EVALUATION OF LOW POWER SONICATION ON ANAEROBIC DIGESTION OF MUNICIPAL WASTE SLUDGE AND ENERGY RECOVERY

by

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ABSTRACT

Ultrasound is one of the emerging pre-treatment technologies to enhance anaerobic digestion process, however high energy input requirement is a concern. This study investigated a low power sonication pre-treatment on anaerobic digestion under thermophilic (55^0C) and mesophilic (35^0C) digester temperatures. Low ultrasonic densities (0.08 to 0.25 W/mL) and specific energies (1,211 to 15,094 kJ/kg total solids (TS)) were applied to thickened waste activated sludge (TWAS) from Kelowna's wastewater treatment plant (WWTP) to determine optimal sludge disintegration (solubilisation) conditions. At 0.25 W/mL and 11,343 kJ/kg TS, maximum solubilisation of organic matters and highest particle size reduction were observed. Following solubilisation, anaerobic digesters utilizing a mixture of primary sludge and pre-treated TWAS indicated that at shorter digestion solid retention times (SRT), the sonication effect on biogas production under mesophilic conditions was more pronounced. In an organic loading rate range of 1.8~6.8 g volatile solids (VS)/L of digester/d), corresponding to SRTs of 20-5 days, thermophilic digesters exhibited process instability with an increase in organic loading as well as ultrasound intensity applied due to reduced microbial diversity of methane formers at elevated temperatures. On the other hand, thermophilic digesters were more successful in fecal coliform destruction than mesophilic digesters due to elevated temperatures. Sonication enhanced dewaterability for mesophilic digesters (sonicated at 4163 and 8153 kJ/Kg TS) at higher SRTs and all thermophilic pre-treated digesters at 20 day SRT. However, sonication did not reduce odour causing volatile sulfur compounds in headspace of digesters. In an energy feasibility study, at 20 day and 10 day SRTs for all the digestion systems, the energy balance came out positive due to higher volume of biogas (methane) generated. Overall, among all digestion systems, the mesophilic digester (sonicated at 4063 kJ/kg TS) showed the highest stability at the shortest SRT of 5 days with significant (80%)

increase in gas production and organic removal efficiency over control (un-pre-treated) digester. However, the results also indicated that at longer (safer) SRTs 10 and 20 days, low power sonication pre-treatment (2042 to 8153 kJ/kg TS) did not represent substantial benefits in terms of organic removals, biogas production, fecal coliform destruction or enhancement in dewaterability.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CFU	Colony forming unit
COD	Chemical oxygen demand
CST	Capillary suction time
EPS	Extracellular polymeric substances
FPS	Fermented primary sludge
M	Mesophilic
MPN	Most probable number
OMRR	Organic matter recycling regulation
OLR	Organic loading rate
SCOD	Soluble chemical oxygen demand
SRT	Sludge retention time
STP	Standard temperature and pressure
TCOD	Total chemical oxygen demand
T	Thermophilic
TS	Total solids
TWAS	Thickened waste activated sludge
TVFA	Total volatile fatty acids
VS	Volatile solids
VSCs	Volatile sulfur compounds
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

GLOSSARY

Solubilisation: Solubilisation of organic matters in sludge refers to the ratio of the organic matter in liquid phase as a result of sludge disintegration and total organic matters in raw sludge.

Thermophilic anaerobic digestion: Thermophilic anaerobic digestion takes place under the optimal temperature conditions of 49 to 57^{0}C, or at elevated temperature up to 70^{0}C. Thermophiles are the primary microorganisms present in thermophilic digesters.

Mesophilic anaerobic digestion: Mesophilic anaerobic digestion takes place under the optimal temperature conditions of 30 to 38^{0}C, or at ambient temperatures in the range of 20 to 45^{0}C. Mesophiles are the primary microorganisms present in mesophilic digesters.

Solid retention time (SRT): Solid retention time is defined as the mean residence time of microorganisms inside the digester. The SRT is related to growth and reproduction rate of the microorganisms and has an effect on the reactions (hydrolysis, fermentation and methanogenesis) of the anaerobic digestion process.

Organic loading rate (OLR): Organic loading rate is defined as the influent mass flow rate of organic materials applied to per unit volume of reactor.

Waste activated sludge (WAS): Sludge generated from the secondary treatment system at a wastewater treatment plant is known as waste activated sludge

Total solids (TS): The weight of dry matter (the remaining material after complete evaporation of water) of sludge sample is represented by total solids and expressed as a percentage of the total weight of sample.

Volatile solids (VS): Volatile solids represent the organic matter in sludge sample measured as solid content minus ash content after complete combustion of sludge waste and expressed as a percentage of the total weight of sample.

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CHAPTER 1: INTRODUCTION

1.1 Background and motivation

There is no denial of the fact that municipal wastewater treatment and sludge management have become a very challenging task across the world. During wastewater treatment processes, sludge production is essentially inevitable. The USA alone produces approximately 6.5 million dry tons of sludge each year. In Canada the wastewater treatment plants (WWTP) generate more than 660,000 tonnes of dry sludge per year (CCME 2012). The dry treated sludge has been termed "biosolids" because of the potential to be beneficially reused. A rough estimate of the rate of sludge production, or biosolids production, in some other countries around the world is presented in Table 1.1.

Table 1.1: Estimated sewage sludge production and populations of different countries

Country	Estimated sewage sludge production (dry metric tons/yr)	Population[1]	Reference
Brazil	372	188078000	UN-HABITAT, 2008
China	2966000	1313974000	UN-HABITAT, 2008
Turkey	580	70414000	UN-HABITAT, 2008
Japan	2000000	127464000	UN-HABITAT, 2008
Slovakia	55	5439000	UN-HABITAT, 2008
Portugal	236.7	10606000	UN-HABITAT, 2008
Finland	150	5231000	UN-HABITAT, 2008
Italy	1000000	58134000	UN-HABITAT, 2008
UK	1640000	60609000	Ludovico Spinosa (2011)
Germany	2000000	82422000	Ludovico Spinosa (2011)
Netherlands	1500000	16491000	Ludovico Spinosa (2011)
Slovenia	57	2010000	UN-HABITAT, 2008
Czech Republic	200	10235000	UN-HABITAT, 2008
Hungary	120	9981000	UN-HABITAT, 2008
USA	6514000	298444000	UN-HABITAT, 2008
Canada	660000	33100000	Ludovico Spinosa (2011)

[1] Source: http://www.infoplease.com//pal/A0004379.html

Table 1.1 suggests that the developed countries with wide-ranging infrastructure and treatment facilities produce more wastewater sludge per person. This implies that as the treatment technology improves, more sludge will be generated, and as countries continue to develop, a steady increases in sludge production is likely to be observed worldwide. Furthermore, environmental legislation has imposed restrictions and bans on the conventional and cheap disposal methods of sludge, such as land application and ocean disposal.

In Canada there are more than four thousand WWTPs regulated by the environmental agencies of provincial governments (UN-HABITAT, 2008). The annual cost of sludge management is around 50% of the total operating cost of wastewater management (Bryden and Langman, 2009). In the USA, sludge management is regulated at the federal level. In Canada, sludge management, and the resulting policies aimed at protecting public health, water quality and the environment are regulated at the provincial and territorial level under a multifaceted controlling system (Ludovico Spinosa, 2011). In Figure 1.1 presents the steps followed in sludge management. Each province manages the production, treatment, processing, handling, storage, transport, end use of biosolids; and the operation and maintenance of WWTPs through relevant standards and regulations. Currently, the commonly practiced sustainable sludge reuse strategies include composting, incineration, and land application in agriculture, mines, forestry, silviculture and other unsettled areas; however, there has been an increasing attention to curb land application which will ultimately put more pressure on municipalities to adopt alternative methods of reusing biosolids. Although biosolids can have beneficial uses, the management of biosolids accounts for the majority of the cost in a WWTP, so treatment processes that minimize biosolid production are favourable.

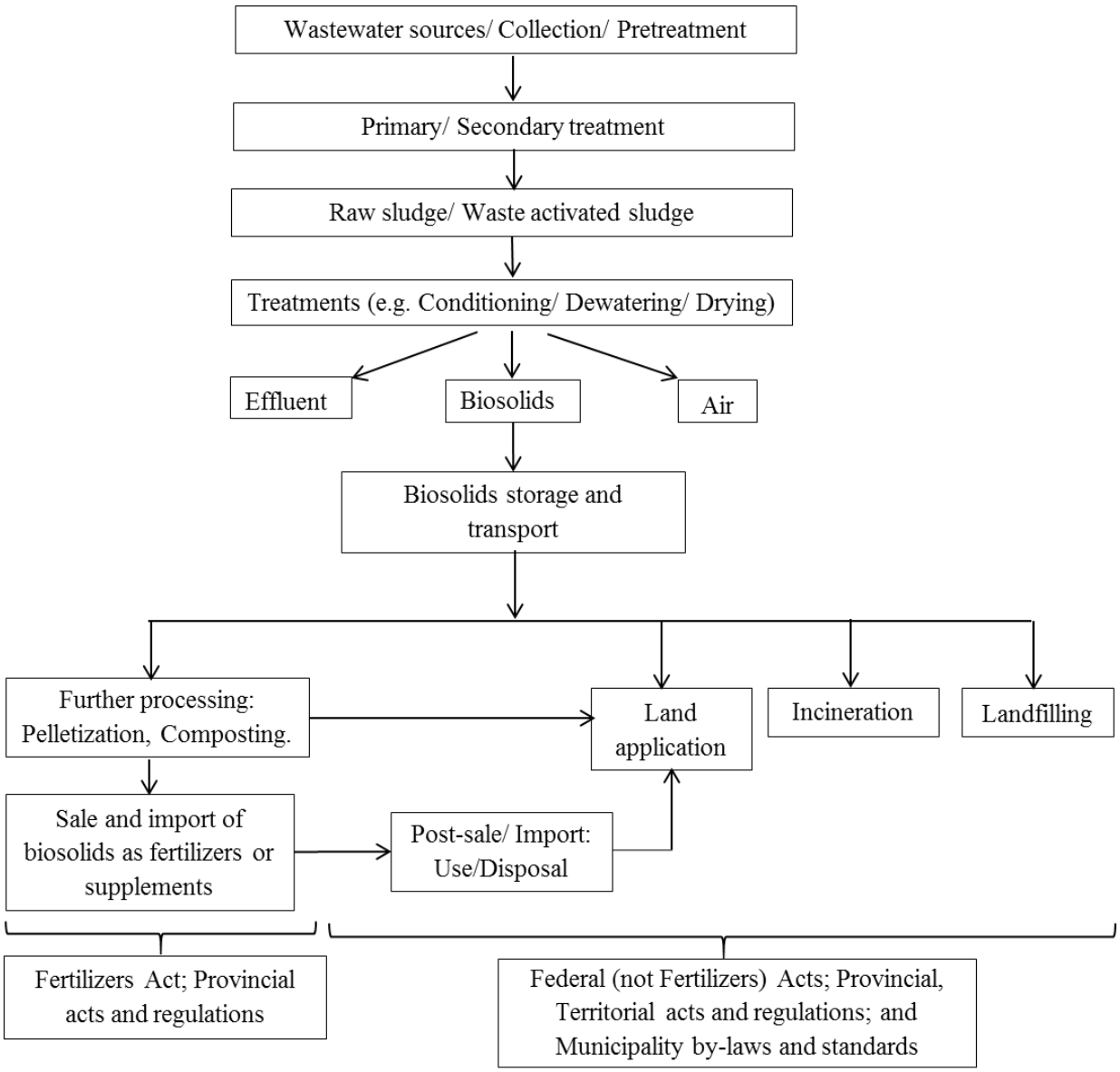


Figure 1.1: Regulations of sludge/biosolids production, treatment and disposal in Canada (redrawn; source: CCME, 2010)

From the above scenario it is clear that this issue should be addressed with consideration of the adverse effects inadequate sludge treatment can have on the social, environmental and health aspects of society. This perspective has led researchers to develop sound sludge management systems that produce reduced amounts of biosolids as well as other economic and sustainable benefits.

During the last decade, anaerobic digestion has been one of the preferred options of sludge stabilisation due to the low volumetric generation of biosolids and the potential to recover energy in the form of methane gas. The process is a highly complex multi-enzymatic biodegradation process with various stages. During the hydrolysis stage, larger suspended particles are being chemically deteriorated into soluble materials that can be metabolized by the bacteria. The hydrolysis process is known to be the rate limiting step of the anaerobic digestion process (Appels et al., 2008). Therefore, several sludge pre-treatment methods, i.e. mechanical (Riaau et al., 2015), thermal (Xue et al., 2015), chemical (Feng et al., 2014), biological (Kavitha et al. 2014) and various combinations of the aforementioned methods (Rani et al., 2014) have been investigated to enhance the hydrolysis of particulate organic matter as well as the efficiency of anaerobic degradation. Among these pre-treatment methods, ultrasonication is an attractive technology for sludge disintegration because of some inherent advantages such as: no chemical addition requirement, short retention time requirement for anaerobic digestion, improved biosolids quality, enhanced biodegradability and more energy recovery potential (at the full-scale). Ultrasonic assisted anaerobic digestion has been extensively studied in the laboratory (Martín et al., 2015; Braguglia et al., 2011; Feng et al., 2009; Zhang et al., 2007;Chu et al. 2001), pilot (Tiehm et al., 1997; Zhanga et al., 2013) and in full-scale applications (Barber et al., 2005; Xie et al., 2007; Perez-Elvira et al., 2009). Nevertheless, little data is available on the effect of low power sonication on subsequent anaerobic digestion performance, especially dewaterability, odour causing volatile sulfur compounds (VSCs) emissions and fecal coliform destruction under both thermophilic and mesophilic digestion conditions. Furthermore, negative energy balance (reported for studies at the laboratory-scale) is an economic bottleneck in the consideration of applying ultrasonication assisted anaerobic digestion in a conventional WWTP.

Thus, with an attempt to establish a cost-effective sonication anaerobic digestion process, this study was carried out to investigate the effect of low power sonication on the anaerobic digestion process under a wide range of organic loading rates (i.e., 1.8, 3.4 and 6.8 g VS L^{-1} of digester d^{-1} at SRTs of 20-d, 10-d and 5-d respectively).

1.2 Objectives

The aim of this study was to evaluate the effect of low power sonication at three different specific ultrasonic energy conditions (at 2042, 4163 and 8153 kJ/kg TS) on anaerobic digestibility of municipal waste sludge under mesophilic and thermophilic digester temperatures. The specific objectives are as follows:

1. Optimization of sonication operating conditions (i.e. sonication time, and sonication density) by investigating solubilisation of chemical oxygen demand (COD) and biopolymers (sugar and protein), particle size reduction, and zeta potential of thickened waste activated sludge (TWAS) before and after sonication.
2. Evaluation and comparison of anaerobic digestibility of control (non pre-treated) and sonication pre-treated anaerobic digestion at three different solid retention times under mesophilic and thermophilic digester temperatures by monitoring the following parameters:
    a. biogas production and composition
    b. solid removals
    c. volatile fatty acids (VFA) accumulation
    d. odour causing VSCs emissions
    e. dewaterability of digester effluents or digestates
    f. fecal coliform densities in digestates
3. To perform an energy feasibility study to evaluate the sonication pre-treatment on energy balance of anaerobic digestion

CHAPTER 2: LITERATURE REVIEW

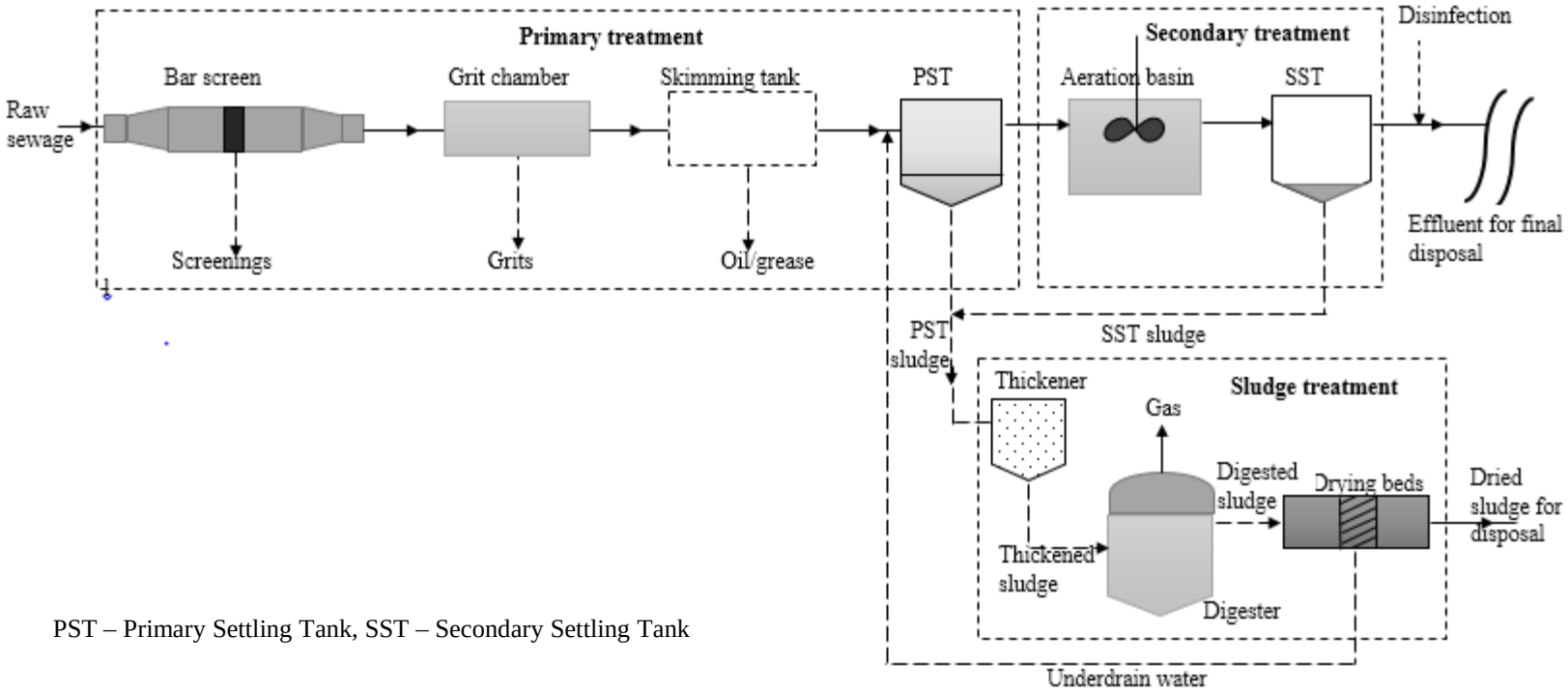
In a global perspective of growing concern over public health, energy demand, greenhouse gas emissions, rising costs and environmental pollution, it is essential to develop strategies for the proper management of wastewater sludge. Though sludge generated in a WWTP represents only 1% to 2% of treated wastewater by volume, its management cost accounts for 20% to 60% of the operating cost of the WWTP (Sperling and Andreoli, 2007).

Wastewater treatment sludge or simply "sludge" contains highly degradable organics, heavy metals, pathogens and trace amount of contaminants that must be subjected to appropriate treatment prior to final disposal. Among various treatment and disposal routes, anaerobic digestion offers a viable option of sludge stabilization by transforming organic matters into a renewable energy in the form of biogas. In addition to the generation of renewable energy, anaerobic digestion has additional advantages which include: reduction of the final biosolid volume, increased nutrient quality in the biosolid, and odor control. Hence, anaerobic digestion optimises the costs of a WWTP and has been extensively recognised as an indispensable part of a modern WWTP (Appels et al., 2008).

This chapter will briefly discuss wastewater treatment, sludge characteristics, sludge floc structure, sludge minimization techniques and anaerobic digestion.

2.1 Wastewater treatment

Wastewater treatment is strongly correlated with the regulations set for the effluent quality. A typical WWTP (Figure 2.1) comprises of several unit processes (physical, chemical or biological) to achieve clean effluent.



PST – Primary Settling Tank, SST – Secondary Settling Tank

Figure 2.1: Schematic diagram of a conventional wastewater treatment plant (adapted from Karia and Christian, 2006; Kroiss et al., 2011)

Depending on the selection of treatment system, either one or a combination of the unit processes are employed to reduce suspended solids, biodegradable organics (e.g. biochemical oxygen demand or BOD), pathogens, nutrients etc. Usually a WWTP will have a combination of the primary, secondary or tertiary (advanced) treatment systems (Karia and Christian, 2006).

2.1.1 Primary treatment

The primary treatment involves a preliminary treatment system and a primary sedimentation tank. The preliminary treatment system is designed to remove suspended material, floating material and large objects that could cause maintenance or operation problems with the equipment. This system includes screening (physical removal of gross objects), grit chamber (removal of suspended settleable grits) and skimming tank (removal of oil and grease from wastewater). Wastewater then passes slowly through a primary sedimentation tank which promotes the gravitational sedimentation of the readily settleable large solid particles. The settled sludge is pumped away for further treatment. The primary sedimentation tank removes around 60-70% of total suspended solids (including 30-32% of the organic fraction) in the wastewater, while the colloidal and dissolved organic content is removed in the secondary treatment system. Sludge generated from primary treatment and sedimentation is called primary sludge.

2.1.2 Secondary treatment

The secondary treatment system utilizes biological growth in which the remaining dissolved organic matter is converted to settleable bacterial flocs. This process is achieved by using microorganisms that consume the soluble organic content of wastewater as food and transform it to water, carbon dioxide (CO_2) and energy for their own growth and reproduction. The biodegradation is then followed by secondary sedimentation for removal of the settleable bacterial

flocs. Sludge generated from the secondary treatment system is known as waste activated sludge (WAS). There are a variety of secondary treatment technologies which use biological activity to transform the dissolved organics into settleable bacterial flocs including oxidation ponds, aerated lagoons, rotating biological contactor, upflow anaerobic sludge blanket, extended aeration system, etc.

2.1.3 Advanced treatment

Tertiary or advanced wastewater treatment is typically employed when secondary effluent is not suitable for final discharge as per regulation requirements. This system is required for further reduction or complete removal (for reuse or recycling) of residual dissolved solids, heavy metals, nitrogen, phosphorus and refractory materials. However, sometimes advanced treatment processes are combined with primary or secondary treatment (e.g., addition of chemicals to primary sedimentation tanks or aeration tanks for phosphorus removal) or applied in place of secondary treatment (e.g., overland flow treatment of primary effluent).

2.2 Sludge and biosolids

The term “sludge” is described as untreated solids produced during wastewater treatment processes. The residual solids are treated to destroy pathogens and heavy metals using one or more technologies including biological (i.e. aerobic or anaerobic digestion, composting), chemical (i.e. alkaline addition) or physical methods (i.e. air drying, heat treatment) and the treated product is termed “biosolids” for its potential to be beneficially reused. Biosolids contain nutrients (nitrogen, phosphorus etc.), organic matter and trace amounts of regulated metals (micronutrients like zinc, molybdenum and copper) which are beneficial for plant growth. Therefore, biosolids are a

beneficial natural resource once they meet regulations and standards set by the federal or provincial legislation.

The U.S. Environmental Protection Agency (EPA) categorized biosolids into Class A and Class B based on fecal coliform densities. When fecal coliforms are reduced to less than 1000 most probable number (MPN)/ g dry TS, or density of *Salmonella* spp. is reduced to less than 3 MPN/ 4 g dry TS, biosolids are considered as Class A biosolids. Class A biosolids are convenient for use on residential grasses, parks, golf courses and botanical gardens. On the other hand, Class B biosolids are treated to reduce the fecal coliforms to site authorized levels before being applied to the land. Class B biosolids usually contain between 1000 - 2,000,000 MPN (fecal coliforms) per g dry TS. Some biosolids end-users like farmers prefer Class B biosolids over Class A as Class B has more plant-available nutrients. In British Columbia under the Organic Matter Recycling Regulation (OMRR), biosolids are classified as Class A or Class B. Class A (fecal coliforms less than 1000 MPN/g dry TS) biosolids meet the most stringent concentration levels of trace element provided by OMRR while Class B biosolids (containing fecal coliforms less than 2,000,000 MPN/g dry TS) undergo less stringent trace constituents and are of lower quality.

2.3 Sludge characteristics

Sludge is a biologically active mixture of water, organic matter, inorganic solids, dead and alive microorganisms (including pathogens) and trace contaminants (chemicals). The sludge generated in a WWTP is usually in the form of a liquid or semisolid, containing 0.25 to 12 percent solids by weight, depending on the treatment operations and processes used (McGhee, 1991). The knowledge of sludge characteristics is crucial in the design of a tertiary digestion system. The sludge characteristics vary based on the geographic location and cultural food consumption of the

population; food to microorganism ratio; and on the source of sludge, whether from primary or secondary treatment systems. Additional factors that influence sludge characteristics include the quality of wastewater, type of treatment used in the primary and secondary treatment systems, and the fate of the sludge. As mentioned in sections 2.1.1 and 2.2.2, the simplest classification of sludge is primary sludge from the primary treatment system and WAS from the secondary treatment system.

Table 2.1: Physical properties of sludge

Parameters	Untreated Primary Sludge		Waste Activated Sludge		References
Basic definition	Grey or light brown, sour odor, contains readily biodegradable organic matters		Dark brown or light grey, flocculent suspension of microbial cells, earthly odor		
Total solid content (%)	2-8%		0.5-2%		Metcalf and Eddy (2003)
Volatile solid content	60-80%		50-70%		Metcalf and Eddy (2003)
Specific gravity	1.02		1.05		Metcalf and Eddy (2003)
Density (kg/m^3)	1000-1003		1000		Turovskiy and Mathai (2006)
SVI and ZSV			80-120 ml/g 2.89±1.26 m/h		Sanin et al.(2011) Jin et al. (2003)
Particle size distribution (%)	>100 µm	66.5%	(< 0.1) µm	28%	Aldin (2010)
	1-100 µm	27.5%	(0.1-1) µm	3%	Levine et al. (1985)
	0.001-1 µm	0.5%	(1–12) µm	20%	
	<0.001µm	5.4%	(> 12) µm	49%	
Shear strength (kN/m^2)	<5		<2		Kiely (1997)

Physical properties of wastewater sludge are characterized by floc or particle size, density and fractal dimension (Wu et al., 1997). Fractal dimension and filament index are the major factors which greatly influence the sludge volume index (SVI) and zone settling velocity (ZSV). SVI and ZSV measurements are the two important characterization tests of sludge settleability, and they are considered as an important economic property in the operation of a biological treatment process because the reduction of sludge volume will result in less operational costs for the WWTP. The

optimum secondary treatment systems will produce a SVI of 80-120 (Sanin et al., 2011). The activated sludge with such low SVI values and high ZSV settles fast parting a clear supernatant. In contrast, an activated sludge with high amounts of filamentous bacteria, corresponding to low fractal dimension exhibits poor settleability and has a high SVI of around 200 (Palm et al., 1980). Table 2.1 represents the typical physical properties of sludge. Physical characteristics of sludge determine, to a great extent, the possibilities and conditions of digestion and disposal of sludge.

Sludge settling and filterability is affected by some other factors, such as the floc size, floc heterogeneity and the amount of polymeric compounds (Schmid et al., 2003). Morgenroth et al. (2002) stated that particle size and particle composition govern the rate and mechanism of hydrolysis and degradation in wastewater treatment. Since it is difficult to measure floc size, the effect of individual particles on sludge settleability and separation has been studied instead of measuring floc size (Sanin et al., 2011). The typical particle size distributions for primary sludge and WAS were presented in Table 2.1.

The amount and composition of extracellular polymeric substances is a sludge characteristic that influences the dewaterability, rheology, floc structure and thermal conductivity of the sludge. Sludge dewatering involves separating water and solids through the use of various methods, such as vacuum filters, centrifuges, geomembrane filtration, filter and belt fibre presses. Depending on the method, the dewatering process removes 15 to 45 percent of the water. The main objective of dewatering is to reduce the sludge volume to make it easier for handling and transportation. Municipal and industrial sludge is hydrated by nature, and will contain water 99.7% - 98% of the time. Particle size of the sludge flocs is one of the important factors that affect the dewaterability of sludge, as the particle size decreases it becomes more difficult to dewater sludge. Sludge

treatment prior to dewatering, especially by aerobic or anaerobic digestion, decreases average particle size, and as a result, digested sludge is more difficult to dewater than raw sludge. The chemical properties of waste sludge include alkalinity, pH, organic matter, nutrients, metals and fatty acids. Alkalinity and pH are the most important chemical parameters that affect sludge conditioning. Table 2.2 lists some of the chemical properties of raw sludge.

Table 2.2: Chemical properties of sludge

Parameters	Primary sludge	Waste Activated Sludge	References
pH	5.0-8.0	6.5-8.0	Metcalf and Eddy (2003)
Alkalinity (mg/L as $CaCO_3$)	500-1500	580-1100	Metcalf and Eddy (2003)
Organic acid (mg/L as HAc)	200-2000	1100-1700	Turovskiy and Mathai (2006)
Nitrogen (% of TS)	1.5 - 4.0	2.4 - 5.0	Metcalf and Eddy (2003)
Phosphorus (% of TS)	0.8 - 2.8	2.8 - 11.0	Metcalf and Eddy (2003)
Energy Content (kJ/kg)	23,300	23,300	Turovskiy and Mathai (2006)

* HAc – Hydrogen acetate

2.4 Structure of waste activated sludge

Activated sludge is one of the biological treatments used in the secondary treatment process. The soluble organic contents in the wastewater are consumed by microbial organisms in the presence of air. In a WWTP, the efficiency of activated sludge treatment is monitored by analyzing the sludge characteristics and quality. Sludge floc structure has a great influence on the performance of sludge solubilisation, bioflocculation, solid-liquid separation and dewaterability (Li and Ganczarczyk, 1990).

2.4.1 Waste activated sludge

The excess sludge that is removed from the treatment process is recycled back into the system in order to maintain a constant biomass (microorganism) to food ratio. This sludge, as described in section 2.1.2 is referred to as WAS. It is widely known that WAS mainly comprises of aggregate

forming flocs having a complex and heterogeneous composition of microorganisms, extracellular and intracellular polymers, organic, inorganic particulates and large quantities of water. Figure 2.2 displays main constituents of an activated sludge floc.

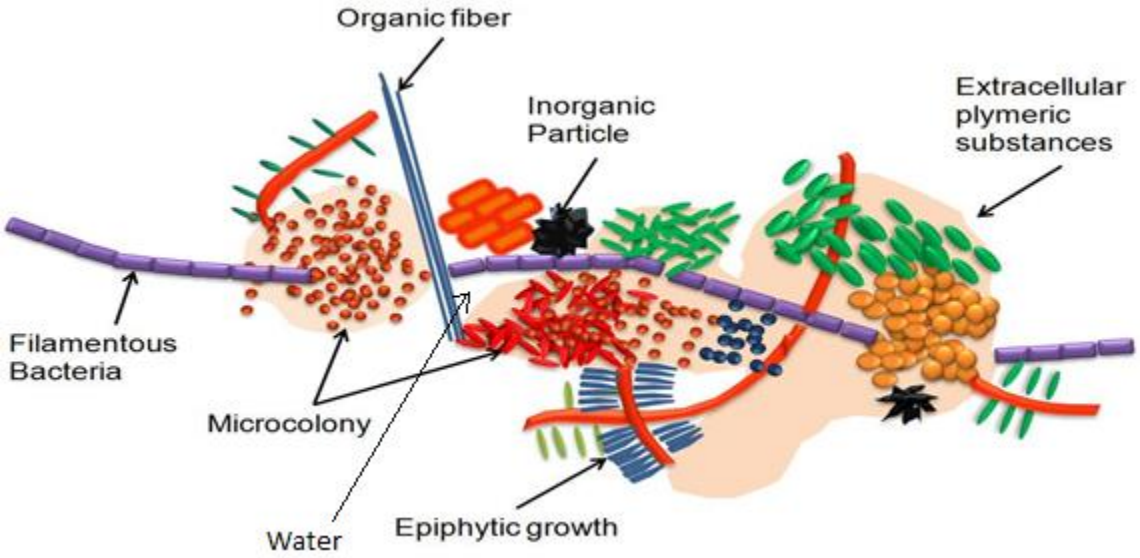


Figure 2.2: The structure of activated sludge floc (redrawn from Nielsen et al., 2012)

2.4.2 Microbial composition

Sludge flocs comprise a broad range of microorganisms like bacteria, protozoa, viruses, fungi and rotifers. The majority of these microorganisms are heterotrophic bacteria. The bacteria are present as single bacteria, floc-forming or filamentous bacteria. Filamentous bacteria serve as a backbone being responsible for mechanical strength and drainage properties of the floc (Ekama et al., 1997). The floc forming bacteria agglomerate onto this backbone (Kins et al., 1986; Wanner 1994). The bacterial cells make up 5-20% of the organic matters in the activated sludge flocs (Fround et al., 1996; Lavellée et al., 2002). The remaining 80-95% of the organic matter exists as dead cells. The inorganic compounds are found to be accountable for many of the colloidal-chemical properties of the floc (Keiding et al., 2001).

