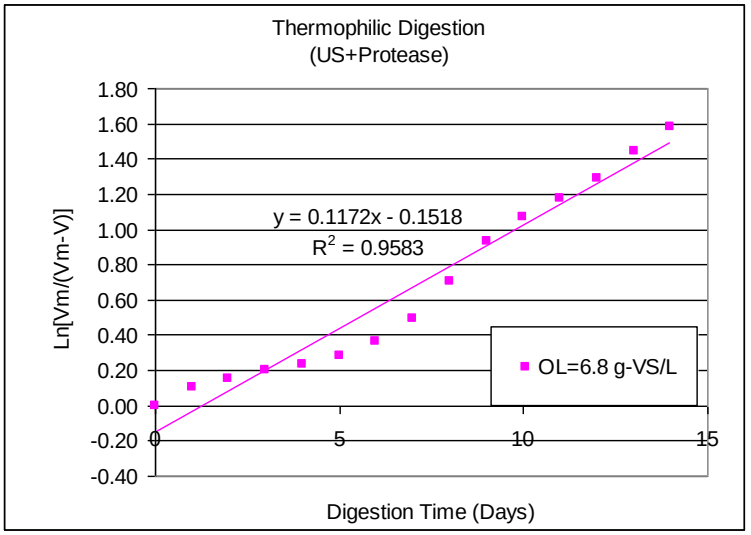




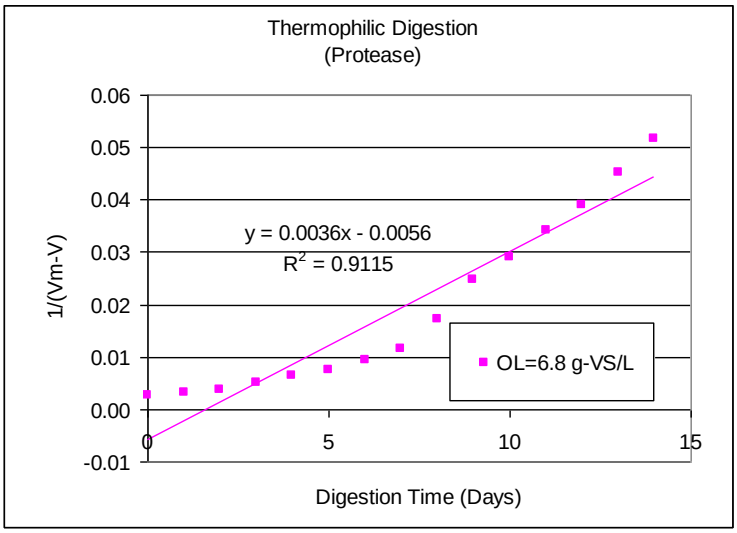
(b)

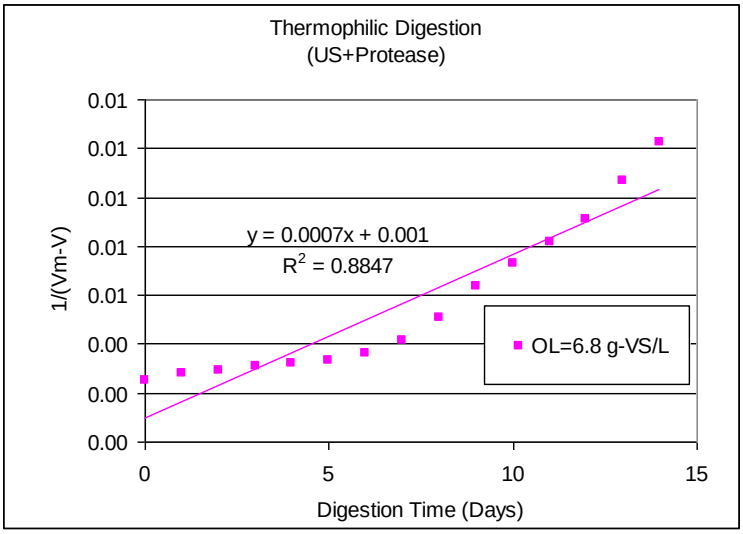
Figure 6.39 Thermophilic total (a) and daily biogas (b) increase from protease and ultrasound/protease treated sludge in compare to WAS feed control





(a)





(b)

Figure 6.40 Reaction kinetics for thermophilic biogas productions from protease and ultrasound/protease treated feed (first order plots (a) and second order plots (b))

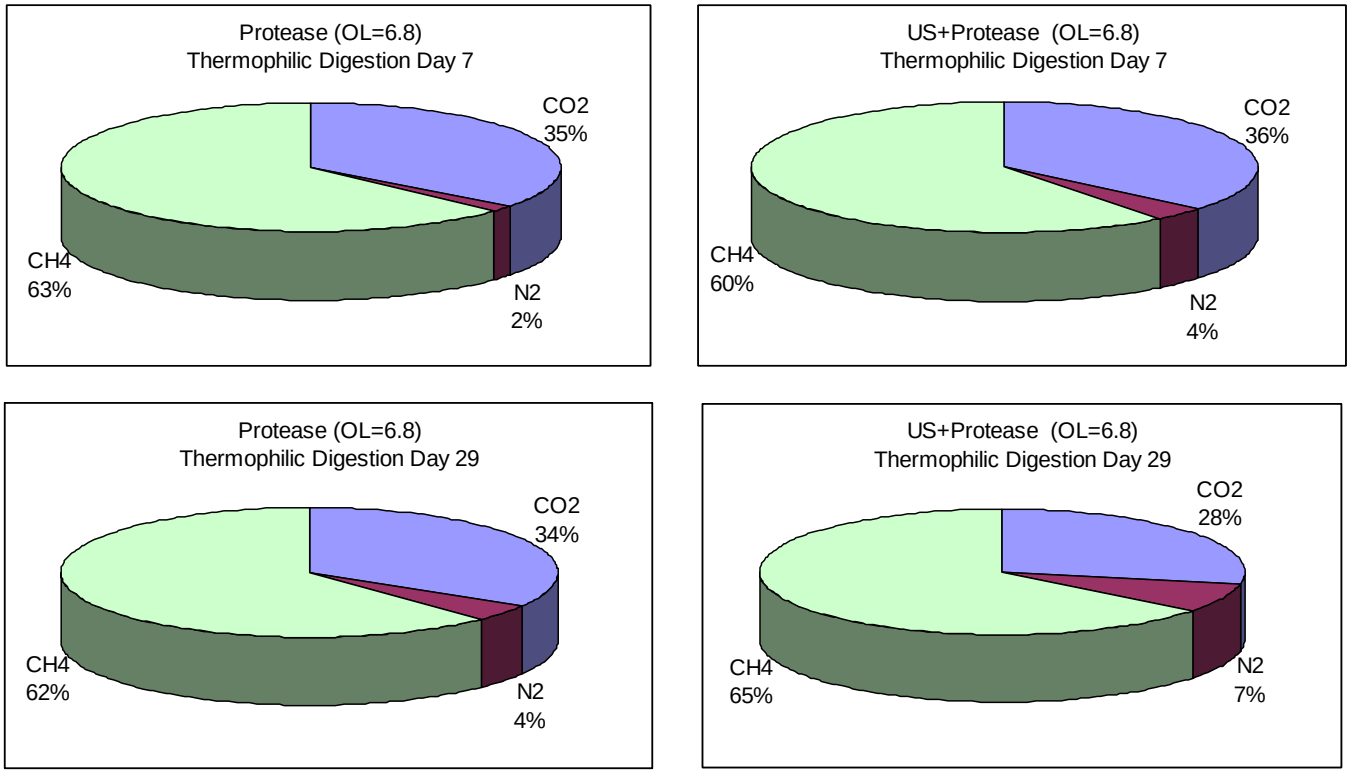


Figure 6.41 Biogas composition from protease and ultrasound/protease treated sludge at thermophilic digestion day 7 and day 29

6.3.2.5 Summary

The results for volatile solids reduction, COD degradable fraction and specific biogas production rate for thermophilic digestion of untreated WAS and for the pretreated feeds are reported in Table 6.2. The VS reductions were in the range of 65.5% to 69.4%. Under the low loading conditions (1.7 g-VS/L) where inhibitions were minor, the biodegradable fraction of COD substrate increased, from 61.1% in the untreated WAS, to 63.7%, 73.1%, and 77.6% in microwave, MW/H_2O_2, and ultrasound treated feed, respectively. The specific biogas production rates also increased, from 1.12 L/g-VS-destroyed with the untreated WAS, to 1.29 and 1.27 L/g-VS-destroyed with MW/H_2O_2 and ultrasound treated feed, respectively. At high loadings condition (6.8 g-VS/L), the biodegradable fraction of COD and specific biogas production rates also increased in various degrees.

In thermophilic digestion overall, biogas production from pretreated feeds was inhibited, likely through VFA accumulation due to the large increases in soluble substrates. Under low organic loading conditions, this inhibition was relatively minor; acclimation took on average 2 days. However, with high organic loadings the inhibition was significant. In the worst case scenario with ultrasound/protease treated feed, the acclimation took 6 days for daily biogas production to start climbing positively over the production rate for untreated WAS. The total biogas production was, however, still higher than the WAS feed control. Due to the inhibition from pretreated feed, the reaction kinetic for thermophilic digestion was better described by the first order reaction rather than by the second order reaction used previously to describe mesophilic digestion. Nonetheless, the thermophilic digestion of untreated WAS feed still fit with the second order kinetics under both loading conditions.

