Ultrasonic Pretreatment: Impact on Solubilization, Biogas Production and Kinetics of Anaerobic Digestion of Conventional and Biofilm Waste Sludges

Peter Roebuck

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Ottawa-Carleton Institute for Environmental Engineering

Department of Civil Engineering

University of Ottawa

Ottawa, Ontario, Canada

ABSTRACT

Anaerobic digestion is a useful method for stabilizing and reducing the waste activated sludges (WAS) produced from biological secondary treatment. Pretreatments can make anaerobic digestion more efficient. However, the study of anaerobic digestion and pretreatments is limited to a focus in treating conventional WAS. Therefore, WAS from three non-conventional municipal wastewater treatment systems, a rotating biological contactor (RBC), a lagoon, and a moving bed bioreactor (MBBR), were digested anaerobically to determine the sludges' biogas potentials compared to a conventional WAS. All three WAS had lower biogas potential normalized per volatile solids than conventional sludge by 46% $\pm$ 6 (MBBR), 63% $\pm$ 6 (RBC), and 77% $\pm$ 7 (lagoon). The four sludges were pretreated with ultrasonic energies of 800 - 6550 kJ/kg TS to illustrate impact of sludge type on biogas production, solubilization, and digestion kinetics. All four sludge types responded uniquely to the same levels of sonication energies. The greatest increase in biogas production over the control of pretreated sludge did not coincide consistently with greater sonication energy but occurred within a solubilization range of 2.9 – 7.4% degree of disintegration (DD) and are as follows: 5% $\pm$ 3 biogas increase for conventional sludge, 12% $\pm$ 9 for lagoon, 15% $\pm$ 2 for MBBR and 20% $\pm$ 2 for RBC. The yield of biogas production related to soluble COD decreases with increased sonication energy. Hence it is likely that sonication produces refractory COD or causes inhibition in biogas production. The effect of sonication on digestion kinetics was inconclusive with the application of Modified Gompertz, Reaction Curve, and First Order models to biogas production. Diauxic growth patterns of biogas production of sonicated conventional waste demonstrates that the active time of digestion can be decreased through the conversion of less preferential substrates into existing, preferential substrates.

RÉUSMÉ

La digestion anaérobique est une technique utile afin de stabiliser et réduire la quantité de boues activées produites par un système de traitement biologique secondaire. Le prétraitement de la boue peut rendre la digestion anaérobique plus efficace. Cependant, l'étude de la digestion anaérobique et du prétraitement des boues est souvent limitée au prétraitement des boues activées traditionnelles. Par conséquent, les boues activées provenant de trois systèmes de traitement des eaux usées municipales non conventionnels furent analysées. Les boues des trois systèmes suivants: un contacteur biologique rotatif (CBR), une lagune et un bioréacteur à lit mobile, ou Moving Bed Biofilm Reactor (MBBR), furent digérées anaérobiquement afin de déterminer les potentiels de production de biogaz des boues par rapport à un système de boues activés conventionnel. Les trois boues activés ont des potentiels de génération de biogaz normalisé par la masse de solides volatiles plus faibles que les boues conventionnelles de 46% ± 6 (MBBR), 63% ± 6 (CBR) et 77% ± 7 (lagune) respectivement. Les quatre boues furent prétraitées avec des énergies ultrasoniques de 800 à 6550 kJ / kg de solides totaux pour illustrer l'impact du type de boues sur la production de biogaz, la solubilisation et la cinétique de la digestion. La plus grande augmentation de production de biogaz par rapport aux boues régulières s'est produite dans une fourchette de solubilisation de 2,9 à 7,4% de degré de désintégration (DD). Les augmentations de biogaz sont les suivantes : 5% ± 3 pour les boues classiques, 12% ± 9 pour les boues provenant de la lagune, 15% ± 2, pour les boues provenant du MBBR et 20% ± 2 pour les boues provenant du CBR. Le rendement de la production de biogaz liée à la DCO soluble diminue avec l'augmentation de l'énergie de sonication. Il est donc probable que la sonication génère une DCO récalcitrante ou bien difficile à consommer par les microorganismes, causant une potentielle inhibition dans la production de biogaz. Les effets de la sonication sur la cinétique de la digestion n'étaient pas concluants suite à l’application de la courbe de réaction de Gompertz modifiée et des modèles de production de premier ordre. Les tendances de croissance diauxique dans la production de biogaz de boues traditionnelles semblent toutefois indiquer que le temps actif de la digestion peut être diminué grâce à la conversion de substrats moins préférentiels en substrats préférentiels à la digestion.

ACKNOWLEDGEMENTS

I would like to express my thankfulness and love for my wonderful Lady who supported me and corrected me throughout this work. She is my home.

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NOMENCLATURE

Abbreviations

AD	Anaerobic Digestion
BG	Biogas
BGP	Biogas production
COD	Chemical oxygen demand
sCOD	Soluble chemical oxygen demand
tCOD	Total chemical oxygen demand
DD	Degree of disintegration
E	Energy
E_D	Energy density
E_{Do}	Energy dose
E_I	Energy intensity
E_S	Specific energy
EPS	Exopolymeric substances
HRT	Hydraulic retention time
MBBR	Moving bed bioreactor
OUR	Oxygen uptake rate
RBC	Rotating biological contactor
SRT	Solids retention time
US	Ultrasound / Ultrasonic
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

CHAPTER 1 INTRODUCTION

1.1 BACKGROUND

Wastewater, defined as the spent water discharged from anthropologic sources, has been collected and treated since the time of the ancient Greek and Roman civilizations (McCarty, 1982). With the current global population increasing at a rate of 83 million people per annum, there is more waste than ever (Gomi and Stinchecum, 1993; United Nations, 2015). As population density increases, the availability of space for wastewater treatment footprints and landfill disposal of by-products decreases. This places an impetus on research to make treatment plants more efficient in dealing with waste without significantly increasing their existing size or energy consumption.

Modern conventional wastewater treatment plants (WWTP) use physical and biological means to convert pollutants to biomass, which, in turn, becomes a new source of waste requiring disposal. While WWTPs offer an effective treatment for wastewater, the disposal of the excess biomass can be costly. Anaerobic digestion (AD) is often used as a successive treatment to reduce the volume and improve the character of the biomass. The by-product of this process is a biogas (BG) of methane and carbon dioxide that can be captured and combusted to offset energy and heating costs. Detractions to AD are the heating requirements for operation and a rate of digestion much slower than that of the aerobic process that produces the biomass (Pavlostathis and Giraldo-Gomez, 1991). Thus, AD requires longer retention times in large capacity vessels that negatively impact the footprint and energy requirements of a WWTP; or incomplete digestion that can negatively impact quantity and quality of sludge thus limiting disposal options.

A pretreatment step can be used before AD to offset the negative attributes of treating excess biomass. Through thermal, mechanical, or chemical processes, biomass can be pretreated to decrease retention time, increase extent of digestion and increase biogas (BG) production from which more energy can be extracted and put back into the system. This is achieved through the disruption of the exopolymeric substances (EPS) matrix that bind cells together and lysing the digestion-resistant cell walls to release easily digested cell contents. Ultrasound (US) is a powerful pretreatment method that disrupts biomass through a combination of thermal, mechanical and chemical effects caused by waves of sound pressure and cavitation. The operation of several WWTPs using US technology indicate that full-scale units can achieve a net-positive production of energy through the energy recoverable from increased biogas production (BGP) (Long and Bullard, 2012; Xie et al., 2007).

The investigation of US as a pretreatment has resulted in a wide range of reported results in literature illustrating variability in the AD of sonicated sludges collected from conventional WWTPs. Reports of solubilization and BG increases vary considerably among articles using different sources of conventional sludges. Beyond conventional sludges, literature is currently lacking US pretreatment research on any other type of sludges, such as those generated by biofilm systems or lagoons. Further investigation could help clarify the impact of origin and sludge type; therefore, this study will focus on the impact of US pretreatment on the AD of non-conventional sludges.

1.2 RESEARCH OBJECTIVES

The purpose of this work is to develop an understanding of the effect of ultrasonic pretreatment on the AD of non-conventional sludges. The specific objectives of this research are as follows:

- Determine anaerobic BG potential of three non-conventional waste sludges from a rotating biological contactor (RBC), moving bed bioreactor (MBBR), and a lagoon relative to a conventional waste sludge
- Investigate the impact of ultrasonic pretreatment of waste sludges by quantifying effect on solubilization, BGP, and kinetics of digestion of non-conventional sludges compared to conventional sludge.

1.3 THESIS STRUCTURE

Chapter 1 presents brief background information on the context of AD and pretreatments to illustrate the significance and objectives of this study. Chapter 2 presents a literature review of AD and available pretreatments with a focus on the impact of ultrasound on increasing sludge solubilization and BGP. The effect of AD and ultrasonic pretreatment on conventional and non-conventional sludges is investigated in Chapter 3. This work will be submitted to Ultrasonics Sonochemistry under the following title: *Effect of ultrasonic pretreatment for anaerobic digestion of biofilm and suspended municipal waste activated sludge* by P. Roebuck, K. Kennedy, R. Delatolla, and D. Kennedy. Chapter 3 contains a comprehensive description of all material, methods and the experimental plan used for the thesis, hence a separate chapter pertaining to materials and methods is not included in this thesis to minimize redundancy. The study investigates the impact of four levels of sonication energy on the AD of four waste sludges from a conventional municipal system, RBC, MBBR, and lagoon. Specifically, impact is measured through changes in solubilization, BGP, and kinetics of digestion. Finally, Chapter 4 presents the conclusions derived from this study in regards to the AD of non-conventional sludges and the use of US as a pretreatment.

1.4 STATEMENT OF CONTRIBUTIONS OF COLLABORATORS

During the course of this work, the following manuscript was developed for submission to peer-reviewed journals. The author's contributions are described below.

Roebuck, P., Kennedy, K., Delatolla, R., Kennedy, D. *Effect of ultrasonic pretreatment for anaerobic digestion of biofilm and suspended municipal waste activated sludge*. In preparation for submission to Ultrasonics Sonochemistry.

P. Roebuck: Conducted literature review, developed and conducted experimental procedure, collected samples, analysed results, and wrote manuscript.

K. Kennedy: Provided expertise, supervision in development of experimental procedure, analysis of results and revision of manuscript.

R. Delatolla: Provided supervision in analysis of results and revision of manuscript.

D. Kennedy: Provided assistance in collection of samples and data.

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CHAPTER 2 LITERATURE SURVEY

2.1 ANAEROBIC DIGESTION

AD serves many purposes in the treatment of excess waste sludges. AD is applied to 1) reduce waste volume through water removal; 2) reduce organic, putrescible content through stabilisation; 3) to aid in disposal of residue. A by-product of the four-stage, sequential degradation of organic material is the production of a renewable, energy-rich biogas. Energy recovery is generally secondary to the main goals listed above, but can be used to offset or even exceed power requirements of the sludge management.

Anaerobic digestion can be described by a sequence of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 2-1). Hydrolysis involves the degradation of complex organics, such as polysaccharides, proteins, lipids and nucleic acids by extra-cellular enzymes into soluble compounds that are able to pass through the acidogenic cell membranes. Here, the compounds are fermented to volatile fatty acids (VFAs), ammonia, carbon dioxide and hydrogen sulphide. Acetogenesis converts complex organic acids and alcohols to acetic acid and additional carbon dioxide and hydrogen gas. Methanogenesis is performed by two types of bacteria. Aceticlastic methanogens account for 65-70% of methane produced in sewage sludge digestors through the reduction of the acetate methyl group. Reductive methanogens reduce CO_2 by H_2 to form methane. This phase regulates acetogenesis through the control of hydrogen partial pressure. Methanogenesis is the most sensitive phase and susceptible to disruption. It has a small range of optimal pH (6.5-7.2) and changes in excess of 1 ºC/day can cause process failure. Production of VFAs in acidogenesis lowers the system's pH but is countered by alkalinity generated during methanogenesis in the form of carbon dioxide, ammonia, and bicarbonate (Appels et al., 2008a; Pavlostathis and Giraldo-Gomez, 1991).

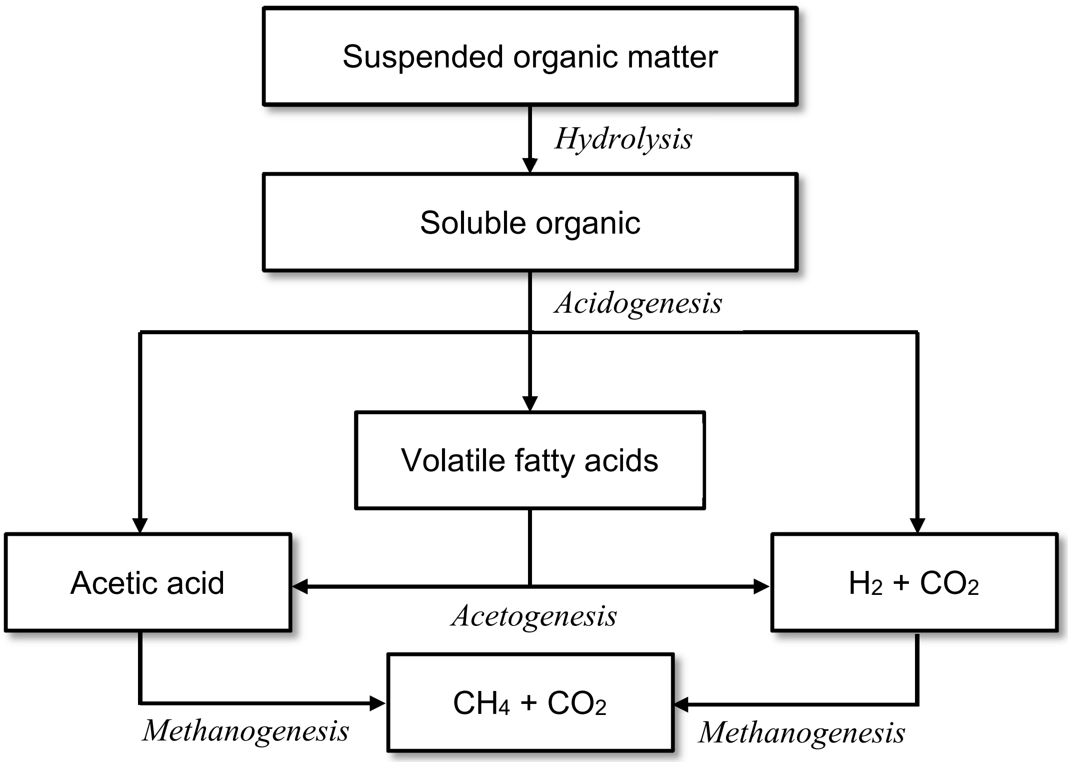

Figure 2-1 - Sequence of anaerobic digestion (Adapted from Appels, 2008a)

Considering the individual kinetics of the steps, the yields and doubling times of methanogenic bacteria responsible for the conversion of acetate (0.032 $^{\text{kg VS}}/_{\text{kg COD}}$, 3.86 d) and hydrogen (0.030 $^{\text{kg VS}}/_{\text{kg COD}}$, 1.15 d) are less efficient than those for hydrolysing carbohydrates (0.350 $^{\text{kg VS}}/_{\text{kg COD}}$, 0.18 d), proteins (0.250 $^{\text{kg VS}}/_{\text{kg COD}}$, 0.43 d), and fats (0.038 $^{\text{kg VS}}/_{\text{kg COD}}$, 3.19 d) (Pavlostathis and Giraldo-Gomez, 1991). Despite this, hydrolysis is considered the rate-limiting step in the digestion of WAS. The hydrolytic enzymes are poor scavengers that require high substrate concentrations to be effective. The organic fraction of WAS is mostly cells conglomerated in flocs surrounded by EPS which can inhibit enzyme contact. The cells themselves are an unfavourable substrate due to tough lignin-reinforced cell walls (Appels et al., 2008a). Without the disruption and solubilization of particulate organic matter before digestion, a fraction of un-hydrolysed material will always be present in an AD system resulting in partial decomposition of 30-50% of the organic fraction to BG (Parkin and Owen, 1986).

