Pretreatment and Conditioning of Municipal Wastewater Secondary Sludge Using Freezing and a Combination of Ultrasound and Freezing

By

Marissa Carrasco

A thesis presented to Lakehead University in partial fulfillment of the thesis requirements for the degree of Master of Science in Environmental Engineering

Thunder Bay, Ontario, Canada, 2013

Supervisor: Dr. W. Gao

Abstract

With the cost of sludge management on the rise, the volume reduction of municipal wastewater sludge is becoming an increasingly important issue for wastewater treatment plants. Current research is focused on pretreatment methods intended to increase the efficiency of anaerobic digestion. Unfortunately, many of the pretreatment methods studied show little to no improvements in dewaterability of the secondary sludge. This research was carried out to investigate the potential of freezing and combined ultrasound-freezing methods for simultaneous sludge pretreatment and conditioning. Three methods of freezing were employed; conventional freeze-thaw (FT), combined ultrasonic-freezing (UF) and progressive ultrasonic freezing (PUF). The solubilisation of sludge organic matter, evaluated by measuring soluble chemical oxygen demand (sCOD), showed significant improvements for all freezing methods compared to the controls. The maximum increase in sCOD was 6.5 times the control for conventional freezing at -30°C and 5 freeze-thaw cycles, 5.3 times the control for combined ultrasonic freezing at 20% amplitude and 12 minutes of sonication and 7.7 times the control for the liquid portion of the progressive ultrasonic freezing samples with a three second sonication pulse for the duration of the freezing. The dewaterability of the freezing methods was also evaluated by measuring sludge volume index (SVI) and capillary suction time (CST). The three freezing methods showed significant improvements in dewaterability with CST ratios ranging from 0.12 – 0.21 for conventional freezing, 0.11- 0.21 for combined ultrasonic freezing and 0.15 – 0.26 for the solid portion of the progressive ultrasonic freezing samples. The freezing methods were compared to three commonly studied pretreatment methods (thermal, microwave and ultrasound) and showed equivalent

or better abilities to solubilise sludge organic matter and improve dewaterability. Further tests revealed that the three freezing treatments also resulted in significantly higher concentrations of proteins as well as increased biodegradability and gas production. The gas production ratio over the control was greatest for conventional freezing (1.52), followed by combined ultrasonic freezing (1.17) and progressive ultrasonic freezing (1.13). The results suggest that freezing could be a very effective pretreatment method as it would be able to simultaneously improve both anaerobic digestion efficiency as well as dewaterability.

Acknowledgements

I would like to thank Dr. Wa Gao, my supervisor, for her guidance and encouragement over the course of this thesis. The time that she has dedicated to me as well as her advice has been very much appreciated.

I would like to extend my appreciation to my thesis examiners, Dr. Kam Leung and Dr. Aincheng Chen, for their time and suggestions.

A very sincere thank you to Rory Ralph for the many hours spent helping me run tests, wash crucibles and keep my work in the lab under control. Special thanks are also due to Mrs. Sharon Young and Mr. Patrick McGuire, Mrs. Michelle Warywoda and the city of Thunder Bay for the use of their experimental equipment, lab space and assistance completing this thesis. Also, to Mr. Conrad Hagstrom, Dr. Miranda Maki, Dr. Kam Leung and Dr. Biaqiao Liao – thank you very much for the use of your equipment.

Last, but certainly not least, to my family and friends – your encouragement and confidence in my abilities was invaluable. Thank you so much for your constant support over the last two years.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
INTRODUCTION

1.1 Problem Statement

As early as 1994, municipal wastewater sludge management was estimated to account for up to 60% of wastewater treatment plants' (WWTP) operating cost (Weemaes and Verstraete, 1998). Since then, environmental awareness continues to increase and regulations regarding the treatment and disposal of municipal sludge are becoming more stringent. At the same time, the amount of sludge requiring treatment is increasing due to the growing population, urbanization and the more frequent use of secondary (biological) wastewater treatment processes in developing countries. For the aforementioned reasons, the cost of sludge management is on the rise.

The primary objectives in sludge management are to stabilize the sludge as well as to decrease the amount of sludge, both in weight and volume. These goals are accomplished through two main techniques: digestion and dewatering. Digestion, either aerobic or anaerobic, is used to stabilize the sludge while simultaneously reducing the sludge organic matter. A major advantage of anaerobic digestion is that the reactions taking place essentially transform the sludge organic matter into methane. Methane is a useful biogas which can be used by the WWTP to produce energy for heating or electricity, thereby offsetting the cost for sludge management.

Once digested, sludge can comprise of up to 97% water (Turovskiy and Mathai, 2006). Disposing of sludge with such high water content would be very expensive and so dewatering becomes an important step to follow digestion. A process known as sludge conditioning is carried out prior to dewatering in order to enhance the removal of water from the sludge. Several methods of sludge conditioning are used in WWTP, the most common widespread being chemical followed by physical methods such as thermal, freeze-thaw and elutriation (Turovskiy and Mathai, 2006).

In recent years, numerous studies have been focused on finding pre-treatment methods which can further enhance the anaerobic digestion efficiency of this secondary sludge. The biological sludge being produced from secondary treatment is made up primarily of biomass. This makes it especially difficult to degrade as most of the organic content required for digestion is trapped within the cell walls of the microorganisms. The greater the extent of digestion, the greater the volume reduction of the sludge and the more methane gas will be produced. Both will lead to a significant reduction in the cost of sludge management. Special interest is being paid to find treatment techniques that can be used within or added to the existing facilities of the municipal wastewater treatment plants. Some of the methods investigated include ultrasound, thermal/thermochemical and microwave treatment. These pre-treatment techniques have shown the ability to significantly solubilise sludge organic matter, but little or no effect on improving sludge dewaterability has been noted (Bougrier et al., 2008; Chang et al., 2011; Chu et al., 2001).

Considering the already expensive cost of sludge management, it would be extremely beneficial to find a pre-treatment method that could simultaneously improve digestion and dewaterability. Research has shown that freezing could be one such method as it has proven results in improving dewaterability as well as shown some indication of releasing organic matter from sludge (Gao, 2011; Hong et al., 1995; Montusiewicz et al., 2010; Ormeci and Vesilind, 2001).

1.2 Objectives

The objectives of this study are:

1. Determine the potential of using freezing as a combined method for pre-treatment and sludge conditioning,
2. Examine the effect of different freezing methods – conventional freezing (FT), combined ultrasonic-freezing (UF) and progressive ultrasonic freezing (PUF) – on solubilisation of sludge organic matter and dewaterability of sludge,
    a. Determine the effect of freezing temperature and FT cycles on the treatment efficiency of the FT method,
    b. Determine the effect of amplitude and sonication time on the treatment efficiency of the UF method,
    c. Determine the effect of pulse time and sonication time on the treatment efficiency of the PUF method,
3. Compare freezing methods to those of ultrasound, thermal and microwave in terms of solubilisation of sludge organic matter and dewaterability, and
4. Investigate the relationship between solubilisation of sludge organic matter, dewaterability and particle size

1.3 References

Bougrier, Claire, Jean Delgenes, and Helene Carrere. "Effects of Thermal Treatments on Five Different Waste Activated Sludge Samples' Solubilisation, Physical Properties and Anaerobic Digestion." *Chemical Engineering Journal* 139, no. 2 (2008): 236-244.

Chang, Chia-Jung, Vinay Kumar Tyagi, and Shang-Lien Lo. "Effects of Microwave and Alkali Induced Pretreatment on Sludge Solubilization and Subsequent Aerobic Digestion." *Bioresource Technology* 102, no. 17 (2011): 7633-7640.

Chu, C. P., Bea-Ven Chang, G. S. Liao, D. S. Jean, and D. J. Lee. "Observations on Changes in Ultrasonically Treated Waste-Activated Sludge." *Water Research* 35, no. 4 (2001): 1038-1046.

Gao, Wa. "Freezing as a Combined Wastewater Sludge Pretreatment and Conditioning Method." *Desalination*, 2011: 170-173.

Hong, S. G., J. D. Young, G. W. Chen, I. L. Chang, W. T. Hung, and D. J. Lee. "Freeze/Thaw Treatment on Waste Activated Sludge: A FTIR Spectroscopic Study." *Journal of Environmental Science & Health Part A: Environmental Science & Engineering & Toxicology* 30, no. 8 (1995): 1717-1726.

Montusiewicz, A., M. Lebiocka, A. Rozej, E. Zacharska, and L. Pawlowski. "Freezing/thawing Effects on Anaerobic Digestion of Mixed Sewage Sludge." *Bioresource Technology* 101 (2010): 3466 - 3473.

Ormeci, Banu, and Aarne P. Vesilind. "Effect of Dissolved Organic Material and Cations on Freeze-Thaw Conditioning of Activated and Alum Sludges." *Water Research* 35, no. 18 (2001): 4299-4306.

Turovskiy, Izeail S., and P. K. Mathai. *Wastewater Sludge Processing.* New Jersey: John Wiley & Sons Inc., 2006.

Weemaes, Marjoleine P. J., and Willy H. Verstraete. "Evaluation of Current Wet Sludge Disintegration Techniques." *Journal of Chemical Technology & Biotechnology* 73, no. 2 (1998): 83-92.

CHAPTER 2
BACKGROUND AND LITERATURE REVIEW

2.1 Conventional Municipal Wastewater Treatment

Municipal wastewater contains wastewater or liquid waste collected in sewers from residential, commercial and institutional establishments. It could also contain some industrial wastewater that may or may not be pre-treated. Municipal wastewater usually has a high concentration of organic matter, nutrients, pathogenic microorganisms and solids.

A conventional municipal wastewater treatment process, shown in Figure 2.1, begins with primary treatment. The influent sewage passes through screens and into a grit chamber where larger pieces of solid waste can be removed. From here, the wastewater will move on to clarifiers or sedimentation tanks where a large percentage of suspended solids is either settled or skimmed off the top in the case of grease and oils. Primary treatment is able to remove 50 – 70% of the suspended solids, 65% of the oil and grease and 25 – 50% of the BOD_5.

Following primary treatment, secondary treatment uses microorganisms to degrade the sewage either using attached growth systems (biological aerated filters, trickling filters, biofilm reactors, rotating biological contactors) or suspended growth systems such as activated sludge. Once again, the biological solid flocs are settled in the secondary clarifier while the effluent is passed on in some cases for nutrient removal followed by disinfection or effluent polishing. The quality of the effluent at this point is generally high enough to be discharged to the receiving body of water.

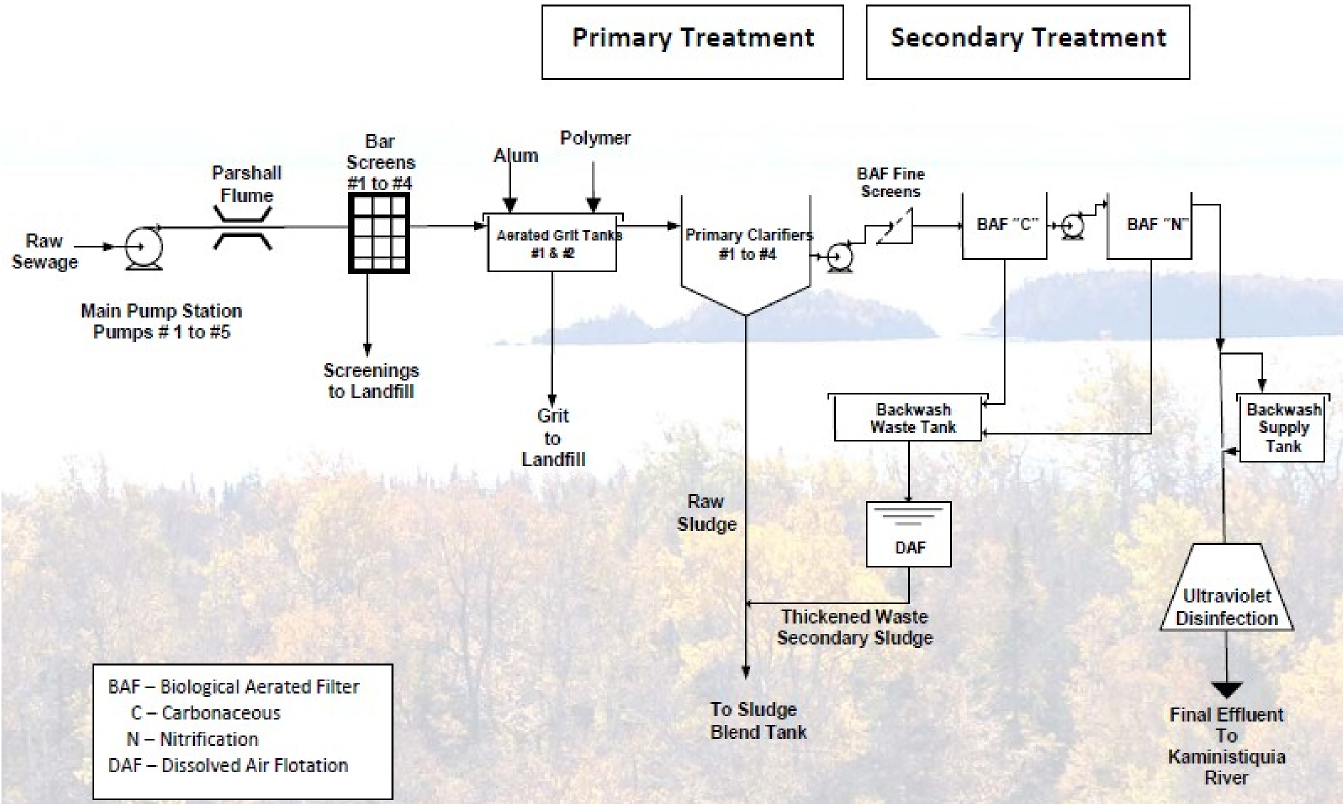

Figure 2.1: Conventional municipal wastewater treatment (Thunder Bay water pollution control plant, 2010)

2.2 Sources and Characteristics of Municipal Wastewater Sludge

2.2.1 Grit Sludge

Grit sludge solids are the first solids to be removed from the influent wastewater. It comprises of heavy solids such as sand, gravel, broken glass, metals and other inorganic materials. It can also consist of larger organic materials such as corn, coffee grinds and other food remains. Due to its heavy nature, grit settles quickly and is generally disposed of by landfill without any further treatment (Turovskiy and Mathai, 2006).

2.2.2 Primary Sludge

The solids settled out of the primary clarifiers are named primary sludge and their total solids concentration can range between 1 – 12% (Liu and Liptak, 1999). Initially, primary sludge is a light grey or brown suspension consisting mainly of organic matter. The high organic content of primary sludge causes it to decay quickly, resulting in the darkening of the suspension as well as foul odours (Turovskiy and Mathai, 2006). Since primary sludge is made up of larger, discrete particles, it is easier to dewater in comparison to biological sludge, although it does not drain well on drying beds (Sanin et al., 2011).

2.2.3 Secondary Sludge

The excess biomass produced by the biological treatment along with some non-biodegradable inorganic matter unable to be removed in the secondary clarifier makes up secondary sludge. Common biological processes used in wastewater treatment include biological aerated filters, trickling filters and the activated sludge process.

The characteristics of the secondary sludge vary depending on the type of biological treatment as well as the organisms present in the sludge (Wang et al., 2007). Generally, secondary sludge has a lower solids concentration compared to primary sludge somewhere between 1 -5% solids (Turovskiy and Mathai, 2006; Wang et al., 2007). After thickening however, it can increase up to as much as 7%. Although primary and secondary sludge contain similar amounts of volatile solids – a good measure of the organic content of the sludge, the organic content of the secondary sludge decays more rapidly than that of primary sludge due to the presence of microorganisms. This results in smaller colloidal particles as well as an increase in bound water – both of which are associated with poor settleability and dewaterability (Turovskiy and Mathai, 2006). Secondary sludge contains less grease/fat and cellulose than primary sludge, but more nitrogen, phosphorus and proteins (Wang et al., 2007).

2.2.4 Tertiary Sludge

Sludge can also be accumulated from advanced wastewater treatment processes such as the removal of BOD, excess solids or the chemical precipitation of nutrients (Spellman, 2003; Stander and Theodore, 2008). For example, the precipitation of phosphorus requires the addition of chemicals such as lime, alum or other iron salts. These chemicals can affect the characteristics of the wastewater, resulting in changes in pH and dewaterability.

2.3 Characteristics of Municipal Wastewater Sludge

Figure 2.2 illustrates that sludge is a mixture of solids and water. The water can be classified as *free water* if it is not part of the solids, *interstitial water* if it is trapped within the solid flocs, *surface water* if it is held on to individual solid particles by surface forces and *bound water* if it is chemically bound to the solid particles (Vesilind and Martel, 1990).

Primary sludge is made up of larger particles the majority of which are greater than 0.2mm. On the other hand, the organic content in biological sludge decays rapidly resulting in finely dispersed and colloidal particles, of which more than 90% are smaller than 0.2mm (Turovskiy and Mathai, 2006). The density of sludge tends to be quite close to that of water, with primary sludge being slightly greater than that of biological sludge (Sanin et al., 2011).

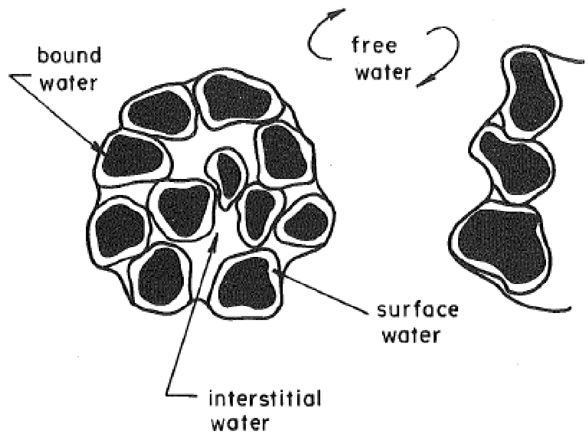

Figure 2.2: Classification of Water in Sludge (Vesilind & Martel, 1990)

The double layer model, shown in Figure 2.3, is used to describe the ionic surroundings of a sludge particle. Sludge colloids are negatively charged, causing a tightly bound layer of counter ions to form at the surface of the colloid. This first layer is known as the Stern layer. The second layer, called the diffuse layer, consists of more loosely bound positive counter ions and some negative ones as well. This double layer of ions ultimately results in the repulsion of other colloid particles and prevents aggregation.

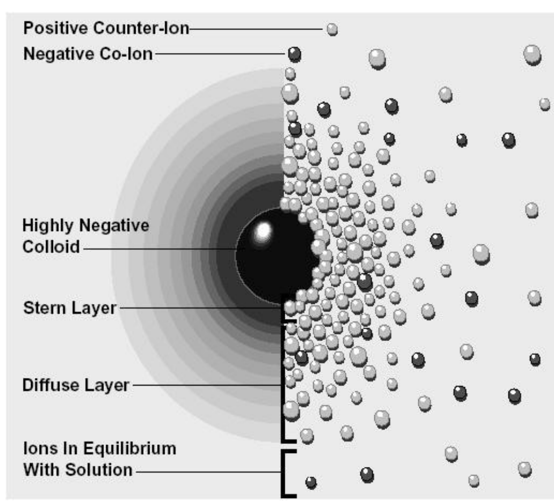

Figure 2.3: Double layer model for sludge colloids (Subramanian et al., 2009)

2.4 Basic Processes for Sludge Treatment

A conventional sludge treatment system, shown in Figure 2.4, begins with a thickening step which improves digestion efficiency, followed by stabilization and finally a conditioning step to enhance sludge dewatering. Each of these steps will briefly be described in the following sections.

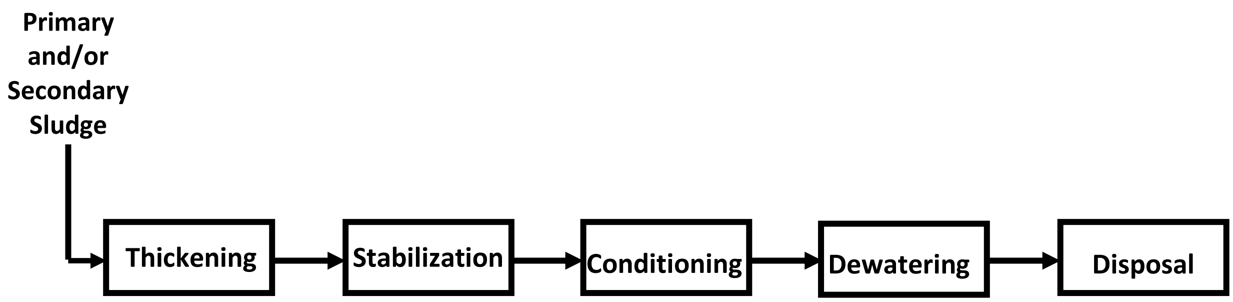

Figure 2.4: Conventional sludge treatment

2.5 Sludge Thickening

Thickening is a process used to increase the solids concentration of the sludge prior to stabilization. It can be performed on a mixture of the primary and secondary sludges, or on the secondary sludge alone, depending on which is more economical for the treatment plant (Thunder Bay Water Pollution Control Plant, 2010; Dentel, 2001). The three most common methods of thickening sludge are by gravity, flotation and belt filtration (Spellman, 2003). Generally thickening is able to decrease the volume of sludge to approximately one-third of its original volume (Appels et al., 2008).

2.6 Sludge Stabilization

Sludge stabilization, as the name suggests, is intended to convert the sludge into a stable product through volume reduction, elimination of odour and pathogen destruction. There are several methods of sludge stabilization such as aerobic digestion, anaerobic digestion, lime stabilization, composting, and thermal drying or incineration (Turovskiy and Mathai, 2006). Of these methods, anaerobic digestion is the most long-standing and commonly used (Lee et al., 2005; Spellman, 2003). It is considered advantageous over aerobic digestion as it does not require the addition of oxygen and results in the production of methane, a useful biogas which can be used to as a source of useful energy for the plant.

2.6.1 Anaerobic Digestion

The earliest use of anaerobic digestion, to break down household sludge, was well over a century ago in France (Gerardi, 2003; Klass, 1984). Soon after, the first large-scale applications were introduced in municipal wastewater treatment plants as the sludge was known to contain high levels of easily degradable organics. Today, anaerobic digestion has become the most common method of sludge stabilization used in medium to large wastewater treatment plants due to its ability to minimize the putrescilbility and degrade organic solids into methane, carbon dioxide and other harmless substances in the absence of oxygen (Tiehm et al., 1997; Zhang et al., 2007).

2.6.1.1 Mechanisms of Anaerobic Digestion

The transformation from organic solids to biogas takes place in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis starts by taking complex, insoluble organic substrates such as carbohydrates, lipids and proteins and breaks them down into their soluble forms of sugar, fatty acids and amino acids respectively. Bacteria can now use these soluble organics in next step that is called acidogenesis. In acidogenesis, the products of hydrolysis are broken down into hydrogen gas (H_2), carbon dioxide (CO_2) and short-chained organic acids, including acetic acid, lactic acid and propionic acid. Acetogenesis, the third step in the process, converts the remaining volatile organic acids into acetic acid and H_2. Finally, in the last step of methanogenesis, methane gas and CO_2 are produced in two separate reactions; one uses the acetic acid as a reactant while the other uses the H_2 (see Figure 2.5).

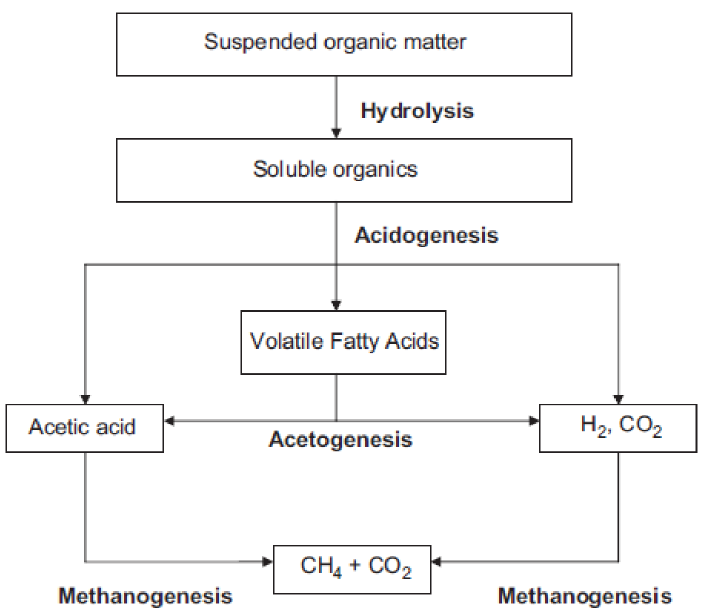

Figure 2.5: Steps in the anaerobic digestion process (Appels et al., 2008)

2.6.1.2 Factors Affecting Anaerobic Digestion

Factors that affect the rate of the reactions in each of the four stages of anaerobic digestion are (Appels et al., 2008; Gerardi, 2003):

- Solids Retention Time
- pH & Alkalinity
- Temperature

Solids Retention Time

Solids retention time (SRT) is the average number of days the solids spend in the digester. When solids are removed from the digester, a portion of the bacterial cells responsible for reactions taking place are also removed. Since steady state is an important requirement for successful anaerobic digestion, the rate of cell reproduction must match the removal rate (Turovskiy and Mathai, 2006). If this minimum SRT, which is suggested to be approximately 12 days, is not met, the anaerobic process will fail (Gerardi, 2003).

pH & Alkalinity

Several groups of micro-organisms are responsible for the various steps of anaerobic digestion and each has a different optimum pH range. The bacteria responsible for the last step of methanogenesis have the strictest pH requirements. Their optimal pH range is between 6.8 – 7.2 and they are extremely vulnerable to pH changes outside of this range (Turovskiy and Mathai, 2006). Nevertheless, as long as acidogenesis and acetogenesis are producing acetate at the same rate as methanogenesis is converting it to methane and carbon dioxide, the pH will remain constant (Appels et al., 2008; Gerardi 2003).

Temperature

Temperature affects the growth rate of the bacteria which in turn affects the rates for the various reactions that take place during anaerobic digestion. Higher temperatures result in greater volatile solids destruction as well as increased methane production (Gerardi, 2003). Experiments have shown that 20 °C appears to be the minimum temperature required for anaerobic digestion to be carried out (Turovskiy and Mathai, 2006).

The majority of methane-forming bacteria are most active in two different temperature ranges: the mesophilic range of 30 – 38 °C and the thermophilic range of 50 – 60 °C. While the rate of thermophilic anaerobic digestion is greater than that of its counterpart, most treatment plants still use mesophilic systems. There are three main reasons for this. First, mesophiles are more common and diverse than thermophiles. Second, the energy requirements are much lower for mesophilic digestion compared to thermophilic. Finally, thermophiles are much more sensitive to changes in temperature compared to mesophiles, making the process potentially unstable (Appels et al., 2008; Gerardi, 2003).

2.6.1.3 Biogas

The gases produced from the process of anaerobic digestion are referred to as digester gas or biogas. Biogas is formed directly from the destruction of volatile solids (Turovskiy and Mathai, 2006). Methane gas, $CH_{4,}$ is an odourless, biogas which has the most economic value as it can be used by the treatment plant as a source of fuel. It accounts for up to 65% of the gas production while CO_2 accounts for 30 – 40% and other trace gases such as water vapour, H_2S and H_2 make up the remainder (Appels et al., 2008; Gerardi, 2003; Turovskiy and Mathai, 2006). Biogas production in municipal wastewater plants is generally somewhere between 0.75 – 1.0 m^3/kg VS (Gerardi, 2003).

Biogas can essentially be used in replacement of natural gas. Most wastewater treatment plants use the gas produced to heat the digester. More often than not, even after doing so, there is gas remaining. Larger wastewater treatment plants use this excess gas in boilers to produce heat for the building, to power engines which can then generate electricity, to fire incinerators used to burn dewatered sludge or it can even be sold to local utility companies (Gerardi, 2003; Turovskiy and Mathai, 2006). If the plant is unable to use the excess gas, it will be flared in order to prevent odour problems.

A cogeneration system is one of the most effective uses of digester gas. It uses the biogas to power a generator which provides electricity. The water used to cool the engine is then used to provide heat to the digester and the building (Turovskiy and Mathai, 2006).

2.6.1.4 Measuring Anaerobic Digestion Efficiency

The most common method of measuring anaerobic digestion is based on biogas production (Muller et al., 2009). The volume of biogas created is generally divided by the volatile solids loading rate in order to account for variations in solids concentrations. This is called biogas yield and it will indicate how successful the digestion process is at converting the volatile solids to biogas.

Total solids destruction as well as volatile solids destruction is also considered to be a very reliable measure of anaerobic digestion (Muller et al., 2009). Additionally, when microorganisms break down proteins, they release ammonium-nitrogen. Increased levels of ammonium in the solution can therefore indicate improved anaerobic digestion (Muller et al., 2009).

2.6.1.5 Enhancing Anaerobic Digestion

Anaerobic digestion requires long digestion times of 10 – 20 days in order to allow for the solubilisation of solids and even still only produces a degradation efficiency of 30 – 50% (Appels et al., 2008). The digestion process will continue so long as the rates for all stages are equal. Unfortunately, it is very common for the first step of hydrolysis to be the rate limiting step (Appels et al., 2008; Gerardi, 2003; Turovskiy and Mathai, 2006).

Primary sludge is degraded much more effortlessly than secondary sludge during anaerobic digestion (Muller et al., 2009). This may be because a large amount of the organic solids required for hydrolysis are contained within the cell walls of microorganisms as well as in extracellular polymeric substances (EPS). Unfortunately, microbial cell walls are quite rigid and protect the cell from lysis making them resistant to biodegradation (Weemaes and Verstraete, 1998). The best way to make their content available for degradable is to rupture the cell walls, allowing the organic solids to be made available for degradation.

2.7 Sludge Conditioning

Sludge conditioning is a process used to help improve the separation between the solids and liquids in the sludge, thereby increasing its dewaterability. Sludge conditioning is usually done prior to dewatering. Since sludge disposal accounts for a major part of a treatment plant's costs, maximizing dewaterability is an important goal (Turovskiy and Mathai, 2006). There are several methods of conditioning used including, but not limited to:

- Chemical
- Heating
- Freeze-Thaw

2.7.1 Chemical Conditioning

Chemical conditioning is the most common method of sludge conditioning (Turovskiy and Mathai, 2006). Its mechanisms are very similar to those of coagulation and flocculation. The chemicals, which can be organic or inorganic, are added in order to neutralize the surface charge of the colloids and allow them to unite. Due to the variation in sludge characteristics, the type and dose of chemicals must be determined on an individual basis (Turovskiy and Mathai, 2006). Inorganic salts such as ferric salts and alum are the most commonly used chemical conditioners. Organic polymers have also become more and more popular since their introduction in the 1960s as they require much smaller doses of chemicals in comparison to inorganic chemicals (Sanin et al., 2011). The cost of the chemicals can be a major proportion of the entire operating cost for dewatering in many treatment plants (Turovskiy and Mathai, 2006).

2.7.2 Thermal Conditioning

Thermal conditioning has been used in sludge treatment as early as the 1900s. The application of heat and pressure has demonstrated the ability to enhance the dewatering of sludge while simultaneously producing biologically stable biosolids (Wang et al., 2007). The temperature generally ranges from 170 to 220°C at pressures of 1.2 to 2.5MPa for anywhere between 15 to 40 minutes (Turovskiy and Mathai, 2006). Although this process does not require the addition of any chemicals, it does involve a high capital cost due to the use of corrosion-resistant materials in the heat exchangers.

2.7.3 Freeze-Thaw Conditioning

Freeze-thaw conditioning has been studied for many years as a method improve the dewaterability of sludge. Several studies focused on understanding the mechanisms of freeze-thaw conditioning in order to improve its efficiency. A conceptual model for sludge freezing was an important first step to understanding the effect of freeze-thaw on dewaterability of sludge.

2.7.3.1 Mechanisms of Freeze-Thaw Conditioning

One theory, proposed by Vesilind and Martel (1990), is that when sludge freezes, the free water is the first to freeze, followed by interstitial water. If the temperature is low enough and freezing time long enough, even the surface water will be added to the crystalline structure (Vesilind et al., 1991). When water which contains impurities freezes, the ice front will advance but rejects all impurities it encounters. This phenomenon, called *gross migration*, pushes the impurities into a more condensed volume.

2.7.3.2 Effect of Freeze-Thaw on Sludge Characteristics

Upon visual inspection of activated sludge treated by freeze-thaw conditioning, it was established that the floc structure of the treated sludge is more compact that that of the control sludge and has better gravitational settling (Hung et al., 1996b; Lee and Hsu, 1994; Parker et al., 1998). Hung et al. (1996b) found that the flocs that show the most change in compactness are the flocs that undergo gross migration. Lee and Hsu (1994) also noted that the weakly bound activated sludge flocs are made up of microflocs with a higher density which can endure fairly strong agitation without losing their structure.

2.7.3.3 Effect of Freeze-Thaw Conditioning on Dewaterability

Freeze-thaw conditioning of activated sludge has a less compressibility than unconditioned sludge, although its sediment height is lower to begin with (Lee and Hsu, 1994). Drying tests have confirmed that freeze-thaw treatment results in less moisture attached to flocs, or in other words, the release of bound water (Hong et al., 1995; Lee and Hsu, 1994; Parker et al., 1998). Using vacuum filtration, Lee and Hsu (1994) were also able to demonstrate that the filtrate flow rate from frozen sludge is much higher than the control sludge; in fact, they found that frozen sludge could almost be dewatered without the aid of a vacuum.

Several studies have attempted to explain the factors affecting the dewaterability of freeze-thawed sludge (Hung et al., 1996b; Jean et al., 2000; Kawasaki and Matsuda, 1995; Ormeci and Vesilind, 2001; Vesilind et al., 1991). Most frequently discussed are:

- Sludge Type
- Freezing rate
- Curing time & Final temperature
- Solids Content
- Electrolyte Concentration
- Dissolved Organic Matter

Sludge Type

Freezing and curing time has a marked effect on dewaterability for activated sludge compared to clay slurry (Jean et al., 2000). This suggests that there is something about the make-up of sludge which gives rise to the effectiveness of freeze-thaw conditioning. Furthermore, when comparing different types of sludge, alum sludge appears to react very favourably to freeze-thaw conditioning while the improvements to activated sludge seem to be temporary (Lee and Hsu, 1994; Ormeci and Vesilind, 2001). The fragilely bound activated sludge flocs cannot endure vigorous mixing while their alum counterparts can (Martel, 2000).

Freezing Rate

Freezing rate seems to play a critical role in the efficiency of freeze-thaw conditioning. Instant freezing has demonstrated to be ineffective (Lee and Hsu, 1994). Several studies found that the lower freezing speed, the greater the filterability (Hung et al., 1996b; Vesilind and Martel, 1990). If the freezing rate is too fast, it has been suggested that the solid particles become entrapped in the ice as opposed to migrating ahead of the ice front (Chu et al., 1997; Hung et al., 1996; Vesilind et al., 1991). This prevents the particles from conglomerating and therefore is suggested to decrease the dewaterability of the sludge (Vesilind and Martel, 1990). However, when migration effects were isolated from freezing rate by freezing very thin layers of alum sludge, results did not correspond and increased freezing rates did not negatively affect filterability (Parker et al., 1998). Additionally, Hung et al. (1996b) found that at low freezing speeds below 11.1 mm/h, both filterability and settleability improved as well as an improvement in floc density and morphology. At high freezing speeds up to approximately 260 mm/h, as long as the sludge is completely frozen, the filterability will improve despite the lack of gross migration, but the settleability will be similar to that of the original sludge as will the floc morphology and density. Consequently, it is important to discuss filterability and settleability separately instead of labeling them both as measurements of dewaterability (Hung et al., 1996b).

Curing Time and Final Temperature

Curing sludge improves dewaterability as it seems to ensure complete freezing (Jean et al., 2000; Parker et al., 1998; Vesilind and Martel, 1990). Vesilind and Martel (1990) determined that longer curing times lead to greater filterability for alum sludge. Other studies demonstrated that curing for times greater than 6-12 hours produced no significant increases in filterability and settleability (Jean et al., 2000; Parker et al., 1998). The effect of the curing temperature was also examined and it was found that improved dewaterability occurred for samples cured at lower temperatures (Vesilind and Martel, 1990).

Solid Content

As sludge freezes and solids migrate, or in some cases are entrapped, the solids content of the melt will vary as a function of height. This occurrence was avoided by Parker et al. (1998) by freezing very thin disks of sludge. They studied the effect of initial solid concentration using 1%, 3%, 5% and 10%. Filterability was highest for the 5% and 10% sludge, with cake solids content consistently reaching over 30%. Parker et al. (1998) suggested that freezing thickened sludge not only produces a dryer filter cake, but it also can more effectively be frozen at high speeds which would decrease energy requirements.

Electrolyte Concentration

The *double* layer model is commonly used to describe sludge particles. An early hypothesis was that the aggregation of sludge particles was due to the increase of ionic strength caused by the build-up of dissolved solids in the thin layer surrounding particles (Vesilind et al., 1991). This build-up was thought to potentially cause a compression of the double layer and neutralize the repulsive forces allowing particles to come together. To test this hypothesis, Vesilind et al. (1991) increased the ionic strength of the fluid by adding various concentrations, up to 2%, of sodium chloride and then measuring the dewaterability of the sludge. They found no change in dewaterability for any of the four types of sludge tested.

Many other studies have since examined the effect of electrolyte concentration on dewaterability of freeze-thawed sludge and the results have been mixed (Jean et al., 2000; Kawasaki and Matsuda, 1995). Kawasaki and Matsuda (1995) found a large increase in dewaterability with additions of NaCl under 0.2 wt%, whereas over 0.2 wt% the settleability and filterability decreased. A study by Jean et al. (2000) used four different electrolytes (Na_2SO_4, KNO_3, $NaNO_3$ and NaCl) and found that although there is a small increase in both the filterability and settleability of activated sludge for amounts less than 0.24%, it is insignificant and safe to say that dewatering is independent of the type of electrolyte as well as the amount added.

As NaCl is added to activated sludge samples, the amount of gross migration decreases, more particles become entrapped and this results in less floc shape transformation (Chu et al., 1997; Kawasaki and Matsuda, 1995). Chu et al. (1997) showed that as the concentration of NaCl increased, there was no change in filterability, but found a significant effect on settleability. In addition, dissolved impurities caused ice crystals to form in branching, finger-like structures called dendrites (Martel, 2000). He found that alum sludge, which usually exhibits a planar ice-water interface, produced dendritic crystal growth if more than 100mg/L of NaCl was added.

Dissolved Organic Matter

The critical distinction between alum sludge and activated sludge is the high concentrations of organic matter, dissolved ions and microorganisms in activated sludge. This led to an investigation of the effect of dissolved organic material on dewaterability of freeze-thaw conditioned sludge. An effect that freeze-thaw seems to have on sludge is that it releases extracellular polymers from activated sludge (Hong et al., 1995; Hung et al., 1996; Ormeci and Vesilind, 2001; Ormeci and Vesilind, 2002). This would appear to account for simultaneous increase in BOD and COD in freeze thaw conditioned sludge (Hong et al., 1995; Lee and Hsu, 1994). It was found that the concentration of proteins, carbohydrates and cations increases significantly following freeze-thaw conditioning for activated sludge while they remain fairly constant for alum sludge (Ormeci and Vesilind, 2001).

When the EPS and ions are removed before conditioning, an improvement in dewatering was observed (Ormeci and Vesilind, 2001; Ormeci and Vesilind, 2002). In a further investigation of the increase of proteins, carbohydrates and cations, it was found that DNA concentration also increases following freeze-thaw treatment. This suggests that as cells expand and contract during the freeze-thaw, they may weaken and eventually burst, causing cell disruption (Jin et al., 2004; Ormeci and Vesilind, 2001). The extracellular polymeric substances (EPS) are believed to aid in floc formation but when their concentrations are too high lead to poor settling (Jin et al., 2004).

2.7.3.4 Effect of Freeze-Thaw Conditioning on Particle Size Distribution

Freeze-thaw conditioning seems to increase the particle size distribution of sludge (Chu et al., 1997; Gao, 2011; Vesilind and Martel, 1990). Vesilind and Martel (1990) proposed that this is due to gross migration. They suggested that as the particles are forced into a condensed volume by the ice front, the solid particles are drawn to each other due to surface attractive forces, resulting in a larger particle size distribution. However, if the ice crystal growth is not planar and instead dendritic, particles are trapped in the ice and there is a 50% decrease in the average particle size of the freeze-thaw conditioned sludge (Martel, 2000). This was supported by the work of Chu et al. (1997) since floc diameter decreased as freezing speed increased and gross migration decreased.

2.8 Sludge Dewatering

Sludge dewatering is the process of removing free and bound water from sludge. In general, the more bound water that exists, the more difficult dewatering is. Dewatering results in a significant reduction in volume which in turn decreases the cost of disposing of the sludge. There are currently four dewatering methods used by wastewater treatment plants: centrifuge, belt filter press, pressure filter press and drying beds.

The centrifuge was first introduced to wastewater treatment in the 1920's; however, the design was poor and inefficient. Better designs emerged in the 1960's and today similar designs are used for sludge treatment in many treatment plants across North America as well as Europe. A force 500 – 3000 times that of gravity is applied to the sludge causing the solids and liquids to separate. The centrifuge is more effective for primary sludge compared to biological sludge since biological sludge contains more bound water and centrifuging is unable to remove bound water. Cake solids concentration ranges between 15 to 36% depending on the type of sludge. While the centrifuge takes up less space and has good odour containment compared to some of the other dewatering methods, it has a relatively high capital cost and requires skilled maintenance personnel when problems arise (Turovskiy and Mathai, 2006).

Drying beds are the most widely used method in municipal treatment plants in North America for sludge dewatering. There are several types of drying beds (sand, paved, artificial media as well as vacuum assisted). In general, 12-15% solids concentration can be reached within 1 day (mostly due to gravity). Within a few days, the concentration increases to 20-25% and if left for 6 -12 months, the solids concentration can increase as high as 60-70%. Due to their simplicity, sand drying beds they have been used for over a century. While drying beds are known for their low cost and maintenance, they require a large area and have a potential for odour problems.

The belt filter press is a continuously-fed machine which has two porous moving belts. It has a gravity drainage zone which removes 60-75% of the moisture followed by a zone with mechanically applied pressure. It is the most widely used dewatering method in the world and was introduced to North America in the mid 1970's. The belt filter press requires the addition of polymers and requires large amounts of water in order to wash the belt. On the other hand, it has relatively low capital, operating and power costs.

A less frequently used dewatering process is the pressure filter process which forces the water out of the sludge using high pressure. It produces the driest cake yet it is the most expensive and has large area requirements. In addition, it requires the use of inorganic chemicals that produce additional solids.

2.9 Sludge Disposal

Following dewatering, sludge solids still require disposal. The three most common methods of sludge disposal are incineration, landfill and agricultural use. Disposal can be an expensive process as energy costs associated with incineration and landfill costs are continuously rising, and environmental regulations regarding the land use of sludge are becoming increasingly stringent (Lee et al., 2005).

Incineration is the most common method of sludge disposal and has been used since 1934 (Liu and Liptak, 1999). Solids are pumped to the incinerator, where they are dried and then ignited. In the process, the organic matter in the sludge is converted to carbon dioxide and water while the inorganic matter remains as ash. Incineration results in the greatest moisture and volume reduction and is suitable in densely populated countries.

The operating and maintenance costs for landfilling are the lowest out of all the disposal options (Liu and Liptak, 1999). The majority of the cost associated with landfill disposal depends on the distance of the treatment plant to the landfill. Major problems arise when nearby landfills fill up and dried sludge must now be transported much further distances. Precautions must also be taken to prevent ground and surface water pollution by the leachate.

Land application is considered an environmentally friendly manner to dispose of dried sewage sludge while recycling nutrients and fertilizing the soil. The sludge being disposed must be rigorously tested in order to ensure it meets government regulations. One of the major benefits of composting is that it can achieve pathogen destruction, stabilization, resource recovery and serve as a method of disposal all at once.

2.10 Sludge Pretreatment

With the cost of sludge management on the rise, it is becoming more important to find ways to optimize sludge treatment. Current research has focused their attention on finding methods to increase the efficiency of anaerobic digestion. These methods being studied are commonly referred to as pre-treatment methods, as they are implemented prior to anaerobic digestion (Figure 2.6). The purpose of pretreatment is to improve hydrolysis and to increase the production of biogas while also reducing pathogens and improving dewaterability (Mudhoo and Sharma, 2011). This is generally accomplished by rupturing the cell wall and allowing the intracellular matter as well as EPS to be released and accessible for degradation (Chang et al., 2011).

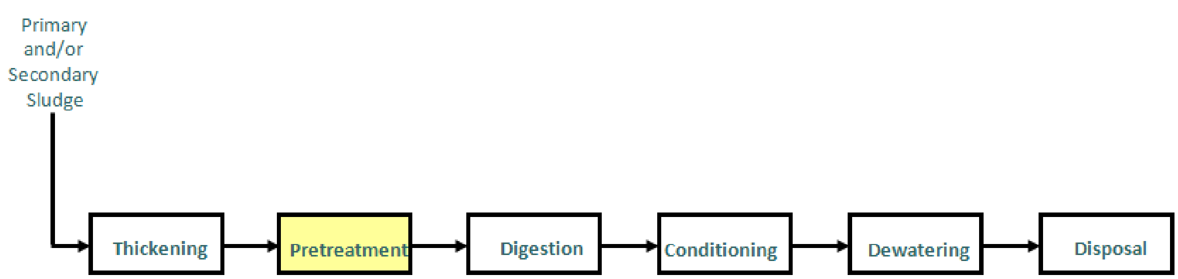

Figure 2.6: Pretreatment within the conventional sludge treatment system

When bacterial cells in sludge are properly disintegrated, the physical, chemical and biological properties of the sludge are changed (Khanal et al., 2007; Pilli et al., 2011). In order to determine the extent of disintegration, several parameters are commonly examined.

Common physical parameters used to evaluate the degree of disintegration are particle size distribution, turbidity, and microscopic evaluations. Ultrasound treatment should decrease the size of flocs that would affect each of the above parameters.

Important chemical parameters are soluble chemical oxygen demand (SCOD), protein concentration, nitrate nitrogen and the release of NH_3. There is a linear relationship between solubilisation of WAS and methane generation (Wang et al., 1999). SCOD increases after sonication due to the increase of solid matter being solubilised as well as the increase of organic matter and EPS in the aqueous phase (Pilli et al., 2011). Similarly, increases in protein should be observed considering in WAS, the majority of EPS is in the form of proteins (Wang et al., 2006). Increases in soluble protein should result in increased efficiency of anaerobic digestion. Lastly, NH_3 increases after sludge in sonicated due to the disintegration of the cells which releases intracellular nitrogen which is hydrolyzed to ammonia.

Biological parameters are the specific oxygen uptake rate (SOUR) and heterotrophic counts. When the bacteria cells are killed, they will no longer use up the oxygen in the sludge. The SOUR would be zero if the bacterial cells were completely disintegrated.