The ortho-phosphate levels in post thermophilic digestion supernatant were shown

on Figure 6.42 (a), and compare to the pre-digestion levels (Chapter 5, Section 5.3.4). The Figure 6.42 (b) shows the student's t-test (95% confidence interval) for group comparison of post thermophilic digestion ortho-phosphate results. The results show that pretreatment (all but microwave) did not improve ortho-phosphate release after thermophilic digestion. This is different than the mesophilic digestion result which shows approximately 20% increase from microwave, MW/H_2O_2 and ultrasound treatment. The reason could be that thermophilic digestion is operating at higher temperature range (55ºC), which could in effect as a thermal treatment on its own. The difference created in pretreatment could be overrided by the effect of a long (30 days) thermal treatment during the digestion.

Table 6.2 Volatile solids reduction, biodegradable COD faction and specific biogas production from thermophilic digestion

	Initial volatile solids	Volatile solids after digestion	Volatile solids reduction	Initial measured total COD	Total biogas production from feed	Theoretical COD substrate for biogas*	Bio-degradable COD fraction	Specific biogas from feed
	g/L	g/L	%	g/L	mL	g/L	%	L/g-VS-destroyed
OL=1.7 g-VS/L								
WAS	34.8	11.5	67.0	57.4	131	35.1	61.1	1.12
MW	36.1	11.4	68.5	61.3	131	39.0	63.7	1.06
MW/H_2O_2	33.9	10.9	67.8	54.0	148	39.5	73.1	1.29
US	34.1	11.1	67.3	55.8	146	43.3	77.6	1.27
OL=6.8 g-VS/L								
WAS	34.8	11.9	65.7	57.4	345	32.5	56.6	0.75
MW	36.1	12.5	65.5	61.3	394	37.1	60.6	0.83
MW/H_2O_2	33.9	11.0	67.6	54.0	398	37.6	69.5	0.87
US	34.1	10.4	69.4	55.8	382	36.0	64.5	0.81
Protease	32.2	10.8	66.5	56.1	382	36.1	64.3	0.89
US+Protease	33.7	11.0	67.3	55.8	397	37.5	67.1	0.88

* Theoretically 0.350 m^3 of methane produced from every 1 kg COD converted (McCarty, 1964)

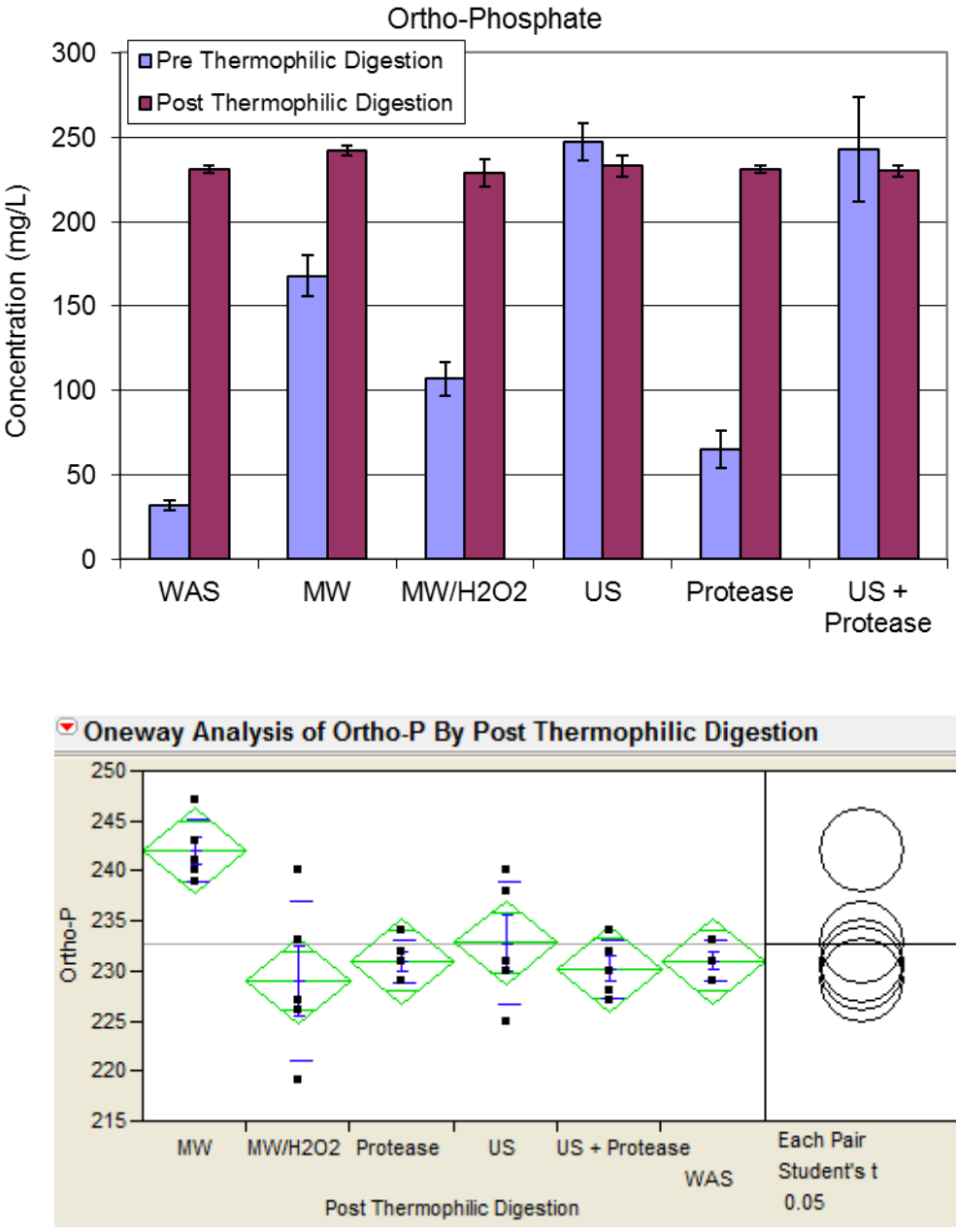


Figure 6.42 Ortho-phosphate results before and post thermophilic digestion (a) and Student's t-Test for group comparisons of post digestion (b)

6.3.3 Correlation of digester performance to various pretreatment methods and parameters

There are many parameters that could be used to describe the extent and efficiency of sludge disintegration. Soluble COD and its derivatives such as SCOD/TCOD%, and degree of disintegration (DD, defined in Section 3.2.4), are by far the most commonly applied ones. The use of protein content has also been suggested as a critical parameter (Schmitz et al., 2000; Pavlostathis and Gossett, 2004; Wang et al., 2006). However, the correlation between the disintegration pretreatment and digestion behavior (biogas production) has rarely been made.

Bougrier et al. (2006) linked methane production (mL-methane/g-COD-added) to percentage COD solubilization (SCOD increase versus initial particulate COD) for ultrasound, ozone and thermal pretreatments. The correlations were made at two data points (usually in close proximity) within each treatment method. Schmitz et al. (2000) have reported that in their ultrasound pretreatment, soluble protein made a better linear correlation to biogas yield than to COD parameters. But in that study, the treatment time was the only variable.

For each treatment method, it is reasonable to expect a linear relationship between treatment extent and biogas production as long as digestion is not limited. The question remains as to whether this is comparable among different pretreatment methods in terms of biogas production or digestion behavior. No attempt has yet been described in the literature to correlate any parameter in various pretreatments to the resulting digestion performance. Many researchers consider that pretreatments are not equal. This is due to differences intrinsic to the mechanisms at work in each pretreatment. Bougrier et al. (2006) made this point by stating that ultrasound pretreatment resulted in weak COD solubilization but had the highest biodegradability (methane/g-COD-added), whereas ozone pretreatment resulted in

weak COD solubilization and weak biodegradability. Thermal pretreatment yielded strong COD solubilization and relatively high biodegradability. In explaining how the highest biodegradability resulted from the lowest COD solubilization, the authors (Bougrier et al., 2006) suspected that particulate COD was made more easily biodegradable due to particle size reduction by ultrasound. However, this explanation could not explain the high biodegradability from an increased particle size that occurred in thermally treated sludge. Bougrier et al. (2006) concluded that the high biodegradability in ultrasound treated sludge was due to particle accessibility, while in thermally treated sludge it was due to COD solubilization.

Indeed, pretreatments are not the same in terms of disintegration and solubilization of sludge particulates, as demonstrated in Chapter 5. But the principle governing the anaerobic digestion of these various feeds is a general one. Batstone et al. (2002, 2003) summarized the biochemical processes in a structured model (Anaerobic Digestion Model No.1, ADM1). In WAS, protein is the dominant component. If protein hydrolysis holds the key (i.e. is rate-limiting, Pavlostathis and Gossett, 2004) and kinetically controls the overall digestion, its improvement by pretreatment methods should correlate with digestion behavior.