2.2 PRETREATMENTS

The purpose of pretreatments are to compensate for the detractions of AD; namely the slow, rate-limiting hydrolysis phase and low organic conversion. Every type of pretreatment meets these goals by treating WAS through disruption of the EPS structure and cell lysis, thus solubilizing the particulates and compounds that may hinder hydrolysis. By improving degradation rate and degradability, digester performance will improve. Better kinetics of digestion will lead to decreased hydraulic retention time (HRT) meaning volume of digesters could decrease or a greater extent of digestion or degradation can be reached with the same volume.

2.2.1 Quantification of Solubilization

The common purpose of pretreatments is to solubilize particulate matter that is resistant to digestion. The extent of solubilization is quantified by measuring the degree of disintegration (DD). DD is the ratio of sCOD (soluble COD) increase to the total possible sCOD increase, representing the change of state of COD from solid to liquid. This is historically calculated through two methods. Earlier papers defined the total possible sCOD as that that could be degraded through the application of 0.5 mol/L NaOH for 22 h at 20 °C (2-1) (Gonze et al., 2003; Nickel and Neis, 2007; Tiehm et al., 2001). More recent papers have relied on a less time intensive method of calculation without addition of NaOH based on total possible sCOD being the total solubilization of tCOD (2-2) (Şahinkaya and Sevimli, 2013; Yagci and Akpinar, 2011; Zhang et al., 2007).

$$DD_{COD} = \frac{sCOD - sCOD_0}{COD_{NaOH} - sCOD_0} \tag{2-1}$$

$$DD_{COD} = \frac{sCOD - sCOD_0}{tCOD - sCOD_0} \tag{2-2}$$

Where

DD_{COD} is degree of disintegration based on COD solubilization
sCOD is the soluble COD of samples after solubilization
$sCOD_0$ is the initial soluble COD of the control or untreated WAS
tCOD is the total COD including soluble and particulate fractions

2.2.2 Proven Pretreatment Techniques

Thermal pretreatments use heat to degrade EPS and disrupt chemical bonding in cell walls and membranes by heating WAS under pressure. Optimal conditions for thermal pretreatment are reported as holding a temperature of 160 – 180 °C for 30 – 60 min at pressures of 600 – 2500 kPa (Carrère et al., 2010). In full-scale treatments, this is achieved through direct steam injection resulting in reported average increases in BGP of 25% (Long and Bullard, 2012). Decreased biodegradability has been reported when thermalization temperatures in excess of 170 °C are used.

Chemical pretreatments can be split into oxidation or alkali treatments that are used to directly hydrolyse cell walls to solubilize cell contents. Chemical oxidants degrade into radicals and attack soluble and particulate fractions of sludge (Bougrier et al., 2006). The most common oxidant is ozone with an optimal dose of 0.1 g O_3 / g COD. Other oxidants can be used such as peroxide in combination with heat at optimal doses of 90 °C, 2 g H_2O_2 / g VSS (Carrère et al., 2010). Alkali pretreatment involves increasing the pH of sludge to a high level while at an elevated temperature. Optimally, temperature is increased to 120-130 °C while sludge pH is increased to 12 through the application of NaOH or KOH (Carrère et al., 2010).

Mechanical pretreatments involve using physical means, such as shear stresses, pressure changes and cavitation to disrupt floc structure and induce cell lysis. These treatments are generally energy intensive requiring more energy than can be recouped through the resultant increased BGP (Cano et al., 2015). One of the few promising mechanical technologies is a lysis centrifuge, which involves the addition of an extra disintegration gear to a classical centrifuge used for sludge dewatering. This technology has been successfully implemented in full scale municipal WWTPs to achieve energy self-sufficient operation with an increase in BGP of 15 – 26% (Carrère et al., 2010; Jenicek et al., 2013).

2.3 ULTRASOUND

A second promising form of mechanical pretreatment already at use in full-scale is the application of ultrasound, or "sonication" to WAS. Floc and cell structures are broken down mainly through cavitation and mechanical agitation. While lab-scale US probes are not efficient, sonication has positive effects on energy balances in full, plant scale operation. A possible 2 – 3 year payback period on equipment cost has been reported by yielding 7 kW of energy from increased methane production for every kilowatt of power used by the US device through the increased solubilization of WAS (Barber, 2005; Cano et al., 2015; Xie et al., 2007).

2.3.1 Mechanism of Ultrasonic Pretreatment

Sound waves with frequencies beyond those audible to humans are typically referred to as "ultra" sound ranging from 20 kHz to greater than 100 GHz. It is the transmission of mechanical energy in the form of a wave field through a medium. In a fluid, this transmission of sound pressure is by cyclic, acoustic waves that cause the fluid to alternate between high pressure compression and low pressure rarefaction phases. The waves can be reflected from surfaces or diffracted by the edges of surfaces or particles. Low intensity US, characterized by low energy and high frequency, is used to transmit energy such as is utilized in the medical imaging field. In contrast, high intensity US, characterized by high energy and low frequency, is used to produce an effect on the medium through which it is passing. Vapourous cavitation occurs with high intensity sonication during the low pressure, low density rarefaction segment of the acoustic wave when the fluid pressure is less than ambient conditions. The fluid media changes phase to create a micro-bubble of gas in the low pressure area which exist at extreme conditions, up to 5000 K and 500 atm (Flint and Suslick, 1991). Contact with the edges of the micro-bubbles can cause the erosion of surfaces or cell walls. Free radicals are also formed by the intense conditions of the bubbles that further promote oxidation and electrolytic corrosion of metals. The formation and violent collapse of cavitation creates high, localized shear stresses in the fluid that can cause the rupture and erosion of surrounding material and particles.

2.3.2 Ultrasound as a Pretreatment

As a pretreatment, sonication is classified as a mechanical process due to stresses induced by cavitation, but will also exhibit some thermal and chemical effects. Mechanical energy is lost to the surrounding fluid through the absorption and attenuation of the acoustic waves by viscous effects which produces heat in the fluid. Chemical effects are caused by the extreme conditions of cavitational bubbles, investigated in the field of sonochemistry. However, Wang et al. (2005) found the main mechanism of solubilisation is from hydromechanical shear stresses through induced cavitation. The slow increase in bulk sludge heat caused by absorption of the acoustic waves is not a significant contributing factor to solubilization. The oxidative effect is contributed by $OH^{\cdot}$ radicals generated by pyrolysis from the extreme nature of cavitational bubbles. The percent of solubilisation from oxidation is negligible at an US density of 0.096 W/mL but can rise to 25.86% at 0.72 W/mL (Wang et al., 2005).

The application of ultrasound as a pretreatment for AD has been studied extensively in scientific literature but with variable results in terms of reported ideal energy application and the extent of increased BGP through digestion (**Error! Reference source not found.**). Early studies illustrated the link between lower frequencies of US leading to increased cavitation and greater solubilization (Grönroos et al., 2005; Tiehm et al., 2001; Wang et al., 2005). However, there has been little consistency in reporting of quantifying the US energies.

Table 2-1 - Summary of reported results concerning the AD of sonicated sludges arranged by year from 1997 to 2013

Sludge Type	Ultrasonic Conditions			AD Conditions			Findings	Source
	Frequency (kHz)	Energy Level*	Duration*	Scale	Temperature	Residence Time (days)	Biogas (BGI) and/or Methane (MI) Increase	
Industrial WAS	20	0.33 W/mL	20 min	Batch	Mesophilic	40	104% MI	(Chu et al., 2002)
						6	290% MI	
Municipal WAS	41	-	150 min	Semi-continuous	Mesophilic	8	41.6% BGI	(Tiehm et al., 2001)
	20	-	30 min	Batch	Mesophilic	33	23% BGI	(Onyeche et al., 2002)
	27	200 – 300 W/L	2.5 – 30 min	Batch	Mesophilic	19	10 – 20% BGI	(Grönroos et al., 2005)
	20	7000 kJ/kg TS		Batch	Mesophilic	16	40% BGI	(Bougrier et al., 2005)
	31	10 W/cm²	90 sec	Pilot	Mesophilic	8	16% BGI	(Nickel and Neis, 2007)
	20	1 W/mL	1 min	Batch	Mesophilic	-	5.6% MI 6.3% BGI	(Şahinkaya and Sevimli, 2013)
TWAS	20	0.52 W/mL	1 min	Batch	Mesophilic	16	53% BGI	(Mao and Show, 2007)
	25	1020 W/L	-	Batch	-	8	40% BGI	(Appels et al., 2008b)
1/3 Primary 2/3 WAS	20	13.7 W/cm²	1.5 sec	Full	-	30	45% BGI	(Xie et al., 2007)
53% Primary 47% WAS	31	-	96 sec	Batch	Mesophilic	28	30% BGI	(Tiehm et al., 1997)
75% Primary 25% WAS	20	11000 kJ/kg TS		Semi-continuous	Mesophilic	20	31% BGI	(Benabdallah El-Hadj et al., 2007)
					Thermophilic	15	16% BGI	
Not specified	-	-	-	14 Full	-	12 – 69	20 – 50% BGI	(Barber, 2005)
	20	10.8 kW/kg TS		Pilot	-	20	42% BGI	(Pérez-Elvira et al., 2009)

*Energy levels and duration reported as shown in Table 2-2.

2.3.3 Ultrasonic Energy Quantification

A universally accepted method for quantifying the application of US energy has not yet been standardized which leads to difficulty in direct comparison of reported results. Authors tend to vary between four different expressions in reporting US energy (Table 2-2). Density refers to the power supplied to a sample volume. Intensity defines the mode of application of energy describing power supplied by US horn or transducer. These two methods require additional time data to be relevant. US dose includes a time term by describing the energy applied per sample volume. More relevant still is to include aspects of the sample volume as well. Specific energy contains more information by including dependent properties of the sample in describing the amount of energy that is supplied to a quantity of sample at known solids concentration. The latter expression of US energy application is preferable, especially for modelling, as it contains more information in a single term (Lambert et al., 2014). More exacting descriptions of energy allow a better correlation between energy and solubilization.

Table 2-2 - Common expressions of US energy quantification in terms of US power applied (P), sonication time (t), sludge volume (v) and the sludge initial total solids (TS_0).

Method	*Expression*	*Common Unit*	*Reference*
US Density	$E_D = \frac{P}{v}$	$[\mathrm{W/L}]$	
US Intensity	$E_I = \frac{P}{A}$	$[\mathrm{W/cm^2}]$	(Tiehm et al., 2001)
US Dose	$E_{Do} = \frac{Pt}{v}$	$[\mathrm{Ws/L}]$ or $[\mathrm{J/L}]$	
Specific Energy	$E_S = \frac{Pt}{vTS_o}$	$[\mathrm{kJ/kg\ TS}]$	(Bougrier et al., 2005)

2.3.4 Solubilization

In studies of solubilization of WAS with US, a staged effect on the sludge solubilization is universally reported, yet with differences in the energy levels at which the stages differentiate (Bougrier et al., 2005; Cho et al., 2012; Feng et al., 2009; Lehne et al., 2001; Show et al., 2007; Wang et al., 2006; Yagci and Akpinar, 2011; Zhang et al., 2007). The first stage is floc disintegration. The mechanical forces of US first act to disrupt the EPS floc structure reducing it to micro-aggregates (Figure 2-2). This stage is characterized by the reduction of floc size and filament destruction (Lehne et al., 2001); the release of binding agents from EPS such as Ca^{2+} and Mg^{2+} (Zhang et al., 2007); and the increase of

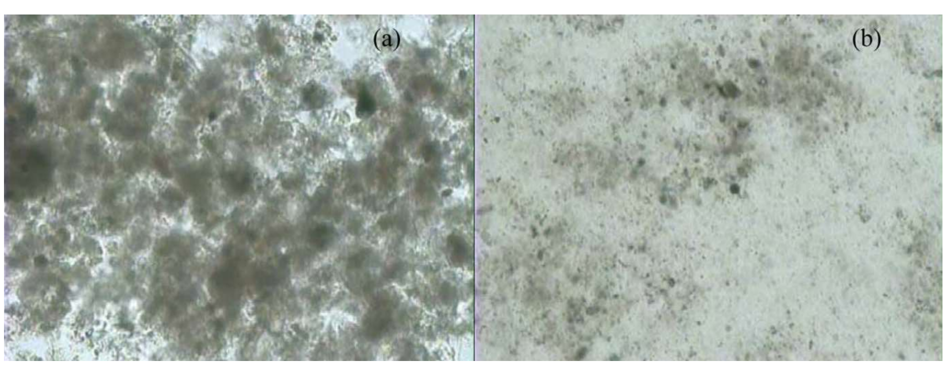

Figure 2-2 - WAS before (a) and after sonication (b) illustrating disruption of floc structure (Yagci, 2011)

microbial activity as measured by oxygen uptake rate (OUR) from the greater capacity of mass transfer due to release of cells from the EPS matrix (Huan et al., 2009). In this phase, disintegration is limited to the reduction of floc size and from disruption of the EPS matrix and, as such, has only a small benefit to increased BGP in AD (Lehne et al., 2001).