While the efficiency of each of these types of pre-treatments varies, it is agreed that sludge pretreatment of one kind or another will eventually become a standard in all wastewater treatment facilities (Kennedy et al., 2007).

Various pretreatment methods have been examined and the methods that seem best able to break apart flocs and destroy cells include:

- Thermal
- Microwave
- Ultrasound
- Mechanical
- Chemical
- Combinations of the above methods

2.10.1 Thermal Pretreatment

Studies have been done for well over 40 years investigating the use of heat in the treatment of sewage sludge. Thermal treatment was initially used as a conditioning method as it demonstrated the ability to improve the dewaterability of primary and WAS sludge (Haug, 1977; Li and Noike, 1992). Two major disadvantages of this conditioning method were odour produced from heating the sludge, as well as the energy requirements (Haug et al., 1978). Thermal pretreatment as opposed to conditioning was an approach that could avoid these problems while offering several additional benefits. Since sludge is not exposed to the atmosphere until after anaerobic digestion, odour would no longer be a problem as the digestion process degrades odour causing compounds (Kepp et al., 2000). The heat applied to the sludge prior to digestion is suitable for both mesophilic and thermophilic digestion and therefore no additional energy is required. Once it was determined that hydrolysis, the rate limiting step of anaerobic digestion, can be accomplished thermally rather than biologically, additional benefits were realized (Graja et al., 2005; Pinnekamp, 1989). The enhanced biogas production can be used by the treatment plant to offset energy costs, while the increased pathogen destruction that occurs during the process is also extremely valuable (Haug et al., 1983; Skiadas et al., 2005; Wilson and Novak, 2009).

2.10.1.1 Effect of Thermal Pretreatment on Disintegration and Digestion

Thermal pretreatment has consistently shown the ability to increase the solubilisation and disintegration of sludge solids (Bougrier et al., 2008; Hiraoka et al., 1985; Li and Noike, 1992; Skiadas et al., 2005). Solubilisation of COD in thermally treated WAS has shown increases of up to six times compared to controls, reaching solubilisation percentages as high as 55% (Li and Noike, 1992). Additionally, the solubilisation of solids has been found to increase, with TSS concentrations in control samples of WAS starting at about 90% of the TS and decreasing to as low as 27% after thermal treatment (Bougrier et al., 2008). Another important indication of disintegration is the increase in volatile organic acids after thermal pretreatment. The total volatile acids of thermally pretreated sludge is five times greater than that of the control (Hiraoka et al., 1985).

As expected, the increase in solubilisation is correlated to increased solids destruction and biogas production. In addition, several studies have also demonstrated that thermal pretreatment allows for a reduction in hydraulic retention type, which enables further energy and cost savings (Jolis, 2008; Li and Noike, 1992). The increase in biogas production of thermally treated WAS samples compared to control samples has shown considerable fluctuation from study to study, ranging anywhere from 40 – 100% (Bougrier et al., 2008).

Several factors appear to vary the effect of thermal pretreatment on disintegration and biodegradability of sludge solids. These include:

- Sludge Type
- Solids Concentration

- Temperature
- Length of Treatment
- pH

Sludge Type

The handful of studies that have examined the effect of thermal pretreatment on primary sludge have found that pretreatment on WAS results in greater increases in COD solubilisation, even though primary sludge is known to start with a greater initial solubilisation (Haug, 1977; Haug et al., 1978; Wilson and Novak, 2009). One study found that while primary sludge started with 18.4% COD solubilisation compared to WAS's 5.1%, after thermal pretreatment, the solubilisation of primary sludge increased to 31.9% whereas WAS was over 9 times its initial solubilisation, at 48.4% (Haug et al., 1978). For this reason, many wastewater treatment plants choose to pre-treat only the WAS, despite anaerobic digestion on the mixed sludge (Wilson and Novak, 2009). Wilson and Novak (2009) suggest that the increases in solubilisation of organic matter are similar for WAS and primary sludge, especially for temperatures above 130 °C. Further investigations on primary sludge should be conducted in order to determine the pros and cons of pretreatment on primary sludge.

In terms of biogas production and solids destruction, an early study by Haug et al. (1978) found that for thermal treatment at 175°C, VS destruction of primary sludge showed no significant change over the control while that of the activated sludge showed increases of up to 22% . When a 1:1 mixture of activated sludge and primary sludge was thermally pretreated, its VS destruction fell somewhere in the middle at 8% (Haug et al., 1978). Another study found that the efficiency of VSS removal was 28% higher than its control for primary sludge whereas it was an astonishing 617% higher for WAS (Skiadas et al., 2005).

Due to the large variation in biogas production reported in numerous research papers, studies were carried out using five different WAS samples in order to determine whether the differences were due to experimental error, or the WAS samples themselves (Bougrier et al., 2008; Carrere et al., 2008). It was found that solubilisation did not depend on the sludge sample, whereas the biogas volume enhancements did; the lower the initial biodegradability, the greater the increase in biogas volume (Bougrier et al., 2008, Carrere et al., 2008).

These findings can be carried over to the results of primary sludge. Raw primary sludge has a higher initial soluble COD and volatile organic acids concentration in comparison with untreated WAS (Haug et al., 1978; Hiraoka et al., 1985; Wilson and Novak, 2009). This is translated into a higher initial biodegradability for primary sludge (Haug et al., 1978; Muller et al., 2009), which may explain why the increases in biodegradability are not as pronounced compared to WAS.

Temperature

Thermal pretreatment in the range of 60-180°C breaks down cell walls and allows organic matter found within cells to become available for degradation (Neyens and Baeyens, 2003). This is ultimately the goal of any pretreatment method. A positive correlation between treatment temperature and COD solubilisation has been established in several studies

(Bougrier et al., 2008; Haug et al., 1978; Li and Noike, 1992). Li and Noike (1992) studied temperatures in the range of 120-175°C and found that the solubilisation ratio of COD was 7 times higher for treatment at 175°C compared to the control. When examining specific organic compounds were examined, the solubilisation ratios differed for proteins, carbohydrates and lipids (Bougrier et al., 2008; Li and Noike, 1992).

Gas production and solids destruction follow a similar trend, with increases in temperature resulting in increased gas production and volatile solids destruction (Bougrier et al., 2008; Haug et al., 1978; Li and Noike, 1992). Bougrier et al. (2008) examined the biogas volume produced from the soluble fraction of sludge compared that with the biogas volume produced from the particulate fraction. They found that the amount of biogas produced from the soluble fraction increases as pretreatment temperature increases, especially at temperatures above 130°C. This confirms the idea that soluble organic matter is more readily biodegradable than particulate matter and that the increase in biogas production is in fact due to the conversion of particulate organic matter into soluble.

When temperatures above 180°C were used, increases in solubilisation of COD and gas production are not as pronounced and do not follow the same pattern as those below 200°C (Bougrier et al., 2008; Haug et al., 1978). Haug et al. (1978) suggested that higher temperatures result in the formation inhibitory or toxic compounds. One such compound is thought to be melanoidins, the product of a reaction between carbohydrates and amino acids at high temperatures (Bougrier et al., 2008; Pinnekamp, 1989; Wilson and Novak, 2009). For this reason, many studies have reported the optimal pretreatment temperature to be between 160 – 180 °C (Haug et al., 1978; Li and Noike, 1992; Pinnekamp, 1989).

Li and Noike (1992) concluded that the increased methane production at temperatures below 100°C to be slight in comparison to higher temperatures. However, due to the rather high cost of thermal pretreatment at elevated temperatures a small number of studies have examined thermal pretreatment at lower temperatures in more detail. Significant increases in solubilisation and gas production were found at temperatures as low as 60°C (Hiraoka et al., 1985; Skiadas et al., 2005). It was noted however, that longer treatment times (greater than 60 minutes) were required to achieve significant improvements in biodegradability (Hiraoka et al., 1985). A study was conducted using thermophilic anaerobic digestion, to treat primary and WAS for 2 days at 70°C before passing it on to digester tanks (Skiadas et al., 2005). Significant increases in solubilisation were observed and VSS removal was 38% greater than the control for the primary sludge and over seven times greater for the WAS.

Solids Concentrations

Thermal pretreatment causes a reduction in viscosity of WAS (Bougrier et al., 2008; Graja et al., 2005; Jolis, 2008). This presents an opportunity to increase the solids concentration for anaerobic digestion as efficient mixing, pumping and heat transfer all require low viscosities (Jolis, 2008). Using temperature-phased anaerobic digestion, Jolis (2008) compared high solids sludge with 7.9% solids concentration to a control with 3.6% solids concentration. They found that the solubilisation of COD increased by 13% for the control after thermal pretreatment whereas it increased 37% for the high solids sludge. However, changes organic loading in a mesophilic digester did not affect the net rate of VS destruction significantly; the ratio of bacteria performing anaerobic digestion simply changed thereby changing the organic matter digested by the same percentage (Haug et al., 1978; Jolis, 2008).

Length of Treatment

Most studies suggested that 30 – 60 minutes of thermal pretreatment is optimal for enhanced biodegradability (Haug et al., 1978; Li and Noike, 1992). For contact times up to one hour solubilisation of COD increased significantly with treatment temperatures greater than 100 °C (Haug et al., 1978). Li and Noike (1992) found that gas production also increased as treatment time increased; however, they only observed significant change for times up to 30 minutes. As mentioned earlier, temperatures below 100 °C often require longer treatment times.

pH

Thermochemical treatment has been studied in order to determine the combined effect of pH and thermal treatment (Haug et al., 1978; Rafique et al., 2010; Vigueras-Carmona et al., 2011). In terms of solubilisation, several studies have found that thermochemical treatment results in increased solubilisation of COD (Delgenes et al., 2000; Haug et al., 1978; Kim et al., 2003). Solubilisation percentages reached as high as 86.5% at a concentration of 9 g/L of NaOH and treatment at 121 °C for 30 minutes (Kim et al., 2003). Many studies have also found increased biogas production during anaerobic digestion following thermochemical pretreatment (Kim et al., 2003; Rafique et al., 2010; Vlyssides and Karlis, 2004). After anaerobic digestion the percentage increases in biogas production have been reported to be up to 97% (Rafique et al., 2010). On the other hand, some studies have suggested that thermochemical pretreatment hinders gas production (Delgenes et al., 2000; Haug et al., 1978). A study by Delgenes et al. (2000) found that only 40% of the initial COD was converted into biogas with or without thermochemical pretreatment. This discrepancy can be explained with the theory that there are two separate hydrolysis reactions that occur at different rates: the hydrolysis of suspended solids and that of soluble solids (Vigueras-Carmona et al., 2011). Vigueras-Carmona et al. (2011) suggest that thermochemical treatment increases the degradation of suspended solids but inhibits the degradation of soluble solids.

2.10.1.2 Effect of Thermal Pretreatment on Dewaterability

Thermal conditioning to enhance dewaterability was used long before the idea of pretreatment to enhance anaerobic digestion. Studies are in agreement that thermal treatment enhances the dewaterability of sludge (Bougrier et al., 2008; Haug et al., 1978; Neyens and Baeyens, 2003). Two main factors affecting the effectiveness of thermal treatment for dewaterability are sludge type and temperatures.

Primary sludge showed the greatest increase in dewaterability following thermal treated compared to WAS or mixed sludge (Haug et al., 1978). In terms of temperature, dewaterability increased as temperature increased between 90 and 210°C (Bougrier et al., 2008; Haug et al., 1978). Bougrier et al. (2008) found that settleability consistently improved up to temperatures of 160°C after which they slowly decreased. Filterability, on the other hand, decreased for temperatures up to 130°C and then rapidly improved from 150 to 190°C.

At lower temperatures, capillary suction time values decreased from 20-60°C then increased to values almost as high as the control when temperature increased from 60 to 80°C (Lin and Shien, 2001).

When using heat as a pretreatment method, it will be applied before digestion and therefore the effect of anaerobic digestion on dewaterability needs to be examined. Preliminary tests by Haug et al. (1978) tested the dewaterability of thermally treated primary, WAS and mixed sludge both before and after anaerobic digestion. Following anaerobic digestion, thermally treated mixed and WAS sludges showed a slight, but insignificant decrease in dewaterability whereas primary sludge showed a large decrease in dewaterability. In all cases, before and after digestion, the dewaterability of sludge with thermal treatment was improved when compared to the control.

2.10.2 Microwave Pretreatment

Of the various pretreatment methods, microwave has recently been studied as a possible superior method to thermal pretreatment. Microwave radiation lies between radio waves and infrared waves on the electromagnetic spectrum. When microwaves are absorbed by a material, the energy carried by the wave is converted to thermal energy within the material and therefore results in an increase in temperature.

The benefits of thermal pretreatment, (increased biogas production, pathogen destruction and dewaterability) are realized through both conventional heating as well as with microwave heating. Additionally, microwave radiation heats objects from within, resulting in very little heat lost through convection and conduction as is the case with conventional heating (Chang et al., 2011; Toreci et al., 2010). In comparison to conventional heating, it is considered advantageous due to its faster, non-contact and more specific heating abilities (Mudhoo and Sharma, 2011).

2.10.2.1 Effect of Microwave Pretreatment on Disintegration and Digestion

Like thermal pretreatment, microwave pretreatment is able to significantly solubilise COD, proteins and sugars in WAS (Ahn et al., 2009; Chang et al., 2011; Toreci et al., 2009). At temperatures of approximately 70°C, soluble COD has shown increases of up to 125% (Hong et al., 2006). Additionally, studies have shown that a 20% disintegration of COD with microwave pretreatment is achievable using 10 times less energy that ultrasound pretreatment (Ahn et al., 2009). Solubilisation of volatile suspended solids has also been impressive reaching values of up to 77% when a combined microwave-alkali treatment was used (Chi et al., 2010).

Similar benefits have been found in terms of solids destruction and biogas production. The increase in gas production varies from 12-46% greater than the control depending on the study (Chi et al., 2010; Hong, 2002; Park et al., 2004). Park et al. (2004) also found that microwave pretreatment could allow for a decrease in hydraulic retention time from fifteen down to eight days.

The factors that seem to have the greatest effect on disintegration and degradation of microwave-treated sludge are:

- Treatment Temperature
- Microwave Intensity
- Solids Concentration
- Sludge Type

Treatment Temperature

One of the first factors to be examined with respect to microwave pretreatment was the effect of different temperatures or treatment times. Kennedy et al. (2007) examined temperatures in the range of 45-85°C and found that as temperature increased, the soluble to total COD ratio of aerobic sequencing batch reactor (SBR) sludge also increased from 1.4% up to a maximum of approximately 7%. WAS increases in sCOD to tCOD ratios have increased from 8% for the control up to 18% at temperatures of 70°C (Hong, 2002) and from 2% for the control up to 19% at temperatures of 91°C (Park et al., 2004). Many other studies have confirmed that an increase in temperature results in increased solubilisation of COD (Ahn et al., 2009; Chang et al., 2011; Qiao et al., 2010; Tang et al., 2010).

While many initial studies limited temperatures to below the boiling point of sludge, recent studies have investigated the effects of temperatures above 100°C on WAS. Mixed results have been obtained. Ahn et al. (2009) found that once the boiling point was achieved, the increase in the ratio of soluble to total COD increased very little, or not at all. Conversely, other studies showed that even above boiling point, increases in temperature continue to increase the solubilisation of COD (Qiao et al., 2010; Toreci et al., 2009; Toreci et al., 2010).

Toreci et al. (2010) found that increasing the temperature from 110°C to 175°C increased the soluble to total COD ratio from 30 to 46%.

Pretreatment temperature has also shown an effect on biodegradability and therefore biogas production; as the temperature increases, the biodegradability is enhanced and biogas production also increases (Ahn et al., 2009; Kennedy et al., 2007; Qiao et al., 2010; Tang et al., 2010). Using biochemical methane potential (BMP) assays, SBR sludge treated to temperatures below 65°C showed no increase in biogas production. However, above 65°C, increases in temperature increased the biogas production to values up to 16.2% greater than the control (Kennedy et al., 2007). The WAS treated by microwave irradiation a the study conducted by Eskicioglu et al. (2007) also showed that the highest temperature (96°C) resulted in the largest improvements over the controls in biogas production regardless of other factors such as sludge concentration.

Microwave Intensity

Another commonly studied factor is the intensity of the microwave radiation, which is can be measured as a percentage of the maximum intensity of the microwave (Eskicioglu et al., 2007; Kennedy et al., 2007) or as the temperature change rate (Toreci et al., 2010). Kennedy et al. (2007) did not find any significant change in sCOD/tCOD ratio with respect to microwave intensity when the intensity was increased between 60 to 100% of the maximum intensity of 1460 W. Likewise, Chang et al. (2011) found similar sCOD values at MW powers of 300, 450 and 600 W. On the other hand, other studies have shown that decreasing the microwave intensity from 7.5 to 3.75°C/min results in a significant improvement in solubilisation of COD while decreasing it further to 1.25°C/min reveals little improvement (Toreci et al., 2009; Toreci et al., 2010).

Solids Concentration

Studies examining the effect of solids concentration on microwave pretreatment found differing results (Chang et al., 2011; Kennedy et al., 2007; Tang et al., 2010). Kennedy et al. (2007) compared various sludge concentrations suggested that solids concentration does not have a significant effect on solubilisation of COD. In agreement, another study found that despite changes in WAS concentration (2-55g/L TSS), COD solubilisation seemed to remain between 8-10% while consistent increases in soluble COD concentration were observed (Chang et al., 2011). On the other hand, studies conducted by Tang et al. (2010) found that at the same energy dose, sludge with higher solids concentrations were able to achieve higher COD solubilisation.

Toreci et al. (2010) suggested that the effect of solids concentrations depended on pretreatment temperature. They suggested that below the boiling temperature, solubilisation of organic matter was greater for low sludge concentration, while above the boiling temperature greater improvements were observed for higher sludge concentrations.

Sludge Type

When examining sludge type, results were similar to those of conventional thermal pretreatment. Although primary sludge began with the greatest amount of soluble COD, its increase was only 16% greater than the control whereas that of the WAS reached 125%

greater than the control with similar treatment times (Hong et al., 2006). When comparing a 1:1 mixture of primary and secondary sludge with secondary sludge alone, increases in biogas production ranged from 13– 20% and 11 – 26% greater than the control for mixed and secondary sludge respectively (Qiao et al., 2010).

2.10.2.2 Effect of Microwave Pretreatment on Dewaterability

Studies that have examined the effect of microwave pretreatment on dewaterability are all in agreement. Several studies have found that up to a certain contact time, sludge filterability can be improved after which it begins to deteriorate rapidly (Chang et al., 2011; Wojciechowska, 2005; Yu et al., 2009). The exact contact time depended on the amount and type of sludge being treated as well as the intensity of the microwave treatment. It was found that greater microwave intensities reached lower CST values faster; however, they also resulted in a faster decline in dewaterability once the optimal treatment time was surpassed (Chang et al., 2011; Yu et al., 2009). Additionally, Yu et al. (2009) found that there is an optimal EPS concentration that will enhance dewaterability. As EPS concentration increased from approximately 750 to 1800 mg/L the filterability increased. Further increases caused a rapid increase in CST and SRF values.

2.10.3 Ultrasonic Pretreatment

Ultrasonic waves are inaudible sound waves with frequencies greater than 20 kHz. This wide range of frequencies is generally divided into two categories. The first is known as diagnostic ultrasound which is low power/high frequency waves with frequencies greater than 2 MHz.

The applications of these high frequency ultrasounds are mostly for analytical purposes for things such as medical imaging, SONAR, and animal communication. The second category is high energy/low frequency waves called power ultrasound. Conventional power ultrasound deals with frequencies between 20 – 100 kHz although in some cases in sonochemistry frequencies can be as high as 2 MHz (Mason and Lorimer, 2002). Power ultrasound is commonly used for cleaning, therapeutics, plastic welding and cell disruption.

The application of power ultrasound is one of the most efficient methods of cell disruption for sludge (Appels et al., 2008). Ultrasonic pretreatment is a process which does not involve the addition of chemicals to the sludge or the creation of toxic compounds. On the contrary, this physical process can actually break down many toxic and organic pollutants into simpler forms (Khanal et al., 2007).

Power ultrasound waves are most commonly generated using one of two types of ultrasonic generators: magnetostrictive and piezoelectric. The magnetostrictive technique uses materials that change shape under magnetization and converts the energy produced by the magnetic field into mechanical energy. The piezoelectric method uses piezoelectric crystals, which experience strain when exposed to an electric charge. They convert electrical energy to mechanical energy and are the more commonly used of the two (Mason and Lorimer, 2002).

Regardless of the method, an ultrasonic probe system used to sonicate sludge consists of three parts. The transducer, or converter, converts the electrical energy to mechanical ultrasound waves. The waves then pass through the booster which increases their amplitude. The horn is responsible for delivering the ultrasonic energy; it is often also used to further amplify the wave (Figure 2.7).

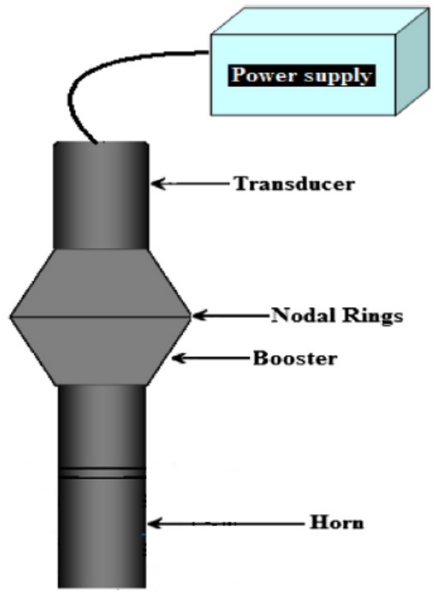

Figure 2.7: Ultrasound Probe System (Pilli et al., 2011)

2.10.3.1 Measuring Ultrasonic Energy

It is important to be able to measure the amount of energy or power required to achieve adequate disintegration of sludge solids. Four commonly used methods are: (1) specific energy input, (2) ultrasonic dose, (3) ultrasonic density and (4) ultrasonic intensity. A summary of these methods can be found in Table 2.1.

2.10.3.2 Mechanisms of Ultrasonic Pretreatment

Ultrasound waves are a longitudinal sound wave which generates a series of compressions and rarefactions in the medium through which they propagate. Rarefactions are essentially areas of large negative pressure which cause microbubbles, known as cavitation bubbles to form. Compressions are areas of positive pressure which cause the growth of the bubbles. When these cavitation bubbles are exposed to the positive pressure of the compressions, they grow to an unstable size and proceed to collapse violently. This process, called cavitation, occurs in just microseconds and is deemed necessary for the successful disintegration of sludge. The collapse causes the temperatures in the immediate region to reach as high as 5000 °C and pressures up to 500 - 1000 atm (Flint and Suslick, 1991; Suslick and Flannigan, 2008).

Table 2.1: Methods for measuring ultrasonic energy for sludge disintegration (Pilli et al., 2011). P = power, t = time, V = Volume of sludge, TS = total solids concentration of sludge, A = surface area of the probe

Parameter	Equation	Units
Specific Energy Input	$Es = \frac{P \times t}{V \times TS}$	kJ/kg TS or kW·s/kg TS
Ultrasound Dose	$Udose = \frac{P \times t}{V}$	J/L
Ultrasound Density	$UD = \frac{P}{V}$	W/L
Ultrasound Intensity	$UI = \frac{P}{A}$	W/cm^2

The explosive collapse disrupts nearby bacterial cells, causing devastation to the cell wall and membranes. The ultrasonic energy applied to the sludge must be greater than the molecular attractive forces for cavitation to occur (Clark and Nujjoo, 2000). The disintegration of sludge is, for the most part, due to the shear forces produced by the cavitation bubbles (Pilli et al. 2011; Zhang et al., 2007). The oxidizing effect of hydroxyl radicals is also thought to influence disintegration, although it is generally considered negligible (Wang et al., 2005). Lower frequencies in the range of 20 – 40 kHz tend to be optimal for cavitation as they allow enough time for cavitation bubbles to form (Capelo-Martinez, 2009; Zhang et al., 2007).

2.10.3.3 Effect of Ultrasonic Pretreatment on Disintegration and Digestion

Breaking the evidence down into physical, chemical and biological, we can see that ultrasonic pretreatment is an effective means of disintegrating sludge solids. Zhang et al. (2007) found that over the course of 30 minutes of ultrasonic treatment, increases in SCOD, supernatant proteins and nucleic acids were 690%, 560% and 1640%, respectively. Muller et al. (2009) carried out ultrasonic treatment prior to digestion as well as in a recycle line in the digester and found that regardless of where the treatment took place, it significantly increased both TS and VS destruction and increased biogas yield. In order to optimize the ultrasonic treatment, several factors have been examined which will be discussed below:

- Sonication Time
- Ultrasonic Energy/Power
- Sludge Solids Content
- Type of Sludge
- pH

Sonication Time

Several studies have found that increased sonication times lead to increases in COD solubilisation for WAS and biological sludge (Clark and Nujjoo, 2000; Kim et al., 2003; Zhang et al., 2007). Additionally, it is thought that the relationship between the sonication time and solubilisation is linear for times less than 20-30 minutes, after which the rate of increase slows down considerably (Clark and Nujjoo, 2000; Wang et al., 2005; Wang et al., 2006). At a high, but unspecified intensity, Clark et al. (2000) sonicated sludge for 60 s to determine if there was an upper limit to the amount of solubilisation possible. They were able to attain 90% solubilisation which seems to indicate that there is no upper limit.

Wang et al. (2006) studied the concentration of protein, DNA and polysaccharides in solution before and after ultrasonication. They found that protein is the main component released when sludge is disintegrated, followed by DNA and polysaccharides. In the first 10 – 20 minutes of ultrasonication, the increase in concentration of proteins was rapid, after which it slowed down (Wang et al., 2006; Zhang et al., 2007).

Sludge Solids Content

Greater sludge solids contents have shown to result in increased solubilisation for both waste activated sludge as well as primary sludge (Clark and Nujjoo, 2000; Wang et al. 2005). Clark et al. (2000) suggest the reason for the increase could be due to the fact that thicker sludges contain more microbial cells and therefore are more likely to be found near cavitation sites.

Type of Sludge

Primary sludge is thought to be made up of readily degradable materials and therefore is not generally pre-treated. However, Clark et al. (2000) tested both WAS and primary sludge. They found that the initial concentrations of SCOD are ten times larger in primary sludge compared to WAS. This was expected as the organic content in primary sludge is not trapped by microbial cells. What was not expected was the significant increase in SCOD following sonication since primary cells are not composed of nearly as many bacterial cells as WAS.

Ultrasound Power/Intensity

It had been observed that increased ultrasonic intensity and density result in increased solubilisation (Wang et al., 2005; Zhang et al., 2007). However, it is important to determine the most energy efficient conditions if ultrasonic treatment is to be considered a realistic pretreatment method. Wang et al. (2006) determined that specific energy consumptions less than 50 kJ/g-TS was optimum for the release of proteins, carbohydrates and DNA.

pH

Wang et al. (2005) studied the effect of pH on SCOD release during ultrasonic treatment by adding H_2SO_4 and NaOH to adjust the pH of WAS to values between 6 and 12. After 30 minutes of sonication, they found that increases in pH resulted in increased solubilisation. Out of all the factors they investigated (pH, sludge concentration, ultrasonic intensity and ultrasonic density), they found that pH had the largest magnitude of an effect on sludge disintegration.

2.10.3.4 Effect of Ultrasonic Pretreatment on Gas Production

When ultrasonically pre-treated sludge undergoes anaerobic digestion, there is a significant increase in biogas production as well as methane yield (Clark and Nujjoo, 2000; Kim et al., 2003; Wang et al., 1999). Increases in SCOD and organic solids found after ultrasonic pretreatment translate linearly to increases in methane gas and solids destruction as well (Wang et al., 1999). Wang (1999) found that methane generation increased 12, 31, and 63% compared to the control for 10, 20 and 30 minutes of ultrasound treatment respectively. This steady increase slowed down after 30 minutes, with 40 minutes representing only 6% more than the 30 minute increase. The organic destruction efficiency on the other hand, was established to increase steadily over the course of 40 minutes from 11 to 46% more than the control.

Clark et al. (2000) found that the greatest increase in methane production, 61%, was at a HRT of 15 days. Retention times greater than that resulted in the percentage increases to decrease while a hydraulic retention time (HRT) of 12 days resulted in washout. Clark et al. (2000) proposed that the largest benefits of ultrasonic pretreatment are apparent at low HRTs since helping with microbial breakdown is most beneficial when the digester is under the greatest stress, at short HRTs.

Kim et al. (2003) found that the volatile solids reduction during anaerobic digestion increased from 20.5% for the control to 38.9% for the WAS that was treated with power ultrasound. While increases in methane yield were observed, the methane content of the biogas remained fairly constant (Muller et al., 2009; Kim et al. 2003).

2.10.3.5 Effect of Ultrasonic Pretreatment on Dewaterability

Results pertaining to the effect of ultrasonic pretreatment on dewaterability have been mixed. Many studies have found that ultrasound treatment decreases the dewaterability of waste activated sludge (Chu et al., 2001; Feng et al., 2009; Muller et al., 2009; Wang et al., 2006). Muller et al. (2009) found that when WAS was treated using power ultrasound, it required more polymer addition in order to achieve adequate dewaterability. Chu et al. (2001) determined that the settleability of sludge remains virtually unchanged despite changes in sonication time and density. They also found that bound water content increased with sonication time and density. Results for CST were varied; in most cases CST also increased with ultrasonic treatment (Chu et al., 2001; Wang et al., 2006). Interestingly, WAS treated for 20 minutes at 0.11 W/mL showed a decrease in CST from 197.4 to 188.2 seconds (Chu et al., 2001). These findings are in agreement with Feng et al. (2009) who found that up to energy doses of 2200 kJ/kg TS, CST will decrease.

Shao et al. (2010) studied the dewaterability of ultrasonically treated sludge during anaerobic digestion. They found that as digestion continues, the dewaterability of the control sludge increased from 2.3 s to 51.4 s over the course of the 47 days of digestion whereas the sonicated sludge started at a higher value of 44.4 s but decreased to 11.1 s by the end of the 47 days. This was an important finding as previous studies only measured the dewaterability of the treated sludge immediately after treatment as opposed to after digestion.

2.10.3.6 Effect of Ultrasonic Pretreatment on Particle Size

It has been established through several studies that the average floc size decreases when treated with power ultrasound (Chu et al., 2001; Feng et al., 2009). Chu et al. (2001) demonstrated that both increased sonication times as well as increased ultrasound densities resulted in a smaller particle size. Particle size dropped from an average of 98.9 µm to 3-4 µm when sonicated for 120 minutes at 0.33 W/mL or 20 minutes at 0.44 W/mL. They noted that the power density had to be greater than 0.11 W/mL in order to see the effect. Results obtained by Feng et al. (2009) confirm this as at energy dosages below 8800 kJ/kg TS, the change in particle size was not significant.

2.11 Freezing as a Combined Pretreatment and Conditioning Method

Freezing has conventionally been used as a conditioning method; however, in studying conditioning, it has been realized that freezing is also an effective technique for disrupting sludge cells and releasing extracellular polymeric substances (Hong et al., 1995; Hung et al., 1996; Ormeci and Vesilind, 2001). This makes freezing an excellent candidate for an effective pre-treatment method.

2.11.1 Mechanisms of Freeze-Thaw Pretreatment

There are three general mechanisms thought to be responsible for the disruption of cells. The first and most significant is thought to be cellular dehydration (Thomashow, 1998). Thomashow (1998) notes that as ice forms in the intercellular regions, water moves out of the cells in an attempt to balance the lower chemical potential of the ice. This dehydration causes membrane lesions. The second mechanism that accounts for cell disruption is the expansion and contraction of the cell membrane as water freezes and thaws (Thomashow, 1998). This expansion and contraction eventually weaken the membrane causing permanent damage to the cells. The expansion can also be due to intracellular water freezing putting pressure on the cell membrane from within (Hu et al., 2011). If the pressure becomes too great, cells may also burst.

2.11.2 Conventional Freeze-Thaw Pretreatment

In comparison to other pre-treatment methods, very few studies have been done to investigate the potential of freeze-thaw as a sludge pre-treatment method. The handful that have carried out experiments have found that conventional freezing is a very effective method of solubilising COD (Gao, 2011; Hu et al., 2011; Montusiewicz et al., 2010). Gao (2011) found that soluble COD concentrations increased up to seven times greater than the control. Similar increases in COD solubilisation were found for experiments done on kitchen waste as opposed to municipal sludge (Ma et al., 2011; Stabnikova et al., 2008).

Factors which have been considered to affect sludge solubilisation are freezing temperature, freeze-thaw cycles, type of sludge and curing time. Gao (2011) found that when sludge was frozen at -10 and -18°C no statistical difference was made to COD solubilisation. However, when the number of freeze-thaw cycles was increased from one to five, the COD solubilisation increased by approximately three times (Gao, 2011). Hu et al. (2011) studied the effect of curing time by freezing sludge at -18°C for 3 – 72 hours. WAS frozen for 72 hours had a solubilisation over 6.5 times greater than that frozen for three hours. In this same study, COD solubilisation for mixed sludge was compared to WAS. Given the same treatment conditions, WAS had a greater COD solubilisation compared to mixed sludge (Hu et al., 2011). Both Gao (2011) and Hu et al. (2011) determined that freeze-thaw is as effective at solubilising COD as thermal pretreatment at approximately 100°C for 30 minutes.

In terms of biodegradability, freeze-thaw pretreatment of municipal sludge has shown the ability to increase the concentration of VFAs and increase gas production compared to the controls (Jan et al., 2008; Montusiewicz et al., 2010; Ting and Lee, 2004). Although results are promising, further studies are needed to confirm the effectiveness of freezing as a pre-treatment method.

2.11.3 Power Ultrasonic Freezing Treatment

The crystallization of water consists of two major events: nucleation and crystal growth (Erickson and Hung, 1997; Kiani et al., 2011). Nucleation can be either primary or secondary (Chow et al., 2005). Primary nucleation is where the first nucleus, sometimes called the seed, is created. It is from this nucleus that the first ice crystals will grow. Secondary nucleation is when new crystals are produced from the fragmentation of other crystals. Power ultrasound in conjunction with freezing has been used due to its ability to promote both primary and secondary ice nucleation as well as crystal growth (Chow et al., 2005; Zheng and Sun, 2006).

2.11.3.1 Sonocrystallisation

Sonocrystallisation is the application of ultrasound in order to control the nucleation of ice crystals. Ultrasonic freezing seems to result in faster, more even nucleation (Li and Sun, 2002; Mason et al., 1996). It has been determined that after the application of ultrasound, the nucleation temperature was consistently higher in comparison to the controls (Chow et al., 2005; Delgado et al., 2009; Kiani et al., 2011). Experiments have also shown that as the power output increases, the temperature of nucleation increases as well as long as the heating effects of the ultrasound do not interfere (Chow et al., 2005). Ultrasonic power greater than 2 W/L at frequencies between 20 – 40 kHz is recommended to initiate nucleation with pulses as short as possible (Acton and Morris, 1992).

In an attempt to understand the mechanisms of sonocrystallisation, studies have been carried out on a single cavitation bubble (Hickling, 1965; Hunt and Jackson, 1966). The most commonly accepted theory suggests that the high pressures resulting from cavitation stimulate the nucleation of ice (Hickling, 1965). While this conventional theory seems like an acceptable explanation, it has been inadequate in completely explaining nucleation in practical cases where several cavitation bubbles are present (Kiani et al., 2011; Zhang et al., 2003). Both Zhang et al. (2003) and Kiani et al. (2011) found that in some samples, there was a delay between sonication and nucleation; this conflicted with Hickling's theory. Flow streams, caused by the bubbles produced during sonication are thought to be a secondary process that also induces nucleation (Chow et al., 2005; Zhang et al., 2003). If this is the case, transient cavitation is not necessarily a requirement for nucleation, as stable cavitation can also produce flow streams that initiate nucleation.

2.11.3.2 Effect of Power Ultrasonic Freezing on Freezing Rate

The flow streams, also referred to as microstreaming, result in a form of agitation in the solution being sonicated. This agitation leads to a reduction in the resistance of heat and mass transfer and therefore, result an increase in freezing rate (Li and Sun, 2002). A study by Sastry et al. (1989) concluded that increased power resulted in higher heat transfer coefficients. However, there appears to be both an upper and lower limit in ultrasonic power in order to achieve the optimal heat transfer when the thermal effects of ultrasound are taken into consideration (Li and Sun, 2002). They determined that ultrasonic power greater than 7.34 W but less than 25.89 W was necessary to obtain a significant increase in freezing rate.

Another factor that affects the freezing rate during ultrasonic freezing is the point at which the ultrasound is delivered. When ultrasound was applied during the phase change period, it resulted in the greatest increase in freezing rate.

2.11.3.3 Effect of Power Ultrasonic Freezing on the Size of Ice Crystals

When freezing rates are high, research has shown that ice crystals formed are smaller (Acton and Morris, 1992; Li and Sun, 2002). Considering this, as well as the fact that ultrasound waves promote the fragmentation of crystals, it makes sense that food that has been frozen with ultrasonic assistance has been found to have smaller, more uniform ice crystals. These smaller ice crystals are beneficial as smaller crystals translate to better quality in many frozen foods, such as ice cream as well as frozen fruits and vegetables (Li and Sun, 2002; Mason et al., 1996; Zheng and Sun, 2005).

2.11.3.4 Effect of Power Ultrasonic Freezing on Cell Structure

Foods frozen in conjunction with ultrasound are subjected to less cell membrane damage (Delgado et al., 2009; Li and Sun, 2002). This is hypothesized to be due to the faster freezing rate and smaller crystals found in ultrasonic freezing (Sun and Li, 2003). Sun and Li (2003) used a cryogenic scanning electron microscope to examine ultrasonically frozen potatoes. They concluded that the microstructure of potatoes frozen with ultrasound experience less cell disruption than those frozen without ultrasound.

2.12 Summary

There has been extensive research done on pre-treatment techniques and their effect on secondary municipal sludge. A brief summary can be found in Table 2.2. A substantial number of the recent studies on pretreatment have focused on methods such as thermal, microwave and ultrasound which have shown very little, if any, improvements in dewaterability. The handful of studies that have examined freezing as a potential pre-treatment method have found it to be a very effective method of solubilising COD and increasing the gas production, however more studies are needed to confirm these findings. There has also been no evaluation of the effect of freezing on other measurements of pretreatment effectiveness such as soluble protein concentration and biodegradability. Another area that has been overlooked is the potential for combined freezing treatments. A combined chemical-freezing technique was investigated in Gao (2011); however, treatments involving a combination of sonication and freezing have yet to be investigated.

Table 2.2: Advantages and disadvantages of currently studied pretreatment methods

	Advantages	Disadvantages	References
Thermal	- Rate limiting step of AD, hydrolysis, can be overcome thermally. - Already used in sludge treatment process as a conditioning method; moving it to a pre-treatment method would eliminate odour problems. - Heat applied prior to digestion is suitable for mesophilic and thermophilic digestion. - Increased pathogen destruction. - Improved dewaterability.	- High energy requirements. - High capital cost due to the use of corrosion resistant materials. - Requires skilled workers to be present at all times. - Scale formation in heat exchangers, pipes and reactors.	- Appels et al., 2008; Graja et al., 2005; Haug et al., 1983; Kepp et al., 2000; Skiadas et al., 2005; Turovskiy and Mathai, 2006
Microwave	- Very similar advantages compared to thermal treatment: - Increased pathogen destruction - Improved dewaterability - Heat applied can be used for digestion - Ability to overcome rate limiting step - Faster more specific heating abilities (compared to thermal). - Non contact heating; very little heat lost through convection and conduction. - Effective at releasing nutrients.	- High energy requirements. - High capital costs. - Has not been used at full scale operation.	- Chang et al., 2011; Mudhoo and Sharma, 2011; Toreci et al., 2010;
Ultrasound	- One of the most efficient methods of cell disruption. - Potentially has no upper limit to the amount of solubilisation of organic matter possible. - Can be implemented easily within the existing wastewater treatment process. - Process can be completely automated	- Decreased dewaterability - High energy requirements. - High capital and operating costs since technology is new. - Has not been used at full scale operation	- Chu et al., 2001; Hogan et al., 2004; Khanal et al., 2007; Muller et al., 2009;
Freezing	- Already used in sludge treatment process as a conditioning method - Improvements in dewaterability - Low energy costs if natural freezing is used - Effective method of cell disruption; shows potential to increase digestion.	- High energy requirements if freezing is not natural. - Very little research done on the effect of FT as a pretreatment method.	- Gao, 2011; Hedstrom and Hanaeus, 1999; Hu et al., 2011; Montusiewicz et al., 2010

2.13 References

Acton, E., and G. J. Morris. Method and Apparatus for the Control of Solidification in Liquids. USA Patent W.O. 99/20420. 1992.

Ahn, Johng-Hwa, Seung Gu Shin, and Seokhwan Hwang. "Effect of Microwave Irradiation on the Disintegration and Acidogenesis of Municipal Secondary Sludge." *Chemical Engineering Journal* 153 (2009): 145-150.

Appels, Lise, Jan Baeyens, Jan Degreve, and Raf Dewil. "Principles and Potential of the Anaerobic Digestion of Waste Activated Sludge." *Progress in Energy and Combustion Science* 34 (2008): 755-781.

Bougrier, Claire, Jean Delgenes, and Helene Carrere. "Effects of Thermal Treatments on Five Different Waste Activated Sludge Samples' Solubilisation, Physical Properties and Anaerobic Digestion." *Chemical Engineering Journal* 139, no. 2 (2008): 236-244.

Capelo-Martinez, Jose-Luis. *Ultrasound in Chemistry.* Weinheim: Wiley-VCH, 2009.

Carrere, Helene, Claire Bougrier, Delphine Castets, and Jean-Phillipe Delgenes. "Impact of Initial Biodegradability on Sludge Anaerobic Digestion Enhancement by Thermal Pretreatment." *Journal of Environmental Science & Health Part A* 43, no. 13 (2008): 1551-1555.

Chang, Chia-Jung, Vinay Kumar Tyagi, and Shang-Lien Lo. "Effects of Microwave and Alkali Induced Pretreatment on Sludge Solubilization and Subsequent Aerobic Digestion." *Bioresource Technology* 102, no. 17 (2011): 7633-7640.

Chi, Yong-Zhi, Xue-Ning Fei, Hong-Ying Yuan, Shao-Po Wang, and Yu-You Li. "Upgrading of Anaerobic Digestion of Thickened WAS by Microwave-Assisted Alkali Pretreatment." *2nd Conference on Environmental Science and Information Application Technology.* 2010. 316-320.

Chow, R., R. Blindt, R. Chivers, and M. Povey. "A Study on the Primary and Secondary Nucleation of Ice by Power Ultrasound." *Ultrasonics* 43, no. 4 (2005): 227-230.

Chu, C. P., Bea-Ven Chang, G. S. Liao, D. S. Jean, and D. J. Lee. "Observations on Changes in Ultrasonically Treated Waste-Activated Sludge." *Water Research* 35, no. 4 (2001): 1038-1046.

Chu, C. P., W. H. Feng, Y. H. Tsai, and D. J. Lee. "Unidirectional Freezing of Waste-Activated Sludge: The Presence of Sodium Chloride." *Environmental Science & Technology* 31, no. 5 (1997): 1512-1517.

Clark, P. B., and I. Nujjoo. "Ultrasonic Sludge Pretreatment for Enhanced Sludge Digestion." *Journal of the Chartered Institution of Water and Environmental Management* 14, no. 1 (2000): 66-71.

Delgado, Adriana E., Liyun Zheng, and Da-Wen Sun. "Influence of Ultrasound on Freezing Rate of Immersion-Frozen Apples." *Food Bioprocess and Technology* 2, no. 3 (2009): 263-270.

Delgenes, J.P., V. Penaud, and R. Moletta. "Investigations on the Changes in Anaerobic Biodegradability and Biotoxicity of an Industrial Microbial Biomass Induced by a Thermochemical Pretreatment." *Water Science and Technology* 41, no. 3 (2000): 137-144.

Dentel, S.K. "Conditioning, Dewatering and Thickening; Research Update/ Research Needs." *Water Science and Technology* 44, no. 10 (2001): 9-18.

Erickson, Marilyn C., and Yen-Con Hung. *Quality in Frozen Food.* New York, NY: Chapman & Hall, 1997.

Eskicioglu, C., K.J. Kennedy, and R.L. Droste. "Enhancement of Batch Waste Activated Sludge Disgestion by Microwave Pretreatment." *Water Environment Federation* 79, no. 11 (2007): 2304-2317.

Eskicioglu, Cigdem, Kevin J. Kennedy, and Ronald L. Droste. "Enhancement of Batch Waste Activated Sludge Digestion by Microwave Pretreatment." *Water Environment Research* 79, no. 11 (2007): 2304-2317.

Feng, Xin, Jinchuan Deng, Hengyi Lei, Tao Bai, Qingjuan Fan, and Zhaoxu Li. "Dewaterability of Waste Activated Sludge With Ultrasound Conditioning." *Bioresource Technology* 100, no. 3 (2009): 1074-1081.

Flint, Edward B., and Kenneth S. Suslick. "The Temperature of Cavitation." *Science - New Series* 253, no. 5026 (1991): 1397 - 1399.

Gao, Wa. "Freezing as a Combined Wastewater Sludge Pretreatment and Conditioning Method." *Desalination*, 2011: 170-173.

Gerardi, M. H. *The Microbiology of Anaerobic Digestion.* New Jersey: John Wiley & Sons, 2003.

Graja, S, J. Chauzy, L. Patria, and D. Cretenot. "Reduction of Sludge Production from WWTP using Thermal Pretreatment and Enhanced Anaerobic Methanisation." *Water Science & Technology* 52, no. 1-2 (2005): 267-273.

Graja, S., J. Chauzy, P. Fernandes, and D. Cretenot. "Reduction of Sludge Production from WWTP Using Thermal Pretreatment and Enhanced Anaerobic Methanisation." *Water Science & Technology* 52, no. 1-2 (2005): 267-273.

Haug, R. T. "Sludge Processing to Optimize Disgestibility and Energy Production." *Journal of Water Pollution Control Federation* 49, no. 7 (1977): 1713-1721.

Haug, Roger T., David C. Stuckey, James M. Gossett, and Perry L. McCarty. "Effect of Thermal Pretreatment on Digestibility and Dewaterability of Organic Sludges." *Journal of Water Pollution Contro Federation* 50, no. 1 (1978): 73-85.

Haug, Roger T., Thomas J. LeBrun, and Liberato D. Tortorici. "Thermal Pretreatment of Sludges - Field Demonstration." *Journal of Water Pollution Control Federation* 55, no. 1 (1983): 23-34.

Hedstrom, Annelle, and Jorgen Hanaeus. "Natural Freezing, Drying and Composting for Treatment of Septic Sludge." *Journal of Cold Regions Engineering* 13 (1999): 167-179.