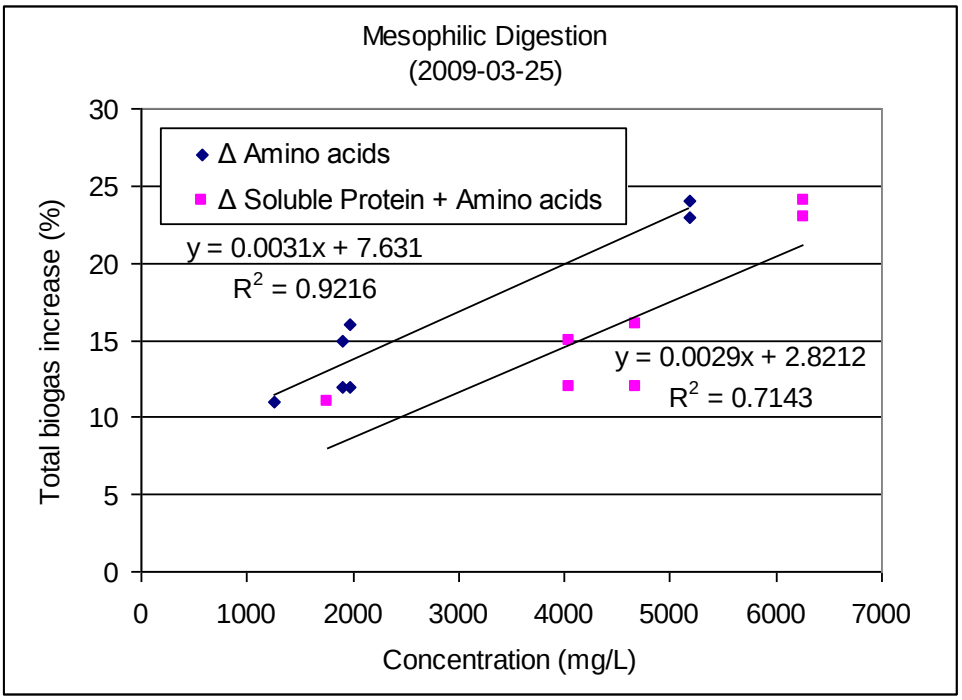
In Figure 6.43(a) the increases in amino acids (the products of protein hydrolysis) and the sum of the increases in soluble protein and amino acids from various pretreatments (Chapter 5) have been plotted against the final total biogas increases (%) from mesophilic digestion. Since no major inhibition was found under either of the loading conditions, the data points were included for both conditions in mesophilic digestion. The final total biogas increase was used because it represents the biodegradability improvement over the untreated WAS control.

With an increase in amino acids, the correlation yielded a reasonable linear fit (R^2=0.9216) to the biogas increase. If the average biogas increases (from the two loading conditions) from microwave and MW/H_2O_2-pretreated feed (13% and 14% respectively) is used, the correlation fit is even higher (R^2=0.9921). In contrast, the increase in soluble protein plus the amino acids had a poor relationship with the increase in biogas (R^2=0.7143).

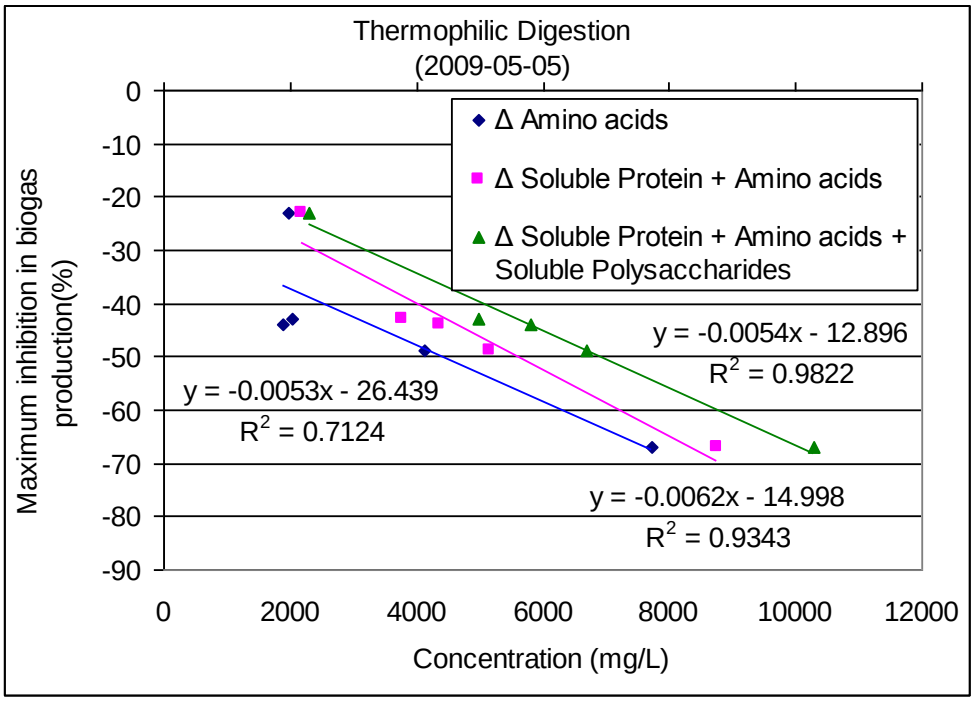
The increase in SCOD, degree of disintegration, soluble protein alone, and plus polysaccharide plus amino acids, all deviated further from the correlation to the increase in biogas. This indicates that it was the products of pretreatment hydrolysis rather than disintegration that largely contributed to the final biogas increase (or biodegradability improvement). The likely scenario could be described in the following way. The pretreatment hydrolysis products (amino acids in this case) were quickly utilized by the abundance of healthy microorganisms. This occurred alongside the normal digestion proceedings of disintegration and hydrolysis of the remaining substrates (particulate and soluble). The digestion of the disintegration products from the various pretreated feeds (namely soluble protein, polysaccharides, or the collective term of SCOD) appeared similar to the digestion of the untreated feed, given that the inoculums were not inhibited.

In thermophilic digestion, the impact of pretreatments was negative during the early days of digestion. However, inhibition was severe, especially with the higher organic loadings. The correlation of pretreated feeds to the maximum inhibition was therefore investigated, since maximum inhibition best represents the impact. The results are reported in Figure 6.43 (b). In this case, amino acids were not the sole contributor to the likely VFA accumulation. The increases in soluble protein, soluble polysaccharides (expressed in glucose, including the hydrolyzed products monosaccharides) and amino acids were lumped together. A

confirmed a close correlation (R^2=0.9822) was confirmed to the maximum inhibition of biogas production. It is thought that, due to the higher reaction rate at thermophilic temperatures, the increased amount of immediately available substrates was quickly degraded to VFAs. This resulted in VFA accumulation, which inhibited the overall digestion. Soluble COD, which includes soluble inerts, has a poorer correlation (R^2=0.878) than the sum of these immediately available substrates.



(a)



(b)

Figure 6.43 Correlation of pretreated substrate and total biogas improvement (a) and maximum inhibition (b)

6.4 Conclusions

In this chapter, the mesophilic and thermophilic anaerobic digestion of microwave, MW/H_2O_2, ultrasound and protease-pretreated WAS were investigated at two levels of organic loading, 1.7 and 6.8 g-VS/L. The biogas production (or overall digestion) from the two types of digestion was drastically different, both in terms of improvements to biodegradability and in the initial inhibition. The digestion reaction kinetics was studied and the correlation between biogas production and pretreatments was also examined. The conclusions from this study are listed as follows.

In mesophilic digestion:

- No major inhibition was found with pretreated or untreated WAS feeds, under both organic loading conditions;
- A short acclimation time (1 day) was needed for all feeds at high organic loading of 6.8 g-VS/L;
- Microwave-pretreated feed yielded 12-15% of final total biogas increase (i.e. biodegradability improvement), whereas, MW/H_2O_2, ultrasound and protease treated feed recorded 12-16%, 25% and 11%, respectively;
- The biogas production reaction (or overall digestion reaction) closely fit second order reaction kinetics;
- The VS reductions increased with pretreated feeds, in the range of 64.2% to 69.5%;
- The specific biogas production rates also increased with pretreated feeds, from 0.83 with untreated WAS, to 0.87, 0.93, 1.04, and 1.07 L/g-VS-destroyed for microwave, MW/H_2O_2, ultrasound and protease treated feed, respectively.

In thermophilic digestion:

- Inhibition was found in pretreated feeds under both organic loading conditions;
- At low loading 1.7 g-VS/L, the acclimation time was 2 days for all pretreated feed;
- At high loading 6.8 g-VS/L, the acclimation took between 2 to 6 days, with protease-pretreated feed suffering the least impact and ultrasound/protease the worst;
- Despite the initial inhibition, all pretreated feeds concluded at approximately 11%-15% of final biogas production increase, Various degrees of volatile solids reduction improvement over the untreated WAS feed were achieved;
- The biogas production reaction (or overall digestion reaction) in pretreated feeds was found to better fit the first order reaction kinetics, due to the inhibition. The digestion of untreated WAS feed (not inhibited) was, however, still better described by second order kinetics;
- The specific biogas production rate (L/g-VS-destroyed) also saw increases from pretreated feeds, under both loading (except microwave treated at low loading);
- Biogas composition was consistent from all feeds with methane in the range of 59% to 65%, which suggests that the digestion process fundamentals remained intact.

The correlations between digestion behavior (biogas production increase or inhibition) and various pretreatment methods (in substrate parameters) were made. It was found that the biogas production increase (biodegradability improvement) in mesophilic digestion was better correlated to the increase in amino acids, the protein hydrolysis products. The impact of various pretreated feeds on thermophilic digestion (maximum inhibition) was likely due to the increase in soluble protein, polysaccharides and amino acids, regardless of the different pretreatment methods used.