In the second phase, the US energy reaches a critical level where it believed to cause cell lysis. This stage is characterized by a sharp increase in solubilization (Bougrier et al., 2005; Lehne et al., 2001) coupled with a decrease in microbial activity and biomass inactivation, either through cell lysis or inhibition of cell metabolisms from chemical reaction (Huan et al., 2009; Zhang et al., 2007). The optimum energy for sonication is that which provides the greatest increase of solubilization and BGP at the lowest energy level. This value is the energy level that typically ends this phase. A final stage is discussed that describes a point of diminishing returns. Increasing the energy level beyond optimum will result in a lower rate of solubilization with little to no increase of BGP resulting in no return on the higher energy expenditures (Bougrier et al., 2005; Lehne et al., 2001; Zhang et al., 2007). The impact of specific energy on the three phases is illustrated by decreasing particle size and the leveling effect on DD in Figure 2-3.

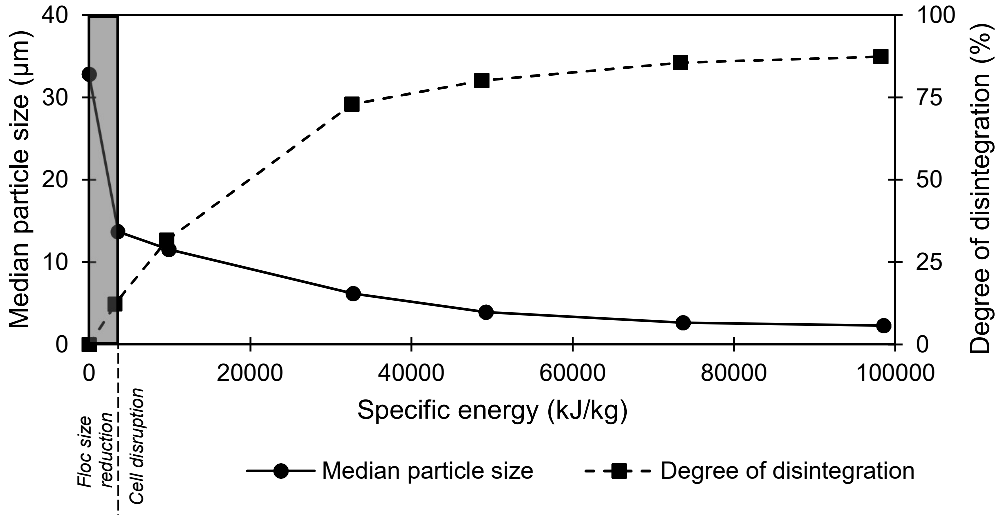

Figure 2-3 - Mean particle size and degree of disintegration as impacted by ultrasound at specific energies of 0 - 100 000 kJ/kg TS (Lehne, 2001)

While the reporting of the three phases is consistent in literature concerning the ultrasonic pretreatment of WAS, the energy level at which these phases are separated varies with each author. Bougrier et al. (2005) determined that the first phase of floc reduction occurred at $E_S < 1000$ kJ/kg TS. Cell lysis would occur beyond this threshold though there would be little benefit to BGP and solubilization after an optimum value of $E_S = 7000$ kJ/kg TS. This contrasts with later work by Benabdallah et al. (2007) who found the specific energy value for the point of decreasing returns on solubilization and BGP to be 11,000 kJ/kg TS. Lehne et al. (2001) considered the floc disruption phase to be from 0 – 3000 kJ/kg TS with cell lysis occurring beyond this threshold. Their data illustrated decreasing returns after approximately 28 000 kJ/kg TS (Figure 2-3).

In fact, although all these tests were performed on conventional WAS, or blends of WAS and primary sludge all from a conventional source, there is a wide range of reported results (**Error! Reference source not found.**) that has lent a lack of confidence in sonication as a pretreatment for AD. The entirety of the research focus on sonication pretreatment in the literature reviewed has been conducted solely with conventional sludges. This means that emerging technologies, such as biofilm systems, or other sources of WAS, such as lagoons, have been neglected and ideal energies applications are unknown.

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CHAPTER 3 EFFECT OF ULTRASONIC PRETREATMENT FOR ANAEROBIC DIGESTION OF BIOFILM AND SUSPENDED MUNICIPAL WASTE ACTIVATED SLUDGE

3.1 SETTING THE CONTEXT

The article presented in chapter 3 is entitled *Effect of ultrasonic pretreatment for anaerobic digestion of biofilm and suspended municipal waste activated sludge* by P. Roebuck, K. Kennedy, R. Delatolla, D. Kennedy. This article is in preparation for submission to Ultrasonics Sonochemistry. This paper describes the impact on solubilization, BGP, and digestion kinetics of WAS from four very different municipal wastewater treatment systems. This chapter addresses the objectives of determining BG potential of three non-conventional sludges and comparing the impact of ultrasonic pretreatment on conventional and non-conventional waste sludges.

3.2 INTRODUCTION

Renewable energy produced from biogas (BG) has grown from 0.20% of the world's global electrical production in 2009 to 0.34% in 2014, representing an increase of 39146 GWh (IEA, 2016). If this increasing trend continues, BG could become an important contributor towards the reduction of greenhouse gases and mitigation of climate change by reducing the reliance on coal and fossil fuels. A focus on increasing the impact and efficiency of renewable BG sources for energy production would be in line with the goals of the United Nations' Paris Agreement that was signed and ratified in 2015 by 125 global parties (United Nations/Framework Convention on Climate Change, 2015). As a case study, Feldheim, Germany achieved energy self-sufficiency in 2012 mainly through the use of wind power and BG from agricultural corn waste and manure (Von Bock Und Polach et al., 2015).

BG is produced by anaerobic fermentation of a variety of biomass through the conversion of putrescible organic matter to a methane-enriched gas. Recovering energy in this form is advantageous to high-waste, organic operations such as wastewater treatment. In the United States, sewage treatment can represent 20% of a municipality's total energy use (Gu et al., 2017). Anaerobic digestion (AD) is used in municipal wastewater treatment systems (WWTS) to treat primary and secondary waste activated sludge (WAS) whose disposal can represent up to 57% of operations and maintenance cost (LeBlanc et al., 2008). However, sewage sludge is an excellent fuel source with a higher methane potential than currently available energy crops, such as beets, sorghum and maize (Appels et al., 2011). It has the additional benefit of being a waste product and does not cause competition for food resources. A single population equivalent can produce 60 – 90 g dry solid (DS) sludge per day yielding 0.590 m^3 CH_4/ kg volatile dry solids (VS) or 0.787 m^3 BG /kg DS (Appels et al., 2011, 2008a). Considering this, the following are reported sewage sludge productions (dry metric tons) for various countries across different demographics and economies: China (2,966,000), Japan (2,000,000), Netherlands (1,500,000), UK (1,500,000), and USA (6,514,000) (LeBlanc et al., 2008). As countries grow, and as more population is connected to new or existing sewage infrastructure, these sludge productions will continue to increase along with the energy requirement for treatment. If anaerobic digestion is fully utilized to manufacture BG, this production of waste could represent a significant renewable energy source to offset treatment costs, thus short-circuiting the water-energy nexus by using the waste generated as the energy source to treat the water.

A current goal in sustainable design is the achievement of net-zero energy WWTS (Gu et al., 2017; Jenicek et al., 2013, 2012; Nowak et al., 2015; Shen et al., 2015a). By 2015, eight wastewater treatment plants (WWTP) across North America and Europe reported 100% energy self-sufficiency with four being net-positive, able to sell 10-20% excess energy (Shen et al., 2015b).There are two main requirements for achieving net-zero operation: (1) reducing power consumption by improving efficiency of existing mechanisms and (2) increasing power generation through optimizing energy recovery from new and existing sources (Gu et al., 2017; Jenicek et al., 2013). The second goal can be met by optimizing AD for increased generation of BG from the waste sludges.

AD is a multistage process involving hydrolysis, acidogenesis, acetogenesis and methanogenesis (Appels et al., 2008a; Pavlostathis and Giraldo-Gomez, 1991). A cost intensive detraction to AD is its long hydraulic retention time (HRT) compared to aerobic digestion resulting in larger capital cost of digestors and longer operation times. For sludge digestion, the rate limiting phase is commonly considered to be hydrolysis. The hydrolytic enzymes are poor scavengers that require high substrate concentrations to overcome mass transfer limitation of degrading sludge particles, resulting in a fraction of un-hydrolysed material always remaining in the digestate (Barber, 2005; Pavlostathis and Giraldo-Gomez, 1991). BG generation can be increased while decreasing HRT in AD through pretreatments designed to disintegrate the tough, cellular material of WAS resulting in a faster hydrolysis phase and a smaller fraction of undigested material in the effluent.

Common sludge pretreatments before AD include thermal, chemical and mechanical techniques. A significant number of studies concerning application on conventional WAS show benefits of various pretreatments to particle size reduction, solubilization and biodegradability (Cano et al., 2015). For net-zero operation, these pretreatments must produce more energy through increased biogas production than they consume. Full-scale thermal pretreatment methods, such as Cambi and EXCELYS show net-positive energy when used with full heat and power integration (Cano et al., 2015; Carrère et al., 2010). Mechanical pretreatments, such as lysate centrifuges, have been implemented as the sludge pretreatment method for net-positive WWTP in Wolfgangse-Ischal and Strauss (Jenicek et al., 2013; Nowak et al., 2015).

The focus of this research is ultrasound (US) as a WAS pretreatment for AD. Although it has thermal, mechanical, and chemical effects, the main mechanism of solubilisation is hydromechanical shear stress caused by induced cavitation which can disrupt sludge flocs and rupture microbial cell walls, thus releasing soluble organic matter and intracellular material (Wang et al., 2005). While not efficient at lab-scale, it is reported as energetically self-sufficient at full, plant scale with manufacturers claiming it can yield 3-10 kW of energy from increased methane production for every kilowatt of power used by the US device (Barber, 2005; Cano et al., 2015; Pérez-Elvira et al., 2009; Xie et al., 2007). Sonicated sludge can also indirectly assist AD by enhancing the buffering capacity of anaerobic phases sensitive to acid accumulation and increasing methanogenic biomass by 45% to 140% with sonication densities of 0.18 – 0.52 W/mL (Mao and Show, 2007). While sonication reduces dewaterability of sludge (Ruiz-Hernando et al., 2015), dewaterability is enhanced post-digestion (Şahinkaya and Sevimli, 2013), thereby reducing cost of residual solids treatment.

Anaerobic digestion and the effects of sonication on conventional WAS sludge from large municipal WWTP has been thoroughly researched (Appels et al., 2008a; Cano et al., 2015). However, there are alternative sludge-producing sewage treatment systems that have not been studied that may yield different methane potentials than conventional activated sludge process. Biofilm type treatments, such as rotating biological contactors (RBC) and moving bed bioreactors (MBBR) produce WAS when sloughing excess pieces of biofilm from the attached film system. The different floc structure and extracellular polymeric substance (EPS) component concentrations of biofilm sludge, as compared to conventional suspended systems, may have an impact on the efficacy of sonication (Martín-Cereceda et al., 2001). In this work, we investigate the methane potential of alternative sludges such as biofilms, and the effect of ultrasonic pretreatment on the increase of their biogas yield.

3.3 Materials and Methods

3.3.1 Sludge Sources

Sludge was collected from four municipal wastewater treatment systems operating suspended and attached growth biological treatment technologies. The inoculum and four waste sludges were characterized for total chemical oxygen demand (tCOD), soluble COD (sCOD), total solids (TS) and volatile solids (VS) (Table 3-1). Thickened conventional waste activated sludge (CAS) was collected from the Robert O. Pickard Environmental Centre (ROPEC), located in Ottawa, Canada. The plant can treat a daily average capacity of 545,000 m^3/day through conventional biological secondary treatment with a solid retention time (SRT) of 5 to 7 days. ROPEC was not designed to achieve nitrification. The bacteriological seed for anaerobic digestion (inoculum) was taken from the mesophilic anaerobic digestors of the ROPEC facility operating at a 48/52 % mixture of primary sludge and TWAS at an SRT of 20 days. The plant uses internal combustion generators fueled by digestor gases to recover electrical and thermal energy (Ontario Ministry of the Environment, 2011). The biofilm sludge source was collected from the Water Pollution Control Plant in Wendover, Canada that conducts secondary, biological treatment through the use of three RBCs with a maximum capacity of 1260 m^3/day of municipal sewage (Ontario Ministry of the Environment, 2013). As the system is an attached growth technology the SRT values are unknown. The sludge of the RBC technology is produced through the sloughing off of excess biogrowth from the rotating contactors. The third and fourth sources were collected from the municipal wastewater treatment of Masson-Angers, Canada that uses a series of four aerated lagoons to treat a combined maximum volume of 230,000 m^3 without nitrification (Aquatech Inc., 2015). Settled waste sludge was harvested by dredging from the fourth lagoon in the treatment series. Sludge removal records from the lagoons indicate that sludge age of the harvested sludge was 5 years. Effluent from the Masson-Angers lagoon fed a temporary post-carbon-removal, nitrifying moving bed biofilm reactor (MBBR) pilot system (Young et al., 2016). As the MBBR pilot is an attached growth technology the SRT of the system is known. Sludge of the nitrifying MBBR technology is produced through the erosion of biofilm carriers that are kept in constant motion in the MBBR basins.