Hickling, Robert. "Nucleation of Freezing by Cavitation Collapse and its Relation to Cavitation Damage." *Nature* 206, no. 4987 (1965): 915-917.

Hiraoka, M., N. Takeda, S. Sakai, and A. Yasuda. "Highly Efficient Anaerobic Digestion with Thermal Pretreatment." *Water Science & Technology* 17, no. 4-5 (1985): 529-539.

Hogan, F., S. Mormede, P. Clark, and M. Crane. "Ultrasonic Sludge Treatment for Enhanced Anaerobic Digestion." *Water Science and Technology* 50, no. 9 (2004): 25-32.

Hong, S. G., J. D. Young, G. W. Chen, I. L. Chang, W. T. Hung, and D. J. Lee. "Freeze/Thaw Treatment on Waste Activated Sludge: A FTIR Spectroscopic Study." *Journal of Environmental Science & Health Part A: Environmental Science & Engineering & Toxicology* 30, no. 8 (1995): 1717-1726.

Hong, SM. "Enhancement of Pathogen Destruction and Anaerobic Digestibility Using Microwaves." ph.D. Thesis, Department of Civil and Environmental Engineering, University of Wisconsin-Madison, 2002.

Hong, SM, JK Park, N Teeradej, YO Lee, YK Cho, and CH Park. "Pretreatment of Sludge with Microwaves for Pathogen Destruction and Improved Anaerobic Digestion Performance." *Water Environment Research* 78, no. 1 (2006): 76-83.

Hu, Kai, jun-Qiu Jiang, Qing-Ling Zhao, Duu-Jong Lee, Kun Wang, and Wei Qiu. "Conditioning of Wastewater Sludge Using Freezing: Role of Curing." *Water Research* 45 (2011): 5969 - 5976.

Hung, W. T., I. L. Chang, D. J. Lee, and S. G. Hong. "Sludge Chemical Composition Changes Under Uni-directional Freezing." *Water Science and Technology* 34, no. 3-4 (1996): 525-531.

Hung, W. T., I. L. Chang, W. W. Lin, and D. J. Lee. "Unidirectional Freezing of Waste-Activated Sludges: Effects of Freezing Speed." *Environmental Science & Technology* 30, no. 7 (1996b): 2391-2396.

Hunt, J. D., and K. A. Jackson. "Nucleation of Solid in an Undercooled Liquid by Cavitation." *Journal of Applied Physics* 37, no. 1 (1966): 254-257.

Jan, Tsai-Wu, Sunil S. Adav, D.J. Lee, R.M. Wu, Ay Su, and Joo-Hwa Tay. "Hydrogen Fermentation and Methane Production from Sludge with Pretreatments." *Energy and Fuels* 22 (2008): 98-102.

Jean, D. S., C. P. Chu, and D. J. Lee. "Effects of Electrolyte and Curing on Freeze/Thaw Treatment of Sludge." *Water Research* 34, no. 5 (2000): 1577-1583.

Jin, Bo, Britt-Marie Wilen, and Paul Lant. "Impacts of Morphological, Physical and Chemical Properties of Sludge Flocs on Dewaterability of Activated Sludge." *Chemical Engineering Journal* 98, no. 1-2 (2004): 115-126.

Jolis, Domenec. "High-Solids Anaerobic Digestion of Municipal Sludge Pretreated by Thermal Hydrolysis." *Water Environment Research* 80, no. 7 (2008): 654-662.

Kawasaki, Kenji, and Akira Matsuda. "Effect of Dissolved Solids Concentration on Freezing and Thawing Treatment of Excess Activated Sludge." *Kagaku Kogaku Ronbunshu* 21, no. 5 (1995): 859-865.

Kennedy, Kevin J., Gabriel Thibault, and Ronald L. Droste. "Microwave Enhanced Digestion of Aerobic SBR Sludge." *Water SA* 33, no. 2 (2007): 261-270.

Kepp, U., I. Machenbach, N. Weisz, and O. E. Solheim. "Enhanced Stabilisation of Sewage Sludge through Thermal Hydrolysis - Three Years of Experience with Full Scale Plant." *Water Science & Technology* 42, no. 9 (2000): 89-96.

Khanal, Samir Kumar, David Grewell, Shihwu Sung, and Hans J. Leeuwen. "Ultrasound Applications in Wastewater Sludge Pretreatment: A Review." *Critical Reviews in Environmental Science and Technology* 37, no. 4 (2007): 277-313.

Kiani, Hossein, Zhihang Zhang, Adriana Delgado, and Da-Wen Sun. "Ultrasound Assisted Nucleation of Some Liquid and Solid Model Foods During Freezing." *Food Research International* 44, no. 9 (2011): 2915-2921.

Kim, Jeonsik, et al. "Effects of Various Pretreatments for Enhanced Anaerobic Digestion with Waste Activated Sludge." *Journal of Bioscience and Bioengineering* 95, no. 3 (2003): 271-275.

Klass, Donald L. "Methane from Anaerobic Fermentation." *Science, New Series* 223, no. 4640 (1984): 1021-1028.

Lee, Duu Jong, and Yuan Hway Hsu. "Fast Freeze/Thaw Treatment on Excess Activated Sludges: Floc Structure and Sludge Dewaterability." *Environmental Science & Technology* 28, no. 8 (1994): 1444-1449.

Lee, Duu-Jong, Joo-Hwa Tay, Yung-Tse Hung, and Pin Jing He. "Introduction to Sludge Treatment." In *Physiochemical Treatment Processes*, by Lawrence K. Wang, Yung-Tse Hung and Iazih K. Shammas. New Jersey: Humana Press Inc., 2005.

Li, Bing, and Da-Wen Sun. "Effect of Power Ultrasound on Freezing Rate During Immersion Freezing of Potatoes." *Journal of Food Engineering* 55, no. 3 (2002): 277-282.

Li, Y. Y., and T. Noike. "Upgrading of Anaerobic Digestion of Waste Activated Sludge by Thermal Pretreatment." *Water Science and Technology* 26, no. 3-4 (1992): 857-866.

Lin, C.F., and Y. Shien. "Sludge Dewatering using Centrifuge with Thermal/Polymer Conditioning." *Water Science and Technology* 44, no. 1 (2001): 321-325.

Liu, David H.F., and Bela G. Liptak. "Wastewater Treatment." In *Environmental Engineer's Handbook*, by C.E Jr. Adams, D.B. Aulenbach and et. al, Chapter 7. Florida: CRC Press LLC, 1999.

Ma, Jingxing, Thu Hang Duong, Marianne Smits, Willy Verstraete, and Marta Carballa. "Enhanced Biomethanation of Kitchen Waste by Different Pretreatments." *Bioresource Technology* 102 (2011): 592-599.

Martel, James C. "Influence of Dissolved Solids on the Mechanism of Freeze-Thaw Conditioning." *Water Research* 34, no. 2 (2000): 657-662.

Mason, T. J., L. Paniwnyk, and J. P. Lorimer. "The Uses of Ultrasound in Food Technology." *Ultrasonics Sonochemistry* 3, no. 3 (1996): S253-260.

Mason, Timothy J., and John P. Lorimer. *Applied Sonochemistry: The Uses of Power Ultrasound in Chemstry and Processing.* Weinh: Wiley-VCH, 2002.

Montusiewicz, A., M. Lebiocka, A. Rozej, E. Zacharska, and L. Pawlowski. "Freezing/thawing Effects on Anaerobic Digestion of Mixed Sewage Sludge." *Bioresource Technology* 101 (2010): 3466 - 3473.

Mudhoo, Ackmez, and Samjay Kumar Sharma. "Microwave Irradiation Technology in Waste Sludge and Wastewater Treatment Research." *Critical Reviews in Environmental Science and Technology* 41, no. 11 (2011): 999-1066.

Muller, Christopher D., Mohammad Abu-Orf, Charles D. Blumenschein, and John T. Novak. "A Comparative Study of Ultrasonic Pretreatment and an Internal Recycle for the Enhancement of Mesophilic Anaerobic Digestion." *Water Environment Research* 81, no. 12 (2009): 2398-2410.

Neyens, E., and J. Baeyens. "A Review of Thermal Sludge Pretreatment Processes to Improve Dewaterability." *Journal of Hazardous Materials* 98, no. 1-3 (2003): 51-67.

Ormeci, B., and P. A. Vesilind. "Effect of Extracellular Polymers on Freeze-Thaw Conditioning of Activated Sludge." *Water Science and Technology* 46, no. 10 (2002): 269-275.

Ormeci, Banu, and Aarne P. Vesilind. "Effect of Dissolved Organic Material and Cations on Freeze-Thaw Conditioning of Activated and Alum Sludges." *Water Research* 35, no. 18 (2001): 4299-4306.

Park, B., JH Ahn, J. Kim, and S. Hwang. "Use of Microwave Pretreatment for Enhanced Anaerobiosis of Secondary Sludge." *Water Science and Technology* 50, no. 9 (2004): 17-23.

Parker, Philip J., Anthony G. Collins, and John P. Dempsey. "Effects of Freezing Rate, Solids Content, and Curing Time on Freeze/Thaw Conditioning of Water Treatment Residuals." *Environmental Science & Technology* 32, no. 3 (1998): 383-387.

Pilli, Sridhar, Puspendu Bhunia, Song Yan, R. J. LeBlanc, R. D. Tyagi, and R. Y. Surampalli. "Ultrasonic Pretreatment of Sludge: A Review." *Ultrasonics Sonochemistry* 18, no. 1 (2011): 1-18.

Pinnekamp, J. "Effects of Thermal Pretreatment of Sewage Sludge on Anaerobic Digestion." *Water Science & Technology* 21, no. 4-5 (1989): 97-108.

Qiao, Wei, Wei Wang, CuiPing Zhu, and ZhongZhi Zhang. "Biogas Recovery from Microwave Heated Sludge by Anaerobic Digestion." *Science China Technological Sciences* 53, no. 1 (2010): 144-149.

Rafique, Rashad, Tjalfe Gorm Poulsen, Abdul-Sattar Nizami, and Zaki-ul-Zaman Asam. "Effect of Thermal, Chemical and Termo-Chemical Pre-treatments to Enhance Methane Production." *Energy* 35, no. 12 (2010): 4556-4561.

Sanin, Dilek F., William W. Clarkson, and Aarne P. Vesilind. *Sludge Engineering: The Treatment and Disposal of Wastewater Sludges.* Lancaster, PA: DEStech Publishings Inc., 2011.

Sastry, S. K., G. Q. Shen, and J. L. Blaisdell. "Effect of Ultrasonic Vibration on Fluid-to-Particle Convective Heat Transfer Coefficients." *Journal of Food Science* 54 (1989): 229-230.

Skiadas, I. V., H. N. Gavala, and B.K. Ahring. "Thermal Pre-Treatment of Primary and Secondary Sludge at 70oC Prior to Anaerobic Digestion." *Water Science & Technology* 52, no. 1-2 (2005): 161-166.

Spellman, Frank R. "Handbook of Water and Wastewater Treatment Plant Operations." Chap. 18. New York: Lewis Publishers, 2003.

Stabnikova, O., X.Y. Liu, and J.Y. Wang. "Digestion of Frozen/Thawed Food Waste in the Hybrid Anaerobic Solid-Liquid System." *Waste Management*, 2008: 1654-1659.

Stander, Leo, and Louis Theodore. *Environmental Regulatory Calculations Handbook.* Hoboken, NJ: John Wiley & Sons, Inc., 2008.

Subramanian, S. Bala, Song Yan, R.D. Tyagi, and R.Y. Surampalli. "Bioflocculants." In *Sustainable Sludge Management: Production of Value Added Products*, by R.D. Tyagi, Rao Y. Surampalli, Song Yan, Tian C. Zhang, C.M. Kao and B.N. Lohani, 146-167. USA: American Society of Civil Engineers, 2009.

Sun, Da-Wen, and Bing Li. "Microstructural Change of Potato Tissues Frozen by Ultrasound-Assisted Immersion Freezing." *Jounal of Food Engineering* 57, no. 4 (2003): 337-345.

Suslick, Kenneth S., and David J. Flannigan. "Inside a Collapsing Bubble: Sonoluminescence and the Conditions During Cavitation." *Annual Review of Physical Chemistry* 59 (2008): 659-683.

Tang, Bing, Linfeng Yu, Shaosong Huang, Jianzhong Luo, and Ying Zhuo. "Energy Efficiency of Pre-Treating Excess Sewage Sludge with Microwave Irradiation." *Bioresource Technology*, 2010: 5092-5097.

Thomashow, Michael F. "Role of Cold-Responsive Genes in Plant Freezing Tolerance." *Plant Physiology* 118 (1998): 1-7.

"Thunder Bay Water Pollution Control Plant - Annual Report." Thunder Bay, 2010.

Tiehm, A., K. Nickel, and U. Neis. "Use of Ultrasound to Accelerate the Anaerobic Digestion of Sewage Sludge." *Water Science and Technology* 36, no. 11 (1997): 121-128.

Ting, C. H., and D. J. Lee. "Production of Hydrogen and Methane from Wastewater Sludge using Anaerobic Fermentation." *Water Science & Technology* 50, no. 9 (2004): 223-228.

Toreci, Isil, Kevin J. Kennedy, and Ronald L. Droste. "Effect of High Temperature Microwave Thickened WAS Pretreatment on Distribution and Digestion of Soluble Organic Matter." *Environmental Engineering Science* 26, no. 5 (2009): 981-991.

Toreci, Isil, Kevin J. Kennedy, and Ronald L. Droste. "Effect of High-Temperature Microwave Irradiation on Municipal Thickened Waste Activated Sludge Solubilization." *Heat Transfer Engineering* 31, no. 9 (2010): 766-773.

Turovskiy, Izeail S., and P. K. Mathai. *Wastewater Sludge Processing.* New Jersey: John Wiley & Sons Inc., 2006.

Vesilind, Aarne P., and James C. Martel. "Freezing of Water and Wastewater Sludges." *Journal of Environmental Engineering* 116, no. 5 (1990): 854-862.

Vesilind, Aarne P., Seppo Wallinmaa, and James C. Martel. "Freeze-thaw sludge conditioning and double layer compression." *Canadian Journal of Civil Engineering* 18, no. 6 (1991): 1078-1083.

Vigueras-Carmona, S.E., F. Ramirez, A. Noyola, and O. Monroy. "Effect of Thermal Alkaline Pretreatment on the Anaerobic Digestion of Waste Activated Sludge." *Water Science and Technology* 64, no. 4 (2011): 953-959.

Vlyssides, A.G., and P.K. Karlis. "Thermal-Alkaline Solubilisation of Waste Activated Sludge as a Pretreatment Stage for Anaerobic Digestion." *Bioresource Technology* 91, no. 2 (2004): 201-206.

Wang, F., M. Ji, and S. Lu. "Influence of Ultrasonic Disintegration on the Dewaterability of Waste Activated Sludge." *Environmental Progress* 25, no. 3 (2006): 257-260.

Wang, Fe, Shan Lu, and Min Ji. "Components of Released Liquid from Ultrasonic Waste Actived Sludge Disintegration." *Ulrasonics Sonochemistry* 13, no. 4 (2006): 334-338.

Wang, Fen, Yong Wang, and Min Ji. "Mechanisms and Kinetics Mondels for Ultrasonic Waste Activated Sludge Disintegration." *Journal of Hazardous Materials* 123, no. 1-3 (2005): 145-150.

Wang, Lawrence K., Nazih K. Shammas, and Yung-Tse Hung, . *Biosolids Treatment Processes.* Totowa, NJ: Humana Press Inc., 2007.

Wang, Qunhui, Masaaki Kuninobu, Kohji Kakimoto, Hiroaki Ogawa, and Yasuhiko Kato. "Upgrading of Anaerobic Digestion of Waste Activated Sludge by Ultrasonic Pretreatment." *Bioresource Technology* 68, no. 3 (1999): 309-313.

Weemaes, Marjoleine P. J., and Willy H. Verstraete. "Evaluation of Current Wet Sludge Disintegration Techniques." *Journal of Chemical Technology & Biotechnology* 73, no. 2 (1998): 83-92.

Wilson, Christopher A., and John T. Novak. "Hydrolysis of Macromolecular Components of Primary and Secondary Wastewater Sludge by Thermal Hydrolytic Pretreatment." *Water Research* 43, no. 18 (2009): 4489-4498.

Wojciechowska, Eva. "Application of microwaves for sewage sludge conditioning." *Water Research* 39 (2005): 4749-4754.

Yu, Qiang, Hengyi Lei, Guangwei Yu, Zhaoxu Li, and Zhicheng Wu. "Influence of Microwave Irradiation on Sludge Dewaterability." *Chemical Engineering Journal*, 2009: 88-93.

Zhang, Panyue, Guangming Zhang, and Wei Wang. "Ultrasonic Treatment of Biological Sludge: Floc Disintegration, Cell Lysis and Inactivation." *Bioresource Technology* 98, no. 1 (2007): 207-210.

Zhang, X., T. Inada, and A. Tezuka. "Ultrasonic-Induced Nucleation of Ice in Water Containing Air Bubbles." *Ultrasonics Sonochemistry* 10, no. 2 (2003): 71-76.

Zheng, L. Y., and D.W. Sun. "Ultrasonic Acceleration of Food Freezing." In *Emerging Technologies for Food Processing*, by D. W. Sun. London: Elsevier Ltd., 2005.

Zheng, Liyun, and Da-Wen Sun. "Innovative Applications of Power Ultrasound During Food Freezing Processes: A Review." *Trends in Food Science & Technology* 17, no. 1 (2006): 16-23.

CHAPTER 3
MATERIALS AND METHODS

3.1 Sludge Samples

3.1.1 Thickened Waste Secondary Sludge

Thickened waste secondary sludge (TWSS) was obtained from the city of Thunder Bay's wastewater treatment plant (WWTP) located in Ontario, Canada. The WWTP uses biological aerated filters as secondary treatment. The thickened sludge samples were collected from dissolved air flotation tanks. The sludge samples obtained from the WWTP varied over the course of the year and a half the experiments were carried out. Characteristics of the TWSS can be found in Table 3.1.

Table 3.1: Characteristics of Thickened Waste Secondary Sludge

Value	Ph	Conductivity (µS/cm)	TS (mg/L)	TSS (mg/L)	tCOD (mg/L)	sCOD (mg/L)	SVI (mL/g)	CST (s)
Average	6.54	435	37,165	35,826	50,529	1,903	29.1	106

3.2 Apparatus

3.2.1 Freezing Apparatus

A temperature controlled room was used to freeze sludge samples (Climatic Testing Systems Inc., Pennsylvania, USA). The temperature of the walk-in freezer could be varied from -40.0°C to 20.0°C and had a control stability of ±0.5°C. It was large enough to hold all necessary sludge samples.

Figure 3.1: Walk-in freezer used to freeze sludge samples

3.2.2 Thermal Apparatus

A Precision Thelco Laboratory Oven (Model 70, Thermo Scientific, Massachusetts, USA) was used to heat TWSS. The maximum power output of the oven is 1200 Watts and it is able to reach a maximum temperature of 250°C. The oven is shown in Figure 3.2.

Figure 3.2: Precision Thelco laboratory oven

3.2.3 Microwave Apparatus

Figure 3.3 shows the Danby microwave-oven used (model DMW607W, Danby Products Ltd., Ontario, Canada) which operates at a frequency of 2450 MHz and has a maximum power output of 700 W.

Figure 3.3: Danby microwave-oven

3.2.4 Ultrasound Apparatus

Sludge samples requiring ultrasound as part of their treatment were sonicated using Sonics Vibra-Cell High Intensity Ultrasonic Processor model VC750 (Sonics & Materials Inc., Connecticut, USA). The processor, shown in Figure 3.4 operates at a frequency of 20 kHz and a maximum power output of 750 W. The sonication power applied to the TWSS can be varied by adjusting the amplitude to values between 0 - 100%. Two different sized probes (13 & 25mm) were used for different treatments.

Figure 3.4: Sonics Vibra-Cell ultrasonic processor

3.2.5 Freezing Bath

Progressive freezing was accomplished using Thomas Programmable Ultra-Low Refrigerating/Heating Circulator (model 9712G11C, Thomas Scientific, New Jersey, USA) shown in Figure 3.5. The freezing bath has a temperature range of -45°C to 200°C and a control stability of 0.01°C. The 13 L reservoir was filled with Motomaster Long-life Premixed Antifreeze which has a freezing point of -33.6°C and a boiling point of 108°C.

Figure 3.5: Thomas programmable freezing bath

3.3 Experimental Design

3.3.1 Conventional Freezing Treatment

Treatment effectiveness of conventional freeze-thaw was evaluated by considering two factors: temperature and freeze-thaw cycles. Two levels of temperature (-15 and -30°C) and three levels of freeze-thaw cycles (1, 3 & 5) were selected. The conventional freeze-thaw experiments were carried out as a 2x3 factorial fully crossed design, with a total of six treatment conditions. Each condition was run in duplicate in order to minimize experimental and random error as well as to improve the precision of the results.

3.3.2 Thermal Treatment

Based on previous studies (Hiraoka et al., 1985; Skiadas et al., 2005), a temperature of 103°C was selected for 2.5 hours of treatment time. These conditions were found to optimize energy requirements while maximizing the solubilisation of organic matter.

3.3.3 Microwave Treatment

The effect of treatment time was investigated for the microwave treatment with times of one and three minutes selected which correspond to temperatures of approximately 46 and 77°C respectively.

3.3.4 Ultrasonic Treatment

The effect of sound intensity and duration of sonication on treatment efficiency was investigated. Two levels of sound intensity (20 and 40% of the ultrasonic processor`s amplitude) and three levels of sonication time (2, 6 and 12 minutes) were tested. Like the conventional freezing experiments, these experiments were carried out as a 2x3 fully crossed factorial design.

3.3.5 Ultrasonic Freezing Treatment

When ultrasound and conventional freezing were tested as a combined treatment technique, two factors were examined: sonication time and sound intensity. These experiments were carried out as a 2x3 fully crossed factorial design with six treatment conditions.

3.3.6 Progressive Ultrasonic Freezing Treatment

Treatment effectiveness of power ultrasonic treatment was assessed in terms of two factors: pulse time and treatment time. Pulse varied between two and three seconds of sonication followed by 30 seconds of rest and total sonication time was either for 12 minutes or the entire duration of the treatment which was approximately 25 minutes.

3.4 Procedures

3.4.1 Sludge Preparation & Storage

Secondary sludge obtained from the WWTP was stored in a closed jar in the refrigerator at 4°C if not immediately treated in order to prevent degradation. All samples were diluted four times for the ease of subsequent testing within 24 hours of obtaining the TWSS. When not being treated or tested, samples were kept covered in the refrigerator.

3.4.2 Conventional Freezing Treatment

The walk-in freezer described in section 3.2.1 was used to freeze 600 mL samples of diluted TWSS in 1L polyethylene beakers. The samples were covered with plastic wrap and frozen for 24 hours at -15 or -30°C and then removed to thaw at room temperature for a further 24 hours. The process was then repeated for those samples requiring multiple freeze-thaw cycles.

3.4.3 Thermal Treatment

600 mL samples of TWSS was put into 1L glass beakers and covered with aluminum foil. They were placed in the oven undisturbed at 103 - 105°C for 2.5 hours which corresponds to a final temperature of approximately 75°C. Samples were allowed to cool at room temperature prior to analysis.

3.4.4 Microwave Treatment

The Danby microwave oven was used to treat 200 mL of TWSS in covered glass containers at maximum power for one and three minutes. This corresponds to a final temperature of approximately 46 and 77°C respectively. Like thermal samples, microwave samples were cooled at room temperature prior to analysis.

3.4.5 Ultrasound and Combined Ultrasonic-Freezing Treatments

600 mL of TWSS were sonicated in 1L glass beakers using the 25 mm probe for ultrasound and ultrasonic-freezing treatments. Approximately 120 glass beads (0.5-1 mm) were added to these samples during sonication in order to promote cell disruption. Sludge was sonicated at 20 and 40% amplitude with sonication times of 2, 6 and 12 minutes. The pulse duration during these sets of experiments was set at 30 seconds on and 59 seconds off.

After sonication, the samples undergoing ultrasonic-freezing treatment were then transferred to 1 L polyethylene beakers. The beakers were placed in the walk-in freezer at -15°C for 24 hours and then removed to thaw for a further 24 hours.

3.4.6 Progressive Ultrasonic Freezing Treatment

250 mL of TWSS was placed in a 500 mL stainless steel container insulated with foil faced bubble wrap. The insulated container was then placed on a platform attached to a pulley and slowly lowered into the freezing bath approximately 40mL at a time. The 13 mm (1/2") probe was used to sonicate the TWSS sample while simultaneously freezing the sample from the bottom up at -15°C in the freezing bath. The ultrasonic processor was set at 20% amplitude and the sonication time and pulse duration was varied. Three treatment conditions were tested: (1) 2 seconds on and 30 seconds off for 12 minutes, (2) 3 seconds on and 30 seconds off for 12 minutes and (3) 3 seconds on and 30 seconds off for the entire duration of the progressive freezing, which took on average 4.5 hours.

Once approximately 200 mL (or 80%) of the TWSS was frozen, the stainless steel bottle containing the sludge was removed from the freezing bath. The unfrozen portion (20%) of the sludge was removed from the container while the frozen portion (80%) was left at room temperature to thaw.

3.5 Sample Analysis

Both untreated and treated sludge samples were measured for pH and conductivity using a symphony electrode (model 14002-850 & 14002-802, VWR International, Ontario, Canada). A portion of the samples was used for total solids (TS), total chemical oxygen demand (tCOD), capillary suction time (CST), particle size (PS), ammonia-nitrogen (NH_4-N), total phosphorus (TP), biodegradability and gas production.

Sludge Volume Index (SVI) was calculated using the settled sludge volume of 1L of sludge at 30 minutes settling time. The total solids concentration (TS) was determined after a 12 hour drying period at 103°C. Total suspended solids concentration (TSS) was obtained by filtering sludge through a 2.7μm glass microfiber filter followed by 12 hours of drying at 103°C. The filtrate from the TSS samples was passed through a second 0.45 μm filter in order to obtain samples for soluble chemical oxygen demand (sCOD). Both tCOD and sCOD samples were added to mercury free reagent COD kits (Model K-7366, CHEMetrics, Virginia, USA), heated in a digester block (Model COD125, Thermo Fisher Scientific Inc., Massachusetts, USA) at 150°C and then measured using a spectrophotometer (Model DR 2800, Hach Company, Colorado, USA) at a wavelength of 620nm.

Capillary suction time (CST), ammonia and total phosphorus tests were completed at the laboratory at the city of Thunder Bay's WWTP. CST was measured with a Triton capillary suction timer (model 304, Triton Electronics, England, UK). Analysis for TS, TSS, COD, CST, SVI, NH_4-N, & TP were all determined according to procedures outlined in the standard methods (APHA, 2005). Particle size was measured using a Mastersizer 2000 (Malvern Canada, Quebec, Canada) able to measure the percent by volume of particles in the range of 0.2 – 2000 μm. It also provided information regarding the 10^{th}, 50^{th} and 90^{th} standard percentile diameters.

Samples were also passed through 0.45 μm filters in order to measure the soluble protein concentration following the modified Lowry protocol (Gerhardt, 1994). Bovine serum albumin (BSA) was used as the standard. Biodegradability and gas production were measured by placing 70 mL sludge samples with 10 mL of anaerobic seed sludge into light-free serum bottles and flushing the headspace with nitrogen to create an anaerobic environment. The bottles were then sealed with rubber stoppers to create a gas-tight environment. Samples were placed in an incubator at 37°C and 150 rpm. Gas samples were measured over the course of 20 days using a 10 mL syringe. After 20 days of digestion, the biodegradability of the samples was measured according to Pham et al. (2010) using the following equation:

$$Biodegradability = \left(1 - \frac{TS\ concentration\ after\ biodegradation}{TS\ concentration\ before\ biodegradation}\right) * 100$$

3.6 Data Analysis

All treated sample concentrations measured were normalized by using concentration ratios (C/C_o), where C_o is the concentration of the control and C is the concentration of the treated samples. Concentration ratios were used in order to allow the comparison of data obtained from different batches of sludge. Statistical analysis was performed using the software environment R (R Development Core Team, 2008).

Analyses of variance (ANOVAs) and the Tukey test were used to determine the factors that most affect sludge solubilisation, dewaterability and particle size for each type of pretreatment method. The significance level for all ANOVA tests was set at 95% ($\alpha = 0.05$). Pearson correlation coefficients were also calculated in R to determine the relationship between particle size, dewaterability and concentration of soluble COD.

CHAPTER 4
SOLUBILISATION AND VOLUME REDUCTION OF MUNICIPAL SLUDGE USING FREEZING AS A TREATMENT METHOD

In this chapter, freezing was used to treat municipal wastewater secondary sludge for the solubilisation of organic matter and dewaterability. The effects of freezing temperature and freeze-thaw cycles were investigated. A combination of ultrasound and freezing was also evaluated as a potential treatment technology, with an investigation into the effects of sonication time and amplitude. The solubilisation of sludge organic matter, evaluated by measuring soluble chemical oxygen demand (sCOD), showed significant improvements for both freezing methods compared to the controls. The maximum increase in sCOD was 6.5 times the control for conventional freezing at -30°C and 5 freeze-thaw cycles and 5.3 times the control for combined ultrasonic freezing at 20% amplitude and 12 minutes of sonication. The dewaterability of the freezing methods was also evaluated by measuring sludge volume index (SVI) and capillary suction time (CST). The two freezing methods showed significant improvements in dewaterability with CST ratios ranging from 0.12 – 0.21 for conventional freezing and 0.11- 0.21 for combined ultrasonic freezing. The two freezing treatments were then compared to other commonly studied pretreatment methods such as microwave, thermal and ultrasound and showed equivalent or better abilities to solubilise sludge organic matter and improve dewaterability. The results suggest that freezing could be a very effective pretreatment method as it would be able to simultaneously improve both anaerobic digestion efficiency as well as dewaterability.

4.1 Introduction

With secondary sludge production on the rise and increasingly more stringent regulations in regards to the disposal of municipal wastewater sludge, it is becoming more important to achieve on-site sludge reduction. Sludge reduction technologies aim to solubilise sludge solids and disintegrate bacterial cells in sludge (Foladori et al., 2010). This increases the amount of organic matter available for anaerobic digestion helping to overcome the rate-limiting step of hydrolysis (Appels et al., 2008; Turovskiy and Mathai 2006). When these reduction technologies are applied within the sludge treatment process, they are commonly referred to as pre-treatment methods. In summary, the pre-treatment of sludge not only results in improved sludge reduction but also enhanced digestion efficiency and increased production of useful biogas.

Various pre-treatment methods have been studied in recent years. These include, but are not limited to:

- Mechanical treatment such as Ultrasound (Muller et al., 2009; Zhang et al., 2007).
- Chemical treatment such as acidification, basification or ozonation (Muller et al., 1998; Ting and Lee, 2004).
- Microwave treatment (Eskicioglu et al., 2006; Kennedy et al., 2007).
- Thermal treatment (Bougrier et al., 2008; Neyens and Baeyens, 2003).
- Combinations of the above such as thermochemical (Vigueras-Carmona et al., 2011).

While these methods are able to solubilise sludge solids and increase digestion efficiency, they show little or no effect on improving sludge dewaterability (Neyens and Baeyens 2003; Wang et al., 2006; Yu et al., 2009). In some cases, sludge dewaterability has even been reported to decrease (Bougrier et al., 2008; Chang et al., 2011; Chu et al. 2001). Since the majority of a wastewater treatment plant's costs are derived from sludge management (Canales et al., 1994), introducing an additional step in the sludge treatment process may further increase costs. Therefore, it would be advantageous to find a pre-treatment method that could simultaneously improve digestion and dewaterability.

Freeze-thaw has typically been considered a conditioning treatment to improve sludge dewaterability (Hellstrom and Kvarnstrom, 1997; Saveyn et al., 2009). Full scale experiments have shown that freeze-thaw conditioning followed by drying is able to increase the dry matter content from 4-6% to 25-95% depending on the depth of the sludge layer (Hedstrom and Hanaeus, 1999). The mechanism that causes increased dewaterability is not well understood (Vesilind and Martel, 1990). However, both the physical and chemical properties of sludge seem to be affected by freezing treatment. While studying freeze-thaw conditioning, Ormeci and Vesilind (2001) found that the concentration of dissolved organic matter increases following freeze-thaw treatment. This was followed up by studies which confirmed that freeze-thaw treatment results in cell disruption and the release of intracellular matter (Chu et al., 1999; Gao, 2011; Hu et al., 2011; Montusiewicz et al., 2010). After freeze-thaw treatment, soluble chemical oxygen demand (sCOD) was reported to be 2 – 9 times greater than the control (Gao, 2011; Hu et al., 2011; Montusiewicz et al., 2010).

To date, only a handful of studies have studied the effectiveness of freezing as a pretreatment method for municipal sludge. Of those that have, the only factors that have been examined are freezing temperature, number of freeze-thaw cycles and curing time (Gao, 2011; Hu et al., 2011). The objectives of this study were to investigate the effectiveness of freezing treatments on both the solubilisation of sludge organic matter and the dewaterability of municipal secondary sludge. Two freezing treatments were examined; conventional freezing, and for the first time, combined ultrasonic-freezing. The effect of freezing temperature (-15 and -30°C) and freeze-thaw cycles (1, 3 and 5) was examined for conventional freezing and the effect of sonication time (2, 6 and 12 minutes) and sonication amplitude (20 and 40%) was examined for combined ultrasonic-freezing treatment. The results from the freezing experiments were compared to those of ultrasound, thermal and microwave, which are currently considered effective pre-treatment methods.

4.2 Materials and Methods

4.2.1 Sludge Samples

Thickened waste secondary sludge (TWSS) was obtained from Thunder Bay's wastewater treatment plant located in Thunder Bay, Ontario, Canada. Sludge was stored in the refrigerator in closed jars at 4°C if not immediately treated. Characteristics of the sludge samples obtained from the WWTP varied over the course of the experiments and can be found in Table 4.1. All samples were diluted four times prior to treatment for the ease of testing.

Table 4.1: Characteristics of TWSS

Value	pH	Conductivity (µS/cm)	TS (mg/L)	TSS (mg/L)	tCOD (mg/L)	sCOD (mg/L)	SVI (mL/g)	CST (s)
Average	6.55	438	37,245	35,826	50,583	1,903	27.4	102.7

4.2.2 Experimental Design

Freezing experiments were divided into two parts, conventional freezing and combined ultrasonic-freezing. In order to compare the effectiveness of freezing as a pretreatment method, another set of experiments was carried out using commonly studied pretreatment methods such as microwave, thermal and ultrasound. Duplicate or triplicate runs were carried out for each treatment in order to minimize experimental and random error as well as to improve the precision of the results.

For conventional freezing (freezing without ultrasound), the effect of freezing temperature and freeze-thaw cycles on solubilisation of sludge organic matter and dewaterability was examined. The freezing tests were carried out with two levels of temperature (-15 and -30°C) and three levels of freeze-thaw cycles (1, 3 and 5), a 2x3 factorial design (with a total of six treatment conditions) was carried out.

The combined ultrasonic-freezing experiments were carried out by sonicating the sludge samples first, followed by 24 hours of freezing at -15°C. These experiments were also a 2x3 fully crossed factorial design with two levels of sonication intensity (20 and 40% of the processor's maximum amplitude) and three levels of sonication time (2, 6 and 12 minutes).

Based on previous studies (Neyens and Baeyens, 2003), one temperature (103°C) and treatment duration (2.5 hours) was chosen for thermal treatment. For microwave treatment, two levels of treatment time (1 and 3 minutes) were selected. The ultrasound treatment examined the same levels of sonication time and amplitude as those selected for the combined ultrasonic-freezing treatment. Table 4.2 summarizes the pretreatment methods and the corresponding experimental conditions used in this study.

Table 4.2: Experimental condition of pretreatment methods investigated

Treatment	Experimental Condition
Conventional Freezing	Freezing at -15 and -30°C for 1, 3 and 5 cycles
Combined Ultrasonic-Freezing	Sonication at 20 and 40% amplitude for 2, 6 and 12 minutes followed by 24 hours of freezing at -15°C
Ultrasound	Sonication at 20 and 40% amplitude for 2, 6 and 12 minutes
Microwave	Microwave at 700 W for 1 and 3 minutes
Thermal	Heated at 103°C for 150 minutes

4.2.3 Experiments

4.2.3.1 Freezing Treatment

A temperature controlled environmental room (Climatic Testing Systems Inc., Pennsylvania, USA) was used to freeze sludge samples at -15 and -30°C. The temperature fluctuation of the freezer was ±0.5°C. The six 600 mL sludge samples were placed in 1 L polyethylene beakers and frozen in the cold room. After 24 hours of freezing, they were removed to thaw at room temperature for a further 24 hours. The process was then repeated for those samples requiring multiple freeze-thaw cycles.

4.2.3.2 Ultrasound and Combined Ultrasonic-Freezing Treatments

Sludge samples requiring ultrasound as part of their treatment were sonicated using a Sonics Vibra-Cell High Intensity Ultrasonic Processor model VC750 (Sonics & Materials Inc., Connecticut, USA). Sludge samples of 600 mL were sonicated using the 2.54 cm (1”) diameter probe at 20 and 40% power with sonication times of 2, 6 and 12 minutes. The combined ultrasonic-freezing samples were then frozen at -15°C in the freezer described in section 4.2.3.1 for 24 hours then thawed at room temperature for another 24 hours.

4.2.3.3 Thermal Treatment

A Precision Thelco Laboratory Oven (Model 70, Thermo Scientific, Massachusetts, USA) was used to heat 600 mL of secondary sludge at 103°C for 2.5 hours. This corresponded to a final temperature of approximately 75°C. The samples were then left to cool to approximately room temperature prior to analysis.

4.2.3.4 Microwave Treatment

A Danby microwave-oven (model DMW607W), was used to microwave 200 mL of sludge samples in sealed glass containers at maximum power for one and three minutes. This corresponds to a final temperature of 46 and 77 °C respectively.

4.2.4 Sample Analysis

pH, total solids (TS), capillary suction time (CST), sludge volume index (SVI), total suspended solids (TSS), total and soluble chemical oxygen demand (tCOD, sCOD) were measured for the sludge samples before and after treatment following the procedures outlined in the Standard Methods for Examination of Water and Wastewater (APHA, 2005).

The effect of pretreatment on the solubilisation of sludge organic matter was evaluated based on pH, COD solubilisation as well as disintegration of suspended solids.

TSS disintegration is defined as:

$$\text{TSS disintegration (\%)} = \left(\frac{\text{TSS}_o - TSS}{\text{TSS}_o}\right) * 100$$

Where TSS_o is the suspended solids concentration of the untreated sludge and TSS is the suspended solids concentration of the treated sludge.

Two measurements were used to assess the dewaterability of the secondary sludge: sludge volume index (SVI) and capillary suction time (CST). SVI, measured using a 1L graduated cylinder, was used as a measure of settleability and capillary suction time (CST) as a measure of filterability.

4.2.5 Data Analysis

Data collected for the treated samples were normalized to that of the controls using ratios (C/C_o), where C_o is the value of the untreated samples and C is the treated value. Concentration ratios were used to allow the comparison of data obtained from different batches of sludge. The ratios were compared using analyses of variance (ANOVAs) in order to determine if any statistical difference occurred between different treatments. The Tukey test was used as a post-hoc test in order to determine which means were statistically different from each other. The significance level for these tests were set at 95% ($\alpha = 0.05$). Statistical analysis was completed using the computing environment R (R Development Core Team, 2008).

4.3 Results and Discussion

4.3.1 Solubilisation of Sludge Organic Matter

The effectiveness of the various pretreatment methods to solubilise sludge organic matter were measured by examining the pH, soluble and total COD concentrations as well as TSS concentrations before and after treatments. The results from the various pretreatment methods are summarized below.

4.3.1.1 Conventional Freezing Treatment

Conventional freezing was able to significantly disintegrate suspended solids to a ratio of 0.886 which corresponds to a TSS disintegration of approximately 11.4%. The solubilisation of COD was 4.7 times greater than the control, which was also found to be statistically greater than the control. On the other hand, the difference between the pH of conventionally frozen sludge and the control remained insignificant ($p > 0.05$).

A) Effect of Freezing Temperature:

When the freezing temperature decreased from -15 to -30°C, the disintegration of suspended solids increased slightly from approximately 10.6 to 12.1%. The soluble COD concentration increased from 4.2 to 5.2 times the control and the pH also showed a slight increase (Table

4.3). The changes for all three of these parameters were found to be statistically insignificant ($p > 0.05$). This same trend was observed for the solubilisation of COD by Gao (2011) when the temperature was lowered from -10 to -18°C. Another study examining the effect of freezing temperature on the viability of microbial cells, found that there was an insignificant difference when freezing temperatures between -7 to -30°C were used (Gao et al., 2009). These same results would therefore be expected for solubilisation of organic matter since it also requires the disruption of cells in order to release organic matter.

Table 4.3: Effect of freezing temperature on TSS, sCOD and pH ratios (C/C_o) for conventional freezing treatment. (C = concentration/value of treated samples, and C_o = concentration/value of the control samples).

	-15°C	-30°C
TSS	0.894 ± 0.019	0.878 ± 0.023
sCOD	4.247 ± 0.232	5.156 ± 0.408
pH	0.973 ± 0.004	0.990 ± 0.011

B) Effect of Freeze-Thaw Cycles:

In terms of freeze-thaw cycles, changes in pH remained insignificant while increasing the number of freeze-thaw cycles from one to three significantly improved the TSS ratio, decreasing it from 0.979 to 0.870. With five freeze-thaw cycles, the TSS ratio decreased further to 0.809, however this decrease was not statistically significant ($p > 0.05$) from that of the three freeze-thaw cycles, indicating a limited effect of freeze-thaw cycles. The effect of freeze-thaw cycles on sCOD showed similar results to those of TSS. Increasing the freeze-thaw cycles from one to three drastically improved the solubilisation of COD by approximately 1.5 times after which point it remained fairly stable. The results are displayed graphically in Figure 4.1.

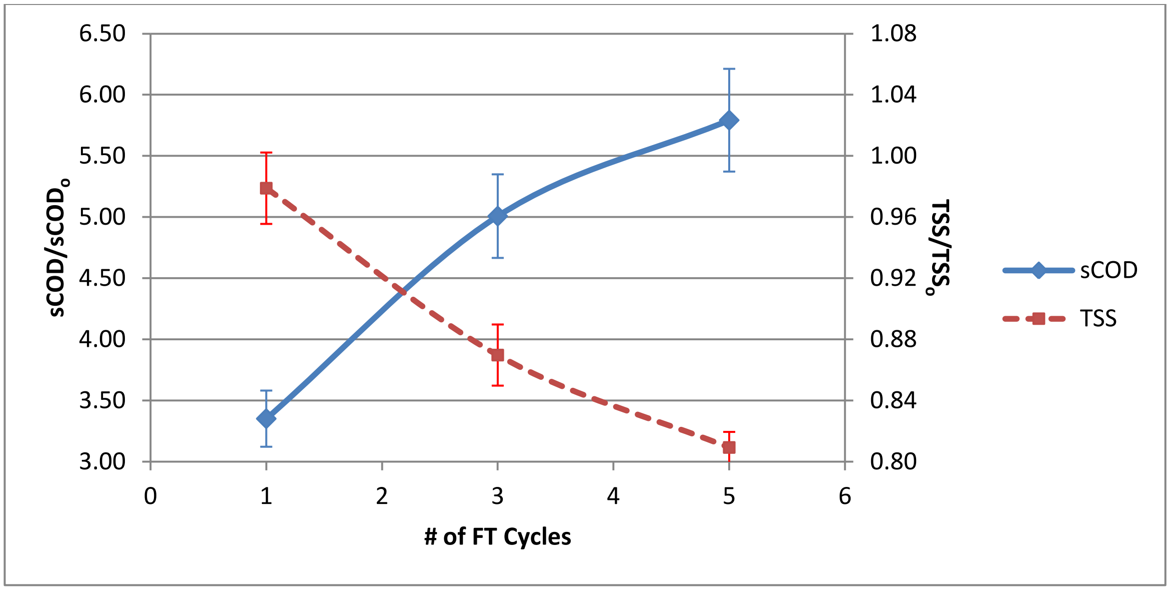

Figure 4.1: Effect of freeze-thaw cycles on soluble COD and TSS ratios for conventional freezing treatment. (sCOD = soluble COD concentration of the treated sample, sCOD$_o$ = soluble COD concentration of the control, TSS = total suspended solids concentration of the treated samples, and TSS$_o$ = total suspended solids concentration of the control)

Freezing of micro-organisms causes both intracellular and intercellular water to freeze (Gao, 2011; Ormeci and Vesilind, 2001; Thomashow, 1998). This results in cell disruption,, either through dehydration, or through the increased pressure that is applied to the cell walls by the ice crystals (Hu et al., 2011; Ormeci and Vesilind, 2001; Stabnikova et al., 2008). It is reasonable that multiple freeze-thaw cycles will result in an increasing number of cells that are ruptured as the more times the cells expand and contract, the more weak they become and the more likely the cell wall will be to burst. Studies investigating the effect of freeze-thaw cycles on the inactivation of micro-organisms found similar results; the greater the number of freeze-thaw cycles, the more cell destruction was noted (Gao et al., 2006; Gao et al., 2009). However, after a certain number of repeated freeze-thaw cycles, three in this study, the effect of freeze-thaw cycles on the solubilisation of sludge organic matter became less obvious.

C) Combined Effect of Temperature and Freeze-Thaw Cycles:

No significant combined effects of freezing temperature and freeze thaw cycles were found for pH, disintegration of suspended solids or solubilisation of COD, as shown in Table 4.4.

Table 4.4: Combined effect of freezing temperature and freeze-thaw cycles on sCOD, TSS and pH Ratios (C/C_o) following conventional freezing treatment, where C = concentration/value of treated samples and C_o = concentration/value of control samples.

Temperature (°C)	1 cycle			3 cycles			5 cycles		
	sCOD	TSS	pH	sCOD	TSS	pH	sCOD	TSS	pH
-15	3.199 ± 0.296	0.967 ± 0.033	0.981 ± 0.006	4.441 ± 0.189	0.883 ± 0.030	0.961 ± 0.010	5.125 ± 0.319	0.831 ± 0.012	0.974 ± 0.006
-30	3.505 ± 0.363	1.000 ± 0.040	0.979 ± 0.010	5.504 ± 0.580	0.864 ± 0.033	0.981 ± 0.016	6.459 ± 0.726	0.787 ± 0.013	1.008 ± 0.029

4.3.1.2 Combined Ultrasonic-Freezing Treatment

Combined ultrasonic-freezing treatment did not significantly affect the pH compared to the control, but it was able to significantly solubilise COD and disintegrate suspended solids. The soluble COD values of the treated sludge were 4.7 times greater than the control and the disintegration of suspended solids reached an average of 10.5%.