Chapter 7 Summary and Overview

This chapter summarizes the findings from the present research work. A discussion of the possible uses of the technology in pilot-scale or field applications is also included.

7.1 Factors and Optimization of Pretreatment Efficiencies

Chapter 2 reports on an investigation into the solubilization effect of the microwave and hydrogen peroxide system on secondary biological sludge. The research conditions were at a low temperature range and relatively low hydrogen peroxide dosages. Results showed that solids content, temperature, and the hydrogen peroxide addition were the three main factors in sludge solubilization. Of these, solids content was the dominant factor. At a higher solids content, microwave and hydrogen peroxide treatment yielded substantial increases in organic matter solubilization (SCOD), nutrient release (ortho-P), and volatile fatty acids production, even at the relatively low temperature range of 40 to 80°C. This suggests that if microwave and hydrogen peroxide treatment were to be used in engineering applications, sludge thickening would be an essential part of the process.

Temperature, which represents the energy cost of the process, is another important factor. Organic solubilization is a linear function of increasing temperature. A temperature range of around 80°C appears to be advantageous in terms of balancing effective solubilization and energy costs. The results for volatile fatty acids also showed that at these conditions, microwave and hydrogen peroxide treatment is mainly a disintegration process. The disintegration / solubilization process results in largely COD solublilization with relatively minor final oxidation of the resulting SCOD.

In addition, the surface response model approach proved to be very useful in both

factor screening and response prediction (at unknown conditions within the experimental range). The use of this type of model will be beneficial, especially from a control perspective in either pilot-scale research or in field practice.

In Chapter 3, the microwave and hydrogen peroxide treatment was subjected to direct comparison with thermal and peroxide treatment. The results showed both the non-thermal and synergistic effects of microwave and hydrogen peroxide treatment. Thermal and peroxide treatment did not showed significant improvement in sludge solubilization even at high dosages of hydrogen peroxide. Temperature was found to be the most influential factor for both treatments. Treatment time was the second most important factor for thermal peroxide treatment.

The comparison of ortho-P release between the two treatments also showed that microwave peroxide outperformed thermal peroxide treatment. This may have been attributed to the different cell membrane rupture mechanisms of the two treatments. However, at temperatures below 80°C, ortho-P release did not increase with a higher peroxide addition. It was therefore suggested that other factors (apart from cell rupturing) should be considered at temperatures below 80°C. These factors may include metabolism and growth uptake of the microorganisms as well as polyphosphate formation.

In Chapter 4, both the ultrasound and microwave peroxide treatment treatments were studied in flow through operation. For both systems, specific energy was found to be the main factor for sludge solubilization. At the same specific energy level, ultrasound treatment achieve better sludge solubilization with a higher power input and shorter treatment time than that with a lower power input and long treatment time. Flow through operation outperformed batch treatment, particularly at lower specific energy conditions.

This is due to the increased power density (W/L) with the flow through cell configuration. From a field application perspective, this means energy savings by using high power input and small pressurized flow through cells to achieve high power density.

An addition of hydrogen peroxide in ultrasound treatment had a positive effect on COD solubilization, but a negative effect on ortho-P release. It was similar to the results from the microwave peroxide treatment. It is likely due to other factors such as poly-P, microorganism metabolism that had a more significant impacts on ortho-P release in low temperatures range (below 55°C for ultrasound treatment, and below 80°C for MW/H_2O_2),

The ultrasound cavitation has an effect on the whole spectrum of sludge organic compounds, from large particulates to the short chain VFAs. The total VFA concentration increased with power, treatment time and hydrogen peroxide addition. However, the overall percentage TVFA to SCOD decreased, due to the larger increase in SCOD. The changes of individual VFAs were determined by their own degradations and other VFAs' transformation. The extent of this transformation was significantly affected by the power input and power density.

For microwave peroxide flow through treatments, temperature and the hydrogen peroxide addition were the two main factors. By injecting peroxide immediately before the microwave irradiation, the flow through system improved over the batch treatment. However, the current flow through operation suffers from significant heat loss and microwave equipment inefficiency. In order to further progress with microwave peroxide treatment research, equipment energy efficiency will need to be addressed.

7.2 Sludge Solubilization and Physical Property Modification

In Chapter 5, the effects of microwave, microwave peroxide, ultrasound, protease and ultrasound protease treatments on sludge solubilization and physical properties were examined and compared. The comparison was based on similar specific energy level of treatment inputs.

The TS, VS and TCOD from all treatments remained relatively constant, indicating that the treatments at these energy levels were mostly via a disintegration and hydrolysis stage. This is appropriate for anaerobic digestion pretreatment purposes. Ultrasound treatment appeared to be more energy efficient.

In protein and polysaccharides solubilization, both microwave and microwave peroxide treatments produced high levels of soluble proteins but were low in amino acids. Ultrasound resulted in high amino acids and overall protein disintegration / hydrolysis. A protease dosage after ultrasound treatment further enhanced amino acids levels. This was not only from the soluble protein, but also from the particulate protein degradation. The low levels of ammonium found after all treatments indicated that protein degradation largely proceeded only to the amino acid stage. The low levels of ammonium should have no immediate toxic impact on subsequent anaerobic digestion. In terms of polysaccharides, the microwave, microwave peroxide and ultrasound treatments all yielded high degrees of solubilization. Protease had little or no effect on polysaccharides.

Soluble protein, amino acids and soluble polysaccharides represent the readily available substrate for anaerobic digestion. As demonstrated in Section 6.3.3, they are the key parameters in evaluating any improvement or inhibition in the anaerobic digestion process. In future pilot-scale studies or field applications of pretreatment technologies,

these key parameters should be closely monitored.

With the exclusion of the protease treatment, all treatments decreased total and individual VFAs. Microwave and microwave peroxide treatments had higher degrees of DNA leakage than ultrasound and protease treatments. This may have been from the non-thermal effects on the cell membranes. All treatments except protease achieved significant levels of ortho-P release.

Particle sizes were reduced by all the treatments. This suggests that the contact surface was enlarged. The particle size distribution pattern remained similar before and after treatment, for the microwave, microwave peroxide and protease treatments. Ultrasound altered this pattern to a further non-uniform distribution. Floc microscopic imaging and Scanning Electron Microscopic imaging revealed that despite changes in the floc structure, wall destruction was not completed by microwave, microwave peroxide, ultrasound or protease treatments at this specific energy level. The ultrasound plus protease treatment yielded the best results in terms of cell wall destruction.

7.3 Mesophilic and Thermophilic Anaerobic Digestion of Pretreated WAS

Chapter 6 reports on the results for the mesophilic and thermophilic digestion of microwave, microwave peroxide, ultrasound and protease pretreated WAS. The pretreatment were done at approximately 5000 kJ/kg-DS energy inputs. And two levels of organic loading conditions, 1.7 and 6.8 g-VS/L, were used in the digestion experiment.

In mesophilic digestion, no significant inhibition was found with any of the pretreated feeds, under either of the organic loading conditions. However, a short acclimation period

(1 day) was needed for all feeds at the higher organic loading rate. Microwave-pretreated feed yielded an increase of approximately 12-15% of the final total biogas production (biodegradability improvement), while microwave peroxide, ultrasound and protease pretreated feed recorded biogas production increases of 12-16%, 25% and 11%, respectively. The VS reductions and specific biogas production rates were also increased with use of the pretreated feeds. The biogas production reaction (or overall digestion reaction) was found to closely fit second order reaction kinetics.

In current engineering practices associated with mesophilic digestion, the organic loading is usually in the range of 1.6-4.8 kg-VS/m^3.d, with typical solids retention times of from 10-20 days (Tchobanoglous et al., 2003). At the present treatment levels (specific energy at approximately 5000 kJ/kg-DS), the application of these pretreated feeds to either pilot or full scale mesophilic digesters is not likely to pose any operational problems, assuming that the digesters are running with a healthy community of microorganisms. Acclimation would not be necessary. However, increasing treatment levels or organic loading above certain upper limits could result in upsets in digester operation. Acclimation may or may not be capable of reversing such problem. It would depend on the how overloaded the digester suffered in each particular case. The first major benefit that would accrue upon implementing these pretreatments would be an improvement in energy recovery through increases in biogas production. (This would be the case even with a very well run digester such as the one in the Lulu Island WWTP.) Two additional potential benefits would include less quantity of sludge or biosolids for final disposal, and an increase in digester throughput.

In thermophilic digestion, inhibitions were found for all pretreated feeds, and at both

organic loadings. Acclimation took a longer time (2 to 6 days) under the higher loading conditions. Among the pretreatments, protease treatment had the least impact, while ultrasound/protease resulted in the greatest process inhibition. Despite the initial inhibition, the results for all pretreated feeds showed an increase of approximately 11%-15% of final biogas production. They also resulted in various degrees of improvement in volatile solids reduction over the untreated WAS feed control. The biogas production reactions (or overall digestion reaction) for all the pretreated feeds were found to be a better fit to first order reaction kinetics due to the inhibitions.

Correlations between digestion behavior (biogas production increase or inhibition) and pretreatments were made. It was found that the biogas production increase in mesophilic digestion was correlated to the amino acids increase in pretreated feeds. The impact of pretreated feeds on thermophilic digestion (maximum inhibition) was linear to the increase in soluble protein, polysaccharides and amino acids, regardless of the different pretreatment methods used.

