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Enhancement of Biohydrogen and Biomethane Production from Wastes Using Ultrasonication

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A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

Enhancement of Biohydrogen and Biomethane Production from Wastes Using Ultrasonication

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Abstract

This thesis demonstrated the feasibility of using ultrasonication to solubilize the particulate matter, suppress the growth of methanogens, and enrich the biohydrogen producers, thus overcoming the main challenge of biohydrogen systems i.e. long-term stability and contamination with methanogens. Furthermore, this work emphasized the benefits of applying ultrasonication inside a bioreactor over using it as a pretreatment for biohydrogen and biomethane production from wastes. The results of this work showed that sonicating hog manure at specific energy (SE) of 500 kJ/kg TS resulted in a 20% increase in methane production and 36% increase in VSS destruction. The viability of using ultrasonication as a pretreatment method for elimination of methane producers and enrichment of hydrogen producers has been confirmed at SE of 79 kJ/g TSS. Moreover, hydrogen production in a novel sonicated biological hydrogen reactor (SBHR), which comprised a continuous stirred tank reactor (CSTR) connected with an ultrasonic probe at the bottom of the reactor, was about 85% higher than that in a conventional CSTR. On the other hand, an extensive comparative study of five different mesophilic systems (single and two-stage with and without sonicated feed, and two-stage; SBHR followed by methane reactor) was undertaken using food waste. The results showed that sonication inside the reactor in the first stage showed superior results compared to all other systems with respect to hydrogen production, methane production, and VSS destruction. The study also confirmed the advantages of two-stage mesophilic digestion of food wastes over single-stage systems, as reflected by VSS destruction efficiencies in the range of 51% - 59% versus 36% - 44% at a short SRT of 7 days.

Keywords: anaerobic digestion, pretreatment, ultrasonication, solubilization, hydrogen, methane, food waste, hog manure, degree of disintegration, batch, CSTR, SBHR.

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Chapter 10: Comparative Study of ultrasonic Effect on the Anaerobic Biodegradability of Food Waste

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To my late (deceased) Mom,

To my wife, Lobna, for her love, patience and support,

To my kids, Maryam, Manar, and AbdAllah for the best feelings ever they gave to me,

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Finally, of course the responsibility for any errors or omissions is mine and mine alone.

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Chapter (1)

General Introduction

1.1 Background

The anaerobic digestion process can convert organic wastes to hydrogen and methane in two distinct stages: acidification (first stage) and methanogenesis (second stage). In the first stage organic wastes are converted to hydrogen and volatile fatty acids via hydrogen-producing bacteria while in the second stage the hydrogen and volatile fatty acids are converted to methane via methanogenesis. In general, the limiting step of anaerobic digestion of organic waste is the first step of hydrolysis or solubilization, where the cell wall is broken down allowing the organic matter inside the cell to be available for biological degradation [1]. The anaerobic digestion process may therefore be improved if hydrolysis can be enhanced. Thus, pretreatment is often required in order to achieve the release of lignocellulosic material and thus accelerate the degradation process by means of waste solubilisation and consequently enhance the biogas production during anaerobic digestion [2]. Various pretreatment methods such as thermal, chemical, physical, and biological have been studied by many researchers [3].

Hydrogen, as an energy carrier, offers numerous advantages over other conventional energy carriers. The major advantage of energy from hydrogen is the absence of polluting emissions since the utilization of hydrogen, either via combustion or via fuel cells, results in pure water [4]. At present, hydrogen is produced mainly from fossil fuels, biomass, and water using chemical or biological processes. Anaerobic (or dark) fermentation and photosynthetic degradation are the two most widely studied biohydrogen production techniques [1]. Anaerobic fermentation is promising for sustainable hydrogen and methane production since organic matter,

including waste products, can be used as a feedstock for the process [5]. However, the rate of biological H_2 production is low and the technology needs further development [6].

Hydrogen partial pressure and the resulting H_2 concentration in the liquid phase are key factors affecting fermentative H_2 production [3]. Generally, high H_2 partial pressure has a negative effect on H_2 production by decreasing the activity of *hydrogenase* and making the H_2 production reaction thermodynamically unfavourable [7]. Various techniques have been used to remove metabolic gases (H_2, CO_2) from the liquid phase [8]. Gas sparging has been the most common method used to decrease the concentrations of dissolved gases in fermentative H_2-producing bioreactors. Other techniques to decrease concentrations of dissolved gases include increased stirring [9], decreasing the reactor headspace pressure i.e. applying a vacuum [10], and using an immersed membrane to directly remove dissolved gases [10]. The disadvantage of the gas sparging is that the sparging gas should be free of CO_2 so as not to inhibit *hydrogenase* [7]. In addition, too much sparger gas dilutes the H_2 content in the headspace and creates problems in the separation and utilization of the hydrogen [11].

Ultrasonication causes a localised pressure drop to below the evaporating pressure in the aqueous phase, resulting in the formation of microbubbles or cavitation bubbles [12]. During cavitation, microbubbles form at various nucleation sites in the fluid and grow during the rarefaction phase of the sound wave [13]. Subsequently, in the compression phase, the bubbles implode and the collapsing bubbles release a violent shock wave that propagates through the medium [14], disrupting biosolids flocs and bacterial cells, releasing intracellular components, subsequently improving the rate of anaerobic degradation due to the solubilisation of the particulate matter, thus decreasing solids retention time (SRT), and improving the overall performance of anaerobic digestion [15]. Furthermore, the use of ultrasonication in the

pretreatment of waste activated sludge (WAS) improved the operational reliability of anaerobic digesters, decreased odor generation and clogging problems, and enhanced sludge dewatering [16]. On the other hand, ultrasonication can enhance hydrogen production when applied inside the bioreactor. The mechanisms for enhancement of hydrogen production by ultrasonication inside the bioreactor include but not limited to one or more of the following: (1) decreasing the dissolved hydrogen concentration, (2) enhancement of the mass transfer, and/or (3) solubilization.