A) Effect of Sonication Time:

When secondary sludge samples were sonicated prior to freezing, an increase in sonication time from 2 to 12 minutes resulted in an increase in disintegration of suspended solids from 9.2 to 13.3%. The soluble COD ratio also increased from 4.5 to 4.9 times that of the control. These increases with sonication time, reported in Table 4.5, were found to be negligible ($p > 0.05$). The pH also remained constant throughout the increase in sonication time.

Table 4.5: Main effect of sonication time on TSS, sCOD and pH ratios (C/C_o) for combined ultrasonic-freezing treatment. (C = concentration/value of treated samples, and C_o = concentration/value of the control samples).

	2 minutes	6 minutes	12 minutes
TSS	0.908 ± 0.019	0.909 ± 0.020	0.867 ± 0.019
sCOD	4.540 ± 0.349	4.618 ± 0.435	4.928 ± 0.524
pH	1.021 ± 0.009	1.022 ± 0.017	1.023 ± 0.011

B) Effect of Sonication Amplitude:

Like sonication time, the amplitude of sonication was also not an important factor in the solubilisation of sludge organic matter for combined ultrasonic freezing. The pH ratios at 20 and 40% were 1.03 and 1.01 respectively. There was a small improvement in TSS ratio from 0.90 to 0.88 when the amplitude increased to 40%. On the other hand, the increase in sonication intensity resulted in a lowering of the soluble COD ratio from 5.0 to 4.4 times that of the control (Figure 4.2). These results are inconsistent with studies that examined ultrasound alone in which both sonication time and ultrasonic intensity were found to significantly affect solubilisation of sludge organic matter (Wang et al., 2005; Zhang et al., 2007). This could possibly indicate that freezing after sonication alters the effects of sonication.

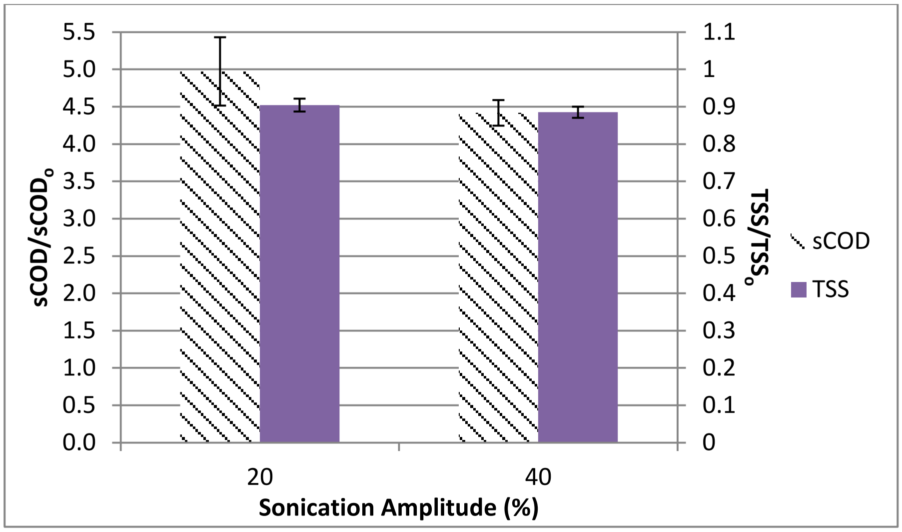

Figure 4.2: Main effect of sonication amplitude on soluble COD and TSS ratios for combined ultrasonic-freezing treatment. (sCOD = soluble COD concentration of treated sample, $sCOD_o$ = soluble COD concentration of control, TSS = total suspended solids concentration of treated samples, and TSS_o = total suspended solids concentration of control)

C) Combined Effect of Sonication Time and Amplitude:

There were no significant combined effects found for sonication time and amplitude in terms of solubilisation of COD, disintegration of suspended solids and pH when TWSS was treated with combined ultrasonic-freezing, as can be seen from Figure 4.3.

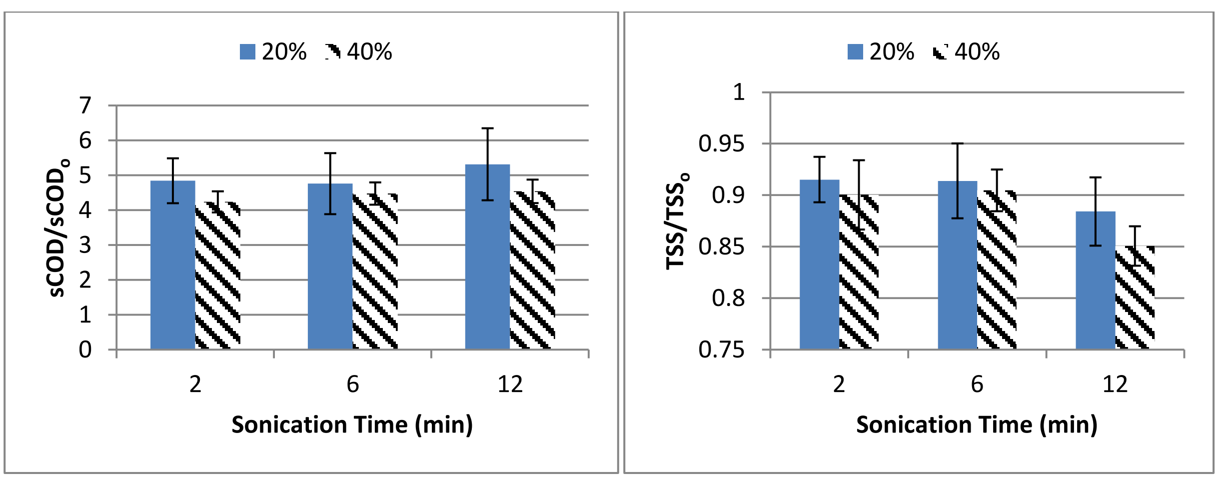

Figure 4.3: Combined effect of sonication time and amplitude on sCOD and TSS ratios for combined ultrasonic-freezing. sCOD = concentration of soluble COD in the treated samples, $sCOD_o$ = soluble COD concentration of control sample, TSS = total suspended solids concentration of the treated samples, TSS_o = total suspended solids concentration of the control.

4.3.1.3 Thermal Treatment

When sludge samples were heated for 2.5 hours at 103°C they reached an average final temperature of 75°C. The pH dropped slightly to an average value of 0.98 times that of the control and the disintegration of suspended solids was on average 10.5%. However, neither of these values was found to be statistically different than the control. The soluble COD concentration of the thermally pretreated sludge was significantly greater than the control at a value almost six times greater than the control. These results are comparable to those of Li and Noike (1992) who measured an increase in soluble COD 5.5 times greater than the control when WAS was thermally pretreated at 120°C for 30 minutes. The results confirm that thermal pre-treatment in the range of 60 - 180°C is able to break cell walls and release intercellular and extracellular matter (Carrere et al., 2008; Neyens and Baeyens, 2003).

4.3.1.4 Microwave Treatment

Overall, microwave was not able to significantly improve the disintegration of suspended solids or the solubilisation of COD. TSS disintegration was 1.06 times greater than the control and sCOD concentrations were on average 2.03 times the control. The pH also remained very close to a ratio of 1.0.

A) Effect of Treatment Time:

When microwaving time increased from one to three minutes, this corresponded to a change in temperature from 46 to 77°C. There have been different findings regarding the change in pH which occurs with increased microwaving time (Toreci et al., 2010). This study found that as treatment time increased from one to three minutes, the pH significantly increased from 0.99 to 1.05 times that of the control. This could possible indicate that some organic acids in the sludge had been reduced. The TSS ratios were 1.03 and 1.08 times that of the control for one and three minutes of microwaving time respectively, showing a slight but insignificant increase following increased microwave treatment. The ratio greater than one and the increase with treatment time is likely due to the evaporation of water that occurred during the treatment process.

The soluble COD concentration was approximately twice that of the control for both treatment conditions. The box-plot, shown in Figure 4.4, shows that the soluble COD ratio increased slightly from a median value of 1.86 to 2.21 times the control with increased treatment time, but the difference was found to be statistically insignificant ($p > 0.05$). Eskilogu et al. (2006) found that COD solubilisation increased from 6% for the control to 15% for WAS microwaved to 92°C. Kennedy et al. (2007) observed an increase in sCOD/tCOD ratio from 1.4% for the control to 6.4% after microwave treatment to 85°C; these values are higher than the findings of this study. It has been suggested that significant improvements in solubilisation do not occur until sludge is brought to temperatures above the boiling point (Toreci et al., 2010) indicating that in order to see better solubilisation of organic matter, a longer treatment time might be required.

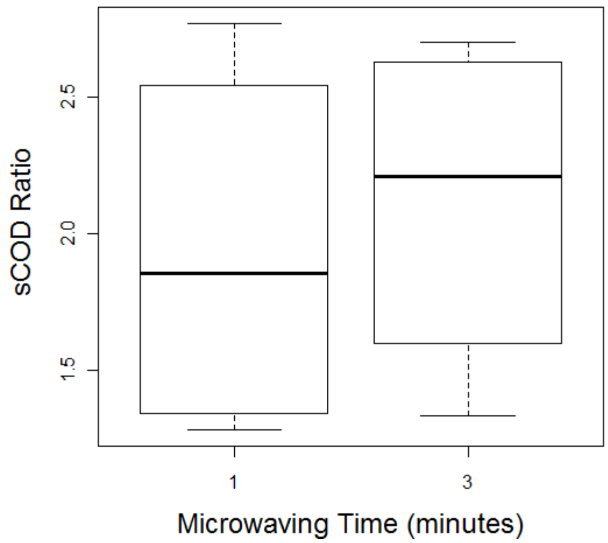

Figure 4.4: Effect of microwave treatment time on sCOD Ratio (sCOD/sCOD$_o$) for microwave treatment. sCOD = soluble COD concentration of the treated samples, sCOD$_o$ = soluble COD concentration of the control samples.

4.3.1.5 Ultrasonic Treatment

Ultrasonic treatment did not significantly affect the pH of the sludge samples compared to the control. It was however, able to significantly increase the disintegration of suspended solids to 9.9% and increase the solubilisation of COD to 2.5 times that of the control.

A) Effect of Sonication Time:

The pH ratio of the ultrasonically treated sludge remained between 1.00 – 1.03 for treatment times of 2, 6 and 12 minutes. However, as sonication time increased from two to twelve minutes, the disintegration of suspended solids increased from 2.9% to 13.7%, representing a significant increase in disintegration. Additionally, the solubilisation of COD more than doubled from 1.6 times that of the control at two minutes to 3.5 times the control for 12 minutes of sonication. It can be seen from Figure 4.5, that the increase in sCOD ratio follows a linear trend for the sonication times investigated. Several other studies have also confirmed that increasing sonication time up to 20 -30 minutes will results in a steady increase in soluble COD (Wang et al., 2006; Zhang et al., 2007). Increased solubilisation of organic matter is expected, as the longer sludge is treated, the more cavitation bubbles will be formed and a greater number of adjacent cell walls will be disrupted (Khanal et al., 2007).

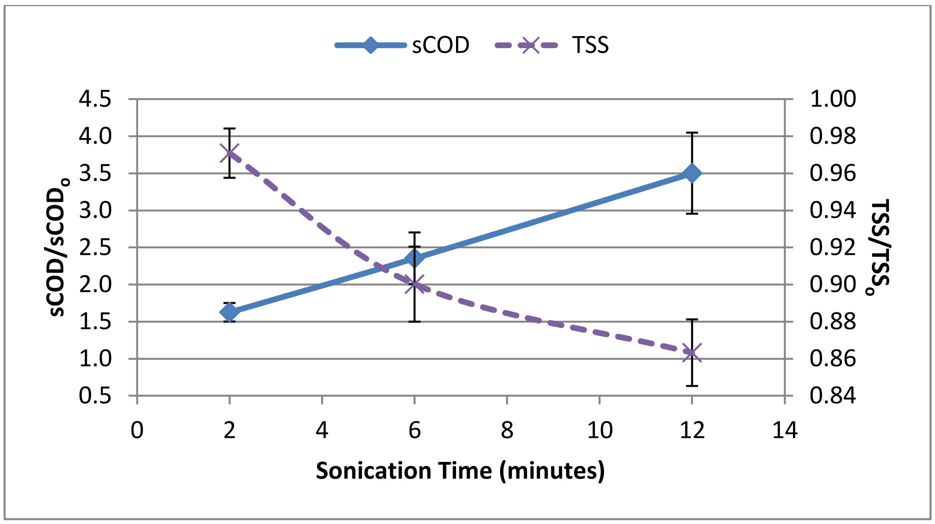

Figure 4.5: Effect of sonication time on soluble COD and TSS ratios for ultrasound treatment. (sCOD = soluble COD concentration of treated samples, $sCOD_o$ = soluble COD concentration of the control, TSS = total suspended solids concentration of treated samples, and TSS_o = total suspended solids concentration of the control)

B) Effect of Sonication Amplitude:

As with treatment time, the pH did not change when the amplitude was increased from 20 to 40%. Doubling the intensity however, more than doubled the disintegration of suspended solids from approximately 5.4% to 13.5%. The soluble COD ratio also significantly increased from 1.8 to 3.1 times the control with the increase in amplitude (Table 4.6). The increase in soluble COD was comparable with studies by Zhang et al. (2007) who also found that an increase from 0.2 W/mL to 0.5 W/mL caused an increase in soluble COD from 1500 mg/L to 3100 mg/L respectively.

Table 4.6: Main effect of sonication amplitude on TSS, sCOD and pH ratios (C/C_o) for ultrasound treatment. (C = concentration/value of treated samples, and C_o = concentration/value of the control samples).

	20%	40%
pH	1.02 ± 0.01	1.02 ± 0.01
TSS	0.946 ± 0.014	0.865 ± 0.015
sCOD	1.84 ± 0.18	3.15 ± 0.42

One of the major disintegration mechanisms during ultrasonic treatment is mechanical shear forces. The increase in solubilisation of organic matter that occurred with the increase in amplitude can be attributed to the greater shear forces caused by the increase in ultrasonic energy (Pilli et al., 2011).

C) Combined Effect of Sonication Time and Amplitude:

There were no combined effects of sonication time and amplitude for pH, disintegration of suspended solids or solubilisation of organic matter for secondary sludge treated with ultrasound (Table 4.7).

Table 4.7: Combined effect of sonication time and amplitude on sCOD, TSS and pH Ratios (C/Co) for ultrasound treatment, where C = concentration/value of treated samples and Co = concentration/value of control samples.

Amplitude (%)	2 minutes			6 minutes			12 minutes		
	sCOD	**TSS**	**pH**	**sCOD**	**TSS**	**pH**	**sCOD**	**TSS**	**pH**
20	1.476 ± 0.116	1.004 ± 0.013	1.029 ± 0.009	1.561 ± 0.255	0.937 ± 0.026	1.017 ± 0.013	2.472 ± 0.365	0.898 ± 0.019	1.008 ± 0.011
40	1.774 ± 0.215	0.927 ± 0.009	1.027 ± 0.013	3.146 ± 0.466	0.851 ± 0.019	1.026 ± 0.007	4.530 ± 0.870	0.816 ± 0.022	1.000 ± 0.008

4.3.2 Dewaterability of Secondary Sludge

The treatment methods examined for their effectiveness solubilising sludge organic matter were also assessed for their effectiveness improving sludge dewaterability. Sludge volume index (SVI) was used as a measure of settleability and capillary suction time (CST) was used to assess filterability. The results from the pretreatment methods are summarized below.

4.3.2.1 Conventional Freezing Treatment

Conventional freezing treatment showed substantial improvements in dewaterability compared to the control. The average SVI ratio following freeze-thaw was 0.154 and CST values fell to 0.160 times the control. These results are slightly lower than those of Gao (2011) whose CST ratios ranged from 0.17-0.34. Freezing has been studied extensively as a conditioning method, and it is essentially agreed that freezing results in a more compact sludge floc with less moisture attached to the flocs (Hong et al., 1995; Hung et al., 1996b; Lee and Hsu, 1994).

A) Effect of Freezing Temperature:

In terms of dewaterability, the results of this study showed a slight, insignificant decrease in both filterability and settleability when sludge was frozen at -30°C compared to -15°C. The SVI and CST ratios increased from 0.15 to 0.16 and 0.14 to 0.18 respectively as the temperature dropped from -15 to -30°C (Figure 4.6). These findings are consistent with several studies using freeze-thaw as a conditioning method (Hung et al., 1996; Lee & Hsu, 1994; Vesilind & Martel, 1990). It is speculated that lower freezing temperatures result in higher freezing rates which prevent gross migration – a phenomena that seems to be necessary for improvement in dewaterability (Chu et al., 1997; Vesilind et al., 1991).

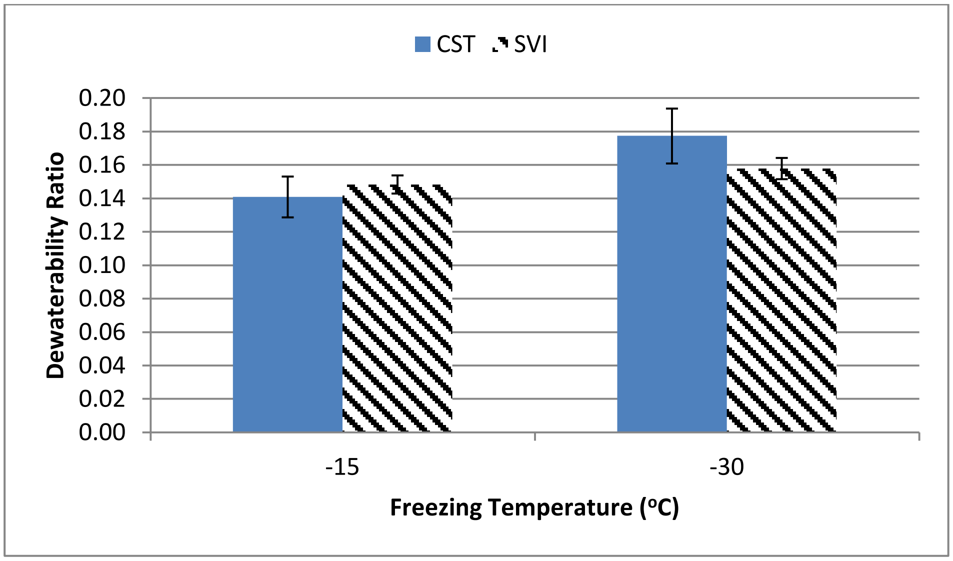

Figure 4.6: Effect of freezing temperature on SVI and CST ratios (C/C_o) for conventional freezing treatment. (C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.)

B) Effect of Freeze-Thaw Cycles:

Increasing the number of freeze thaw cycles from one to three to five resulted in an insignificant improvement in dewaterability in terms of filterability and settleability. The SVI ratio decreased from 0.16 to 0.15 as the number of cycles increased from one to five, whereas the CST ratio decreased from 0.19 to 0.14 for the same increase in cycles (Table 4.8). These values are also consistent with those of Gao (2011) whose CST ratios ranged from 0.17 – 0.34 for various numbers of freeze-thaw cycles.

Table 4.8: Effect of freeze-thaw cycles on SVI and CST ratios (C/C_o) for conventional freezing treatment. (C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.)

	1 cycle	3 cycles	5 cycles
SVI	0.163 ± 0.005	0.154 ± 0.009	0.145 ± 0.007
CST	0.187 ± 0.025	0.152 ± 0.014	0.139 ± 0.009

C) Combined Effect of Freezing Temperature and Freeze-Thaw Cycles:

There were no combined effects of freezing temperature and freeze-thaw cycles on the dewaterability ratios for sludge exposed to conventional freezing treatment (Figure 4.7).

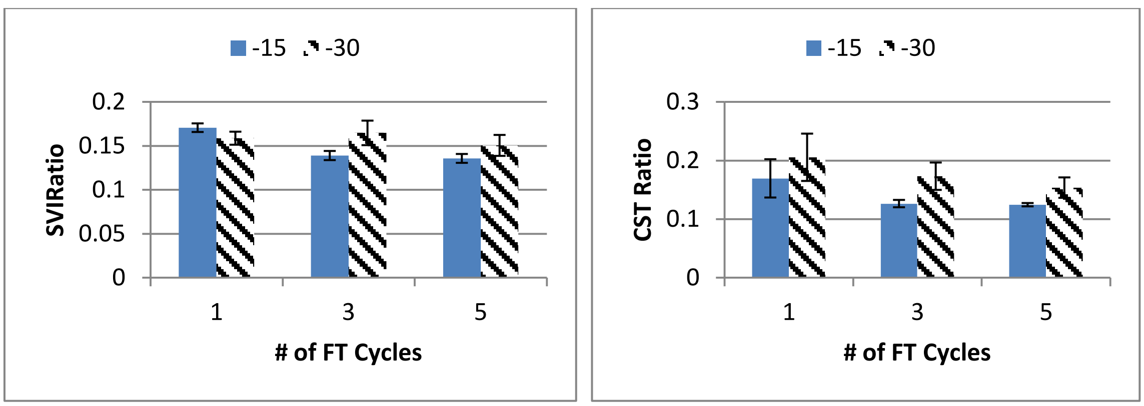

Figure 4.7: Combined effect of freezing temperature and freeze thaw cycles on SVI and CST ratios (C/C_o) following freezing treatment. (C = SVI or CST value of the treated samples, C_o = CVI or CST value of the control sample)

4.3.2.2 Combined Ultrasonic-Freezing Treatment

Both the filterability and settleability of the TWSS were significantly improved with ratios more than five times smaller than the control. The average SVI ratio was 0.192 and the CST ratio was found to be 0.166. The improvement in dewaterability is most likely due to an improvement in floc structure caused by the freezing and thawing of the sludge (Hong et al., 1995).

A) Effect of Sonication Time:

There was no significant difference in CST or SVI ratios for combined ultrasonic-freezing as the sonication time increased. The CST ratios varied between 0.15 – 0.19 whereas the SVI ratio remained stable at 0.19 as sonication time increased from 2 to 12 minutes. Results are shown in Table 4.9. These results were somewhat unexpected as an increase in sonication time has often been linked with a decline in dewaterability (Chu et al., 2001). It is likely that the freezing that occurs after the sonication negates the effect the increased sonication time would typically have on dewaterability.

Table 4.9: Main effect of sonication time on SVI and CST ratios (C/C_o) for combined ultrasonic-freezing treatment. (C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.)

	2 minutes	**6 minutes**	**12 minutes**
SVI	0.192 ± 0.008	0.191 ± 0.011	0.193 ± 0.011
CST	0.161 ± 0.017	0.185 ± 0.027	0.152 ± 0.018

B) Effect of Sonication Amplitude:

When sludge was frozen after sonication, an increase in amplitude resulted in a significant improvement in dewaterability. The CST and SVI ratios improved by 46 and 17% respectively (Figure 4.8). It is possible that after the sludge is frozen, the water that is trapped in the smaller sludge flocs gets released through gross migration (Vesilind and Martel, 1990).

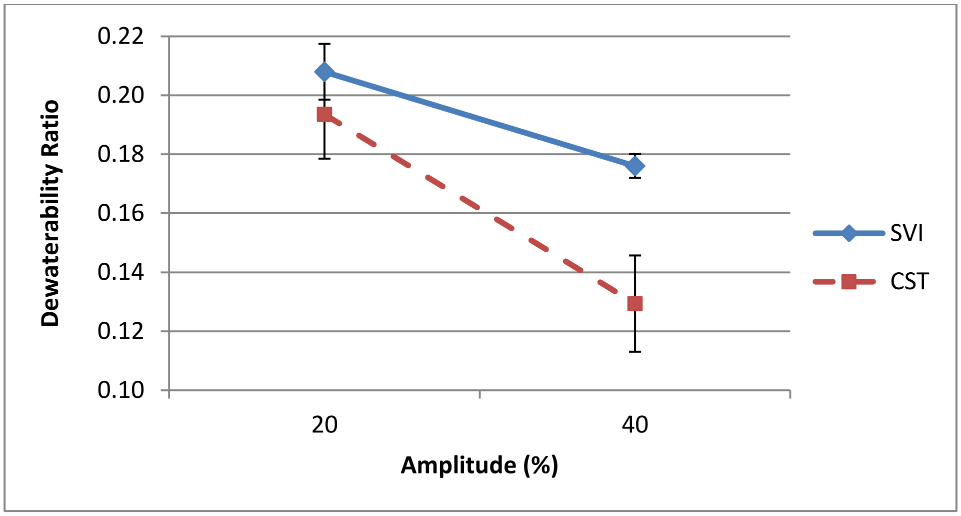

Figure 4.8: Effect of sonication amplitude on SVI and CST ratios (C/C_o) for combined ultrasonic-freezing treatment. (C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.)

C) Combined Effect of Sonication Time and Intensity on Dewaterability:

There were no significant effects of sonication time and intensity on the dewaterability ratios for secondary sludge treated with combined ultrasonic-freezing (Table 4.10).

Table 4.10: Combined effect of sonication time & amplitude on SVI and CST ratios (C/C_o) following combined ultrasonic-freezing treatment.
(C = SVI or CST value of the treated samples, C_o = CVI or CST value of control samples)

Amplitude (%)	2 minutes		6 minutes		12 minutes	
	SVI	CST	SVI	CST	SVI	CST
20	0.202 ± 0.017	0.190 ± 0.022	0.213 ± 0.018	0.211 ± 0.033	0.208 ± 0.018	0.179 ± 0.024
40	0.182 ± 0.002	0.122 ± 0.021	0.168 ± 0.003	0.149 ± 0.043	0.177 ± 0.011	0.117 ± 0.018

4.3.2.3 Thermal Treatment

The filterability, as measured by CST, took approximately twice as long after thermal pretreatment as the control. This has been suggested to be caused by the increased number of small particles following thermal treatment (Bougrier et al., 2008; Neyens & Baeyens, 2003). The results of this study are in agreement with other studies that have found that increases in filterability can only be found at temperatures below 60°C or those above 130°C (Bougrier et al., 2008; Lin & Shien, 2001).

Conversely, the settleability showed a slight improvement over the control (Figure 4.9). Bougrier et al. (2008) suggested that a modification of sludge structure accounted for the improvement in sludge settleability. When extracellular polymers are solubilised during thermal pretreatment, a large quantity of bound water is released resulting in improved settleability (Bougrier et al., 2008).

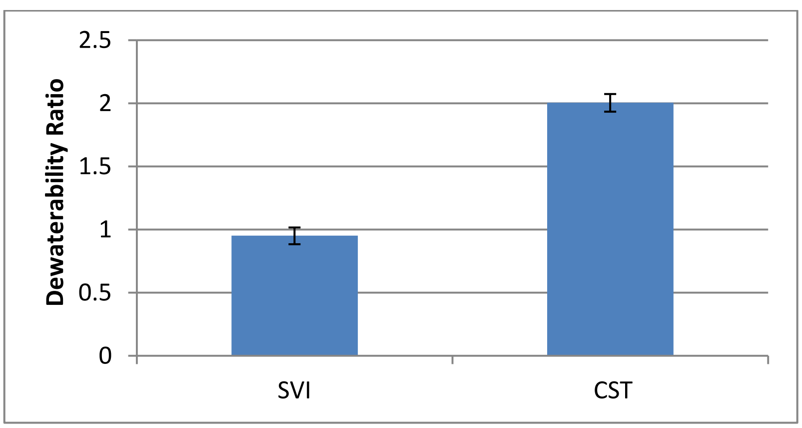

Figure 4.9: Effect of thermal pretreatment on SVI and CST ratios (C/C_o) where C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.

4.3.2.4 Microwave Treatment

As with thermal treatment, the dewaterability of the microwave treated sludge showed mixed results compared to the control in terms of settleability and filterability. The settleability showed a small insignificant improvement with an average SVI ratio of 0.93 and the filterability was significantly worsened to an average CST ratio of 1.75 times the control following microwave treatment.

A) Effect of Treatment Time:

An increase in treatment time from one to three minutes did not significantly affect SVI or CST ratios. The SVI ratio showed a small decrease from 0.97 to 0.90 with the increase in treatment time while the CST increased from 1.49 to 2.01 (Figure 4.10). These results correspond well with those of Chang et al. (2011) who reported increases in CST over three times that of the control for a treatment time of two minutes for microwave treatment of WAS. Like with thermal pretreatment, this increase in filterability is thought to be due to the solubilisation of EPS that occurs with heating secondary sludge (Bougrier et al. 2008). The results of this study did conflict with many studies that have reported an initial improvement in filterability for small contact times followed by increasing CST values as treatment time increases (Chang et al., 2011; Kennedy et al., 2007; Wojciechowska, 2005). It is possible that in order to see the improvement in filterability, smaller contact times than the ones investigated here are necessary.

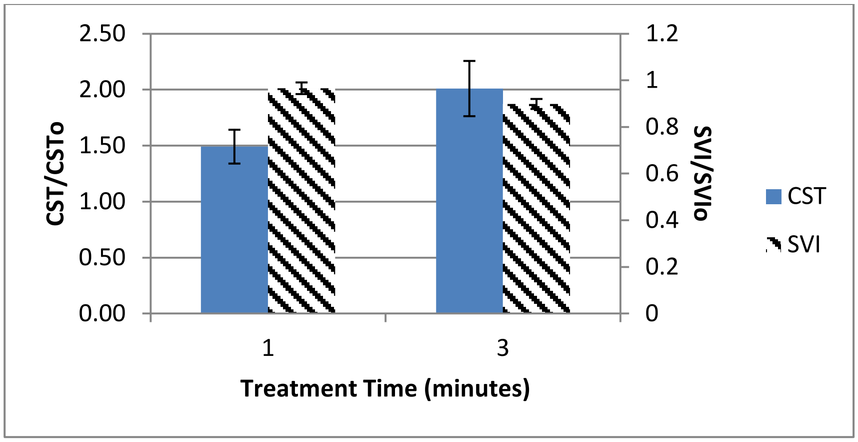

Figure 4.10: Effect of treatment time on SVI and CST ratios (C/C_o) for microwave treatment. (C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.)

4.3.2.5 Ultrasonic Treatment

Unfortunately, the significant improvement in solubilisation of sludge organic matter following ultrasonic treatment was accompanied by an equally significant decline in dewaterability. The SVI and CST ratios were 1.15 and 1.92 times the control respectively. These results are in agreement with several other studies that have studied the effect of ultrasonic treatment on sludge dewaterability (Chu et al., 2001; Muller et al., 2009; Wang et al., 2006).

A) Effect of Sonication Time:

The settleability was very close to that of the control for two minutes of sonication time, but by 12 minutes the average value for the SVI significantly increased to 1.22 times the control. The filterability followed the same trend with CST ratio values increasing significantly, almost 44%, from 1.59 to 2.27 times the control as sonication time increased from 2-12 minutes (Table 4.11). Chu et al. (2001) attributed the decrease in dewaterability to the increase in small particles following sonication which now provide a larger surface area for retaining water.

Table 4.11: Effect of sonication time on SVI and CST ratios (C/C_o) for ultrasound treatment. (C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.)

	2 minutes	6 minutes	12 minutes
SVI	1.057 ± 0.017	1.163 ± 0.027	1.217 ± 0.028
CST	1.585 ± 0.074	1.927 ± 0.134	2.273 ± 0.192

B) Effect of Sonication Amplitude:

An increase in sonication amplitude resulted in a greater degree of disintegration of sludge flocs which in turn resulted in a decrease in dewaterability. The decrease in settleability was significant with an increase in SVI ratio from 1.10 to 1.19 times the control. The filterability showed a very small, insignificant increase in CST ratio from 1.91 and 1.93 as the amplitude increased from 20 to 40% (Figure 4.11). These results differ from those of Chu et al. (2001) who found a significant decline in filterability as sonication intensity increased. This is likely due to the fact that their intensity range was lower than the range in this study and ultrasound has very little effect on dewaterability at low intensities (Chu et al., 2001; Feng et al., 2009).

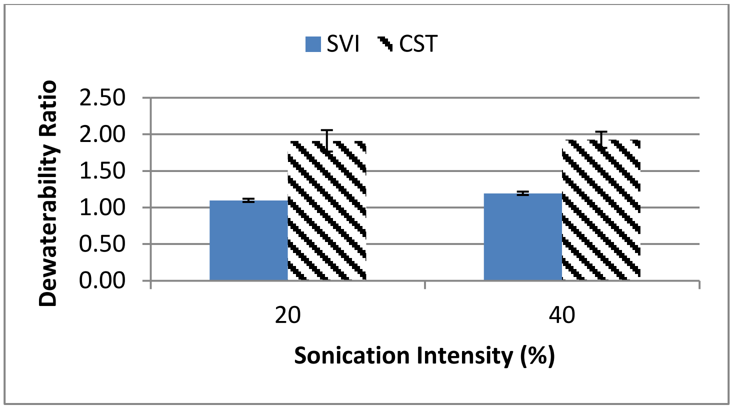

Figure 4.11: Effect of sonication amplitude on SVI and CST ratios (C/C_o) for ultrasound treatment. (C = CST or SVI value of the treated sample, C_o = CST or SVI value of the control sample.)

C) Combined Effect of Sonication Time and Amplitude:

There were no significant combined effects of sonication time and amplitude for dewaterability ratios of secondary sludge treated with ultrasound (Figure 4.12).

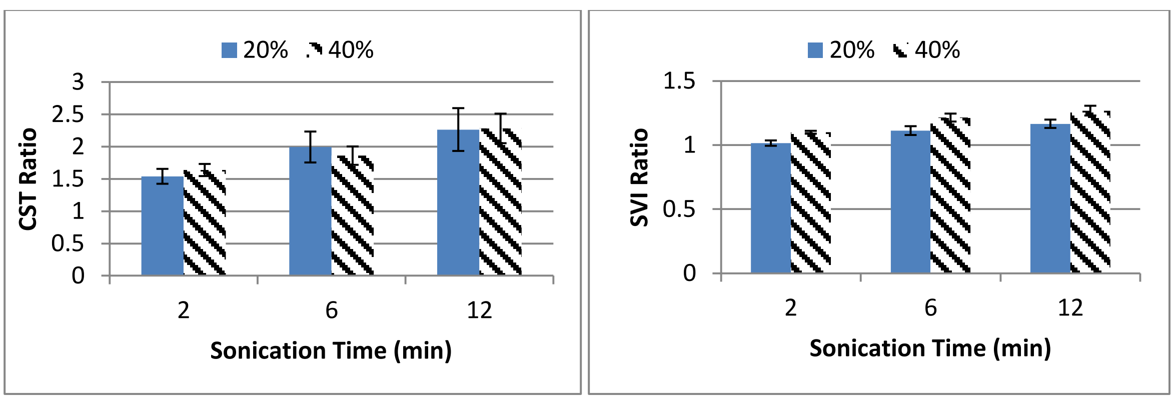

Figure 4.12: Combined effect of sonication time and amplitude on SVI and CST ratios (C/C_o) following ultrasound treatment. (C = SVI or CST value of the treated samples, C_o = CVI or CST value of the control sample)

4.3.3 Comparison of Conventional Freezing and Combined Ultrasonic-Freezing to Ultrasound, Thermal and Microwave Treatments

4.3.3.1 Solubilisation of Sludge Organic Matter

When sludge was conventionally frozen at -15°C for one freeze-thaw cycle, the TSS disintegration was just over 3% and the sCOD ratio was 3.2 times of the control. The combined ultrasonic-freezing treatment increased the disintegration of suspended solids to 4-14% and the sCOD ratio ranged from 4.2-5.3 depending on the sonication time. The addition of just two minutes of sonication at 40% amplitude prior to freezing significantly improved the soluble COD ratio (4.23) compared to one cycle of conventional freezing alone (3.20). The increase in soluble COD for combined ultrasonic-freezing at 20% amplitude compared to conventional freezing was for the most part insignificant ($p > 0.05$).

When comparing the TSS ratios for ultrasound alone to combined ultrasonic-freezing treatment, the difference between each level measured was statistically insignificant ($p > 0.05$). The disintegration of suspended solids ranged from approximately 0-18% and 4-15% for ultrasound and combined ultrasonic freezing respectively. On the other hand, soluble COD ratios were significantly higher for all combined ultrasonic-freezing treatments at 20% amplitude compared to their ultrasound-only treated counterparts (Figure 4.13).

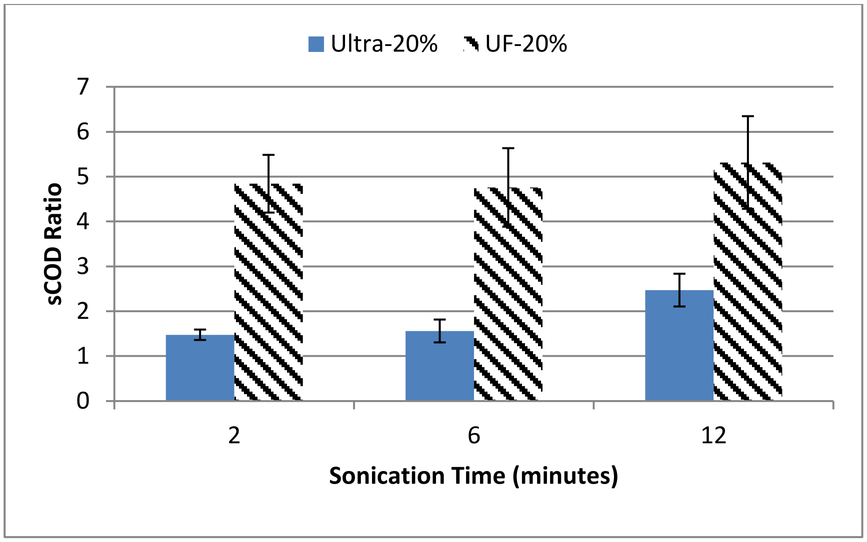

Figure 4.13: Comparison of sCOD ratio at an amplitude of 20% between ultrasound (Ultra-20%) and combined ultrasonic freezing (UF-20%), where sCOD = soluble COD concentration of the treated samples and $sCOD_o$ = soluble COD concentration of the control samples.

When comparing all the treatments examined, it is apparent that the disintegration of suspended solids is similar for all methods. In terms of solubilisation of COD, thermal treatment was the most effective with the treated sCOD almost six times that of the control. However, conventional freezing and combined ultrasonic-freezing were not far behind with their average sCOD ratios falling on the higher end of the spectrum both at 4.7 times (Figure 4.14). There was no statistical significance between the sCOD ratios of thermal, conventional freezing and combined ultrasonic-freezing treatments. These three treatments however, all had sCOD ratios significantly greater than those of ultrasound and microwave.

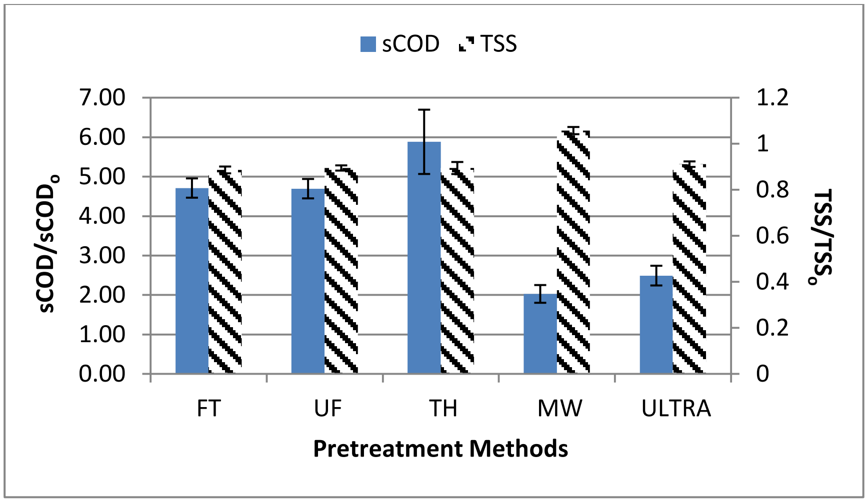

Figure 4.14: Comparison of sCOD and TSS ratios for conventional freezing (FT) and combined ultrasonic-freezing (UF) compared to thermal (TH), Microwave (MW) and Ultrasound (ULTRA). sCOD = soluble COD concentration of the treated samples, sCOD$_o$ = soluble COD concentration of the control, TSS = suspended solids concentration of the treated samples, TSS$_o$ = suspended solids concentration of the control.

4.3.3.2 Dewaterability of TWSS

In terms of settleability and filterability, the sludge samples subjected to conventional freezing and combined ultrasonic-freezing treatments showed significant improvements compared to the other treatment methods (Figure 4.15).

There was up to a 549% increase in settleability for sludge treated with freezing, compared to 5 and 7% for thermal and microwave respectively. Ultrasound, on the other hand, showed a decrease in settleability of 13%. The same degree of improvement was noted in terms of filterability. In fact, the two treatment methods involving freezing were the only ones that were capable of improving the filterability of the sludge.

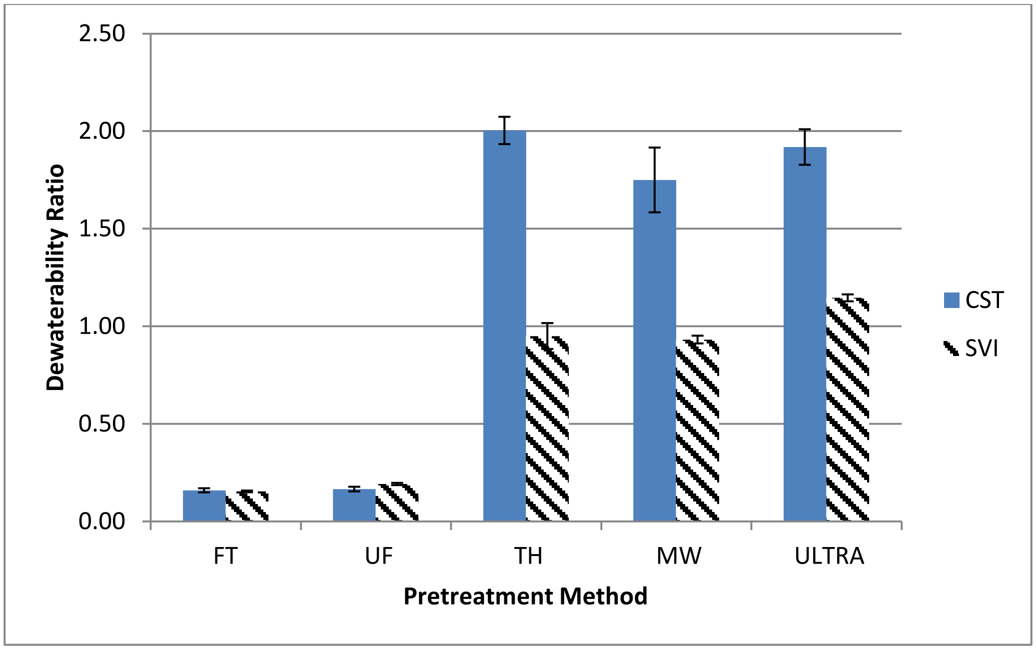

Figure 4.15: Comparison of CST and SVI ratios (C/C_o) for conventional freezing (FT) and combined ultrasonic-freezing (UF) compared to thermal (TH), Microwave (MW) and Ultrasound (ULTRA). C = CST/SVI value of the treated samples, C_o = CST/SVI value of the control samples.

It has been noted, that capillary suction time may not be an accurate measurement of filterability when freeze-thaw is employed as it causes interstitial water to be released as free water (Ormeci and Vesilind, 2001). As a result, the CST values of sludge treated by freezing are essentially the same as those of distilled water. In order to more accurately assess the filterability of the freeze-thawed sludge, a different test, such as specific resistance to filtration, should be conducted to ensure the validity of the data.

4.4 Conclusions

The following conclusions can be drawn from this study:

- Conventional freezing is an effective method in solubilising sludge organic matter. The disintegration of sludge solids reached a maximum of 21% and concentration of soluble COD was 6.5 times that of the control with five freeze-thaw cycles and a freezing temperature of -30°C.
    - The freezing temperatures examined (-15 and -30°C) did not significantly affect the solubilisation of sludge organic matter. However, an increase in the number of freeze-thaw cycles from one to three resulted in a significant increase in both TSS disintegration and COD solubilisation. The increase in sCOD and TSS ratios from a further increase to five cycles was statistically insignificant.
- Combined ultrasonic-freezing was also effective at solubilising sludge organic matter. However, neither sonication time nor amplitude affected the disintegration of suspended solids or the solubilisation of COD. The disintegration of suspended solids ranged from 4-14% while the solubilisation of COD was ranged between 4.3-5.3 times the control.
- There was no statistical difference between solubilisation of organic matter for one cycle of conventional freezing at -15°C and combined ultrasonic-freezing at 20%. On the other hand, when the sonication amplitude was increased to 40%,

just two minutes of sonication prior to freezing significantly improved the solubilisation of COD compared to conventional freezing alone.

- Conventional freezing and combined ultrasonic-freezing are comparable to other treatment techniques such as ultrasound, thermal and microwave in terms of solubilisation of sludge organic matter. TSS disintegration was comparable for all the treatment methods with the greatest value being 11% for conventional freezing. COD solubilisation was highest for thermal treatment at 5.9 times the control followed closely by conventional freezing and combined ultrasonic-freezing at 4.7 times the control.

- Both treatments involving freezing (conventional freezing and combined ultrasonic-freezing) are far more effective at enhancing the filterability and settleability of the secondary sludge. Conventional freezing and combined ultrasonic-freezing showed improvements 4-5 times greater than the control for both filterability and settleability.

- Freezing shows much potential as an effective pre-treatment method that can simultaneously improve solubilisation of sludge organic matter and sludge dewaterability.

4.5 References

Appels, Lise, Jan Baeyens, Jan Degreve, and Raf Dewil. "Principles and Potential of the Anaerobic Digestion of Waste Activated Sludge." *Progress in Energy and Combustion Science* 34 (2008): 755-781.

Bougrier, Claire, Jean Delgenes, and Helene Carrere. "Effects of Thermal Treatments on Five Different Waste Activated Sludge Samples' Solubilisation, Physical Properties and Anaerobic Digestion." *Chemical Engineering Journal* 139, no. 2 (2008): 236-244.

Canales, Angel, Alain Pareilleux, Jean Luc Rols, Gerard Goma, and Alain Huyard. "Decreased Sludge Production Strategy for Domestic Wastewater Treatment." *Water Science & Technology* 30, no. 8 (1994): 97-106.

Carrere, Helene, Claire Bougrier, Delphine Castets, and Jean-Phillipe Delgenes. "Impact of Initial Biodegradability on Sludge Anaerobic Digestion Enhancement by Thermal Pretreatment." *Journal of Environmental Science & Health Part A* 43, no. 13 (2008): 1551-1555.

Chang, Chia-Jung, Vinay Kumar Tyagi, and Shang-Lien Lo. "Effects of Microwave and Alkali Induced Pretreatment on Sludge Solubilization and Subsequent Aerobic Digestion." *Bioresource Technology* 102, no. 17 (2011): 7633-7640.