Thermophilic digestion, although not as common as mesophilic digestion, offers significant advantages. These include increased pathogen destruction and faster reaction rates. However, thermophilic digestion process is also a more delicate one due to its less versatile microorganism community. This makes it more susceptible to either the overall loading increase or an immediate substrate increase. For pretreatments to work with thermophilic digestion, acclimation could be critical. With careful acclimation, same benefits could also be achieved in thermophilic digestion, since there is no fundamental difference in the digestion process itself.

Even though the various pretreatments proceed with different mechanisms and vary

significantly in sludge solubilization, a common linkage to anaerobic digestion performance does exist. The present research shows that this connection is the readily available substrates. Most importantly, the hydrolysis products of protein, namely amino acids are the key in secondary biological sludge anaerobic digestion.

7.4 Research Work Limitations

There are a number of limitations in the present research work that should be noted, for future research or field application.

The first one is related to the site specific sludge used for the research. It has been well documented in academic and engineering practice that sludge characteristics have a substantial impact on anaerobic digestibility. The liquid stream treatment process (attached or suspended growth) and operational conditions (such as sludge age, SRTs) will determine the anaerobic digester performance to a large degree. And almost certainly, it will affect the pretreated sludge digestion.

There were two types of biological sludge used in the present research. They were aerobic sludge (or mixed liquor) from the aerobic tank of the UBC wastewater treatment pilot plant, and thickened waste activated sludge (WAS) from the trickling filter and solids contact process in the Lulu Island Wastewater Treatment Plant.

The solids retention time (SRT, or sludge age) in UBC pilot plant aerobic tank is approximately 12 days. This sludge was used for the experiments described in Chapter 2 and Chapter 3. The thickened WAS from Lulu Island WWTP is from the trickling filter and solids contact (TF/SC) tank process. The SRT in the solids contact tank is approximately 1

to 2.5 days. This sludge was used for the pretreatment experiment and the anaerobic digestion tests described in Chapter 4, Chapter 5 and Chapter 6. Both of these types of sludge are considered “young” sludge. They are relatively easier to digest than the “old” sludge (produced from the long SRT processes such as extended aeration). For example, the Lulu Island WWTP digesters achieve a high level of organic reduction at approximately 64% with the “young” sludge feed. With additional pretreatment step, the organic reduction level could reach 71-80% (a 12-25% improvement shown in Chapter 6). For other treatment plant processes that produce “old” sludge, the overall organic reduction level in anaerobic digestion will be lower than 64%, but the improvement will be greater than 12-25% with pretreatment step. This is one of the research topics recommended for further work (Chapter 8).

The second limitation is the lack of liquid portion analysis and biogas partitioning analysis during the first five days of anaerobic digestion. The liquid potion sampling during the BMP test can be achieved by sacrificing a few digestion bottles for sampling. This early liquid portion and biogas analysis can greatly reduce the need for speculations about the factor affecting the biodegradation processes (Parker, W.J, review comments). For future work that uses the same digestion test (BMP) procedure, the liquid portion analysis and biogas partitioning in the first five digestion days are recommended.

The third limitation is the relatively low temperature range (40-80 ^{0}C) tested in the microwave and MW/H2O2 pretreatment experiments. At higher temperature of 120 ^{0}C or above, microwave and MW/H2O2 appear to have better treatment results in terms of COD and orthophosphate solubilization (Liao et al., 2005b, Wong et al., 2006a, 2007; Eskicioglu et al., 2008), than at a lower temperature range. However, operating at high temperature

will inevitably result in a high energy cost. Therefore, the microwave equipment development, in terms of energy efficiency, is on the critical path for potential application of microwave and MW/H2O2 in sludge pre-digestion treatment.

One other limitation is the inconclusive work regarding the poly-P interference in microwave and MW/H2O2 pretreatments. During the experiment, the DAPI (4, 6-diamidino-2-phenylindole) fluorescence detection method (Aschar-Sobbi et al., 2008) was used in an attempt to quantify the poly-P in the solution. However, it was not successful, and the possible cause could be the multiple interferences by DNA and other components in sludge. Further research on the poly-P interference will certainly benefit the phosphate recovery application. It is therefore also recommended for the future works (Chapter 8).

Chapter 8 Conclusions and Recommendations for Future Work

The research work presented in this dissertation investigated a broad range of sludge disintegration technologies. These included thermal treatment, microwave irradiation, microwave hydrogen peroxide treatment, physical ultrasound treatment and biological enzyme protease treatment. The mechanisms and factors influencing these pretreatment technologies were identified and studied under both batch and flow through operations. Sludge solubilization and physical property modifications due to the pretreatments were examined and compared. The benefits or inhibitions from the pretreatments on both mesophilic and thermophilic anaerobic digestion were explored and correlated. General conclusions from this research program are offered in Section 8.1. Recommendations for future research work are made in Section 8.2.

8.1 General Conclusions

This research work found that the degree of sludge solubilization by various treatment methods depends on the following operating parameters: specific energy, solids content, temperature, power input, power density, treatment time and specific oxidant dosage. In general, specific energy was the dominant factor. Sludge thickening was found to be very beneficial.

In the cases of microwave and microwave hydrogen peroxide treatment, temperature and hydrogen peroxide dosage determines the treatment results to a large degree. For ultrasound treatment, the power input and power density were the most important ones. A high power input and small flow-through cell system will yield best results.

Chemical oxygen demand (COD), a collective term for organic material in wastewater sludge, can have substantial improvements in solubilization from all pretreatments. At relatively low temperature and low energy input conditions, final oxidation was at a minimum.

The examination of amino acids was first introduced to sludge pretreatment research in this study. It was proved to be very important. As an indicator of sludge protein hydrolysis improvement, it can be directly correlated to the anaerobic digestion improvements. Volatile fatty acids production increased with pretreatments, but only represented a small fraction of the soluble COD. The transformation of individual VFAs was largely dependent on the treatment mechanisms and the extent of treatment.

The different treatment mechanisms also resulted in various degrees of biomass cell destruction. Evidences for this were the deoxyribonucleic acids (DNA) leakage and scanning electron microscopic (SEM) imaging.

Mesophilic digestion using pretreated feeds had various degrees of biodegradability improvements (in total biogas production, specific biogas production rate, and volatile organic reduction), depending on the treatment methods. At approximately 5000-7000 kJ/kg-DS energy levels, the results for all pretreated feeds indicated no negative effects on mesophilic digestion for both low and high loading conditions (1.7 and 6.8 g-VS/L). The biogas production reaction (or overall digestion reaction) was found to closely fit second order reaction kinetics.

Thermophilic digestion, without pre-acclimation, was inhibited by the large increase in soluble substrates in the pretreated feeds. Higher organic loading magnified the inhibition. Despite the early inhibition, the overall biodegradability at the end of digestion

period still showed improvement with the pretreated feed.

Regardless of the different pretreatment methods used, the biodegradability increase in mesophilic digestion can be correlated to the increase in amino acids, but not the increase in overall soluble COD. The inhibition in thermophilic digestion was found linear to the increases in soluble protein, polysaccharides and amino acids.

Based on the comparison on similar energy input level, ultrasound appears to be better in terms of overall improvement in anaerobic digestion process for secondary biological sludge.

8.2 Recommendations for Future Work

The following recommendations are provided for further research to expand current knowledge and understanding of sludge pretreatment subjects:

1. Quantification of the free hydroxyl radicals generated by microwave hydrogen peroxide system, with spin- trapping and electron spin resonance (ESR) techniques;
2. Exploration of the energy efficient options for microwave hydrogen peroxide system;
3. Investigation of the polyphosphate transformations in sludge treatment for better ortho-P recovery.
4. Further investigation of the options for increasing amino acids from pretreatments and the role amino acids in mesophilic and thermophilic anaerobic digestion. Amino acids appeared to be the controlling factor in biodegradability improvements. Therefore, it is of great interest to the sludge pretreatment practice.

5. Examination of the improvement or inhibition of pretreated feeds in specifically acclimated digestion conditions.
6. Liquid portion and biogas partitioning analysis in the first several days of anaerobic digestion, to confirm the factors affecting the biodegradation process.
7. Economic feasibility study of the potential capital and O&M costs to implement ultrasound treatment on a real life system, to determine if the benefits outweigh the additional costs.
8. If proven economically feasibility, pilot scale and / or demonstration scale experiment of flow through digestion, to bridge the research finding to engineering practice.

References

Abbassi, B., Dullstein, S. and Rabiger, N (1999) Minimization of excess sludge production by increase of oxygen concentration in activated sludge flocs: experimental and theoretical approach. *Wat. Res.* 34(1), 139-146

Alves, M.M., Mota Viera, J.M., Pereira Alvares, R.M., Pereira, M.A. and Mota, M. (2001) Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: oleic acid toxicity and biodegradability. *Water Res.* 35, 264–270.

Am. Public Health Assoc. (1995) *Standard Methods for the Examination of Water and Wastewater.* 19th Ed. Water Environment Federation; Alexandria, VA.

Angelidaki, I. and Ahring, B.K. (1992) Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Appl Microbiol Bio.* 37, 808–812.