Nielsen et al. (2004) postulated that microbial communities are able to determine floc properties (e.g. floc strength and stability) and sludge properties (e.g. flocculation, dewaterability and settling) as they possess different properties like floc density, metabolic reaction etc. Different floc-forming bacteria exhibit different floc strengths and sensitivities to shear applied. From the aforementioned (Nielsen et al., 2004) study, it was observed that when shear was applied some bacterial groups deflocculated under anaerobic conditions while some other groups were found to be reduced in size under aerobic conditions.

2.4.3 Water content of WAS

Water associated with extracellular polymers and other chemical substances is the main component of the waste activated sludge floc. All types of water within the sludge do not possess identical properties due to the existence of solid particles (Vesilind, 1994) and therefore it is not very easy to completely dewater the solids. For a better understanding of sludge dewatering, it is necessary to understand the structuring of water molecules as well and various water fractions (Vesilind, 1994).

The earlier researchers classified the water content of sludge into two categories, “free” water and “bound” water. Vesilind investigated four types of water depending on their attachment to the solid particles existing in the sludge matrix (Figure 2.3) (Vesilind and Hsu, 1997; Kopp and Dichtl, 2000). The types of water are as followed:

I. **Free (bulk) water**: Water that is not related with and not influenced by the suspended solid particles. It can be removed by drainage, thickening or mechanical treatment.
II. **Interstitial water**: This type of water is entrapped in interstitial spaces of the flocs and can be released only by destruction or compression of the floc structures using

sufficient mechanical energy. Sometimes interstitial water may become free water when the cells are damaged.

III. **Surface or vicinal water:** Surface water represents the water very closely attached to the surface of particles in manifold layers. This type of water cannot move freely. It cannot be removed mechanically unless the sludge is polymer conditioned.

IV. **Intracellular water or water of hydration**: Water that is chemically bound to the solids and can only be removed with the application of thermal energy.

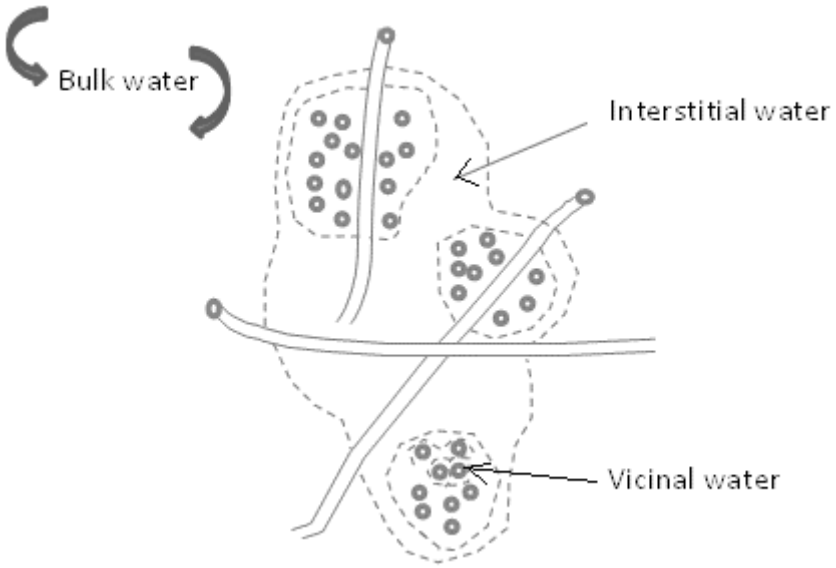


Figure 2.3: Representation of water contents of sludge (adapted from Vesilind, 1994)

From the above classification system, it can be stated that the sludge dewatering will be successfully accomplished only when all types of water are removed. Although, it is difficult to completely remove hydration water, the modification of the sludge structure can enhance its removal.

2.4.4 Extracellular polymeric substances

In WAS, the largest fraction (80-95%) of organic matter other than bacteria are extracellular polymeric substances (EPSs). An EPS is a three-dimensional matrix consisting of various biopolymers such as polysaccharides, proteins, glycoproteins, lipids, phospholipids, nucleic acids

and humic substances. EPSs are gel-like, highly-hydrated and often highly-charged networks that keep the microbes together in flocs. EPSs are produced as a result of lysis and metabolism of microbial cells in combination with the adsorption of dissolved and particulate substances from the surrounding environment.

In the understanding of the structure, function, properties and development of flocs, EPSs are the key components (Wingender et al., 1999; Cloete et al., 2001). There are voids and channels in the EPS matrix which function to construct a large surface area for absorption of nutrients and expedite water and nutrient carriage to the cells (Liss et al., 1996; Daims et al., 2001; Chu and Lee, 2004). Several additional functions of EPS are mentioned in Table 2.3.

Table 2.3: Functions of EPS (adapted from Flemming and Wingender, 2010; Flemming et al., 2007)

Functions	The key EPS component
Adhesion of biofilms to surfaces	Polysaccharides, amyloids, proteins
Accumulation of bacterial cells	Polysaccharides, proteins and DNA
Protection of bacteria against harmful effects during infection	Polysaccharides, proteins
Mechanical stabilization of flocs through the EPS structure or multivalent cations bridges	Neutral and charged polysaccharides, amyloids
Retention of water	Hydrophilic polysaccharides
Sorption of exogenous organic and inorganic compounds	Charged or hydrophobic polysaccharides and proteins
Enzymatic activities for degradation of EPS resulting the release of bacteria from biofilms	Enzymes
Acts as a source of C, N, P containing nutrients for utilization of microbial cells	All polymers

2.4.5 Composition of EPS

The composition and quantity of EPS is greatly affected by biofilm growth conditions as well as the wastewater composition (Eriksson and Alm, 1991; Nielsen et al., 1996). Protein is the main constituent (more than 43% of EPS) (Wilen et al., 2003; Liao et al., 2001; Bura et al., 1998; Frølund

et al., {1994;1996}; Urbain et al., 1993; Goodwin and Foster, 1985); whereas Horan et al. (1986) found that polysaccharides are the major components (65% of extracellular matrix). Humic substances were found to be the next largest constituent, and they hold approximately 15–42% and 10–18% of the EPS matrix respectively. Uronic acid (1-2%), DNA (1-6%) and RNA constitute a moderately smaller organic fraction of the EPS (Wilen et al., 2003).

2.4.6 Structure of waste activated sludge: size and shape

On the basis of visual inspection and some physical measurements, floc structure can be divided into two categories termed micro-structure and macro-structure (Sezgin et al., 1978). Sludge flocs are not spherical in shape, rather they have their own extremely irregular forms. In a waste activated sludge floc, the components may be separated into two key parts, flocs and primary particles. Primary particles which contain bacteria and other floc components are around 0.5–5 μm whereas the floc sizes range between 25-1000 μm (diameter) (Li et al., 1990; Snidaro et al., 1997). According to Andreadakis (1993), the flocs fall in a typical range of 10-70 μm having floc densities of 1.015-1.034 g/cm^3. He observed that floc density decreases as the floc size increases.

In an activated sludge process using a relatively young sludge age (retention time of biomass spent in bioreactor) only floc-forming bacteria exist. At this stage the flocs are small, compact and spherical. With the increase of sludge age, the filamentous organisms grow and tend to elongate. Filamentous bacteria provide macro-structure and the floc particles become larger and the spherical shape becomes irregular.

2.4.7 Stratification structure of waste activated sludge

Yu et al. (2008) described a stratification of sludge flocs to investigate the distribution patterns of proteins and polysaccharides in the different layers and to identify the factors that influence

sludge dewaterability. EPSs in sludge flocs can be divided into soluble EPSs (i.e., slime) and bound EPSs. Slime or soluble EPSs are not directly linked with the cell whereas bound EPS is closely attached to the cell. On basis of the extraction methodology, bound EPS again have been characterized as loosely bound EPSs (LB-EPSs) and tightly bound EPSs (TB-EPSs). After EPSs have been extracted, the leftover cells form a pellet. Therefore, sludge flocs hold a multilayered structure (from their outer surfaces to the nuclei of their granules) consisting of supernatant (bulk solution), slime, LB-EPS, TB-EPS, and pellet (Figure 2.4).

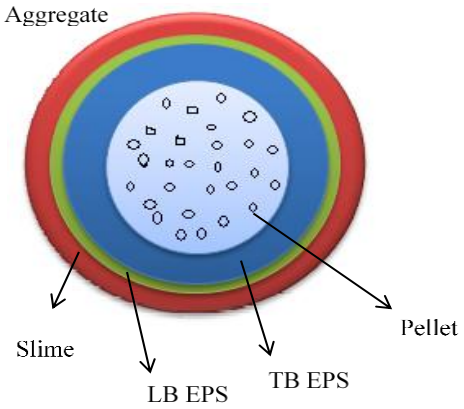


Figure 2.4: Proposed stratification multilayered structure of sludge flocs (adapted from Yu et al., 2008)

2.4.8 Cations in sludge

In waste activated sludge, EPSs are negatively charged. The negative charge is caused by functional groups such as the carboxyl groups. Cations (usually multivalent) play a substantial role in binding the EPSs to the cell by their high bridging capacities which ultimately form electrostatic sludge flocs (Bruus et al., 1992; Higgins and Novak, 1997a; Urbain et al., 1993). Divalent cations lead to improved flocculation and dewatering properties. On the contrary, monovalent cations cause deterioration in settling and dewatering properties. Trivalent cations (Al^{3+} and Fe^{3+})

contribute to floc formation by neutralizing the negatively charged functional groups and by cationic bridging in the sludge. Kara et al. (2008) postulated that sodium ions have a greater negative impact on floc stability and dewatering properties of sludge and effluent in comparison with potassium. Higgins and Novak (1997b) investigated the effects of cations on sludge properties and revealed that both the concentration and ratio of cations in the feed govern the settling and dewatering properties of the waste activated sludge. In addition, they suggested that the ratio of monovalent and divalent cations (M/D) should be kept in the range of 2 to 1. If M/D ratio exceeds 2, the settling and dewatering properties become unfavourable.

2.5 Sludge treatment technologies

There is a variety of biological sludge treatment methods used in a WWTP. The goal of these treatment methods is to minimize sludge generation. Perez-Elvira et al. (2006) presented an overview of the existing sludge treatment processes, organized based on the nature of the treatment and the location of the plant where sludge minimization will be applied (Figure 2.5). Three key approaches have been identified as follows:

I. Processes in the water line
II. Processes in the sludge line
III. Processes in the final waste line

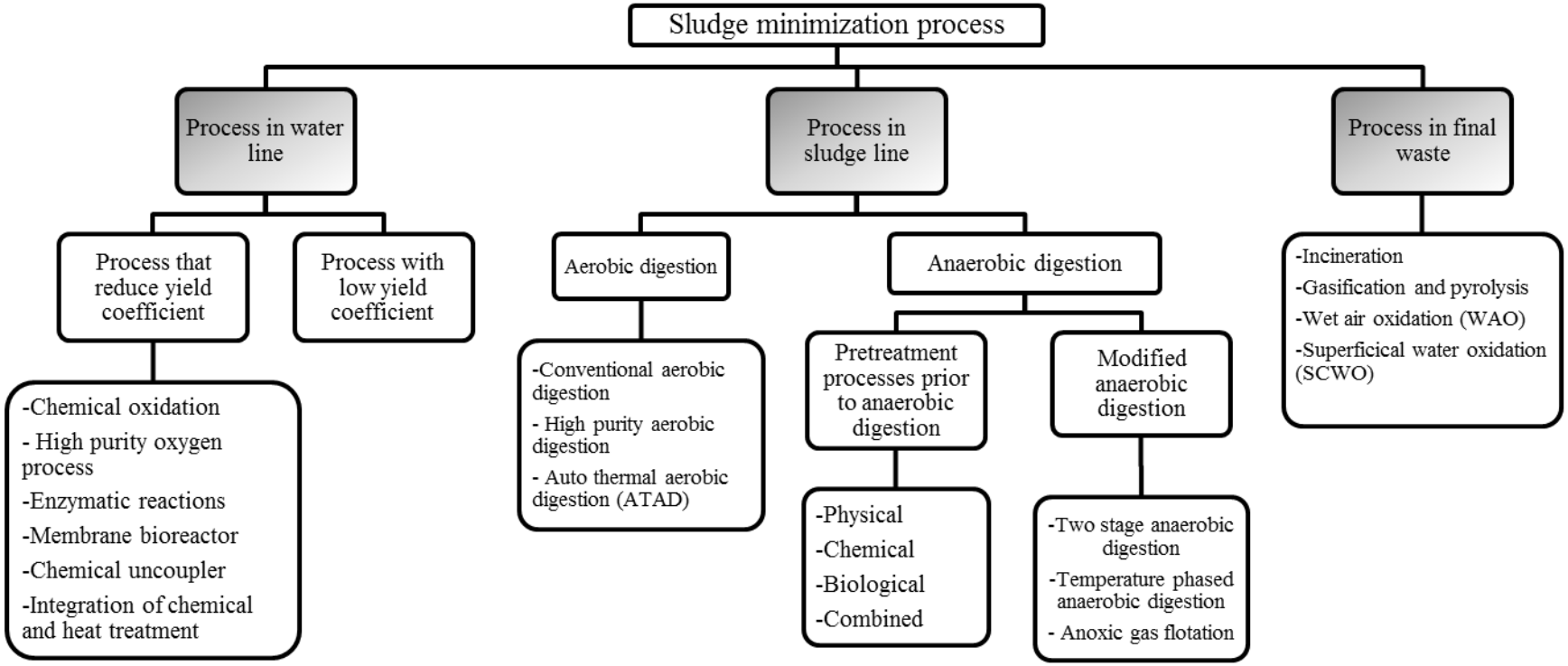


Figure 2.5: Sludge minimization technologies (adapted from Perez-Elvira et al., 2006)

2.5.1 Processes in the water line

The processes in water include lysis cryptic growth, maintenance and uncoupling metabolism, anaerobic-aerobic digestion processes and predation on bacteria that reduce sludge generation by lowering the cellular yield coefficient.

2.5.2 Processes in the sludge line

The anaerobic digestion process can be applied to the sludge line in order to reduce the volume of undigested solids coming from the primary and secondary treatment processes. The anaerobic digestion process can be enhanced by implementing a variety of pre-treatment technologies. When sludge pre-treatment technologies are applied before anaerobic digestion, the process is commonly known as advanced anaerobic digestion or modified anaerobic digestion. The pre-treatment methods applied prior to anaerobic digestion are matters of increasing interest and will be discussed later in this chapter.

2.5.3 Processes in the final waste line

The processes applied to the final waste line present an opportunity for the final disposal of sludge (incineration produces sludge ash, not dewatered biosolids). Among these processes incineration, gasification, pyrolysis, wet air oxidation and superficial water oxidation are popular.

2.6 Anaerobic digestion

The anaerobic digestion process involves a series of several symbiotic, complex and parallel bio-chemical reactions that take place in a sealed environment, free from contact with the atmosphere. During this process the products derived from one group of the microbial population serve as the substrates for the subsequent reactions resulting in a mixture of methane (CH_4) and

carbon dioxide (CO_2) gas (Noykova et al., 2002; Pavlostathis and Giraldo-Gomez, 1991; Gujer and Zehnder, 1983). Figure 2.6 shows a simplified anaerobic digestion process consisting of four key stages:

1. **Hydrolysis**: During hydrolysis, biopolymers and other organic materials are broken down to soluble organic molecules (e.g., monosaccharaides, amino acids and fatty acids) with the help of extracellular enzymes secreted by fermentative bacteria. Hydrolysis is considered as the rate limiting step in the anaerobic digestion process (Appels et al., 2008). During hydrolysis, the conversion of polymeric carbohydrates, proteins and fats occur by cellulases, proteases and lipases (Stryer, 1995). Eastman and Ferguson (1981) analyzed the aggregate effects on hydrolysis kinetics and concluded that the reactions proceeded according to single first order kinetics. Literature suggests that anaerobic digestion operating conditions such as temperature, pH, particle size, SRT and mainly food to inoculum (microorganism) (F/I) ratio govern the hydrolysis kinetic coefficient (k_h).

2. **Fermentation**: The components formed in hydrolysis are fragmented into short chain organic acids. Acetate is the main end product, but volatile fatty acids (VFAs), carbon dioxide, hydrogen (H_2) and other by-products are also produced. The growth of acidogenic bacteria is relatively faster. Moreover, they are less sensitive to pH variations than acetogens and methanogens. Zeikus (1980) reported that acidogens population commonly embodies around 90% of the total bacterial population existing in anaerobic digestion system.

3. **Acetogenesis**: In this step, volatile acids derived from fermentation are transformed to acetic acid as well as CO_2 and H_2 by obliged H_2 producing acetogenic bacteria.

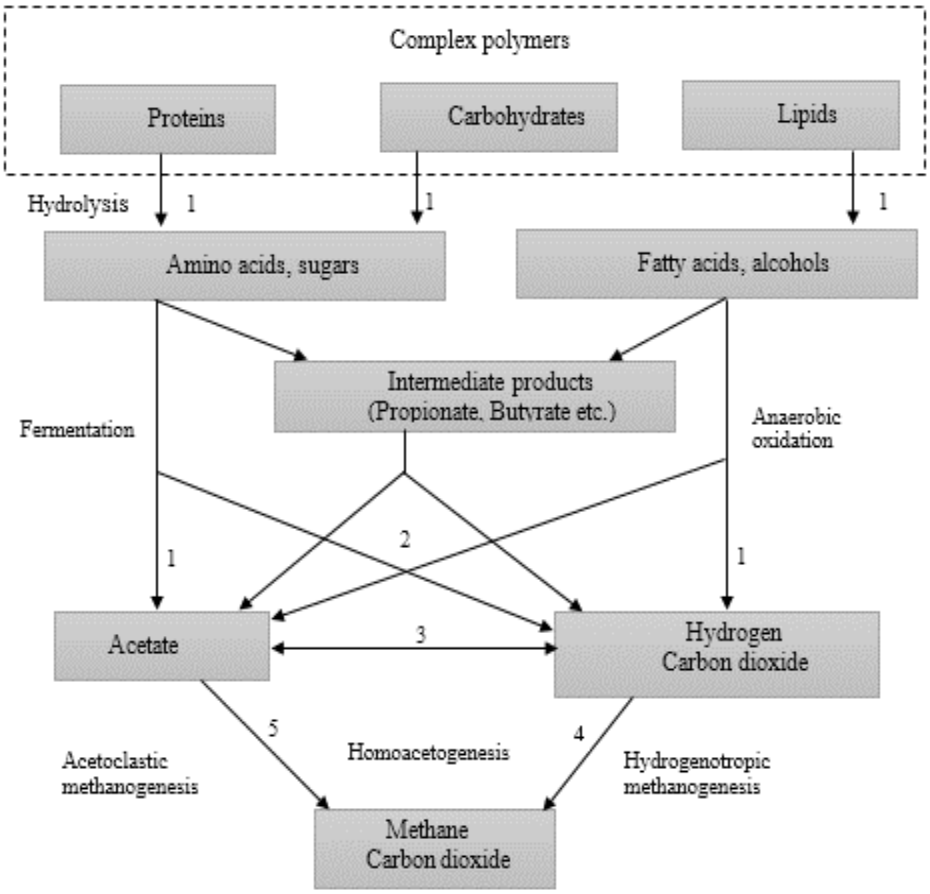


Figure 2.6: Schematic diagram of a simplified anaerobic process. Numbers specify the bacterial groups involved in the metabolic reactions: 1. Hydrolytic and fermentative bacteria, 2. Acetogenic bacteria, 3. Homo-acetogenic bacteria, 4. Hydrogenotrophic methanogens, 5. Acetoclastic methanogens (adapted from van Lier et al., 2008)

4. **Methanogenesis**: In the final step, one group of methanogenic bacteria converts acetic acid into CH_4 and CO_2 in one reaction (acetoclastic pathway) and in another reaction (hydrogenotropic pathway) another group use CO_2 and H_2 to produce methane. Most of CH_4 (nearly 70%) is derived from acetate (acetoclastic pathway) (Wilson, 2009).

2.6.1 Factors affecting anaerobic digestion process

Various important factors that control the rates of the different steps of the anaerobic digestion process are discussed in the following subsections.

2.6.1.1 Temperature

Temperature is a critical parameter in the determination of the rate of anaerobic digestion. The hydrolysis and methanogenesis steps involved in the anaerobic digestion process are particularly sensitive to temperature (Metcalf and Eddy, 2003). As a result of the sensitivity to temperature, it is important to maintain a stable operating temperature in anaerobic digester. Optimum anaerobic degradation rates can be achieved by operating digesters at temperatures between 30 to 40^{0}C for mesophilic microorganisms and 50 to 60^{0}C for thermophilic microorganisms. Generally, temperature changes greater than 1^{0}C/day affect the degradation rate and therefore, changes in temperature of more than 0.6^{0}C/day should be avoided (Turovskiy and Mathai, 2006). Additionally, temperature influences sludge characteristics and the metabolism rate of dead or inactive microorganisms.

2.6.1.2 Solid retention time (SRT)

SRT is defined as the mean residence time of microorganisms inside the digester. The SRT is related to growth and reproduction rate of the microorganisms and has an effect on the reactions (hydrolysis, fermentation and methanogenesis) of the anaerobic digestion process. For each reaction involved in the anaerobic digestion process, a minimum SRT is required in order for the complete anaerobic degradation process to be achieved. If the SRT is too short, bacteria will not have sufficient time to grow and ultimately the systematic process will fail (WEF, 1998). To achieve stable, reliable performance at 35^{0}C, an SRT of 15-20 days is generally required. Many high rate anaerobic digestion processes have SRTs in excess of 30-50 days and, on occasion, the SRT can reach 100 days.

2.6.1.3 pH, alkalinity and VFA/alkalinity ratio

Each group of microorganisms has a different optimum pH range. Methanogenic bacteria are extremely sensitive to pH with an optimum between 6.5 and 7.2. In contrast, the growth of fermentative organisms is much less sensitive, having an optimal pH between 4 and 8.5. A low pH is an indicator of an upset digester. The addition of a buffer solution is desirable for pH adjustment.

The anaerobic digestion process produces carbon dioxide, ammonia and bicarbonate which are the reflection of alkalinity requirements in the digester. A healthy digester should have a total alkalinity of 2000 to 5000 mg/L (WEF, 1996).

The ratio between the bicarbonate alkalinity and the VFA concentrations is an excellent process indicator. For a stable and well buffered digestion process 1.4:1 of bicarbonate/VFA should be maintained (STORA, 1985).

2.7 Biogas: Renewable energy source

Biogas is a by-product of the anaerobic digestion process, representing a clean and renewable source of energy in the form of methane gas. It is a great substitute to the conventional sources of energy (i.e. fossil fuels, oil, coal etc.) which have become a threat to the environment (Santosh et al., 2004). The predominant constituents of biogas are methane (CH_4) and carbon dioxide (CO_2), small amounts of water vapor, nitrogen (N_2), oxygen (O_2), H_2, hydrogen sulfide (H_2S) and ammonia (NH_3) are also present. Table 2.4 represents the typical biogas composition.

Table 2.4: Composition of biogas (Seadi et al., 2008)

Compound	Chemical symbol	Content (% v/v)
Methane	CH_4	50-75
Carbon dioxide	CO_2	25-45
Water vapour	H_2O	2 (20^0C) -7 (40^0C)
Oxygen	O_2	<2
Nitrogen	N_2	<2
Ammonia	NH_3	<1
Hydrogen	H_2	<1
Hydrogen sulphide	H_2S	<1

The methane in biogas can be burned to generate heat and electricity in a combined heat and power (CHP) engine. 1 m^3 of biogas generates 6~6.5 kW of electricity (Table 2.5). At present, this electricity is mainly used for domestic use and the waste heat generated is used to provide heat to the digesters, but the excess electricity can be used for a variety of purposes.

Table 2.5: Typical details of biogas (adapted from Deublein and Steinhauser, 2008)

Parameter	Range
Energy content (kW/m^3)	6.0-6.5
Fuel equivalent (L oil/m^3)	0.6-0.65
Biogas explosion limits	6-12% biogas in air
Ignition temperature (^{0}C)	650-750
Critical pressure (bar)	75-89
Critical temperature (^{0}C)	-82.5
Normal density (kg/m^3)	1.2
Odor	Like rotten eggs

2.7.1 Biogas: Reduction of greenhouse gas potentials

As the main component of biogas, methane has a greenhouse gas (GHG) potential 23 times higher than CO_2 and 296 times than nitrous oxide (N_2O). Though combustion of biogas produces

CO_2, the difference between the carbon in biogas and conventional fossil fuels (e.g. lignite coal, crude oil, natural gas etc.) is that biogas combustion is carbon neutral. The process of generation and burning of biogas is a closed carbon cycle. CO_2, released during biogas burning, is stored in vegetation through photosynthesis and in this way, the carbon cycle of biogas is closed within a short period (between one and several years). Therefore, by maximising the extraction of methane gas from the anaerobic digestion process, a substantial reduction in GHG emissions is possible, contributing to the mitigation of climate change effects.

2.8 Sludge pre-treatment methods

Pre-treatment methods are developed to cause lysis in the dead or inactive cells existing in the waste activated sludge. Lysis is desirable because intracellular components that act as food are shielded from the anaerobic microorganisms. Through lysis, the intracellular constituents are broken free from the cell membrane and become readily degradable for anaerobic microorganisms. A wide range of pre-treatment techniques have been applied at the lab scale and among them only a few are industrially applicable. The aim of all pre-treatment methods is to improve anaerobic digestion performance either by increasing the conversion rate of organics or inherent biodegradability of waste sludge (Carrere et al., 2010). This section focuses on different pre-treatment methods available for enhancement of anaerobic digestion process.

2.8.1 Biological pre-treatment

Biological pre-treatment is based on enzymatic activity that involves the solubilisation of EPSs in waste activated sludge. Biological pre-treatment may be categorized as auto-enzymatic or external enzymatic and incorporates both the aerobic and anaerobic processes. In an auto-enzymatic process hydrolytic enzymes are released from the microbial cells within the system and

enhance the hydrolysis reaction rate which in turn facilitates faster anaerobic degradation and faster biogas production. As the enzymes are soluble and easily able to reach to the substrates, the rate of hydrolysis reaction can be increased either by adding microorganisms or by adding directly hydrolytic enzymes to the reactor.

2.8.2 Chemical pre-treatment

The complex compounds of sludge can be destroyed by the addition of acids, alkali, ozone, chelating, and cation binding agents. Among the chemical pre-treatment techniques, adding ozone is of special interest because there is no chemical addition.

2.8.2.1 Oxidation pre-treatment

Among oxidative pre-treatment processes, the addition of ozone is one of the most effective and extensively used sludge disintegration methods used due to the high oxidation potential of ozone. Ozone is capable of achieving more than a 90% conversion of waste activated sludge to readily degradable solubilized compounds (Déléris et al., 2000). Ozone is a powerful oxidizing agent that reacts in both direct and indirect ways. The direct reaction of ozone usually occurs at unsaturated bonds whereas the indirect one is related with oxidation of organic matters by hydroxyl radicals. In sludge pre-treatment applications, ozone aims to partial oxidation of organic matters.

Ozone dose has a great impact on sludge biodegradation. According to Yeom et al. (2002), for ozonated sludge at 0.1 g O_3/g suspended solids (SS), anaerobic biodegradability was 2-3 times greater compared to raw sludge and it increased with ozone dosage up to 0.2 O_3/g SS. Weemaes et al. (2000) found the optimal ozone dose as 0.1 g O_3/g COD of sludge. At 0.1 g O_3/g COD, methane production enhancement was 1.8 times and total removal of organic matter achieved was 68% but a negative effect on dewaterability was observed at this dose.

Carballa et al. (2007) studied the effects of ozonation on removal of pharmaceutical and personal care products from wastewater sludge during anaerobic digestion and no significant removal efficiency was observed except for carbamazepine which is known to be a highly stable pharmaceutical with less than 10% degradation under conventional treatment processes.

2.8.2.2 Acid or alkaline pre-treatment

Although the effects of both strong acids and bases on sludge disintegration efficiency have been extensively studied, the use of alkaline is being emphasised more than that of acids. Knezevic et al. (1995) studied the effect of alkaline hydrolysis on anaerobic digestibility by adding lime ($Ca(OH)_2$) and sodium hydroxide (NaOH) at different doses (10, 12.5, and 15 meq/L). For both chemicals, WAS solubilisation was improved, but NaOH (at dosage of 12.5 meq/L) showed the best results. The anaerobic digestion performance had better results in terms of organic removals and gas production in case of NaOH pre-treatment.

Kim et al. (2003) assessed the effects of various bases (NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$.) on anaerobic digestibility of WAS and 7 g/L NaOH was found as the optimum dose at which solid reduction for pre-treated sludge (29.8%) was higher over control (20.5%) at an SRT of 7 days.

2.8.3 Thermal pre-treatment

Thermal pre-treatment accelerates sludge degradation by exposing sludge to elevated temperatures. Thermal hydrolysis enhances the anaerobic digestion process because of the partial solubilisation of the sludge (Carrere et al., 2008). Initially, thermal pre-treatment was applied for sludge dewaterability enhancement (Haug et al., 1978). Some other advantages exist when considering thermal pre-treatment, which include sludge sanitation via destruction of pathogenic microorganisms (Potts, 2007); sludge viscosity reduction (Lin and Shien, 2001) and the potential

to reuse the heat energy to heat the anaerobic digestion process to mesophilic or thermophilic temperatures, in some cases there is a positive energy balance (Perez-Elvira et al., 2008).

2.8.3.1 Conventional heating

Thermal pre-treatment disrupts the cell walls and makes the intracellular substances in sludge available for subsequent biodegradation. Extensive research has been carried out on thermal pre-treatment of secondary sludge using a wide range of temperatures, including ranges between 60 to 270^{0}C (Climent et al., 2007) with 160 to 180^{0}C being reported as the optimum temperature (Carrère et al., 2010). The formation of refractory compounds has been resulted at temperature above 200^{0}C. Climent et al. (2007) considered the treatments occurred at temperature less than 100^{0}C as low temperature thermal treatments. They observed that since thermal treatment around 70^{0}C fails to break the cell parts by the action of heat, they may be considered as more biological hydrolysis or pre-digestion rather that thermal hydrolysis. Some research findings of conventional heat pre-treatment of sludge at different temperatures are presented in Table 2.6.