1.2 Objectives

The main goal of this study is to investigate the applicability of ultrasonication to solubilisation of particulate matter and enhancement of hydrogen and methane production from wastes. The specific objectives are as follows:

1. Evaluation of the impact of ultrasonication on solubilisation and anaerobic biodegradability of hog manure and food wastes.
2. Correlating easy-to-measure solubilisation parameters with the laborious and expensive degree of disintegration method.
3. Studying the effect of ultrasonication on odor reduction, specifically the removal of bound protein and hydrogen sulfide from the headspace of continuous-flow anaerobic digesters.
4. Comparative evaluation of the effect of individual and combined pretreatment methods (ultrasonic with heat shock, ultrasonic with acid, and ultrasonic with base), on the solubilisation of food waste and biohydrogen production.

5. Development of a novel sonicated biological hydrogen reactor (SBHR) for hydrogen production and compare it with the most common bioreactor, the continuous stirred tank reactor (CSTR).
6. Comparative assessment of single and two-stage anaerobic digestion processes utilizing ultrasonication for food wastes.

1.3 Thesis organization

This thesis comprises eleven chapters and conforms to the "integrated-article" format as outlined in the Thesis Regulation Guide by the School of Graduate and Postdoctoral Studies (SGPS) of the University of Western Ontario. The thesis consists of the follows chapters:

Chapter 1 presents general introduction and the objectives of this study.

Chapter 2 presents a review of literature on anaerobic digestion, methane production, hydrogen production, and ultrasonication pretreatment.

Chapter 3 discusses the impact of ultrasonication on solubilisation and anaerobic biodegradability of hog manure and ensuing enhancement of methane production.

Chapter 4 presents the impact of ultrasonication of hog manure on the performance of anaerobic digestion and its effect on odor precursors reduction, specifically the removal of bound proteins and gaseous hydrogen sulfide.

Chapter 5 discusses the effect of ultrasonication on food waste solubilisation and therefore enhancement of biohydrogen production.

Chapter 6 demonstrates the impact of ultrasonication on biomethane and biohydrogen production.

Chapter 7 presents the impact of four individual pretreatment methods (ultrasonic, heat shock, acid, and base) and three combined pretreatment methods (ultrasonic with heat shock, ultrasonic with acid, and ultrasonic with base), on the solubilisation of food waste and biohydrogen production.

Chapter 8 introduces the novel sonicated biological hydrogen reactor (SBHR) for biohydrogen production and compares it with the most common bioreactor, the continuous stirred tank reactor (CSTR).

Chapter 9 discusses the applicability of ultrasonication to food wastes and compares the hydrogen production from three different systems employing various approaches for ultrasonication (inside and outside the reactor).

Chapter 10 presents a comparison of single and two-stage anaerobic digestion processes utilizing ultrasonication for food waste degradation, specifically evaluating the impact of ultrasonication on solubilisation, and hydrogen and methane production.

Chapter 11 summarizes the major conclusions of this research and provides recommendations for future research directions based on the findings of this study.

1.4 Contribution of Thesis

Biogas production from wastes provides an environmentally-friendly waste management technique as well as a sustainable approach producing renewable energy. Although anaerobic digestion is a very old process, significant research efforts are currently underway to enhance the biological conversion process performance for methane and hydrogen production. The two most important contributions of this work are: first, introducing the ultrasonication pretreatment, as a novel pretreatment for enhancement of biohydrogen production, which doubled biohydrogen

production from glucose. Second, developing the sonicated biological hydrogen reactor (SBHR) followed by a methane bioreactor (US patent-pending). This novel system has multi functions: a) solubilisation of particulate organics, and b) removal of dissolved gases, thus improving mass transfer and biohydrogen yield, and increasing the microorganisms' growth rate. The results from this novel system using the source separated organics solid waste obtained from the Dufferin Organics Processing Facility (DOPF) in Toronto, Ontario, emphatically revealed the benefits of using the SBHR which doubled biogas production and affected more than 60% increase in solids reduction efficiency, thereby reducing off-site transportation costs and associated GHG emissions. Moreover, this novel system has the potential to mitigate the solid waste problems through diversion of the organic fraction to produce "green" biogas.

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CHAPTER (2)

Literature Review[1]

2.1. Introduction

Energy is the most important element for the development of nations. World energy consumption is projected to expand by 49 percent from 2007 to 2035 [1]. The rapid growth of the world population combined with concomitant economic development exerts drastic increase in global energy demand. Currently, the majority of the world energy needs are supplied through carbon-containing fossil fuel sources such as coal, natural gas, and oil. The widespread use of these fossil fuels had a significant impact of industrialized societies; since the side effects of using fossil fuel are detrimental to the environment and human health. Fossil fuels come from non-renewable sources, and combustion of these fossil fuels is considered as the largest contributing factor to the release of greenhouse gases such as CO_2 into the atmosphere and associated climate change [2]. Furthermore, in recent years, due to the economic conditions such as increasing oil prices as well as the negative environmental impacts, government initiatives in many countries are focusing on the increased use of various renewable energies including solar, wind, biomass, hydro-power, tidal energy, and energy from waste.

Protecting and restoring the environmental damage has become a global concern now. Due to changes in life style as well as the industrial development, large quantities of domestic, industrial and agricultural wastes are being generated all over the world. The proper management of these wastes continues to be a major concern due to the risk of air, water and soil pollution.

[1] A version of this chapter is in press as a chapter entitled "State of the Art of Biogas Production from Solid Waste and Wastewater" in Handbook of Biogas published by *Nova Science Publishers, Inc.*

Over 1.8 billion tonnes of waste including households, industry and agriculture, etc. are generated each year in Europe alone [3]. With such vast quantities of waste being produced, resource and energy recovery is an integrated part of an efficient waste management program [4]. Biogas production from waste provides an environmentally friendly way for waste management as well as production of sustainable renewable energy (Figure 2.1).