Applels, L., Baeyens, J., Degreve, J. and Dewil, R. (2008) Principles and potential of anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science.* 34, 755-781

Appleton, T.J., Colder, R.I., Kingman, S.W., Lowndes, I.S., and Read, A.G. (2005) Microwave technology for energy-efficiency processing of waste. *Applied Energy* 81(1), 85-113

Aschar-Sobbi, R. Abramov, A.Y., Diao, C. Kargacin, M.E., Kargacin, G.J. French, R.J. and Pavlov E. (2008) High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. *J. of Fluoresc.* 18, 859-866

Baier, U. and Schmidheiny P. (1997). Enhance anaerobic degradation of mechanically disintegrated sludge. *Wat. Sci. Tech.* 36 (11), 137-143

Balmer, P. (2001). Possibilities to improve the quality of wastewater sludges. *Wat. Sci. Tech.,* 44(10), 19–26.

Banik, S., Bandyopadhyay, S., and Ganguly, S. (2003) Bioeffects of microwave – a brief review. *Bioresource Technology* 87, 155-159

Batstone, D.J, Keller J., Newell RB, and Newland M. (2000) Modelling anaerobic degradation of complex wastewater. I: model development. *Bioresour Technol* 75, 67–74.

Batstone, D.J, Keller J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H. and Vavilin, V.A. (2002) The IWA anaerobic digestion model No 1 (ADM1). *Wat. Sci. Tech.* 45 (10), 65-73

Battimelli, A., Millet, C., Delgenes, J.P., and Moletta, R. (2003) Anaerobic digestion of waste activated sludge combined with ozone post-treatment and recycling. Water *Sci Technol*, 48, 61–68.

Beltra, A.P., Iniesta, J., Gras, L., Gallud, F., Montiel, V., Aldaz, A., and Canals, A. (2003) Development of a fully automatic microwave assisted chemical oxygen demand (COD) measurement device. *Instrumentation science and technology* 31(3), 249-259

Bishop, D.F., Stern, G., Fleischman, M. and Marshall, L.S. (1968) Hydrogen peroxide catalytic oxidation of refractory organics in municipal waste waters. *Ind. Eng. Chem. Process Des. Dev.*, 7 (1), 110–117

Brunk, C.F., Jones, K.C. and James, T.W. (1978) Assay for nanogram quantities of DNA in cellular homogenates. *Analytical Biochemistry*. 92, 497-500.

Brunkhorst, C., Ciotti, D., Fredd, E., Wilson, J.R., Geveke, D.J. and Kozempel, M. (2000) Development of process equipment to separate nonthermal and thermal effects of RF

energy on microorganisms. *Journal of Microwave Power and Electromagnetic Energy*. 35(1), 44-50.

Bougrier, C., Degenes, J.P., and Carre´ re, H. (2007) Impacts of thermal pre-treatments on the semi-continuous anaerobic digestion of waste activated sludge. *Biochem Eng J*, 34, 20–27.

Buffler C.R. (1993) *Microwave Cooking and Processing: Engineering Fundamentals for the Food Scientist. 1st ed.* Van Nostrand Reinhold, New York, USA.

Burgess J.E. and Pletschke B.I. (2008) Hydrolytic enzymes in sewage sludge treatment: A mini-review. *Water SA.* 34 (3), 343-350

Cadoret, A., Conrad, A. and Block, J-C. (2002) Availability of low and high molecular weight substrates to extracellular enzymes in whole and dispersed activated sludges. *Enzyme Microb. Technol.* 31, 179-186.

Canales, A., Pareilleux, A, Rols, J.L., Goma, G. and Huyard A. (1994) Decrease sludge production stragtegy for domestic wastewater treatment. *Wat. Sci. Tech.* 30(8), 97-106

Carballa, M., Omil, F., and Lema J.M. (2004) Improvement of anaerobic digestion operation and digested sludge characteristics using chemical and thermal pretreatment. *In: 10th world congress of Anaerobic Digestion,* Montreal, Canada, 23 August–2 September,

Champomier-Verges, MC, Maguin E, Mistou MY, Anglade P, and Chich JF. (2002) Lactic acid bacteria and proteomics:current knowledge and perspectives. *Journal of Chromatography B.* 771 (1/2), 329-342.

Chan, W.I., Wong, W.T., Liao, P.H. and Lo, K.V 2007 Sewage sludge nutrient solubilization using a single-stage microwave treatment. *J. Environ. Sci. Health Pt. A.* 42 (1), 59-63

Chen Y, Cheng JJ, and Creamer KS. (2008) Inhibition of anaerobic digestion process: a review. *Bioresour Technol* 99, 4044–4064.

Chiu, Y.C., Chang, C.N., Lin, J.G. and Huang S.J. (1997) Alkaline and ultrasonic pretreatment of sludge before anaerobic digestion. *Wat. Sci. Tech.* 36 (11), 155-162

Chu, C.P., Chang, B.V., Liao, G.S., Jean, D.S. and Lee, D.J. (2001) Observations on changes in ultrasonically treated waste-activated sludge. *Wat. Res.* 35 (4), 1038-1046

Choi, H.B., Hwang, K.Y., and Shin, E.B. (1997) Effects on anaerobic digestion of waste activated sludge pre-treatment. Water Sci Technol, 35, 207–11.

Climent, M., Ferrer, I., Baeza, M.D., Artola, A., Vazquez, F. and Font, X. (2007) Effects of thermal and mechanical pretreatments of secondary sludge on biogas production under thermophilic conditions. *Chem Eng J.* 133, 335–342.

Datta, A.K. and Davidson, P.M. (2000) Microwave and radio frequency processing. *Journal of Food Science – Supplement. Kinetics of Microbial Inactivation for Alternative Food Processing Technologies* 65 (Suppl.), 32–41

Dewil, R., Appels, L. and Baeyens, J. (2006) Energy use of biogas hampered by the presence of siloxanes. *Energy Convers Manage*. 47, 1711–1722.

Dreyfuss, MS. and Chipley, J.R. (1980) Comparison of effects of sublethal microwave radiation and conventional heating on the metabolic activity of *Staphylococcus aureus*. *Applied and Environmental Microbiology.* 39(1), 13-16.

Driver, J., Lijmbach, D. and Steen, I. (1999) Why recover phosphorus for recycling, and how? *Environmental Technology*. 20, 651-662.

Dubois, M.G., Gilles, K.A., Hamilton, J.A., Rebers, P.A., Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 330–356.

Eastman J. A. and Ferguson J. F. (1981) Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *JWPCF* 53 (3), 352 – 366.

Eder, B. and Gunthert, F.W. (2002). Practical experience of sewage sludge disintegration by ultrasound. *TU Hamburg-Harburg Reports on Sanitary Engineering*. 35, 173-188

Emanual, N.M. (1968). Present state and main trends of research on liquid-phase oxidation of organic compounds. In *Oxidation of Organic Compounds*, Volume I, Edited by F.R. Mayo, Advances in Chemistry, Series 75, American Chemical Society, Washington, D.C.

Eskicioglu, C., Kennedy, K.J., and Droste, R.L. (2006) Characterization of soluble organic matter of waste activated sludge before and after thermal pretreatment Characterization of . Wat. Res. 40, 3725-3736

Eskicioglu, C., Terzian, N., Kennedy, K.J., Droste, R.L., and Hamoda, M. (2007) Athermal microwave effects for enhancing digestibility of waste activated sludge. Wat. Res. 41, 2457-2466

Eskicioglu, C., Prorot, A., Marin, J., Droste, R.L., and Kennedy, K.J., (2008) Synergetic pretreatment of sewage sludge by microwave irradiation in presence of H_2O_2 for enhanced anaerobic digestion. Wat. Res. 42, 4674-4682

Fang, H.H.P. and Jia, X.S. (1996) Extraction of extracellular polymer from anaerobic sludges. *Biotech. Tech.* 10 (11) 803-808

Fellows, P.J. (2000) *Food Processing Technology Principles and Practice.* 2nd ed.

Woodhead Publishing Limited. Cambridge, England.

Frolund, B., Palmgren, R., Keiding, K. and Nielsen P.H. (1995a) Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Wat. Res.* 30(8), 1749-1758.

Frolund, B., Griebe, T. and Nielsen P.H. (1995b) Enzymatic activity in the activated-sludge floc matrix. *Appl. Microbiol. Biotechnol.* 43, 755-761.

Goel, R., Mino, T., Satoh, H. and Matsuo, T. (1998) Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. *Water Res.* 32 (7), 2081-2088.

Goel, R., Tokutomi, T. and Yasui, H. (2003) Anaerobic digestion of excess activated sludge with ozone pre-treatment. *Water Sci Technol*. 47, 207–14.

Gronroos, A., Kyllonen, H., Korpijarvi, K., Pirkonen, P., Paavola, T., Jokela, J. and Rintala J. (2005) Ultrasound assisted method to increase soluble chemical oxygen demand (SCOD) of sewage sludge for digestion. *Ultrasonics Sonochemistry.* 12, 115-120

Guellil, A., Boualam, M., Quiquampoix, H., Ginestet, P., Audic, J.M. and Block, J.C. (2001) Hydrolysis of wastewater colloidal organic matter by extracellular enzymes extracted from activated sludge flocs. *Water Sci. Technol.* 43 (6) 33-40.

Hansen KH, Angelidaki I, and Ahring BK. (1998) Anaerobic digestion of swine manure: inhibition of ammonia. *Water Res* 32, 5–12.