Table 2.6: Conventional heating pre-treatment studies on anaerobic digestion performance*

Sludge type	Treatment conditions	Results	References
WAS	170^0C, 30 min	Methane production increased by 51% (anaerobic digestion in CSTR, 20 days)	Bougrier et al. (2006a)
WAS	170^0C, 30 min	Methane production enhancement by 76% (batch anaerobic digestion, 24 days)	Bougrier et al. (2007)
WAS	190^0C , 0.24 - 1 hr	For temperatures above 150 ^{o}C dewaterability was improved. At 190 ^{o}C, CST of sludge decreased from 1330 s to 31 s	Bougrier et al. (2008)
Mixed sludge	170^0C, 30 min	Dewaterability improved for thermally pre-treated sludge almost two fold than that of fresh sludge. Biogas production increased by 40% in half time in comparison with a conventional digester	Perez-Elvira et al. (2010)
WAS	170-180^0C 1.0 hr	Biogas production increased by 75%, anaerobic biodegradability (in terms of COD) enhanced from 33% to 51%.	Wett et al. (2010)
Six different WAS samples	190^0C, 30 min; 170^0C, 30 min	Biogas production improved by 23% at 190 ^{o}C pre-treatment for one sludge; methane production increased by 78% at 170 ^{o}C for another sludge	Carrere et al. (2008)
WAS	LTHP: 60^0C, 70^0C, 80^0C, and 90^0C, 24 hr; HTHP: 120^0C, 140^0C, 160^0C and 180^0C, 3 hr	SRT could be reduced from 18-20 days to 12-14 days under 140 ^{o}C-160 ^{o}C based on acceleration of methane production	Xue et al. (2015)

*LTHP: Low temperature thermal hydrolysis process, HTHP: High temperature thermal hydrolysis process, CSTR: Continuous stirred-tank reactor; CST: Capillary suction time, COD: Chemical oxygen demand, SRT: Sludge retention time, WAS: Waste activated sludge, MS: mixed sludge.

2.8.3.2 Microwave pre-treatment

Microwave pre-treatment prior to anaerobic digestion has been proven to be more efficient than conventional heating due to its rapid and selective heating and enhanced yields (Wu, 2008). Due to high water content, sludge readily absorbs microwave irradiation. Considerable research has been done on the optimization of microwave pre-treatment for sludge solubilisation, biogas yield and in some cases for pathogen destruction (Table 2.7).

Table 2.7: Microwave pre-treatment studies*

Sludge type	Treatment conditions	Anaerobic digestion conditions	Results	References
Secondary sludge	N/A	SRT 10 d, 15 d	COD removal and methane production increased by 64% and 79% respectively	Park et al. (2004)
MS	Microwave irradiation (2450 MHz, 1000 Watts, 60-65^{0}C) for 110 sec; Conventional heating (70^{0}C) for 960 sec	Semi-continuous feeding, HRT 25 d, 35^{0}C	17% and 6% increase in biogas yield compared with untreated and conventionally heated controls. Dewaterability and fecal coliform removal also enhanced.	Pino Jelcic et al. (2006)
Activated sludge	Microwave irradiation (2.45 GHz, 1250 Watts, 175^{0}C)	Batch, 18 days, 33^{0}C	31% increase in methane production	Eskicioglu et al. (2009)
Primary sludge	Microwave irradiation (2.45 GHz, 1460 Watts, 35-90^{0}C)	Batch, 18 days, 33^{0}C	Increase of degradation rates No improvement on ultimate methane production	Zheng et al. (2009)
WAS	Microwave irradiation (2.45 GHz, 1250 Watts, 96^{0}C)	Batch, 37^{0}C	16 ± 4% higher biogas production after 15 d of digestion	Eskicioglu et al. (2007)
MS	Microwave irradiation (2.45 GHz, 1000 Watts, 78^{0}C) for sludge with 1.46% TSS (w/w)	Batch, 33 days, 33^{0}C	84% increase in cumulative biogas production with compared to the controls	Elagroudya and El-Goharyb (2013)
WAS	Microwave irradiation (800 Watts, 336 kJ/ kg sludge, 80^{0}C for 3.5 min)	Pilot scale, semi continuous feeding, 80^{0}C, 20-d SRT	An increase in biogas production by 50%	Appels et al. (2013)

*WAS: Waste activated sludge, MS: mixed sludge, COD: Chemical oxygen demand, SRT: Sludge retention time, HRT: Hydraulic retention time.

Eskicioglu et al. (2007) elucidated that microwave heating had no athermal effects on COD solubilisation of sludge as both conventional heating pre-treatment and microwave heating pre-treatment resulted the same level of solubilisation of WAS. But improved biogas production (16 ± 4% at 96^{0}C for acclimated sludge) from microwave pre-treated samples indicated that

microwave heating had positive athermal effect on anaerobic digestibility. In the study by Eskicioglu et al. (2007), microwave irradiation (0–1250W, 2450 MHz) and conventional heating (in glass volumetric flask equipped with a rubber stopper) were applied to TWAS at three different temperatures of 50, 75 and 96^{0}C at identical heating rates. Athermal effects are not associated with temperature increase and are likely caused by macromolecules (polarized) aligning with the poles of electromagnetic field, which may cause breakage of the hydrogen bonds (Loupy 2002).

In a recent study by Kuglarz et al. (2013), microwave irradiation (900 W, frequency of 2.45 GHz, 60-70^{0}C) turned out to be a better option in comparison to another microwave irradiation (700 W, frequency of 2.45 GHz, 30–100^{0}C) in terms of energy input requirement. Furthermore, a thermal pre-treatment was done in closed vessels equipped with a heating coil (900 W, 30–100^{0}C). The results from the study demonstrated that microwave irradiation (900 W, at 60-70 ^{0}C) substantially increased the methane yield by 35% when comparing the control (un-treated sludge). In addition, microware pre-treatment achieved better pathogen destruction (67-71%) over control (55%) and was found to be more sustainable in terms of energy efficiency when considering the thermal pre-treatment.

2.8.4 Mechanical pre-treatments

The application of mechanical force causes the cell wall rupture and release of the cell compounds that act as food for the anaerobic microorganisms. There is a wide range of methods to mechanically pre-treat sludge, including high pressure homogenizer (Wahidunnabi and Eskicioglu, 2014), lysate thickening centrifuge (Zábranská et al., 2006), pulse power technology, ultrasonication (Martín et al, 2015), freezing and thawing (Montusiewicz et al., 2010), stirred ball mills (Strunkmann et al., 2006), mechanical jet technique (Nah et al., 2000) etc.

2.8.5 Ultrasonication

The main purpose of applying ultrasonication (also known as sonication or ultrasound) pre-treatment is to rupture sludge flocs and bacterial cells leading to release of intracellular and extracellular material into solution for enhanced biodegradation during the anaerobic digestion process. Ultrasound is generated using magnetostrictive and piezoelectric techniques. During sonication, the transducer converts the electrical energy to mechanical sound waves which are amplified by the booster and the mechanical waves are delivered by the horn into liquid. Like any other sound wave, ultrasound passes through sludge medium by generating a series of compression (exerting positive pressure) and rarefaction (exerting negative pressure) cycles (Figure 2.7). When large enough, negative pressure in rarefaction zone exceeds the molecular attractive forces in the liquid, liquid breaks down and voids (cavitation bubbles) are formed.

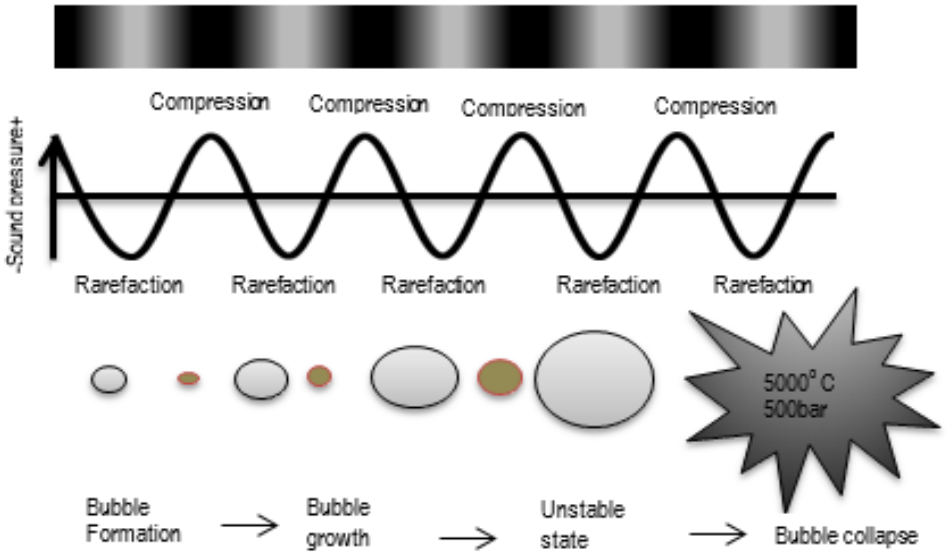


Figure 2.7: Cavitation bubbles development and collapse (adapted from Pilli et al., 2011)

Heat generation, high dynamic shear forces, and sono-chemical effects are held responsible for sludge disintegration and are related to the cavitation phenomena. According to Show et al. (2010),

cavitation releases sufficient energy to cause thermolysis of substances along with the formation of highly reactive radicals ($H^{\cdot}$, $OH^{\cdot}$). The highly reactive radicals are then utilized in sono-chemical reactions (i.e. $OH^{\cdot}$, $H^{\cdot}$ and $HO_2^{\cdot}$). Hydrogen in peroxide also plays a role in the ultrasonic destruction of sludge (Tiehm et al., 2001).

Ultrasonication performances for sludge pre-treatment depend on some important parameters like specific energy input, sonication intensity, duration, frequency, and sludge characteristics. Hua and Hoffman (1997) stated that ultrasound frequency is of paramount importance for efficacy of ultrasonic systems involved with sludge pre-treatment as the critical size of micro bubbles is governed by the operating frequency. At high frequency operation (of the order of MHz), the formation of cavitation bubbles is more difficult than operation at a low frequency (of the order of kHz), and lower frequency operation (20-40 kHz) has been proven to be more effective to achieve desired sludge disintegration (Perez-Elvira et al., 2009; Carrere et al., 2010).

To evaluate the disintegration efficiency, the concentrations of soluble chemical oxygen demand (SCOD), protein, lipids, humic acids and carbohydrates are very important parameters. But comparison of sludge disintegration results is difficult as important factors, such as, specific energy input, ultrasound frequency, density, sonication time, sludge types, TS content of sludge was not kept constant or reported by all the previous studies (Pilli et al., 2011). Braguglia et al. (2012) stated that sludge solubilisation is a function of specific energy input (sonication duration and power applied to the system) though sonication does not maintain a linear relationship with specific energy (Tyagi et al., 2014). Since sonication has no effect on total COD (TCOD) representing total organic compounds present, the release of organic substances in aqueous phase is represented by the ratio of SCOD/TCOD (Pilli et al., 2011). Show et al. (2010) confirmed that

after sonication the increase of SCOD in sludge has a beneficial impact on its biodegradability during the anaerobic digestion process. The effect of the nature of sludge on solubilisation was studied by Mao et al. (2004). In his research, SCOD solubilisation in secondary sludge (7.7 times) was found to be higher than that in primary sludge (4 times), and for both types of sludge sonication time maintained a linear relationship with SCOD.

Previous studies reported that sonication has a significant impact on the particle size distribution of sludge. Particle size is reduced significantly at high ultrasonic power (at 0.33 and 0.44 W/mL of sludge sample) and longer period of sonication (20, 40 and 60 min) (Chu et al., 2001), and a high specific energy input (i.e. 14,550 kJ/kg TS) also contributes to reduction in particle size (Bougrier et al., 2005). Particle size reduction and sludge solubilisation during sonication is also dependent on sludge TS concentration. Show et al. (2007) reported that 2.3~3.2% TS is the optimal range of solid content for effective sonication of sludge.

Table 2.8 summarizes results from previous sonication pre-treatment studies. In general, higher sludge solubilisation increases the hydrolysis rate during anaerobic digestion leading to improved biogas generation and increased solids removal. Regarding the effect of sonication pre-treatment on the dewaterability of sludge, conflicting results have been reported. Sludge dewaterability deteriorated at high power density (at 0.33 W/mL) and longer sonication time (60 min) (Wang et al., 2006; Chu et al, 2001). Feng et al. (2009) observed good dewatering performance at lower specific energy of 1000 kJ/kg TS but above 5000 kJ/kg TS, the dewaterability started to worsen.

Table 2.8: Ultrasound pre-treatment studies

Sludge type	Treatment conditions	Anaerobic digestion conditions	Results	References
WAS	150 min, 41 kHz	Semi-continuous, SRT 25 d, 37^{0}C	VS removal increased by 56.7%	Tiehm et al. (2001)
MS	20 kHz, 60s	Batch, HRT 28 d, 35^{0}C	20-24% increase in biogas yield, organic removal efficiency 45-47%	Bien et al. (2004)
WAS	20 kHz, 108,000 kJ/kg TS	Batch, 50 days, 37^{0}C	An increase in biogas production by 84%	Salsabil et al. (2009)
WAS	5000 kJ/kg TS	Semi-continuous, SRT 20 d, 37^{0}C	An increase in biogas production by 36%	Braguglia et al. (2009)
WAS	5000 kJ/kg TS, 24 kHz	Semi continuous, 37^{0}C, 10-d SRT	An increase in biogas production and VS reduction by 30% and 20.6% respectively	Braguglia et al. (2011)
WAS	24 kHz, 0.51 W/mL	Semi-continuous, SRT 15-d, 35^{0}C	An increase in biogas production and VS reduction by 49% and 24.6% respectively	Apul and Sanin (2010)
WAS	30 kW/h/m^3	Pilot scale, semi continuous, 80^{0}C, 20-d SRT	An increase in biogas production by 50%	Perez-Elvira et al. (2009)
WAS (2.5% TS), pulp and paper mill sludge	20 kHz, 117,719 kJ/kg TS	Batch, 33^{0}C, 43-d SRT	An increase in specific methane production by 51%	Saha et al. (2011)
WAS	2500 kJ/kg TS, 24 kHz	Semi continuous, 37^{0}C, 1-d SRT	An increase in biogas production and VS reduction by 26% and 19% respectively	Braguglia et al. (2012)
MS	0-26,000 kJ/kg TS, 150 W	Batch, 35^{0}C, 100 days	Methane yield improved by 95% and biodegradability was 81% (in VS)	Martín et al. (2015)

2.8.6 Full-scale application

Ultrasound assisted anaerobic digestion has been extensively investigated both in the laboratory and in the field. Table 2.9 summarizes the results from full-scale applications of ultrasound pre-treatment followed by anaerobic sludge digestion.

Table 2.9: Ultrasonic application studies at full-scale

Sludge type	Power of sonotrode	Sonication treatment conditions	Anaerobic digestion conditions	Findings	References
	N/A	14 installation up to 75,000 p.e.	12-69 days SRT	22% improvement in biogas production and VS removal, 7% improvement in dewaterability	Barber (2005)
TWAS	6 kW	20 kHz, Power density 13.7 W/cm^2	Continuous flow , 30-d SRT	45% increase in daily biogas production, 30% increase in solids removal	Xie et al. (2007)
WAS	2 kW	20 kHz, 25-50 W/cm^2, sonication time 30 s	Continuous flow	COD solubilisation increased up to 45% for 70 Wh/L energy input	Nickel and Neis (2007)

Hogan et al. (2004) studied the outcomes from several demonstration and full-scale plants using Sonix^{TM} technology for enhancing anaerobic digestion methane recovery, solids removal, and dewaterability. This technology appeared to be efficient in terms of enhanced biogas generation (up to 50% increase), better VS removal (54-70%), and improved sludge dewatering properties. It ended up providing a relatively short payback period of two years or less.

In another similar study by Barber (2005), the outcomes of several ultrasound WWTP (full-scale, pre-treatment of partial sludge stream) in Germany, Austria, Switzerland, Italy and Japan have been reported. He demonstrated that a 22% increase in both biogas generation and VS destruction and 7% improvement in dewaterability were observed in a typical full-scale WWTP.

After performing an energy and mass balance calculation of a typical anaerobic digester, he established that 7 kW of electrical energy (after losses) will be generated at the cost of 1 kW of applied ultrasound energy.

The most common users of ultrasound technology as sludge pre-treatment prior to anaerobic digestion are: BioSonator (Ultrawaves, Germany), Sonix (Sonico, UK), IWE Tec, Smart DMS (Weber Ultrasonics), Sonolyzer (Ovivo) and Hielscher (Germany). The key difference among the techniques applied by these suppliers lies in design of the horn which delivers the mechanical sound energy into the liquid (Elliott and Mahmood, 2007). There are great differences between lab-scale and full-scale devices, regarding the relationship between the disintegration achieved and the energy supplied. Based on economic aspects, most of the full-scale plants use partial-stream instead of the full-stream sonication, which affects biogas production and sludge dewatering characteristics. (Pérez-Elvira et al., 2010).

The main difference between lab-scale and full-scale application of ultrasound technology is efficiency. Lab-scale applications appear to be inefficient as the net energy balance from energy production out of biogas and ultrasound energy consumed is positive for full-scale application and negative for lab-scale application. Table 2.10 below highlights a comparative study between lab-scale and full-scale (three main suppliers) application of ultrasound pre-treatment. Different full-scale studies presented that the ratio of net energy gain (NEG) to electrical energy consumption due to ultrasonication falls in the range of 2.5 to 7 (Barber 2005, Xie et al. 2007).

Table 2.10: Comparison between lab-scale and full-scale application of sonication and digestion operation (adapted from Pérez-Elvira et al., 2009)

Parameter	Sonix	WAVE	IWE Tec.	Laboratory scale
Frequency (kHz)	20 kHz	20 kHz	20 kHz	20 kHz
Power intensity (W/cm^2)	15-50	20-50	<200	N/A
Power density(kW/L)	4	0.17	0.5-2	0.4-3
Power per sonotrode (kW)	6	2	8	0.2-3.6
Liquid volume(L)	4	29	4-5	0.1-1
Sludge TS content (%)	9%	<8%	10%	0.5-2%
Biogas generation increase (%)	35-55%	30-45%	20-50%	25-100%
Energy supplied (kJ/L)	4-5	23	18-36	205-900
Net energy balance	5-10	3-7	<5	Negative

2.9 Comparison of treatment methods

Although all pre-treatment methods, summarized above, are intended to provide an enhancement to the anaerobic digestion process. The extent to which the purpose is achieved depends greatly on the technique/intensity applied and the sludge characteristics to which it is applied. Therefore, the selection of an appropriate pre-treatment technique in terms of efficiency, energy balance, economic analysis and environmental impacts such as dewaterability, odor removal, pathogen removal, sustainability assessment for any given circumstance is very difficult.

Bougrier et al. (2006b) evaluated the effects of three different pre-treatment methods (ultrasound, thermal hydrolysis and ozonation) on WAS followed by batch mesophilic anaerobic digestion. Thermal treatment (at 170 or 190^{0}C) appeared to be the most efficient in terms of solubilisation and filterability rate (39 ± 1 s at 170^{0}C and 29 ± 4 s at 190^{0}C) but failed to enhance biodegradability of particulate fractions. On the other hand, ultrasound (6250 or 9350 kJ/kg TS) provided significant improvement in biodegradability of particulates. In regards to improved biogas generation, thermal hydrolysis and sonication offered better results than ozonation (0.1 and

0.16 g O_3 g^{-1} TS). Salsabil et al. (2010) studied the effects of thermal, ozonation and sonication pre-treatment methods have on TSS removal during subsequent batch anaerobic digestion. TSS removal was increased by 30% with sonication whereas both ozonation (0.1 g O_3 g TS^{-1}) and thermal pre-treatments resulted in 20% enhancement at 90^0C and 120^0C. Kim et al. (2003) made a comparison among the effects of thermal (121^0C) , chemical (7 g L^{-1} NaOH), ultrasonic (42 kHz, 120 min) and thermochemical (121^0C, 7 g L^{-1} NaOH) pre-treatment on enhancement of batch anaerobic digestion of WAS and obtained the following order in terms of biogas production:

Thermal (3390 L CH_4 m^{-3}) > Thermochemical (3367 L CH_4 m^{-3}) > Ultrasound (3007 L CH_4 m^{-3}) > Chemical (2827 L CH_4 m^{-3}) > Control (2507 L CH_4 m^{-3})

Muller (2001) made a comparison of different pre-treatment methods based on net energy gain and the results of sludge disintegration effect on subsequent treatment stages (Table 2.11).

Table 2.11: Comparison of different pre-treatment methods (adapted from Muller, 2001)

	Mechanical					Thermal	Chemical Ozone	Chemical Acid/ Alkali	Biological	Freeze/ Thaw
Sludge degradation rate	++					+	+	+	+	O
Degree of sludge degradation	+					O	++	O	O	O
Dewatering performance	O					+	O	+	O	++
Flocculants requirement	-					O	- -	-	-	O
Odor generation	O					- -	-	-	-	O
Disinfection	+					++	+	+	O	+
Low energy demand	LC +	HPP +	HPH O	SBM O	UD -	-[TH]	O	++	++	- -

++ Excellent, + Good, O Moderate, - Poor, - - Very poor, - TH unless using waste heat
LC: Lysat centrifuge, HPP: High pressure homogenizer, HPH: High pulse power, SBM: Stirred Ball mill, UD: Ultrasonic disintegrator

Carballa et al. (2011) evaluated the economic and environmental effects of seven different pre-treatments on two different types of waste (municipal sludge and kitchen waste) by using the life cycle assessment method. The results show that mechanical (pressurize-depressurize) and chemical (acid or alkaline) methods might be recommended as the most sustainable choices whereas thermal and ozonation necessitate energy efficiency optimization to diminish their environmental encumbrances.

2.10 Summary

It is apparent that although there are a wide range of pre-treatment methods established in the laboratory, only few mechanical, sonication, thermal and thermochemical techniques are being successfully applied at full-scale. Among these methods, ultrasonication is a powerful pre-treatment technology for sludge floc disintegration. This method has a great potential in sludge management as it has shown significant improvements in anaerobic digestibility in the last decade. However, there is a concern that high-power ultrasound pre-treatment is highly energy intensive and may cause deterioration in sludge dewaterability. Furthermore, there is a lack of information about low-power sonication effect on mesophilic and thermophilic anaerobic digestion performance of waste sludge from a biological nutrient treatment (BNR) process, such as one employed at Kelowna's municipal WWTP, especially in terms of sludge characteristics and energy assessment. Hence, this study was carried out to investigate the effect of low-ultrasound application (at specific energies of 2042, 4163 and 8153 kJ/kg TS) on the subsequent anaerobic sludge digestion of Kelowna - BNR waste sludge operated under a wide range of SRTs (20, 10 and 5 days) corresponding to organic loading rates (2.1, 8.2 g TS L^{-1} of digester d^{-1}).

CHAPTER 3: MATERIALS AND METHODS

This study was intended to see the effect of ultrasound pre-treatment of TWAS obtained from a BNR plant on subsequent anaerobic digestion. For this purpose, the research work was conducted into two main phases: 1) optimization of sonication operating conditions (i.e. sonication time, ultrasonic density) in terms of specific energy to achieve efficient sonication disintegration; 2) evaluation of mesophilic and thermophilic anaerobic digestion performance of control (without sonication) and pre-treated (at three different sonication conditions) reactors operating at three different SRTs. The research plan frame work is shown in Figure 3.1.

3.1 Waste sludge

The waste sludge (Fermented primary sludge (FPS) and TWAS) used in this study was obtained from Kelowna Wastewater Treatment Facility (WWTF) which is currently serving 80% of Kelowna's population with a capacity of treating 42 million litres of sewage per day. The Kelowna WWTF is a modified Bardenpho type BNR plant which utilizes a UV disinfection system and advanced nutrient and carbonaceous removal systems. In the treatment plant, the primary sludge from the primary clarifiers is pumped to a gravity thickener/fermenter and WAS from the secondary process is pumped to a dissolved air floatation (DAF) unit. The thickened sludge from both the fermenter and DAF unit is then mixed in line prior to dewatering through centrifugation. Finally, the dewatered sludge cake is transported to a composting facility near Vernon (BC). For this study, the primary sludge and TWAS samples were collected every two weeks from the effluent line of the gravity thickener and DAF units, respectively. The samples were characterized on the same day that sampling was done and then preserved in a cold storage at 4^0C.

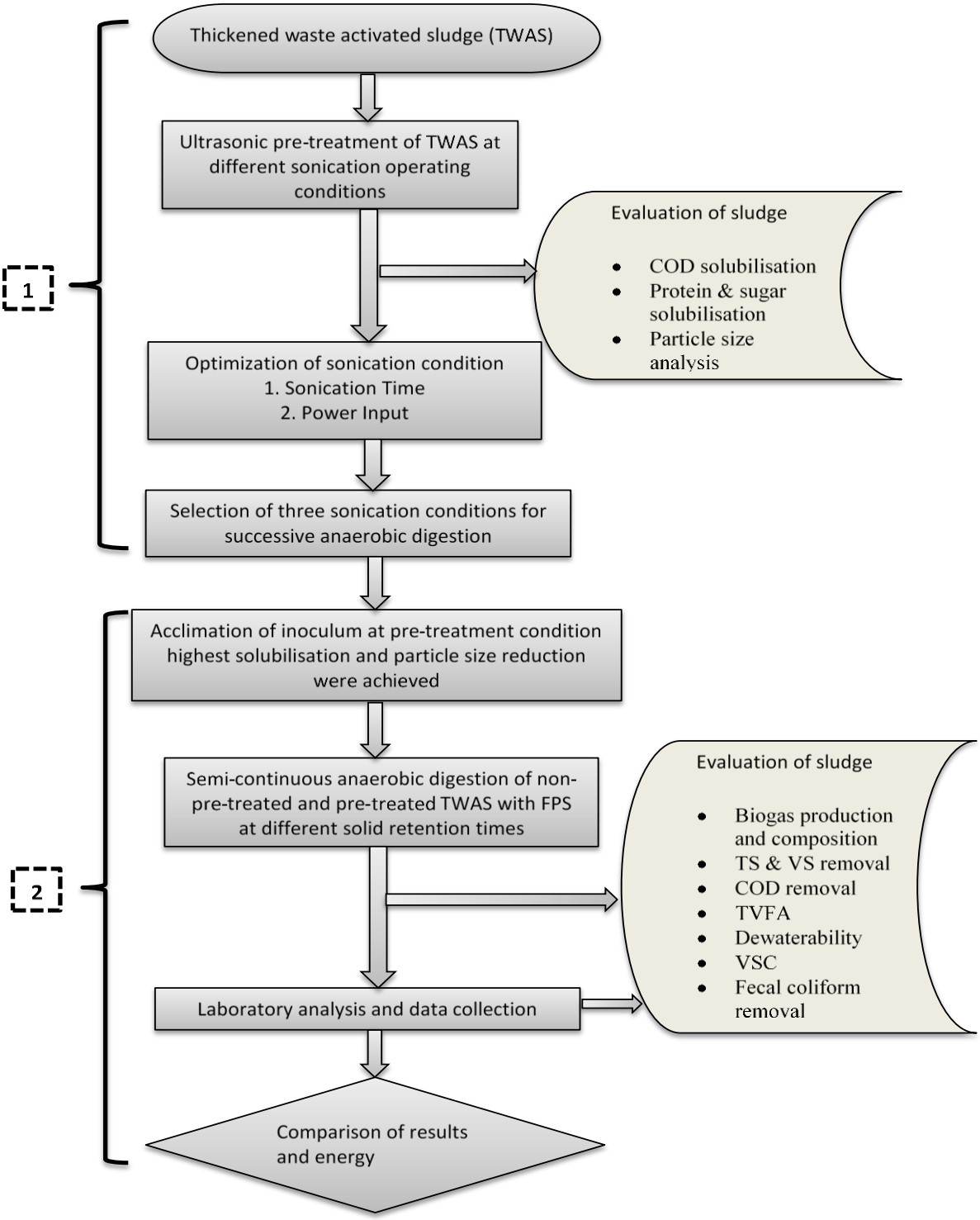


Figure 3.1: The flow chart of research plan work (FPS: fermented primary sludge; COD: chemical oxygen demand; TS & VS: total and volatile solids; TVFA: total volatile fatty acids; VSC: volatile sulfur compounds)

3.2 Ultrasound pre-treatment

For this study, only secondary sludge (TWAS) was subjected to sonication and then mixed with FPS at a 67:33% volume ratio before being fed to anaerobic reactors. Since primary sludge is more readily biodegradable due to lower concentration of microbial cells and EPS compared to secondary sludge, in this research, ultrasonic pre-treatment was only applied to TWAS to reduce energy input and hence enabling cost effective anaerobic digestion. Some previous studies also elucidated that after sonication, secondary sludge has a more remarkable impact on substantial reduction in particle size and improved sludge disintegration over the primary and mixed sludge (Mao et al., 2004; Show et al., 2007).

The apparatus used for carrying out preliminary experiments on ultrasound pre-treatment was the Branson Fisher Scientific Sonic Dismembrator model 500 (400 W, 20 KHz) equipped with a replaceable probe tip of 3/4" diameter (Figure 3.2). Sonication was performed at different sonication times applying different amplitudes to provide different values of specific energies ranging from 1,000 to 15,000 kJ/kg TS. Figure 3.3 portrays a relationship among sonication time, amplitude and specific energy.

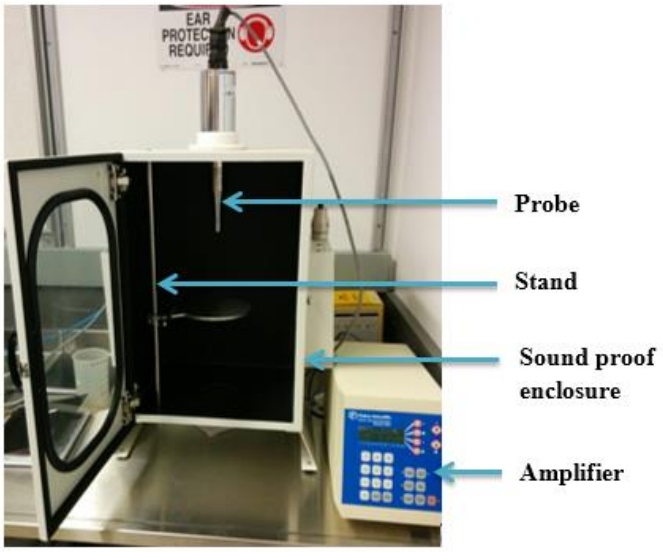


Figure 3.2: Ultrasonic Dismembrator

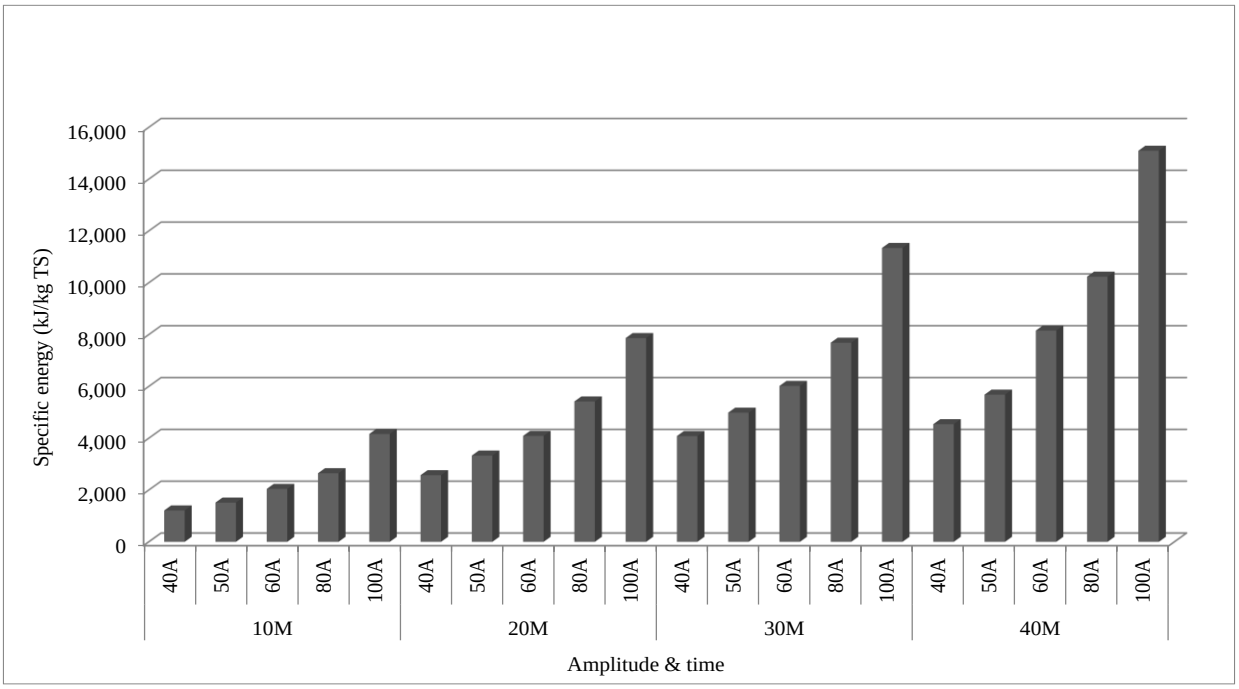


Figure 3.3: Specific energy at different sonication time and amplitude (A: sonication amplitude in %; M: sonication time in minutes)

400 mL of TWAS was taken in a 500 mL glass beaker and the beaker was placed in a cooling bath to minimize the temperature increment during sonication. Table 3.1 presents the sonication

operating conditions at which a series of sonication experiments were done to determine the optimum sonication conditions for subsequent anaerobic digestion.