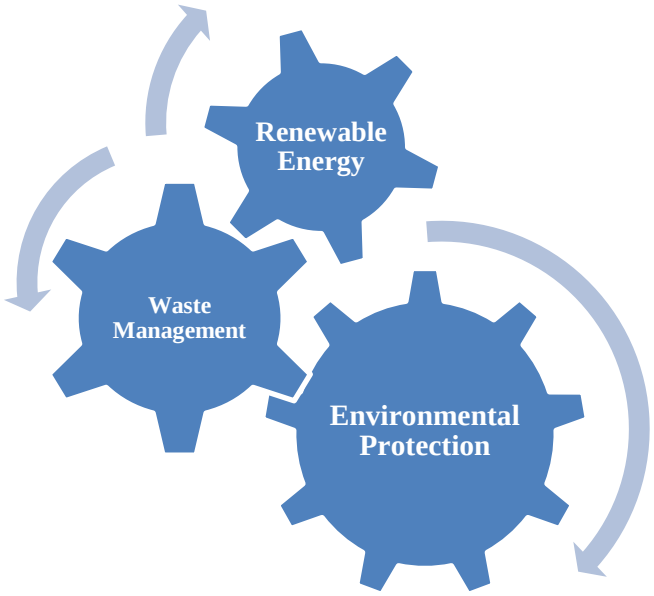

Figure 2.1 Benefits of Biogas Production.

Biogas is produced through anaerobic digestion (AD) of organic wastes. AD has been in use for centuries [5]. Anaerobic digestion of waste by microorganisms is a widely accepted and well established technology for processing variety of wastes in absence of oxygen at low temperatures and pressures, mostly at ambient conditions. Moreover, these technologies are suitable for decentralized energy production with small-scale installations. The major biogases produced from the biological conversion of waste are methane and hydrogen.

Although anaerobic digestion is a very old process, significant research efforts are underway to enhance the biological conversion process performance for methane and hydrogen production. Until recently, only biological methane production from waste has been widely practiced, although hydrogen is also an important intermediate product in biological methane production, which needs decoupling and separation from methane production. Although biological hydrogen from wastes has been demonstrated at the lab scale [6], and pilot-scale [7] levels, further research is needed for practical and commercial applications.

This chapter addresses the technical overview of biological methane and hydrogen production from various types of wastes. The basics of anaerobic digestion for bio-methane production, related process parameters, various digester technologies and recent advances, benefits of bio-methane production are outlined in section 2 of this chapter. Section 3 provides the technical overview of the biohydrogen production from waste. Section 4 provides the mechanisms and parameters affecting the use of ultrasonication pretreatment for enhancement of bio-hydrogen and bio-methane production.

2.2. Bio-methane

Bio-methane is produced through the anaerobic degradation of organic content of wastes by a diverse group of microorganisms. Depending on the feedstock, the biogas produced from anaerobic digestion of waste usually contains 40-70% methane (CH_4) and 30-50% carbon dioxide (CO_2). In addition, biogas also contains significant amounts of undesirable compounds such as hydrogen sulfide, ammonia, mercaptans, siloxanes etc. and needs to be cleaned before it is used as fuel in boilers and combustion engines [8]. Typical biogas composition and its

contaminants' concentrations are shown in Table 2.1 [9]. Presence of hydrogen sulfide above 100 ppm requires installation of additional hydrogen sulfide removal processes [8].

Table 2.1 *Typical Biogas Composition and Contaminants [9].*

Compounds	Unit	Composition
Methane (CH_4)	(Volume %)	40-70
Carbon-dioxide (CO_2)	(Volume %)	30-50
Nitrogen (N_2)	(Volume %)	0-20
Oxygen (O_2)	(Volume %)	0-5
Hydrogen Sulfide (H_2S)	(ppm_v)	0-2000
Mercaptans	(ppm_v)	0-100
Siloxanes	(ppm_v)	0-100
Halogenated hydrocarbon	(ppm_v)	0-100

2.2.1 Bio-methane Production from Waste

Anaerobic Digestion (AD) is defined as a multi-step biochemical process in which organic waste materials are broken down in by a causation of facultative and anaerobic microorganisms an oxygen-free environment. The basic steps involved in anaerobic digestion shown in Figure 2.2 are hydrolysis, acidogenesis, acetogenesis, and methanogenesis [10]. Microorganisms are not able to take up non-soluble and particulate substrates that are too large to pass through the cell membrane and therefore extra-cellular enzymes (cellulases, amylases,

proteases, lipases) are released to cleave polymers into smaller substrate molecules. This process is the bacterial hydrolysis where insoluble organic polymers (carbohydrates, lipids and proteins) are solubilized making them available for biological degradation. Hydrolysis is regarded as the rate limiting step for insoluble polymers [10]. The second step is the acidogenesis which is the first energy-yielding step, where the products of hydrolysis further degrade to form volatile fatty acids (VFAs such as acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acids etc.), ammonia, hydrogen sulfide, carbon dioxide, and other by-products. The next step, known as acetogenesis, involves acetogenic bacteria which convert organic acids into acetic acid, hydrogen, and carbon dioxide. The final stage of anaerobic digestion is methanogenesis wherein methane is produced by two groups of methanogenic organisms: acetoclastic methanogens which degrade acetate into methane and carbon dioxide, and hydrogenophilic methanogens which use hydrogen as electron donor and carbon dioxide as acceptor to produce methane [8, 11].

Approximately 70% of the total methane is produced from the conversion of acetic acid (CH_3COOH) to methane (CH_4) by acetoclastic methanogens.

$$4CH_3COOH \rightarrow CH_4 + 2CO_2 \qquad (2.1)$$

The remaining 30% comes from the reduction of carbon dioxide (CO_2) and hydrogen (H_2) by hydrogen utilizing/ CO_2-reducing methanogens.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (2.2)$$

2.2.2 Process Kinetics

Based on the biochemistry and microbiology of the anaerobic process, process kinetics play an important role in the development and operation of anaerobic systems. Process kinetics provides a rational basis for analysis, control, and design. The understanding of process kinetics

is also essential for predicting system stability, effluent quality, and waste stabilization efficiency [13]. For a waste material, the amount of organic content usually present as chemical oxygen demand (COD) is anaerobically broken down during digestion by anaerobic microorganisms and converted to biogas.