Table 3-1 – Characterisation of harvested sludges and inoculum

Source	tCOD (mg/L)	sCOD (mg/L)	TS (%)	VS (%)	VS/TS (%)
Inoculum	21200 ± 400	11300 ± 100	1.48 ± 0.01	0.88 ± 0.01	59.5 ± 0.7
CAS	73200 ± 1100	15400 ± 300	6.67 ± 0.02	4.43 ± 0.03	66.4 ± 0.5
Lagoon	24100 ± 1200	1200 ± 200	3.64 ± 0.05	1.51 ± 0.03	42 ± 1
RBC	25200 ± 200	420 ± 10	3.02 ± 0.02	1.70 ± 0.01	56.3 ± 0.5
MBBR	20800 ± 2200	515 ± 3	2.18 ± 0.02	1.53 ± 0.01	70.2 ± 0.8

Values given in table are the sample mean ± standard deviation, based on n ≥ 3

3.3.2 Sonication

RBC, lagoon and MBBR sludge samples were gravity settled, centrifuged and diluted with supernatant to an initial concentration of 6.5% TS in order to be similar to the CAS samples collected from ROPEC. All samples were further diluted to 4.5% TS with a buffer/micronutrient medium, to maintain pH and ensure anaerobic growth would not be limited by a lack of trace nutrients (Kennedy and Droste, 1985). Sonication was performed with a 450 Branson Digital Sonifier (Emerson Industrial, Connecticut), with a probe diameter of 13 mm, operating at 20 kHz and peak capability of 400 W. Sonication power (E_s, in kJ/kg) was quantified using specific energy (Equation 1):

$$E_S = \frac{Pt}{vTS_o} \qquad \text{(Equation 1)}$$

Where P is the power (J), t is the duration of sonication (seconds), v is the sample volume (L) and TS_o is the initial total solids (g/L).

Samples were sonicated in 200 mL batches for 1, 2, 5, and 10 minutes which correspond to specific energies of 800 ± 40, 1550 ± 130, 3770 ± 300, and 6550 ± 530 kJ/kg TS. These values bracket the range of sonication power as reported by Bougrier et al. which defines low power (E_S < 1000 kJ/kg TS) as the level at which disintegration is limited to floc disruption and high power (1000 kJ/kg TS < E_S < 7000 kJ/kg TS) as the level at which cell lysis occurs (Bougrier et al., 2005). Beyond E_S = 7000 kJ/kg TS, there is little reported benefit to increasing BG production and the rate of solubilisation will decrease (Bougrier et al., 2005).

Solubilization was quantified through the degree of sludge disintegration (DD) calculated as the ratio of sCOD increase after sonication to the total possible sCOD increase where $sCOD_0$ represents unsonicated or 0 min sludge (Equation 2) (Bougrier et al., 2006; Zhang et al., 2007). Sonication did not affect tCOD, thus tCOD is consistent for each sludge type after sonication.

$$DD = \frac{sCOD - sCOD_0}{tCOD - sCOD_0} \qquad \text{(Equation 2)}$$

Where sCOD is soluble COD, $sCOD_{0\ \text{is initial}}$ soluble COD of untreated sludge, tCOD is total COD.

3.3.3 Bioassays

The bioassay tests were conducted based on the procedure by Owen et al. (Owen et al., 1979) as single stage mesophilic (35°C) assays on sludges of 4.5% TS to test the effect of sonication on biogas production (BGP). In order to normalize and compare results, inoculum was not acclimatized to any specific substrate. A substrate to inoculum ratio of 5 ($mL_{substrate}/mL_{inoculum}$) was used for all reactors. Bottles were purged with N_2 gas for 2 minutes to displace oxygen in the headspace.

CAS assays were conducted in triplicate utilizing Bioprocess Control's AMPTS II in a 35°C water bath. Quantity of produced BG was automatically recorded hourly. The 500 mL reactor vessels contained 300 mL substrate and 60 mL inoculum. Due to sample volume limitations, all other assays were conducted at a 1:10 scale to the CAS assays. RBC, lagoon and MBBR tests were conducted in replicates of 4 in 50 mL reaction vessels containing 30 ml substrate and 6 ml inoculum. Bottles were closed with butyl rubber stoppers and sealed with an aluminium crimp. Samples were incubated and shaken at 35°C in a Psycrotherm Controlled Environment Incubator Shaker of New Brunswick Scientific Co. Inc. BGP was sampled daily and measured by manometer.

3.3.4 Analytical Methods

TS and VS were determined as per standard method 2540 (APHA, WEF, 2012). tCOD was measured by first homogenizing the samples and subsequently analysing with Hach's TNTplus 823 COD testing kit (Method 10212) (Hach Company, 2012). Measurements were conducted with a Hach DR6000 spectrophotometer (Loveland, CO). To mitigate the potential interference of filamentous bacteria causing bias during filtration, soluble COD (sCOD) was separated by centrifuging sludge samples at 8,000 g for 20 minutes (Feng et al., 2009). The resultant supernatant was then analyzed with Hach's TNTplus 822 COD testing kit (Method 8000) (Hach Company, 2014).

3.3.5 Data Analysis

Three non-linear models for estimation of performance parameters were compared to empirical data for biogas production (Table 3-2). The following models were shown by Donoso-Bravo et al., and Li et al. as being effective at modelling production parameters in batch systems (Donoso-Bravo et al., 2010; Li et al., 2012). Experimental ultimate biogas production (B_o), maximum production rate (R_m), and lag time (λ) parameters were determined as described by Lay (Lay et al., 1996). Cumulative biogas yield, B (mL/g VS) and time of digestion t (h) are independent variables. The modified Gompertz Equation (GM) (Equation 1Equation 3) has been used successfully to model multiple biogases in AD systems. The transference function, or Reaction Curve model (RC), is based on control principles by considering the process as a system receiving inputs and generating outputs to predict maximum gas production. A first order kinetic model (FO) (Equation 5) based on substrate degradation is used to find the coefficient of the limiting rate (k_H), which, for AD is assumed to be hydrolysis. Non-linear optimization and statistical analysis were conducted using GraphPad Prism 6.01 and MS Excel 2013.

Table 3-2 - Models for determination of biogas production parameters

Model	Equation		Reference
Modified Gompertz (GM)	$B = B_o \cdot \exp\left(-\exp\left(\frac{R_m e}{B_o}(\lambda - t) + 1\right)\right)$	(Equation 3)	(Donoso-Bravo et al., 2010; Lay et al., 1996)
Reaction Curve (RC)	$B = B_o\left(1 - \exp\left(-\frac{R_m(\lambda - t)}{B_o}\right)\right)$	(Equation 4)	(Alqaralleh et al., 2016; Donoso-Bravo et al., 2010)
First Order (FO)	$B = B_o\left(1 - e^{-k_H t}\right)$	(Equation 5)	(Pavlostathis and Giraldo-Gomez, 1991; Redzwan and Banks, 2004)

3.4 RESULTS AND DISCUSSION

3.4.1 Digestion

For a wastewater treatment train utilizing pretreatments to be energetically self-sufficient, the energy obtained from the increased BGP of digestion of pretreated sludge must be able to cover the energy cost of the pretreatment step. The effect of sonication on normalized cumulative BGP (mL BG/ g sludge) is illustrated in Figure 3-1. The ultimate BGP was statistically greater than the control for all sonicated samples. To compare the effects of sonication on ultimate BGP, one way ANOVAs were conducted on ultimate BGP, normalized per total mass of sludge digested for 0, 1, 2, 5, and 10 minutes of sonication.

For BG produced from CAS sludge, there was not a significant effect of sonication on BGP at the $p < 0.05$ level for the 5 conditions [$F(4, 10) = 2.623$, $p = 0.0985$]; however, a t-test performed on 0 min and 1 min BGP at a lower confidence of 90% determined there is a significant difference [$t(3) = 2.967$, $p = 0.0592$] of ultimate BGP between the control, 0 min ($BGP_m = 7.6$, $SD = 0.2$) and 1 min ($BGP_m = 8.00$, $SD = 0.09$) sonication levels representing a 5% ± 3 increase in BGP over the control. While the DD increased with increased sonication, BGP from 1 min sonicated samples did not differ significantly ($p < 0.1$) from 2, 5 and 10 min sonicated samples concomitantly the biogas yield (Y_{sCOD}) based on soluble COD decreased. The fact that there was little difference in cumulative BG productions, as well as the rate of BG production discussed in next section, suggests that there was a balance between the increased yield, based on tCOD, and decreased yield due to inhibition based on sCOD yield discussed in the next section. The extent of pretreatment energy may have been too little for the sludge to achieve cell lysis.

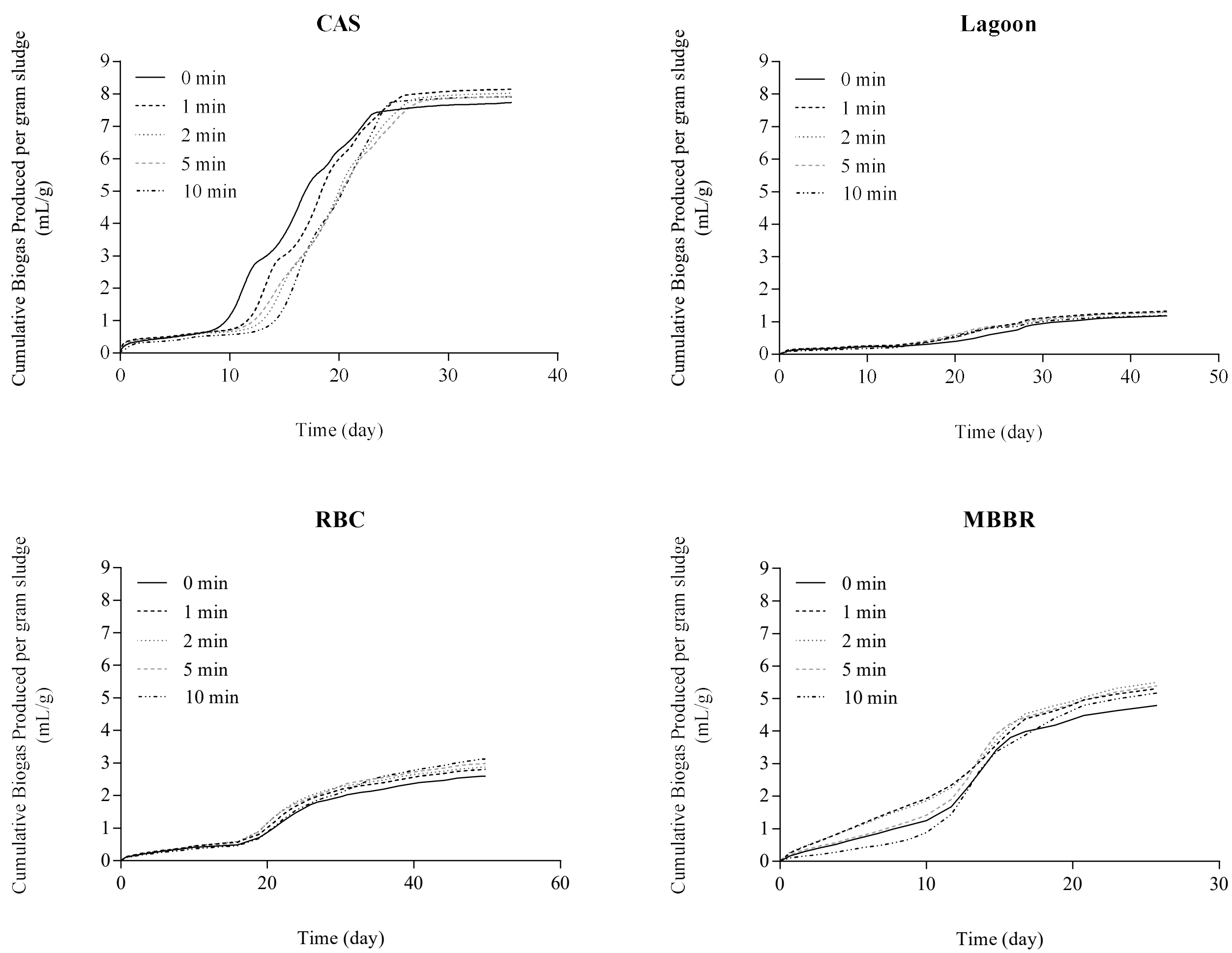


Figure 3-1 Normalized cumulative biogas production for all sludge types and sonication times

An ANOVA conducted on ultimate BGP of lagoon sludges indicated that sonication had a significant effect at the $p < 0.05$ level for all 5 applied energy levels [$F(4, 15) = 5.994$, $p = 0.0044$]. 1 min of sonication produced the greatest final quantity of BG ($BGP_m = 1.326$, SD = 0.003) for all lagoon sludges, increasing BGP over the control by 12% ± 9. A subsequent post hoc comparison using Tukey HSD test found BGP from 1 min sonicated sludge was significantly greater ($p < 0.05$) than the control, 0 min ($BGP_m = 1.2$, SD = 0.1) and 10 min ($BGP_m = 1.19$, SD = 0.02) ultimate BGP, yet not significantly different ($p > 0.05$) than 2 min ($BGP_m = 1.28$, SD = 0.04) and 5 min ($BGP_m = 1.29$, SD = 0.03). The overall low ultimate BGP of 1.19-1.32 mL/g sludge suggests that a majority of the biodegradable organics were already digested over the 5 years the sludge has accumulated in the lagoon. The majority of the starting tCOD was refractory and not susceptible to sonication pretreatment

An ANOVA conducted on ultimate BGP of RBC sludges indicated that sonication had a significant effect on ultimate BGP at the $p < 0.05$ level for this sludge type [$F(4, 15) = 49.75$, $p < 0.0001$]. In this case, 10 min of sonication produced the greatest final volume of BG ($BGP_m = 3.12$, SD = 0.01) for RBC sludges, increasing BGP over the control by 20% ± 2. A post hoc comparison found BGP from 10 min sonicated sludge significantly greater ($p < 0.05$) than 0 min ($BGP_m = 2.59$, SD = 0.04), 1 min ($BGP_m = 2.80$, SD = 0.06), 2 min ($BGP_m = 2.87$, SD = 0.04), and 5 min ($BGP_m = 2.98$, SD = 0.09). This is the only case within the four sludge types tested where increased sonication energy correlated directly with increased BGP. Sonicating RBC sludge for 10 min exhibited the greatest increase in biogas, 20% ± 2 compared to the other sludges (Figure 3-1). The fact that biofilm sludge has a high component of extracellular polysaccharide material the sonication pretreatment may have a very positive impact on solubilizing the EPS and making it more readily available for digestion.

An ANOVA conducted on ultimate BGP of MBBR sludges indicated that sonication again had a significant effect at the $p < 0.05$ level for all levels of sonication [$F(4, 10) = 19.33$, $p = 0.0001$]. The greatest increase of BGP production over the control, 15% ± 2, occurred for the 2 min sonicated sludge ($BGP_m = 5.49$, SD = 0.2) which was significantly greater ($p < 0.05$) than 0 min ($BGP_m = 4.79$, SD = 0.06) and 10 min ($BGP_m = 5.17$, SD = 0.07), yet not significantly different ($p > 0.05$) from 1 min ($BGP_m = 5.30$, SD = 0.04) and 5 min ($BGP_m = 5.4$, SD = 0.2) BGP.