Table 3.1: Operating conditions for ultrasonication

Parameters	Range
Ultrasonic Frequency (kHz)	20 kHz, constant
TS content of TWAS (%)	3.9~4.1
Amplitude, A (%)	40, 50, 60, 80, 100 A
Sonication duration (min)	10, 20, 30, 40
Probe immersed	2.5 cm
Sludge volume (mL)	400

3.3 Acclimation of inocula

To avoid a lag-phase in the beginning of anaerobic digestion, the seed microbial inocula had been acclimated for approximately 72 days. For acclimation, four laboratory scale semi-continuous anaerobic digesters (two mesophilic and two thermophilic) with 1.5 L effective volume were operated at a 20-d SRT at 35 ± 2^0C and 55 ± 2^0C, respectively. The mesophilic inoculum was collected from an automated (7 L New Brunswick) fermenter operated in the same laboratory. The automated fermenter was being fed with Kelowna WWTF mixed sludge (a mixture of TWAS and FPS at 67:33% v/v ratio) at a 20-d SRT since 2012.

On the other hand, the thermophilic inoculum was obtained from full scale thermophilic digesters operated at Annacis Island WWTP, Vancouver, Canada. Each acclimation digester was acclimated to a mixture of FPS and pre-treated TWAS at 33:67% v/v ratio. Previous studies indicated that the severity of initial or acute inhibition of anaerobic inocula increased with the intensity of the sludge pre-treatment (Eskicioglu et al., 2007b). Therefore, sonication for 30 min at 0.25 W/mL ultrasonic density was chosen as the pre-treatment condition for acclimation phase because at this operating condition, the highest solubilisation and particle size reduction of TWAS

were achieved during preliminary pre-treatment studies. The digester feed characteristics during the acclimation phase were as follows:

Table 3.2: Sludge feed and organic loading characteristics for acclimation phase

Parameters	Range
TS content (% by weight)	4.57 ± 0.38
†OLR (g TS/L of reactor-d)	2.29 ± 0.19
OLR (g VS/L of reactor-d)	1.95 ± 0.13
OLR (g TCOD/L of reactor-d)	2.64 ± 0.2

†OLR: Organic loading rate

3.4 Experimental plan for advanced anaerobic digesters with ultrasound pre-treatment

As shown in the experimental plan (Figure 3.4), eight laboratory scale anaerobic digesters were fabricated with ultrasound acclimated inocula. To evaluate the effect of sonication on anaerobic digester performance, three sonication operating conditions were identified and termed as S1 (Sonicated at 10 min and 60% A), S2 (Sonicated at 10 min and 100% A) and S3 (Sonicated at 40 min and 60% A) during preliminary solubilisation experiments. The specific energy inputs and ultrasonic densities for these sonication options (S1, S2 and S3) were 2042, 4163, 8153 kJ/kg TS and 0.14, 0.275, 0.14 W/mL, respectively. Although at sonication time 30 min and 100% A, the highest COD solubilisation and particle size reduction were achieved, this pre-treatment condition for digestion testing was not selected due to an extremely high specific energy input of 11,342 kJ/kg TS. At this energy input level, enhancement in methane production or output energy would not be high enough (based on theoretical methane yield) to achieve an overall energy positive (cost-effective) anaerobic digester operation. Therefore digestion experiments incorporated low energy sonication pre-treatments (i.e. 0-8153 kJ/kg TS).

The configuration of the anaerobic digestion set-up (Figure 3.4) was followed in a manner that four digesters were operated under mesophilic conditions (MC, MS1, MS2 and MS3) and the other

four were run under thermophilic conditions (TC, TS1, TS2 and TS3) in semi-continuous mode at three different SRTs (20 days, 10 days and 5 days). The digesters were fed daily in a draw and fill manner with a mixture of TWAS and FPS sludge (at 67:33 % v/v ratio). The operation of the advanced anaerobic digesters with pre-treatments started at a 20-d SRT with feed sludge at concentration of 5~5.5% TS. But right after start-up of the advanced anaerobic digesters at a 20-d SRT, the thermophilic control and sonicated TS3 digesters with ultrasound intensity of 8153 kJ/kg TS started experiencing process upsets which resulted in lower daily biogas productions than those of other digesters. The pH and VFA were monitored regularly. Although pH was above 7, VFA accumulations were very high for both cases which was likely due to sensitivity of thermophilic inoculum to the high OLR at around 5.5% TS feed concentration (2.1-2.35 g VS/L/d at 5.5% TS). Therefore, after operation of 40 days at high OLR, the feed concentration was decreased to 4~4.6% TS (OLR of 1.8~6.8 g VS/L/d) and this feed sludge concentration was maintained throughout the study period. After the concentration had been changed, the thermophilic control digester started to perform well while thermophilic sonicated TS3 continued the previous condition. Since, the pH was above 7, no alkalinity was added to TS3 digester.

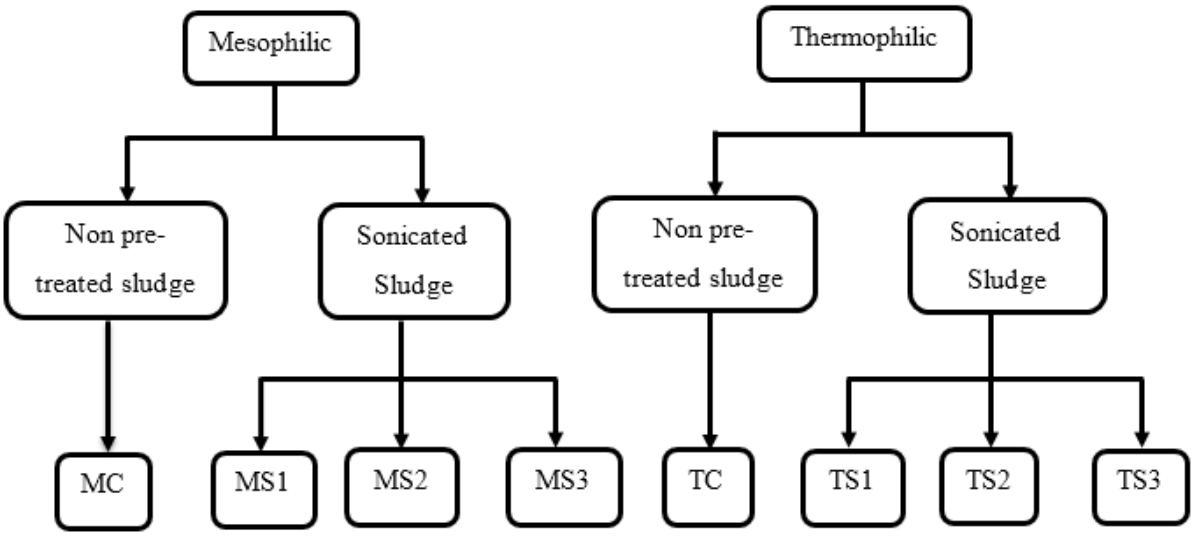


Figure 3.4: Experimental set up of the digesters (M- Meso; T- Thermo; S1- Sonicated at 10 min and 60% A; S2- Sonicated at 10 min and 100% A; S3- Sonicated at 40 min and 60% A; A-amplitude)

After all digesters (except TS3) had reached at steady state condition, they were run for almost 3×SRT days. Then the SRT was reduced to 10-d. At this SRT, with the increase of OLR to around 3.6 g VS/L/d, TS3 digester experienced further instability and digester pH started to decline. To control the pH, feeding was stopped for a few days and alkalinity (mixture of sodium and potassium bicarbonate) was added to this digester and as a result, it started to recover and perform well. At SRT of 10 days, the digesters were operated for 4×SRT days after the steady state condition was achieved. Furthermore, at the lowest SRT of 5 days, the duration of digestion operation was 5×SRT days for thermophilic and 6×SRT days for mesophilic digesters during steady state period. Overall, at this SRT among all the digestion systems the mesophilic sonicated MS2 performed well till the end. During operation of digesters, biogas production and composition, pH, volatile sulfur compounds in biogas, TS/VS and COD removal, VFA accumulation, alkalinity, ammonia, dewaterability and fecal coliform counts in effluents were monitored to assess digester performance.

3.4.1 Bench scale anaerobic digester configuration

Figure 3.5 displays one of the eight bench scale semi-continuous flow anaerobic digesters used in this experiment. Anaerobic digestion was carried out using a thick-walled side-arm Erlenmeyer style flask of 1 L. In each flask, 500 mL mesophilic or thermophilic anaerobic inoculum was placed for start-up. During digester set-up, O_2 was removed from the headspace in the flask using N_2 purging for about 2 min. Then, the flask was sealed with a rubber stopper leaving provisions for one outlet to collect effluents and another for biogas. To collect biogas, Tedlar (2 L) bags were used. A small piece of hose (tubing) connected to the side port of the flask acted as the feed line of the digester (Figure 3.5). The digesters were kept at two large capacity shakers maintaining temperatures of 37 ± 1^{0}C and 55 ± 1^{0}C to carry out mesophilic and thermophilic digestion, respectively. These shakers provided mixing at 90 rpm to provide homogeneous contact between feed sludge and inoculum to simulate complete-mix reactor conditions.

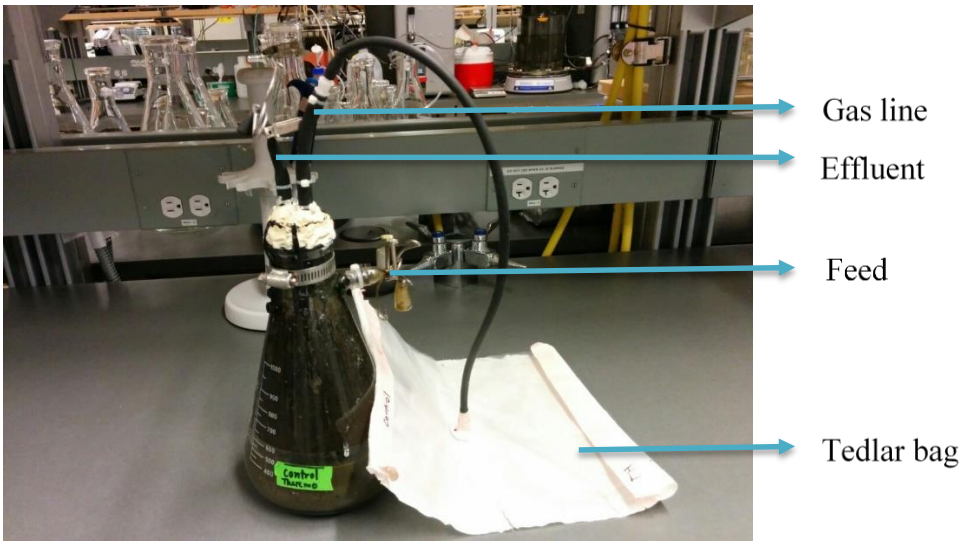


Figure 3.5: Configuration of an anaerobic digester (lab scale)

3.5 Analytical methods

3.5.1 Total solids and volatile solids

Total solids and VS measurements of sludge samples were done according to Standard methods 2540 B and 2540 E (APHA, 2005). A well-mixed sample was poured in a weighed evaporating dish and dried in an oven at 103 to 105^{0}C for overnight to constant weight. After weighing the dried dish with sample, the difference in weight over that of bare dish represents TS. Then the residue was ignited at 550^{0}C in a muffle furnace to constant weight for approximately 1 hour and the weight lost in this process is represented by VS. For calculation of TS and VS, the two equations given below were followed:

% Total solids by weight = 100 (B-W)/ (A-W)

% Volatile solids by weight = 100 (B-C)/ (A-W)

Where,

W = Mass of empty dish

A = Mass of dish with wet sludge

B = Mass of dish with dried (at 104^{0}C) sludge and

C = Mass of dish with ignited (at 550^{0}C) sludge

3.5.2 Chemical oxygen demand (COD)

Closed Reflux Colorimetric Method (Standard Method 5220 D) (APHA, 2005) was followed to measure COD for both total and soluble fractions of sludge samples. For SCOD determination, sample was prepared by filtering the supernatant (collected after centrifugation in Sorval Legend XT at 8000 rpm for 20 minutes) through a 0.45 mm pore size filter paper. After dilution, the sample was mixed with digestion solution (a mixture of dried 20.532 g potassium dichromate ($K_2Cr_2O_7$),

334 ml sulfuric acid (H_2SO_4) and 34 g mercury (II) sulfate ($HgSO_4$) in 1 L distilled water) and catalyst (22 g silver sulfate (Ag_2SO_4) in 4.08 kg H_2SO_4). Then the mixture was digested at 150^0C for 2 hrs. During digestion, dichromate ion reacts with COD matters and reduces the chromium ion from hexavalent form to trivalent form. A calibration curve for COD concentrations ranging from 100 to 700 mg/L was used (Appendix A, Figure A.1)

3.5.3 Alkalinity

For alkalinity measurement, according to Standard Method 2320B (APHA, 2005), sample supernatant (prepared by centrifuging at 8000 rpm for 20 minutes) was titrated with 0.1 N sulphuric acid until the pH of solution reached 4.6. In this method, a XL25 dual channel pH/ion meter with Accumet probe was used to measure pH.

3.5.4 Ammonia

Standard Method 4500-NH_3 D (Ammonia-Selective Electrode Method) was followed to measure dissolved ammonia in supernatant of sludge sample. In this method, strong base NaOH (10 N) was added to the sample to raise pH above 11 which converts dissolved ammonia ($NH_{3\,(aq)}$ and NH_4^+) to $NH_{3\,(aq)}$. $NH_{3\,(aq)}$ diffuses through a membrane in an ammonia probe and changes the pH of an internal solution (ammonium chloride) that is sensed by a pH electrode. A dual channel pH/ion meter (Accumet Excell XL 25) with ammonia probe was used for this analysis. A calibration curve for ammonia-N with a range of 10-1000 mg/L was used and included in Appendix A (Figure A.2).

3.5.5 Volatile fatty acid analysis

Volatile fatty acid (VFA) composition, in terms of acetic, propionic and butyric acids, was analyzed using a gas chromatograph (GC) (Agilent 7890A). The gas chromatograph used a flame ionization detector (FID) and a polyethylene capillary column (HP-FFAP, 25 m, 0.32 mm ID) and an auto sampler. Helium was used as the carrier gas at 25 mL/min flowrate. The samples were prepared by filtering the sludge supernatant through 0.22 µm membrane and iso-butyric acid was added as an internal standard before analysis.

3.5.6 Sugars

Sugars in sludge samples were measured based on a technique developed by Dubois et al. (1956). In this analysis, 1 mL of diluted sludge sample was mixed with 1 mL of phenol and 5 mL of sulphuric acid (98%) in a disposable borosilicate glass tube. The mixture was then allowed to react for 10 minutes at room temperature followed by heating at 30^0C for 20 minutes in the oven. The solution was placed in the well of a polymer base (0.4 m well) tray microplate (96 well Optical Bottom Plates) and absorbance was measured by a BioTek Synergy HT (multimode micro plate, Gen5 2.0) reader at 490 nm. A calibration curve (Appendix A, Figure A.3) was prepared using glucose as standard.

3.5.7 Proteins

For determination of proteins in sample, a modified Lowry protein assay (Frølund et al., 1995) was followed. In this experiment, 0.5 mL of diluted sludge sample was mixed with 2.5 mL of reagents (provided in Appendix B) in a disposable borosilicate glass tube. The mixture was then allowed to incubate at room temperature for 10 minutes prior to addition of 0.25 mL of folin reagent. The glass tubes were stored in the dark again for 30 minutes at room temperature to give

time for the colour to develop. Solution was placed in the well of a polymer base (0.4 m well) tray microplate (96 well Optical Bottom Plates) and absorbance was measured by a BioTek Synergy HT (multimode micro plate, Gen5 2.0) reader at 750 nm. The calculation procedure is explained in detail in Appendix B2. Standard curves (Appendix A, Figures A.4, A.5) were prepared for proteins and humic acids by bovine serum albumin (BSA) and humic acid standard solutions, respectively.

3.5.8 Dewaterability

A Capillary Suction Timer (Model 440, Fann Instrument Company, TX, USA) was used to asses dewaterability of digestate sludges. According to Standard Methods Procedure 2710 G (APHA, 2005), an aliquot of 5 mL digestate sample was injected to a cylinder placed on a piece of chromatography paper (57 mm × 57 mm). When sludge comes into contact with the paper, they water from sludge will start to wet the paper due to capillary suction pressure. The time required for water to pass between two electrodes is then recorded on a digital timer. The recorded time is denoted by capillary suction time or CST in seconds. Additionally, TS of the tested sample was also measured to calculate normalized CST as suggested in Standard Methods.

3.5.9 Gas composition

An Agilent 7820A GC equipped with a packed column (Agilent G3591-8003/80002) and a thermal conductivity detector (TCD) was used to determine biogas composition in terms of CH_4, CO_2, O_2 and N_2 percentages in headspace of the anaerobic reactors. A method developed by van Huyssteen (1967) was followed for determination of gas composition. Helium was used as the carrier gas at 25 mL/min flowrate.

3.5.10 Volatile sulfur compounds

An Agilent 7890A GC equipped with a flame photometric detector (FPD) was used to measure VSCs in the headspace of the anaerobic digesters. The GC system can detect eight odorous VSCs compounds (hydrogen sulphide, methyl mercaptan, ethyl mercaptan, dimethyl sulphide, carbon disulphide, ethyl methyl sulfide, 1-propanehiol and dimethyl disulphide) in biogas. N_2 was used as a balance gas for dilution of the headspace biogas samples so that the amounts of the VSCs fall within the range detectable by the GC-FPD method.

3.5.11 Particle size distribution

The particle size analysis for both unsonicated and sonicated sludge samples was carried out by a Malvern Mastersizer 3000 capable of measuring particle sizes ranging from 10 nm up to 3.5 mm. The Mastersizer 3000 works on the principle of laser light scattering and a particle size distribution is calculated from measurements of the angular intensity of the scattered light produced by a sample.

In this study, fresh sludge sample at room temperature was pumped to Hydro LV, a liquid dispersion unit and each sample was run for 10 times. Mastersizer 3000 software provides the graph of volume frequency (%) versus the particle size over a wide range. Another representation of mean particle diameter at Dv10, Dv50, Dv90 give the 10th, 50th and 90th percentile of particle size distribution, respectively.

3.5.12 Zeta potential

Zeta potential of sludge samples were measured by a Zetasizer Nano series Nano-Zs (Malvern Instruments Ltd.) which is capable of measuring particle size ranging from 0.3 nm to 10 microns

and zeta potential with a range of > +/-500 mV. Zetasizer Nano uses the Laser Doppler Micro-electrophoresis method to measure zeta potential. For this analysis, the raw or pre-treated sludge sample was filtered through a 1 µm filter paper to remove large particles outside the colloidal range that may clog the instrument and the filtered sample was transferred into a disposable folded capillary cell (zeta potential cuvette). The cuvette was then inserted in to the Zetasizer to obtain the measurement results.

3.5.13 Fecal coliform test

Fecal coliform densities in digested sludge samples can be determined using either the multiple tube or membrane filtration (MF) technique. Standard Method 9222D (APHA, 2005) for the MF technique was followed for this project. For cultivating fecal coliforms, mFC Nutrient Pad Sets supplied by Sartorius Stedim Biotech, Germany were used. These nutrient pad sets were dehydrated culture medium available in petri dishes with 0.45 µm green membrane filters. To saturate the nutrient pad, 3-3.5 ml of sterile, distilled water was added to the petri dish. After filtration of the appropriate volume of each diluted sludge sample, the membrane filter was placed on the nutrient pad without entrapping any air bubbles. Then the culture dishes were incubated at 44.5 ± 0.2^{0}C for 18-24 h. The dark blue colonies grown as a result of incubation and strong lactose fermentation were enumerated for determination of fecal coliform densities. In the laboratory, before use, all the glassware, tweezers, vacuum filtration units, and distilled water were sterilized in an autoclave at 120 - 130^{0}C for 1 hour.

CHAPTER 4: RESULTS AND DISCUSSIONS

In this chapter, the results obtained from laboratory experiments involving sludge sonication pre-treatment and the subsequent anaerobic digestion process are presented and discussed. The results from the ultrasound pre-treatment were conferred to optimize the sonication operating conditions based on the efficiency of sludge solubilisation and particle size reduction. Furthermore, the experimental results from the anaerobic digestion stage are analyzed to investigate the effect low power sonication pre-treatment has on the operational parameters of anaerobic digestion.

4.1 Characterization of waste sludge

In order to assess the effect sonication pre-treatment has on sludge characteristics, a characterization of waste sludge before and after ultrasound disintegration is important**.** In Table 4.1, the waste sludge characteristics of TWAS and FPS from Kelowna's WWTF are reported.

Table 4.1: Characteristics of waste sludge streams

Parameters	TWAS	FPS
pH (-)	6.1 (0.1; 6)†	4.7 (0.3; 3)
TS (% w/w)	3.3 (0.18; 15)	7.5 (2.4; 9)
VS (% w/w)	2.7 (0.13; 15)	5.7 (1.2; 9)
(VS/TS) ×100 (%)	81.8	76.0
Ammonia (mg/L)	196 (16; 6)	150 (15; 6)
Alkalinity (mg as $CaCO_3$/L)	485 (25; 6)	317 (45; 6)
TCOD (mg/L)	35316 (3638; 9)	74805 (18793; 6)
SCOD (mg/L)	1721 (870; 9)	5588 (1938; 6)
(SCOD/TCOD)×100 (%)	4.8	7.4
TVFA (mg/L)	454.5 (272.5; 7)	2609.9 (1139.9; 7)

*TWAS: thickened waste activated sludge, FPS: Fermented primary sludge, TS: total solids, VS: volatile solids, TCOD: total chemical oxygen demand, SCOD: soluble chemical oxygen demand, TVFA = total volatile fatty acids.
†Data represent arithmetic mean of replicates (standard deviation; number of replicates)

From the Table 4.1, it is apparent that FPS was slightly acidic (~4.72), whereas TWAS (~6.12) had a more neutral pH value. In both of the sludge samples, alkalinity and ammonia concentrations were low, but between the two sludge streams, FPS had a slightly lower ammonia and alkalinity concentration. It is well known that a low ammonia concentration in the sludge feed is preferred for stable operation of the anaerobic digestion process. In comparison with TWAS, total volatile fatty acids (TVFA) concentration in the FPS stream was significantly higher (2,610 mg/L), which was expected because primary sludge is fermented within the primary settling tank at the Kelowna WWTF to produce a carbon source as TVFA for biological nutrient removal in the modified Bardenpho process.

Both total solid (TS) and VFA concentrations of FPS varied over the entire sampling period (June, 2013 to June 2014). The variation was a result of a change in mixing regime in the gravity fermenters at the Kelowna WWTF. In order to mitigate the inconsistency in sludge characteristics within the researching time period, the sludge concentrations were controlled via laboratory thickening or dilution to maintain a steady homogeneous sludge feed (FPS:TWAS at 33:67% volume ratio), with a TS content of 4.00~4.60% by weight.

4.2 Effect of ultrasound application on sludge properties

In this section, the effect of sonication pre-treatment on sludge solubilisation is evaluated. Specific parameters such as SCOD, protein concentrations, sugar concentrations, particle size distribution and zeta potential are analyzed.

4.2.1 Sludge solubilisation

4.2.1.1 Chemical oxygen demand (COD)

Figure 4.1 depicts the percentage of increase in the ratio of SCOD over total COD multiplied by one hundred at various specific energies of sonication, using an untreated control sludge as a reference. From the graph, it is observed that the solubilisation of COD is not a linear function of specific energy input, also reported by Tyagi et al. (2014). The maximum increase in solubilisation (10.8 times) over control was achieved at 30 min sonication time for a specific energy input of 11,343 kJ/kg TS. At 11,343 kJ/kg TS, the SCOD in the supernatant of the TWAS increased by approximately 91%, from 1050 mg/L to 11,889 mg/L. In an extensive study by Pérez-Elvira et al. (2010), a 2.4% to 34.3% increase in SCOD of TWAS was observed by varying the specific energy (0 ~ 25,700 kJ/kg TS) input to the sludge that contained 3.33% TS. However, in their study the sonication duration was ranging only from 0 to 2 minutes which explains the lower yields in solubilisation (2.4-34.3%) when comparing this study (up to 91%).

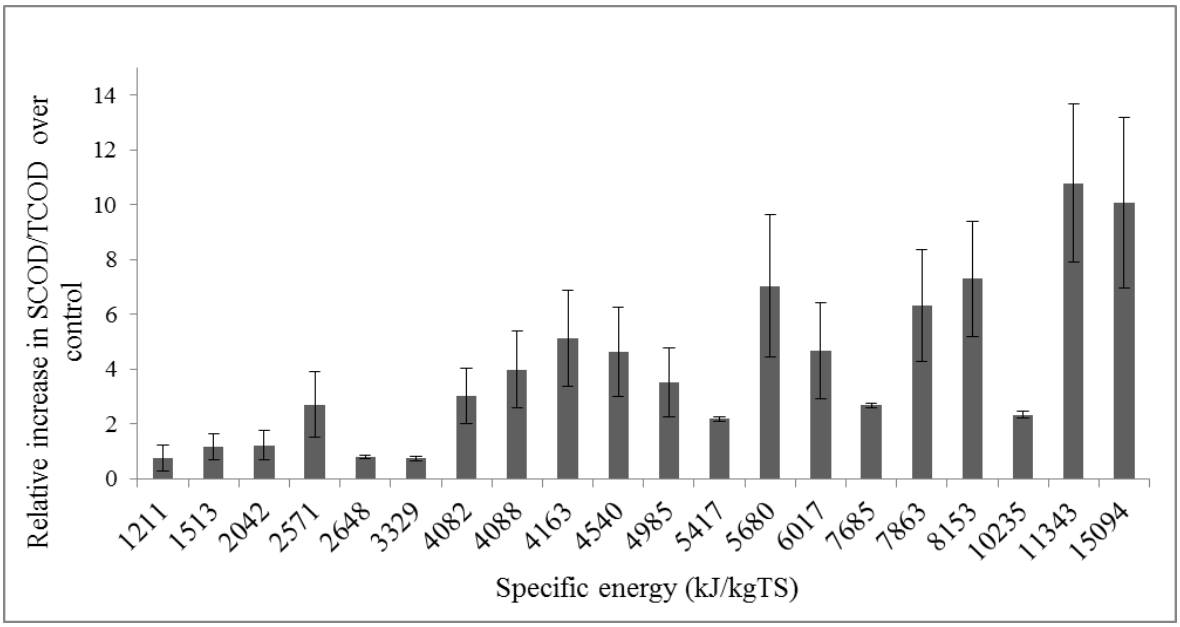


Figure 4.1: Relative increase in solubilisation of COD over control (data represent arithmetic mean and error bars represent standard deviation of the three replicates)

The effects of sonication time and ultrasound density on sludge COD solubilisation (%), calculated as SCOD/TCOD of samples multiplied by one hundred, were shown in Figure 4.2.

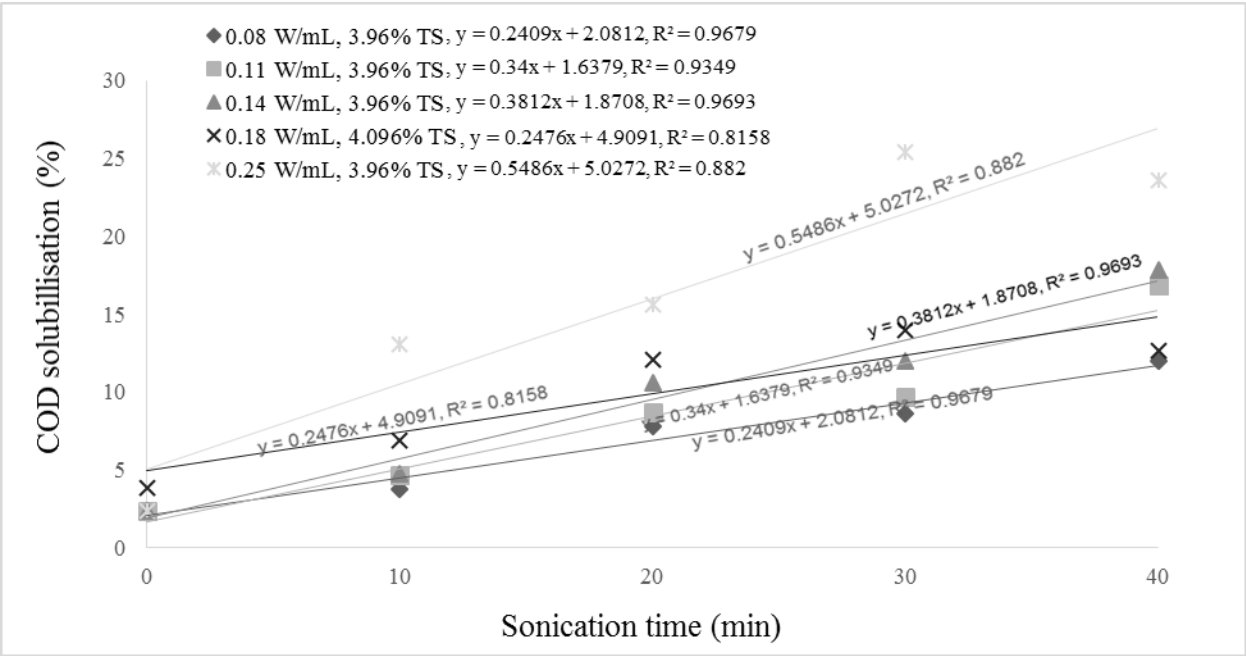


Figure 4.2: COD solubilisation of control and sonicated sludge (at different sonication time and densities)

In this study, at an ultrasonic power of 0.14 W/mL, COD solubilisation increased from 2.3% to 4.8%, 10.6%, 12.0% and 17.8% for 10, 20, 30 and 40 min of sonication, respectively. Similarly, an increasing trend was observed in solubilisation as the ultrasonic power was increased at constant treatment times. At a constant 20 minute sonication period and different ultrasonic densities of 0.08, 0.11, 0.14, 0.18 and 0.25 W/mL, COD solubilisations of TWAS were 7.8%, 8%, 10.6%, 12.0% and 15.6%, respectively. Similar observations were reported by Huan et al. (2009). From their research it was observed that the rate of COD solubilisation became slower as sonication density and time increased. For example, at ultrasonic densities of 0.6 and 0.8 W/mL and after 10

minute sonication duration, sludge disintegration degrees were 0.35 and 0.4 respectively, and after sonication periods of 25 minutes the disintegration degrees were 0.5 and 0.48 respectively.