Harold, F.M. (1960) Accumulation of inorganic polyphosphate in mutants of Neurospora crassa. *Biochim. Biophys. Acta* 45, 172–188.

Heddleson RA. and Doores, S. (1994) Factors affecting microwave heating of foods and microwave induced destruction of foodborne pathogens-A review. Journal *of Food Protection.* 57(11), 1025-1037.

Higuchi, Y., Ohashi, A., Imachi, H. and Harada, H (2005) Hydrolytic activity of alpha-amylase in anaerobic digested sludge. *Water Sci. Technol.* 52 (1-2), 259-266.

Hiraoka, M., Takeda, N., Sakai, S. and Yasuda, A. (1989) Highly efficient anaerobic digestion with thermal pre-treatment. *Water Sci Technol* 17, 54.

Hong, S.M., Park, J.K., and Lee, Y.O. (2004) Mechanisms of microwave irradiation involved in the destruction of fecal coliforms from biosolids. *Wat. Res.* 38, 1615-1625

Hwang, M.H., Jang, N.J., Hyum, S.H. and Kim, I.S. (2004) Anaerobic bio-hydrogen production from ethanol fermentation: the role of pH. *J Biotechnol*,111, 297–309.

Inagaki, N., Suzuki, S., Takemura, K. and Miyata, A. (1997) Enhancement of anaerobic sludge digestion by thermal alkaline pre-treatment. *In: Proceedings of the eighth international conference on Anaerobic Digestion*, Sendai, Japan, May 25–29,.

Iranpour, R., and Cox, H.H.J (2007) Evaluation of thermophilic anaerobic digestion processes for full-scale Class A biosolids disinfection at Hyperion Treatment Plant. *Biotech. and Bioeng.* 97(1), 19-39

Jaeger, M. and Mayer, M. (2000). The Noell Conversion Process – a gasification process for the pollutantfree disposal of sewage sludge and the recovery of energy and materials. *Wat. Sci. Tech.,* 41(8), 37–44.

Jain, S., Lala, A.K., Bhatia, S.K. and Kudchadker, A.P. (1992) Modelling of hydrolysis controlled anaerobic digestion. *J. Chem. Technol. Biotechnol.* 53, 337-344.

Kappe, C. O. (2004) Controlled Microwave Heating in Modern Organic Synthesis. *Angew. Chem. Int. Ed.* 43, 6250

Kapuscinski, J and Skoczylas, B. (1977) Simple and rapid fluorimetric method for DNA microassay. *Analytical Biochemistry*, 83, 252-57.

Keleti, G. and Lederer, W.H. (1974) *Handbook of Micromethods for the Biological Sciences.* van Nostand Reinhold Inc., New York.

Khalil, H. and Villota, R. (1985) A comparative study on the thermal inactivation of Bacillus stearothermophilus spores in microwave and conventional heating. *In: Fourth International Congress on Engineered Food.* Applied Science Publishers, Essex, England.

Khalil, H.M. and Villota, R. (1988) Comparative study on injury and recovery of Staphyloccocus aureus using microwaves and conventional heating. *Journal of Food Protection*. 51(3), 181-186.

Kim, J., Novak, J.T. and Higgins, M.J. (2011) Multistaged anaerobic sludge digestion processes. *J. of Env. Eng. – ASCE,* 137(8), 746-753

Knezevic, Z., Mavinic, D.S. and Anderson, B.C. (1995) Pilot scale evaluation of anaerobic codigestion of primary and pretreated waste activated sludge. *Water Environ Res.* 67, 835–841.

Kopp, J, Muller,. J., Dichtl, N. and Schwedes, J. (1997) Anaerobic digestion and dewatering characteristics of mechanically disintegrated excess sludge. *Water Sci Technol* 36, 129–136.

Koutchma, T., and Ramaswamy, H.S. (2000) Combined effects of microwave heating and hydrogen peroxide on the destruction of Escherichia coli *Lebensm.-Wiss. u.-Technol* 33,

30-36

Kozempel, M.F., Annous, B.A., Cook, R.D., Scullen, O.J. and Whitting, R.C. (1998) Inactivation of microorganisms with microwaves at reduced temperatures. *J. Food Prot.* 61, 582–585.

Kozempel, M., Cook, R.D., Scullen, O.J. and Annous, B.A. (2000) Development of a process for detecting nonthermal effects of microwave energy on microorganisms at low temperature*. Journal of Food Processing and Preservation.* 24(4), 287-301.

Kuroda, A., Takiguchi, N., Gotanda, T., Nomura, K., Kato, J., Ikeda, T., and Ohtake, H. (2002) A simple method to release polyphosphate from activated sludge for phosphorus reuse and recycling. *Biotechnol. Bioeng.* 78, 333–338

Leal, MCMR., Freire, DMG., Cammarota, MC. and Sant'anna (Jr) GL. (2006) Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater. *Process Biochem.* 41 (5), 1173-1178.

Li, D.H. and Ganzarczyk, J. J. (1990) Structure of activated sludge flocs. *Biotechnol. Bioeng.* 35, 57-65.

Lin, J.G., Chang, C.N. and Chang, S.C. (1997). Enhancement of anaerobic digestion of waste activated sludge by alkaline solubilization. *Biores. Technol.,* 62, 85–90.

Liao P.H., Wong W.T. and Lo K.V. (2005a) Release of phosphorus from sewage sludge using microwave technology*. J. Environ. Eng. Sci.* 4 (1), 77-81.

Liao, P.H., Wong, T.W. and Lo, K.V. (2005b). Advance oxidation process using hydrogen peroxide/microwave system for solubilization of phosphate. *J. Environ. Sci. Health Pt. A.* 40 (9), 1753-1761

Liao, P.H., Lo, K.V., Chan, W.I. and Wong, T.W. (2007) Sludge reduction and volatile fatty acid recovery using microwave advanced oxidation process. *J. Environ. Sci. Health Pt. A.* 42 (5), 633-639

Liu T. and Sung S. (2002) Ammonia inhibition on thermophilic aceticlastic methanogens. *Water Sci Tech.* 45 (10),113–120..

Liu, H. and Fang, H.H.P. (2002) Extraction of extracellular polymeric substances (EPS) of sludges. *J. Biotech.* 95, 249-256

Low, E.W. and Chase, H.A (1999) The effect of maintenance energy requirements on biomass production during wastewater treatment. *Wat. Res.* 33(3), 847-853

Lowry, O.H., Rosebrough, N.J., Farr, A.L.. and Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193. 265-275.

Luts, D., Devoldere, K., Laethem, B., Bartholomeeusen, W. and Ockier, P. (2000). Co-incineration of dried sewage sludge in coal-fired power plants: a case study. *Wat. Sci. Tech.,* 42(9), 259–268.

Madigan, M.T., Martinko, J.M. and Parker, J. (1997) *Brock Biology of Microorganisms, 8th ed.*, Prentice-Hall, Ulpper Saddle River, NJ.

Martin-Cereceda, M., Jorand, F., Guinea, A. and Block, J. C. (2001) Characterization of extracellular polymeric substances in rotating biological contactors and activated sludge flocs. *Env. Tech.*, 22(8), 951-959

Masse, D.I. and Droste, R.L. (2000) Comprehensive model of anaerobic digestion of swine manure slurry in an sequencing batch reactor. *Water Res*. 34, 3087–3106.

McCarty P.L. (1964) *Anaerobic Waste Treatment Fundamentals. Part 1. Public Works.* N.Y. September 1964, p.107

McCarty, P.L. and Smith, D.P. (1986) Anaerobic wastewater treatment. *Env. Sci. Tech.* 20, 1200-1226

Mechichi, T. and Sayadi, S. (2005) Evaluating process imbalance of anaerobic digestion of olive mill wastewaters. *Process Biochem* 40, 139–145.

Metaxas, A.C. and Meredith, R.J. (1983) *Industrial microwave heating. 1st ed.* Peter Peregrinus Ltd. London. UK.

Moore, S. and Stein, W.H. (1948) Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.* 176. 367-388.

Moore, S. and Stein, W.H. (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.* 211. 907-913.

Morgan, J.W., Forster, C.F. and Evison, L.M. (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Research*, 24(6), 743–750.

Nybroe, O., Jorgensen, P.R. and Henze, M. (1992) Enzyme activities in waste water and activated sludge. *Water Res.* 26 (5), 579-584.

Nah, I.W., Kang, Y.W., Hwang, K.Y. and Song, W.K. (2000) Mechanical pre-treatment of waste activated sludge for anaerobic digestion process. *Water Res.* 34, 2362–2368.

Neyens, E. and Baeyens, J. (2003) A review of classic Fenton's peroxidation as an advanced oxidation technique. *J Hazard Mater*. 98, 33-50.

Neis, U., Nickel, K. and Tiehm, A. (2000) Enhancement of anaerobic sludge digestion by ultrasonic disintegration. *Wat. Sci. Tech.* 42 (9): 73-80

Nielsen, B. and Peterson, G. (2000) Thermophilic anaerobic digestion and pasteurization. Practical experience from Danish wastewater treatment plants. *Wat. Sci. Tech.* 42 (9), 65-72

Qasim, S.R. (1999) *Wastewater Treatment Plants: Planning, Design and Operation. 2nd ed.* Boca Raton: CRC Press.