The effects of sonication on solubilization of sludge and increase of BGP appear to vary according to the sludge source tested. When Bougrier et al. established the effective sonication energy ranges of floc disruption and cell lysis mentioned previously, the sludge source was from a secondary municipal WTTP using high-load aeration which was floatation-thickened to 1.85% TS (Bougrier et al., 2005). Other researchers using different secondary sludge sources found the same phases of floc disruption and cell lysis, yet at differing energy levels (Benabdallah El-Hadj et al., 2007; Lehne et al., 2001; Zhang et al., 2007) The present study shows that sludges react uniquely to ultrasound based on their source and treatment technology. The level of solubilisation due to sonication and peak BGP vary according to sludge type. However, peak BGP occurred when the DD was within a small range of 2.9- 7.4 % (Figure 3-2). The sonication energy level that achieved the peak BGP was unique for each different sludge type. Each sludge had a unique value of sonication energy for the point of diminishing returns where DD may increase, but BGP is inhibited. For CAS sludge, the possibility exists that this point was exceeded by 1 min of

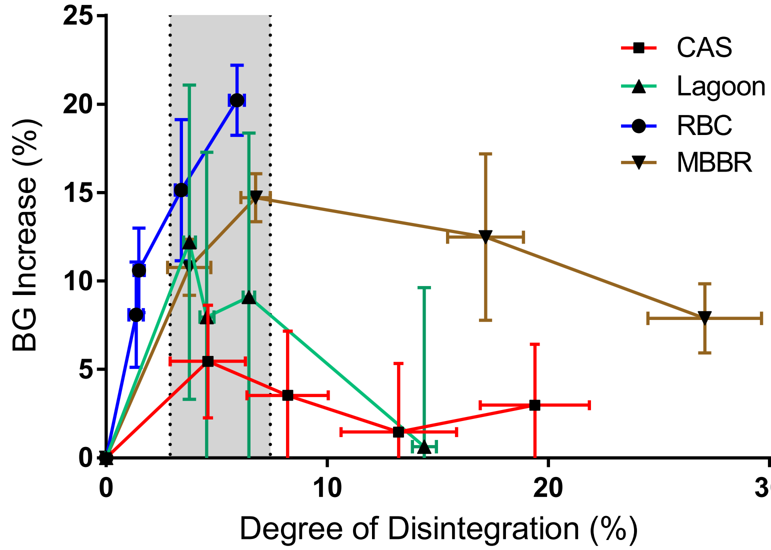


Figure 3-2 Biogas increase vs Solubilization as Degree of Disintegration for all sludge types. Shaded area represents region of maximum biogas increases for 4 sludge types tested.

sonication, hence the lack of significant differences in BGP. These results could explain the large range of literature values for the increased BGP from sonication pretreatment of 19 – 79% (Appels et al., 2008a; Carrère et al., 2010). Although most reported results are from AD of sonicated WAS, variations in treatment style, starting sCOD, sludge age, influent concentrations unique to each source sludge studied could explain the wide variation in BGP increases (Wang et al., 2005).

3.4.2 Solubilization

Concurrent to increased BGP, another purpose of sonication pretreatment is to solubilize particulate and cellular material to shorten the limiting hydrolysis phase of AD, thus increasing the over-all rate of treatment and potentially the extent of stabilization. Sonication can result in both the solubilisation of particulate material as well as production of smaller particulate matter. The degree of sludge disintegration can be used to determine the extent of solubilization caused by pretreatment. Solubilisation was shown to increase with sonication time for all sludge source samples analysed in this study (Figure 3-). A one-way ANOVA comparing the differing sludge types and DD was conducted for 1 and 10 min levels of sonication. The differences in sludge type had a significant effect on mean DD at 1 min [$F(3, 50) = 14.05$, $p < 0.0001$] and at 10 min [$F(3, 50) = 171.9$, $p < 0.0001$]. Post hoc comparison using the Tukey's HSD test indicate that there is only a significant difference between 1 min RBC sludge ($DD_m = 1.36\%$, $SD = 0.32$) and the three other samples of 1 min MBBR ($DD_m = 3.76\%$, $SD = 0.97$), 1 min lagoon ($DD_m = 3.77\%$, $SD = 0.27$), and 1 min CAS ($DD_m = 4.60\%$, $SD = 1.70$). There is no significant difference in 1 min DD between MBBR, lagoon and CAS at this minimum level of sonication. At the highest applied level of sonication (10 min), results indicate that DD of 10 min CAS ($DD_m = 19.39\%$, $SD = 2.47$), 10 min lagoon ($DD_m = 14.39\%$, $SD = 0.54$), 10 min RBC ($DD_m = 5.92\%$, $SD = 0.34$) and 10 min MBBR ($DD_m = 27.07\%$, $SD = 2.57$) are all unique and significantly different from each other. This suggests that sludge source can be a cause of significant variation in disintegration; the effect becoming more pronounced at higher levels of sonication. This is contrary to previous research that simply modeled DD linearly by sonication time alone (Zhang et al., 2007).

In this case, BG yield was measured as BG produced per mass of sCOD consumed during digestion. As DD increased and more sCOD was available for digestion, the yield decreased (Figure 3-). A comparison of yield (Y_{sCOD}) at 0 and 1 min sonication for each sludge type was conducted by t-test to determine the impact of low-powered sonication. There was a significant Y_{sCOD} decrease ($p < 0.0001$) between 0 min and 1 min across all sludge types: CAS (0 min $Y_{sCOD} = 1.54$, $SD = 0.11$; 1 min $Y_{sCOD} = 0.98$, $SD = 0.09$), lagoon (0 min $Y_{sCOD} = 8.36$, $SD = 2.56$; 1 min $Y_{sCOD} = 1.64$, $SD = 0.34$), RBC (0 min Y_{sCOD} =

61.64, SD = 7.81; 1 min Y_{sCOD} = 6.18, SD = 0.60) and MBBR (0 min Y_{sCOD} = 1.59, SD = 0.04; 1 min Y_{sCOD} = 1.17, SD = 0.05). The decrease in biogas yield continued as the degree of sonication was increased to 10 minutes. In particular the RBC sludge was effected most as biogas yield decreased 10 and 60 fold from 62 to 6.2 and from 62 to 1.2 for 1 and 10 minute sonication times.

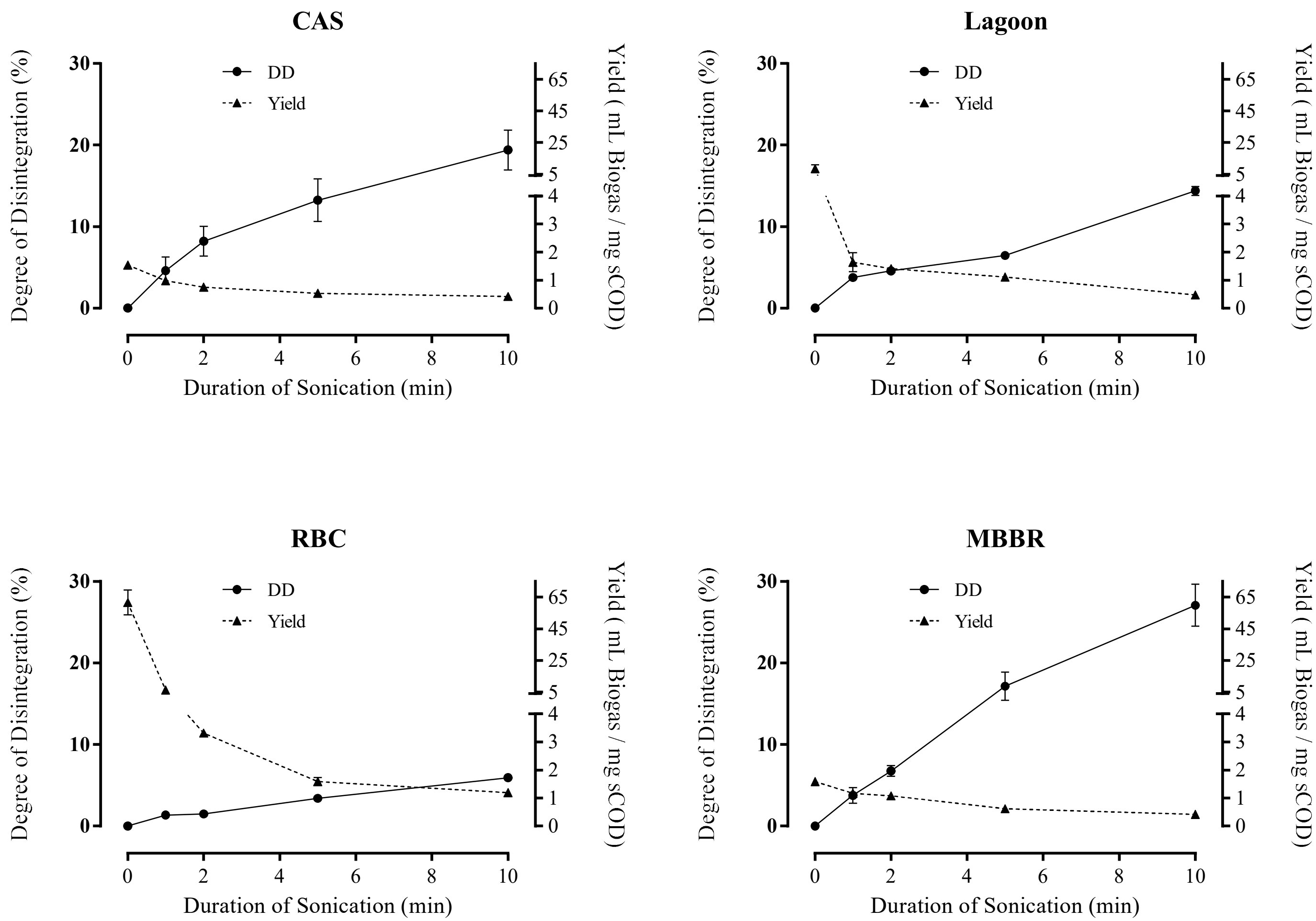


Figure 3-3 - Solubilization and yield (Y_{sCOD}) vs. sonication times for all sludges

The statistically significant difference in Y_{sCOD} caused by sonication may be caused by similar effects that have been noted in thermal pretreatments using high temperatures. Inhibition to BGP has been noted in high temperature thermalizations greater than 170°C (Carrère et al., 2010; Kim and Lee, 2012; Şahinkaya and Sevimli, 2013). The cause is thought to be the caramelization or burning of substrates and the conversion of carbohydrates and amino acids through Maillard reactions to melanoidins that are difficult or impossible to degrade. Although the bulk temperature of 1 min sonicated samples increased by only 8 °C, sonication can still cause high temperature effects in the sludge through the extreme local conditions of cavitation where the bubbles can have temperatures up to 5000 K (Flint and Suslick, 1991; Tiehm et al., 2001). It was previously reported that similar by-product inhibition was caused by high power sonication (Appels et al., 2008b; Kim and Lee, 2012). However, considering the significant decrease in Y_{sCOD} of all 4 types of sludge at the lowest power tested (1 min, E_S = 800 kJ/kg TS), any level of sonication may exhibit an inhibitory effect akin to high temperature thermalization.

3.4.3 Modeling

Three kinetic models previously used for methane production to describe the AD process and critical digestion performance parameters were tested against the BGP results of this study (Alqaralleh et al., 2016; Donoso-Bravo et al., 2010). Overall, the models showed strong correlation (r^2 = 0.920 – 0.999) with the data and are deemed useful for the accurate determination and comparison of design parameters. As an example, Figure 3-4 illustrates the results of the non-linear regression using the three models for 1 min sonication times. Additional results of non-linear regression can be found in Appendix A. Parameter values derived for all 3 models are found in Table 3-3. The GM model had overall stronger fit ($r^2 \geq 0.961$) and could accommodate a larger data set that had significant lag times better than RC (r^2: 0.920 – 0.995) and FO (r^2: 0.917 – 0.992), but tended to underestimate the max rate of biogas production (slope) for the curve as noted by Donoso-Bravo et al. (Donoso-Bravo et al., 2010).

Table 3-3 - Experimental and Non-linear regression analysis of three models for all sludge types and levels of sonication. Maximum (blue) and minimum (red) values are given.