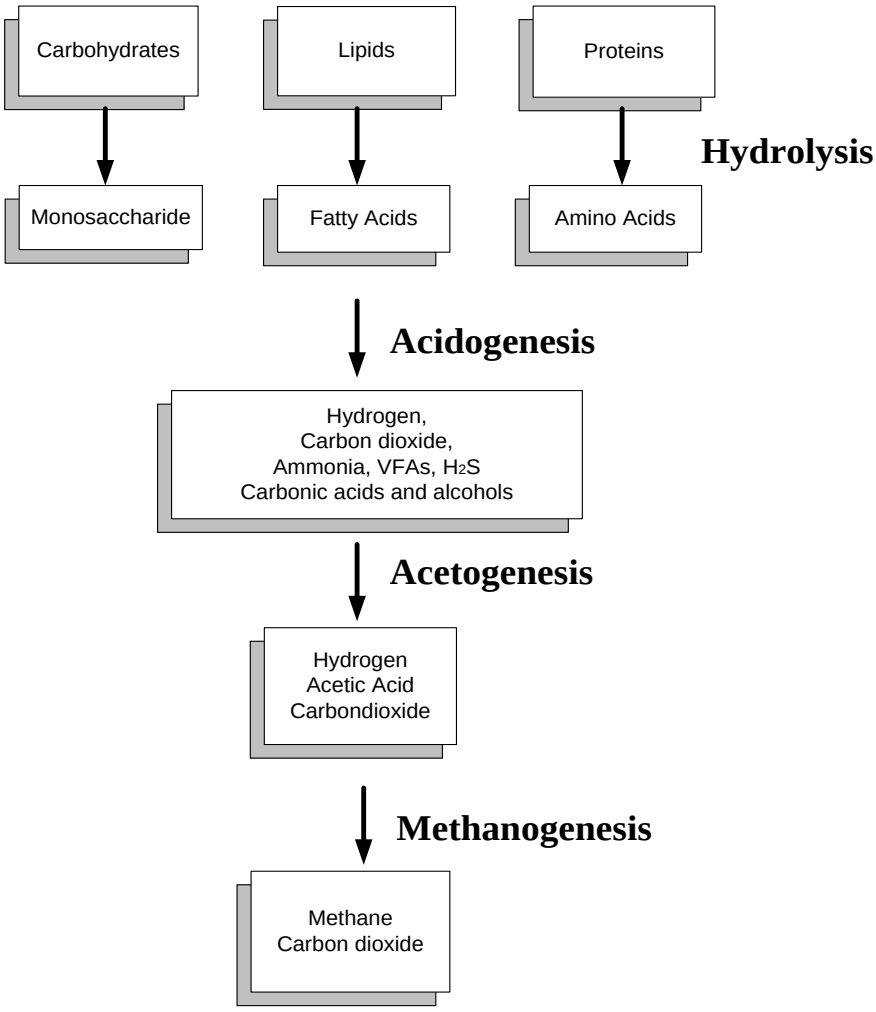

Figure 2.2 Basic Steps of Anaerobic Digestion [12].

Conversion rates during anaerobic treatment of soluble substrates are generally described by Monod kinetics [14, 15] as shown below.

$$\frac{dx}{dt} = \frac{\mu_m XS}{(K_s+S)} \quad (2.3)$$

$$\frac{dX}{dt} = Y\frac{ds}{dt} - K_b X \quad (2.4)$$

Where,

S = substrate concentrations (mg/L)

X = biomass concentrations (mg/L)

μ_m = maximum substrate consumption rate (mg COD/mg VSS-d)

K_s = half-saturation concentration (mg COD/L)

Y = yield of biomass to substrate (mg VSS/mg COD)

K_b= decay constant d^{-1}

Hydrolysis was found to be of paramount importance in the overall process kinetics, even in cases where acidogenesis or methanogenesis were considered to be rate limiting [10, 14]. Hydrolysis of organic polymers is often described by a first-order kinetic model:

$$r_S = K_h S \quad (2.5)$$

Where, K_h is the hydrolytic constant and S is the substrate concentration. Extensive research has been conducted on the kinetics of anaerobic digestion and excellent reviews are provided by Mata-Alvarez et al. [16] and Gunaseelan [17].

2.2.3 Advantages and Disadvantages of Anaerobic Digestion

The major advantages of anaerobic digestion as a waste management process are [8, 18, 19]: i) reduction of waste volume and pathogen content, ii) not only sludge production is much lower than aerobic processes but also anaerobically digested sludge can be used as fertilizer, iii)

unlike aerobic treatment processes which are limited by oxygen transfer, anaerobic digesters can sustain very high organic loading and are thus economical for high strength waste, iv) the relatively low operating costs may be offset by the energy recovered, and v) anaerobic digesters can be restarted after a long starvation time and thus are suitable for the treatment of seasonal wastes. On the other hand, the major drawbacks of the anaerobic digestion process are i) slow process requiring long residence time and large reactor volumes, ii) longer start-up time due to low growth rate of methanogenic bacteria, iii) alkali addition may be required to maintain an acceptable pH, and (iv) produce odors and corrosive volatile sulfur compounds.

2.2.4 Feedstock for Bio-methane Production

Various types of biodegradable wastes can be processed anaerobically. For anaerobic digestion to be cost-competitive, the minimum waste chemical oxygen demand (COD) should be above 1500 to 2000 mg/L [8]. However, the process performance such as methane yield, solids reduction efficiency primarily depends on the level of biodegradable organics in the waste, or biochemical oxygen demand (BOD). Anaerobic treatment can be applied to a range of industrial wastewaters, especially in the agro-processing industry, which typically produce wastewaters containing high concentrations of readily biodegradable organic material in the form of carbohydrates, protein and fats. Carbohydrate content of a process wastewater stream often accounts for the majority of the organic load. In some industries, however, protein is also a major part of the organic load [20]. For example, the protein component of a dairy wastewater stream can account for more than 40% of the total chemical oxygen demand [21]. Other processing industries such as abattoir, whey, cheese, casein, fish and certain vegetable processing also typically produce wastewater containing significant amounts of proteins [20]. Lipids, which can

be traditionally characterized as fat, greases, and oils, are widely found in industrial and municipal wastewaters [22].