Chu, C. P., Bea-Ven Chang, G. S. Liao, D. S. Jean, and D. J. Lee. "Observations on Changes in Ultrasonically Treated Waste-Activated Sludge." *Water Research* 35, no. 4 (2001): 1038-1046.

Chu, C. P., W. H. Feng, Y. H. Tsai, and D. J. Lee. "Unidirectional Freezing of Waste-Activated Sludge: The Presence of Sodium Chloride." *Environmental Science & Technology* 31, no. 5 (1997): 1512-1517.

Chu, C.P, W.C. Feng, Bea-Ven Chang, C.H. Chou, and D.J. Lee. "Reduction of Microbial Density Level in Wastewater Activated Sludge Via Freezing and Thawing." *Water Research* 33, no. 16 (1999): 3532-3535.

Clark, P. B., and I. Nujjoo. "Ultrasonic Sludge Pretreatment for Enhanced Sludge Digestion." *Journal of the Chartered Institution of Water and Environmental Management* 14, no. 1 (2000): 66-71.

Eskicioglu, Cigdem, Kevin J. Kennedy, and Ronald L. Droste. "Characterization of Soluble Organic Matter of Waste Activated Sludge Before and After Thermal Pretreatment." *Water Research* 40, no. 20 (2006): 3725-3736.

Feng, Xin, Jinchuan Deng, Hengyi Lei, Tao Bai, Qingjuan Fan, and Zhaoxu Li. "Dewaterability of Waste Activated Sludge With Ultrasound Conditioning." *Bioresource Technology* 100, no. 3 (2009): 1074-1081.

Foladori, Paola, Gianni Andreottola, and Giuliano Ziglio. *Sludge Reduction Technologies in Wastewater Treatment Plants.* London: IWA Publishing, 2010.

Gao, W, D.W. Smith, and Y. Li. "Natural Freezing as a Wastewater Treatment Method: E.Coli Inactivation Capacity." *Water Research* 40 (2006): 2321-2326.

Gao, W, K Leung, and N Hawdon. "Freezing Inactivation of Escherichia Coli and Enterococcus Faecalis in Water: Response of Different Strains." *Water Environment Research* 81, no. 8 (2009): 824-830.

Gao, Wa. "Freezing as a Combined Wastewater Sludge Pretreatment and Conditioning Method." *Desalination*, 2011: 170-173.

Hedstrom, Annelle, and Jorgen Hanaeus. "Natural Freezing, Drying and Composting for Treatment of Septic Sludge." *Journal of Cold Regions Engineering* 13 (1999): 167-179.

Hellstrom, Daniel. "Natural Sludge Dewatering II: Thawing-Drying Process in Full-Scale Sludge Freezing Ditches." *Jounal of Cold Regions Engineering* 11, no. 1 (1997): 15-29.

Hellstrom, Daniel, and Elisabeth Kvarnstrom. "Natural Sludge Dewatering I: Combination of Freezing, Thawing and Drying as Dewatering Methods." *Journal of Cold Regions Engineering* 11, no. 1 (1997): 1-14.

Hong, S. G., J. D. Young, G. W. Chen, I. L. Chang, W. T. Hung, and D. J. Lee. "Freeze/Thaw Treatment on Waste Activated Sludge: A FTIR Spectroscopic Study." *Journal of Environmental Science & Health Part A: Environmental Science & Engineering & Toxicology* 30, no. 8 (1995): 1717-1726.

Hong, SM, JK Park, N Teeradej, YO Lee, YK Cho, and CH Park. "Pretreatment of Sludge with Microwaves for Pathogen Destruction and Improved Anaerobic Digestion Performance." *Water Environment Research* 78, no. 1 (2006): 76-83.

Hu, Kai, Jun-Qiu Jiang, Qing-Liang Zhao, Duu-Jong Lee, Kun Wang, and Wei Qiu. "Conditioning of Wastewater Sludge using Freezing and Thawing: Role of Curing." *Water Research*, 2011: 5969-5976.

Hu, Kai, jun-Qiu Jiang, Qing-Ling Zhao, Duu-Jong Lee, Kun Wang, and Wei Qiu. "Conditioning of Wastewater Sludge Using Freezing: Role of Curing." *Water Research* 45 (2011): 5969 - 5976.

Hung, W. T., I. L. Chang, W. W. Lin, and D. J. Lee. "Unidirectional Freezing of Waste-Activated Sludges: Effects of Freezing Speed." *Environmental Science & Technology* 30, no. 7 (1996b): 2391-2396.

Kennedy, Kevin J., Gabriel Thibault, and Ronald L. Droste. "Microwave Enhanced Digestion of Aerobic SBR Sludge." *Water SA* 33, no. 2 (2007): 261-270.

Khanal, Samir Kumar, David Grewell, Shihwu Sung, and Hans J. Leeuwen. "Ultrasound Applications in Wastewater Sludge Pretreatment: A Review." *Critical Reviews in Environmental Science and Technology* 37, no. 4 (2007): 277-313.

Lee, Duu Jong, and Yuan Hway Hsu. "Fast Freeze/Thaw Treatment on Excess Activated Sludges: Floc Structure and Sludge Dewaterability." *Environmental Science & Technology* 28, no. 8 (1994): 1444-1449.

Li, Y. Y., and T. Noike. "Upgrading of Anaerobic Digestion of Waste Activated Sludge by Thermal Pretreatment." *Water Science and Technology* 26, no. 3-4 (1992): 857-866.

Lin, C.F., and Y. Shien. "Sludge Dewatering using Centrifuge with Thermal/Polymer Conditioning." *Water Science and Technology* 44, no. 1 (2001): 321-325.

Ma, Jingxing, Thu Hang Duong, Marianne Smits, Willy Verstraete, and Marta Carballa. "Enhanced Biomethanation of Kitchen Waste by Different Pretreatments." *Bioresource Technology* 102 (2011): 592-599.

Montusiewicz, A., M. Lebiocka, A. Rozej, E. Zacharska, and L. Pawlowski. "Freezing/thawing Effects on Anaerobic Digestion of Mixed Sewage Sludge." *Bioresource Technology* 101 (2010): 3466 - 3473.

Muller, Christopher D., Mohammad Abu-Orf, Charles D. Blumenschein, and John T. Novak. "A Comparative Study of Ultrasonic Pretreatment and an Internal Recycle for the Enhancement of Mesophilic Anaerobic Digestion." *Water Environment Research* 81, no. 12 (2009): 2398-2410.

Muller, J, et al. "Disintegration of Sewage Sludges and Influence on Anaerobic Digestion." *Water Science & Technology* 38, no. 8-9 (1998): 425-433.

Neyens, E., and J. Baeyens. "A Review of Thermal Sludge Pretreatment Processes to Improve Dewaterability." *Journal of Hazardous Materials* 98, no. 1-3 (2003): 51-67.

Ormeci, Banu, and Aarne P. Vesilind. "Effect of Dissolved Organic Material and Cations on Freeze-Thaw Conditioning of Activated and Alum Sludges." *Water Research* 35, no. 18 (2001): 4299-4306.

Pilli, Sridhar, Puspendu Bhunia, Song Yan, R. J. LeBlanc, R. D. Tyagi, and R. Y. Surampalli. "Ultrasonic Pretreatment of Sludge: A Review." *Ultrasonics Sonochemistry* 18, no. 1 (2011): 1-18.

Prestamo, Guadalupe, Carmen Fuster, and Carmen Risueno. "Effects of Blanching and Freezing on the Structure of Carrots Cells and their Implications for Food Processing." *Journal of the Science of Food and Agriculture* 77 (1998): 223-229.

Saveyn, H., D. Curvers, R. Jacobsen, and P. Van der Meeren. "Improved Dewatering by Freeze-Thawing of Predewatered Sludge Cakes." *Asia-PacificJournal of Chemical Engineering* 5 (2009): 798-803.

Stabnikova, O., X.Y. Liu, and J.Y. Wang. "Digestion of Frozen/Thawed Food Waste in the Hybrid Anaerobic Solid-Liquid System." *Waste Management*, 2008: 1654-1659.

Thomashow, Michael F. "Role of Cold-Responsive Genes in Plant Freezing Tolerance." *Plant Physiology* 118 (1998): 1-7.

Ting, C. H., and D. J. Lee. "Production of Hydrogen and Methane from Wastewater Sludge using Anaerobic Fermentation." *Water Science & Technology* 50, no. 9 (2004): 223-228.

Toreci, Isil, Kevin J. Kennedy, and Ronald L. Droste. "Effect of High-Temperature Microwave Irradiation on Municipal Thickened Waste Activated Sludge Solubilization." *Heat Transfer Engineering* 31, no. 9 (2010): 766-773.

Turovskiy, Izeail S., and P. K. Mathai. *Wastewater Sludge Processing.* New Jersey: John Wiley & Sons Inc., 2006.

Vesilind, Aarne P., and James C. Martel. "Freezing of Water and Wastewater Sludges." *Journal of Environmental Engineering* 116, no. 5 (1990): 854-862.

Vesilind, Aarne P., Seppo Wallinmaa, and James C. Martel. "Freeze-thaw sludge conditioning and double layer compression." *Canadian Journal of Civil Engineering* 18, no. 6 (1991): 1078-1083.

Vigueras-Carmona, S.E., F. Ramirez, A. Noyola, and O. Monroy. "Effect of Thermal Alkaline Pretreatment on the Anaerobic Digestion of Waste Activated Sludge." *Water Science and Technology* 64, no. 4 (2011): 953-959.

Wang, F., M. Ji, and S. Lu. "Influence of Ultrasonic Disintegration on the Dewaterability of Waste Activated Sludge." *Environmental Progress* 25, no. 3 (2006): 257-260.

Wang, Fen, Shan Lu, and Min Ji. "Components of Released Liquid from Ultrasonic Waste Activated Sludge Disintegration." *Ultrasonics Sonochemistry* 13 (2006): 334-338.

Wang, Fen, Yong Wang, and Min Ji. "Mechanisms and Kinetics Mondels for Ultrasonic Waste Activated Sludge Disintegration." *Journal of Hazardous Materials* 123, no. 1-3 (2005): 145-150.

Williams, A., W. Gao, and K.T Leung. "Effects of Freezing of UV Inactivation of Waterborne Microorganisms." *Journal of Environmental Engineering* 138 (2012): 470-474.

Wojciechowska, Eva. "Application of microwaves for sewage sludge conditioning." *Water Research* 39 (2005): 4749-4754.

Yu, Qiang, Hengyi Lei, Guangwei Yu, Zhaoxu Li, and Zhicheng Wu. "Influence of Microwave Irradiation on Sludge Dewaterability." *Chemical Engineering Journal*, 2009: 88-93.

Zhang, Panyue, Guangming Zhang, and Wei Wang. "Ultrasonic Treatment of Biological Sludge: Floc Disintegration, Cell Lysis and Inactivation." *Bioresource Technology* 98, no. 1 (2007): 207-210.

CHAPTER 5
PROGRESSIVE ULTRASONIC FREEZING AND FURTHER INVESTIGATION OF THE EFFECTIVENESS OF FREEZING AS A PRETREATMENT METHOD

This chapter explores another treatment involving freezing, progressive ultrasonic freezing (PUF), for the solubilisation of sludge organic matter and dewaterability of municipal wastewater secondary sludge. The effect of pulse time and sonication time were investigated. The solubilisation of sludge organic matter, evaluated by measuring soluble chemical oxygen demand (sCOD), showed significant improvements compared to the controls. The maximum increase in sCOD was 7.7 times the control for the liquid portion of the progressive ultrasonic freezing samples with a three second sonication pulse for the duration of the freezing. The dewaterability of the PUF method was also evaluated by measuring capillary suction time (CST). The solid portion of the PUF samples showed significant improvements in dewaterability with CST ratios ranging from 0.15 – 0.26. Subsequently, the most effective conditions from each of the freezing treatments from both chapter 4 and 5 were selected and further investigated for soluble protein concentration, biodegradability and gas production. These tests revealed that the three freezing treatments also resulted in significantly higher concentrations of proteins as well as increased biodegradability and gas production. The gas production ratio over the control was greatest for conventional freezing (1.52), followed by combined ultrasonic freezing (1.17) and progressive ultrasonic freezing (1.13). The results suggest that freezing could be a very effective pretreatment method as it would be able to simultaneously improve both anaerobic digestion efficiency as well as dewaterability.

5.1 Introduction

Power ultrasound to promote ice nucleation and crystal growth has been studied in theory for almost 80 years, but only in recent years are we seeing its increasing application, most often in the food industry (Chow et al., 2005; Zheng and Sun, 2006). It has been considered in the freezing of foods since the combination of freezing and ultrasound has shown the ability to cause faster, more even nucleation (Mason et al.,1996). This is likely due to the flow streams that act as a form of agitation resulting in a reduction in the resistance of heat and mass transfer and therefore an increase in freezing rate (Li and Sun, 2002). Another major benefit of power ultrasonic freezing in the food industry is that it results in the formation of smaller ice crystals which ultimately reduces cell damage (Acton and Morris, 1992; Mason et al., 1996).

Progressive freezing concentration is a treatment technology used to concentrate solutes. Its applications include the concentration of fruit juices and dairy products in the food industry (Liu et al., 1999, Sanchez et al., 2009), contaminant removal for wastewater treatment (Gao et al., 2008; Gao and Shao, 2009; Muller and Sekoulov, 1992) and sludge dewatering (Chu et al., 1997; Halde, 1980; Hung et al., 1996). The idea behind progressive freeze concentration is that as ice begins to form, the solute, impurities or particles get driven by the advancing ice front and end up in a concentrated unfrozen portion of the solution which can now easily be separated from the pure ice.

In Chapter 4, results showed that freezing is a suitable pre-treatment method for both the solubilisation of organic matter as well as the dewaterability of municipal secondary sludge. Additionally, when sludge was frozen following only two minutes of sonication, improvements in solubilisation of COD and disintegration of suspended solids were observed. In order to further investigate the effect of power ultrasound combined with freezing on the solubilisation of organic matter and dewaterability, progressive ultrasonic freezing (PUF) will be examined as a treatment technique. Additionally, the most effective of each freezing treatment conditions (conventional freezing, combined ultrasonic freezing and progressive ultrasonic freezing) will be tested for soluble protein concentration, biodegradability and gas production in order to provide a more thorough examination of their effectiveness as pretreatment methods.

5.2 Materials and Methods

5.2.1 Sludge Samples

Thickened waste secondary sludge (TWSS) was obtained from Thunder Bay's wastewater treatment plant located in Thunder Bay, Ontario, Canada. Sludge was stored in the refrigerator in closed jars at 4°C if not immediately treated. Characteristics of the sludge samples obtained from the WWTP varied over the course of the experiments and can be found in Table 5.1. All samples were diluted four times prior to treatment for the ease of testing.

Table 5.1: Characteristics of TWSS

Value	pH	TS (mg/L)	TSS (mg/L)	tCOD (mg/L)	sCOD (mg/L)	SVI (mL/g)	CST (s)
Average	6.50	32,100	31,672	47,119	1,450	28.8	57.6

During the last set of experiments (measuring proteins, biodegradability and gas production), Thunder Bay's wastewater treatment plant experienced some difficulty and TWSS was not available for sampling. In order to complete the experiment, WAS from Abitibi Bowater's pulp and paper plant was used. The characteristics of the sludge samples from Bowater were very different from that of the TWSS and can be found in Table 5.2. Results from this run will be summarized separately from the runs using municipal sludge. These samples were diluted two times prior to treatment.

Table 5.2: Characteristics of Pulp and Paper WAS

Value	pH	TS (mg/L)	tCOD (mg/L)	sCOD (mg/L)
Average	6.93	8,954	10,633	551

5.2.2 Experimental Design

A new combination of ultrasound and freezing, progressive ultrasonic freezing, was used as a treatment for the solubilisation of sludge organic matter and dewaterability of TWSS. Three different treatment conditions were carried out: (a) 2 seconds on, 30 seconds off for 12 minutes of sonication, (b) 3 seconds on, 30 seconds off for 12 minutes and (c) 3 seconds on, 30 seconds off for the entire duration of progressive freezing (approximately 25 minutes of sonication). These three treatment conditions allowed the investigation of the effect of sonication pulse and duration of sonication on the solubilisation of sludge organic matter and dewaterability of municipal secondary sludge. Duplicate runs were carried out for each treatment in order to minimize experimental and random error as well as to increase the precision of the results.

For each of the freezing methods, the treatment conditions that resulted in the greatest solubilisation of matter and the most improved dewaterability over the control were selected for further analysis of soluble protein concentration, biodegradability and gas production. For conventional freezing (freezing without ultrasound), two treatment conditions were chosen (a) -15°C for 1 freeze-thaw cycle and (b) -15°C for 3 freeze-thaw cycles. The treatments chosen for combined ultrasonic freezing were (a) 40% amplitude with 2 minutes of sonication and (b) 40% sonication with 12 minutes of sonication. Only one treatment condition was tested for progressive ultrasonic freezing; 3 second on, 30 seconds off for the entire duration of the freezing.

5.2.3 Experiments

5.2.3.1 Progressive Ultrasonic Freezing

250 mL of TWSS was placed into 500 mL stainless steel containers covered in foil faced bubble wrap. The insulated containers were then placed on a platform attached to a pulley and lowered into a -15°C freezing bath in approximately 40mL intervals (Figure 5.1). The 13mm (1/2”) diameter probe was used to sonicate the samples at 20% amplitude while they were in the freezing bath. When the 40 mL of TWSS was completely frozen, the platform was lowered another 40mL. This was repeated until 80% of the volume (approximately 200mL of TWSS) was frozen. The unfrozen portion, 20% or 50mL, was poured out into a separate beaker and 30 mL of pure water used to rinse the ice water interface. The water was then added to beaker containing the unfrozen portion. The frozen portion was then left in the stainless steel container to thaw at room temperature.

Figure 5.1: Set-up of probe and freezing bath for progressive ultrasonic freezing experiments.

5.2.3.2 Conventional Freezing Treatment

Conventional freezing treatments were carried out at -15°C as described in section 4.2.3.1.

5.2.3.3 Combined Ultrasonic Freezing Treatment

Combined ultrasonic-freezing was carried our using the 2.54 cm (1”) diameter probe at 40% amplitude for two and twelve minutes. Details of the experimental method can be found in section 4.2.3.2.

5.2.4 Sample Analysis

5.2.4.1 Progressive Ultrasonic Freezing

pH, total solids (TS), total suspended solids (TSS), total and soluble chemical oxygen demand (tCOD, sCOD) and capillary suction time (CST) were measured for the sludge samples before and after progressive ultrasonic freezing for both the frozen and unfrozen portions. All of the above measurements were completed following the procedures outlined in the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005).

The effect of progressive ultrasonic freezing on the solubilisation of sludge organic matter was evaluated based on pH, solubilisation of COD and disintegration of suspended solids. TSS disintegration is defined as:

$$\text{TSS disintegration }(\%)=\left(\frac{\text{TSS}_{\text{o}}\text{- TSS}_{\text{f}}}{\text{TSS}_{\text{o}}}\right)*100$$

Where TSS_o is the suspended solids concentration of the untreated sludge and TSS_f is the suspended solids concentration of the treated sludge.

Capillary suction time (CST) was used to assess the dewaterability, or more specifically filterability, of the secondary sludge. The sludge samples obtained were not large enough to test for sludge volume index (SVI).

5.2.4.2 Further Analysis of Pretreatment Methods

In order to further assess the effectiveness of the pretreatment methods, an analysis was done on the soluble proteins, biodegradability and gas production. Soluble protein concentrations were measured to get a more detailed analysis of the content of the soluble organic matter. The samples were passed through 0.45µm filters in order to obtain soluble protein concentrations. The modified Lowry protocol (Gerhardt, 1994) was followed using bovine serum albumin (BSA) as a standard.

Biodegradability and gas production were measured in order to assess the efficiency of anaerobic digestion by placing 25 mL of sludge samples with 5 mL of anaerobic seed sludge into light-free serum bottles. The head space was flushed with nitrogen to create and anaerobic environment. The serum bottles were then sealed with rubber stoppers to create a gas-tight environment. Samples were placed in an incubator at 37°C and 150rpm. Gas samples were measured over the course of 20 days using a syringe. After 20 days of digestion, the biodegradability of the samples was measured according to the following equation used by Pham et al. (2010):

$$Biodegradability = \left(1 - \frac{TS\ concentration\ after\ biodegradation}{TS\ concentration\ before\ biodegradation}\right) * 100$$

5.2.5 Data Analysis

Data collected for the samples treated with progressive ultrasonic freezing were normalized to that of the controls using ratios (C/C_o), where C is the value of the treated samples and C_o is the value of the control samples. The concentration ratios were used to allow the comparison of data obtained from different batches of sludge. The ratios were compared using analyses of variance (ANOVAs) in order to determine if any statistical difference occurred between different treatments. The Tukey test was used as a post-hoc test in order to determine which means were statistically different from each other. The significance level for these tests were set at 95% (α = 0.05). All statistical analysis was completed using the computing environment R (R Development Core Team, 2008, Vienna, Austria).

Although the sludge samples used for measuring the proteins, biodegradability and gas production were taken from two different treatment plants and were not statistically compared, ratios to the control were still used in order to keep all results uniform. One run was completed using secondary municipal sludge and a second was completed using pulp and paper WAS.

5.3 Results and Discussion

5.3.1 Effect of Progressive Ultrasonic Freezing on Solubilisation of Sludge Organic Matter

Both solid and liquid portions of the samples exposed to progressive ultrasonic freezing (PUF) showed a small increase in pH compared to the control. This is could be due to the oxidizing effect of the hydroxyl radicals that occurs during sonication (Khanal et al., 2007; Wang et al., 2005). There was no difference in pH between the solid and liquid portion of the samples with both their pH ratios being approximately 1.06. The suspended solids concentration of the PUF sludge was lower than that of the control with TSS ratios of 0.84 and 0.81 for ice and liquid respectively. The difference between the solid and liquid values was not as large as expected. This is likely due to the fact that it was very difficult to achieve complete bottom up freezing with the apparatus used in this study due to issues with the insulation. A certain amount of radial freezing was still occurring despite the insulation around the container. It is hypothesized that the tray that was holding the stainless steel container was preventing the circulation of cold antifreeze, thereby acting as an insulation layer for the bottom of the container.

The sCOD ratios for the treated sludge were significantly greater than the controls. The sCOD ratio of the liquid portion was 6.7 times greater than the control while that of the solid portion was 3.7 times the control. It is expected that as sludge freezes, the dissolved solids are rejected by the ice front (Vesilind and Martel, 1990), which could explain the increased sCOD content in the top liquid portion of the PUF samples. However, it is unclear whether the difference in sCOD ratios between the solid and the liquid portion of the samples was due to progressive freeze concentrations or to the fact that the liquid portion (top 20%) was sonicated for a much longer time than the lower frozen portion. In order to accurately determine the cause for the difference in sCOD ratios between the liquid and the solid, an additional treatment with no ultrasound, but some form of mixing, should be done to eliminate the effect of the sonication.

5.3.1.1 Effect of Pulse Time

When the ultrasonic pulse was increased from two to three seconds, the pH ratio showed an insignificant decrease for both liquid and ice from 1.08 to 1.05 and 1.07 to 1.06 respectively. The difference in TSS and sCOD ratio for the liquid portion and solid portion were also found to be insignificant (see Table 5.3).

Table 5.3: Effect of pulse time on sCOD and TSS ratio (C/C_o) of solid and liquid portions for progressive ultrasonic freezing treatment. C = concentration of treated samples, C_o = concentration of control samples

	2 second pulse		3 second pulse	
	Liquid	**Solid**	**Liquid**	**Solid**
sCOD	6.226 ± 1.023	3.694 ± 0.177	5.566 ± 0.865	3.965 ± 0.308
TSS	0.848 ± 0.046	0.843 ± 0.028	0.809 ± 0.041	0.845 ± 0.079

5.3.1.2 Effect of Sonication Time

As with pulse time, a change in the duration of sonication did not have an effect on pH. Samples sonicated for the entire duration of freezing and those sonicated for just 12 minutes all had a pH of 1.04-1.06 times the control. There was a greater difference between the TSS ratios of the solid and liquid portions when the sonication was carried out for the entire freezing time (0.85 for the liquid portion versus 0.76 for the solid) compared to the 12 minutes (0.82 for the liquid portion versus 0.81 for the solid). This indicates a better solid-liquid separation for samples with sonication carried out for the duration of the entire freezing, most likely due to the fact that sonication acts as a form of mixing (Li and Sun, 2002). Gao et al. (2008) also found that mixing during freezing results in a significant improvement of the separation of contaminants.

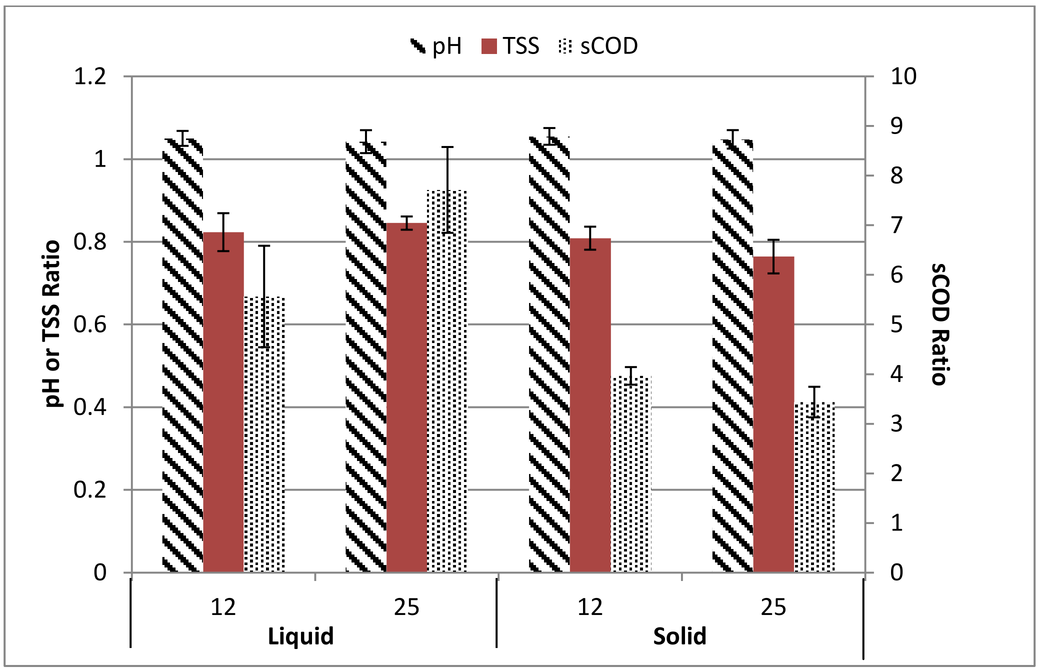

Figure 5.2: Effect of sonication time on pH, TSS & sCOD (C/C_o) ratios of the solid and liquid portion for progressive ultrasonic freezing treatment. C = concentration/value of the treated samples, C_o = concentration/value of the control samples

In terms of sCOD, the ratio of the liquid portion increased from 5.6 to 7.7 times the control as the sonication time increased from 12 to 25 minutes. This was expected as the previous chapter and several other studies have found that an increased sonication time results in increased release of organic matter (Wang et al., 2006; Zhang et al., 2007). There was very little difference in the sCOD ratios of the solid portions; the ratio decreased from 3.97 to 3.44 with the increase in sonication time. This makes sense as only the liquid portion (top 20%) is receiving the additional sonication.

5.3.2 Effect of Progressive Ultrasonic Freezing on Dewaterability

As expected, the filterability of the liquid portion of the treated sludge which was exposed to the longest amount of sonication showed a decline in filterability compared to the control with an average CST ratio of 2.9. This was comparable to the results of the previous section which found that 12 minutes of sonication time at 20% amplitude resulted in an average CST ratio of 2.2 times the control. The filterability of the solid portion of the sample, essentially exposed to one freeze-thaw cycle showed an improvement in CST with a ratio 0.21 times of the control. These results were also similar to the results found in the Chapter 4 for secondary sludge treated with combined ultrasonic-freezing which had an average CST ratio of 0.18.

5.3.2.1 Effect of Pulse Time

The increase in ultrasound pulse time from two to three seconds resulted in an increase of CST ratio from 1.70 to 1.82 times the control while the solid portion decreased from 0.22 to 0.15. These changes, shown in Table 5.4 were found to be statistically insignificant ($p > 0.05$).

Table 5.4: Effect of pulse time on CST ratio (CST/CST_o) of liquid and solid portion for progressive ultrasonic freezing treatment. CST = CST value of treated samples, CST_o = CST value of control samples.

	2 seconds		3 seconds	
	Liquid	Solid	Liquid	Solid
CST/CST_o	1.6984	0.2211	1.8194	0.1548

5.3.2.2 Effect of Sonication Time

Increasing the sonication time from 12 to approximately 25 minutes resulted in a significant decline in dewaterability for the liquid portion of the treated samples. The CST ratio more than doubled from 1.82 to 4.39 times the control. This increase is most likely due to the effect of ultrasound breaking up larger particles into smaller ones and thereby increasing the surface area available for water to adhere to (Chu et al., 2001). The deterioration in dewaterability is not worrisome as the liquid portion of the samples only account for 20% of the total volume. If this treatment were to be used in the municipal sludge treatment process, it is likely that after undergoing digestion, this liquid portion would be returned to the beginning of the wastewater treatment plant as a recycle stream. The more important issue is the dewaterability of the other 80% which gets frozen. The difference between the CST ratios for the solid portion was 0.15 and 0.26 for 12 and 25 minutes of sonication respectively. This difference was found to be statistically insignificant (Figure 5.3).

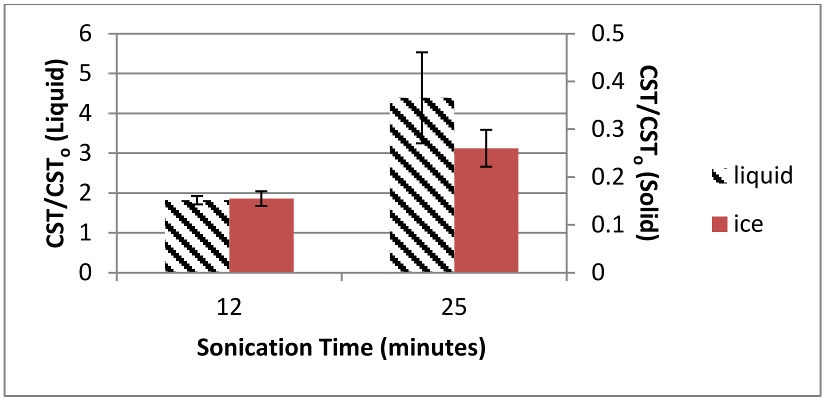

Figure 5.3: Effect of sonication time on CST ratio (CST/CST$_o$) of solid and liquid portion for progressive ultrasonic freezing. CST = CST value of treated sludge, CST$_o$ = CST value of the control sludge.

5.3.3 Comparison of Progressive Ultrasonic Freezing Treatment to Conventional Freezing and Combined Ultrasonic Freezing

5.3.3.1 Solubilisation of Sludge Organic Matter

In comparison to the other freezing methods the TSS ratio was significantly lower for the solid portion of the PUF treated sludge with a value of 0.806 compared to 0.886 and 0.895 for conventional freezing (FT) and combined ultrasonic freezing (UF) respectively. While a part of this decrease in TSS ratio for the PUF-S sludge could be due to the disintegration of suspended solids, part of it is also due to the migration of suspended solids to the liquid portion as they get rejected by the ice front. Given this, the TSS ratio for the solid portion of the PUF sludge may not be a very good indicator of increased solubilisation.

The sCOD ratio of the liquid portion of the PUF sludge was significantly greater compared to conventional freezing and combined ultrasonic freezing treatments, while that of the solid difference showed no statistical difference (Figure 5.4). In order to more accurately assess whether progressive ultrasonic freezing is actually an improvement compared to the other freezing treatments, it would be necessary to modify the experimental set-up in order to ensure that bottom-up freezing was more effective. Additionally, further experiments including the use of progressive freezing without any sonication would need to be conducted in order to determine if the cause of the high COD ratios was due to sonication, progressive freezing or a combination of both.

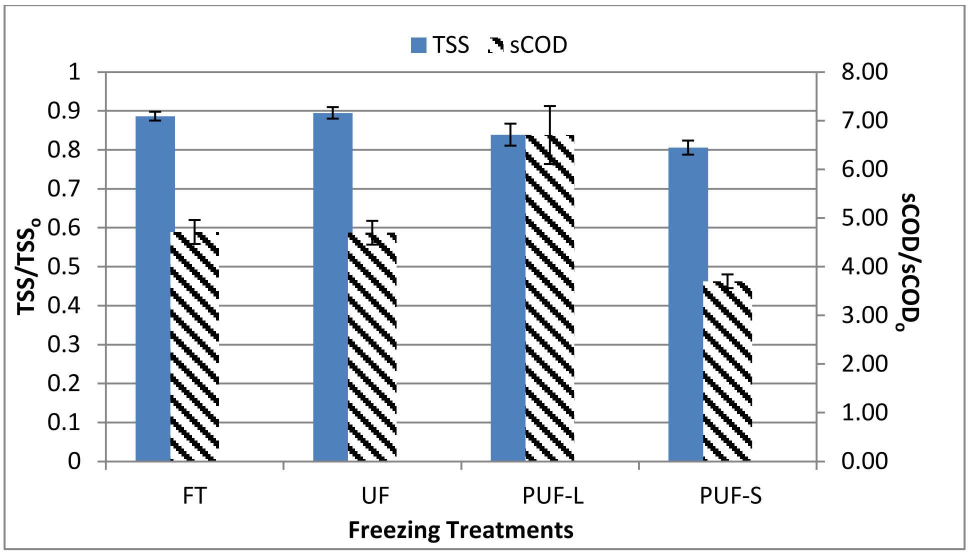

Figure 5.4: Comparison of TSS and sCOD ratio for conventional freezing (FT), combined ultrasonic-freezing (UF), liquid portion of progressive ultrasonic freezing (PUF-L) and solid portion of progressive ultrasonic freezing (PUF-S). Where TSS = TSS concentration of treated samples, TSS_o = TSS concentration of control, sCOD = soluble COD concentration of treated samples, $sCOD_o$ = soluble COD concentration of controls

5.3.3.2 Dewaterability

In terms of dewaterability, only the value for the CST ratio of the solid portion of PUF treated sludge was compared to the other freezing methods as the liquid portion would not undergo dewatering and simply be returned to wastewater treatment plant. When comparing the three freezing treatments, no statistical difference between their CST ratios was found (Figure 5.5).

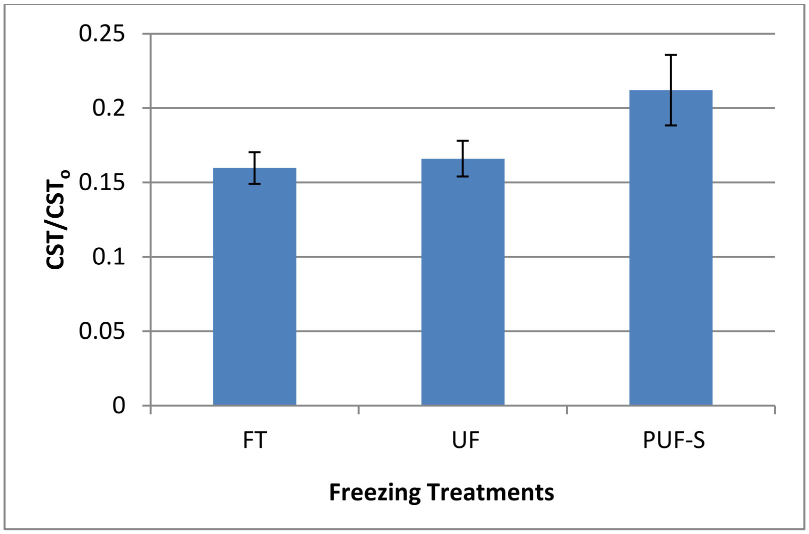

Figure 5.5: Comparison of CST ratio for conventional freezing (FT), combined ultrasonic-freezing (UF), liquid portion of progressive ultrasonic freezing (PUF-L) and solid portion of progressive ultrasonic freezing (PUF-S). Where CST = CST value of treated samples, CST_o = CST value of controls

5.3.4 Protein, Biodegradability and Gas Production of Freezing Treatments

The results presented in this section are from a single run where samples were tested for soluble protein concentration, biodegradability and gas production. Another duplicate run was to be carried out; however, the water treatment plant which provided the municipal secondary sludge samples over the course of the experiments experienced some difficulties and secondary treatment was no longer being performed. In order to complete the experiments, WAS from Bowater's pulp and paper plant in Thunder Bay, Ontario was used to complete a second run. Considering the substantial difference in organic matter between the two types of sludge, it is not possible to directly compare the results of this section. Instead, results from each of the runs (one with municipal secondary sludge and the other with pulp and paper WAS) will be summarized separately. Considering the lack in a true duplicate run, future studies should be carried out in order to obtain more accurate results.

The values of soluble protein, biodegradability and gas production for the controls from the TWSS and pulp and paper WAS are given in Table 5.5. Both the soluble protein and gas production have been divided by the TS solids concentration in order to account for variation in the solids concentration of the samples.

Table 5.5: Average soluble protein concentration, biodegradability and gas production values for control

a)TWSS

Soluble Protein (mg soluble protein/kg TS)	Biodegradability (%)	Gas Production (mL/kg TS)
5,319	9.9	575

b) pulp and paper WAS

Soluble Protein (mg soluble protein/kg TS)	Biodegradability (%)
14,600	4.4

5.3.4.1 Conventional Freezing

The conventional freezing experimental conditions chosen for further study were one and three freeze-thaw cycles at a freezing temperature of -15°C. For the soluble protein ratio, conventional freezing of municipal sludge with one freeze thaw cycle resulted in a soluble protein ratio of 4.30 where as an increase to three freezing cycles resulted in a larger soluble protein concentration of 7.42 times. The results using the pulp and paper sludge also resulted in an increase in soluble protein ratio; however, the increase was not as pronounced. One cycle resulted in an average soluble protein ratio of 1.75 whereas an increase to three cycles produced a ratio of 2.03.

An increase in protein is often associated with improved digestion (Appels et al., 2008; Weemaes and Verstraete, 1998). However, the biodegradability ratio decreased from 2.87 to 1.91 and from 4.54 to 4.04 times the control when the number of cycles increased from one to three for municipal and pulp and paper sludge respectively. It is possible that the high concentrations of soluble protein resulted in the formation of melanoidins, which have been shown to cause a decrease in biodegradability (Liu et al., 2012). As the number of freeze thaw cycles increased from one to three, the gas production increased from a ratio of 1.36 to 1.68 times the control (Figure 5.6). These gas production values are very similar to those of Montusiewics et al. (2010) who found that one cycle of freezing mixed sludge at -25°C resulted in an increased gas production 1.5 times that of the control.

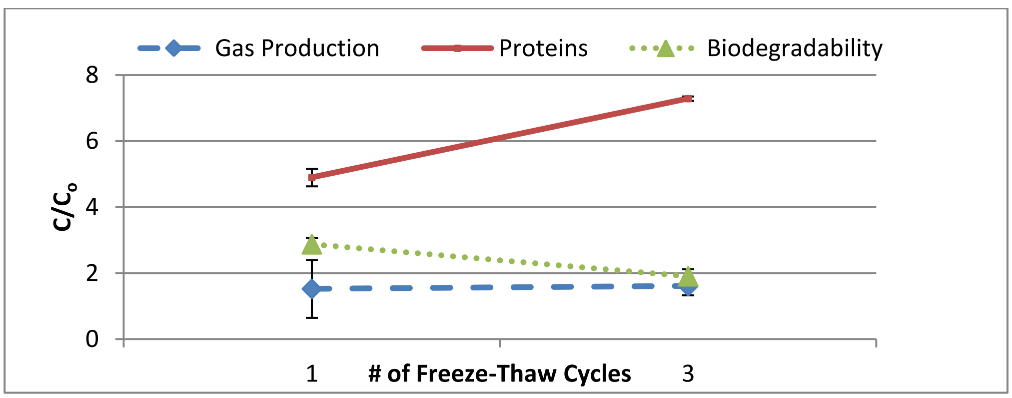

Figure 5.6: Effect of cycles on gas production, soluble protein and biodegradability ratios (C/C_o) for conventional freezing treatment. C = concentration of treated samples, C_o = concentration of control samples.

5.3.4.2 Combined Ultrasonic Freezing

The combined ultrasonic freezing conditions chosen to further investigate were 2 and 12 minutes at 40% amplitude. As the sonication time increased from two to twelve minutes, the soluble protein ratio increased from 4.74 to 5.70 for municipal sludge and from 2.20 to 2.58

for pulp and paper sludge. Considering the only difference between the two treatments is the length of sonication, this seems reasonable as it has been shown that an increase in sonication time also leads to an increase in soluble proteins (Feng et al., 2009; Pilli et al., 2011).

Like with conventional freezing, the increase in soluble proteins achieved with the increase in sonication time was not associated with an increase in biodegradability. In fact, the biodegradability ratio fell from 3.08 to 1.94 times the control when the sonication time increased from 2 to 12 minutes (Figure 5.6). The opposite was found for pulp and paper sludge in which biodegradability increased from 4.41 to 5.07 times the control as the sonication time increased. This could very well be due to either the difference in organic matter between the two types of sludge or experimental error as duplicate runs were not performed.

The gas production showed a small increase from 1.03 to 1.31 times the control with the increase in sonication time of municipal secondary sludge.

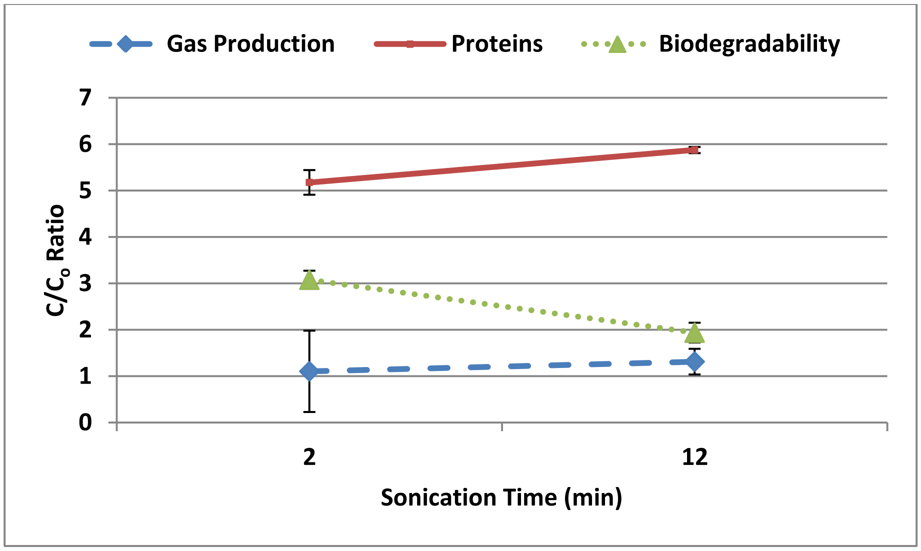

Figure 5.7: Effect of sonication time on gas production, soluble protein and biodegradability ratios (C/C_o) for combined ultrasonic-freezing treatment. C = concentration/value of treated samples, C_o = concentration/value of control samples.

5.3.4.3 Progressive Ultrasonic Freezing

The only experimental condition tested for progressive ultrasonic freezing was a pulse time of 3 seconds on and 30 seconds off for the entire duration of the freezing. The soluble protein concentration in the liquid portion of the municipal sludge samples was 5.58 times greater than the control whereas the soluble protein ratio of the solid portion was slightly higher at 5.95. For pulp and paper sludge, there was a decrease between the liquid and solid portions with soluble protein ratios of 4.19 and 3.60 respectively. Again, it is unclear whether the difference in behaviour of the pulp and paper sludge and municipal sludge is due to their differences in organic matter or experimental error.

The liquid portion of the PUF sample was tested for biodegradability. The biodegradability ratio was 2.19 and 3.55 for municipal secondary sludge and pulp and paper WAS respectively.

For the municipal sludge, this increase in biodegradability corresponded to a very small increase in gas production with a ratio 1.01 times greater than the control.

5.3.4.4 Comparison of Freezing Methods

Looking only at the municipal secondary sludge samples, all three freezing treatments showed comparable soluble protein and biodegradability ratios (Figure 5.8). The soluble protein ratio was highest for the liquid portion of the PUF treatment (6.16), followed by conventional freezing (6.09) and finally combined ultrasonic freezing (5.53). Despite having the least increase in soluble proteins, combined ultrasonic freezing had the greatest increase in biodegradability with an average ratio of 2.51, followed by conventional freezing and progressive ultrasonic freezing at 2.39 and 2.19 respectively.

In terms of gas production, conventional freezing had the greatest increase in gas production, with an average ratio of 1.56, followed by progressive ultrasonic freezing at 1.32 and finally combined ultrasonic freezing at 1.21. These results seem to suggest that conventional freezing on its own it just as effective as the combined methods at solubilising organic matter and increasing the anaerobic digestion efficiency of secondary municipal sludge. If this is the case, conventional freezing would be a preferred treatment as it has lower energy requirements compared to the combined treatments. That being said, due to the limited availability of secondary sludge samples the tests conducted in this section of the chapter did not have duplicate runs and further studies on the gas production and biodegradability capabilities of these treated sludges are required before any definite conclusions can be made.

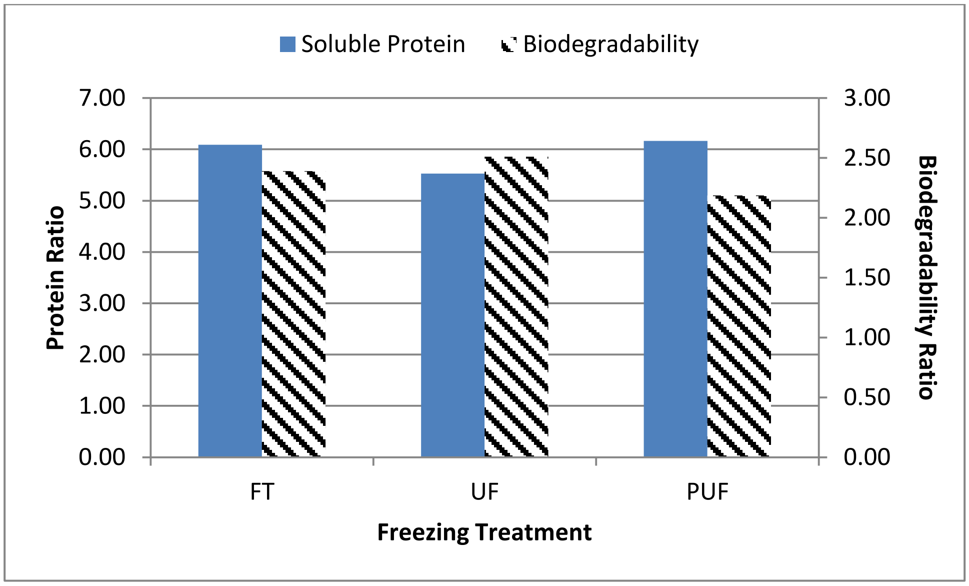

Figure 5.8: Comparison of soluble protein and biodegradability ratios (C/Co) for conventional freezing (FT), combined ultrasonic-freezing (UF) and progressive ultrasonic freezing (PUF). C = concentration/value of the treated samples, Co = concentration/value of the control samples.