Sludge Type	Experimental			Modified Gompertz (GM)							Reaction Curve (RC)							First Order (FO)				
	Cum BG	Rm	λ	Bo	SEM	Rm	SEM	λ	SEM	R^2	Bo	SEM	Rm	SEM	λ	SEM	R^2	Bo	SEM	k_H	SEM	R^2
	[mL/g VS]	[mL/g VS·h]	[h]	[mL/g VS]		[mL/g VS·h]		[h]			[mL/g VS]		[mL/g VS·h]		[h]			[mL/g VS]		[10^{-3} h^{-1}]	[10^{-3}]	
CAS																						
0 min	284.9	1.301	274	290.8	0.4	0.941	0.005	222.9	0.7	0.9934	313	1	1.36	0.01	231.6	0.7	0.9752	326	1	3.90	0.04	0.9664
1 min	310.5	1.444	323	319.3	0.4	1.080	0.005	275.5	0.6	0.9949	349	1	1.51	0.01	280.7	0.7	0.9748	368	2	3.75	0.05	0.9646
2 min	321.9	1.352	477	334.0	0.6	1.140	0.006	312.3	0.7	0.9937	363	2	1.64	0.02	326.4	0.8	0.9700	375	2	4.29	0.05	0.9655
5 min	312.4	1.154	465	328.7	0.6	0.994	0.005	295.6	0.8	0.9937	369	2	1.36	0.01	309.0	0.8	0.9715	391	3	3.34	0.05	0.9660
10 min	306.4	1.533	393	315.4	0.4	1.302	0.007	333.1	0.6	0.9948	339	1	1.83	0.02	339.9	0.6	0.9699	356	2	4.60	0.07	0.9551
RBC																						
0 min	108	0.287	355	112	2	0.178	0.006	264	10	0.9834	115	1	0.309	0.008	393	3	0.9871	117	1	2.93	0.09	0.9859
1 min	119	0.339	356	122	2	0.195	0.006	250	10	0.9844	124	1	0.342	0.009	397	3	0.9868	127	1	3.04	0.09	0.9845
2 min	121	0.403	358	124	1	0.202	0.006	235	10	0.9858	125	1	0.370	0.008	393	3	0.9893	126	1	3.42	0.09	0.9880
5 min	125	0.410	361	130	2	0.203	0.006	241	9	0.9885	133	1	0.358	0.008	390	3	0.9910	134	1	3.09	0.07	0.9904
10 min	127	0.319	370	136	2	0.202	0.005	292	7	0.9916	149	2	0.303	0.006	402	3	0.9946	157	2	1.97	0.06	0.9918
Lagoon																						
0 min	73	0.291	492	85	4	0.119	0.007	300	17	0.9608	94	9	0.175	0.017	424	11	0.9195	100	10	2.0	0.4	0.9169
1 min	79	0.240	413	86	2	0.140	0.005	282	10	0.9872	88	2	0.226	0.009	418	4	0.9830	90	2	3.1	0.2	0.9813
2 min	74	0.223	409	78	2	0.133	0.006	302	11	0.9825	80	2	0.241	0.009	409	1	0.9724	79	2	3.8	0.2	0.9725
5 min	74	0.173	274	81	2	0.116	0.003	227	9	0.9896	83	2	0.184	0.009	399	6	0.9773	81	2	3.3	0.2	0.9756
10 min	60	0.167	396	64	1	0.112	0.004	252	9	0.9882	66	2	0.178	0.011	394	7	0.9671	64	1	4.1	0.3	0.9623
MBBR																						
0 min	159	0.926	226	172	2	0.80	0.03	240	4	0.9873	198	3	0.98	0.04	298	2	0.9888	193	3	9.9	0.6	0.9800
1 min	192	0.752	173	216	2	0.65	0.02	221	3	0.9934	216	2	1.02	0.04	303	2	0.9906	214	2	9.7	0.5	0.9879
2 min	187	0.805	189	207	2	0.72	0.02	236	2	0.9948	209	2	1.06	0.04	306	1	0.9906	209	2	9.9	0.4	0.9903
5 min	186	1.289	238	197	2	0.94	0.04	241	3	0.9895	199	3	1.34	0.09	302	2	0.9654	197	3	14.2	1.0	0.9599
10 min	157	1.112	246	180	2	0.82	0.02	237	2	0.9952	191	3	0.98	0.04	298	2	0.9888	186	3	9.9	0.6	0.9800

Parameters and Standard Error of the Mean (SEM) with following degrees of freedom: CAS: GM (2217), RC/FO (1944); RBC GM (113). RC/FO (89); Lagoon GM (69), RC/FO (45); MBBR GM (55), RC/FO (35)

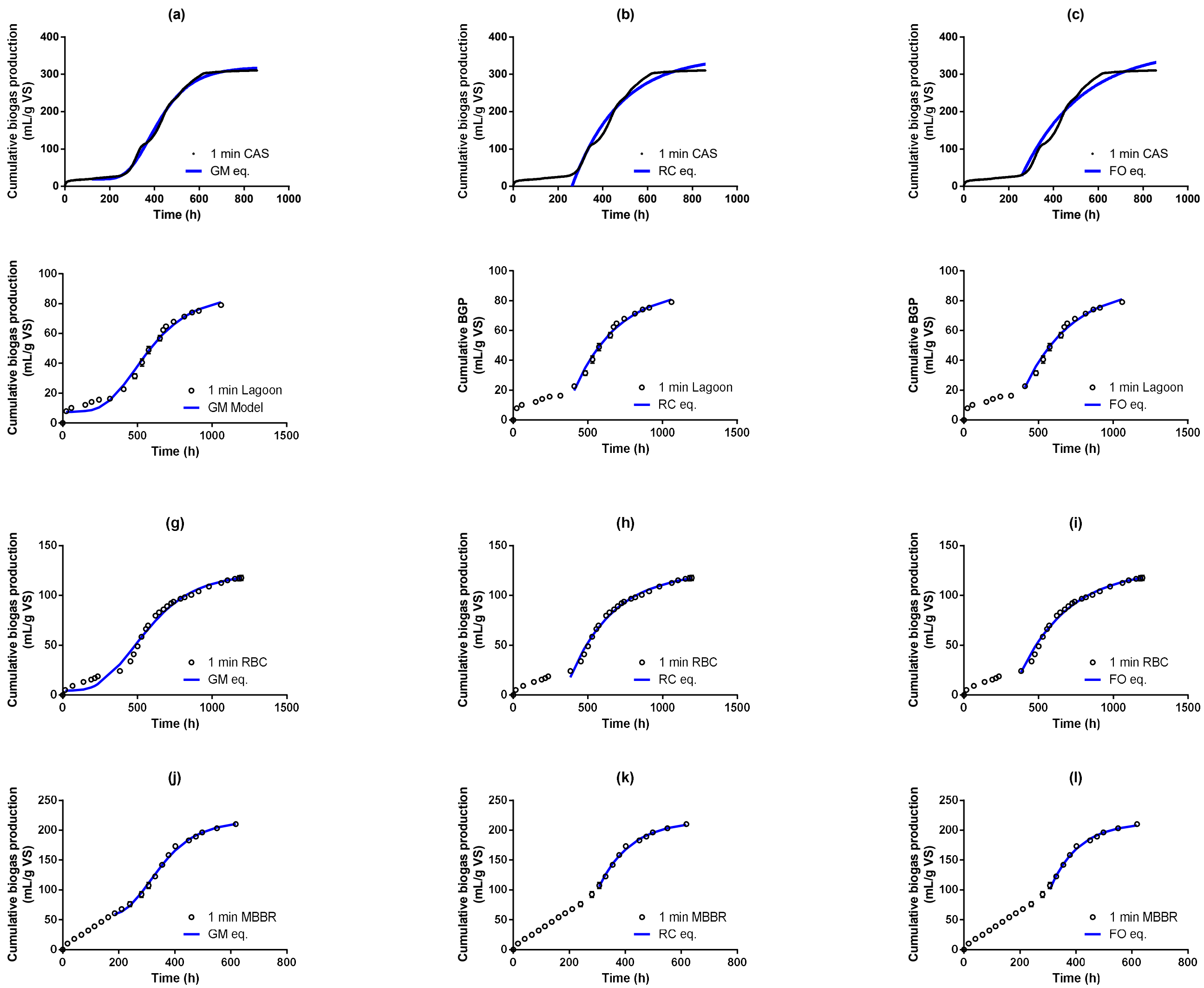
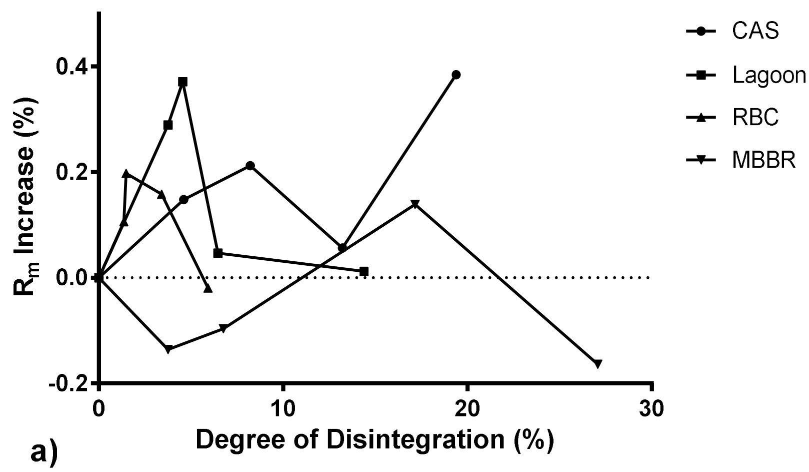


Figure 3-4 Modified Gompertz (GM), Reaction Curve (RC) and First Order models fit to mean cumulative biogas production for 1 min of sonication for CAS (a-c), lagoon (d-f), RBC (g-i), MBBR (j-l). Standard deviation of biogas production is not included for CAS.

A one way ANOVA was conducted on each model parameter to compare the effects of sonication on B_o, R_m, Lag, and k_H for 0, 1, 2, 5, and 10 minutes of sonication pretreatment. All 32 tests showed a significant effect of sonication on the individual design parameters at the $p < 0.05$ level. There is no case in which the control sludge without sonication pretreatment has the greatest modeled maximum (R_m) or overall (k_H) kinetic rate (Table 3). There is no case in which the highest BGP occurs at the same sonication level as the highest maximum (R_m) or overall (k_H) rate.

Ultimate BGP has already been discussed in the previous section. Since there are two values available for R_m, a comparison of the standard deviation of residuals ($S_{y.x}$) for GM and RC models was conducted over the same, truncated data set as used by the RC model to determine which model deviated the least from experimental values and could thus more accurately portray R_m. For CAS sludge, the better parameter is derived from the GM model while RBC, Lagoon and MBBR R_m are better predicted by the RC model. A comparison of R_m and k_H modelled parameters with DD were found to be significantly different ($p < 0.05$) based on separate ANOVA tests, yet there is no clear effect of DD on R_m or k_H (Figure 3-1), which is concurrent with Donoso-Bravo et al's findings (Donoso-Bravo et al., 2010). It is possible that the increased initial sCOD generated from sonication pretreatment has no effect on the maximum rate of digestion or the overall apparent hydrolysis coefficient while still significantly being able to impact the ultimate BG yield.



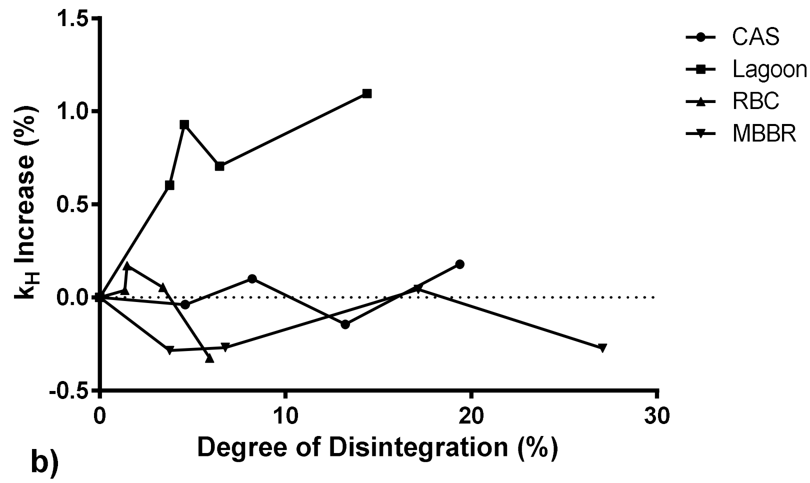


Figure 3-1 Change in modelled parameters of maximum rate of biogas production (a) and apparent hydrolysis rate (b) with respect to solubilization as degree of disintegration

Concerning the lag time before active digestion, previous studies show that inoculum microbes require time to adjust ribosome and enzyme production to accommodate the available substrates (Rolfe et al., 2012). Since inoculum was not acclimatized to the substrates before digestion, a significant lag phase was expected, but is usually neglected in kinetic analysis due to highly variable and uncertain lag phase length in batch experiments (Lay et al., 1996). Experimental lag as reported in Table 3-3 was determined by extrapolating a line from the point of R_m to the axis with slope of R_m (Lay et al., 1996). However, CAS data shows apparent diauxic characteristics where multi-phasic BGP is evident, separated by multiple, short duration plateaus in the profile (Figure 3-), making the aforementioned method unreliable and inconsistent since the maximum BGP rate could occur in different phases resulting in a nonsensical value for lag. In this case, a better separation between lag and growth phases was determined by the time when BGP first exceeded 2 mL/day. The BGP varies in the lag phase yet, when it reaches 2 mL/h, it does not decrease again. Using this estimation for the time when lag phase ends matches with the graphical data (Figure 3-, Figure 3-6) and will be used to delineate between lag phase and the start of the active digestion phase, which ends when BGP again decreases below 2 mL/h.

In general, except in the case of 1 min, 2 min and 10 min sonication pretreatment of MBBR sludge, sonication does increase the maximum rate of digestion, but not in a clearly definable pattern. Ideal sonication energy may be unique for each sludge type and dependent on system requirements, whether the system will be designed for increased kinetics or ultimate biogas production, but both may not be possible.

3.4.4 Multi-phasic Biogas Production

The hourly resolution of the CAS BG data illustrates trends that may not be evident with daily manual BG collection methods such as those used in this study for RBC, Lagoon, and MBBR data collection. The cumulative BGP curves for CAS show multiple production phases separated by short duration, mini-plateaus (Figure 3-2, **Error! Reference source not found.**Figure 3-6) that seem to represent diauxic performance. Diauxic growth patterns become evident when a preferential substrate becomes exhausted, requiring a short lag in metabolic activity while enzymes are synthesised to metabolize less-preferred substrates (Hamilton et al., 2005; Marin et al., 2010; Tonon et al., 2017). Most studies commenting on diauxic growth patterns study the BGP and substrate utilization for single types of bacteria (Hamilton et al., 2005) or singular substrate sources such as seaweed (Kim et al., 2014), or duckweed (Tonon et al., 2017). Kim et al. showed multiphasic biogas production with anaerobic digestion of various food wastes at a daily BGP resolution (Kim and Kim, 2017). The present study differs from previously mentioned studies by using hourly BGP data resolution and use of municipal sewages sludges and inoculum. Rather than known concentrations of single substrates, the municipal WAS used in this study are unknown amalgamations of organic matter and microbial flora representing variable sources of substrates which, for simplicity, are defined as single constituents by representing them as sCOD, tCOD, TS and VS.