2.2.5 Process Parameters

pH and alkalinity: pH is a very important for digesters, as methanogens are very sensitive to pH. The acceptable pH range for anaerobic digestion is 6.8-7.2, and lower pH can inhibit the methanogenesis [8, 23]. To maintain the desired pH in the digester, base or buffer can be added. Adequate alkalinity is needed to maintain the stable pH, as alkalinity serves as buffer to prevent the rapid change in pH. The initial pH of digester usually decreases due to the production of volatile fatty acids. The pH of the digester increases and stabilizes with the consumption of volatile fatty acids (VFAs) by methanogens as well as the production of alkalinity. A VFA/alkalinity ratio of ~0.5 is needed for the stable operation of the digester [24]. Digestion also produces alkalinity in the form of ammonium bicarbonate through the degradation of nitrogen containing protein. It is suggested that optimum alkalinity of digester is around 2000-5000 mg as $CaCO_3$/L [8].

Carbon to Nitrogen (C/N) Ratio: Carbon to nitrogen (C/N) ratio of the waste is one of the major considerations for anaerobic digestion, as during anaerobic digestion microorganisms consume carbon 25-30 times faster than nitrogen [25]. At high C/N ratio, the rapid consumption of nitrogen by methanogenic bacteria results in lower biogas production, while lower C/N ratios create toxic environment for methanogenic bacteria such as ammonia accumulation and increase in pH values (≥8.5) [26]. The optimum C/N ratio for biogas production is 20-30 [27]. To

maintain the optimum C/N ratio as well as the efficient operation of anaerobic digesters, regular feedstock can be mixed with materials of high or low C/N ratios.

Mixing: Proper mixing in anaerobic digester is required for optimum performance, as it provides intimate contact between the substrate and active microorganisms. It also helps to maintain the uniformity of temperature throughout the digester [11]. However, excessive mixing can reduce gas production [23]. Mixing can be accomplished by using external pumped recirculation, internal gas mixing or mechanical mixing [28].

Retention time: Retention time of waste in the digester is a very important parameter for designing anaerobic digester. Two types of retention times are used in anaerobic digester operation: hydraulic retention time (HRT) and solids retention time (SRT). HRT indicates the average time the waste or wastewater remains in the digester in contact with the microorganisms; while SRT indicates the time that biomass (solids) remains in the reactor to achieve a given degree of stabilization. Higher SRT can be achieved by either by increasing reactor volume or by increasing the concentration of solids. At very short SRT (below 48 h), methanogens will wash out from the bioreactor [29]. The operating SRT is inversely related to the digester temperature. Long SRTs provide several benefits such preventing biomass washout and greater stabilization of digested waste [30]. However, increasing SRT will also increase the reactor volume as well as the capital cost. The required HRT depends on the types of waste. For readily biodegradable wastes, digester can be operated at shorter HRT; while anaerobic digestion of less biodegradable wastes required high HRT. For completely mixed system with no recycle, SRT is equal to the

HRT, while with recycle, SRT is significantly different from HRT. HRT controls the conversion of volatile solids to biogas [30].

Nutrients: Similar to other biological systems, to maintain optimum microbial activity as well as the digester performance, the two major nutrients or macronutrients required for anaerobic microorganisms are nitrogen and phosphorous. Macronutrients requirement are directly related to the microbial cell growth, and can be calculated based on the empirical equation of the microbial cell ($C_5H_7O_2NP_{0.06}$) [31], and based on the volatile solids converted to bacterial cell, nitrogen and phosphorous requirements for bacterial growth are 12% and 2% by weight of volatile suspended solids, respectively. Lettinga et al. [32] have also suggested an equation for calculating minimum nutrient requirements:

$$N_r = S_0 \mathrm{Y} N_{bac} \left(\frac{TSS}{VSS}\right) \quad (2.6)$$

Where,

N_r= nutrient requirement (g/L)

S_o= concentration of influent COD (g/L)

Y= yield coefficient (g VSS/g COD)

N_{bac}= concentration of the nutrient in the bacterial cell (g/g VSS)

TSS/VSS= total suspended solids/volatile suspended solids in bacterial cell.

Methane forming microorganisms also require several micronutrients in trace quantities such as iron, copper, zinc, nickel, cobalt, manganese, potassium, calcium, manganese, sodium, sulfur, molybdenum, vanadium [30, 31]. Sometimes yeast extract can be used to provide micronutrients to the microorganisms [30]. Besides, co-digestion can be another option to overcome the nutrient limitations.

Toxicity: Excessive concentrations of several organic and inorganic compounds such as VFA, ammonia, sulfide, heavy metal, salts can causes toxicity to the anaerobic digester [30]. At higher pH (~7.4) total ammonia concentrations in the range of 1500-3000 mg/L may cause digester failure [33]. Sulphate is a competitive inhibitor to methanogenic anaerobic digestion as sulphate-reducing bacteria compete for H_2 and acetate, and are generally more energetically efficient in the use of these intermediate anaerobic substrates. This is because sulphate-reducing bacteria have a higher maximum specific growth rate (μ_m) and a lower half saturation value (Ks), giving them a kinetic advantage [34]. Once sulphate reduction becomes established the toxicity of soluble sulphides further depresses methanogenic activity. This usually starts to occur when there is an initial sulphate concentration greater than 1.0 g /L and tends to total inhibition when sulphate concentrations exceed 4.5 g/L [35]. It is generally accepted that inhibition will occur when the dissolved hydrogen sulphide (H_2S) concentration exceeds 200 mg/L [36].