A two-way analysis of variance (ANOVA) was performed to see the effect of pre-treatment conditions on COD solubilisation (SCOD/TCOD). In the analysis, sonication duration and ultrasonic density were considered as the factors and COD solubilisation (SCOD/TCOD) was the response. In a two-way ANOVA, a p-value of < 0.05 is used to indicate whether a factor has a significant effect on the measured response; a significant factor with a p-value less than 0.05 will have a 95% certainty that the effect that the factor has on the response is caused by the factor rather than being caused by errors or noise in the experiment. In this case, the sonication duration was not a significant factor affecting COD solubilisation. But ultrasonic density and the interaction between ultrasonic density and sonication duration were statistically significant in the solubilisation response. The interaction effect was found to be the most effective factor effecting the solubilisation of COD, have a p-value of 0.0004; however, experimental results indicate that the effect is negative at higher sonication power densities (0.18 and 0.25 W/ml of TWAS) after a certain period of sonication (30 min). This was also confirmed by reduction in coefficient of determination (R^2) values to 0.82-0.88 (at 0.18 and 0.25 W/ml) from 0.93-0.96 (at 0.08-0.14 W/ml) when COD solubilisations (%) were fit into linear models (Figure 4.2). The output table of the two-way ANOVA results for COD solubilisation is included in Table C.1 (Appendix C).

4.2.1.2 Proteins

As mentioned earlier, sludge disintegration efficiency can also be evaluated based on protein release into the liquid phase. Although sonication power density had a great influence on disintegration in terms of COD solubilisation, protein release was not considerably affected by

ultrasonic density in this research, especially at the low ultrasonic densities. Akin et al. (2006) noted that at higher solid concentrations (at 4% and 6% TS), sludge disintegration efficiency is significantly reduced. The decrease in cavitation within the sludge is held responsible for the low release of proteins at higher solid contents. From the Figure 4.3, it was observed that the solubilisation of protein into the liquid phase remained insignificant until the ultrasonic power density exceeded 0.18 W/mL. The protein release is calculated by the ratio of soluble protein to total protein concentration and multiplied by one hundred. The highest protein release observed was calculated to be 47.5%, which was at 0.25 W/mL sonication power density and a specific energy of 11,343 kJ/kg TS after a 30 minute sonication period (Figures 4.3 and 4.4). At the mentioned parameter levels, the soluble protein concentration increased by 380%, from 342 mg/L to 1639 mg/L. It is important to note that in this study, the highest COD solubilisation was also achieved at this sonication condition.

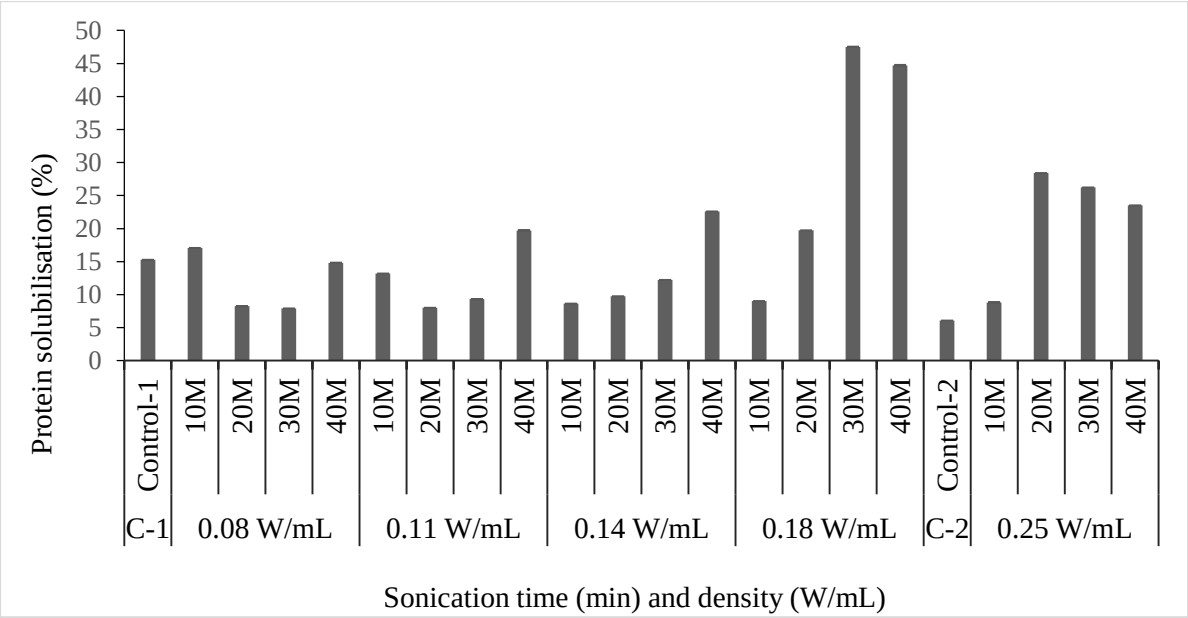


Figure 4.3: Protein solubilisation of control and sonicated sludge (at different sonication time and densities) (data represent arithmetic mean and error bars represent standard deviation of the three replicates, C-1 and C-2 indicate TWAS sampled at different times from Kelowna WWTP)

From a two-way ANOVA analysis of protein solubilisation, it was also apparent that only the interaction between ultrasonic density and sonication duration is found to be significant on protein release. The main effects of either of the factors is found to be insignificant on protein solubilisation. The results from the two-way ANOVA analysis for protein solubilisation are included in Table C.2 (Appendix C).

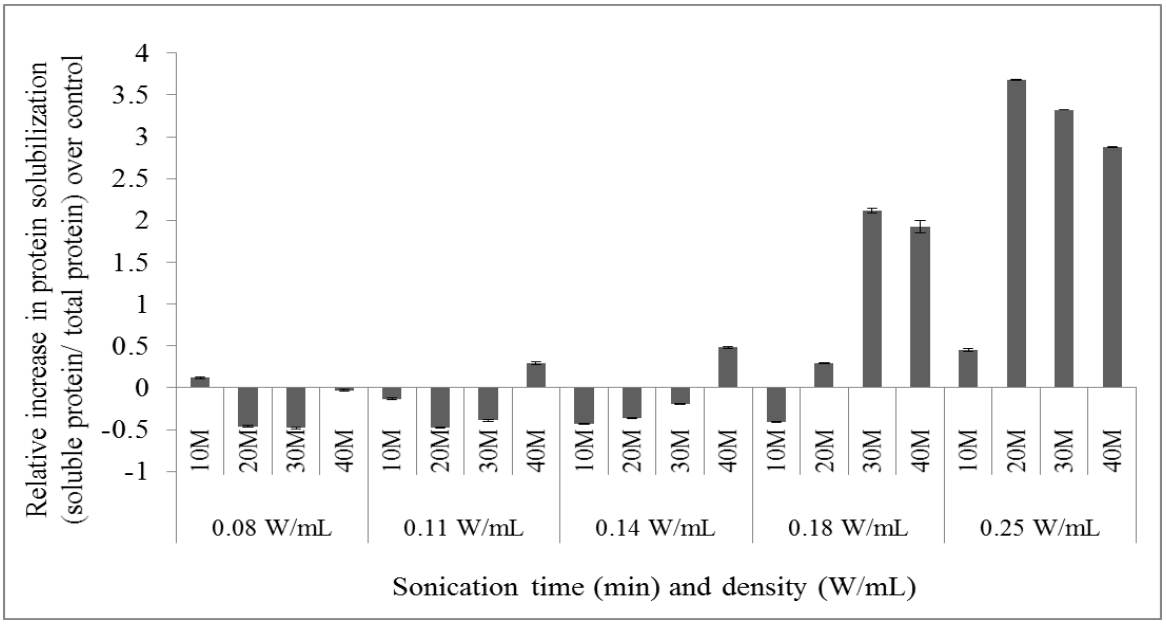


Figure 4.4: Relative increase in protein solubilisation of non-pre-treated controls (C-1 and C-2) and sonicated sludge (at different sonication time and densities. M: minutes (data represent arithmetic mean and error bars represent standard deviation of the three replicates)

4.2.1.3 Sugars

In addition to COD and protein solubilisation, sugar is also an important parameter in the evaluation of sludge disintegration efficiency of sonication pre-treatment. Wang et al. (2006) reported that the release rate of protein and sugar is different during sonication. This observation is also apparent from this research. Figure 4.5 depicts the relative increase in sugar solubilisation of sonicated TWAS. From the figure, it was apparent that sugar solubilisation increased with sonication duration within the first 30 minutes of pre-treatment. However, beyond 30 minutes,

sugar solubilisation decreased. The highest sugar solubilisation of 22.5%, calculated by the ratio of soluble to total sugar concentrations and multiplied by one hundred, was achieved at the highest ultrasonic power density of 0.25 W/mL after a 30 min sonication period. Protein and COD solubilisations previously presented were also the highest at the same operating condition. The statistical analysis results (Table C.3, Appendix C) indicate that both sonication time and ultrasonic density had statistically significant effects on sugar solubilisation ($p<0.05$). Their interaction term was also statistically significant.

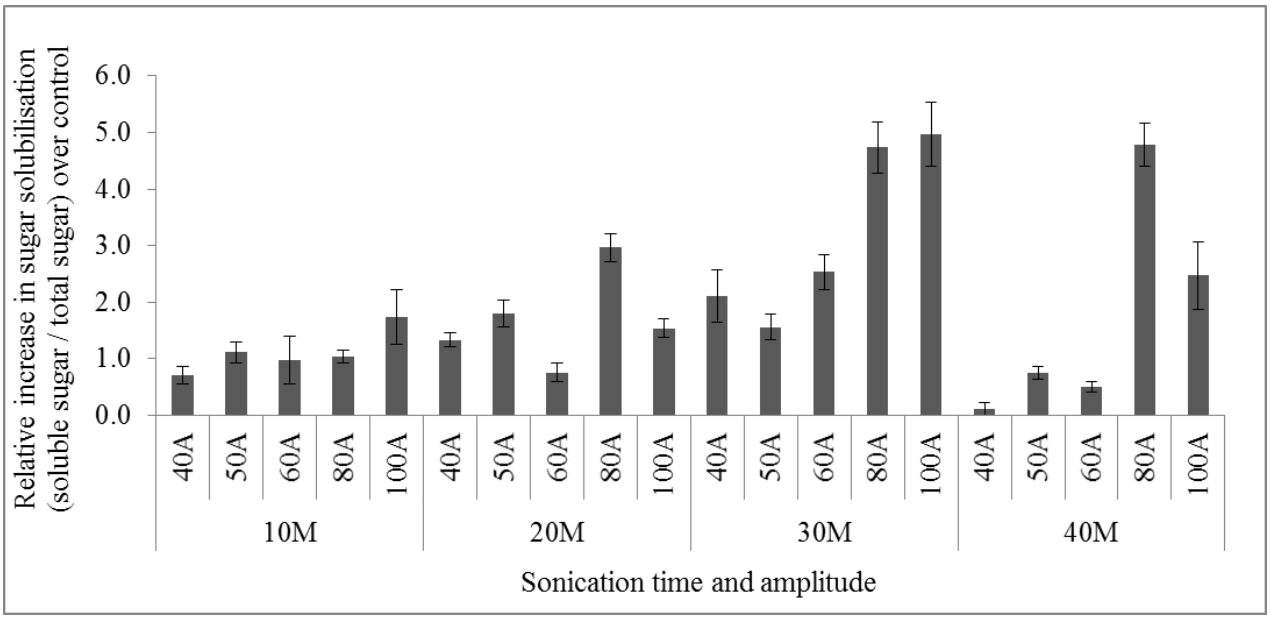
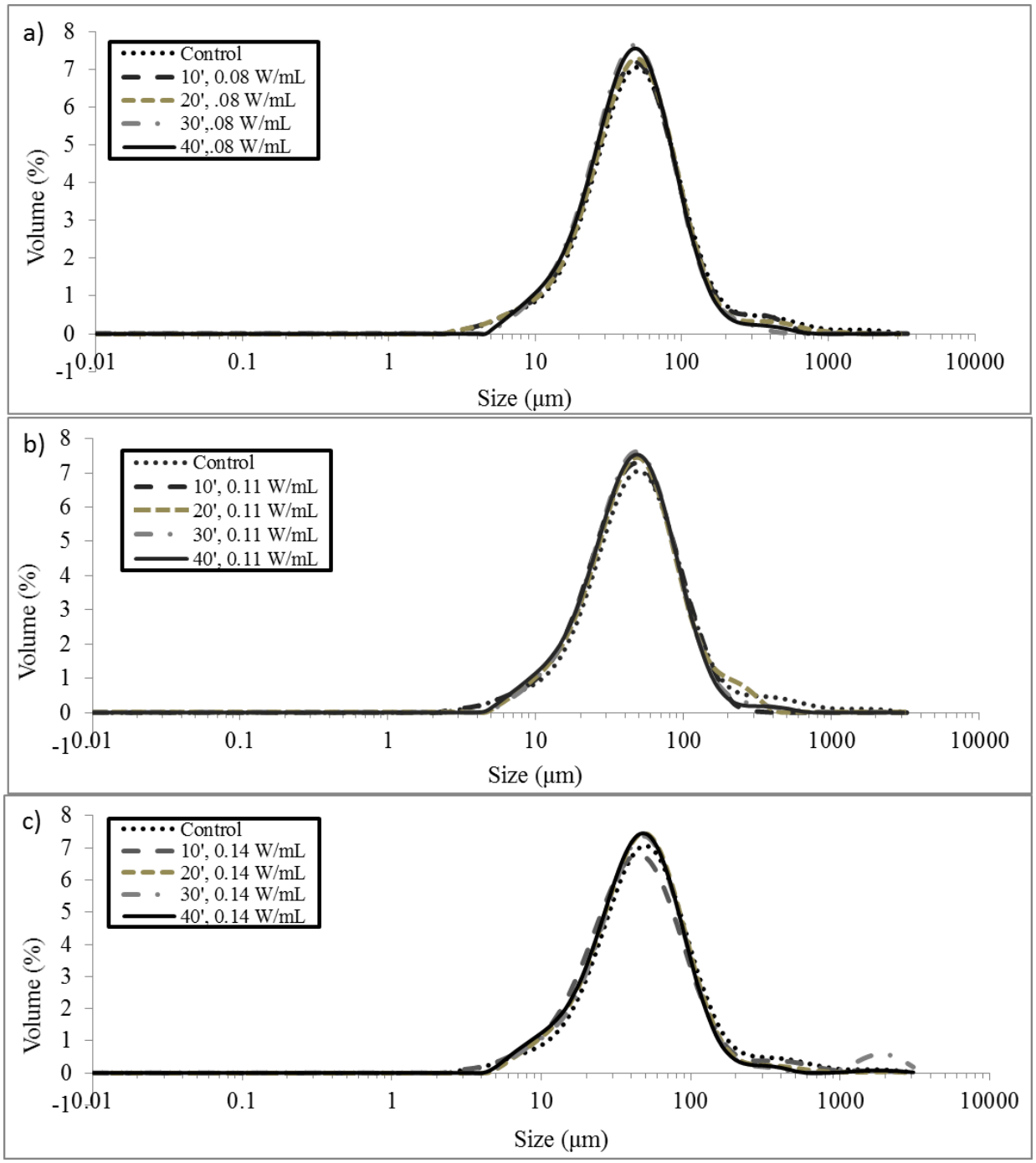


Figure 4.5: Relative increase in sugar solubilisation over control (data represent arithmetic mean and error bars represent standard deviation of the three replicates)

4.2.2 Particle size

Based on literature, sonication is expected to have a great impact on the particle size of sludge. During sonication, the mechanical (hydraulic) shear forces enhance the volume engaged by particulate matter and highly porous sludge flocs are disrupted into micro flocs. The particle size distribution of TWAS before and after sonication at different power densities and duration is presented in Figure 4.6. Figure 4.6 illustrates that at lower densities (0.08-0.18 W/mL) sonication

had no significant effect on floc size reduction. Only at the highest power level of 0.25 W/mL (Figure 4.6e), the distribution curve shifted to left considerably for extensive sonication duration of 30 and 40 minutes indicating an overall decrease in particle size.



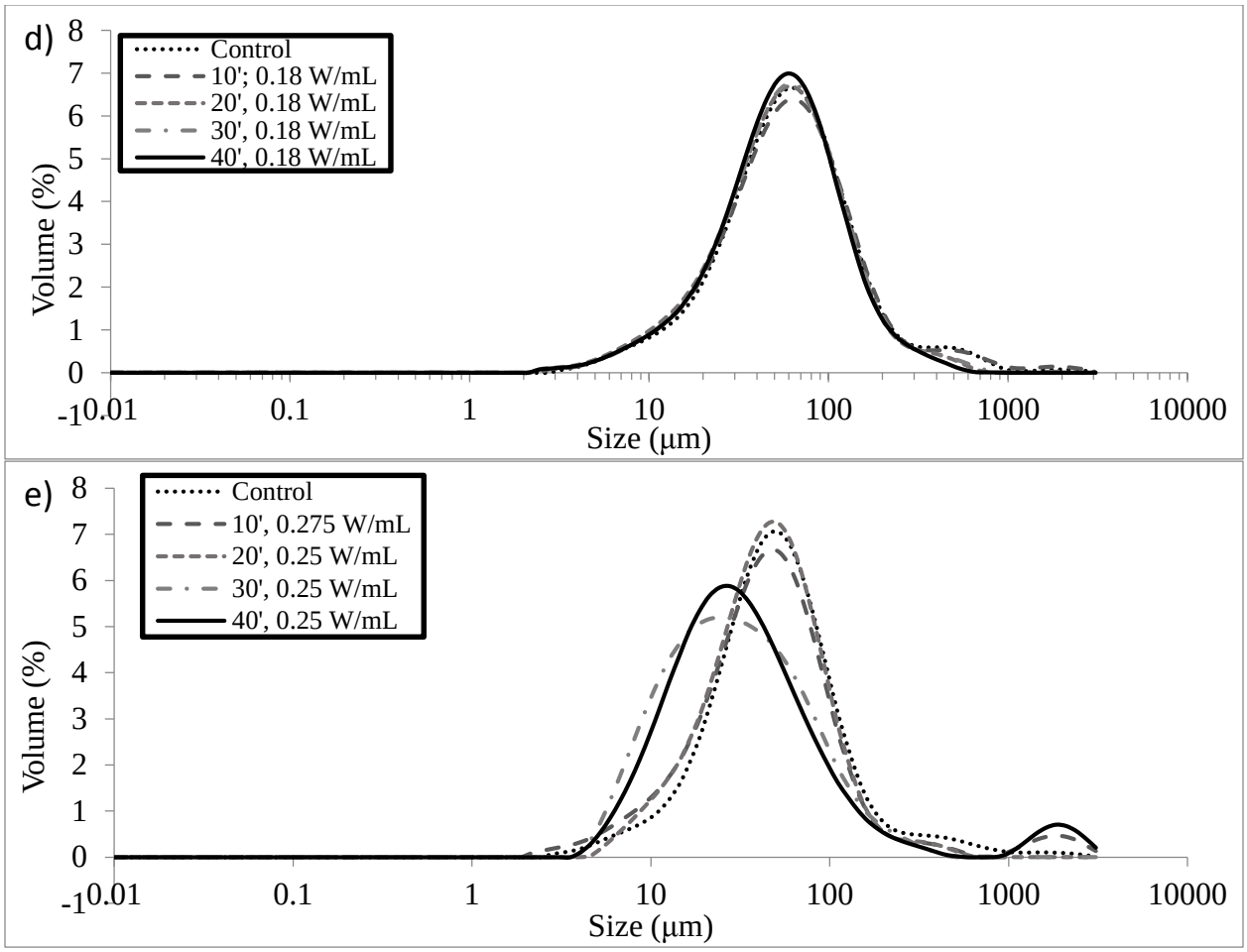


Figure 4.6: Particle size distribution of TWAS before and after sonication at different durations and ultrasonic densities of a) 0.08 W/mL, b) 0.11 W/mL, c) 0.14 W/mL, d) 0.18 W/mL and e) 0.25 W/mL

In a similar study by Chu et al. (2001), it was observed that the apparent reduction in particle size started when the ultrasonic density level exceeded 0.22 W/mL. At 0.33 W/mL and 0.44 W/mL, the particles significantly reduced from 99 μm to 22 μm and 3 μm respectively after 20 min sonication. Figure 4.7 displays the relative reduction in mean particle diameter (D_{50}) of TWAS with respect to a control at different operating conditions. In this analysis, the sonication test at 0.25 W/mL experienced the highest particle size reduction (41%) from D_{50} of 51.7 μm to 30.31 μm after 30 min disintegration. Both the higher power level and longer sonication time played a vital role in achieving the size reduction in particles. Some researchers reported that sludge floc cells

are completely ruptured after sonication of 30 min (Khanal et al, 2007; Cao et al, 2006) whereas Chu et al. (2001) observed the complete break-up of flocs after 40 min sonication. In the study reported by Chu et al. (2001), the reduced particle sizes were much smaller than the reduced particle sizes achieved in this research due to the higher ultrasonic density (0.33 W/mL) used. They mentioned that a critical power level exists beyond which sludge structure might be adequately degenerated.

It is relevant to mention that the highest COD, protein and sugar solubilisations of TWAS is observed at a sonication period of 30 minutes with an applied sonication power density of 0.25 W/mL (specific energy of 11,342 kJ/kg TS). Maximum particle size reduction was also achieved at these operating parameters.

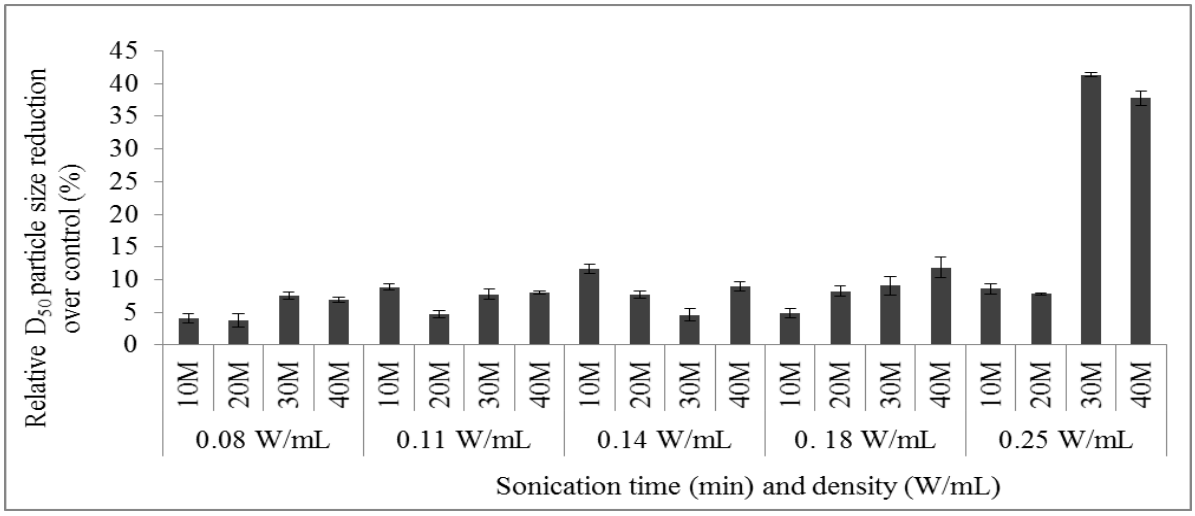


Figure 4.7: Relative particle size reduction (D_{50}) over control at different sonication times and densities (M: minutes) (data represent arithmetic mean and error bars represent standard deviation of the 10-20 replicates)

4.2.3 Zeta potential

Zeta potential is an important index to determine the electric surface charge of sludge particles and tends to decrease as the negative charge of sludge surface decreases. It gives an indication of

potential stability of the colloidal system (Zhang et al., 2007). From the plot of the zeta potential (Figure 4.8) of colloidal particles in TWAS samples against specific energy, it is revealed that flocs were negatively charged at a neutral pH, and the change in zeta potential due to sonication pre-treatment did not follow any specific trend. Wang et al. (2010) reported that ultrasound application had no effect on the zeta potential of suspended particles. The author explained that when a floc cell ruptures, the concentrations of Ca^{2+} and Mg^{2+} ions in the supernatant increase significantly, where these ions are considered as the essential components to associate the constituent particles.

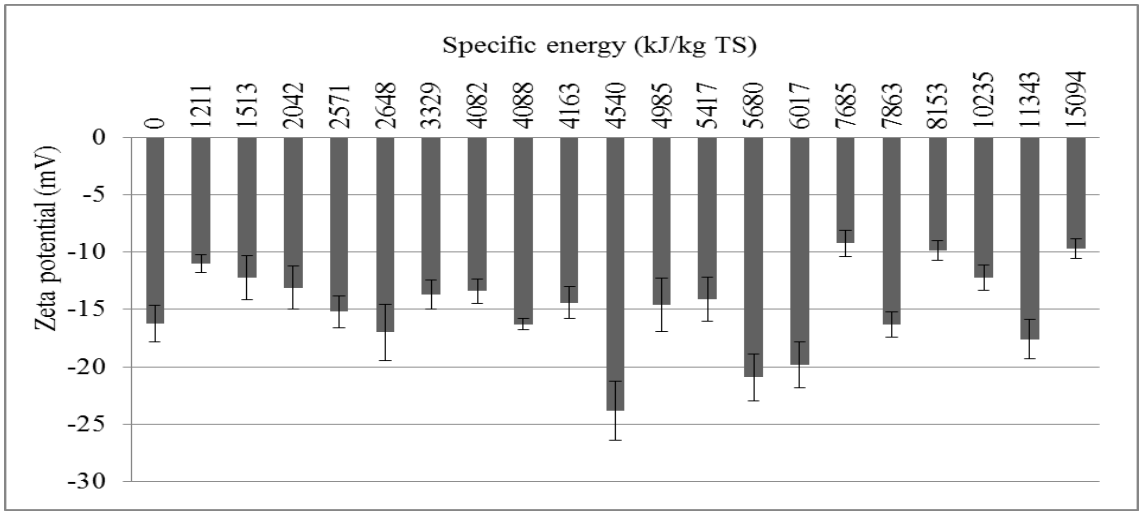


Figure 4.8: Zeta potential of sludge flocs in TWAS samples before and after sonication (data represent arithmetic mean and error bars represent standard deviation of 10-20 replicates)

Generally, carboxyl, phosphate and amino groups in the extracellular polymeric substance (EPS) are accountable for the density of surface charge. The Figure 4.8 demonstrated that the zeta potential of control (non pre-treated) sludge was -16.25 mV and the zeta potential for sonicated TWAS flocs varied from -9.23 to -23.8 mV. These results are in agreement with the usual range (-10 mV to -30 mV) of zeta potential previously reported for waste sludge (Forster, 1985; Forster, 1968; Valin and Sutherland, 1982).

4.3 Effect of sonication on anaerobic digestibility

Based on the solubilisation results, it was concluded that the optimum sonication pre-treatment was a 30 minute duration with a power density of 0.25 W/mL, corresponding to a specific energy of 11,342 kJ/kg TS. At this operating condition, the highest solubilisation and particle size reduction of TWAS was achieved. Therefore, the acclimation of inocula for the anaerobic digestion studies was performed with a feed sludge that has been pre-treated at the aforementioned sonication parameter levels in order to avoid any inhibition of microbial cultures that may be caused if alterations to the substrate (TWAS) were made. The steady state results obtained during the acclimation phase are presented in Table 4.2.

Table 4.2: Steady-state results from acclimation digesters*

Parameters	Mesophilic	Thermophilic
Loading conditions		
Solid retention time (d)	20	20
OLR (g VS/$L_{reactor}$-d)	1.95 ± 0.13†	1.95 ± 0.13
OLR (g TCOD/$L_{reactor}$-d)	2.64 ± 0.20	2.64 ± 0.20
Removal efficiency		
VS (%)	52.31 ± 0.02	52.46 ± 0.01
TS (%)	44.43 ± 0.02	44.80 ± 0.00
TCOD (%)	48.92 ± 0.04	57.33 ± 0.04
Biogas production		
Methane (CH_4) in biogas (%)	65.35 ± 1.65	64.23 ± 0.86
Specific biogas (L/g VS_{added})	0.52 ± 0.05	0.51 ± 0.06
Specific methane (L CH_4/g VS_{added})	0.34 ± 0.04	0.33 ± 0.04
Effluent characteristics		
pH	7.5 ± 0.2	7.8 ± 0.1
SCOD (mg/L)	1040 ± 352	2407 ± 116
NH_3-N (mg/L)	1047 ± 19	1457 ± 31
Alkalinity (mg $CaCO_3$/L)	4352 ± 16	5275 ± 94
TVFA (mg/L)	16 ± 19	35 ± 11

* OLR-Organic loading rate, TS-Total solids, VS- Volatile solids, TCOD- Total chemical oxygen demand, SCOD- Soluble chemical oxygen demand, TVFA- Total volatile fatty acid

† Data represent arithmetic mean of replicates ± standard deviation of 6 to 60 replicates

However, since the operating condition of 11,342 kJ/kg is a high energy intensive input, three lower specific energies that also provided COD solubilisation with potential positive net energy

outcome were selected for the subsequent advanced anaerobic digestion testing with an aim to lower input energy (Table 4.3).

Table 4.3: Sonication operating options for subsequent advanced anaerobic digestion

Sonication option	Sonication time (min)	Amplitude, A (%)	Ultrasonic density (W/mL)	Specific energy (kJ/kg TS)
S1	10	60	0.14	2042
S2	10	100	0.25	4163
S3	40	60	0.14	8153

As mentioned earlier (in Chapter 3), only TWAS (3.9~4.1% TS) was subjected to sonication and the anaerobic digesters were fed with mixed sludge (TWAS: FPS at 67:33% at v/v ratio). The feed sludge TS concentration was maintained at 4~4.6 % TS throughout the period of research involving advanced anaerobic digestion. Table 4.4 displays the characteristics of the mixed sludge used as the digester feed. The performance results of the advanced anaerobic digestion process obtained during steady state at the SRTs of 20-d, 10-d and 5-d were tabulated and included in Appendix D (Table D.1, Table D.2 and Table D.3, respectively). The following sections will highlight some of the important process performance results.