5.4 Conclusion

The following conclusions can be drawn from this study:

- Progressive ultrasonic freezing resulted in TSS ratios significantly lower than the control for both the solid and liquid portions of the samples. Solids separation was not properly achieved by this study as the suspended solids concentration in the solid and liquid portion of the PUF treated samples were very close.
- The soluble COD concentration ratios for both solid and liquid portions of the PUF samples were significantly higher than the control. Additionally, the soluble COD concentration of the liquid portion of the sludge was significantly greater than the

frozen portion. It is unclear whether this difference is due to the progressive freeze concentrations or due to the excess amount of sonication that the top 20% (liquid) part receives. In order to resolve this, experiments need to be conducted without any sonication but some form of mixing to replace the agitation that occurs due to the flow streams from sonication.

- Increasing the pulse time from two to three seconds did not have any effect on the solubilisation of sludge organic matter or dewaterability. An increase in sonication time resulted in higher sCOD and CST ratios for the liquid portion of the sample, which is expected as sonication is known to release organic matter from secondary sludge, increased the soluble COD and deteriorating the filterability. The dewaterability of the liquid portion of the PUF samples is not crucial to the effectiveness of this treatment, as this part would likely be digested and then returned to the wastewater treatment plant instead of continuing the sludge treatment process.
- All three freezing treatments had a similar result in terms of soluble protein concentration, biodegradability and gas production. Conventional freezing and combined ultrasonic-freezing both showed some form of inhibition as protein increases resulted in a decrease in biodegradability.

5.5 References

Acton, E., and G. J. Morris. Method and Apparatus for the Control of Solidification in Liquids. USA Patent W.O. 99/20420. 1992.

Appels, Lise, Jan Baeyens, Jan Degreve, and Raf Dewil. "Principles and Potential of the Anaerobic Digestion of Waste Activated Sludge." *Progress in Energy and Combustion Science* 34 (2008): 755-781.

Chow, R., R. Blindt, R. Chivers, and M. Povey. "A Study on the Primary and Secondary Nucleation of Ice by Power Ultrasound." *Ultrasonics* 43, no. 4 (2005): 227-230.

Chu, C. P., W. H. Feng, Y. H. Tsai, and D. J. Lee. "Unidirectional Freezing of Waste-Activated Sludge: The Presence of Sodium Chloride." *Environmental Science & Technology* 31, no. 5 (1997): 1512-1517.

Feng, Xin, Jinchuan Deng, Hengyi Lei, Tao Bai, Qingjuan Fan, and Zhaoxu Li. "Dewaterability of Waste Activated Sludge With Ultrasound Conditioning." *Bioresource Technology* 100, no. 3 (2009): 1074-1081.

Gao, W., and Y. Shao. "Freeze Concentration for Removal of Pharmaceutically Active Compounds in Water." *Desalination* 249 (2009): 398-402.

Gao, W., D.W. Smith, and M. Habib. "Petroleum Refinery Secondary Effluent Polishing Using Freezing Processes - Toxicity and Organic Contaminant Removal." *Water Environment Research* 80, no. 6 (2008): 517-523.

Gerhardt, Philipp. *Methods for General and Molecular Bacteriology.* Washington: ASM, 1994.

Halde, Rolf. "Concentration of Impuritites by Progressive Freezing." *Water Research* 14, no. 6 (1980): 575-580.

Hung, W. T., I. L. Chang, D. J. Lee, and S. G. Hong. "Sludge Chemical Composition Changes Under Uni-directional Freezing." *Water Science and Technology* 34, no. 3-4 (1996): 525-531.

Hung, W. T., I. L. Chang, W. W. Lin, and D. J. Lee. "Unidirectional Freezing of Waste-Activated Sludges: Effects of Freezing Speed." *Environmental Science & Technology* 30, no. 7 (1996b): 2391-2396.

Khanal, Samir Kumar, David Grewell, Shihwu Sung, and Hans J. Leeuwen. "Ultrasound Applications in Wastewater Sludge Pretreatment: A Review." *Critical Reviews in Environmental Science and Technology* 37, no. 4 (2007): 277-313.

Li, Bing, and Da-Wen Sun. "Effect of Power Ultrasound on Freezing Rate During Immersion Freezing of Potatoes." *Journal of Food Engineering* 55, no. 3 (2002): 277-282.

Liu, Ling, Osato Miyawaki, and Kiro Hayakawa. "Progressive Freeze-Concentration of Tomato Juice." *Food Science and Technology* 5, no. 1 (1999): 108-112.

Liu, Xiao, Wei Wang, Xingbao Gao, Yingjun Zhou, and Renjie Shen. "Effect of Thermal Pretreatment on the Physical and Chemical Properties of Municipal Biomass Waste." *Waste Management* 32, no. 2 (2012): 249-255.

Mason, T. J., L. Paniwnyk, and J. P. Lorimer. "The Uses of Ultrasound in Food Technology." *Ultrasonics Sonochemistry* 3, no. 3 (1996): S253-260.

Montusiewicz, A., M. Lebiocka, A. Rozej, E. Zacharska, and L. Pawlowski. "Freezing/thawing Effects on Anaerobic Digestion of Mixed Sewage Sludge." *Bioresource Technology* 101 (2010): 3466 - 3473.

Muller, M, and I. Sekoulov. "Waste Water Reuse By Freeze Concentration with a Falling Film Reactor." *Water Science & Technology* 26, no. 7-8 (1992): 1475-1482.

Pilli, Sridhar, Puspendu Bhunia, Song Yan, R. J. LeBlanc, R. D. Tyagi, and R. Y. Surampalli. "Ultrasonic Pretreatment of Sludge: A Review." *Ultrasonics Sonochemistry* 18, no. 1 (2011): 1-18.

Sanchez, J., Y. Ruiz, J.M. Auleda, E. Hernandez, and M. Raventos. "Review: Freeze Concentration in Fruit Juices Industry." *Food Science and Technology International* 15, no. 4 (2009): 303-315.

Wang, F., M. Ji, and S. Lu. "Influence of Ultrasonic Disintegration on the Dewaterability of Waste Activated Sludge." *Environmental Progress* 25, no. 3 (2006): 257-260.

Wang, Fen, Yong Wang, and Min Ji. "Mechanisms and Kinetics Mondels for Ultrasonic Waste Activated Sludge Disintegration." *Journal of Hazardous Materials* 123, no. 1-3 (2005): 145-150.

Weemaes, Marjoleine P. J., and Willy H. Verstraete. "Evaluation of Current Wet Sludge Disintegration Techniques." *Journal of Chemical Technology & Biotechnology* 73, no. 2 (1998): 83-92.

Zhang, Panyue, Guangming Zhang, and Wei Wang. "Ultrasonic Treatment of Biological Sludge: Floc Disintegration, Cell Lysis and Inactivation." *Bioresource Technology* 98, no. 1 (2007): 207-210.

Zheng, Liyun, and Da-Wen Sun. "Innovative Applications of Power Ultrasound During Food Freezing Processes: A Review." *Trends in Food Science & Technology* 17, no. 1 (2006): 16-23.

CHAPTER 6
RELATIONSHIP BETWEEN SOLUBILISATION OF COD, DEWATERABILITY AND PARTICLE SIZE FOR SECONDARY MUNICIPAL SLUDGE

In this chapter, different pretreatment techniques and their effect on sludge particle size were investigated. The relationship between solubilisation of COD, dewaterability (both filterability and settleability) and particle size was then investigated for secondary municipal sludge following the various pre-treatment methods. The pre-treatment methods applied were freeze-thaw, ultrasonic freezing, ultrasound, thermal and microwave. The relationship between particle size and dewaterability remained fairly constant across all treatments with increases in particle size correlated to improved dewaterability. On the other hand, the relationship between particle size and solubilisation of COD seemed to depend on the treatment method. For freezing treatments, particle size was positively correlated to sCOD concentrations. Pearson's correlation coefficient between average particle size and sCOD was 0.5705 and 0.8241 for conventional freezing and combined ultrasonic freezing respectively. Conversely, for ultrasound treatment the Pearson correlation coefficient was -0.6388 indicating that a decrease in particle size resulted in an increase in sCOD.

6.1 Introduction

Secondary sludge treatment has become the norm for many wastewater treatment plants. These biological processes produce a large amount of waste sludge which is known to be difficult to both digest and dewater. Since a considerable portion of a wastewater treatment plant`s costs originate from sludge management (Canales et al., 1994), digestion and dewatering are two key processes as they achieve sludge volume reduction (Andreottola and

Foladori, 2006; Lawler et al., 1986). While conditioning methods have long been used to help improve dewaterability of secondary sludge, recent studies in the field of sludge management are focusing on pretreatment methods that are intended to increase the digestion efficiency of secondary sludge. Many pretreatment techniques require the addition of a treatment step prior to digestion (Andreottola and Foladori, 2006). With sludge management already accounting for up to 60% of wastewater treatment plants operating costs (Weemaes and Verstraete, 1998), it would be very beneficial to incorporate a method that could improve sludge dewaterability and digestibility at once.

Several studies examining the effect of particle size on dewaterability following sonication, microwave treatment, conventional freezing and anaerobic digestion have found that as particle size decreases, dewaterability decreases as well (Apul et al., 2010; Chu et al., 2001; Yu et al., 2009; Karr and Keinath, 1978; Lawler et al., 1986). Moreover, it has been shown that supracolloidal particles (those between 1 - 100μm) have the greatest negative effect on sludge dewaterability (Karr and Keinath, 1978; Kennedy et al., 2007). Conversely, solubilisation of organic matter has shown to improve as sludge flocs are broken down and particle size decreases (Feng et al., 2009; Kennedy et al., 2007). If this is in fact true for all treatment methods, it is problematic as it suggests that the goal of finding a combined treatment for enhanced digestion and dewaterability would not be achievable.

The objective of this study is to determine the relationship between particle size, dewaterability and solubilisation of COD. Secondary sludge will be subjected to various pretreatment methods such as conventional freeze-thaw, ultrasonic freezing, ultrasound, thermal and microwave. The effect of these treatments on the overall particle size distribution as well as standard percentile particle sizes will be determined. Additionally, the relationship between particle size, soluble COD concentration and dewaterability of the sludge will be assessed by finding the correlation coefficients between each of the given parameters.

6.2 Materials & Methods

6.2.1 Sludge Samples

Thickened waste secondary sludge (TWSS) was obtained from Thunder Bay's wastewater treatment plant located in Thunder Bay, Ontario, Canada. Sludge was stored in the refrigerator in closed jars at 4°C if not immediately treated. Characteristics of the TWSS can be found in Table 6.1. All samples were diluted four times prior to treatment for the ease of testing.

Table 6.1: Characteristics of TWSS

Value	pH	TS (mg/L)	TSS (mg/L)	tCOD (mg/L)	sCOD (mg/L)	SVI (mL/g)	CST (s)
Average	6.55	37,245	35,826	50,583	1,903	27.4	102.7

6.2.2 Experimental Design

TWSS was exposed to five different potential pre-treatment methods: conventional freezing, combined ultrasonic-freezing, thermal, microwave and ultrasound. The effect of these treatment methods on particle size was examined. Duplicate runs were carried out for each treatment in order to minimize experimental and random error as well as to increase the precision of the results.

For conventional freezing, the effect of temperature and freeze-thaw cycles on particle size was examined. Two levels of temperature (-15 and -30°C) and three levels of freeze-thaw cycles (1, 3 and 5) were selected in a 2x3 fully crossed factorial design.

Combined ultrasonic-freezing experiments were also conducted as a 2x3 fully crossed factorial with two levels of sonication intensity (20 and 40%) and three levels of sonication time (2, 6 and 12 minutes). After sonication, the 600mL sludge samples were frozen for 24 hours at -15°C.

Ultrasound treatment examined the same treatment conditions as ultrasonic freezing, without freezing sludge after treatment. Microwave treatment consisted of heating 200mL sludge samples for one or three minutes and thermal treatment involved 600mL sludge samples being heated at 103°C for 2.5 hours. All treatment conditions were run in duplicates and are summarized in Table 6.2.

Table 6.2: Experimental condition of pretreatment methods investigated

Treatment	Experimental Condition
Freeze-Thaw	Freezing at -15 and -30°C for 1, 3 and 5 cycles
Combined Ultrasonic-Freezing	Sonication at 20 and 40% amplitude for 2, 6 and 12 minutes followed by 24 hours of freezing at -15°C
Ultrasound	Sonication at 20 and 40% amplitude for 2, 6 and 12 minutes
Microwave	Microwave at 700 W for 1 and 3 minutes
Thermal	Heated at 103°C for 150 minutes

6.2.3 Experiments

6.2.3.1 Freezing Treatment

A temperature controlled environmental room (Climatic Testing Systems Inc., Pennsylvania, USA) was used to freeze sludge samples at -15 and -30°C. The temperature fluctuation of the freezer was ±0.5°C. The six 600mL sludge samples were placed in 1L polyethylene beakers and frozen in the cold room. After 24 hours of freezing, they were removed to thaw at room temperature for a further 24 hours. The process was then repeated for those samples requiring multiple freeze-thaw cycles.

6.2.3.2 Ultrasound and Combined Ultrasonic-Freezing Treatments

Sludge samples requiring ultrasound as part of their treatment were sonicated using a Sonics Vibra-Cell High Intensity Ultrasonic Processor model VC750 (Sonics & Materials Inc., Connecticut, USA). Sludge samples of 600mL were sonicated using the 2.54 cm (1") diameter probe at 20 and 40 percent power with sonication times of 2, 6 and 12 minutes. The ultrasonic-freezing samples were then frozen at -15°C in the freezer described in section 6.2.3.1 for 24 hours then thawed at room temperature for another 24 hours.

6.2.3.3 Thermal Treatment

A Precision Thelco Laboratory Oven (Model 70, Thermo Scientific, Massachusetts, USA) was used to heat 600 mL of secondary sludge at 103°C for 2.5 hours. This corresponded to a final temperature of approximately 75°C. The samples were then left to cool to approximately room temperature prior to analysis.

6.2.3.4 Microwave Treatment

A Danby microwave-oven (model DMW607W), was used to microwave 200mL of sludge samples in sealed glass containers at maximum power for one and three minutes. This corresponds to a final temperature of 46 and 77 °C respectively.

6.2.4 Sample Analysis

The particle size distributions of the sludge samples were determined using a Mastersizer 2000 (Malvern Canada, Quebec, Canada) particle size analyzer which is able to measure the percent by volume of particles in the range of 0.02 – 2000μm. Overall particle distributions were obtained as well as standard percentile readings at 10, 50 and 90% by volume of the particles' diameters, expressed as d10, d50 and d90 respectively.

Sludge dewaterability was assessed in terms of settleability and filterability. Using a 1L graduated cylinder, sludge volume index (SVI) was used as a measure of settleability while capillary suction time (CST) was taken as a measure of filterability. Total and soluble chemical oxygen demand (tCOD, sCOD) were measured as an indication of solubilisation of sludge organic matter. All measurements were taken before and after treatment following the procedures outlined in the *Standard Methods for Examination of Water and Wastewater* (APHA, 2005).

6.2.5 Data Analysis

The data collected for the treated samples were normalized to those of the controls using ratios, C/C_o, where C_o is the value of the control samples and C is the value of the treated samples. Concentration ratios allowed for the comparison of data obtained from different batches of sludge samples collected.

In order to determine if the effect of the pretreatment methods on the standard percentile of sludge particle size readings was significant, the ratio of their sizes were compared using analyses of variance (ANOVAs). The Tukey test (a post-hoc test) was used in order to determine which means were statistically different from each other. The significance level for these tests were set at 95% ($\alpha = 0.05$).

The strength and direction of the relationship between particle size, solubilisation of COD and dewaterability was measured using Pearson's correlation coefficient, r. The correlation coefficient was calculated for each combination of parameters using the raw data from each set of runs. The computing environment R (R Development Core Team, 2008; Vienna, Austria) was used to complete the statistical analysis.

6.3 Results and Discussion

6.3.1 Conventional Freezing Treatment

6.3.1.1 Effect of Freezing Treatment on Particle Size

Secondary sludge treated with conventional freezing showed an increase in the particle size leaving a smaller volume of particles in the supracolloidal range and a much greater volume above 100 µm as shown in Figure 6.1 and 6.2. These results are in agreement with those of other researchers who have reported that freezing resulted in larger and more compact flocs (Chu et al., 1999; Gao, 2011; Jean et al., 2000; Vesilind and Martel, 1990).

In terms of standard percentiles, freezing showed a significant increase in particle size for d10, d50 and 90, with ratios of 3.39, 4.17 and 2.59 respectively.

Effect of Freezing Temperature

When the freezing temperature decreased from -15 to -30°C, the particle size decreased from a maximum of 7.1 to 6.0 times of the control at approximately 400 µm (Figure 6.1). This is consistent with the results reported by Chu et al. (1999) and Hung et al. (1996b) who found that increased freezing speeds, which can be achieved by colder freezing temperatures, resulted in decreased particle size. Chu et al. (1999) suggested that slower freezing promotes gross migration of sludge flocs which allows for particles to aggregate and therefore increase in size. Particles that were trapped in the ice front were 1.3 times greater than the control, whereas those that underwent gross migration were up to 2.7 times greater than the control (Chu et al., 1999).

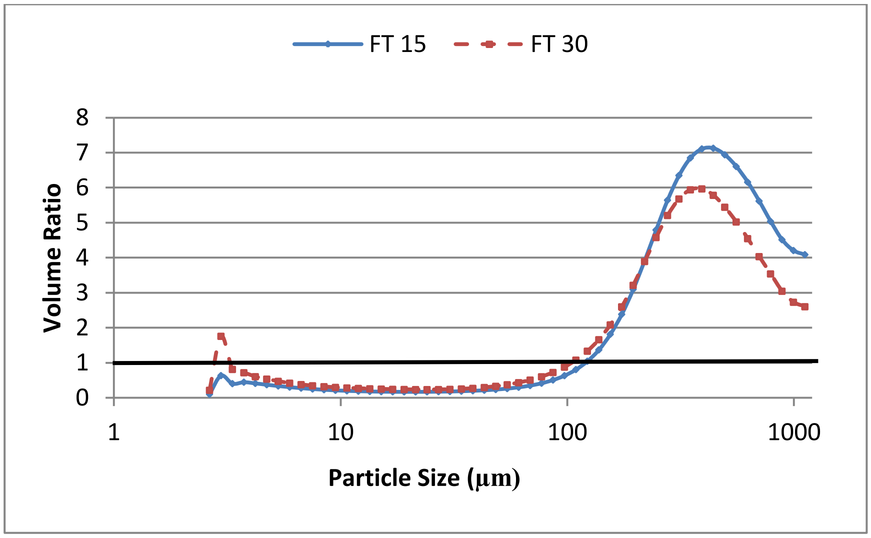

Figure 6.1: Effect of freezing temperature on particle size following conventional freezing treatment

Lowering the temperature from -15 to -30°C lowered the ratios for d10, d50 and d90 from 3.9 to 2.9, 4.6 to 3.7 and 2.8 to 2.4 respectively (Table 6.3). However, this decrease was only significant for d10.

Table 6.3: Effect of freezing temperature on standard percentile measurement ratios (C/C_o), where C = particle size of treated samples and C_o = particle size of the control samples.

Temperature	$d10/d10_o$	$d50/d50_o$	d90/d90
-15°C	3.89 ± 0.21	4.63 ± 0.26	2.75 ± 0.18
-30°C	2.92 ± 0.18	3.73 ± 0.35	2.45 ± 0.23

Effect of Freeze-Thaw Cycles

Increasing the freeze-thaw cycles from one to three to five also resulted in a less pronounced increase in particle size as shown in Figure 6.2. Sludge frozen for one cycle had a maximum increase of 7.3 times that of the control whereas sludge frozen and thawed five times only increased by a maximum of 5.8 times the control. It has been noted that freezing can cause microbial cell disruption due to the increased pressure put on the cell walls by the ice crystals (Ormeci and Vesilind, 2001; Stabnikova et al., 2008). Multiple freezing cycles would likely cause greater cell disruption and could very well account for the decrease in particle size found with multiple freeze-thaw cycles. In terms of freeze-thaw cycles, there was a small decrease in particle size as the number of freeze thaw cycles increased, but none were found to be significant ($p > 0.05$).

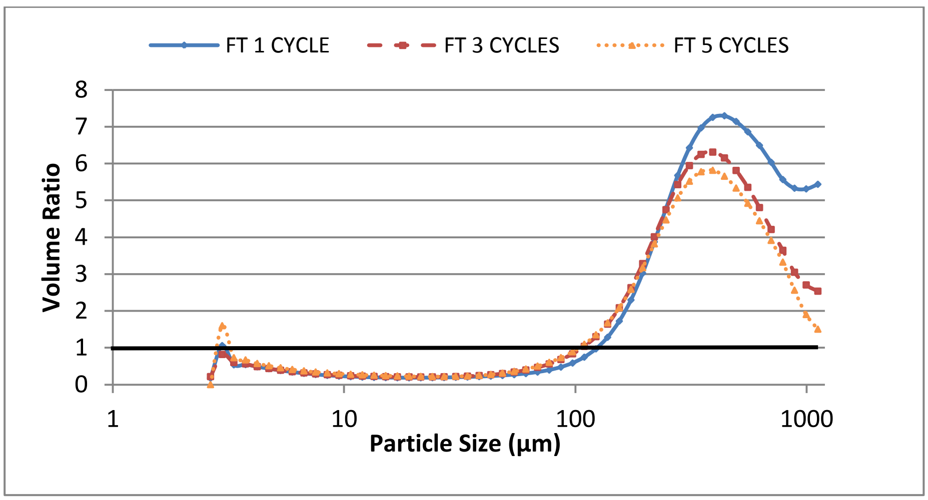

Figure 6.2: Effect of freeze-thaw cycles on particle size for conventional freezing treatment

6.3.1.2 Correlating Particle Size, Dewaterability and Solubilisation of Sludge Organic Matter for Freeze-Thaw Treatment

Table 6.4 lists the correlation coefficients between CST, SVI, sCOD and particle sizes. As shown from the magnitude and size of the Pearson coefficients, an increase in particle size (d50 or d90) corresponded to a slight increase in soluble COD and decrease in SVI and CST values. This is contrary to the belief that a decrease in particle size distribution leads to a greater extent of solubilisation of organic matter (Kennedy et al., 2007) but in agreement with Karr and Keinath (1978) who stated that filterability deteriorated with a decrease in particle size. There was also a significant ($p > 0.05$) negative correlation of -0.8406 and -0.5590 between solubilisation of COD and SVI and CST values respectively. For secondary sludge exposed to conventional freezing, as dewaterability improved (values of CST and SVI got smaller), there was an increase in soluble COD.

Table 6.4: Pearson correlation coefficients between SVI, sCOD, CST, d50 and d90 for conventional freezing

	sCOD	d50	d90
SVI	-0.8406	-0.8255	-0.7769
CST	-0.5590	-0.7021	-0.6626
sCOD	---	0.5705	0.5316

6.3.2 Ultrasonic Freezing Treatment

6.3.2.1 Effect of Ultrasonic Freezing on Particle Size

Ultrasonic freezing significantly increased the particle size of secondary sludge with standard percentiles (d10, d50 and d90) all showing significant increases compared to the control. Combined ultrasonic-freezing also resulted in a much smaller volume of particles in the supracolloidal range and a much larger volume above this range (Figure 6.3 and 6.4). All standard percentile volumes were significantly larger than the controls for secondary sludge exposed to combined ultrasonic-freezing treatment. Like conventional freezing, the increase in particle size is likely due to the aggregation of particles that occurs through gross migration during the freezing process (Chu et al., 1999).

Effect of Sonication Amplitude

Increasing the amplitude from 20 to 40% did not have a substantial effect on the supracolloidal particles but resulted in larger ratios for particles sizes greater than 100 µm (see Figure 6.2). Vesilind & Martel (1990) suggested that freezing of smaller particles can be more effective at creating stable larger flocs. It is possible that the increase in sonication power from 20 to 40% resulted in smaller particles which were more effectively aggregated into larger particles during the freezing process.

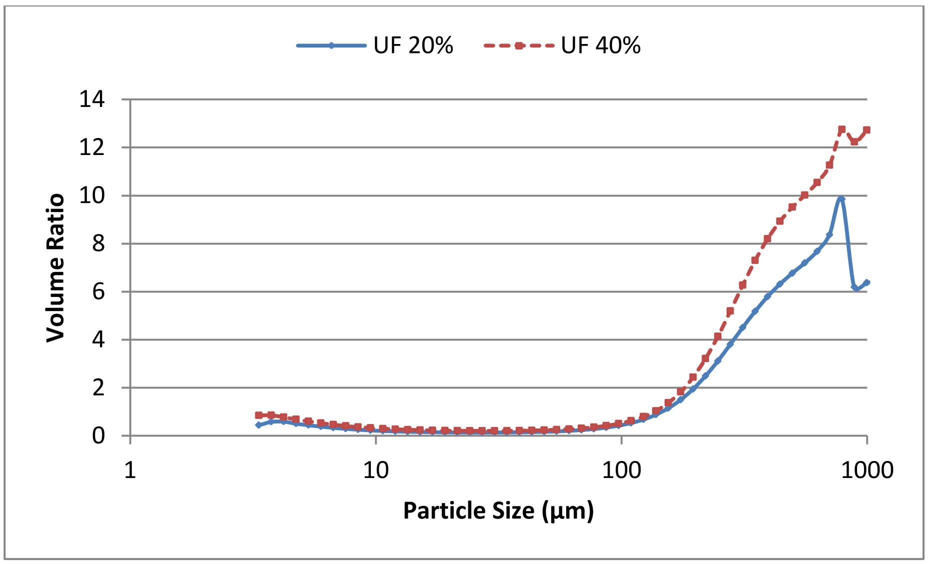

Figure 6.3: Effect of sonication amplitude on particle size for combined ultrasonic freezing treatment

Increasing the amplitude from 20 to 40% resulted in a significant decrease in ratio for d10 and a significant increase for d90 (Figure 6.4). The increase found for d50 was insignificant ($p > 0.05$).

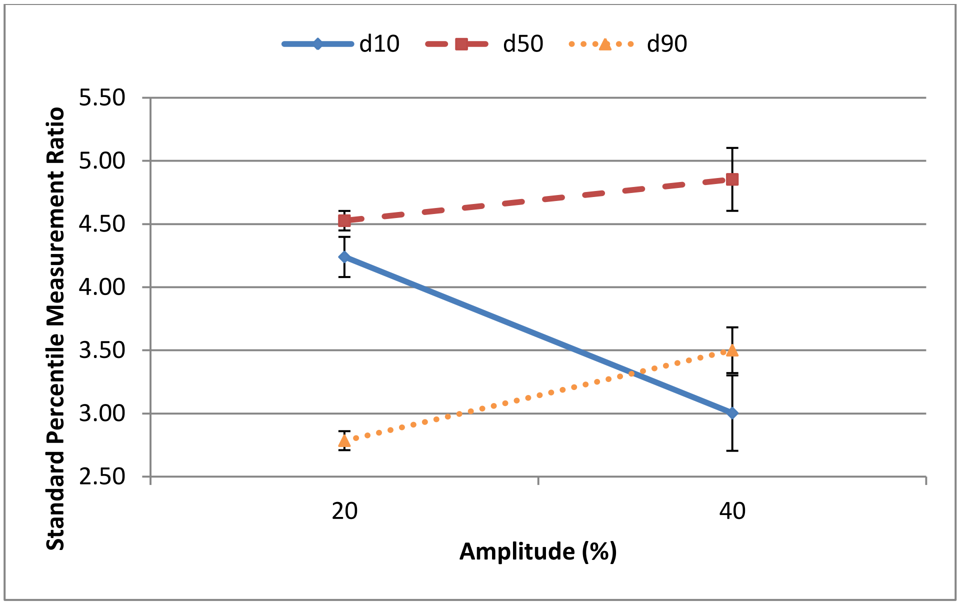

Figure 6.4: Effect of sonication amplitude on standard percentile measurement ratios (C/C_o) following combined ultrasonic-freezing, where C = particle size of treated samples and C_o = particle size of the control.

Effect of Sonication Time

An increase in sonication time also had very little effect on particle sizes below approximately 350 µm after which point an increase in sonication time resulted in a small reduction in the volume ratios (see Figure 6.5). Consequently, as sonication time increased, there was an insignificant decrease in ratio for all standard percentile volumes. This reduction is in agreement with the findings of Halde (1980) who suggest that smaller particles are more likely to get trapped in the ice front. However, it contradicts the findings of the previous section and of Vesilind & Martel (1990) which suggest that smaller particles are more effective at undergoing gross migration and forming larger aggregated particles. It is possible that there is a limit to the number or size of small particles that will be beneficial to increasing particle size.

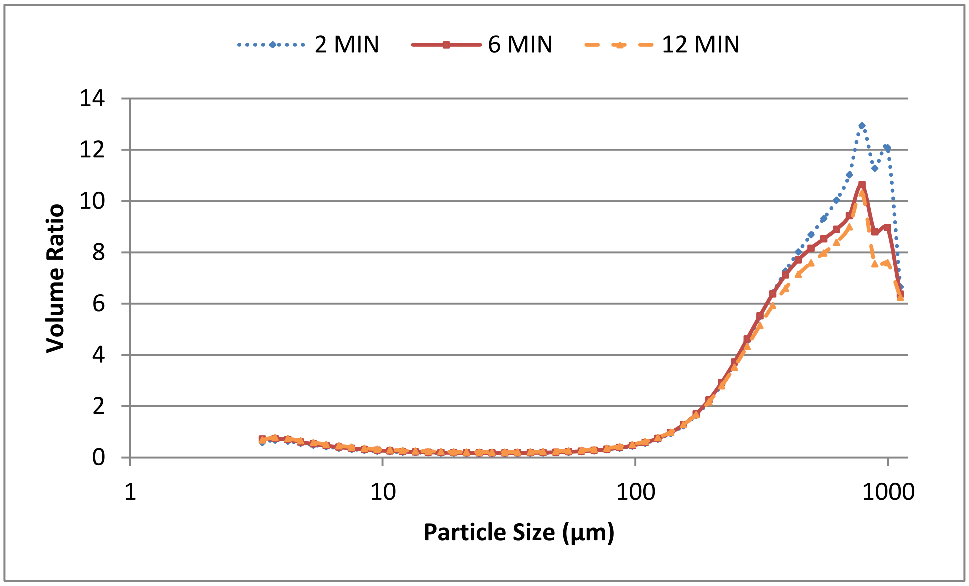

Figure 6.5: Effect of sonication time on particle size following combined ultrasonic-freezing treatment

6.3.2.2 Correlating Particle Size, Dewaterability and Solubilisation of Sludge Organic Matter for Combined Ultrasonic-Freezing Treatment

The correlation between particle size and dewaterability was very strong for secondary sludge treated by combined ultrasonic-freezing. The Pearson correlation coefficient was -0.9272 and -0.8063 for average particle size and SVI and CST respectively, supporting the findings of Karr and Keinath (1978). The correlation strengthened slightly between d90 and settleability to -0.9671 and remained about the same between filterability and d90.

There was also a strong positive correlation between particle size (both d50 and d90) and soluble COD. Like conventional freezing, an increase in particle size was correlated to an increase in solubilisation of organic matter. Both measurements of dewaterability, settleability and filterability, were negatively correlated to soluble COD concentration. As the values of SVI and CST decreased, dewaterability improved and soluble COD concentration increased. Results are summarized below in Table 6.5.

Table 6.5: Pearson correlation coefficients between SVI, sCOD, CST, d50 & d90 for combined ultrasonic-freezing.

	sCOD	d50	d90
SVI	-0.8770	-0.9272	-0.9671
CST	-0.8075	-0.8063	-0.7905
sCOD	---	0.8241	0.8642

6.3.3 Ultrasound Treatment

6.3.3.1 Effect of Ultrasound Treatment on Particle Size

Ultrasound treatment increased the volume of particles below approximately 30 µm. Above 30 µm, the volume of treated particles was less than the control. This is consistent with many other studies done on the effect of sonication on particle size, for example, Akin et al., 2006; Chu et al., 2001; and Feng et al., 2009. It is known that sonication results in cavitation, which is a major contributor to disintegration of sludge cells (Pilli et al., 2011; Zhanget al., 2007). It is the disintegration of sludge cells which results in the decrease in particle size. Overall, there was no significant effect of ultrasound on any of the standard percentile values examined ($p > 0.05$).

Effect of Sonication Amplitude

Sonication amplitude had very little overall effect on particle size, as shown in Figure 6.6. As amplitude increased from 20 to 40%, the volume ratio for particles below approximately 10 µm increased; however, beyond that, the effects were very small. There was also no significant difference found between the standard percentile ratios at sonication intensities of 20 and 40%. This is contrary to findings in previous studies that found that an increase in sonication density should result in a decrease in particle size (Akin et al., 2006; Chu et al., 2001). It may be explained by Feng et al. (2009) who suggest that a minimum energy dose of 8800 kJ/kg TS is required before significant reductions can be noticed. The only treatment that was above this energy dose in our treatment conditions was that of the sludge sonicated for 12 minutes at 40% amplitude.

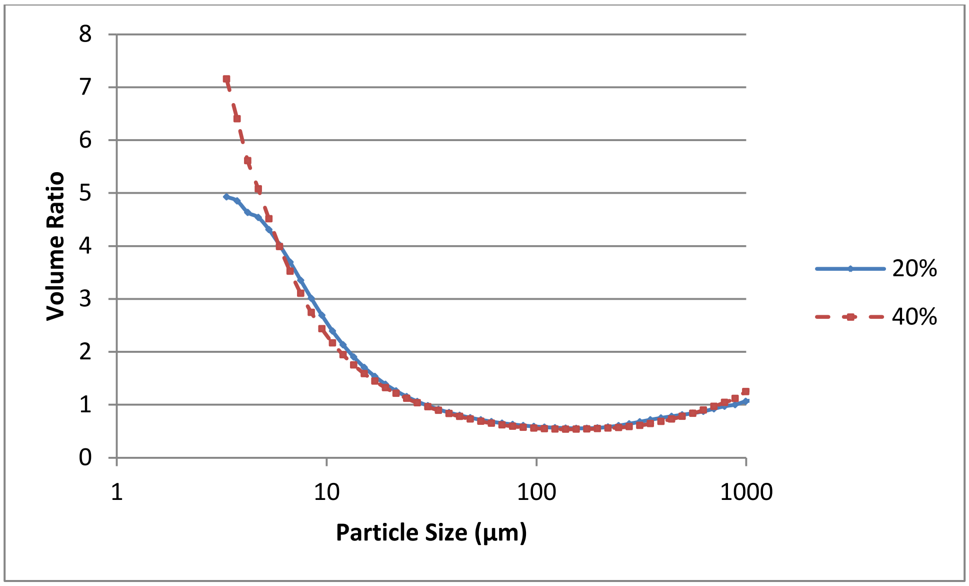

Figure 6.6: Effect of sonication amplitude on particle size following ultrasound treatment.

Effect of Sonication Time

Like sonication amplitude, sonication time had very little effect on the particle size. As shown in Figure 6.7, increasing treatment time, from two to twelve minutes, resulted in an increase in the volume ratio of particles below approximately 30 µm. When examining the standard percentile volumes, increasing the treatment time from two to six minutes significantly lowered the volume ratio for all standard percentiles. A further increase to twelve minutes resulted in an additional decrease in volume ratio; however, the decline was found to be insignificant except for the 90th percentile (Figure 6.8).

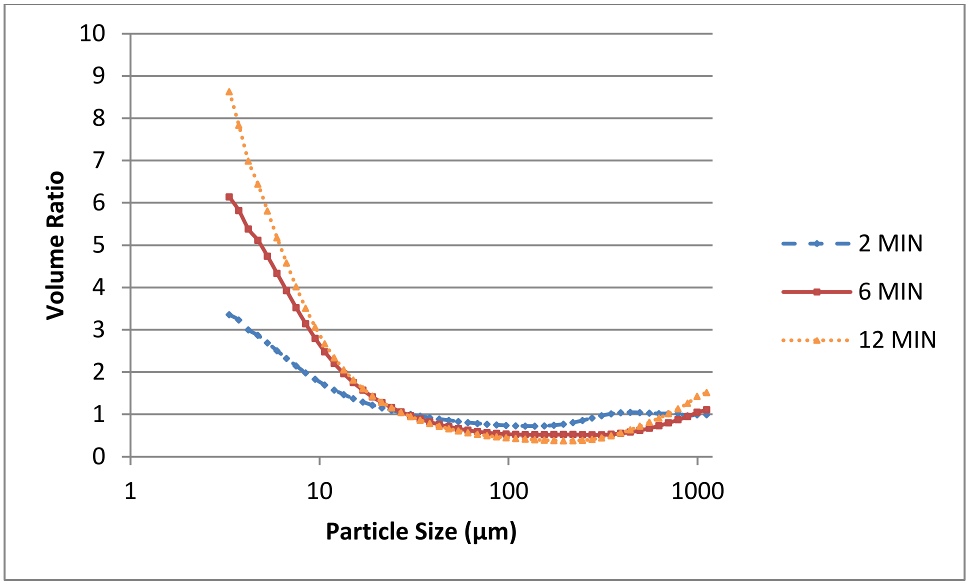

Figure 6.7: Effect of sonication time on particle size following ultrasound treatment.

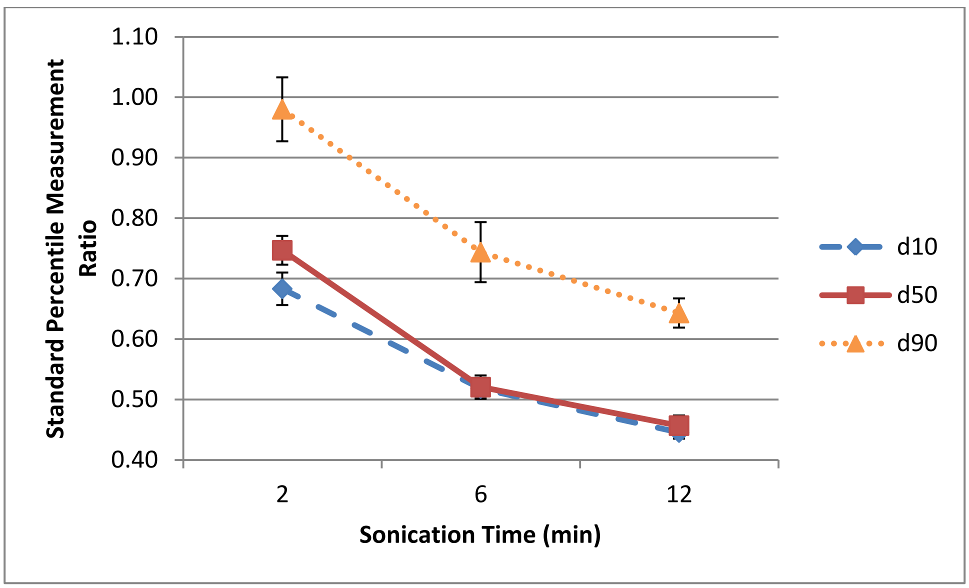

Figure 6.8: Effect of sonication time on standard percentile measurement ratios (C/C_o) following ultrasound treatment, where C = particle size of the treated sample and C_o = particle size of the control.

6.3.3.2 Correlating Particle Size, Dewaterability and Solubilisation of Sludge Organic Matter for Ultrasound Treatment

Average particle size showed a negative correlation of -0.6837 and -0.5557 between settleability and filterability respectively (Table 6.6). An increase in particle size correlated to the reduction of SVI and CST values, meaning that dewaterability improved. The strength of the correlation increased for settleability to -0.7373 and decreased slightly for filterability to -0.4072 between d90. The correlation between d90 and CST are not as strong as that found by Feng et al. (2009) in their study in which the r value for the correlation between d90 and CST was -0.9436.

Kennedy et al. (2007) suggested that particle size reduction is generally accompanied by an increase in solubilisation of organic matter which appears to have also been found in this study. Particle size was negatively correlated to sCOD concentration with correlation coefficients of -0.6388 and -0.5090 for d50 and d90 respectively. Unfortunately, an increase in soluble COD was also correlated with an increase in SVI and CST values. This means that as soluble COD concentrations increased, the dewaterability decreased.

Table 6.6: Pearson correlation coefficients between SVI, sCOD, CST, d50 and d90 for ultrasound treatment

	sCOD	d50	d90
SVI	0.7206	-0.6837	-0.7373
CST	0.7254	-0.5557	-0.4072
sCOD	---	-0.6388	-0.5090

6.3.4 Microwave Treatment

6.3.4.1 Effect of Microwave Treatment on Particle Size

When secondary sludge was exposed to microwave treatment, the volume ratio across all particle sizes, other than the extremities where the volume of particles is at its lowest, stayed fairly close to 1.0 (Figure 6.9). This was especially true for particles in the supracolloidal range (between 1-100 µm). That being said, the particles below approximately 100 µm were slightly below that of the control while above 100 µm it was consistently greater than the control. This suggests that microwave treatment up to three minutes may slightly increase the particle size for secondary sludge. There was no statistical significance between any of the standard percentile measurements and the control ($p > 0.05$).

Effect of Treatment Time

Increasing the treatment time from one to three minutes had very little effect on particle size (Figure 6.9). These findings differed from those of Yu et al. (2009) who found that particle size initially increased for treatment times up to one minute, and then decreased to values below the control beyond two minutes. Kennedy et al. (2007) determined that sludge heated to 85°C resulted in a redistribution of particles greater than 100µm into smaller sizes, but below this temperature, similar changes were not observed. Since the maximum temperature attained by the microwave treatment in this study was 77°C, this may explain why similar results were not found.

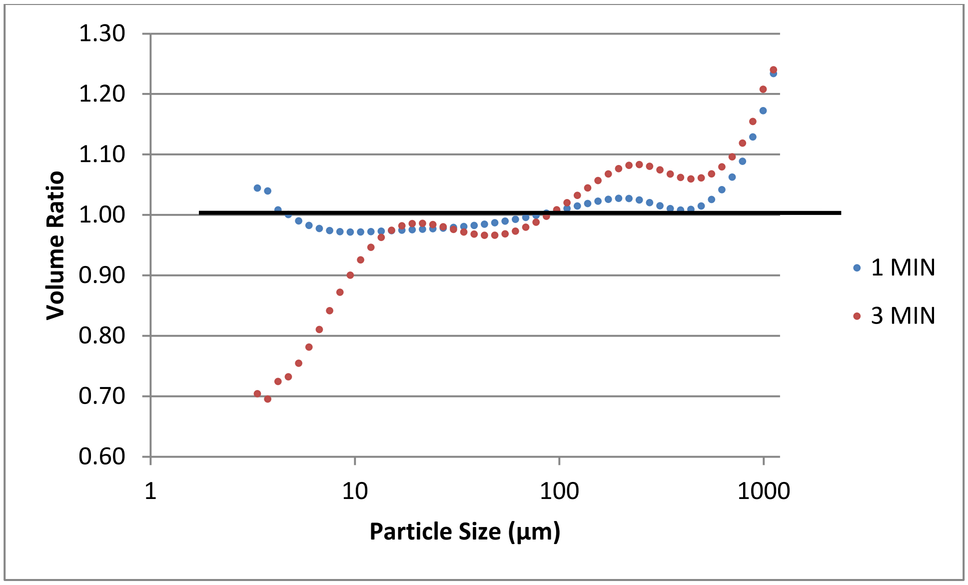

Figure 6.9: Effect of MW treatment time on particle size following microwave treatment.

When investigating the effect of microwave treatment time on particle size in terms of their standard percentiles (shown in Figure 6.10), it was found that the increase in particle size associated with a microwave time of one minute was insignificant compared to the control. Increasing the treatment time from one to three minutes significantly increased the d10 ratio from 1.02 to 1.06 and the d50 ratios from 1.02 to 1.05 times that of the control, but had no significant effect on d90 ratio. Yu et al. (2009) found that one minute of microwave time resulted in an increase in the d90; however, treatment over two minutes resulted in decreases to values less than the control. The difference in results could be due to the difference in temperature, although temperature was not reported in Yu et al. (2009).

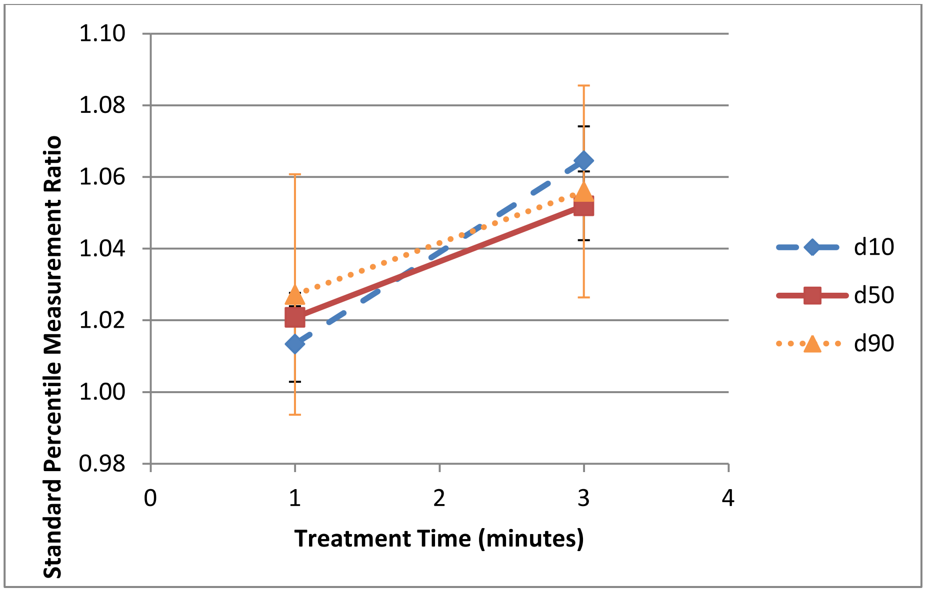

Figure 6.10: Effect of treatment time on standard percentile measurement ratios (C/C_o) following microwave treatment, where C = particle size of the treated sample and C_o = particle size of the control.