2.2.6 Process Options

Anaerobic digestion systems can be divided into high rate and low rate systems. High rate systems with biomass retention use relatively short HRT with long SRT, while low rate systems without biomass retention use long HRTs. For low rate systems, HRT is the same as the SRT. Low rate systems are usually used for solid wastes, while high rate systems are suitable for low suspended solids wastewaters. The common digesters used for low rate systems are continuously stirred tank reactor (CSTR), and high rate systems are upflow anaerobic sludge bed (UASB), expanded granular sludge bed (EGSB), fluidized bed bioreactor (FBR), anaerobic membrane bioreactor (AnMBR) etc. [37]. Although high rate anaerobic digesters such as Upflow

anaerobic sludge bed (UASB), anaerobic membrane bioreactor (AnMBR), expanded granular sludge bed (EGSB), fluidized bed bioreactor (FBR) are not suitable for high solids or thickened wastes, high rate systems can be used as a part of multi-stage system for treating high solids wastes [38].

Based on the water content in the waste, the following two process options are available for anaerobic digestion: wet and dry fermentation. Dry fermentation is usually used for wastes containing 55%-75% water, while wet fermentation is used for waste containing more than 85% water [39]. Table 2.2 shows a comparison between wet and dry fermentation.

Table 2.2 *Comparisons between wet and dry fermentations.*

	Dry fermentation	Wet fermentation
Total solid	High	Low
Reactor volume	Large	Small
Degradation rate	Lower	Higher
Mixing	Difficult	Easy
Variety of wastes	Low	High
Liquid-Solid Separation	Inexpensive	Expensive

Temperature is closely related to the economics as well as the feasibility of the anaerobic digestion [8]. Anaerobic digesters can be operated at thermophilic and mesophilic operating conditions. High temperature ranging from 50-60^{o}C (thermophilic condition) can enhance the biological growth rate, solids reduction, and pathogen destruction. Maintaining stable operating conditions is critical for process performance as major fluctuations in temperature have an adverse effect on methanogens [11]. Thermophilic digestion rate is almost four times higher than

mesophilic digestion rates. Although thermophilic operation is more advantageous compared to mesophilic operation, it requires high energy input. Application of thermophilic digestion is very limited due to poor process stability compared to mesophilic digestion [40]. However, optimum temperature should be selected depending on the type of waste. The optimum mesophilic and thermophilic temperature are 35^{o}C and 55^{o}C, respectively.

A comparison between thermophilic and mesophilic digestion is shown in Table 2.3. Anaerobic digesters can be operated in batch or continuous-flow mode. Operating and capital cost of batch digesters are lower than those of continuous-flow digester, and their design and operation is also simple. For batch digestion, the digester is loaded once with feedstock and inoculums (anaerobically digested sludge from another reactor) for a given retention time. Once digestion is complete, the digestate is removed from the system. Batch digesters can be operated as single stage or sequential batch mode. For continuous-flow operation, digesters require regular loading and discharge of waste [8]. Continuous-flow digestion can be carried at single or multi-stage. Batch digestion is usually used for small scale operation, while continuous-flow digesters are suitable for large scale operation. Retention time of batch digester is significantly higher than continuous digesters.

Table 2.3 *Comparisons between mesophilic and thermophilic fermentations.*

	Thermophilic Digestion	Mesophilic Digestion
Temperature range (^{o}C)	50-60	30-40
Process stability	Low	High
Retention time	Low	High
Temperature sensitivity	High	Low
Energy requirement	High	Low
Pathogen destruction	High	Low

Degradation rate	High	Low

Anaerobic digestion of the mixture of several types of waste or co-digestion can be another process option. The main advantages of co-digestion technology are improved methane yield because of the supply of additional nutrients from the co-digestates [41]. Co-digestion of organic wastes with municipal wastewater sludge can increase digester gas production and provide savings in the overall energy costs of plant operations. Wastes most often used for co-digestion (co-digestates) with the major wastes are agricultural materials such as energy crops and woody materials, industrial wastes such as confectionery byproducts and enzyme industry wastes, farm wastes such as chicken manure (CM), waste milk (WM), and municipal wastes such as food and vegetable waste (FVW). However, the ratio of various wastes should be optimized for co-digestion. The common objectives of co-digestion are [41]:

- To achieve optimum C/N ratio (municipal solid waste with animal manure)
- To facilitate handling (dry solid waste with wastewater)
- To avoid ammonia toxicity (high protein containing waste with low protein containing waste)
- To improve microbial diversity and essential nutrients (domestic wastewater with manure)

2.2.7 Major Challenges and Available Solutions

Anaerobic digestion is a very slow process due to the rate-limiting hydrolysis step, resulting in large reactor volume and long retention time of wastes in the digester. Besides, the anaerobic digestibility and the typical digestion performances such as solids destruction

efficiency and methane yield are very poor for waste containing difficult to-biodegrade constituents. For example, municipal waste activated sludge is very difficult to digest compared to other wastes. The most widely used approach to enhance the anaerobic digestibility of waste is to use pretreatment including chemical, mechanical, thermal, biological, and combined techniques [42].

The microbiology of anaerobic digestion is very complicated, as anaerobic process is involved with a diverse group of microorganisms such as saccharide, amino acid fermenters, VFA oxidizers, and methane forming bacteria [43]. Among them methane forming bacteria are very sensitive and slow growers. When methanogens are inhibited, the anaerobic digestion process is blocked at acidogenesis step [44]. Biomass retention is an essential feature of high rate anaerobic bioreactors. One of the principal reasons behind the failure of digester is the biomass washout [45]. Significant research and development effort have been devoted at maintaining a high concentration of useful microorganisms in the bioreactors to make the process more effective and rapid [45]. The most common anaerobic bioreactor designs that provide biomass retention are the upflow anaerobic sludge bed (UASB), expanded granular sludge bed (EGSB), fluidized bed bioreactor (FBR), and anaerobic membrane bioreactor (AnMBR). Reactor designs that do not provide biomass retention are the completely stirred tank reactor (CSTR) and plug-flow reactor (PFR) with suspended biomass.