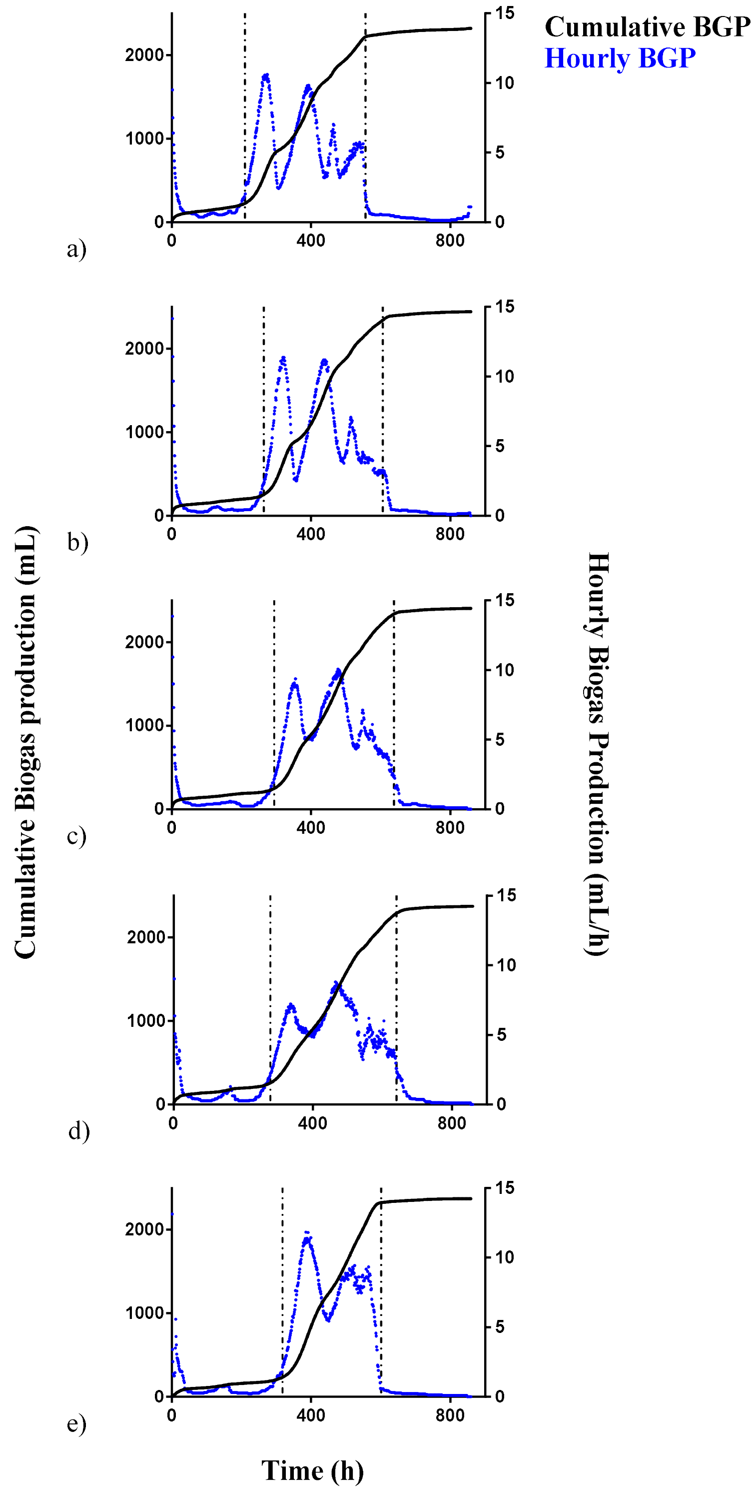


Figure 3-2 Cumulative and hourly BGP with bracketed active digestion times for CAS sludges with sonication of a) 0 min, b) 1 min, c) 2 min, d) 5 min, e) 10 min

The CAS control sludge without pretreatment has four active phases of BGP separated by short lag times (Figure 3-2a) in 345 hours ± 2h of active digestion and a FO modelled hydrolysis coefficient of $3.90 \cdot 10^{-3}$ h^{-1}. CAS 10 min sonicated sludge has 3 phases of BGP resulting in a significantly shorter [$t(4) = 37.74$, $p < 0.0001$] active digestion time of 272 hours ± 2h and a modelled hydrolysis coefficient of $4.60 \cdot 10^{-3}$ h^{-1}.

A one way ANOVA was conducted on all CAS sludge tests to compare the effect of sonication on the active digestion time for 0, 1, 2, 5, and 10 minutes of sonication. There was a significant effect sonication on active digestion time for the 5 conditions [$F(4, 10) = 7.654$, $p = 0.0043$]. The 10 min sonicated sludge had the shortest active digestion time in hours ($Dt_m = 272$, SD = 2) for all CAS sludges which is 22.88% ± 0.01 less than the control without pretreatment. A subsequent post hoc comparison using Tukey HSD test found that the active digestion time for 10 min CAS sludge was significantly less ($p < 0.05$) than the control, 0 min ($Dt_m = 345$, SD = 2), 1 min ($Dt_m = 340$, SD = 20), 2 min ($Dt_m = 345$, SD = 9), and 5 min ($Dt_m = 360$, SD = 40) sonicated sludges, which all had 4 phases of BGP.

The multiphasic trends in the hourly BGP curves of unsonicated CAS sludge implies a hierarchy of preferential substrates (Figure 3-2). As the duration of sonication pretreatment increases, the height and duration of the third and fourth peaks decrease until the fourth peak is non-existent in the BGP curves for 10 min CAS sludge. If the diauxic patterns in the BGP are representative of diauxic patterns in substrate utilization, as shown by Kim et al (Kim et al., 2014), then sonication pretreatment is effective at homogenizing the most recalcitrant substrates that compose the fourth BGP peak into ones that can be digested with other more preferable, more easily digestible substrates, thus shortening active digestion time through the removal of an additional lag phase. This matches previous studies that reported reduced digestion times for sludges sonically pretreated (Nickel and Neis, 2007; Tiehm et al., 1997).

3.5 CONCLUSIONS

In this work, sonication pretreatment was proven to significantly increase tCOD biogas yield in mesophilic batch assays of the four sludge types tested. It had a greater impact on increasing BGP from biofilm type sludge (15-20 % for MBBR and RBC) as compared to conventional, suspended growth technologies (5-12% for CAS and Lagoon). The different sludge types tested responded uniquely to the same levels of sonication energies. An optimal specific energy for the greatest production of BG was not found that coincided for all sludges. Instead, optimal specific energy was unique for each sludge, but the peak BGP for all sludges occurred within a small solubilization range of 2.9-7.4 % DD. Sonication pretreatment exhibited significant inhibition relative to sCOD BG, even at the lowest applied energy levels of 800 kJ/kg TS. In most cases, there was no significant difference ($p < 0.05$) in increased biogas production between low (1 min, 800 kJ/kg TS) and high (10 min, 6550 kJ/kg TS) energy levels.

Three models were used to fit experimental data and determine ultimate biogas production, maximum rate of digestion, lag time and rate of hydrolysis coefficient. GM, RC and FO models show strong correlations with sonicated waste sludge BGP. The GM model was useful for fitting experimental data with significant lag time. R_m and k_H parameters were successfully determined with RC and FO models when data sets were truncated to remove lag time. Sonication had no clear effect on R_m or k_H which were shown to be poor indicators of the effect of sonication pretreatment on digestion kinetics based on the data set of this study.

The use of automated BG logging revealed diauxic growth patterns in the BGP of CAS WAS. The duration of the active phase of BGP decreased significantly in the AD of sludge sonicated for 10 min (6550 kJ/kg TS) where the number of active phases of BGP was reduced from 4 to 3.

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CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

WAS from four types of WWTPs were subjected to ultrasonic pretreatment and subsequently digested anaerobically to determine the effect of sonication on increasing sludge solubilization, BGP, and digestion kinetics. The non-conventional waste sludges all had lower BG potentials than conventional sludges. Compared to conventional sludge, RBC sludge had a 63% $\pm$ 6 lower potential, lagoon sludge had 85% $\pm$ 5 lower potential and MBBR sludge had 46% $\pm$ 6 lower potential. While these sludges are anaerobically digestible, a material and energy balance should be conducted on the non-conventional sludges to determine if the low potentials make anaerobic digestion worthwhile.

Concerning the application of US pretreatment on the four varying waste sludges, the optimal sonication energy is unique for each sludge type. This may explain the diversity in reported optimal sonication energies (Table 2-1) as each author tested on sludges unique to their own study. Within the energy range tested, sonication energy should be chosen to achieve a solubilization of 5% DD for optimal BGP. The application of increased energies may increase solubilization, but will also result in an increased inhibition due to decreased sCOD yield. Before applying sonication as a pretreatment for AD, pilot studies and energy balances should be conducted to determine efficacy of sonication effects for the unique sludge source to be digested. Variability in sludge composition through geography, treatment technology, EPS composition and sludge age may have an effect on digestibility and BGP.

4.2 Future Recommendations

The following recommendations are proposed to assist researchers in the further development of the field of anaerobic digestion and ultrasonic pretreatment:

- Energy and material balances should be conducted in the investigation of biogas potential sludges with acclimated inoculums to determine viability of anaerobic digestion as a disposal and stabilization method of non-conventional WAS.
- The attributes of sludges that are the cause of the varied effects of sonication are not known. Repeating the tests conducted in this work with more exhaustive characterization could identify differences in the sludges allowing the creation of models to more accurately predict effects of sonication on solubilization and BGP.
- The impact of sludge age on the results of sonication are unknown but may be a factor. Multiple conventional systems fed with a controlled synthetic wastewater with differing SRTs as a variable could be developed to determine how sonication and AD of sludges are impacted by sludge age.

APPENDIX A: MODEL APPLICATION TO CUMULATIVE BGP

The following section contains additional supporting material. Figures provide the GM, RC and FO model results applied to mean cumulative biogas production for 0 min (Figure A-1), 2 min (Figure A-2), 5 min (Figure A-3), and 10 min (Figure A-4) sonication times. Standard deviation of mean cumulative biogas for CAS tests is not included for the purpose of maintaining clarity.

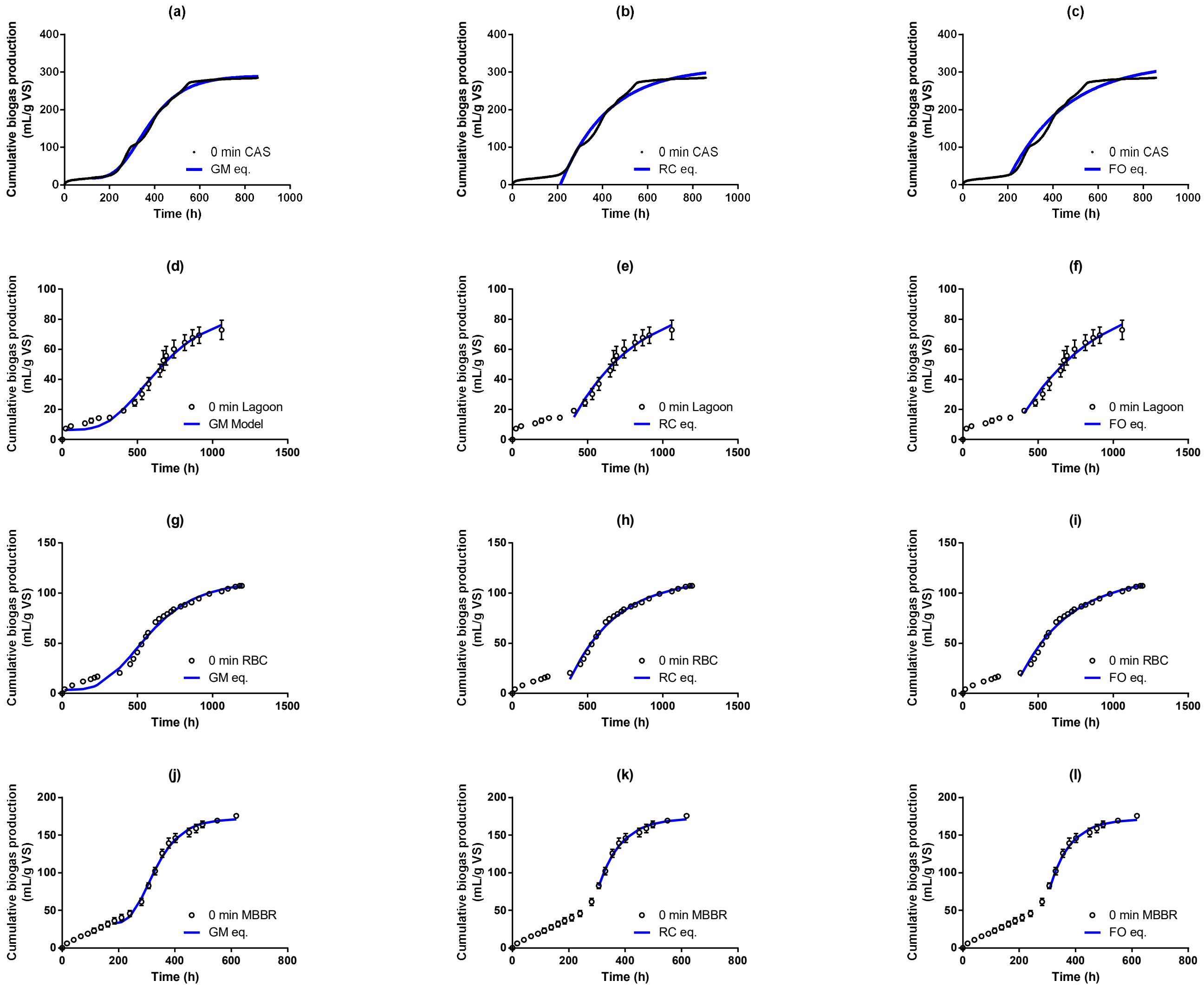

Figure A-1 Modified Gompertz (GM), Reaction Curve (RC) and First Order models fit to mean cumulative biogas production for 0 min of sonication for CAS (a-c), lagoon (d-f), RBC (g-i), MBBR (j-l). Standard deviation of biogas production is not included for CAS.