6.3.4.2 Correlating Particle Size, Dewaterability and Solubilisation of Sludge Organic Matter for Microwave Treatment

An investigation of the correlation between particle size (d50 and d90), filterability (CST), settleability (SVI) and solubilisation of COD (sCOD) for sludge that had undergone microwave treatment was conducted. A table of the correlation coefficients can be found in Table 6.7. A correlation coefficient of 0.6433 was obtained between average particle size (d50) and filterability. This indicates that an increase in average particle size, corresponded to an increase in CST values and hence a decrease in filterability. This is not in alignment with results of Karr and Keinath (1978) who determined that a decrease in particle size results in a deterioration of filterability. For the 90th percentile, the correlation coefficient for between filterability and particle size decreased to 0.4404 which was found to be insignificant ($p > 0.05$). These results were in opposition to previous research by Yu et al. (2009) who found a strong positive correlation ($r = 0.8596$) between filterability and d90. The discrepancy between these results could be due to the fact that the temperature range examined in this study was lower than that of Yu et al. (2009).

There was almost no correlation between settleability and average particles size, with the correlation coefficient being 0.0565. The correlation between d90 and SVI became negative (r = -0.2406), but was also found to be insignificant.

Solubilisation of sludge organic matter showed an insignificant positive correlation to particle size with correlation coefficients of 0.4321 and 0.3728 for d50 and d90 respectively. In terms of dewaterability, an increase in soluble COD was strongly correlated to a decrease in filterability (r = 0.8654). The correlations between many of the parameters examined for microwave treatment were very low compared to other treatment methods. This could be due to the fact that only two treatment times were examined (one and three minutes) and very little difference was found between these treatment times. As suggested by Kennedy et al. (2007), a minimum temperature of 85°C may be required in order to see any significant changes in parameters.

Table 6.7: Pearson correlation coefficients between SVI, sCOD, CST, d50 and d90 for microwave treatment

	sCOD	**d50**	**d90**
SVI	-0.4046	0.0565	-0.2406
CST	0.8654	0.6433	0.4404
sCOD	---	0.4321	0.3728

6.3.5 Thermal Treatment

6.3.5.1 Effect of Thermal Treatment on Particle Size

Thermal treatment had a mixed effect on particle size distribution. There was an increase of very fine particles (smaller than 30 µm) and decrease in very large particles (greater than 700) while the particles in between remained between 0.9 and 1.1 times the control (Figure 6.11). Although there was a small decrease in all standard percentile measurements, the decrease was found to be insignificant ($p > 0.05$).

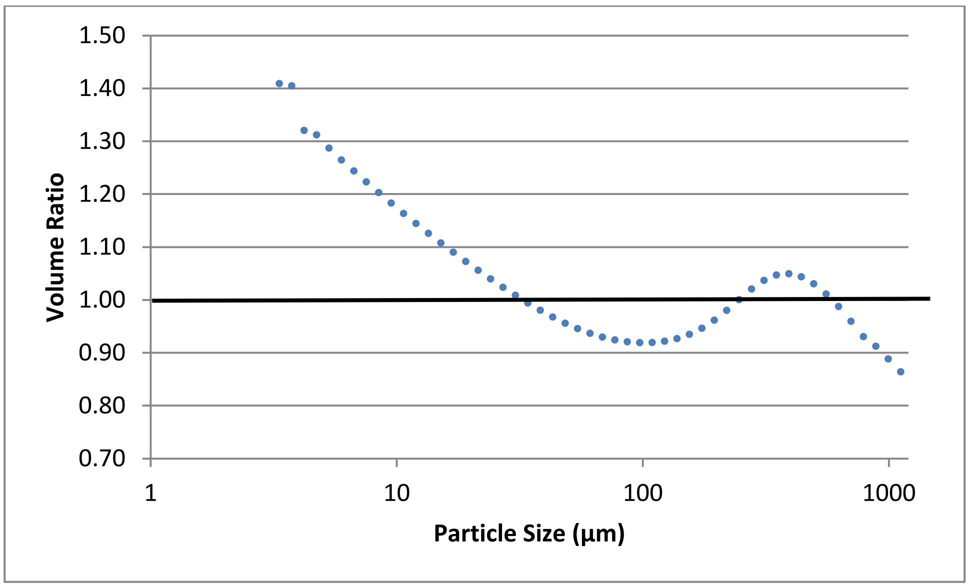

Figure 6.11: Effect of thermal treatment on particle size

6.3.5.2 Correlating Particle Size, Dewaterability and Solubilisation of Sludge Organic Matter for Thermal Treatment

There was a strong positive correlation between the average particle size (d50) and SVI, indicating that an increase in average particle size resulted in a deterioration in settleability. The relationship reversed for the 90th percentile; an increase in the 90th percentile size resulted in improved settleability. The exact opposite was true for CST, increase in average particle size improved filterability, while no significant correlation was found between the CST and d90. The only other correlation that was found to be significant ($p < 0.05$) was between soluble COD concentration and CST ($r = 0.8790$). As with sludge exposed to microwave, an increase in sCOD was correlated with higher CST values, hence worsening the filterability.

Table 6.8: Pearson correlation coefficients between SVI, sCOD, CST, d50 and d90 for thermal treatment

	sCOD	d50	d90
SVI	-0.4887	0.8173	-0.6978
CST	0.8790	-0.7387	0.5592
sCOD	---	-0.5018	0.2383

6.3.6 Comparison of the Effect of Different Treatments on Sludge Particle Size

Secondary sludge treated with freezing had a significant increase in their particle size compared to those that did not. Combined ultrasonic freezing treatment showed the largest increase in particle size for d10, d50 and d90 (3.62, 4.69, and 3.12), followed by freezing (3.39, 4.17 and 2.59), microwave (1.04. 0.93 and 1.04), thermal (0.91, 0.93 and 1.00) and finally ultrasound (0.55, 0.57 and 0.79). It is suspected that combined ultrasonic freezing results greater standard percentile ratios compared to freezing because smaller particles are more effective at aggregating during freeze-thaw compared to larger particles (Vesilind and Martel, 1990).

When comparing the correlation tables of the various treatments, it can be observed that conventional freezing and ultrasonic freezing are the only treatments that show a correlation between an improvement in filterability and an increase in sCOD concentration (Table 6.9).

The correlation between settleability and sCOD concentration for treatments subjected to freezing were also markedly larger than the other treatments.

Table 6.9: Significant correlations between particle size, dewaterability and sCOD for FT, UF, ULTRA, TH and MW

		sCOD	d50	d90
FT	SVI	-0.8406	-0.8255	-0.7769
	CST	-0.5590	-0.7021	-0.6626
	sCOD	---	0.5705	0.5316
UF	SVI	-0.8770	-0.9272	-0.9671
	CST	-0.8075	-0.8063	-0.7905
	sCOD	---	0.8241	0.8642
ULTRA	SVI	0.7206	-0.6837	-0.7373
	CST	0.7254	-0.5557	-0.4072
	sCOD	---	-0.6388	-0.5090
TH	SVI	---	0.8173	-0.6978
	CST	---	-0.7387	---
	sCOD	---	---	---
MW	SVI	---	---	---
	CST	0.8654	0.6433	---
	sCOD	---	---	---

As average particle size increased, mixed results were found in terms of concentration of soluble COD. Conventional freezing and ultrasonic treatment all had positive correlations between average particle size and concentration of sCOD. On the other hand, thermal and ultrasonic treatments showed a decrease in soluble COD concentration was correlated to an increase in the average particle size.

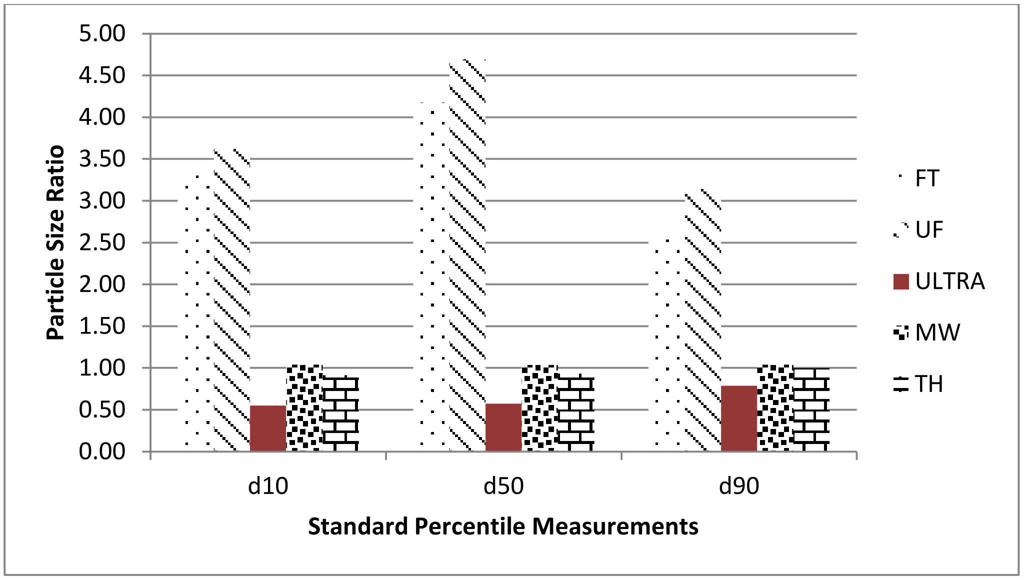

Figure 6.12: Comparison of the effect of conventional freezing (FT), combined ultrasonic-freezing (UF), ultrasound (ULTRA), microwave (MW) and thermal (TH) treatment on standard percentile ratios (C/C_o), where C = particle size of the treated Sludge, and C_o = particle size of the control sludge.

6.4 Conclusion

The following conclusions can be drawn from this chapter:

- Conventional freezing significantly increased the particles size of TWSS, resulting in a smaller volume of supracolloidal particles and a much greater volume of particles above 100 µm. The average particle size increased to 4.17 times of the control.
  - A decrease in freezing temperature from -15 to -30°C resulted in an overall decrease in particle size for particles greater than 400 µm. The decrease in standard percentile measurements was only significant for d10.
  - Increasing the number of freeze thaw cycles from one to three to five decreased the particle size; however, these results are insignificant for d10, d50 and d90.

- Combined ultrasonic-freezing resulted in the greatest increase in particle size of TWSS with the average particle size increased 4.69 times compared to the control.
    - Increase in amplitude from 20 to 40% results in an increased particle size for particles greater than approximately 100 µm. The d10 size at 20% is statistically smaller than that at 40%, while there was no significant difference between the d50s. The d90 ratio at 40% amplitude is significantly larger than at 20%.
    - Increase in sonication time from two to twelve minutes decreased particle size for particles greater than 350 µm. This decrease is statistically insignificant for d10, d50 and d90.
- Ultrasound treatment increased the volume of particles below 30 µm. Above 30 µm, the volume of the treated particles was less than the control. Overall, ultrasonic treatment had no effect on standard percentile ratios for d10, d50 or d90.
    - Increasing the amplitude from 20 to 40% had no significant effect on particle size distribution for d10, d50 and d90.
    - Increasing sonication from 2 to 6 minutes significantly decreased all standard percentile ratios, whereas a further increase to 12 minutes of sonication time only results in a significant decrease of the d90 ratio.
- Microwave treatment had an insignificant effect on particle size.
    - Increasing the treatment time from one to three minutes significantly increased the d10 (1.02 to 1.06) and the d50 (1.02 to 1.05) but had little effect on the d90. These results are contradictory to results found in other studies,

likely due to the fact that the temperature associated with the maximum treatment time of three minutes was below those of the other studies.

- Thermal treatment resulted in a reduced d10 and d50, with ratios of 0.91 and 0.93 respectively. The effect on the d90 ratio was statistically insignificant.
- The data collected in this study suggests that the correlation between
    - particle size and dewaterability is for the most part constant across various treatments; an increase in particle size is correlated to an improvement in dewaterability.
    - soluble COD and particle size varies from one treatment technique to another.
- Treatments that involve freezing are able to simultaneously improve dewaterability and soluble COD which could prove very useful if used as a pretreatment technique.

6.5 References

Akin, Beril, Samir Kumar Khanal, Shihwu Sung, David Grewell, and Hans van Leeuwen. "Ultrasound Pretreatment of Waste Activated Sludge." *Water Science & Technology* 6, no. 6 (2006): 35-42.

Andreottola, Gianni, and Paola Foladori. "A Review and Assessment of Emerging Technologies for the Minimization of Excess Sludge Production in Wastewater Treatment Plants." *Journal of Environmental Science and Health, Part A* 41, no. 9 (2006): 1853-1872.

Apul, O. Guven, Ilgin Atalar, Gozde Zorba, and F. Dilek Sanin. "The Dewaterability of Disintegrated Sludge Samples Before and After Anaerobic Digestion." *Drying Technology* 28, no. 7 (2010): 901-909.

Canales, Angel, Alain Pareilleux, Jean Luc Rols, Gerard Goma, and Alain Huyard. "Decreased Sludge Production Strategy for Domestic Wastewater Treatment." *Water Science & Technology* 30, no. 8 (1994): 97-106.

Chu, C. P., Bea-Ven Chang, G. S. Liao, D. S. Jean, and D. J. Lee. "Observations on Changes in Ultrasonically Treated Waste-Activated Sludge." *Water Research* 35, no. 4 (2001): 1038-1046.

Chu, C.P, W.C. Feng, Bea-Ven Chang, C.H. Chou, and D.J. Lee. "Reduction of Microbial Density Level in Wastewater Activated Sludge Via Freezing and Thawing." *Water Research* 33, no. 16 (1999): 3532-3535.

Feng, Xin, Jinchuan Deng, Hengyi Lei, Tao Bai, Qingjuan Fan, and Zhaoxu Li. "Dewaterability of Waste Activated Sludge With Ultrasound Conditioning." *Bioresource Technology* 100, no. 3 (2009): 1074-1081.

Gao, Wa. "Freezing as a Combined Wastewater Sludge Pretreatment and Conditioning Method." *Desalination*, 2011: 170-173.

Halde, Rolf. "Concentration of Impuritites by Progressive Freezing." *Water Research* 14, no. 6 (1980): 575-580.

Hung, W. T., I. L. Chang, W. W. Lin, and D. J. Lee. "Unidirectional Freezing of Waste-Activated Sludges: Effects of Freezing Speed." *Environmental Science & Technology* 30, no. 7 (1996b): 2391-2396.

Jean, D. S., C. P. Chu, and D. J. Lee. "Effects of Electrolyte and Curing on Freeze/Thaw Treatment of Sludge." *Water Research* 34, no. 5 (2000): 1577-1583.

Karr, P.R., and T.M. Keinath. "Influence of the Particle Size on Sludge Dewaterability." *Journal of the Water Pollution Control Federation* 50, no. 8 (1978): 1911-1930.

Kennedy, Kevin J., Gabriel Thibault, and Ronald L. Droste. "Microwave Enhanced Digestion of Aerobic SBR Sludge." *Water SA* 33, no. 2 (2007): 261-270.

Lawler, Desmond F., Yoon Jin Chung, Shiaw-Jy Hwang, and Barbara A. Hull. "Anaerobic Digestion: Effects on Particle Size and Dewaterability." *Water Pollution Control Federation* 58, no. 12 (1986): 1107-1117.

Ormeci, Banu, and Aarne P. Vesilind. "Effect of Dissolved Organic Material and Cations on Freeze-Thaw Conditioning of Activated and Alum Sludges." *Water Research* 35, no. 18 (2001): 4299-4306.

Pilli, Sridhar, Puspendu Bhunia, Song Yan, R. J. LeBlanc, R. D. Tyagi, and R. Y. Surampalli. "Ultrasonic Pretreatment of Sludge: A Review." *Ultrasonics Sonochemistry* 18, no. 1 (2011): 1-18.

Stabnikova, O., X.Y. Liu, and J.Y. Wang. "Digestion of Frozen/Thawed Food Waste in the Hybrid Anaerobic Solid-Liquid System." *Waste Management*, 2008: 1654-1659.

Vesilind, Aarne P., and James C. Martel. "Freezing of Water and Wastewater Sludges." *Journal of Environmental Engineering* 116, no. 5 (1990): 854-862.

Weemaes, Marjoleine P. J., and Willy H. Verstraete. "Evaluation of Current Wet Sludge Disintegration Techniques." *Journal of Chemical Technology & Biotechnology* 73, no. 2 (1998): 83-92.

Yu, Qiang, Hengyi Lei, Guangwei Yu, Zhaoxu Li, and Zhicheng Wu. "Influence of Microwave Irradiation on Sludge Dewaterability." *Chemical Engineering Journal*, 2009: 88-93.

Zhang, Panyue, Guangming Zhang, and Wei Wang. "Ultrasonic Treatment of Biological Sludge: Floc Disintegration, Cell Lysis and Inactivation." *Bioresource Technology* 98, no. 1 (2007): 207-210.

CHAPTER 7
CONCLUSIONS

The pretreatment of secondary municipal sludge with conventional freezing resulted in substantial solubilisation of sludge organic matter as well as significant improvements in dewaterability compared to control samples. Freezing temperature, -15 and -30°C was not a factor in the solubilisation of organic matter or dewaterability while freeze-thaw cycles had a limited effect on solubilisation of COD and disintegration of TSS. Increasing the number of freeze-thaw cycles from one to three significantly increased the TSS and sCOD ratios, but a further increase to five cycles had no significant effect. The effect of freeze-thaw cycles on dewaterability was negligible.

This study was the first to investigate the combination of ultrasound and freezing as a treatment method. Combined ultrasonic-freezing treatment, where sludge was frozen after being sonicated was also found to be an effective method of solubilisation of sludge organic matter and dewaterability. Sonication time did not affect the solubilisation of organic matter or the dewaterability of the treated sludge. Increasing the amplitude from 20 to 40% had no effect on solubilisation of organic matter, but improved the dewaterability of the sludge samples.

Compared to ultrasound, microwave and thermal treatment, other commonly studied pretreatment methods, the freezing treatments ranked second in terms of solubilisation of COD (behind thermal treatment) and first in terms of enhanced dewaterability. This provides evidence that freezing would make a very good combined pretreatment and conditioning method. When comparing one cycle of conventional freezing to combined ultrasonic-freezing treatment, it was found that sonicating sludge for just two minutes at 40% amplitude resulted in significantly greater solubilisation of organic matter than freezing alone.

Progressive ultrasonic freezing was attempted as a second combined ultrasound and freezing treatment method. Although bottom-up freezing was not perfectly attained, the treated sludge still showed significant increases in soluble COD and TSS disintegration. The frozen portion of the samples showed large improvements in dewaterability while the unfrozen liquid portion showed decreased filterability. Both pulse time and sonication time were found have an insignificant effect on solubilisation of sludge organic matter while sonication time significantly deteriorated the filterability of the liquid portion of the treated samples.

When further tests were conducted, it was found that all three freezing treatments also resulted in a similar significant increase in soluble protein as well as increased biodegradability and gas production compared to the control samples. In terms of gas production, the most common method of measuring the efficiency of anaerobic digestion, conventional freezing had the greatest increase in gas production, followed by the liquid portion of the progressive ultrasonic freezing samples and finally combined ultrasonic freezing samples.

An investigation into the effect of treatment methods on particle size found that conventional freezing and combined ultrasonic freezing resulted in an increase in standard percentile measurements of d10, d50 and d90 while the other methods investigated (thermal, microwave and ultrasound) all resulted in insignificant changes. For most treatments, an increase in particle size was strongly correlated to an improvement in filterability and settleability. On the other hand, the correlation between particle size and soluble COD concentration depended on the treatment type. For both freezing treatments investigated, conventional freezing and combined ultrasonic freezing, an increase in particle size was correlated to an increase in soluble COD concentrations. This was contrary to ultrasound in which a decrease in particle size was correlated to an increase in soluble COD concentration. Both microwave and thermal treatments showed insignificant correlations between particle size and soluble COD. These findings suggest that the relationship between soluble COD and particle size depends on the type of treatment method used.

In summary, treatments that involved freezing showed the ability to significantly solubilise sludge organic matter while simultaneously improving dewaterability (Table 7.1). Furthermore, sonicating sludge for just two minutes prior to one cycle of freezing, resulted in a significant improvement in solubilisation of sludge organic matter. The implication of these results on municipal sludge treatment could be considerable as major sludge reduction could be achieved in one single treatment step.

Table 7.1: Average ratios for sCOD, TSS, CST & SVI following various pre-treatment methods.
* indicates that treatment was significantly different than the control values

Treatments	sCOD	TSS	CST	SVI
FT	4.7*	0.886*	0.160*	0.154*
UF	4.7*	0.895*	0.166*	0.192*
PUF-liquid	6.3*	0.839	2.937	---
PUF-solid	3.7*	0.806	0.212	---
TH	5.9*	0.895	2.004*	0.950
MW	2.0	1.057	1.75*	0.932
ULTRA	2.5*	0.911*	1.919*	1.146*

7.1 Recommendations for Future Work

The results of this study showed that freezing can be used to solubilise sludge organic matter and increase the digestion efficiency of secondary municipal sludge. Additionally, for the first time, results showed that the addition of ultrasound prior to, or during freezing could increase the solubilisation of COD. It is recommended that additional bench or pilot scale tests are done in order to further investigate the factors affecting freezing and ultrasonic-freezing as well as to validate the results found in this study. Additional experiments are especially needed to verify the findings of the soluble protein, biodegradability and gas production analysis as duplicate runs were not able to be carried out.

In order to properly assess the feasibility of progressive ultrasonic freezing, further tests should also be carried out in which better bottom-up freezing is achieved. This can be done by better insulating the outside of the container and modifying the apparatus so that the tray is not used. Additionally, in order to determine the effect of sonication on the treatment, control samples should be progressively frozen without ultrasound, but some form of mixing and compared to the sonicated samples. Without these tests, it is difficult to determine whether increases in soluble organic matter in the liquid portion of the samples are due to the increased sonication they receive or the effect of the dissolved solids being rejected by the ice structure.

Finally, the measurements for filterability and settleability were taken after treatment, but before digestion. In order to replicate the municipal wastewater treatment process, dewaterability tests should be conducted after digestion, as digestion could have an effect on the dewaterability of the sludge samples.

APPENDIX A: EXPERIMENTAL RESULTS

Table A.1: Results of pH, TSS, sCOD, SVI and CST ratios (C/C_o) following conventional freezing treatment. C = concentration/value of the treated samples, C_o = concentration/value of the control samples.

	Conventional Freezing (-15°C)								
	Cycle 1			Cycle 3			Cycle 5		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
pH	0.9813	0.0140	0.0057	0.9613	0.0232	0.0104	0.9738	0.0144	0.0059
TSS	0.9669	0.0944	0.0334	0.8832	0.0785	0.0297	0.8313	0.0343	0.0121
sCOD	3.1993	0.8375	0.2961	4.4408	0.4994	0.1887	5.1253	0.9026	0.3191
SVI	0.1706	0.0098	0.0049	0.1389	0.0104	0.0052	0.1356	0.0101	0.0050
CST	0.1694	0.0800	0.0327	0.1264	0.0140	0.0063	0.1245	0.0069	0.0028
	Conventional Freezing (-30°C)								
	Cycle 1			Cycle 3			Cycle 5		
pH	0.9794	0.0249	0.0102	0.9809	0.0392	0.0160	1.0085	0.0719	0.0294
TSS	0.9909	0.0972	0.0344	0.8580	0.0799	0.0282	0.7873	0.0356	0.0126
sCOD	3.5046	1.0267	0.3630	5.5041	1.6393	0.5796	6.4590	2.0541	0.7262
SVI	0.1586	0.0182	0.0074	0.1647	0.0341	0.0139	0.1504	0.0292	0.0119
CST	0.2054	0.0989	0.0404	0.1731	0.0572	0.0234	0.1536	0.0427	0.0174

Table A.2: Results of pH, TSS, sCOD, SVI and CST ratios (C/C_o) following combined ultrasonic-freezing treatment. C = concentration/value of the treated samples, C_o = concentration/value of the control samples.

	Combined Ultrasonic-Freezing (20 %)								
	2 minutes			6 minutes			12 minutes		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
pH	1.0315	0.0231	0.0082	1.0278	0.0601	0.0212	1.0450	0.0421	0.0149
TSS	0.9152	0.0540	0.0221	0.9139	0.0891	0.0364	0.8841	0.0812	0.0332
sCOD	4.8430	1.2873	0.6437	4.7602	1.7504	0.8752	5.3160	2.0651	1.0325
SVI	0.2023	0.0405	0.0165	0.2132	0.0430	0.0176	0.2084	0.0436	0.0178
CST	0.1901	0.0608	0.0215	0.2116	0.0930	0.0329	0.1788	0.0689	0.0244
	Combined Ultrasonic-Freezing (40 %)								
	2 minutes			6 minutes			12 minutes		
pH	1.0079	0.0457	0.0187	1.0135	0.0712	0.0291	1.0021	0.0216	0.0088
TSS	0.9003	0.0824	0.0337	0.9047	0.0496	0.0203	0.8507	0.0469	0.0191
sCOD	4.2379	0.6051	0.3026	4.4762	0.6399	0.3199	4.5393	0.6734	0.3367
SVI	0.1823	0.0049	0.0020	0.1684	0.0073	0.0030	0.1773	0.0283	0.0116
CST	0.1222	0.0519	0.0212	0.1493	0.1044	0.0426	0.1166	0.0445	0.0182

Table A.3: Results of pH, TSS, sCOD, SVI and CST ratios (C/C_o) following ultrasound treatment. C= concentration/value of the treated samples, C_o = concentration/value of the control samples.

	Ultrasound (20 %)								
	2 minutes			6 minutes			12 minutes		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
pH	1.0289	0.0232	0.0095	1.0165	0.0330	0.0135	1.0083	0.0260	0.0106
TSS	1.0040	0.0367	0.0130	0.9367	0.0748	0.0264	0.8985	0.0548	0.0194
sCOD	1.4761	0.2839	0.1159	1.5607	0.6244	0.2549	2.4715	0.8949	0.3654
SVI	1.0150	0.0516	0.0211	1.1127	0.0842	0.0344	1.1659	0.0800	0.0327
CST	1.5396	0.3055	0.1155	1.9937	0.5871	0.2397	2.2643	0.8121	0.3315
	Ultrasound (40 %)								
	2 minutes			**6 minutes**			**12 minutes**		
pH	1.0267	0.0179	0.0073	1.0259	0.0175	0.0071	1.0005	0.0194	0.0079
TSS	0.9266	0.0212	0.0086	0.8515	0.0460	0.0188	0.8161	0.0537	0.0219
sCOD	1.7737	0.5272	0.2152	3.1456	1.1414	0.4660	4.5301	2.1317	0.8703
SVI	1.0997	0.0274	0.0112	1.2143	0.0757	0.0309	1.2684	0.0934	0.0381
CST	1.6376	0.2300	0.0939	1.8598	0.3489	0.1425	2.2816	0.5608	0.2289

Table A.4: Results of pH, TSS, sCOD, SVI and CST ratios (C/C_o) following microwave treatment. C= concentration/value of the treated samples, C_o = concentration/value of the control samples.

	Microwave					
	1 minute			3 minutes		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
pH	0.9977	0.0060	0.0030	1.0560	0.0166	0.0083
TSS	1.0325	0.0505	0.0253	1.0820	0.0195	0.0097
sCOD	1.9432	0.7190	0.3595	2.1147	0.6365	0.3182
SVI	0.9659	0.0500	0.0250	0.8985	0.0429	0.0214
CST	1.4907	0.3019	0.1510	2.0095	0.4935	0.2468

Table A.5: Results of pH, TSS, sCOD, SVI and CST ratios (C/C_o) following microwave treatment. C= concentration/value of the treated samples, C_o = concentration/value of the control samples.

	Thermal		
	103°C for 2.5 hours		
	Mean	Std. Dev	Std. Err
pH	0.9843	0.025546	0.010429
TSS	0.8947	0.064088	0.026164
sCOD	5.8825	1.995579	0.814692
SVI	0.9504	0.162516	0.066347
CST	2.0037	0.171243	0.06991

Table A.5: Comparison of average pH, TSS, sCOD, CST and SVI ratios (C/C_o) for conventional freezing, combined ultrasonic freezing, ultrasound, thermal and microwave treatment. C = concentration/value of the treated samples; C_o = concentration/value of the control samples.

	Conventional Freezing			Ultrasonic Freezing			Ultrasound			Thermal			Microwave		
	Mean	Std.Dev	Std.Err	Mean	Std.Dev	Std.Err	Mean	Std.Dev	Std.Err	Mean	Std.Dev	Std.Err	Mean	Std.Dev	Std.Err
pH	0.9814	0.0374	0.0063	1.0232	0.0465	0.0072	1.0188	0.0238	0.0040	0.9843	0.0256	0.0104	1.0269	0.0333	0.0118
TSS	0.8863	0.1017	0.0148	0.8948	0.0681	0.0114	0.9114	0.0780	0.0120	0.8947	0.0641	0.0262	1.0572	0.0442	0.0156
sCOD	4.7111	1.6754	0.2444	4.6954	1.2053	0.2460	2.4930	1.5020	0.2503	5.8825	1.9956	0.8147	2.0290	0.6353	0.2246
CST	0.1597	0.0630	0.0106	0.1660	0.0778	0.0120	1.9189	0.5545	0.0912	2.0037	0.1712	0.0699	1.7501	0.4694	0.1660
SVI	0.1541	0.0241	0.0044	0.1920	0.0344	0.0057	1.1460	0.1068	0.0178	0.9504	0.1625	0.0663	0.9322	0.0562	0.0199

Table A.6: Results of pH, TSS, sCOD and CST ratios (C/C_o) following progressive ultrasonic treatment. C= concentration/value of the treated samples, C_o = concentration/value of the control samples.

	Progressive Ultrasonic Freezing (Liquid)								
	2 second pulse; 12 minutes			3 second pulse; 12 minutes			3 second pulse; 25 minutes		
	Mean	Std.Dev	Std.Err	Mean	Std.Dev	Std.Err	Mean	Std.Dev	Std.Err
pH	1.0829	0.0362	0.0181	1.0503	0.0555	0.0278	1.0425	0.0217	0.0108
TSS	0.8477	0.0918	0.0459	0.8235	0.0321	0.0160	0.8454	0.1589	0.0794
sCOD	6.2258	2.0453	1.0227	5.5656	1.7307	0.8654	7.7136	0.3542	0.2505
CST	1.6984	0.1262	0.0893	1.8193	0.2118	0.1059	4.3882	2.2847	1.1423
	Progressive Ultrasonic Freezing (Solid)								
	2 second pulse; 12 minutes			3 second pulse; 12 minutes			3 second pulse; 25 minutes		
pH	1.0683	0.0405	0.0202	1.0551	0.0449	0.0224	1.0479	0.0152	0.0076
TSS	0.8436	0.0558	0.0279	0.8089	0.0813	0.0407	0.7643	0.0209	0.0105
sCOD	3.6943	0.3545	0.1772	3.9655	0.6160	0.3080	3.4374	0.4329	0.2165
CST	0.2211	0.1010	0.0505	0.1548	0.0308	0.0154	0.2602	0.0772	0.0386

Table A.7: Comparison of pH, TSS, sCOD and CST ratios (C/C_o) for solid and liquid portions of progressive ultrasonic freezing. C= concentration/value of treated samples; C_o = concentration/value of control samples.

	Progressive Ultrasonic Freezing					
	Liquid			Solid		
	Mean	Std.Dev	Std.Err	Mean	Std.Dev	Std.Err
pH	1.0586	0.0407	0.0118	1.0571	0.0337	0.0097
TSS	0.8389	0.0979	0.0283	0.8056	0.0626	0.0181
sCOD	6.2593	1.7582	0.5560	3.6991	0.4894	0.1413
CST	2.9369	1.8331	0.5527	0.2120	0.0820	0.0237

Table A.8: Results of soluble protein, biodegradation & gas production ratios (C/C_o) following conventional freezing treatment with TWSS.
C= concentration/value of the treated samples; C_o = concentration/value of the control samples.

	Conventional Freezing (-15°C)					
	1 cycle			3 cycles		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
Soluble Protein	4.8950	0.3756	0.2656	7.2850	0.0939	0.0664
Biodegradation	2.8742	0.2733	0.1933	1.9054	0.3008	0.2127
Gas Production	1.5235	1.2395	0.8765	1.6059	0.3910	0.2765

Table A.9: Results of soluble protein, biodegradation & gas production ratios (C/C_o) following combined ultrasonic-freezing treatment with TWSS.
C= concentration/value of treated samples; C_o = concentration/value of control samples.

	Combined Ultrasonic Freezing (20%)					
	2 minutes			12 minutes		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
Soluble Protein	5.1772	0.1174	0.0830	5.8743	0.0601	0.0212
Biodegradation	3.0808	1.2261	0.8670	1.9404	0.1626	0.1150
Gas Production	1.1059	0.5657	0.4000	1.3147	0.3619	0.2559

Table A.10: Results of soluble protein, biodegradation & gas production ratios (C/C_o) following progressive ultrasonic-freezing treatment with TWSS.
C= concentration/value of treated samples; C_o = concentration/value of control samples.

	Progressive Ultrasonic Freezing					
	Liquid			Solid		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
Soluble Protein	7.2034	0.2801	0.1981	5.1259	0.0939	0.0664
Biodegradation	2.1861	---	---	---	---	---
Gas Production	1.3176	---	---	---	---	---

Table A.11: Results of soluble protein and biodegradation ratios (C/C_o) following conventional freezing treatment with pulp and paper WAS.
C= concentration/value of the treated samples; C_o = concentration/value of the control samples.

	Conventional Freezing (-15°C)					
	1 cycle			3 cycles		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
Soluble Protein	1.9764	0.0384	0.0271	2.2237	0.1322	0.0935
Biodegradation	4.5417	0.4591	0.3247	4.0417	0.0777	0.0549

Table A.12: Results of soluble protein and biodegradation ratios (C/C_o) following combined ultrasonic-freezing treatment with pulp and paper WAS.
C= concentration/value of treated samples; C_o = concentration/value of control samples.

	Combined Ultrasonic Freezing (20 %)					
	2 minutes			12 minutes		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
Soluble Protein	2.4680	---	---	2.7877	---	---
Biodegradation	4.4077	0.2607	0.1844	5.0698	0.2459	0.1739

Table A.13: Results of soluble protein and biodegradation ratios (C/C_o) following progressive ultrasonic-freezing treatment with pulp and paper WAS.
C= concentration/value of treated samples; C_o = concentration/value of control samples.

	Progressive Ultrasonic Freezing					
	Liquid			Solid		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
Soluble Protein	5.8280	0.3668	0.2594	2.2810	0.3071	0.2172
Biodegradation	3.5518	---	---	---	---	---

Table A.14: Results of d10, d50 and d90 ratios (C/C_o) following conventional freezing treatment. C = size of the treated samples, C_o = size of the control samples.

	Conventional Freezing (-15°C)								
	Cycle 1			Cycle 3			Cycle 5		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
d10	4.2262	1.1816	0.4178	3.8617	0.8906	0.3366	3.4889	0.5320	0.2172
d50	5.2851	1.0841	0.3833	4.5709	1.1684	0.4416	3.8409	1.0235	0.4178
d90	3.0219	0.8756	0.3096	2.6600	0.8769	0.3315	2.4936	0.6590	0.2690
	Conventional Freezing (-30°C)								
	Cycle 1			**Cycle 3**			**Cycle 5**		
d10	3.1466	0.6981	0.2468	2.9167	0.9963	0.3522	2.6158	0.8046	0.3285
d50	4.3706	1.7616	0.6228	3.5303	1.4661	0.5183	3.1560	1.7358	0.7086
d90	2.6961	1.2432	0.4395	2.3726	0.9891	0.3497	2.2095	1.1559	0.4719

Table A.15: Results of d10, d50 and d90 ratios (C/C_o) following combined ultrasonic-freezing treatment. C = size of the treated samples, C_o = size of the control samples.

	Ultrasonic Freeze Thaw (20 %)								
	2 minutes			6 minutes			12 minutes		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
d10	4.1629	0.1123	0.0562	4.6730	0.4181	0.2091	3.8830	0.7054	0.3527
d50	4.4389	0.1512	0.0756	4.6065	0.3304	0.1652	4.5351	0.3353	0.1676
d90	2.7874	0.2598	0.1299	2.7715	0.2853	0.1426	2.7948	0.3153	0.1577
	Ultrasonic Freeze Thaw (40 %)								
	2 minutes			6 minutes			12 minutes		
d10	3.6117	1.0337	0.5168	2.6419	0.8055	0.4028	2.7551	1.2081	0.6041
d50	5.6410	0.8545	0.4272	4.7466	0.6686	0.3343	4.1743	0.3144	0.1572
d90	3.8307	0.7306	0.3653	3.4054	0.6593	0.3297	3.2674	0.4953	0.2476

Table A.16: Results of d10, d50 and d90 ratios (C/C_o) following ultrasound treatment. C = size of the treated samples, C_o = size of the control samples.

	Ultrasound (20 %)								
	2 minutes			6 minutes			12 minutes		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
d10	0.7688	0.0304	0.0124	0.5356	0.0514	0.0210	0.4588	0.0463	0.0189
d50	0.8210	0.0321	0.0131	0.5506	0.0524	0.0214	0.4513	0.0294	0.0120
d90	0.9609	0.1965	0.0802	0.8181	0.1779	0.0726	0.6404	0.0698	0.0285
	Ultrasound (40 %)								
	2 minutes			6 minutes			12 minutes		
d10	0.5971	0.0220	0.0090	0.5002	0.0391	0.0160	0.4315	0.0111	0.0045
d50	0.6724	0.0275	0.0112	0.4898	0.0705	0.0288	0.4619	0.0809	0.0330
d90	0.9991	0.1854	0.0757	0.6692	0.1422	0.0580	0.6455	0.1027	0.0419

Table A.17: Results of d10, d50 and d90 ratios (C/C_o) following microwave treatment. C = size of the treated samples, C_o = size of the control samples.

	Microwave					
	1 minute			3 minute		
	Mean	Std. Dev	Std. Err	Mean	Std. Dev	Std. Err
d10	1.0134	0.0211	0.0105	1.0645	0.0192	0.0096
d50	1.0208	0.0136	0.0068	1.0520	0.0192	0.0096
d90	1.0272	0.0671	0.0335	1.0560	0.0591	0.0296

Table A.18: Results of d10, d50 and d90 ratios (C/C_o) following thermal treatment. C = size of the treated samples, C_o = size of the control samples.

	Thermal		
	103°C for 2.5 hours		
	Mean	**Std.Dev**	**Std.Err**
d10	0.9122	0.030673	0.015337
d50	0.9337	0.034978	0.017489
d90	0.9975	0.123312	0.061656

APPENDIX B: RESULTS OF STATISTICAL ANALYSIS

CONVENTIONAL FREEZING

Table B.1: Two way ANOVA comparing the effect of temperature and cycles on pH ratio for conventional freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Temperature	2	0.0052	0.0026	2.4010	1.04E-01
Cycles	2	0.0023	0.0011	1.0713	3.53E-01
Temperature : Cycles	2	0.0020	0.0010	0.9440	0.3980
Residual	38	0.0408	0.0011		
Total	44	0.0503			

Table B.2: Pair-wise multiple comparisons for Table B.1 - Tukey HSD test.

Comparison for factor: Temperature					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
30	**15**	0.0168	-0.0101	0.0438	0.2928
ctrl	**15**	0.0272	-0.0045	0.0590	0.1055
ctrl	**30**	0.0104	-0.0210	0.0418	0.7030
Comparison for factor: Cycles					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
3	**1**	-0.0084	-0.0456	0.0289	0.9307
5	**1**	0.0108	-0.0256	0.0472	0.8561
Ctrl	**1**	0.0196	-0.0185	0.0578	0.5206
5	**3**	0.0192	-0.0180	0.0564	0.5186
Ctrl	**3**	0.0280	-0.0110	0.0670	0.2336
Ctrl	**5**	0.0088	-0.0294	0.0470	0.9255

Table B.3: Two way ANOVA comparing the effect of temperature and cycles on TSS ratio for conventional freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Temperature	2	0.0716	0.0358	6.9836	2.20E-03
Cycles	2	0.2118	0.1059	20.6591	3.96E-07
Temperature : Cycles	2	0.0254	0.0127	2.4786	0.0950
Residual	46	0.2357	0.0051		
Total	52	0.5445			

Table B.4: Pair-wise multiple comparisons for Table B.3 - Tukey HSD test.

Comparison for factor: Temperature					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
30	15	-0.0155	-0.0841	0.0530	0.8485
Ctrl	15	0.1057	-0.0019	0.2134	0.0553
Ctrl	30	0.1213	0.0140	0.2285	0.0231
Comparison for factor: Cycles					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
3	1	-0.1274	-0.2003	-0.0545	0.0001
5	1	-0.1497	-0.2168	-0.0826	0.0000
Ctrl	1	0.0211	-0.0724	0.1146	0.9313
5	3	-0.0223	-0.0933	0.0488	0.8385
Ctrl	3	0.1485	0.0521	0.2449	0.0009
Ctrl	5	0.1708	0.0787	0.2628	0.0001

Table B.5: Two way ANOVA comparing the effect of temperature and cycles on sCOD ratio for FT.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Temperature	2	141.3590	70.6800	54.1852	1.92E-13
Cycles	2	49.3210	24.6600	18.9053	6.75E-07
Temperature : Cycles	2	2.2660	1.1330	0.8687	0.4255
Residual	52	67.8290	1.3040		
Total	58	260.7750			

Table B.6: Pair-wise multiple comparisons for Table B.5 - Tukey HSD test.

Comparison for factor: Temperature					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
30	15	0.9090	-0.1169	1.9349	0.0923
Ctrl	15	-3.2470	-4.4990	-1.9950	0.0000
Ctrl	30	-4.1560	-5.3990	-2.9130	0.0000
Comparison for factor: Cycles					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
3	1	1.6560	0.5110	2.8011	0.0018
5	1	2.4402	1.3138	3.5667	0.0000
Ctrl	1	-2.3517	-3.5686	-1.1352	0.0000
5	3	0.7842	-0.3609	1.9292	0.2777
Ctrl	3	-4.0079	-5.2419	-2.7740	0.0000
Ctrl	5	-4.7921	-6.0088	-3.5754	0.0000

Table B.7: Two way ANOVA comparing the effect of temperature and cycles on SVI ratio for conventional freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Temperature	2	6.1340	3.0670	8478.4347	2.00E-16
Cycles	2	0.0018	0.0009	2.4652	0.0996
Temperature : Cycles	2	0.0018	0.0009	2.5002	0.0966
Residual	35	0.0127	0.0004		
Total	41	6.1503			

Table B.8: Pair-wise multiple comparisons for Table B.7 - Tukey HSD test.

Comparison for factor: Temperature					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
30	15	0.0095	-0.0090	0.0281	0.4301
ctrl	15	0.8516	0.8313	0.8719	0.0000
ctrl	30	0.8421	0.8236	0.8606	0.0000
Comparison for factor: Cycles					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
3	1	-0.0090	-0.0330	0.0150	0.7447
5	1	-0.0189	-0.0428	0.0051	0.1664
ctrl	1	0.8366	0.8137	0.8596	0.0000
5	3	-0.0099	-0.0338	0.0141	0.6879
ctrl	3	0.8456	0.8227	0.8686	0.0000
ctrl	5	0.8555	0.8325	0.8784	0.0000

Table B.9: Two way ANOVA comparing the effect of temperature and cycles on CST ratio for conventional freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	P Value
Temperature	2	6.3208	3.1604	1180.5201	2.00E-16
Cycles	2	0.0152	0.0076	2.8411	0.0702
Temperature : Cycles	2	0.0005	0.0002	0.0842	0.9194
Residual	40	0.1071	0.0027		
Total	46	6.4436			

Table B.10: Pair-wise multiple comparisons for Table B.9 - Tukey HSD test.