One of the biggest factors limiting the use of biogas is the presence of volatile sulfur compounds, as they are very corrosive and toxic. The major volatile sulfur compounds (VSCs) are hydrogen sulfide (H_2S), and other organosulfur compounds (methyl mercaptan (CH_4S), dimethyl sulfide (C_2H_6S), dimethyl disulfide ($C_2H_6S_2$) etc.) [46]. During digestion, sulfur can be produced from the microbial sulfur reduction by sulfate reducing bacteria (SRB). Besides,

microorganisms degrade sulfur containing proteins associated with waste and produce odorous sulfur compounds. The removal of VSCs is very important for utilization of digester gas. Different types of biogas purification processes such as adsorption, chemical scrubbing, biofilter, bioscrubber, biotrickling filter etc. can be used to remove the VSCs [9]. Besides, several techniques have been used to decrease the sulfur generation potential during digestion. Different types of sulfate reducing bacteria (SRB) inhibitors (molybdate, chromate, tungstate, selenate, nitrite etc.) have been used for sulfate reduction control [47, 48]. Recent studies have shown that chemical and mechanical pretreatment of waste prior to the digestion can also reduce the volatile sulfur compounds generation potential during digestion [49, 50, 51]. Usually volatile sulfide is converted into stable form of sulfide such as ferrous sulfide and elemental sulfur to decrease the H_2S generation potential. Micro-aerobic processes can be also used to remove H_2S in anaerobic digestion [52]. As the predominance of elemental sulfur or sulfate as the final oxidation product depends on the oxygen accessibility; thus, in limited oxygen conditions (microaerobic conditions at very low dissolved oxygen concentrations), elemental sulfur is the main product [53]. The biological technologies to remove hydrogen sulfide are mainly bioscrubbers [54] and biotrickling filters [55, 56] that employ pure cultures (*Acidithiobacillus*) developed in the presence of hydrogen sulfide, oxygen and nutrients.

2.2.8 Digester Design

The most common digester designs available for low rate digestion are continuously-stirred tank reactor (CSTR) and plug flow reactor (PFR). CSTR is one of the most flexible and widely used anaerobic digesters (Figure 2.3(a)). Anaerobic CSTR reactors provide uniform distribution of heat and nutrient with the biogas yield usually related to temperature are generally

used for treating wastes with 2%-10% (by weight) total solids (TS) [37]. Anaerobic plug flow reactors (PFR), a long narrow insulated reactors are used for treating wastes with 10%-12% TS (by weight) [37]. In light of lack of solid/liquid separation, for both CSTR and PFR, SRT is equal to HRT.

Recently, prospects of multi-phase digestion have become more promising compared to single stage digestion. In conventional single phase digestion, the acidogenic and methanogenic microorganisms are kept in a single reactor. Both groups of microorganisms are different in terms of physiology, pH requirement, nutrient requirement, growth kinetics, and ability to tolerate environmental conditions [57, 58]. Favorable operating conditions such as shorter HRT and lower pH for acid-forming bacteria are not suitable for methane-forming bacteria [58]. Therefore, it is very difficult to provide an optimum condition for different groups of microorganisms in a conventional single stage digester.

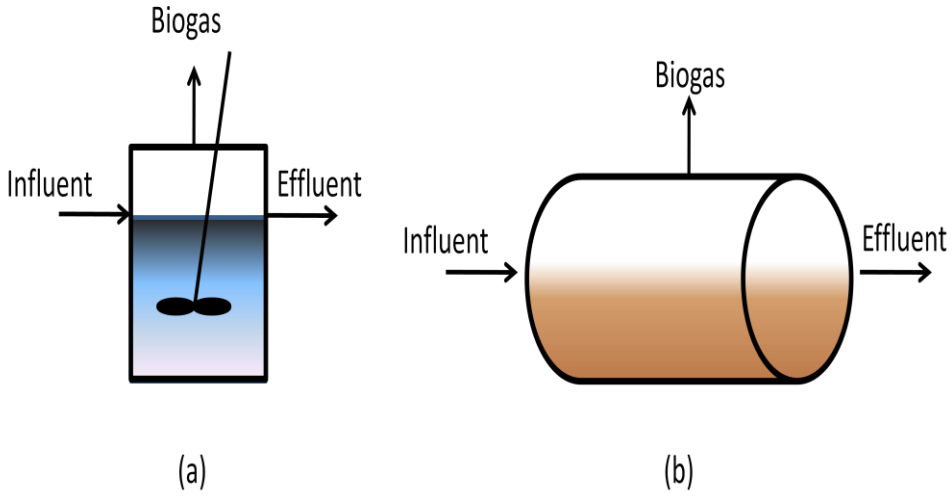

Figure 2.3 Low rate digesters (a) Continuously-Stirred Tank Reactor (CSTR), (b) Plug Flow Reactor (PFR).

Pohland and Ghosh [59] have first proposed that the physical separation of the process into an acidogenic and a methanogenic stage would allow for optimization of each stage independently without interference with the other stage (Figure 2.4), which also allows for a higher organic loading rate (OLR). This concept of two-phase digestion is also known as acid phase digestion. The first phase is usually operated at a short SRT (4-12hr) and at lower pH (≤6), while methane formation stage is operated at long SRTs (10-30 days) and at neutral pH to maintain favorable environment for methanogenic bacteria. Either phase can be operated at thermophilic or mesophilic conditions [8]. Two-phase digestion has several advantages over conventional single stage digestion such as increased stability, process control, and optimization of the digestion process [59]. Two-phase digestion also enhances the performance of the anaerobic digestion process including increased solids reduction and biogas production [59]. However, application of two-phase digestion leads to an increase in capital and operating costs compared to single-stage conventional digester. A significant amount of research has been carried out on two-phase digestion. Two-stage digestion has been applied to various types of waste and wastewaters such as distillery wastewater [60], landfill leachate [61], coffee waste [62, 63], cheese whey and dairy waste [64, 65], pulp and paper mill sludge [66], municipal sludge [67] etc., and performance of two-phase digestion for various wastes has been well documented by Shuizhou and Zhou [68].