Table 4.4: Digester feed (mixed sludge) characteristics*

Parameters	Control	Sonicated S1[a]	Sonicated S2 [a]	Sonicated S3[a]
pH (-)	[†]5.6 ± 0.10	5.6 ± 0.1	5.6 ± 0.09	5.6 ± 0.10
TS (% w/w)	4.2 ± 0.2	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.2
VS (% w/w)	3.6 ± 0.2	3.6 ± 0.3	3.60 ± 0.3	3.6 ± 0.2
VS/TS (-)	0.85	0.85	0.84	0.84
Ammonia (mg/L)	463 ± 208	484 ± 205	618 ± 279	591 ± 254
Alkalinity (mg as $CaCO_3$/L)	1007 ± 294	1159 ± 225	1205 ± 325	1149 ± 354
TCOD (mg/L)	58,557 ± 5166	58,575 ± 5061	56,623 ± 7645	56,630 ± 5482
SCOD (mg/L)	3199 ±319	4766 ± 625.7	6413 ± 361.5	7575 ± 285.47
SCOD/TCOD*100	5	8	11	13
TVFA (mg/L)	2088 ± 337	2315± 549	2366 ± 390	2275 ± 496

*TS-Total solids, VS- Volatile solids, TCOD- Total chemical oxygen demand,
SCOD- Soluble chemical oxygen demand, TVFA- Total volatile fatty acid,
[a]S1- Sonicated at 10′ and 60% A; S2- Sonicated at 10′ and 100 %A; S3- Sonicated at 40′ and 60% A,
[†] Data represent arithmetic mean of replicates ± standard deviation of 8 to 30 replicates

4.3.1 Biogas production

A multi factor of variance (ANOVA) was performed to see the effect of digestion temperature (thermophilic and mesophilic), SRT (20, 10 and 5 days) and specific energy input (2042, 4163 and 8153 kJ/kg TS) on specific biogas production for the digesters. The analysis results (Appendix C, Table C.5) indicated that all three factors and their interaction terms had statistically significant effect ($p < 0.05$) on specific biogas production. Among all of the factors, digestion temperature was the most important factor.

Daily specific biogas production for all the digesters (pre-treated and not pre-treated or control) corrected for standard temperature and pressure (STP) of 0^0C and 1 atm., at three different SRTs of 20-d, 15-d and 5-d are presented in Figures 4.9 (a), (b) and (c) respectively. Furthermore, the average specific biogas production (L/g VS_{added}) for each SRT was plotted in Figure 4.10. In general, digesters produced in a range of 0.45 to 0.65 L of biogas per gram VS added at SRTs of 10 and 20 days (OLR of 1.7 – 3.8 g VS/$L_{reactor}$-d)). These results correspond to 1 - 1.3 L biogas per g VS removed at these SRTs which are comparable to established values in literature for the digestion of mixed sludge of 0.8 – 1.1 L of biogas per gram of VS removed (Turovskiy and Mathai, 2006). Due to a much higher, therefore challenging loading at an SRT of 5 days (ORL of 6.5-6.9 g VS/$L_{reactor}$-d), the specific biogas/methane values ranged from 0.08 – 0.4 L of biogas per gram VS added (Figure 4.10). Furthermore, among the eight digesters operated (4 under mesophilic, 4 under thermophilic conditions), mesophilic digesters performed better than thermophilic digesters in terms of biogas production and in terms of process stability. This can be attributed to lower microbial diversity present at elevated thermophilic digester temperatures (55^0C) in comparison to the microbial diversity present at mesophilic digester temperatures (35^0C). Process instability

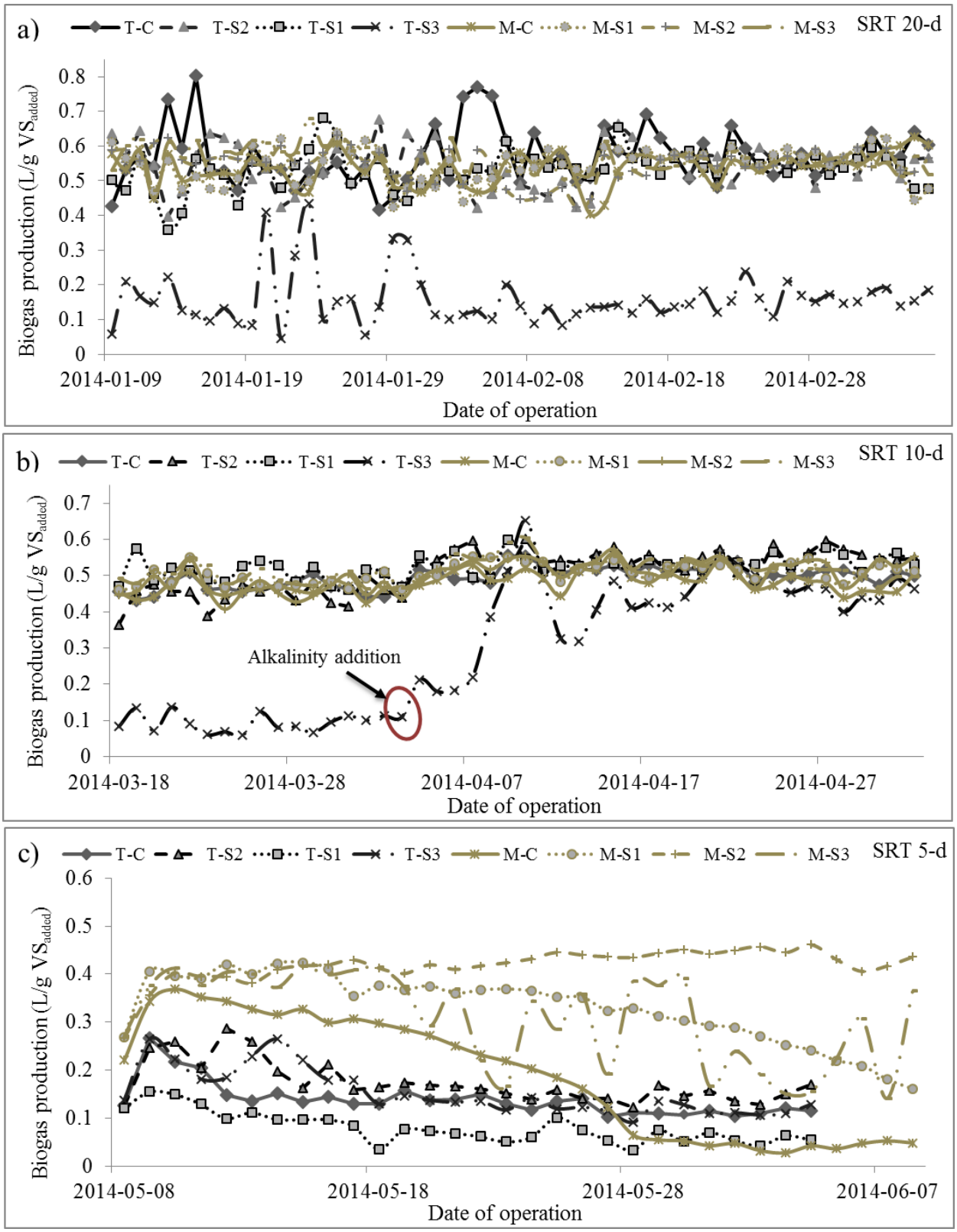


Figure 4.9: Daily specific biogas production from digestion systems during steady state at different SRTs (a. 20-d SRT, b. 10-d SRT and c. 5-d SRT) (T: thermophilic, M: mesophilic, C: control, S1: sonicated at 10′ and 60% A; S2: sonicated at 10′ and 100 % A; S3: sonicated at 40′ and 60% A)

under organic overloading causes VFA accumulation (pH drop) and may reflect false inhibition effects of ultrasound pre-treatment. Furthermore, improvements in biogas production (over the control digesters) as a result of low-energy input sonication was more pronounced for mesophilic digesters rather than thermophilic digesters and only at shorter SRTs (Figure 4.10). Benabdallah E. Hadj et al. (2007) also observed that mesophilic digestion was more influenced in comparison to thermophilic digestion when the digester feed was pre-treated with ultrasound. De la Rubia et al. (2002) described that as hydrolysis of organics is more pronounced at elevated temperatures during thermophilic digestion in control digesters, sludge solubilisation and enhanced hydrolysis (due to sonication pre-treatment) would yield higher improvement on mesophilic digestion performance.

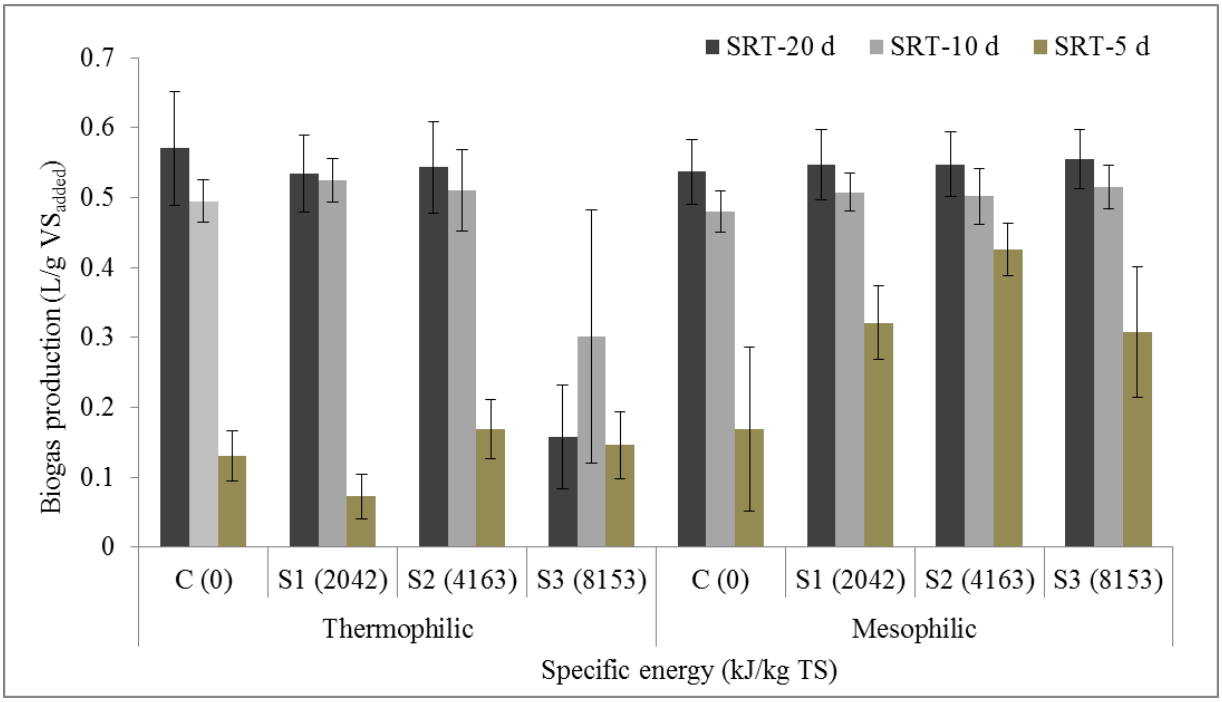


Figure 4.10: Average specific biogas production for all digestion systems during steady state (data represent arithmetic mean and error bars represent standard deviation of 28-65 replicates)

From the Figure 4.10, at an SRT of 20-d the ultrasound pre-treated thermophilic digesters at specific energies of 2042 to 8153 kJ/kg TS did not show any improvement in biogas production

over the control digester (zero specific ultrasound energy). At the shorter SRT of 10-d, pre-treated TS1 (2042 kJ/kg TS) and TS2 (4163 kJ/kg TS) digesters achieved only 6% and 3% higher biogas production over the thermophilic control, respectively. On the other hand, the third thermophilic digester (TS3), sonicated with the highest specific energy (8153 kJ/kg TS) experienced process instability since the start up and through the 20-d SRT, consequently yielding lower daily specific biogas compared to the thermophilic control, TS1 and TS2 digesters. The reasons behind this process upset are currently unknown. There are studies in the literature stating that as pre-treatment intensity increases, the acute inhibition (observed mostly under batch studies) on methane producing cultures increases (Eskicioglu et al., 2007). However, in this study, both thermophilic and mesophilic inocula were acclimated to even more intense ultrasound pre-treatment conditions at specific energy of 11,342 kJ/kg TS. Therefore, it is difficult to conclude that the process instability was due to denaturation of TWAS as a result of ultrasound pre-treatment. Since rest of the digesters (control and pre-treated) were also applied at the same OLR of 1.8 g VS/L/d at a safe SRT of 20 days, an organic overloading outside a typical anaerobic sludge digestion operation (OLRs of 1-4 g VS/L/d) was not the case. When the SRT was reduced to 10 d (OLR of 3.6 g VS/L/d), the pH of the TS3 digester dropped below 7 and external alkalinity addition was applied which resulted in recovery in biogas production (Figure 4.9b).

Similarly, under mesophilic conditions, at the highest SRT of 20-d, sonication led to only 4.5 to 7.8% increase in biogas production over the mesophilic control digester. Similar results were also obtained at the 10-d SRT. These results also match with those obtained from another study by Samaras et al. (2012). In their study, the sonication effect (0.25 W/mL, specific energy of 23,000 kJ/kg) was evaluated on subsequent mesophilic anaerobic digestion utilizing a mixture of primary and secondary sludge at 50:50% volume ratio with 4.18 % TS concentration at 20-d SRT. The

results indicated that ultrasonic pre-treatment had no substantial effect on biogas generation. According to the authors, high organic loading rate of 1.62 g VS/L/d and high percentage of primary sludge (50%) in the mixed sludge digested attributed to these results, which was also a factor in this study with 33% FPS in mixed sludge used as digester feed.

On the other hand, Onyeche et al. (2002) observed high biogas production increase (138%) in mesophilic anaerobic digestion (batch operation mode) fed with sludge of high solid content (5.4% TS). In this study, the input energy was too high compared to the energy produced in the form of biogas and all the systems were proved economically infeasible. These results confirm that it is difficult to make a direct comparison between different studies because of the variation in operational parameters such as specific energy (low vs. high), sludge characteristics (i.e. TS content, PS: WAS volumetric ratio) and digester feeding regime (batch vs. continuous-flow). However, the common conclusion is that at high specific ultrasound energy values, the net energy gain from the systems will be negative.

As expected, during 5-d SRT, biogas production dropped radically for all of the thermophilic digesters as well as the control mesophilic digester due to very high organic loading (OLR of 6.7 g VS/L/d) outside the typical digester operation OLRs of 1-4 g VS/L/d, causing wash out of methane forming microorganisms and reduced time for thorough hydrolysis (Figure 4.9c). It was interesting to observe that between the mesophilic and thermophilic digesters, thermophilic digesters were affected more by the overloading of 6.7 g VS/L/d, resulting in lower biogas productions then the mesophilic digesters (Figures 4.9b and 4.10). In addition to the mesophilic control, the lowest intensity of sonication pre-treatment (2042 kJ/kg TS) sludge fed to the MS1 digester led to accumulation of VFAs (1253 mg/l) and a drop in pH (6.6), in turn inhibiting its

biogas production. At the shortest SRT, the only stable digester with nearly constant biogas production of 0.4 L/g VS added was MS2 fed with sludge that was pre-treated with a specific energy of 4163 kJ/kg TS (Figure 4.9c); MS2 attained a remarkable enhancement of 79.8% in biogas production over the mesophilic control. Although the MS3 digester, fed with sludge pre-treated at specific energy of 8153 kJ/kg TS, exhibited a significant increase of 43.1% in biogas production over the control, yet MS3 never reached a steady state at the shortest SRT (Figure 14.9c). These results are in agreement with other pre-treatment studies reporting that the enhancements in biogas production over the control digesters become more pronounced at shorter SRTs (< 7 days). This is due to the difficulty the control digesters face under high OLRs; as OLRs increase, the retention time of the microorganisms available for completion of hydrolysis decreases and methane conversion is reduced which lowers the biogas production, whereas the pre-treated sludge has less retention time requirement because of the disintegrated sludge particles that do not need to go through the hydrolysis process (Nickel and Neis, 2007).

4.3.2 Biogas composition

Figure 4.11 demonstrates methane content, the main component of biogas, during the steady state period for all digestion systems at different SRTs. At SRTs of 20-d and 10-d, CH_4 content (64-67%) in biogas from the pre-treated and the control digesters did not differ considerably. The only exception was TS3 (8153 kJ/kg TS, 0.14 W/mL). This digester produced relatively lower biogas since its start-up and simultaneously the average methane content was also lower (49%) in its biogas during steady state period of 20-d SRT. But, at 10-d SRT the methane content from this digester started to increase (reaching to 62%), especially when biogas production recovered as a result of the addition of external alkalinity. Among all the digesters, the sonicated mesophilic

digester MS3 (8153 kJ/kg TS, 0.14 W/mL) produced the highest average methane content (67%) during 20-d SRT operation.

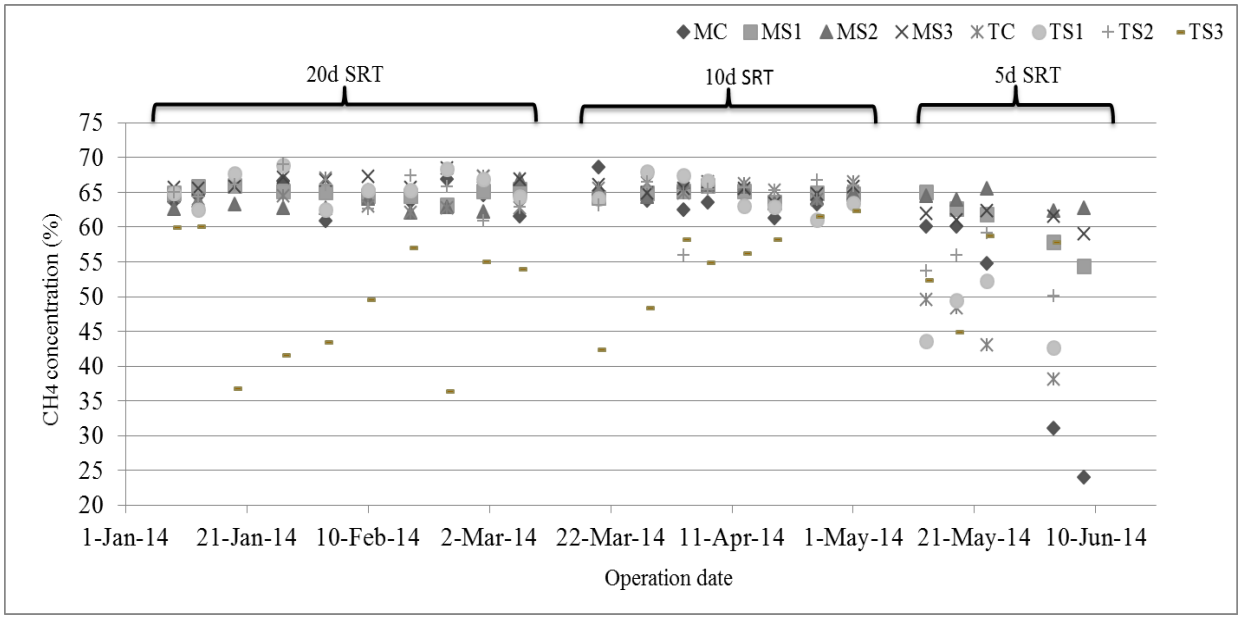


Figure 4.11: Methane composition in biogas generated by digesters (T: thermophilic, M: mesophilic, C: control, S1: sonicated at 10′ and 60% A; S2: sonicated at 10′ and 100 % A; S3: sonicated at 40′ and 60% A)

During 5-d SRT, methane content plunged drastically for all of the thermophilic digesters (ranging from 38-58%) as well as the control mesophilic digester (below 24%) which was in agreement of the biogas production results presented in Figure 4.9c. Among the mesophilic sonicated digesters, the methane content in biogas from MS1 started to drop after 25 days of operation (during steady state). Being the best performing digester at the 5-d SRT, methane content of MS2 was the highest (64%).

4.3.3 Volatile solid removal

The VS, indicating organic solids removal efficiency for all the digestion systems was presented in Figure 4.12. As expected, in general, organic or VS removal efficiency of digesters

increased as SRT was increased due to longer residence time provided for acid and methane forming bacteria to convert VS to biogas. The only exception to this statement was observed with TS3 digesters pre-treated at 8153 kJ/kg TS and experienced process instability at 20-d SRT. At SRTs of 20 and 10 days (OLRs of 1.8-3.6 g VS/L/d), VS removals from mesophilic and thermophilic digesters ranged from 48 to 57%. These results are in agreement with values reported in literature for mixed sludge digestion at similar SRTs (Braguglia et al., 2011). However, when the SRT was reduced to 5-d, under a higher OLR of 6.7 g VS/L/d, all the digesters were challenged and VS removals ranged from 20 to 40% (Figure 4.12). Between the mesophilic and thermophilic digesters, at SRTs of 20 and 10 days, no significant differences were observed with pre-treated thermophilic digesters (TS1 and TS2) achieving slightly higher VS removals. However, at the shortest SRT of 5 days, due to higher instability experienced by thermophilic digesters, as expected from biogas production results (Figure 4.10), mesophilic digesters yielded higher removals (30-42%) than those of thermophilic digesters (20-30%).

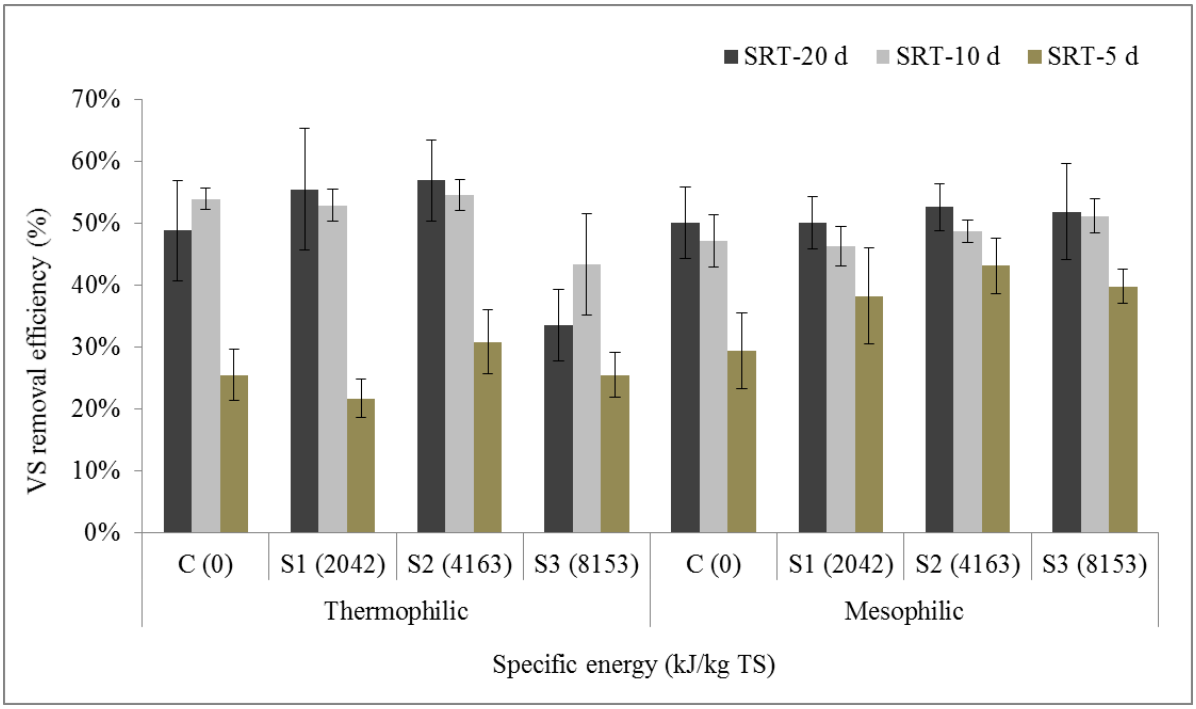


Figure 4.12: VS removal efficiency from all digestion systems during steady state (data represent arithmetic mean and error bars represent standard deviation of 10-26 replicates)

In terms of improvements in VS removal as a result of pre-treatment, similar to biogas production results, at SRTs of 20 and 10 days, the improvements were marginal. At SRT of 20 days, TS1 and TS2 digesters achieved 14 and 17% higher VS removals over the thermophilic control while TS3 yielded less VS removals compared to control due to process instability. On the other hand, at 10-d SRT these digesters did not show any substantial improvement over control. Similarly, under mesophilic conditions, at a SRT of 20-d, the MS2 digester achieved only 5% higher VS removal (over control). These results were in agreement with the results obtained in a prior study by Braguglia et al. (2011). In their study, an 8% improvement in VS removal was observed during semi-continuous mesophilic anaerobic digestion (at 20-d SRT) of full stream sonicated WAS (2.3% TS) at 5000 kJ/kg TS. In another similar study, Samaras et al. (2012) did not observe any substantial difference in VS removal among pre-treated and control digesters. The authors described that the high OLR and high percentage of primary sludge in mixed sludge feed

attributed to these results. In this study, digestion at a 10-d SRT resulted in a similar improvement in solid removal for pre-treated over the control digester. But at the shortest SRT of 5-d, all of the mesophilic pre-treated digesters achieved significant enhancement (30 to 47%) in VS reduction over the mesophilic control. The highest VS removal efficiency of 43% was observed in the digester (MS2) pre-treated at 4163 kJ/kg TS, as expected from the biogas production results (Figure 4.12).

4.3.4 TCOD removal

In addition to VS removal data, TCOD removal data are also useful to assess organic removal efficiency in a digester. Although a significant number of studies were carried out to assess the effect of sonication on biogas generation and solids reduction, only a few articles reported COD removal efficiency in both mesophilic and thermophilic semi-continuous anaerobic digestion following sonication. In Figure 4.13 at different specific ultrasound energies TCOD removal efficiencies were plotted.

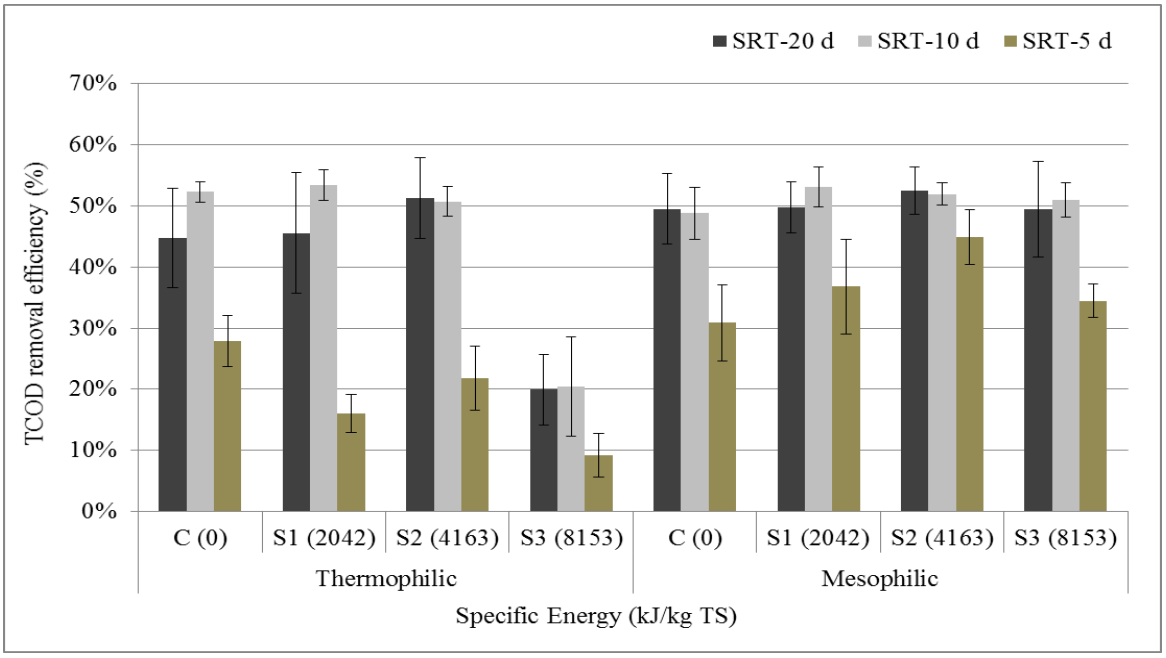


Figure 4.13: TCOD removal efficiency from all digestion systems (data represent arithmetic mean and error bars represent standard deviation of 10-24 replicates)

Similar to the VS removal trend displayed in Figure 4.12, Figure 4.13 revealed that at SRTs of 20 and 10 days, all digesters with the exception of TS3, achieved 44 to 53% TCOD removals. However when the SRT was reduced to 5-d, all digesters were challenged under the high OLR, resulting in much lower TCOD removal efficiencies (9-42%). Between the mesophilic and thermophilic digester groups, the mesophilic digesters achieved higher COD removals over the control than the thermophilic digester achieved (especially at the SRT of 5 days) which was in agreement with the results reported by Benabdallah E. Hadj et al. (2007). The authors studied the effect of sonication (applied at 11,000 kJ/kg TS) on thermophilic (at 15-d SRT) and mesophilic (at 20-d SRT) anaerobic digestion of raw sewage sludge (a mixture of primary and secondary sludge at a ratio of 3:1). They observed 16-21% improvement in COD removals for mesophilic and 5-8% for thermophilic conditions over control.

In this study, similar to biogas production and VS removal results, among all the systems, the mesophilic digester (MS2) fed with sonicated (at 4163 kJ/kg TS) sludge achieved the highest enhancement in TCOD removal by 6-46% over control at all SRTs. The other two mesophilic sonicated systems (MS1 and MS3) achieved 0-19% and 0-12% improvements in comparison with control at 2042 kJ/kg TS and 8153 kJ/kg TS, respectively.

On the other hand, thermophilic digesters did not show any discernable improvement in TCOD removal over control. Only, at 20-d SRT the thermophilic digester, TS2, fed with sonicated sludge at 4163 kJ/kg TS yielded 15% increase in TCOD removal over the conventional digestion system (control). A multi factor of variance (ANOVA) was performed to see the effect of digestion temperature, SRT and specific energy input on TCOD removal efficiency for the digesters. The analysis results indicated that all the three factors of digestion temperature, specific energy input and SRT had statistical significance on TCOD removal ($p < 0.05$) and among these factors, digestion temperature is the most important one (with the highest F value of 51.4). A strong correlation ($p = 0.0001 < .05$) between the two factors of specific energy and digestion temperature was also observed from the results (Appendix C, Table C.6).

4.3.5 Dewaterability

It is established in the literature that aerobic and anaerobic digestion causes a reduction in the dewatering rate of sludge. The rate of dewatering sludge is measured as capillary suction time (CST) and it is found to be several orders of magnitude large in digested sludge in compared to undigested sludge (Novak et al., 2003). This is believed to be due to the presence of biopolymer colloids which are released into the digested solution (with potential to clog filters during CST testing or in the field) (Murthy et al., 2000). In this study, CST results were used to evaluate the

dewaterability of digested sludge with and without ultrasound pre-treatment. As the CST results represent the rate at which a sample releases its water content, a decline of CST value indicates the improvement of the dewaterability (Yu et al., 2008). Figure 4.14 represents the variations of normalized CST (in unit of second per % of TS in the sample by weight) for both mesophilic and thermophilic digested sludge at different applied specific energies.