Odegaard, H. (2004). Sludge minimization technologies – an overview. *Wat. Sci. Tech.* 49 (10), 31-40

Park, B., Ahn, J.H., Kim, J. and Hwang, S. (2004) Use of microwave pretreatment for enhanced anaerobiosis of secondary sludge. *Wat. Sci. Tech*. 50 (9), 17-23

Perez-Cid, B., Fernandez Albores, A. Fernandez Gomez, E., and Falque Lopez, E. (2001) Use of microwave single extractions for metal fractionation in sewage sludge samples. *Analytica Chimica Acta* 431, 209-218

Perez-Cid, B., Lavilla, I., and Bendicho, C. (1999) Application of microwave extraction for partitioning of heavy metals in sewage sludge. *Analytica Chimica Acta* 378, 201-210

Pertrucci, E., Di Palma, L., and Merli, C. (2003) Oxidation of phosphorus compounds by Fenton's reagent. *Ann. Chim.* 93, 935–945.

Posadas, V.V.G., Marco, R., Martin, J.M.R., Frias, C.R., Martin, J.L.J., and Pascual, C.M. (2001) Irradiators for the study of microwave sterilization effects *Microwave and optical technology letters* 30(6), 404-406

Riau, V., De la Rubia M.A. and Perez, M. (2010) Temperature-phased anaerobic digestion (TPAD) to obtain Class A biosolids: A semi-continuous study. *Bioresource Tech.* 101(8), 2706-2712.

Rivard, C.J. and Nagle, N.J. (1996) Pre-treatment technology for the beneficial reuse of municipal sewage sludges. *Appl Bioch Biotechnol*, 57–58, 983–991.

Rubio-Loza, L.A. and Noyala, A. (2010) Two-phase (acidogenic-methanogenic) anaerobic thermophilic/mesophilic digestion system for producing Class A biosolids from municipal sludge. *Bioresource Tech.* 101(2), 576-585

Rudd, T., Sterrit, R. M. and Lester, J. W. (1983) Extraction of extracellular polymers from activated sludge. *Biotechnol. Lett.* 5, 327-332.

Rulkens W.H (2004) Sustainable sludge management – what are the challenges for the future? *Wat. Sci. Tech.* 49(10), 11-19

Sall, J., Creighton, L., and Lehman, A. (2005) *JMP Start Statistics: A Guide to Statistics and Data Analysis using JMP and JMP IN Software.* Third Edition. Brooks/Cole -Thomason Learning. Belmont, California.

Sanz, J., Lombrana, J.I., De Luis, A.M., Verona, F. and Ortueta, M. (2002) Study and comparison of advanced oxidation techniques in the treatment of contaminated effluents. *AFINIDAD LIX,* 59, 542–552.

Shanableh, A. (1999) Production of useful organic matter from sludge using hydro-thermal treatment. *Wat. Res.* 34, 945-951.

Shanableh, A. and Jomaa S (2001) Production and transformation of volatile fatty acids from sludge subjected to hydrothermal treatment. *Wat. Sci. Tech.* 44 (10), 128-135

Shimizu T., Kudo K. and Nasu Y. (1993) Anaerobic wasteactivated sludge digestion - a bioconversion and kinetic model. *Biotechnol. Bioengng.* 41, 1082 – 1091.

Siegert, I. and Banks, C. (2005) The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochem* 40, 3412–3418.

Stevenson, F.J. (1994). *Humus Chemistry: Genesis, Composition, Reactions.* John Wiley & Sons, New York.

Stolarek, P. and Ledakowicz, S. (2001). Thermal processing of sewage sludge by drying, pyrolysis, gasification and combustion. *Wat. Sci. Tech.,* 44(10), 333–340.

Strack, J.T. (1996) *Microwaves for processing environmental waste: Phase I report* Canadian Electricity Association

Sung S, and Liu T. (2003) Ammonia inhibition on thermophilic anaerobic digestion. *Chemosphere* 53, 43–52.

Suslick K. S. (1994) *"The chemistry of ultrasound" The Yearbook of Science & the future 1994.* Encyclopaedia Britanica, Chicago: 138-155

Szewczyk, K.W. and Bukowski, J. (2008) Modelling of a batch anaerobic digester. *Polish Journal of Chemical Technology,* 10 (1), 45-48

Tanaka, S., Kobayashi, T., Kamiyama, K., and Bildan, MLS. (1997) Effects of thermochemical pre-treatment on the anaerobic digestion of waste activated sludge. *Water Sci Technol*. 8, 209–215.

Tanaka, S., and Kamiyama, K. (2002) Thermochemical pre-treatment in the anaerobic digestion of waste activated sludge. *Water Sci Technol*. 46, 173–9.

Tchobanoglous, G., Burton, F.L. and Stensel, H.D. (2003) *Wastewater Engineering Treatment and Reuse, 4th ed.* Metcalf and Eddy Inc. McGraw-Hill, New York.

Tiehm, A. , Nickel, K. and Neis, U. (1997). Accelerate the anaerobic digestion of sewage sludge. *Wat. Sci. Tech.* 36 (11), 121-128

Tiehm, A., Nickel, K., Zellhorn, M. and Neis, U. (2001) Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Wat. Res.* 35 (8), 2003-2009

Turovskiy, I.S. and Mathai, P.K. (2006) Wastewater sludge processing. New York: Wiley;

Ueno, Y and M. Fujii (2001). Three years experience of operating and selling recovered struvite from full-scale plant. *Environmental Technology,* 22(11), 1373-1381.

Vasavada, P.C. (1986) Effect of microwave energy on bacteria. *Journal of Microwave Power*. 21(3), 187-188.

Valo, A., Carre´ re, H., and Delgene´, J. (2004) Thermal, chemical and thermo-chemical pretreatment of waste activated sludge for anaerobic digestion. *J Chem Technol Biotechnol.* 79, 1197–203.

Vavilin, V.A., Rytov, S.V. and Lokshina, L.Y. (1996) A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresour. Technol.* 56, 229-237.

Wang, Q., Kuninobu, M., Kamimoto, K., Ogawa, H.I. and Kato, Y. (1999) Upgrading of anaerobic digestion of waste activated sludge by ultrasonic pre-treatment. Bioresource Technol 68,309

Wang, F., Wang, Y., and Ji, M. (2005) Mechanisms and kinetic models for ultrasonic wasteactivated sludge disintegration, *J. Hazard. Mater.* B123, 145–150,

Water Environment Federation (2004) *High performance anaerobic digestion, white paper.*

Wawrzynczyk, J., Recktenwald, M., Norrlow, O. and Dey, E.S. (2007) Solubilisation of sludge by combined chemical and enzymatic treatment. *Afr. J. Biotechnol.* 6 (17), 1994-1999.

Wawrzynczyk, J., Recktenwald, M., Norrlow, O. and Dey, E.S. (2008) The function of cation-binding agents in the enzymatic treatment of municipal sludge. *Water Res.* 42 (6-7), 1555-1562.

Weemaes, M.P.J. and Verstraete, W. (1998) Evaluation of current wet sludge disintegration techniques. *J Chem Technol Biotechnol.*73, 83–92.

Weemaes, M., Grootaerd, H., Simoens, F. and Verstraete, W. (2000) Anaerobic digestion of ozonized biosolids. *Water Res* 34, 2330–2336.

Wei, Y., Van Houten R. T., Borger, A.R. Eikeboom, D.H. and Fan T. (2003) Minimization of excess sludge production for biological wastewater treatment. *Wat. Res.* 37, 4453-4467

Wong, W.T., Chan, W.I., Liao, P.H., Lo, K.V. and Mavinic, D.S. (2006a) Exploring the role of hydrogen peroxide in the microwave advanced oxidation process: solubilization of ammonia and phosphates *J. Environ. Eng. Sci.* 5 (6), 459-465

Wong, W.T., Chan, W.I., Liao, P.H. and Lo, K.V (2006b) A hydrogen peroxide/ microwave advanced oxidation process for sewage sludge treatment. *J. Environ. Sci. Health Pt. A.* 41 (11), 2623-2633.

Yan, S., Miyanaga, K., Xing, X-H. and Tan, Y. (2008) Succession of bacterial community and enzymatic activities of activated sludge by heat-treatment for reduction of excess sludge. *Biochem. Eng. J.* 39 (3), 598-603.

Yoon, S.H., Kim, H.S. and Lee, S. (2004) Incorporation of ultrasonic cell disintegration into a membrane bioreactor for zero sludge production. *Process Biochemistry* 39, 1923-1929

Yu, G.H., He, P.J., Shao L.M. and Lee, D.J. (2007) Enzyme activity in activated sludge flocs. *Applied Microbiology and Biotechnology.* 77(3), 605-612.

Yu, G.H., He, P.J., Shao L.M. and Lee, D.J. (2008) Extracellular enzymes in sludge flocs collected at 14 full-scale wastewater treatment plants. *J. of Chem. Tech. and Biotech.* 83(12), 1717-1725.

Yu, Y., Lo. I.W., Chan, W.W., Liao, P.H. and Lo. K.V. (2010) Nutrient release from extracted activated sludge cells using the microwave enhanced advanced oxidation process. *J. Environ. Sci. Health Pt. A.* 45 (9), 1071-1075.

Zimmermann, U., Pilwat, G. and Riemann F. (1974) Dielectric breakdown of cell membranes. *Biophysical Journal.* 14(11): 881-899.