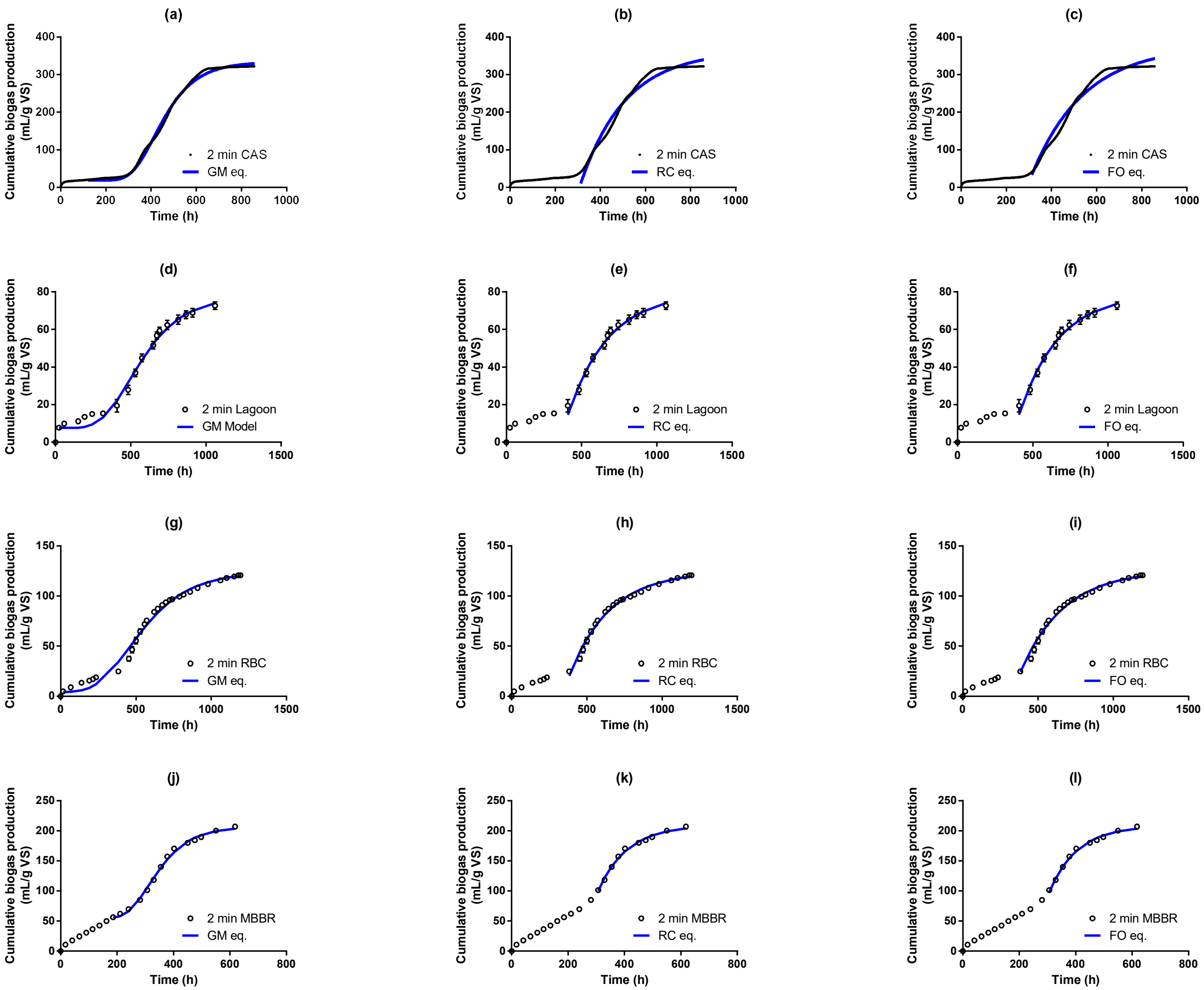

Figure A-2 Modified Gompertz (GM), Reaction Curve (RC) and First Order models fit to mean cumulative biogas production for 2 min of sonication for CAS (a-c), lagoon (d-f), RBC (g-i), MBBR (j-l). Standard deviation of biogas production is not included for CAS.

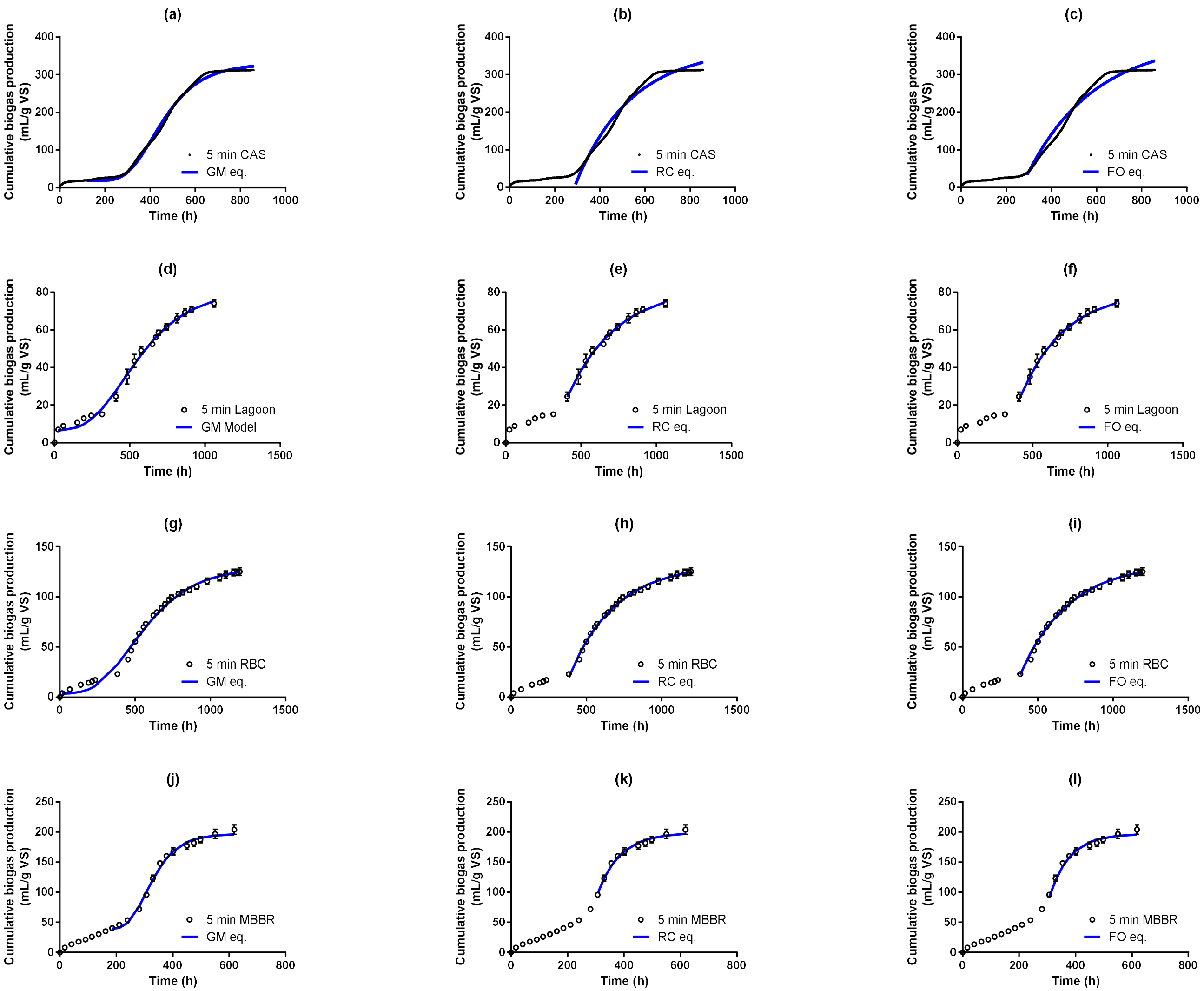

Figure A-3 Modified Gompertz (GM), Reaction Curve (RC) and First Order models fit to mean cumulative biogas production for 5 min of sonication for CAS (a-c), lagoon (d-f), RBC (g-i), MBBR (j-l). Standard deviation of biogas production is not included for CAS.

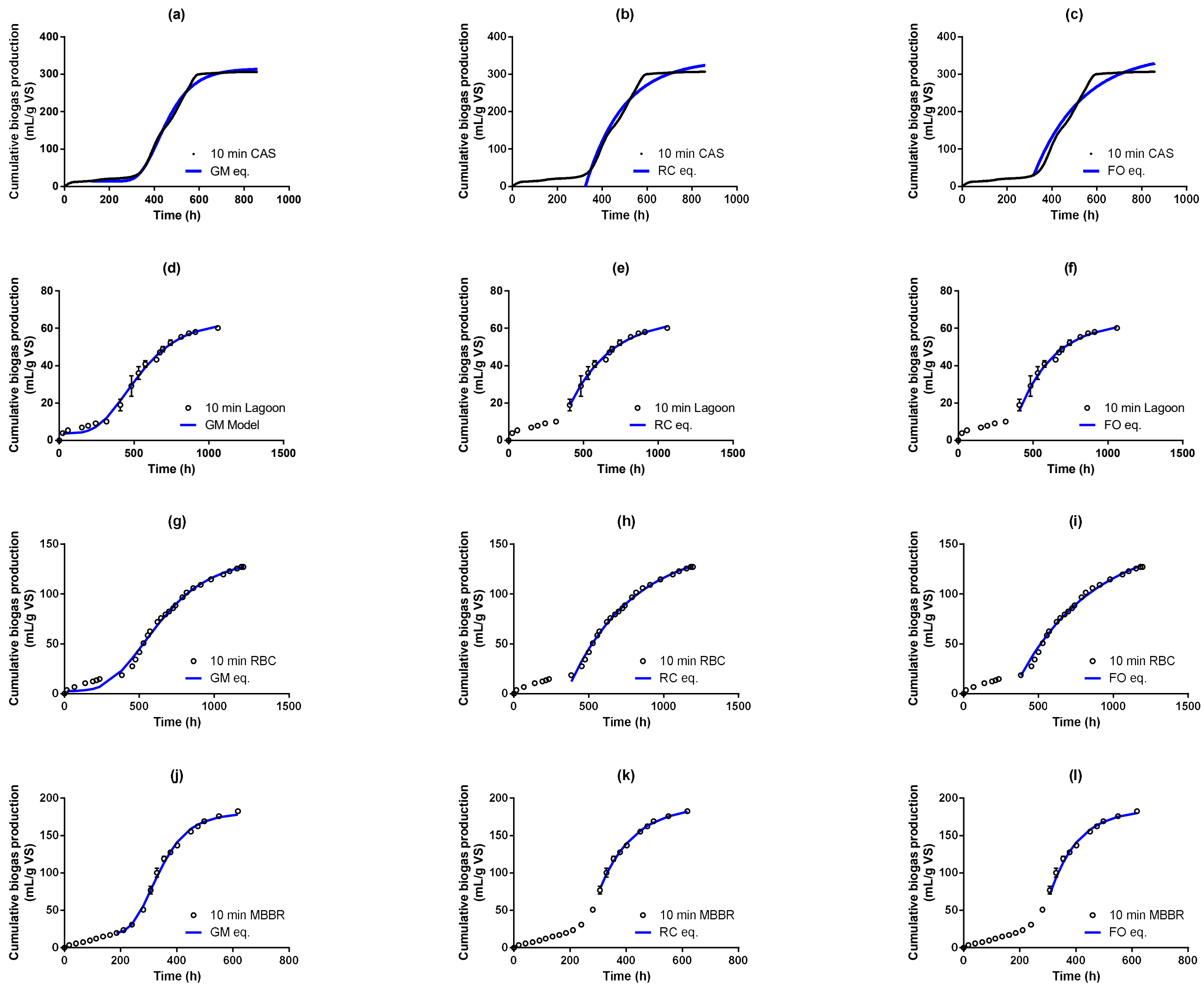

Figure A-4 Modified Gompertz (GM), Reaction Curve (RC) and First Order models fit to mean cumulative biogas production for 10 min of sonication for CAS (a-c), lagoon (d-f), RBC (g-i), MBBR (j-l). Standard deviation of biogas production is not included for CAS.

APPENDIX B: SAMPLE CALCULATIONS

The following section contains additional supporting material on the derivation of some of the values used in this paper.

Table B-1 Values of BGP normalized per gram sludge for CAS and calculated values for Biogas Increase (BGI) over the control, 0 min, for each sonication time and Standard Deviations (Sn).

Sonication Time	**0 min** (mL/g)	**1 min** (mL/g)	**2 min** (mL/g)	**5 min** (mL/g)	**10 min** (mL/g)
Replicate 1	7.351	7.903	7.918	7.475	7.653
Replicate 2	7.603	7.993	7.969	7.769	7.850
Replicate 3	7.794	8.092	7.664	7.836	7.922
Average	7.582	7.996	7.850	7.693	7.808
Sn	0.222	0.095	0.163	0.192	0.139
BGI fraction	-	0.0545	0.0353	0.0146	0.0298
Sn	-	0.0333	0.0248	0.0330	0.0316

The following are sample calculations for finding the BGI and its standard deviation through propagation of error for 1 min sonication. BGP is the mean biogas production normalized per gram sludge, x denotes 0 min, and y denotes 1 min of sonication.

$$\mathrm{BGI} = \frac{\mathrm{BGP}_y - \mathrm{BGP}_x}{\mathrm{BGP}_x} = \frac{7.996 - 7.582}{7.582} = 0.055 \text{ or } 5.5\%$$

$$Sn_{\mathrm{BGI}} = \left(\frac{\mathrm{BGP}_y}{\mathrm{BGP}_x}\right)\sqrt{\left(\frac{Sn_y}{\mathrm{BGP}_y}\right)^2 + \left(\frac{Sn_x}{\mathrm{BGP}_x}\right)^2} = \left(\frac{7.996}{7.582}\right)\sqrt{\left(\frac{0.095}{7.996}\right)^2 + \left(\frac{0.222}{7.582}\right)^2} = 0.033 \text{ or } 3.3\%$$

The following is a representation of One-way ANOVA results from GraphPad comparing all replicate, normalized BGP values for conventional sludge (Table B-1) to determine if sonication has a significant impact on BGP.

ANOVA summary					
F	2.623				
P value	0.0985				
P value summary	ns				
Are differences among means statistically significant? (P < 0.05)	No				
R square	0.512				
Brown-Forsythe test					
F (DFn, DFd)	0.2110 (4, 10)				
P value	0.9264				
P value summary	ns				
Significantly different standard deviations? (P < 0.05)	No				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.2962	4	0.07404	F (4, 10) = 2.623	P = 0.0985
Residual (within columns)	0.2823	10	0.02823		
Total	0.5784	14			
Data summary					
Number of treatments (columns)	5				
Number of values (total)	15				

The results are represented in the text as [$F(4, 10) = 2.623$, $p = 0.0985$] indicating the calculated F-value (2.623) for a degree of freedom between groups (4) and a degree of freedom within groups (10). The P-value, indicating significance, is greater than 0.05; therefore, there is not a significant effect of sonication on the BGP of conventional sludge with 95% confidence.