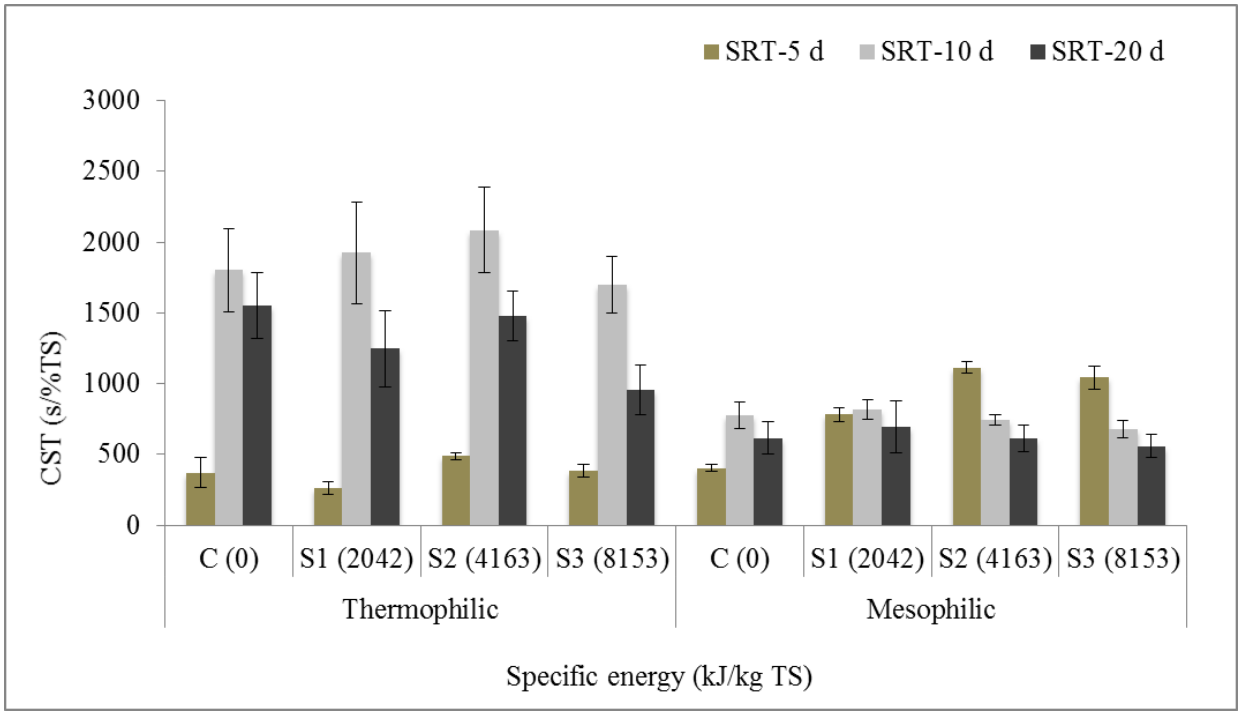


Figure 4.14: Specific capillary suction time for digester effluents during steady state (data represent arithmetic mean and error bars represent standard deviation of 9-15 replicates)

The multi factor ANOVA results (Appendix C, Table C.7) showed that digestion temperature, SRT and ultrasound specific energy including their interaction terms had statistically significant effect ($p < 0.05$) on dewaterability of digested sludge, with digestion temperature being the most significant factor. From the normalized CST values (Figure 4.14), it was also clearly observed that thermophilic digested sludges were slower (900-2200 s/%TS) and therefore more difficult to dewater in comparison with mesophilic digestates (500-1100 s/%TS). The increase of digestion temperature contributes to the rapid release of EPSs into the sludge due to hydrolysis (Novak et

al., 2003), and the higher level of soluble biopolymers (in comparison with the mesophilic digestates) might contribute to the clogging of CST filter paper, resulting in deterioration in dewaterability within the thermophilic digestate.

In terms of the effect of sonication pre-treatment on the digestate dewaterability, no clear trend was observed. The CST results were more dependent on the SRT. At the highest SRT of 20-d, the rate of dewaterability was faster for all thermophilic sonicated digesters over control. The normalized CST values decreased from 1553 s/%TS (thermophilic control) to 1247.5 s/%TS, 1478.8 s/%TS and 956.4376 s/%TS for thermophilic digesters sonicated at specific energies of 2042, 4163 and 8153 kJ/kg TS, respectively. Simultaneously, mesophilic digesters (sonicated at 4163 and 8153 kJ/kg TS) also showed improvements in dewaterability over the mesophilic control at higher SRTs of 20-d and 10-d. Xu et al. (2011) also found the beneficial effect of ultrasound pre-treatment on the dewaterability anaerobic digestate. The authors reported that although immediately after sonication, sludge dewaterability was deteriorated, however succeeding the anaerobic digestion process the dewaterability of the sludge improved. The decrement of proteins in loosely bound fractions likely attributed to the improvement of the dewaterability of pre-treated digested sludge (Xu et al., 2011). Compared to longer SRTs of 20 and 10 days, at the shortest SRT of 5-d, anaerobic digestion was incomplete or partially complete (especially in all the unstable thermophilic digesters as well as mesophilic control) which resulted in lower concentration of biopolymer colloids, therefore much faster dewaterability (200-500 s/%TS).

4.3.6 Fecal coliform counts

According to OMRR (2008) that regulates the land application of biosolids or anaerobic digestate, when fecal coliforms in treated biosolids are less than 1000 MPN per gram of TS (dry

weight basis), biosolids are considered as Class A biosolids. In Class B biosolids, fecal coliform density must be lower than 2 million MPN per gram of TS (on a dry weight basis). Class A biosolids are the most desirable and have the least restrictions, while Class B biosolids have increased restrictions in terms of public access, crop harvesting and animal grazing.

Fecal coliform analysis results are reported as MPN and CFU estimates of the true fecal coliform concentration. CFU is a count of distinguishable bacterial colonies that form on an agar plate or filter disk after filtration and incubation of the diluted sample whereas MPN is a statistical estimate of the numbers of fecal coliforms in a sample. CFU values are generally more conservative and less variable than MPN (Gronewold and Wolpert 2008).

Ultrasound is a pressure as well as acoustic wave that transmits through a medium with huge energy dissipation. It is subjected to sludge prior to digestion to achieve extremely high pressures and temperatures. This results in collapse of gas and vapor bubbles at high velocity and produces shear forces that break up the floc structures; bacterial cells are disinfected acceptably. The bulk temperature rise during sonication also contributes to inactivation of bacteria (Chiu et al., 1997). But cell lysis occurs only after a certain period of sonication (Jorand et al., 1995). Chu et al (2001) also explored that there is a critical sonication power level above which floc disintegration happens effectively.

A multi factor ANOVA was performed to see the effect of digestion temperature, SRT and specific energy on fecal coliform densities reduction. The analysis (Appendix C, Table C.8) indicated that all the factors had statistical significance ($p < 0.05$) on fecal coliform counts. The interaction of the factors was also found to be statistically significant with the exception of the interaction between temperature and specific energy. Digestion temperature was the most

significant factor (with an F value of 43.92) and it was also observed in Figure 4.15. The figure presents the fecal coliform densities in effluents for all anaerobic digestion systems. Thermophilic digestion at 55^0C was found to be more efficient in killing pathogens than mesophilic digestion at 35^0C. This result is expected and supports previous studies. The thermophilic system at a specific energy of 4163 kJ/kg TS showed the best performance (below detection limit of the method) as it produced Class A biosolids at all SRTs (Figure 4.15) in terms of fecal coliform content. The other thermophilic digestion systems also produced Class A biosolids with an exception of the shortest SRT of 5 days. From Figure 4.15, it was also observed that as the OLR increased as a result of reduction in the SRT, fecal coliform densities in effluents increased due to partially complete digestion. This inadequate digestion consequently attributed to higher fecal coliforms in the digested sludge.

Among mesophilic digesters, similar to thermophilic digesters, fecal coliform concentrations increased with a decrease in digester SRT. The only exception was with the mesophilic control digester. As reported earlier, as soon as the SRT was reduced to 5 days, a steady decline in pH (in addition to high VFA accumulation and low biogas generation) was observed in the mesophilic control digester. The digester was no longer stable and the acidic conditions were also not favorable for the survival of the coliforms. As a consequence, the fecal coliform population in digested sludge also dropped significantly. All mesophilic digestion systems produced Class B biosolids (Figure 4.15).

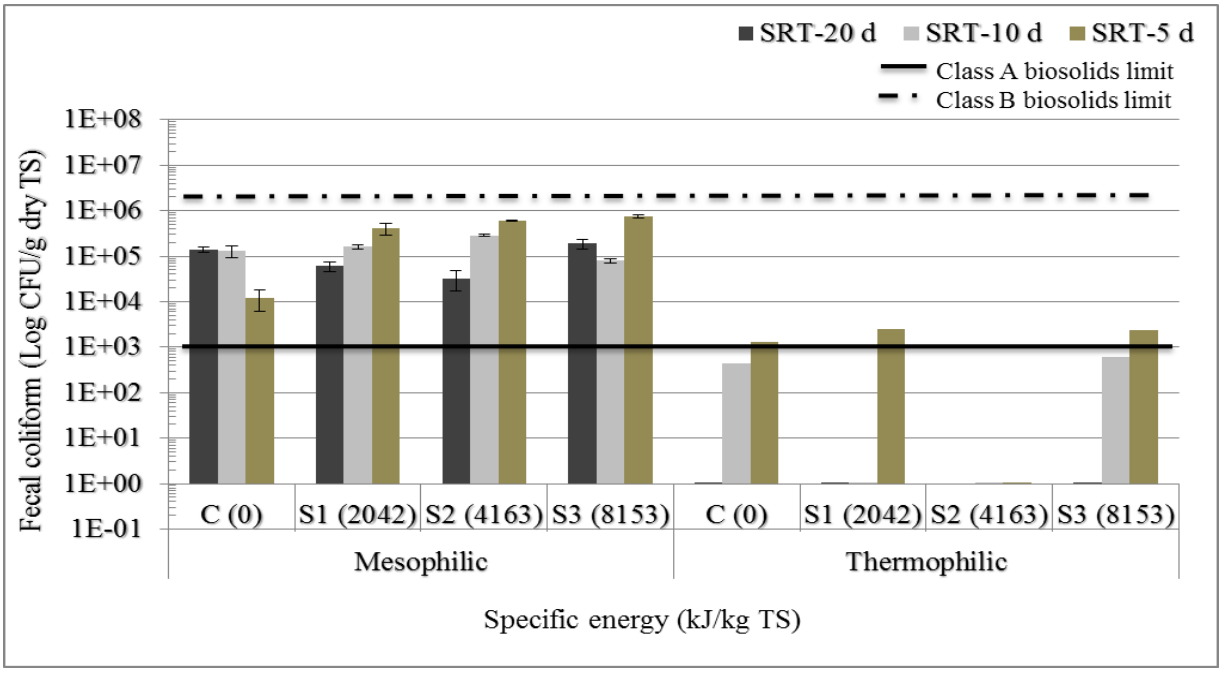


Figure 4.15: Fecal coliform densities in digested effluents (data represent arithmetic mean and error bars represent standard deviation of 4-10 replicates)

In terms of the effect of ultrasound pre-treatment on fecal coliforms of digested biosolids, there was not a clear trend. For example, at the highest SRT of 20-d, the pre-treated systems (at 2042 and 4163 kJ/kg TS) achieved 57% and 77% reduction in coliforms, respectively over the mesophilic control. But at the lower SRTs, the digesters did not show any significant improvements in pathogen removal over the control. Rather, for some operating conditions (MS2, MS3), the fecal coliforms counts were higher in sonicated effluents than those in the un-pre-treated effluent. Trouqué and Forster (2002) also did not observe any significant improvement of sonication pre-treatment on fecal coliform reduction in mesophilic digested effluent. Although in their research, sonication duration was much shorter (90 sec) than the durations used in this project, it is important to note that fecal coliform concentration in sonicated influent reported by the researchers was lower than in the control feed. After mesophilic digestion, the control digester produced relatively lower fecal coliform counts (Trouqué and Forster, 2002). As mentioned previously, cell lysis would occur only after sludge is exposed to a minimum period of sonication.

Thus, low power sonication and the bulk temperature control in pre-treated sludge are likely to attribute to insignificant effects of ultrasound on fecal coliform removal efficiency (for pre-treated digester effluents) under mesophilic conditions.

4.3.7 Volatile sulfur compounds

In this research, a total of eight odor-causing compounds including hydrogen sulphide, methyl mercaptan, ethyl mercaptan, dimethyl sulphide, carbon disulphide, ethyl methyl sulfide, 1-propanehiol and dimethyl disulphide in biogas from the anaerobic digesters were measured and Figure 4.16 presents the total VSC (TVSC) emissions (summation of eight compounds) at different SRTs under mesophilic and thermophilic digester temperatures.

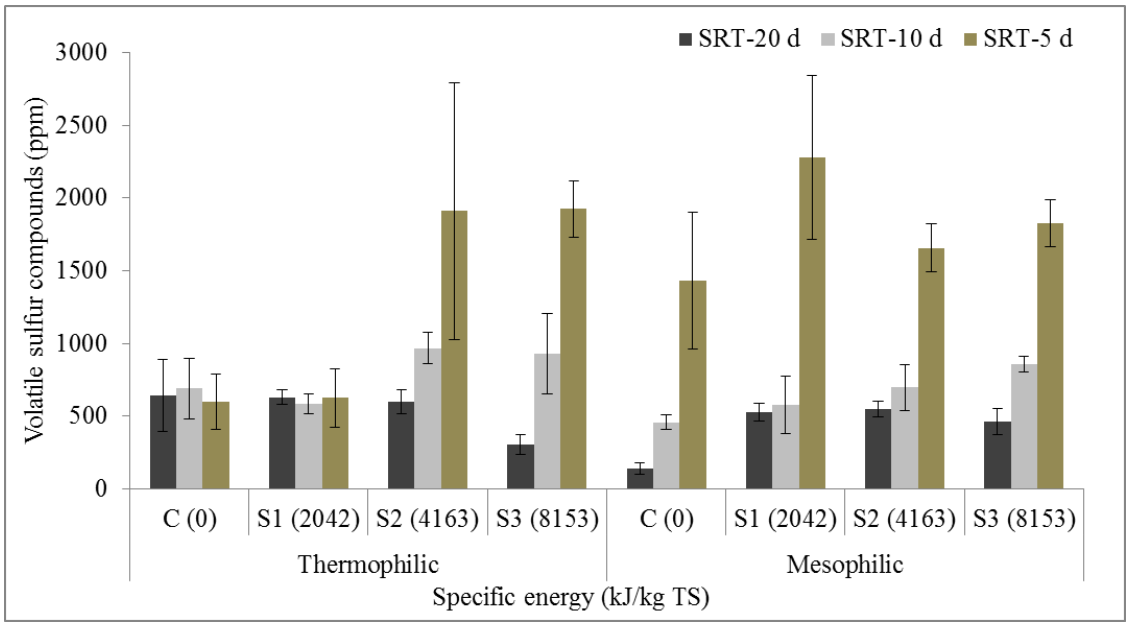


Figure 4.16: Total volatile sulfur compounds in headspace of all the digestion systems (data represent arithmetic mean and error bars represent standard deviation of 7-10 replicates)

From the multi factor ANOVA (Appendix C, Table C.9), it was observed that digestion temperature and SRT had statistically significant effect on VSC removal efficiency, whereas specific energy input was found statistically insignificant in this study at 95% confidence limits.

The analysis also revealed that all the interaction terms of the factors including temperature, specific energy and SRT had statistical significance ($p < 0.05$) on VSC emissions in the headspace of the digestion systems. From the ANOVA results, it was confirmed that SRT was the most significant individual factor. From the figure it is also observed that at the shortest SRT of 5 days, as a result of much higher organic loading rates necessitating more protein degradations, TVSC concentrations in the headspace of all mesophilic and some of the pre-treated thermophilic digesters increased. In general, with the increase of SRT, VSC emissions decreased which were in agreement with the findings stated by Higgins et al. (2008b).

The results also suggested that TVSC emissions between the pre-treated and non-pre-treated digestion systems did not follow any specific trend. Under the studied conditions, no improvement was observed in TVSC removal in all mesophilic sonicated (at all SRTs) and thermophilic sonicated (at 10-d and 5-d SRTs) systems over the controls. It is important to mention that at these sonication conditions (prior to anaerobic digestion) protein solubilisation was lower than that of control. Besides, only at the SRT of 20-d, thermophilic sonication systems showed better performance in reducing odor compounds from biogas over the control. This was likely due to higher protein destruction under thermophilic conditions (Higgins et al., 2006). It is reported that degradation of protein components like the sulfur-containing amino acids (specifically methionine and cysteine) is the main cause of odorous VSCs in biogas (Higgins et al., 2008a). Unfortunately, there are limited studies performed on odor generation in digesters following sonication pre-treatment. Thus, further investigation is essential on destruction of various protein fractions (specially bound protein) during the digestion process.

4.4 Energy Feasibility Study

An energy balance was performed to evaluate the effect of sonication intensity on energy requirement of anaerobic digestion of municipal sludge. Figure 4.17 displays a typical energy balance over a digester with ultrasonic treatment of sludge feed. Specific energy (H_1 in units of kJ/kg TS) was calculated using equation (1) below,

$$H_1 = \frac{E}{V \times TS} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, E = Amount of acoustic energy delivered to probe of sonicator (J)

V = Sample volume (L)

TS = Total solids concentration of sample pre-treated (g/L)

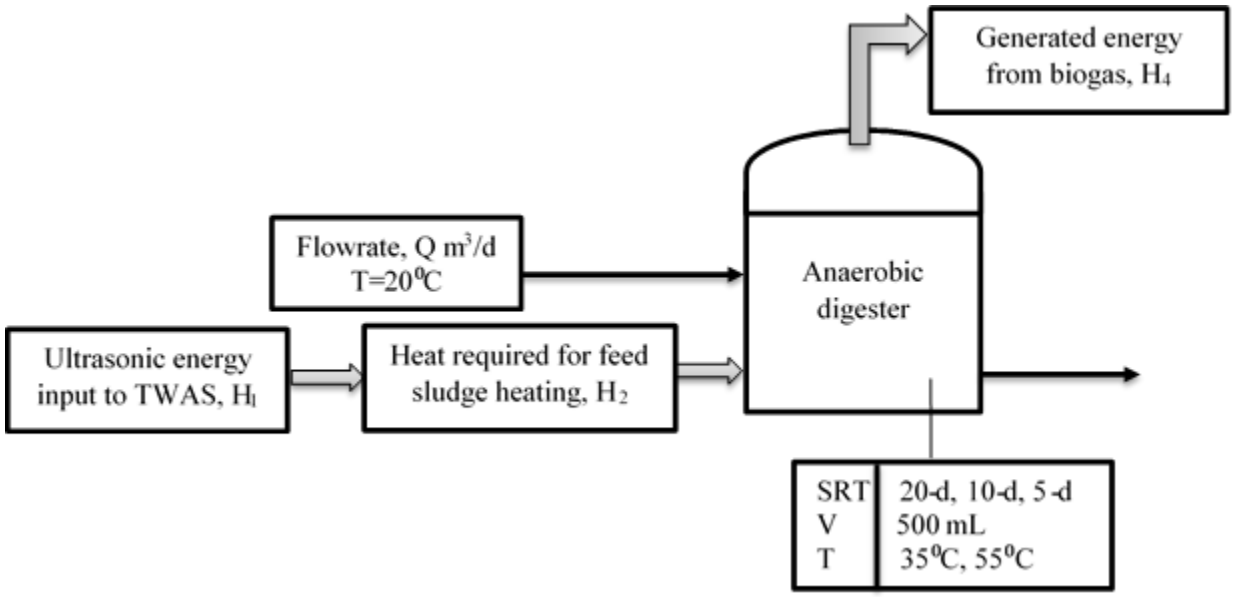


Figure 4.17: Energy balance over a digester fed with sonicated sludge

In the energy balance, the other energy requirements are associated with energy demand for sludge heating to digestion temperature and ambient heat losses from digester. During

ultrasonication of TWAS, the temperature of sludge increased to around 30^0C and then sonicated TWAS was mixed with FPS kept at room temperature. Therefore, instead of room temperature (20^0C), initial temperature of mixed sludge was calculated as 27^0C at 33:67% FPS: TWAS volumetric ratio. Assuming the heat capacity of feed sludge is equal to water due to high water content (96%) of mixed sludge samples (Braguglia et al., 2011), the energy requirement for sludge heating was calculated from the following equation (2),

$$H_2 \text{ (kJ/d)} = m \times S \times (T-T_0) \quad (2)$$

Where, m = mass of feed (mixed sludge) (kg/d)

S = Specific heat capacity of feed (4.18 kJ/kg/K)

T_0 = Initial mixed sludge temperature (27^0C)

T = Digestion temperature (35^0C and 55^0C for mesophilic and thermophilic conditions, respectively)

Ambient heat lost to surroundings was calculated by assuming that anaerobic digestion temperature had undergone a drop of 0.56^0C (1^0F) per day (Braguglia et al., 2011) and therefore,

$$H_3 \text{ (kJ/d)} = 4.184 \times V_{digester} \times 0.56 \quad (3)$$

Where, V = Volume of digester (L)

All the heat energies required for the system were normalized on basis of feed TS content for reproducibility of these results by other researchers.

The normalized energy production (kJ/kg TS_{fed}) was calculated using the methane content (varying from 60-68%) in biogas generated from both control and pre-treated sludge digestion. Methane within biogas has an inferior calorific power (ICP) of 37 MJ/m^3 (Droste, 1997), thus the energy from biogas is,

$$H_4 \text{ (kJ/d)} = V_{biogas} \times \%CH_4 \times ICP \text{ (kJ/m}^3\text{)} \quad (4)$$

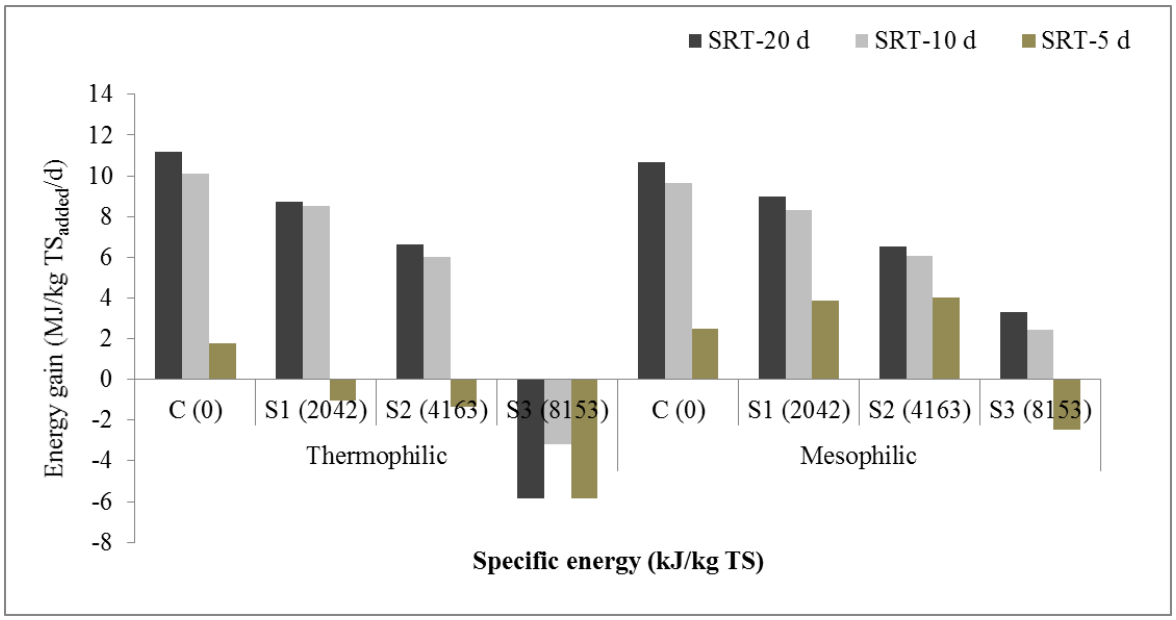


Figure 4.18: Net energy gain from digestion systems with sludge feed (4~4.6% TS)

From Figure 4.18, it can be clearly said that the energy produced from biogas with both sonicated and untreated sludge will be sufficient to meet the energy requirement for all the systems studied in this experiment at 20-d and 10-d SRTs (with an exception for thermophilic sonicated, TS3). However, under both mesophilic and thermophilic conditions and at all SRTs, control digesters yielded the highest net energy production (Figure 4.18) indicating that there was no advantage from an energy point of view to apply sonication. At a SRT of 5-d, all thermophilic sonicated and MS3 generated negative energy balance due to low amount of biogas generation as a result of process instability and higher energy input requirement to heat the digesters (Appendix D, Table D.4).

On the other hand, the performance of TS3 was inhibited from the start-up period, and consequently it resulted in a negative energy balance due to low biogas generation. In this study, the high feed sludge concentration (4-4.6% TS) contributed to positive energy balance by reducing the energy requirement to heat up the sludge to incubation temperature, as is also in concert with

a previous study report (Nickel et al, 1999). Barber (2002) observed that thickening of digester feed to 7% dry solids (DS) led to an increase in energy production by three fold compared with that from a sludge of only 3% TS. Mannheim, a full-scale WWTP digesting feed sludge of 10% TS, also experienced an increase of 35% in biogas generation preceding ultrasound pre-treatment. Therefore, from an economic point of view, positive energy gain is achievable by applying sonication to highly concentrated sludge (i.e. > 7% TS) at the full-scale, which is challenging to simulate at the laboratory scale due to limitation with the ultrasound probe.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this research, ultrasound pre-treatment was applied to TWAS (3.9%~4.0% TS) at very low power levels to see the effect on subsequent anaerobic digestion performance under mesophilic and thermophilic conditions. Firstly, the impact of sonication time and specific energy input on sludge solubilisation was examined to optimize the operating conditions. Secondly, based on solubilisation results, the analysis of the anaerobic digestion processes of untreated and pre-treated TWAS mixed with FPS in semi-continuous mode at three different SRTs was accomplished. The following conclusions have been inferred from all the research work:

Both longer sonication time and higher ultrasonic density had profound impact on effective sludge solubilisation. At 0.25 w/mL density, specific energy of 11,343 kJ/kg TS, and 30 min sonication the maximum solubilisation of COD, protein and sugar was observed with values of 25%, 47% and 23%, respectively. The utmost particle size reduction (51.7 μm to 30.31 μm) was also achieved at this condition. However, taking the cost efficacy into consideration three other sonication options that were also successful in disintegrating TWAS at lower specific energies of 2042, 4063, 8153 kJ/kg TS were selected as pre-treatment for the pre-treatment of sludge used to study the anaerobic digestion process.

a) At higher SRTs, the pre-treated digesters did not show any significant improvement in specific biogas production over control. However, mesophilic digestion systems fed with pre-treated sludge performed better than thermophilic sonicated digesters due to higher level of tolerance to denatured organics by pre-treatment that may contribute to inhibition and high organic loadings. At a 5-d SRT, mesophilic sonicated (at 4063 kJ/kg TS) was the

only stable digester which showed a remarkable increase (around 80%) in biogas generation over control. This also indicates that sonication aids in reducing the retention time required for complete anaerobic digestion to occur under mesophilic conditions.

b) Regarding organic removals, as SRT decreased, relative removal efficiency (in comparison with control) increased. At higher SRTs of 20-d and 10-d, the TCOD removal efficiencies were nearly the same and not significantly improved for mesophilic pre-treated reactors in comparison with control. But at 5-d SRT, the mesophilic digesters utilizing pre-treated sludge yielded the highest COD and VS removals. Among all the digestion systems, mesophilic pre-treated reactors showed the best performance (47%) in organic removals.

c) At low power sonication treatment (2042 to 8153 kJ/kg TS), fecal coliform reduction efficiency of mesophilic digested sludge was relatively lower over control. Besides, under the studied conditions, ultrasound application did not show any substantial positive effect on VSCs reduction from the digestion systems. But at a higher SRT of 20-d, the performance of thermophilic sonicated digesters in terms of VSCs removal efficiency were better in reducing odorous compounds from the biogas.

d) In this study, sonication had a positive impact on the dewaterability of anaerobic digestate at higher SRTs. In all mesophilic sonicated digesters, lower CST values were obtained at SRTs of 20-d and 10-d. Improved dewaterability was observed at 20-d SRT under thermophilic pre-treated conditions.

e) Based on energy feasibility study, at 20-d and 10-d SRTs all the digestion systems were cost effective. Only the thermophilic digester fed with sonicated sludge at 8153 kJ/kg TS generated negative energy balance at all SRTs as it had been experiencing a ‘sour’ condition since its start-up. At 5-d SRT, net energy generation from all digesters declined.

From overall results, it is obvious that among all the digestion systems mesophilic sonicated (at 4163 kJ/kg TS) digestion exhibited the paramount performance especially at shortest SRT of 5-d. Since long SRTs (i.e. >15-20 days) requiring large digester volumes is a bottleneck that limits the wide use of anaerobic digestion, this mesophilic sonicated digestion would be an option for sludge stabilization at the full scale application that needs to operate under higher organic loadings rates than the typical loadings (1-4 g VS/L/d). However, considering the fact that most full scale

digesters prefer an operation with a safety factor, an SRT of 5 days would be a risky loading rate for a long term process stability, which needs to be verified at a larger scale ultrasound pre-treated anaerobic digester. These results indicate that at longer (safer) SRTs 10 and 20 days, low power sonication pre-treatment (2042 to 8153 kJ/kg TS) do not represent substantial benefits in terms of organic removals, biogas production, fecal coliform destruction or enhancement in dewaterability.

5.2 Recommendations for further work

Based on the results of this study, some recommendations are offered for further investigation:

a. Considering equipment efficiency, the working scale, operation and maintenance cost, a comprehensive economic sustainability study is necessary. Since, the efficiency of a laboratory device used in this study was significantly low, reckoning of the experimental outcomes (obtained at lab scale) to full scale is necessary.
b. Ultrasound pre-treatment might be incorporated with temperature phased anaerobic digestion (TPAD) which has a thermophilic acid phase and a mesophilic methane phase digester as it may offer better anaerobic digestion performance in terms of improved biogas production as well as organic removal efficiency based on previous studies.
c. Since degradation of protein components like the sulfur-containing amino acids (specifically methionine and cysteine) is the main cause of odorous VSCs in biogas, further research should be conducted to investigate the effect of protein (especially bound proteins) on sulfur bearing odorous compounds followed by anaerobic digestion.