Comparison for factor: Temperature					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
30	**15**	0.0363	-0.0070	0.0797	0.1161
Ctrl	**15**	0.8589	0.8106	0.9072	0.0000
Ctrl	**30**	0.8226	0.7747	0.8704	0.0000
Comparison for factor: Cycles					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
3	**1**	-0.0355	-0.0943	0.0232	0.3815
5	**1**	-0.0482	-0.1057	0.0093	0.1290
Ctrl	**1**	0.8126	0.7551	0.8701	0.0000
5	**3**	-0.0127	-0.0714	0.0461	0.9389
Ctrl	**3**	0.8481	0.7893	0.9069	0.0000
Ctrl	**5**	0.8608	0.8033	0.9182	0.0000

COMBINED ULTRASONIC-FREEZING

Table B.11: Two way ANOVA comparing the effect of sonication amplitude and time on pH ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	3	0.0137	0.0046	2.5823	6.2780E-02
Time	2	0.0002	0.0001	0.0682	9.3415E-01
Amplitude : Time	2	0.0014	0.0007	0.4119	0.6644
Residual	54	0.0953	0.0018		
Total	61	0.1106			

Table B.12: Pair-wise multiple comparisons for Table B.11 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	**20**	-0.0269	-0.0607	0.0068	0.1613
Ctrl	**20**	-0.0348	-0.0755	0.0059	0.1199
Ctrl	**40**	-0.0078	-0.0505	0.0348	0.9620
Comparison for factor: Time					
Comparison		Mean	95% Confidence Interval		P
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	**12**	-0.0052	-0.0508	0.0403	0.9975
6	**12**	-0.0049	-0.0504	0.0406	0.9981
Ctrl	**12**	-0.0266	-0.0765	0.0233	0.5650
6	**2**	0.0003	-0.0452	0.0459	1.0000
Ctrl	**2**	-0.0214	-0.0712	0.0285	0.7473
Ctrl	**6**	-0.0178	-0.0716	0.0282	0.7366

Table B.13: Two way ANOVA comparing the effect of sonication amplitude and time on TSS ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	3	0.0783	0.0261	6.5870	9.21E-04
Time	2	0.0135	0.0068	1.7084	1.93E-01
Amplitude : Time	2	0.0010	0.0005	0.1216	0.8858
Residual	43	0.1703	0.0040		
Total	50	0.2631			

Table B.14: Pair-wise multiple comparisons for Table B.13 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	**20**	-0.0192	-0.0749	0.0365	0.7951
ctrl	**20**	0.0955	0.0169	0.1743	0.0116
ctrl	**40**	0.1148	0.0361	0.1935	0.0018
Comparison for factor: Time					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	**12**	0.0403	-0.0311	0.1117	0.5030
6	**12**	0.0419	-0.0295	0.1133	0.4648
ctrl	**12**	0.1326	0.0451	0.2201	0.0008
6	**2**	0.0016	-0.0698	0.0730	1.0000
ctrl	**2**	0.0923	0.0048	0.1797	0.0340
ctrl	**6**	0.0907	0.0033	0.1782	0.0386

Table B.15: Two way ANOVA comparing the effect of sonication amplitude and time on sCOD ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	3	69.7260	23.2420	20.5252	9.0650E-08
Time	2	0.6710	0.3356	0.2964	7.4540E-01
Amplitude : Time	2	0.2500	0.1251	0.1105	0.8957
Residual	34	38.5000	1.1324		
Total	41	109.1470			

Table B.16: Pair-wise multiple comparisons for Table B.15 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	20	-0.5553	-1.6723	0.5618	0.5467
Ctrl	20	-3.9731	-5.3412	-2.6049	0.0000
Ctrl	40	-3.4178	-4.7859	-2.0497	0.0000
Comparison for factor: Time					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	12	-0.3872	-1.8887	1.1144	0.9458
6	12	-0.3095	-1.8110	1.1921	0.9756
Ctrl	12	-3.9277	-5.5495	-2.3058	0.0000
6	2	0.0777	-1.4239	1.5792	0.9999
Ctrl	2	-3.5405	-5.1624	-1.9186	0.0000
Ctrl	6	-3.6182	-5.2400	-1.9963	0.0000

Table B.17: Two way ANOVA comparing the effect of sonication amplitude and time on SVI ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	P Value
Amplitude	3	4.5185	1.5061	1833.0585	2.0000E-16
Time	2	0.0000	0.0000	0.0167	9.8340E-01
Amplitude : Time	2	0.0009	0.0005	0.5640	0.5730
Residual	44	0.0362	0.0008		
Total	51	4.5556			

Table B.18: Pair-wise multiple comparisons for Table B.17 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	**20**	-0.0320	-0.0566	-0.0073	0.0063
Ctrl	**20**	0.7920	0.7606	0.8235	0.0000
Ctrl	**40**	0.8240	0.7925	0.8554	0.0000
Comparison for factor: Time					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	**12**	-0.0006	-0.0369	0.0358	1.0000
6	**12**	-0.0021	-0.0384	0.0343	0.9998
Ctrl	**12**	0.8071	0.7665	0.8477	0.0000
6	**2**	-0.0015	-0.0378	0.0348	1.0000
Ctrl	**2**	-0.8077	0.7671	0.8483	0.0000
Ctrl	**6**	-0.8091	-0.7686	0.8499	0.0000

Table B.19: Two way ANOVA comparing the effect of sonication amplitude & time on CST ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	3	4.8862	1.6287	333.2973	2.00E-16
Time	2	0.0080	0.0040	0.8228	4.45E-01
Amplitude : Time	2	0.0001	0.0000	0.0075	0.9925
Residual	55	0.2688	0.0049		
Total	62	5.1631			

Table B.20: Pair-wise multiple comparisons for Table B.19 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean	95% Confidence Interval		P
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	**20**	-0.0642	-0.1206	-0.0077	0.0199
Ctrl	**20**	0.8065	0.7325	0.8804	0.0000
Ctrl	**40**	0.8706	0.7937	0.9476	0.0000
Comparison for factor: Time					
Comparison		Mean	95% Confidence Interval		P
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	**12**	0.0088	-0.0691	0.0868	0.9977
6	**12**	0.0328	-0.0452	0.1107	0.7609
Ctrl	**12**	0.8478	0.7564	0.9392	0.0000
6	**2**	0.0239	-0.0540	0.1019	0.9089
Ctrl	**2**	0.8390	0.7476	0.9304	0.0000
Ctrl	**6**	0.8151	0.7237	0.9065	0.0000

ULTRASOUND

Table B.21: Two way ANOVA comparing the effect of sonication amplitude and time on pH ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	0.0014	0.0007	1.4328	2.5270E-01
Time	2	0.0035	0.0017	3.5890	3.8530E-02
Amplitude : Time	2	0.0005	0.0002	0.4735	0.6269
Residual	34	0.0165	0.0005		
Total	40	0.0219			

Table B.22: Pair-wise multiple comparisons for Table B.21 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		**Mean Diff (I-J)**	**95% Confidence Interval**		**p Value**
Group I	**Group J**		**Lower Bound**	**Upper Bound**	
40	**20**	-0.0002	-0.0190	0.0186	0.9996
Ctrl	**20**	-0.0179	-0.0465	0.0107	0.2900
Ctrl	**40**	-0.0180	-0.0463	0.0109	0.2983
Comparison for factor: Time					
Comparison		**Mean Diff (I-J)**	**95% Confidence Interval**		**p Value**
Group I	**Group J**		**Lower Bound**	**Upper Bound**	
2	**12**	0.0234	-0.0001	0.0469	0.0519
6	**12**	0.0168	-0.0067	0.0403	0.2379
Ctrl	**12**	-0.0044	-0.0351	0.0262	0.9799
6	**2**	-0.0066	-0.0301	0.0169	0.8740
Ctrl	**2**	-0.0278	-0.0585	0.0029	0.0874
Ctrl	**6**	-0.0212	-0.0519	0.0095	0.2630

Table B.0.23: Two way ANOVA comparing the effect of sonication amplitude and time on TSS ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	0.1097	0.0549	23.2342	1.80E-07
Time	2	0.0837	0.0419	17.7238	2.84E-06
Amplitude : Time	2	0.0001	0.0001	0.0231	0.9771
Residual	41	0.0968	0.0024		
Total	47	0.2903			

Table B.24: Pair-wise multiple comparisons for Table B.23 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
40	**20**	-0.0816	-0.1295	-0.0337	0.0004
Ctrl	**20**	0.0536	-0.0164	0.1237	0.1639
Ctrl	**40**	0.1352	0.0629	0.2076	0.0001
Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
2	**12**	0.1076	0.0457	0.1695	0.0002
6	**12**	0.0370	-0.0249	0.0989	0.3918
Ctrl	**12**	0.1368	0.0569	0.2167	0.0002
6	**2**	-0.0706	-0.1325	-0.0088	0.0196
Ctrl	**2**	0.0292	-0.0507	0.1091	0.7641
Ctrl	**6**	0.0998	0.0199	0.1797	0.0091

Table B.25: Two way ANOVA comparing the effect of amplitude & time on sCOD ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	P Value
Amplitude	2	26.9960	13.4982	12.7750	6.83E-05
Time	2	21.4670	10.7333	10.1582	3.00E-04
Amplitude : Time	2	4.9820	2.4912	2.3577	0.1095
Residual	35	36.9820	1.0566		
Total	41	90.4270			

Table B.26: Pair-wise multiple comparisons for Table B.25 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean Diff (I-J)	95% Confidence Interval		P Value
Group I	Group J		Lower Bound	Upper Bound	
40	**20**	1.3138	0.2781	2.3494	0.0101
Ctrl	**20**	-0.8361	-2.3008	0.6286	0.3555
Ctrl	**40**	-2.1498	-3.6145	-0.6851	0.0027
Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		P Value
Group I	Group J		Lower Bound	Upper Bound	
2	**12**	-1.8759	-3.2250	-0.5268	0.0033
6	**12**	-1.1477	-2.4967	0.2014	0.1195
Ctrl	**12**	-2.5008	-4.1531	-0.8485	0.0013
6	**2**	0.7283	-0.6208	2.0774	0.4769
Ctrl	**2**	-0.6249	-2.2772	1.0274	0.7411
Ctrl	**6**	-1.3532	-3.0054	0.2991	0.1416

Table B.27: Two way ANOVA comparing the effect of sonication amplitude and time on SVI ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	0.1931	0.0965	21.5520	7.9320E-07
Time	2	0.1588	0.0794	12.7220	4.8290E-06
Amplitude : Time	2	0.0006	0.0003	0.0680	0.9344
Residual	35	0.1568	0.0045		
Total	41	0.5093			

Table B.28: Pair-wise multiple comparisons for Table B.27 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		**Mean Diff (I-J)**	**95% Confidence Interval**		**p Value**
Group I	**Group J**		**Lower Bound**	**Upper Bound**	
40	**20**	0.0963	0.0232	0.1964	0.0074
Ctrl	**20**	-0.0977	-0.2013	0.0055	0.0668
Ctrl	**40**	-0.1942	-0.2976	-0.0907	0.0001
Comparison for factor: Time					
Comparison		**Mean Diff (I-J)**	**95% Confidence Interval**		**p Value**
Group I	**Group J**		**Lower Bound**	**Upper Bound**	
2	**12**	-0.1598	-0.2471	-0.0725	0.0001
6	**12**	-0.0537	-0.1410	0.0336	0.3628
Ctrl	**12**	-0.2172	-0.3241	-0.1102	0.0000
6	**2**	0.1061	0.0188	0.1935	0.0119
Ctrl	**2**	-0.0574	-0.1643	0.0496	0.4825
Ctrl	**6**	-0.1635	-0.2704	-0.0566	0.0011

Table B.29: Two way ANOVA comparing the effect of sonication amplitude and time on CST ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	4.3610	2.1805	9.7789	4.0550E-04
Time	2	2.9537	1.4769	6.6234	3.5535E-03
Amplitude : Time	2	0.0856	0.0428	0.1920	0.8562
Residual	36	0.0272	0.2230		
Total	42	7.4275			

Table B.30: Pair-wise multiple comparisons for Table B.29 - Tukey HSD test.

Comparison for factor: Amplitude					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
40	**20**	0.0145	-0.4066	0.4356	0.9961
ctrl	**20**	-0.9118	-1.5113	-0.3123	0.0018
ctrl	**40**	-0.9263	-1.5280	-0.3228	0.0017
Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
2	**12**	-0.6881	-1.1780	-0.1981	0.0029
6	**12**	-0.3462	-0.8458	0.1535	0.2621
ctrl	**12**	-1.2729	-1.8848	-0.6610	0.0000
6	**2**	0.3419	-0.1480	0.8318	0.2563
ctrl	**2**	-0.5848	-1.1889	0.0192	0.0608

MICROWAVE

Table B.31: One way ANOVA comparing the effect of treatment time on pH ratio for microwave.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	2	0.0087	0.0044	41.9200	2.75E-05
Residual	9	0.0009	0.0001		
Total	11	0.0096			

Table B.32: Pair-wise multiple comparisons for Table B.31 - Tukey HSD test.

Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
3	**1**	0.0584	0.0382	0.0785	0.0001
Ctrl	**1**	0.0023	-0.0179	0.0225	0.9459
Ctrl	**3**	-0.0561	-0.0762	-0.0359	0.0001

Table B.33: One way ANOVA comparing the effect of treatment time on TSS ratio for microwave.

Source of Variation	DF	Sum Sq	Mean Sq	F	P Value
Time	2	0.0136	0.0068	6.9679	1.49E-02
Residual	9	0.0088	0.0010		
Total	11	0.0224	0.0078		

Table B.34: Pair-wise multiple comparisons for Table B.33 - Tukey HSD test.

Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
3	1	0.0494	-0.0123	0.1112	0.1179
ctrl	1	-0.0325	-0.0942	0.0292	0.3488
ctrl	3	-0.0820	-0.1437	-0.0202	0.0122

Table B.35: One way ANOVA comparing the effect of treatment time on sCOD ratio for microwave.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	2	2.8823	1.4412	4.6892	4.03E-02
Residual	9	2.7660	0.3073		
Total	11	5.6483			

Table B.36: Pair-wise multiple comparisons for Table B.35 - Tukey HSD test.

Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
3	1	0.1716	-0.9229	1.2660	0.9009
Ctrl	1	-0.9432	-2.0377	0.1513	0.0910
Ctrl	3	-1.1148	-2.2092	-0.0203	0.0461

Table B.37: One way ANOVA comparing the effect of treatment time on SVI ratio for microwave.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	2	0.0213	0.0107	7.3778	1.27E-02
Residual	9	0.0130	0.0014		
Total	11	0.0343			

Table B.38: Pair-wise multiple comparisons for Table B.37 - Tukey HSD test.

Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
3	1	-0.0674	-0.1424	0.0077	0.0779
ctrl	1	0.0341	-0.0410	0.1091	0.4465
ctrl	3	0.1015	0.0264	0.1765	0.0110

Table B.0.39: One way ANOVA comparing the effect of treatment time on CST ratio for microwave.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	2	2.0386	1.0193	9.1356	6.81E-03
Residual	9	1.0042	0.1116		
Total	11	3.0428			

Table B.40: Pair-wise multiple comparisons for Table B.39 - Tukey HSD test.

Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
3	**1**	0.5188	-0.1407	1.1782	0.1253
Ctrl	**1**	-0.4907	-1.1502	0.1688	0.1497
Ctrl	**3**	-1.0095	-1.6689	-0.3500	0.0053

COMPARISON OF TREATMENTS ON SOLUBILISATION OF SLUDGE ORGANIC MATTER & DEWATERABILITY

Table B.41: One way ANOVA comparing the effect of treatment type on pH ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Treatment	5	0.0448	0.0090	6.9880	8.11E-06
Residual	131	0.1678	0.0013		
Total	136	0.2126	0.0102		

Table B.42: Pairwise Multiple Comparison for Table 41 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
FT	**CTRL**	-0.0186	-0.0557	0.0186	0.6985
MW	**CTRL**	0.0269	-0.0222	0.0760	0.6113
TH	**CTRL**	-0.0157	-0.0692	0.0377	0.9573
UF	**CTRL**	0.0232	-0.0132	0.0597	0.4413
ULTRA	**CTRL**	0.0178	-0.0192	0.0548	0.7323
MW	**FT**	0.0454	0.0049	0.0860	0.0186
TH	**FT**	0.0028	-0.0429	0.0486	1.0000
UF	**FT**	0.0418	0.0181	0.0655	0.0000
ULTRA	**FT**	0.0364	0.0118	0.0609	0.0005
TH	**MW**	-0.0426	-0.0985	0.0133	0.2434
UF	**MW**	-0.0036	-0.0436	0.0363	0.9998
ULTRA	**MW**	-0.0091	-0.0495	0.0314	0.9870
UF	**TH**	0.0389	-0.0062	0.0841	0.1337
ULTRA	**TH**	0.0335	-0.0121	0.0792	0.2818
ULTRA	**UF**	-0.0054	-0.0289	0.0181	0.9851

Table B.43: One way ANOVA comparing the effect of treatment type on TSS ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	P Value
Treatment	5	0.2840	0.0568	8.3410	6.07E-07
Residual	143	0.9739	0.0068		
Total	148	1.2579	0.0636		

Table B.44: Pairwise Multiple Comparison for Table 43 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
FT	**CTRL**	-0.1112	-0.1942	-0.0282	0.0022
MW	**CTRL**	0.0574	-0.0557	0.1705	0.6866
TH	**CTRL**	-0.1053	-0.2284	0.0178	0.1396
UF	**CTRL**	-0.1052	-0.1904	-0.0200	0.0064
ULTRA	**CTRL**	-0.0886	-0.1725	-0.0048	0.0317
MW	**FT**	0.1686	0.0774	0.2598	0.0000
TH	**FT**	0.0059	-0.0975	0.1092	1.0000
UF	**FT**	0.0060	-0.0468	0.0588	0.9995
ULTRA	**FT**	0.0226	-0.0280	0.0732	0.7912
TH	**MW**	-0.1627	-0.2915	-0.0340	0.0048
UF	**MW**	-0.1626	-0.2558	-0.0694	0.0000
ULTRA	**MW**	-0.1460	-0.2380	-0.0541	0.0001
UF	**TH**	0.0001	-0.1050	0.1053	1.0000
ULTRA	**TH**	0.0167	-0.0874	0.1207	0.9973
ULTRA	**UF**	0.0166	-0.0376	0.0707	0.9500

Table B.45: One way ANOVA comparing the effect of treatment type on sCOD ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Treatment	5	248.1000	49.6200	23.4700	2.00E-16
Residual	125	264.2000	2.1100		
Total	130	512.3000	51.7300		

Table B.46: Pairwise Multiple Comparison for Table 45 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
FT	CTRL	**3.7111**	2.2456	5.1766	0.0000
MW	CTRL	**1.0290**	-0.9672	3.0251	0.6700
TH	CTRL	**4.8827**	2.7095	7.0558	0.0000
UF	CTRL	**3.6954**	2.1115	5.2794	0.0000
ULTRA	CTRL	**1.4929**	-0.0113	2.9972	0.0530
MW	FT	**-2.6822**	-4.2916	-1.0727	0.0001
TH	FT	**1.1715**	-0.6528	2.9959	0.4325
UF	FT	**-0.0157**	-1.0715	1.0401	1.0000
ULTRA	FT	**-2.2182**	-3.1503	-1.2862	0.0000
TH	MW	**3.8537**	1.5810	6.1264	0.0000
UF	MW	**2.6665**	0.9485	4.3845	0.0002
ULTRA	MW	**0.4640**	-1.1809	2.1088	0.9640
UF	TH	**-1.1872**	-3.1080	0.7336	0.4766
ULTRA	TH	**-3.3897**	-5.2454	-1.5341	0.0000
ULTRA	UF	**-2.2025**	-3.3115	-1.0935	0.0000

Table B.47: One way ANOVA comparing the effect of treatment type on CST ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Treatment	5	93.1800	18.6360	181.5000	2.00E-16
Residual	128	13.1400	0.1030		
Total	133	106.3200	18.7390		

Table B.48: Pairwise Multiple Comparison for Table 47 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
FT	CTRL	**-0.8403**	-1.2500	-0.4307	0.0000
MW	CTRL	**0.7501**	0.2494	1.2507	0.0004
TH	CTRL	**1.0037**	0.4684	1.5389	0.0000
UF	CTRL	**-0.8340**	-1.2386	-0.4294	0.0000
ULTRA	CTRL	**0.9189**	0.5109	1.3269	0.0000
MW	FT	**1.5904**	1.2271	1.9537	0.0000
TH	FT	**1.8440**	1.4344	2.2536	0.0000
UF	FT	**0.0064**	-0.2058	0.2185	1.0000
ULTRA	FT	**1.7592**	1.5406	1.9778	0.0000
TH	MW	**0.2536**	-0.2471	0.7542	0.6867
UF	MW	**-1.5841**	-1.9417	-1.2264	0.0000
ULTRA	MW	**0.1688**	-0.1927	0.5302	0.7558
UF	TH	**-1.8376**	-2.2422	-1.4330	0.0000
ULTRA	TH	**-0.0848**	-0.4928	0.3232	0.9908
ULTRA	UF	**1.7528**	1.5438	1.9619	0.0000

Table B.49: One way ANOVA comparing the effect of treatment type on SVI ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Treatment	5	25.2560	5.0510	957.2000	2.00E-16
Residual	116	0.6120	0.0050		
Total	121	25.8680	5.0560		

Table B.50: Pairwise Multiple Comparison for Table 49 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean Diff (I-J)	95% Confidence Interval		P Value
Group I	Group J		Lower Bound	Upper Bound	
FT	CTRL	-0.8459	-0.9401	-0.7518	0.0000
MW	CTRL	-0.0678	-0.1815	0.0459	0.5168
TH	CTRL	-0.0496	-0.1711	0.0719	0.8443
UF	CTRL	-0.8080	-0.9008	-0.7152	0.0000
ULTRA	CTRL	0.1460	0.0532	0.2388	0.0002
MW	FT	0.7781	0.6944	0.8619	0.0000
TH	FT	0.7963	0.7022	0.8905	0.0000
UF	FT	0.0379	-0.0141	0.0899	0.2891
ULTRA	FT	0.9919	0.9399	1.0439	0.0000
TH	MW	0.0182	-0.0955	0.1319	0.9973
UF	MW	-0.7402	-0.8225	-0.6580	0.0000
ULTRA	MW	0.2137	0.1315	0.2960	0.0000
UF	TH	-0.7584	-0.8512	-0.6656	0.0000
ULTRA	TH	0.1956	0.1028	0.2884	0.0000
ULTRA	UF	0.9540	0.9044	1.0036	0.0000

Table B.51: T-test for Equality of Means for TSS of Ultrasonic Freezing and Freezing (1 cycle)

	t	df	95% Confidence Interval of the Difference		p-Value
FT - UF 20-2	1.2923	11.402	-0.036	0.1394	0.2218
FT - UF 20-6	1.0729	11.266	-0.0554	0.1613	0.3057
FT - UF 20-12	1.7582	11.692	-0.0201	0.1856	0.1048
FT - UF 40-2	1.4049	11.636	-0.037	0.1703	0.1862
FT - UF 40-6	1.593	11.012	-0.0237	0.1482	0.1394
FT - UF 40-12	3.0192	10.3735	0.0312	0.2012	0.012

Table B.52: T-test for Equality of Means for sCOD of Ultrasonic Freezing and Freezing (1 cycle)

	t	Df	95% Confidence Interval of the Difference		p-Value
FT - UF 20-2	-2.3201	4.3210	-3.5546	0.2670	0.0763
FT - UF 20-6	-1.6895	3.7050	-4.2086	1.0868	0.1721
FT - UF 20-12	-1.9706	3.5040	-5.2733	1.0398	0.1301
FT - UF 40-2	-2.4535	8.2540	-2.0097	-0.0676	0.0388
FT - UF 40-6	-2.9292	7.8670	-2.2851	-0.2687	0.0194
FT - UF 40-12	-2.9888	7.5100	-2.3859	-0.2943	0.0187

Table B.53: T-test for Equality of Means for TSS of Ultrasonic Freezing and Ultrasound

	t	df	95% Confidence Interval of the Difference		p-Value
UF 20-2 - Ultra 20-2	-3.4685	8.3480	-0.1474	-0.0302	0.0079
UF 20-6 - Ultra 20-6	-0.5066	9.7370	-0.1233	0.0778	0.6237
UF 20-12 - Ultra 20-12	-0.3736	8.3050	-0.1024	0.0737	0.7180
UF 40-2 - Ultra 40-2	-0.7585	5.6560	-0.1126	0.0599	0.4786
UF 40-6 - Ultra 40-6	1.9257	9.9430	-0.0084	0.1148	0.0832
UF 40-12 - Ultra 40-12	1.1861	9.8220	-0.0305	0.0995	0.2635

Table B.54: T-test for Equality of Means for sCOD of Ultrasonic Freezing and Ultrasound

	t	df	95% Confidence Interval of the Difference		p-Value
UF 20-2 - Ultra 20-2	5.1482	3.1960	1.3559	5.3781	0.0121
UF 20-6 - Ultra 20-6	3.5100	3.5150	0.5250	5.8741	0.0304
UF 20-12 - Ultra 20-12	2.5971	3.7630	-0.2735	5.9625	0.0640
UF 40-2 - Ultra 40-2	6.6368	5.8980	1.5519	1.7737	0.0006
UF 40-6 - Ultra 40-6	2.3539	7.8990	0.0242	2.6369	0.0468
UF 40-12 - Ultra 40-12	0.0099	6.3710	-2.2422	2.2606	0.9924

PROGRESSIVE ULTRASONIC FREEZING

Table B.55: One way ANOVA comparing the effect of pulse time on pH ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	0.0182	0.0046	3.0230	0.0492
Residual	16	0.0241	0.0015		
Total	20	0.0423			

Table B.56: Pair-wise multiple comparisons for Table B.55 - Tukey HSD test.

Comparison for factor: Pulse Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
2S	2L	-0.0145	-0.0987	0.0696	0.9830
3L	2L	-0.0325	-0.1167	0.0516	0.7599
Ctrl	2L	-0.0829	-0.1627	-0.0030	0.0400
3S	2S	-0.0132	-0.0973	0.0709	0.9881
Ctrl	2S	-0.0683	-0.1481	0.0115	0.1128
3S	3L	0.0048	-0.0793	0.0889	0.9998
Ctrl	3L	-0.0503	-0.1301	0.0295	0.3410
Ctrl	3S	-0.0551	-0.1349	0.0247	0.2608

Table B.57: One way ANOVA comparing the effect of pulse time on TSS ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	0.1128	0.0282	7.8438	0.0010
Residual	16	0.0575	0.0036		
Total	20	0.1703			

Table B.58: Pair-wise multiple comparisons for Table B.57 - Tukey HSD test.

Comparison for factor: Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
2S	2L	-0.0041	-0.1340	0.1258	1.0000
3L	2L	-0.0242	-0.1541	0.1057	0.9777
Ctrl	2L	0.1523	0.0291	0.2756	0.0121
3S	2S	-0.0347	-0.1646	0.0952	0.9211
Ctrl	2S	0.1564	0.0332	0.2796	0.0099
3S	3L	-0.0146	-0.1445	0.1153	0.9966
Ctrl	3L	0.1765	0.0532	0.2997	0.0036
Ctrl	3S	0.1911	0.0679	0.3143	0.0018

Table B.59: One way ANOVA comparing the effect of pulse time on sCOD ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	86.4390	21.6097	27.8930	1.55E-06
Residual	14	10.8460	0.7747		
Total	18	97.2850			

Table B.60: Pair-wise multiple comparisons for Table B.59 – Tukey HSD test.

Comparison for factor: Pulse Time					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
2S	2L	-4.2744	-6.6495	-1.8992	0.0005
3L	2L	-2.4031	-4.7782	-0.0279	0.0468
Ctrl	2L	-6.9687	-9.2633	-4.6741	0.0000
3S	2S	0.2712	-1.6682	2.2105	0.9917
Ctrl	2S	-2.6943	-4.5341	-0.8545	0.0034
3S	3L	-1.6002	-3.5395	0.3391	0.1300
Ctrl	3L	-4.5657	-6.4055	-2.7258	0.0000
Ctrl	3S	-2.9655	-4.8053	-1.1257	0.0015

Table B.61: One way ANOVA comparing the effect of pulse time on CST ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	8.7701	2.1925	166.8700	1.21E-11
Residual	14	0.1840	0.0131		
Total	18	8.9541			

Table B.62: Pair-wise multiple comparisons for Table B.61 - Tukey HSD test.

Comparison for factor: Pulse Time					
Comparison			95% Confidence Interval		p Value
Group I	Group J	Mean Diff (I-J)	Lower Bound	Upper Bound	
2S	2L	-1.4773	-1.7866	-1.1680	0.0000
3L	2L	0.1209	-0.1884	0.4302	0.7415
Ctrl	2L	-0.6984	-0.9972	-0.3996	0.0000
3S	2S	-0.0663	-0.3189	0.1863	0.9207
Ctrl	2S	0.7789	0.5393	1.0185	0.0000
3S	3L	-1.6645	-1.9171	-1.4120	0.0000
Ctrl	3L	-0.8193	-1.0589	-0.5797	0.0000
Ctrl	3S	0.8452	0.6056	1.0848	0.0000

Table B.63: One way ANOVA comparing the effect of sonication time on pH ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	0.0095	0.0024	2.1770	0.1181
Residual	16	0.0174	0.0011		
Total	20	0.0269			

Table B.64: One way ANOVA comparing the effect of sonication time on TSS ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	0.1508	0.0377	6.0360	0.0037
Residual	16	0.1000	0.0062		
Total	20	0.2508			

Table B.65: Pair-wise multiple comparisons for Table B.64 - Tukey HSD test.

Comparison for factor: Sonication Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
12S	12L	-0.0146	-0.1858	0.1566	0.9989
25L	12L	0.0219	-0.1493	0.1931	0.9945
ctrl	12L	0.1765	0.0140	0.3389	0.0300
25S	12S	-0.0446	-0.2158	0.1267	0.9276
ctrl	12S	0.1911	0.0287	0.3535	0.0174
25S	25L	-0.0811	-0.2523	0.0902	0.6062
ctrl	25L	0.1546	-0.0078	0.3170	0.0661
ctrl	25S	0.2357	0.0732	0.3981	0.0032

Table B.66: One way ANOVA comparing the effect of sonication time on sCOD ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	82.9380	20.7346	26.8480	1.95E-06
Residual	14	10.8120	0.7723		
Total	18	93.7500			

Table B.67: Pair-wise multiple comparisons for Table B.66 - Tukey HSD test.

Comparison for factor: Sonication Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
12S	12L	-1.6002	-3.5364	0.3361	0.1291
25L	12L	2.1480	-0.2234	4.5194	0.0845
ctrl	12L	-4.5657	-6.4026	-2.7287	0.0000
25S	12S	-0.5281	-2.4643	1.4082	0.9102
ctrl	12S	-2.9655	-4.8024	-1.1286	0.0015
25S	25L	-4.2763	-6.6477	-1.9048	0.0005
ctrl	25L	-6.7137	-9.0047	-4.4226	0.0000
ctrl	25S	-2.4374	-4.2743	-0.6005	0.0075

Table B.68: One way ANOVA comparing the effect of sonication time on CST ratio for progressive ultrasonic freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Time	4	12.2989	3.0747	175.3700	8.63E-12
Residual	14	0.2455	0.0175		
Total	18	12.5444			

Table B.69: Pair-wise multiple comparisons for Table B.68 - Tukey HSD test.

Comparison for factor: Sonication Time					
Comparison			**95% Confidence Interval**		
Group I	**Group J**	**Mean Diff (I-J)**	**Lower Bound**	**Upper Bound**	**p Value**
12S	**12L**	-1.6645	-1.9563	-1.3728	0.0000
25L	**12L**	0.6918	0.3345	1.0491	0.0003
Ctrl	**12L**	-0.8193	-1.0961	-0.5426	0.0000
25S	**12S**	0.1054	-0.1864	0.3971	0.7910
Ctrl	**12S**	0.8452	0.5684	1.1220	0.0000
25S	**25L**	-2.2510	-2.6083	-1.8936	0.0000
Ctrl	**25L**	-1.5111	-1.8563	-1.1659	0.0000
Ctrl	**25S**	0.7399	0.4631	1.0166	0.0000

PARTICLE SIZE – CONVENTIONAL FREEZING

Table B.70: Two way ANOVA comparing the effect of temperature and cycles on d10 ratio for conventional freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Temperature	2	64.0360	32.0180	52.0213	9.6080E-13
Cycles	2	2.7630	1.3810	2.2444	1.1700E-01
Temperature : Cylces	2	0.0780	0.0390	0.0636	0.9385
Residual	48	29.5430	0.6150		
Total	54	96.4200			

Table B.71: Pair-wise multiple comparisons for Table B.70 - Tukey HSD test.

Comparison for factor: Freezing Temperature					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
30	15	-0.9758	-1.5567	-0.3950	0.0005
Ctrl	15	-2.8941	-3.5830	-2.2051	0.0000
Ctrl	30	-1.9182	-2.6015	-1.2350	0.0000
Comparison for factor: Cycles					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
3	1	-0.3287	-1.1722	0.5148	0.7298
5	1	-0.6341	-1.5303	0.2622	0.2498
Ctrl	1	-2.6864	-3.5827	-1.7902	0.000
5	3	-0.3054	-1.2143	0.6036	0.8089
Ctrl	3	-2.3577	-3.2667	-1.4487	0.0000
Ctrl	5	-2.0523	-3.0105	-1.0942	0.0000

Table B.72: Two way ANOVA comparing the effect of temperature and cycles on d50 ratio for FT.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Temperature	2	103.2080	51.6040	33.7051	7.18E-10
Cycles	2	12.5580	6.2790	4.1013	2.27E-02
Temperature : Cycles	2	0.2130	0.1070	0.0696	0.9329
Residual	48	73.4900	1.5310		
Total	54	189.4690			

Table B.73: Pair-wise multiple comparisons for Table B.72 - Tukey HSD test.

Comparison for factor: Freezing Temperature					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
30	15	-0.9006	-1.8486	0.0474	0.0658
Ctrl	15	-3.6344	-4.7589	-2.5099	0.0000
Ctrl	30	-2.7338	-3.8489	-1.6187	0.0000
Comparison for factor: Cycles					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
3	1	-0.8119	-2.0243	0.4005	0.2953
5	1	-1.3294	-2.6176	-0.0411	0.0407
Ctrl	1	-3.8279	-5.1161	-2.5396	0.0000
5	3	-0.5175	-1.8240	0.7891	0.7199
Ctrl	3	-3.0159	-4.3225	-1.7094	0.0000
Ctrl	5	-2.4985	-3.8757	-1.1213	0.0001

Table B.74: Two way ANOVA comparing the effect of temperature and cycles on d90 ratio for conventional freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Temperature	2	24.8490	12.4247	16.3387	3.87E-06
Cycles	2	1.9180	0.9590	1.2611	2.93E-01
Temperature : Cylces	2	0.0040	0.0020	0.0026	0.9974
Residual	48	36.5010	0.7604		
Total	54	63.2720			

Table B.75: Pair-wise multiple comparisons for Table B.74 - Tukey HSD test.

Comparison for factor: Freezing Temperature					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
30	15	-0.3046	-0.9372	0.3281	0.4814
Ctrl	15	-1.7503	-2.5008	-0.9998	0.0000
Ctrl	30	-1.4458	-2.1900	-0.7015	0.0001
Comparison for factor: Cycles					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
3	1	-0.3523	-1.1705	0.4659	0.6646
5	1	-0.5075	-1.3769	0.3619	0.4158
Ctrl	1	-1.8590	-2.7284	-0.9896	0.0000
5	3	-0.1552	-1.0369	0.7266	0.9659
Ctrl	3	-1.5067	-2.3884	-0.6250	0.0002
Ctrl	5	-1.3516	-2.2810	-0.4221	0.0018

PARTICLE SIZE – COMBINED ULTRASONIC FREEZING

Table B.76: Two way ANOVA comparing the effect of sonication amplitude and time on d10 ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	32.7340	16.3672	29.6666	7.62E-07
Time	2	1.3070	0.6536	1.1847	3.26E-01
Amplitude : Time	2	2.2250	1.1127	2.0169	0.1580
Residual	21	11.5860	0.5517		
Total	27	47.8520			

Table B.77: Pair-wise multiple comparisons for Table B.76 - Tukey HSD test.

Comparison for factor: Sonication Amplitude					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	**20**	-1.2367	-2.0275	-0.4459	0.0018
Ctrl	**20**	-3.2396	-4.3579	-2.1213	0.0000
Ctrl	**40**	-2.0029	-3.1212	-0.8846	0.0004
Comparison for factor: Sonication Time					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	**12**	0.5682	-0.7817	1.9181	0.6563
6	**12**	0.3384	-1.0115	1.6883	0.8993
Ctrl	**12**	-2.3191	-3.9724	-0.6658	0.0038
6	**2**	-0.2299	-1.5798	1.1201	0.9650
Ctrl	**2**	-2.8873	-4.5406	-1.2340	0.0004
Ctrl	**6**	-2.6574	-4.3107	-1.0041	0.0009

Table B.78: Two way ANOVA comparing the effect of sonication amplitude and time on d50 ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	47.3360	23.6679	108.9592	8.16E-12
Time	2	1.8810	0.9403	4.3286	2.67E-02
Amplitude : Time	2	2.5480	1.2738	5.8640	0.0095
Residual	21	4.5620	0.2172		
Total	27	56.3270			

Table B.79: Pair-wise multiple comparisons for Table B.78 - Tukey HSD test.

Comparison for factor: Sonication Amplitude					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	**20**	0.3272	-0.2826	0.9369	0.3889
ctrl	**20**	-3.5268	-4.3892	-2.6645	0.0000
ctrl	**40**	-3.8540	-4.7163	-2.9916	0.0000
Comparison for factor: Sonication Time					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	**12**	0.6852	-0.0986	1.4691	0.1018
6	**12**	0.3218	-0.4620	1.1057	0.6735
ctrl	**12**	-3.3547	-4.3147	-2.3947	0.0000
6	**2**	-0.3634	-1.1473	0.4205	0.5846
ctrl	**2**	-4.0399	-5.0000	-3.0799	0.0000
ctrl	**6**	-3.6765	-4.6366	-2.7165	0.0000

Table B.80: Two way ANOVA comparing the effect of sonication amplitude and time on d90 ratio for combined ultrasonic-freezing.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	18.8247	9.4123	45.0623	2.53E-08
Time	2	0.3444	0.1722	0.8245	4.52E-01
Amplitude : Time	2	0.3462	0.1731	0.8288	0.4503
Residual	21	4.3864	0.2089		
Total	27	23.9017			

Table B.81: Pair-wise multiple comparisons for Table B.80 - Tukey HSD test.

Comparison for factor: Sonication Amplitude					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
40	**20**	0.7166	0.2584	1.1749	0.0018
ctrl	**20**	-1.7846	-2.4326	-1.1365	0.0000
ctrl	**40**	-2.5012	-3.1492	-1.8531	0.0000
Comparison for factor: Sonication Time					
Comparison		Mean	95% Confidence Interval		p
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	Value
2	**12**	0.2779	-0.5091	1.0649	0.7653
6	**12**	0.0573	-0.7297	0.8443	0.9970
ctrl	**12**	-2.0311	-2.9950	-1.0672	0.0000
6	**2**	-0.2206	-1.0076	0.5664	0.8658
ctrl	**2**	-2.3090	-3.2729	-1.3451	0.0000
ctrl	**6**	-2.0884	-3.0523	-1.1245	0.0000

PARTICLE SIZE – ULTRASOUND

Table B.82: Two way ANOVA comparing the effect of sonication amplitude and time on d10 ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	1.5216	0.7608	736.8700	2.2000E-16
Time	2	0.3563	0.1782	172.5450	2.2000E-16
Amplitude : Time	2	0.0395	0.0197	19.1210	1.80E-06
Residual	38	0.0392	0.0010		
Total	44	1.9566			

Table B.83: Pair-wise multiple comparisons for Table B.82 - Tukey HSD test.

Comparison for factor: Sonication Amplitude					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
40	**20**	-0.0782	-0.1606	0.0043	0.0662
ctrl	**20**	0.4123	0.3113	0.5132	0.0000
ctrl	**40**	0.4904	0.3895	0.5913	0.0000
Comparison for factor: Sonication Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
2	**12**	0.2378	0.1754	0.3002	0.0000
6	**12**	0.0727	0.0103	0.1351	0.0168
ctrl	**12**	0.5548	0.4874	0.6223	0.0000
6	**2**	-0.1651	-0.2275	-0.1026	1.00E-07
ctrl	**2**	0.3171	0.2496	0.3845	0.0000
ctrl	**6**	0.4821	0.4147	0.5495	0.0000

Table B.84: Two way ANOVA comparing the effect of sonication amplitude and time on d50 ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	1.3431	0.6715	301.6533	2.2e-16
Time	2	0.5580	0.2790	125.3289	2.2e-16
Amplitude : Time	2	0.0381	0.0191	8.5626	0.0009
Residual	38	0.0846	0.0022		
Total	44	2.0238			

Table B.85: Pair-wise multiple comparisons for Table B.84 - Tukey HSD test.

Comparison for factor: Sonication Amplitude					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
40	**20**	-0.0663	-0.1694	0.0368	0.2734
Ctrl	**20**	0.3924	0.2661	0.5186	0.0000
Ctrl	**40**	0.4586	0.3324	0.5849	0.0000
Comparison for factor: Sonication Time					
Comparison		Mean Diff (I-J)	95% Confidence Interval		p Value
Group I	Group J		Lower Bound	Upper Bound	
2	**12**	0.2901	0.2213	0.3589	0.0000
6	**12**	0.0636	-0.0051	0.1324	0.0786
Ctrl	**12**	0.5434	0.4691	0.6177	0.0000
6	**2**	-0.2265	-0.2952	-0.1577	0.0000
Ctrl	**2**	0.2533	0.1790	0.3276	0.0000
Ctrl	**6**	0.4798	0.4055	0.5541	0.0000

Table B.86: Two way ANOVA comparing the effect of sonication amplitude and time on d90 ratio for ultrasound.

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Amplitude	2	0.3321	0.1661	8.9946	6.34E-04
Time	2	0.7186	0.3593	19.4598	1.52E-06
Amplitude : Time	2	0.0599	0.0299	1.6212	0.2110
Residual	38	0.7016	0.0185		
Total	44	1.8121			

Table B.87: Pair-wise multiple comparisons for Table B.86 - Tukey HSD test.

Comparison for factor: Sonication Amplitude					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
40	20	-0.0352	-0.1872	0.1168	0.8406
ctrl	20	0.1935	0.0074	0.3797	0.0401
ctrl	40	0.2287	0.0426	0.4149	0.0128
Comparison for factor: Sonication Time					
Comparison		Mean	95% Confidence Interval		p Value
Group I	Group J	Diff (I-J)	Lower Bound	Upper Bound	
2	12	0.3371	0.1870	0.4871	2.40E-06
6	12	0.1007	-0.0494	0.2507	0.2896
ctrl	12	0.3571	0.1950	0.5191	3.50E-06
6	2	-0.2364	-0.3864	-0.0863	0.0007
ctrl	2	0.0200	-0.1421	0.1821	0.9874
ctrl	6	0.2564	0.0943	0.4185	0.0007

PEARSON CORRELATION COEFFICIENTS

Table B.88: Pearson's correlation coefficients between SVI, CST, sCOD, d50 and d90 for conventional freezing treatment

		sCOD	d50	d90
SVI	r	-0.8406	-0.8255	-0.7769
	p-value	7.76E-14	5.27E-13	8.50E-11
	df	46	46	46
CST	r	-0.5590	-0.7021	-0.6626
	p-value	3.64E-05	2.68E-08	2.90E-07
	df	46	46	46
sCOD	r	---	0.5705	0.5316
	p-value	---	2.30E-05	1.00E-04
	df	---	46	46

Table B.89: Pearson's correlation coefficients between SVI, CST, sCOD, d50 and d90 for ultrasound treatment

		sCOD	d50	d90
SVI	r	0.7206	-0.6837	-0.7373
	p-value	1.08E-07	8.36E-07	3.84E-08
	df	39	39	39
CST	r	0.7254	-0.5557	-0.4072
	p-value	8.09E-08	2.00E-04	8.20E-03
	df	39	39	39
sCOD	r	---	-0.6388	-0.5090
	p-value	---	6.97E-06	6.77E-04
	df	---	39	39

Table B.0.90: Pearson's correlation coefficients between SVI, CST, sCOD, d50 and d90 for ultrasonic freezing treatment

		sCOD	d50	d90
SVI	r	-0.8770	-0.0927	-0.9671
	p-value	5.19E-13	2.20E-16	2.20E-16
	df	36	36	36
CST	r	-0.8075	-0.8063	-0.7905
	p-value	9.01E-10	9.94E-10	3.55E-09
	df	36	36	36
sCOD	r	---	0.8241	0.8642
	p-value	---	2.06E-07	2.77E-12
	df	---	36	36

Table B.91: Pearson's correlation coefficients between SVI, CST, sCOD, d50 and d90 for microwave treatment

		sCOD	d50	d90
SVI	r	-0.4046	0.0565	0.4542
	p-value	0.1702	0.8546	0.1189
	df	11	11	11
CST	r	0.8654	0.6433	-0.4131
	p-value	0.0001	0.0177	0.1607
	df	11	11	11
sCOD	r	---	0.4321	-0.3347
	p-value	---	0.1403	0.2636
	df	---	11	11

Table B.92: Pearson's correlation coefficients between SVI, CST, sCOD, d50 and d90 for thermal treatment

		sCOD	d50	d90
SVI	r	-0.4887	0.8173	-0.6978
	p-value	0.1819	7.10E-03	3.66E-02
	df	7	7	7
CST	r	0.8790	-0.7387	0.5592
	p-value	1.80E-03	2.30E-02	1.18E-01
	df	7	7	7
sCOD	r	---	-0.5018	0.2383
	p-value	---	1.69E-01	5.37E-01
	df	---	7	7

COMPARISON OF TREATMENT METHODS ON PARTICLE SIZE

Table B.93: One way ANOVA comparing the effect treatment type on d10 ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Treatment	5	239.85	47.97	84.68	2.00E-16
Residual	119	67.41	.57		
Total	124	307.26			

Table B.94: Pairwise Multiple Comparison for Table B.93 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean Diff	95% Confidence Interval		P Value
Group I	Group J	(I-J)	Lower Bound	Upper Bound	
FT	**CTRL**	2.394795	1.427733	3.361858	0
MW	**CTRL**	0.038975	-1.15945	1.237397	0.999999
TH	**CTRL**	-0.08783	-1.52021	1.344564	0.999975
UF	**CTRL**	2.621267	1.608415	3.634118	0
ULTRA	**CTRL**	-0.45133	-1.42984	0.527177	0.763919
MW	**FT**	-2.35582	-3.21024	-1.5014	0
TH	**FT**	-2.48262	-3.6426	-1.32264	1E-07
UF	**FT**	0.226471	-0.33894	0.791882	0.854297
ULTRA	**FT**	-2.84613	-3.34742	-2.34483	0
TH	**MW**	-0.1268	-1.48568	1.232083	0.999799
UF	**MW**	2.582292	1.67637	3.488214	0
ULTRA	**MW**	-0.49031	-1.35766	0.377049	0.575057
UF	**TH**	2.709092	1.51067	3.907514	0
ULTRA	**TH**	-0.36351	-1.53305	0.806035	0.945559
ULTRA	**UF**	-3.0726	-3.65737	-2.48783	0

Table B.95: One way ANOVA comparing the effect treatment type on d50 ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Treatment	5	405.8	81.16	88.6	2.00E-16
Residual	115	105.3	.92		
Total	120	511.1			

Table B.96: Pairwise Multiple Comparison for Table B.95 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean Diff (I-J)	95% Confidence Interval		P Value
Group I	Group J		Lower Bound	Upper Bound	
FT	CTRL	3.1736	1.9647	4.3825	0.0000
MW	CTRL	-0.0663	-1.5644	1.4318	1.0000
TH	CTRL	-0.0663	-1.8569	1.7243	1.0000
UF	CTRL	3.6904	2.4243	4.9565	0.0000
ULTRA	CTRL	-0.4255	-1.6487	0.7977	0.9144
MW	FT	-3.2399	-4.3080	-2.1719	0.0000
TH	FT	-3.2399	-4.6900	-1.7899	0.0000
UF	FT	0.5168	-0.1900	1.2236	0.2849
ULTRA	FT	-3.5991	-4.2258	-2.9725	0.0000
TH	MW	0.0000	-1.6987	1.6987	1.0000
UF	MW	3.7567	2.6242	4.8891	0.0000
ULTRA	MW	-0.3592	-1.4434	0.7250	0.9294
UF	TH	3.7567	2.2586	5.2548	0.0000
ULTRA	TH	-0.3592	-1.8212	1.1028	0.9801
ULTRA	UF	-4.1159	-4.8469	-3.3849	0.0000

Table B.97: One way ANOVA comparing the effect treatment type on d90 ratio

Source of Variation	DF	Sum Sq	Mean Sq	F	p Value
Treatment	5	116.15	23.231	54.37	2.00E-16
Residual	115	49.14	0.427		
Total	120	165.29			

Table B.98: Pairwise Multiple Comparison for Table B.97 - Tukey HSD Test

Comparison for factor: Treatment Type					
Comparison		Mean Diff (I-J)	95% Confidence Interval		P Value
Group I	Group J		Lower Bound	Upper Bound	
FT	CTRL	1.5945	0.7688	2.4202	0.0000
MW	CTRL	0.0416	-0.9816	1.0648	1.0000
TH	CTRL	-0.0025	-1.2255	1.2204	1.0000
UF	CTRL	2.1429	1.2781	3.0076	0.0000
ULTRA	CTRL	-0.2111	-1.0466	0.6243	0.9775
MW	FT	-1.5529	-2.2824	-0.8234	0.0000
TH	FT	-1.5970	-2.5874	-0.6066	0.0001
UF	FT	0.5484	0.0656	1.0311	0.0163
ULTRA	FT	-1.8056	-2.2336	-1.3776	0.0000
TH	MW	-0.0441	-1.2043	1.1161	1.0000
UF	MW	2.1013	1.3278	2.8748	0.0000
ULTRA	MW	-0.2527	-0.9933	0.4878	0.9206
UF	TH	2.1454	1.1222	3.1686	0.0000
ULTRA	TH	-0.2086	-1.2072	0.7899	0.9905
ULTRA	UF	-2.3540	-2.8533	-1.8547	0.0000