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APPENDICES

Appendix A: Calibration curves

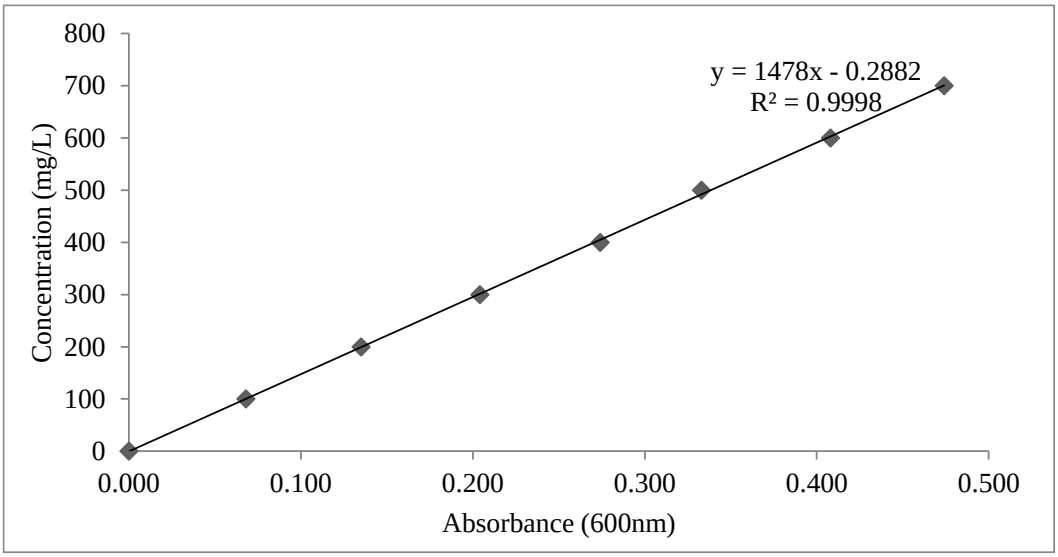

Figure A.1: Calibration curve for COD determination

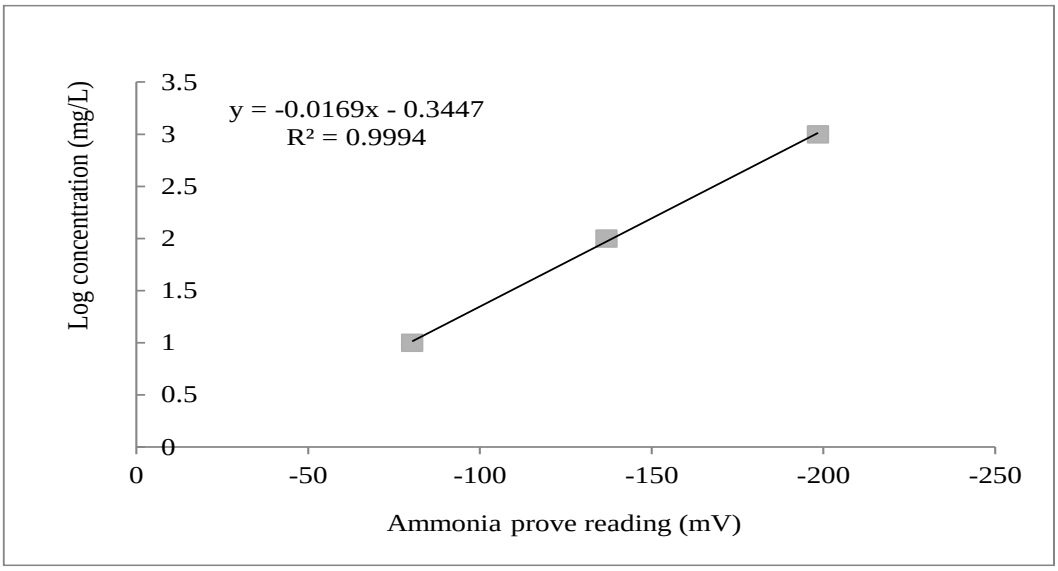

Figure A.2: Calibration curve for ammonia concentration determination

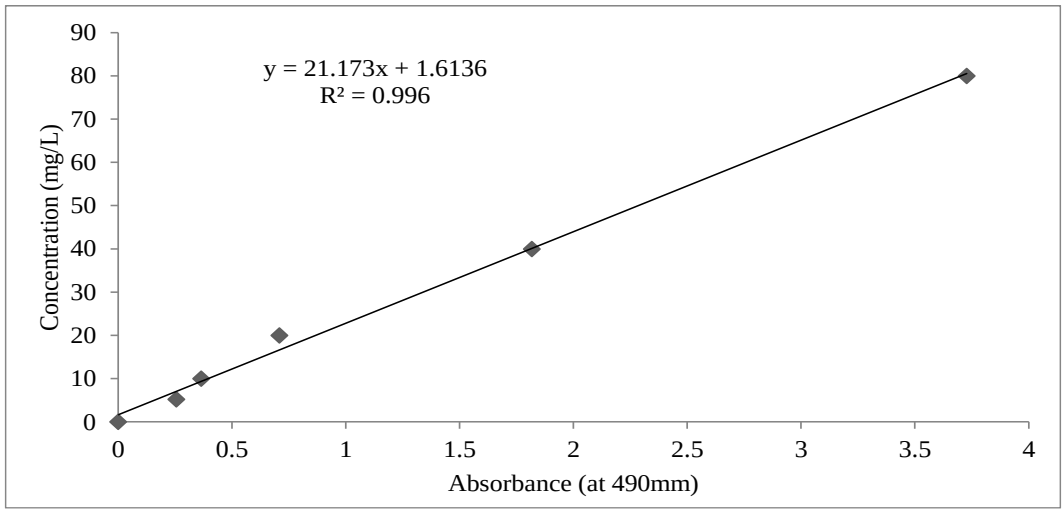

Figure A.3: Calibration curve for sugar concentration determination

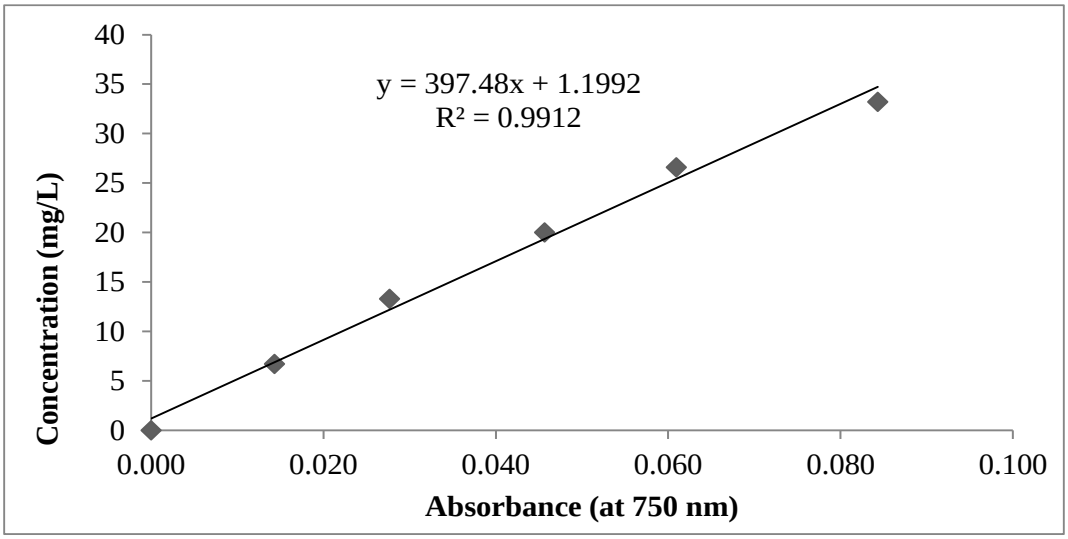

Figure A.4: Calibration curve for protein concentration determination

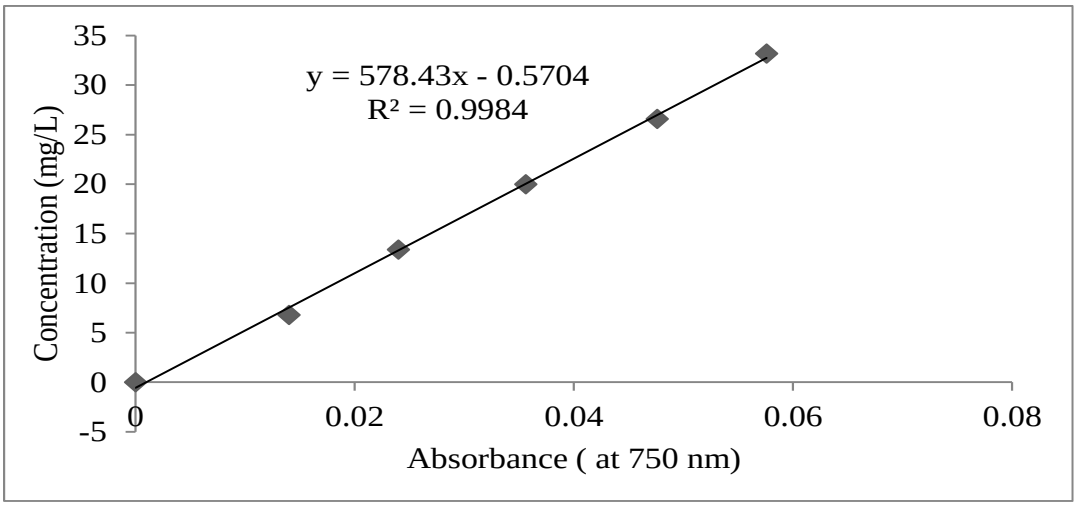

Figure A.5: Calibration curve for humic acid concentration determination

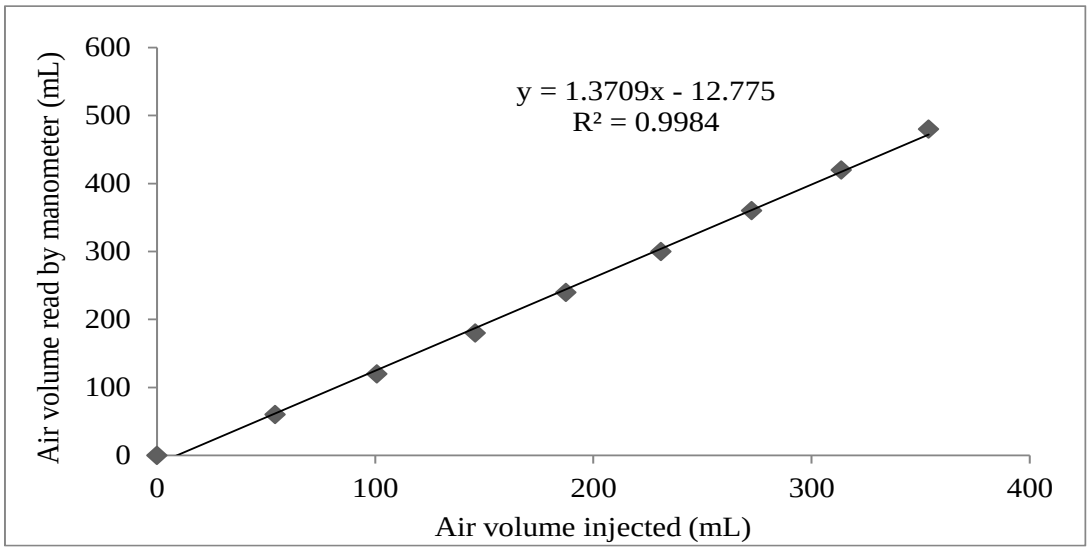

Figure A.6: Calibration curve for biogas measurement by manometer (at STP)

Appendix B: Reagents for protein determination and calculation

B1. Reagents for protein determination

- A: 20g Na_2CO_3 in 1000 mL of NaOH 0.1N
- B1: 0.25 g $CuSO_4$ in 50 mL distilled water
- B1: 0.5 g Tartrate K, Na in 50 mL distilled water (DW)
- C1: 48 mL of A+1 mL B1+1 mL B2
- C1: 48 mL of A+1 mL DW+1 mL B2
- Stock solution-1: 20 mg BSA in 100 mL DW (final conc.= 0.2 g/l)
- Stock solution-2: 20 mg humic acids in 100 mL DW (final conc.= 0.2 g/l)

B2. Calculation

Proteins concentrations are calculated according to the equations developed by Frølund et al. (1995)

$$A_{total} = A_{Protenes} + A_{humiques} \quad (1)$$

$$A_{blanc} = 0.2\ A_{Protenes} + A_{humiques} \quad (2)$$

$$A_{Protenes} = 1.25\ (A_{total} - A_{blanc}) \quad (3)$$

$$A_{humiques} = A_{blanc} - 0.2\ A_{Protenes} \quad (4)$$

Where,

A_{total} = Absorbance total with $CuSO_4$

A_{blanc} = Absorbance total without $CuSO_4$

$A_{Protene}$ = Proteins absorbance

$A_{humiques}$ = Humic acid absorbance

Appendix C: Results of analysis of variance (ANOVA)

Table C.1: Two way analysis of variance for COD solubilisation

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	0.23	14	0.016	130.17	< 0.0001	significant
A-Sonication duration	5.41E-07	1	5.41E-07	4.28E-03	0.9481	
B-Ultrasonic density	6.43E-04	1	6.43E-04	5.09	0.0284	
AB	1.85E-03	1	1.85E-03	14.63	0.0004	
Residual	6.45E-03	51	1.26E-04			
Lack of Fit	4.43E-03	6	7.38E-04	16.45	< 0.0001	significant
Pure Error	2.02E-03	45	4.49E-05			
Corrected Total	0.24	65				

Table C.2: Two way analysis of variance for protein solubilisation

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	0.73	14	0.052	38.76	< 0.0001	significant
A-Sonication duration	5.886E-004	1	5.886E-004	0.44	0.5103	
B-Ultrasonic density	1.193E-003	1	1.193E-003	0.89	0.3496	
AB	0.039	1	0.039	29.29	< 0.0001	
Residual	0.068	51	1.339E-003			
Lack of Fit	0.044	6	7.258E-003	13.20	< 0.0001	significant
Pure Error	0.025	45	5.497E-004			
Corrected Total	0.79	65				

Table C.3: Two way analysis of variance for relative increase in sugar solubilisation over control

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	94.36	9	10.48	16.95	< 0.0001	significant
A-Sonication duration	26.24	1	26.24	42.44	< 0.0001	
B-Ultrasonic density	14.29	1	14.29	23.11	< 0.0001	
AB	7.42	1	7.42	11.99	0.0011	
Residual	30.92	50	0.62			
Lack of Fit	24.64	10	2.46	15.68	< 0.0001	significant
Pure Error	6.28	40	0.16			
Corrected Total	125.29	59				

Table C.4: Multi ANOVA results for VS removal from anaerobic digesters

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	0.90	6	0.15	18.08	< 0.0001	significant
A- Specific Energy	0.01	1	0.013	1.62	0.2043	
B- Digestion temperature	0.05	1	0.046	5.62	0.018	
C-SRT	0.52	1	0.52	62.99	<0.0001	
AB	0.18	1	0.18	21.31	<0.0001	
AC	0.003	1	0.068	8.19	0.0046	
BC		1	0.003	0.4	0.5262	
Residual	1.74	210	0.008			
Lack of Fit	1.06	17	0.062	17.73	< 0.0001	significant
Pure Error	0.68	193	0.003			
Corrected Total	2.63	216				

Table C.5: Multi ANOVA results for specific biogas production for anaerobic digestion systems

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	15.78	6	2.63	196.04	< 0.0001	Significant
A- Digestion temperature	3.41	1	3.41	254.17	< 0.0001	
B-Specific Energy	0.99	1	0.99	73.87	< 0.0001	
C-SRT	1.14	1	1.14	85.24	< 0.0001	
AB	2.00	1	2.00	149.00	< 0.0001	
AC	0.078	1	0.078	5.78	0.0164	
BC	1.46	1	1.46	108.54	< 0.0001	
Residual	14.45	1077	0.013			
Lack of Fit	9.65	16	0.60	133.32	< 0.0001	Significant
Pure Error	4.80	1061	4.523E-003			
Corrected Total	30.22	1083				

Table C.6: Multi ANOVA results for TCOD removal efficiency for anaerobic digested sludges

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	1.38	6	0.23	22.3	< 0.0001	Significant
A- Specific energy	0.27	1	0.28	26.6	< 0.0001	
B- Digestion temperature	0.53	1	0.53	51.4	< 0.0001	
C-SRT	0.38	1	0.38	36.7	< 0.0001	
AB	0.34	1	0.34	32.95	< 0.0001	
AC	0.00	1	0.00	0.12	0.71	
BC	0.01	1	0.01	1.05	0.320	
Residual	1.36	132	0.01			
Lack of Fit	0.92	17	0.05	14.29	< 0.0001	significant
Pure Error	0.44	115	3.8E-03			
Corrected Total	2.75	138				

Table C.7: Multi ANOVA results for dewaterability for anaerobic digested sludges

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	2.645E+007	6	4.409E+006	26.18	< 0.0001	Significant
A-SRT	2.425E+006	1	2.425E+006	14.40	0.0002	
B- Digestion temperature	9.384E+006	1	9.384E+006	55.72	< 0.0001	
C-Specific Energy	9.237E+005	1	9.237E+005	5.48	0.0201	
AB	3.674E+006	1	3.674E+006	21.82	< 0.0001	
AC	1.681E+006	1	1.681E+006	9.98	0.0018	
BC	8.814E+005	1	8.814E+005	5.23	0.0232	
Residual	3.453E+007	205	1.684E+005			
Lack of Fit	2.893E+007	17	1.702E+006	57.12	< 0.0001	Significant
Pure Error	5.600E+006	188	29788.82			
Corrected Total	6.098E+007	211				

Table C.8: Multi ANOVA results for fecal coliform counts in digester effluents

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	1.19E+12	6	1.99E+11	13.46	< 0.0001	significant
A-Digestion temperature	6.48E+11	1	6.48E+11	43.92	< 0.0001	
B-Specific energy	2.18E+11	1	2.18E+11	14.75	0.0004	
C-SRT	4.70E+11	1	4.70E+11	31.88	< 0.0001	
AB	5.52E+10	1	5.52E+10	3.74	0.0595	
AC	1.56E+11	1	1.56E+11	10.57	0.0022	
BC	1.17E+11	1	1.17E+11	7.91	0.0073	
Residual	6.64E+11	45	1.48E+10			
Lack of Fit	6.03E+11	17	3.55E+10	16.28	< 0.0001	significant
Pure Error	6.10E+10	28	2.18E+09			
Corrected Total	1.86E+12	51	1.99E+11		< 0.0001	

Table C.9: Multi ANOVA results for volatile sulfur compounds emissions for anaerobic digestion systems

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value Prob > F	
Model	3.281E+007	6	5.468E+006	31.47	< 0.0001	Significant
A-Temperature	1.716E+006	1	1.716E+006	9.88	0.0020	
B-Specific Energy	1.186E+005	1	1.186E+005	0.68	0.4099	
C-SRT	1.683E+007	1	1.683E+007	96.88	< 0.0001	
AB	7.607E+005	1	7.607E+005	4.38	0.0379	
AC	3.204E+006	1	3.204E+006	18.44	< 0.0001	
BC	2.200E+006	1	2.200E+006	12.66	0.0005	
Residual	3.006E+007	173	1.737E+005			
Lack of Fit	1.929E+007	17	1.135E+006	16.45	< 0.0001	significant
Pure Error	1.076E+007	156	68981.41			
Corrected Total	6.287E+007	179				

Appendix D: Steady state results and energy balance

Table D.1: Steady state results for actual digesters at 20-d SRT

Parameter	Thermophilic				Mesophilic			
	Control	TS1	TS2	TS3	Control	MS1	MS2	MS3
OLR (g VS/L/d)	1.81 (0.07, 16)	1.83 (0.12, 16)	1.82 (0.13, 16)	1.80 (0.05, 16)	1.81 (0.07, 16)	1.83 (0.12, 16)	1.82 (0.13, 16)	1.80 (0.05, 16)
OLR (TCOD/L/d)	2.97 (0.29, 12)	2.9 (0.30, 12)	2.92 (0.38, 12)	2.92 (0.33, 12)	2.97 (0.29, 12)	2.9 (0.30, 12)	2.92 (0.38, 12)	2.92 (0.33, 12)
Removal Efficiency								
VS (%)	49 (0.06,26)	55.5 (0.03,26)	56.93 (0.05,26)	33.5 (0.159,26)	50.18 (0.03,26)	50.18 (0.04,26)	52.64 (0.08,26)	51.9 (0.04,26)
TS (%)	41 (0.07,28)	44.97 (0.06,28)	48.43 (0.05,28)	22.73 (0.12,28)	41.5 (0.05,28)	38.7 (0.05,28)	44.37 (0.08,28)	41.4 (0.06,28)
TCOD (%)	45 (0.08, 24)	46 (0.09, 24)	51 (0.07, 24)	20 (0.06, 24)	50 (0.06, 24)	50 (0.04, 24)	53 (0.04, 24)	50 (0.08, 24)
Biogas Production								
Specific Biogas Production (L/g VS added-d)	0.57 (0.08, 65)	0.53 (0.05, 65)	0.54 (0.06, 65)	0.157 (0.04, 65)	0.54 (0.05, 65)	0.55 (0.05, 65)	0.55 (0.05, 65)	0.56 (0.04, 65)
TVSC emissions (mg/L)	639.9 (249.15, 10)	628.26 (50.97, 10)	598.86 (82.5, 10)	303.64 (67.7, 10)	139 (38.8, 10)	524.2 (61.7, 10)	548.14 (53.8, 10)	460.7 (88, 10)
CH4 (%)	64.25 (2.14, 10)	65.7 (1.85, 10)	65.06 (2.3, 10)	49.4 (7.25, 10)	64.48 (2.09, 10)	65 (0.73, 10)	63.4 (21.64, 10)	67.72 (0.85, 10)
Effluent Characteristics								
pH(-)	7.58 (0.15, 34)	7.76 (0.02, 34)	7.72 (0.04, 34)	7.30 (0.05, 34)	7.25 (0.04, 34)	7.27 (0.03, 34)	7.28 (0.04, 34)	7.29 (0.02, 34)
NH3-N (mg/L)	2128 (495,8)	2044 (270,8)	2067 (372,8)	2175 (385,8)	1439 (337,8)	1607 (466,8)	1684 (483,8)	1795 (401,8)
Alkalinity (mg $CaCO_3$/L)	4753 (447,8)	5307 (282,8)	5438 (261,8)	4471 (130,8)	4399 (167,8)	4737 (163,8)	4754 (168,8)	4792 (109,8)
TVFA (mg/L)	1677.5 (1118,12)	319.5 (124.3,12)	774 (270,12)	2411 (106,12)	22.07 (11.6,12)	18.4 (6.3,12)	15.3 (6.6,12)	23.34 (24.52,12)
CST (s/%TS)	1553 (232,15)	1247 (271,15)	1478 (176,15)	956 (172,15)	615 (115,15)	693 (181,15)	613.5 (96,15)	559 (82,15)
Fecal coliform counts (mg/L)	0<1	0<1	0<1	0<1	142,054 (19221,10)	60,840 (15139,10)	32,537 (15330,10)	190,706 (48350,10)

* T-Thermophilic, M-Mesophilic, S1- Sonicated at 10′ and 60% A; S2- Sonicated at 10′ and 100 %A; S3- Sonicated at 40′ and 60% A, STP: standard temperature and pressure (0°C, 1 atm), OLR: organic loading rate, TS: total solids, VS: volatile solids, TCOD: total chemical oxygen demand, TVSC-Total volatile sulfur compounds, TVFA: total volatile fatty acids (summation of acetic, propionic and butyric acids), CST-Capillary suction time, CFU- Colony forming unit,

† Data represent arithmetic mean of data (standard deviation, number of data points)

Table D.2: Steady state results for actual digesters at 10-d SRT

Parameter	Thermophilic				Mesophilic			
	Control	TS1	TS2	TS3	Control	MS1	MS2	MS3
OLR (g VS/L/d)	3.67 (0.16,10)	3.57 (0.16, 6)	3.59 (0.23, 6)	3.62 (0.18, 6)	3.67 (0.16,10)	3.57 (0.16, 6)	3.59 (0.23, 6)	3.62 (0.18, 6)
OLR (g TCOD/L/d)	5.55 (0.21, 9)	6.00 (0.33, 9)	5.37 (0.80, 9)	5.55 (0.15, 9)	5.55 (0.21, 9)	6.00 (0.33, 9)	5.37 (0.80, 9)	5.55 (0.15, 9)
Removal Efficiency								
VS (%)	53.95 (0.03,18)	52.95 (0.025,18)	54.95 (0.03,18)	49.7 (0.194,18)	47.18 (0.03,18)	46.31 (0.04,18)	48.68 (0.05,18)	51.2 (0.03,18)
TS (%)	45.5 (0.03,18)	44.3 (0.03,18)	46.4 (0.03,18)	34.85 (0.07,18)	40.65 (0.03,18)	38.9 (0.03,18)	40.3 (0.06,18)	42.8 (0.02,18)
TCOD (%)	52 (0.02, 15)	53 (0.03, 15)	51 (0.03, 15)	20 (0.08, 15)	49 (0.04, 15)	53 (0.03, 15)	52 (0.02, 15)	51 (0.03, 15)
Biogas Production								
Specific Biogas Production (L/g VS added-d)	0.49 (0.03, 40)	0.53 (0.03, 40)	0.51 (0.06, 40)	0.30 (0.18, 40)	0.48 (0.03, 40)	0.51 (0.03, 40)	0.50 (0.04, 40)	0.52 (0.03, 40)
TVSC emissions (mg/L)	690.3 (207.5, 10)	586.57 (67.63, 10)	968.17 (104.88, 10)	931.28 (277.88, 10)	457.65 (51.56, 10)	574.05 (197.28, 10)	696.41 (157.18, 10)	856.7 (51.88, 10)
CH4 (%)	65.59 (0.78, 8)	64.6 (2.29, 8)	64.37 (3.34, 8)	55.34 (6.34, 8)	64.06 (2, 8)	64.85 (.67, 8)	65.15 (1, 8)	65.59 (0.87, 8)
Effluent Characteristics								
pH (-)	7.749 (0.05, 24)	7.799 (0.06, 24)	7.674 (0.04, 24)	7.17 (0.17, 24)	7.18 (0.06, 24)	7.20 (0.06, 24)	7.23 (0.06, 24)	7.24 (0.06, 24)
NH3-N (mg/L)	1444 (58,6)	1472 (106.8,6)	1528 (149.5,6)	1500 (145.8,6)	885 (162,6)	1019 (192,6)	1005 (203,6)	1002 (256,6)
Alkalinity (mg $CaCO_3$/L)	5057 (83,6)	5124 (37,6)	5115 (177,6)	4889 (178,6)	3585 (155.6,6)	3904 (152,4)	4180 (97.3,4)	4283 (103,4)
TVFA (mg/L)	345.6 (249,10)	72 (4.64,10)	438.8 (141,10)	2142.3 (550.7,14)	29.37 (21.48,12)	20.10 (8.77,10)	16.37 (3.29,10)	18.606 (6.58,10)
CST (mg/L)	1800 (297,12)	1925 (358,12)	2085 (304,12)	1700 (203,12)	773 (93,12)	816 (69,12)	741 (37,12)	677 (58,12)
Fecal coliform counts (mg/L)	439.96	0<1	0<1	597.9	132,250 (39021,4)	161,965 (17630,4)	287,841 (20480,4)	79,325 (9150,4)

* T-Thermophilic, M-Mesophilic, S1- Sonicated at 10′ and 60% A; S2- Sonicated at 10′ and 100 %A; S3- Sonicated at 40′ and 60% A, STP: standard temperature and pressure (0°C, 1 atm), OLR: organic loading rate, TS: total solids, VS: volatile solids, TCOD: total chemical oxygen demand, TVSC-Total volatile sulfur compounds, TVFA: total volatile fatty acids (summation of acetic, propionic and butyric acids), CST-Capillary suction time, CFU- Colony forming unit

† Data represent arithmetic mean of data (standard deviation, number of data points)

Table D.3: Steady state results for actual digesters at 5-d SRT

Parameter	Thermophilic				Mesophilic			
	Control	TS1	TS2	TS3	Control	MS1	MS2	MS3
OLR (g VS/L/d)	6.64 (0.07, 6)	6.64 (0.07, 6)	6.74 (0.14, 6)	6.64 (0.24, 6)	6.64 (0.07, 6)	6.64 (0.07, 6)	6.74 (0.14, 6)	6.64 (0.24, 6)
OLR (g TCOD/L/d)	11.59 (0.45, 7)	11.57 (0.33, 7)	11.11 (0.31, 7)	10.89 (0.60, 7)	11.59 (0.45, 7)	11.57 (0.33, 7)	11.11 (0.31, 7)	10.89 (0.60, 7)
Removal Efficiency								
VS (%)	26 (0.08, 10)	22 (0.09, 10)	31 (0.12, 10)	26 (0.08, 10)	29 (0.07, 10)	38 (0.04, 10)	43 (0.02, 10)	40 (0.03, 10)
TS (%)	22 (0.06, 10)	19 (0.08, 10)	26 (0.09, 10)	21 (0.06, 10)	31 (0.07, 10)	32 (0.04, 10)	35 (0.03, 10)	33 (0.03, 10)
TCOD (%)	28 (0.04, 10)	16 (0.03, 10)	22 (0.05, 10)	9 (0.04, 10)	49 (0.06, 10)	53 (0.08, 10)	45 (0.04, 10)	34 (0.03, 10)
Biogas Production								
Specific Biogas Production (L/g VS added-d)	0.13 (0.03, 28)	0.07 (0.03, 28)	0.17 (0.05, 28)	0.15 (0.05, 28)	0.17 (0.11, 33)	0.32 (0.05, 33)	0.43 (0.04, 33)	0.3 (0.09, 33)
TVSC emissions (mg/L)	598.25 (193.25, 7)	623.88 (203.44, 7)	1911.34 (885.18, 7)	1926.11 (194.67, 7)	1434.4 (471.9, 7)	2280 (567.15, 7)	1657 (166.55, 7)	1827.75 (164.5, 7)
CH4 (%)	44.83 (4.55, 5)	47.01 (4.55, 5)	54.74 (4.55, 5)	53.53 (4.55, 5)	46.04 (4.55, 5)	60.34 (4.55, 5)	63.86 (4.55, 5)	61.22 (4.55, 5)
Effluent Characteristics								
pH (-)	6.65 (0.4, 15)	6.39 (0.37, 15)	6.86 (0.13, 15)	6.82 (0.25, 15)	6.00 (0.81, 18)	6.73 (0.27, 18)	7.02 (0.06, 18)	6.8 (0.16, 18)
NH3-N (mg/L)	1094 (29,4)	1202 (155,4)	990 (45.7,4)	1036 (33.8,4)	583.6 (53,5)	628.4 (8.33,5)	647 (24.4,5)	702 (36,5)
Alkalinity (mg $CaCO_3$/L)	3248 (160,4)	3216 (144,4)	3605 (123,4)	3585 (113,4)	2258 (219,4)	2594 (181,4)	2999 (95,4)	2919 (178,4)
TVFA (mg/L)	2406.9 (391.6,8)	3537.4 (362.87,8)	2423.6 (150.46,8)	2200.9 (65.03,8)	3078.6 (1985.9,10)	1486.6 (1038.6,10)	249.96 (214.41,10)	1013.8 (238.63,10)
CST (mg/L)	372 (103,9)	263 (42,9)	489 (24,9)	382 (45,9)	403 (25,9)	781 (49,9)	1111 (41,9)	1041 (78,9)
Fecal coliform counts (CFU/g dry TS)	1313.5	2514.3	0<1	2323.2	12,060 (5959,4)	411,208 (124434,4)	601,839 (10689,4)	741,719 (67845,4)

* T-Thermophilic, M-Mesophilic, S1- Sonicated at 10′ and 60% A; S2- Sonicated at 10′ and 100 %A; S3- Sonicated at 40′ and 60% A, STP: standard temperature and pressure (0°C, 1 atm), OLR: organic loading rate, TS: total solids, VS: volatile solids, TCOD: total chemical oxygen demand, TVSC-Total volatile sulfur compounds, TVFA: total volatile fatty acids (summation of acetic, propionic and butyric acids), CST-Capillary suction time, CFU- Colony forming unit

† Data represent arithmetic mean of data (standard deviation, number of data points)

Table D.4: Energy balance for all digesters at all SRTs

	Thermophilic				Mesophilic			
Parameters	C	S1	S2	S3	C	S1	S2	S3
At 20-d SRT								
Energy gained from biogas (kJ/d)	11346	10884	10921	2417	10715	11045	10726	11497
Ultrasonic energy input (kJ/kg TS)	0	2042	4163	8153	0	2042	4163	8153
Energy required to heat sludge (kJ/kg TS)	146.3	146.3	146.3	146.3	62.7	62.7	62.7	62.7
Energy loss from digester (kJ)	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Net energy gain (MJ/kg TS added-d)	11.2	8.7	6.61	-5.88	10.65	8.94	6.5	3.28
At 10-d SRT								
Energy gained from biogas (kJ/d)	10254	10694	10307	5107	9720	10376	10281	10626
Ultrasonic energy input (kJ/kg TS)	0	2042	4163	8153	0	2042	4163	8153
Energy required to heat sludge (kJ/kg TS)	146.3	146.3	146.3	146.3	62.7	62.7	62.7	62.7
Energy loss from digester (kJ)	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Net energy gain (MJ/kg TS added-d)	10.11	8.50	5.99	-3.19	9.66	8.27	6.05	2.41
At 5-d SRT								
Energy gained from biogas (kJ/d)	1904.5	1114	2922	2420	2553	5938	8220	5713
Ultrasonic energy input (kJ/kg TS)	0	2042	4163	8153	0	2042	4163	8153
Energy required to heat sludge (kJ/kg TS)	146.3	146.3	146.3	146.3	62.7	62.7	62.7	62.7
Energy loss from digester (kJ)	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Net energy gain (MJ/kg TS added-d)	1.76	-1.07	-1.39	-5.88	2.49	3.83	3.99	-2.5