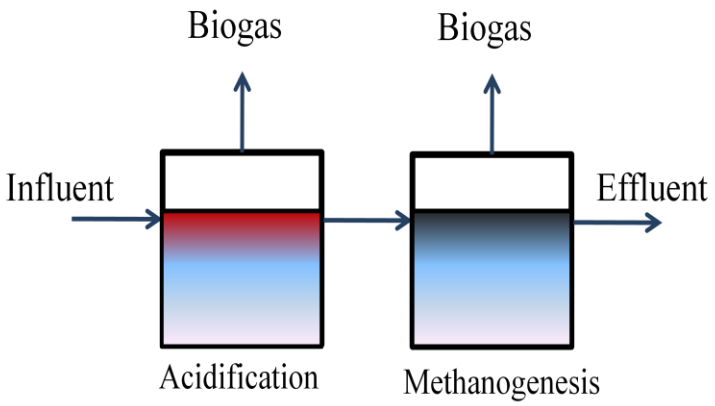

Figure 2.4 Acid Phase Digestion.

Several studies have shown that two-phase digestion is more effective for high suspended solids (>10%) waste [16, 69, 70, 71]. Parkin and Owen [72] suggested that the phase separation will be more beneficial for less degradable wastes, as it would not offer any significant advantages for readily biodegradable wastes.

Temperature-phased anaerobic digestion (TPAD) has been developed to combine the advantages of both mesophilic and thermophilic digestion as well as to improve the digestion performance of waste. TPAD systems use thermophilic and mesophilic digesters in series (Figure 2.5). In the first phase, a thermophilic digester is operated at short SRT (1-3 days), the second phase is mesophilic digestion, usually operated at longer SRT (10-20 days) [73]. The elevated temperature in the thermophilic digester enhances the hydrolysis rate. TPAD provides more solids and pathogen reduction compared to single-stage thermophilic or mesophilic digestion. TPAD system is also capable of producing Class A biosolids [8]. TPAD is more feasible in terms of energy efficiency compared to single stage thermophilic or mesophilic digestion [74]. However, TPAD is not widely used for full scale operation as it is a relatively new concept [75].

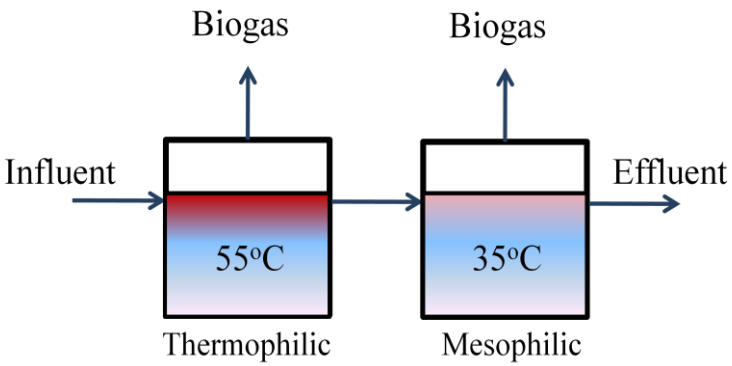

Figure 2.5 Temperature-phased anaerobic digester (TPAD).

The upflow anaerobic sludge bed (UASB) is the most widely used high rate digester, and a significant numbers of UASB reactors are now in operation for treating various types of wastewaters throughout the world. In the early 1970's, the UASB technology has been developed by Dr. Gatze Lettinga and coworkers in Wageningen University in The Netherlands for high rate anaerobic treatment of wastewater in sugar industries. Due to very simple and compact design, installation of UASB needs very small space. A schematic diagram of UASB reactor is shown in Figure 2.6. In UASB, waste enters at the bottom of the bioreactor, and passes through a granular sludge bed. The heart of UASB reactor is the dense granular sludge bed. The sludge bed is formed by the accumulation of incoming suspended solids and microbial growth [76]. In the granular sludge bed, the biological conversion of organic compounds takes place, and SCOD of the waste or wastewater is converted into biogas. At the top of UASB reactor, the biogas and solids are separated from the liquid using a three phase gas-liquid-solid separator. One of the major advantages of UASB is the ability to maintain high concentrations of biomass inside the digester. Besides, UASB reactor can be operated at short hydraulic retention times. More than 65% of the anaerobic industrial wastewater systems worldwide are using UASB technology [77]. Based on the UASB technology, several commercial anaerobic digestion systems such as

Biothane®, BIOPAQ® IC (Internal Circulation) technology have been developed and are used for anaerobic treatment of various wastewaters. The expanded granular sludge bed (EGSB) is another promising version of UASB. In EGSB reactor, high recycle ratio and elevated height/width ratio provide higher upflow velocity (>4 m/h) compared to conventional UASB (0.5-2 m/h) [77]. The higher upflow velocity achieves expansion of the granular sludge bed as well as better mixing between sludge and wastewater. EGSB can also be used for low strength wastewater containing 1-2 g /L of COD [78]. Demirbas [77] has reported that EGSB is gradually replacing conventional UASB due to additional benefits. The advantage of EGSB system over UASB system is higher biomass accumulation since higher ULV will expand the sludge bed layer upward through the reactor's height [79].

Recently, the anaerobic membrane bioreactor (AnMBR) using micro and ultra filtration [80] has proved to be an attractive process for the treatment of municipal and industrial wastewaters as it prevents the biomass washout from the digester and provides very low suspended solids concentrations in the treated effluent. Application of membrane technology for anaerobic treatment of wastewater was first reported by Grethlein [81]. Based on the position of the membrane in the anaerobic digestion system, two configurations are available: side-stream and submerged anaerobic membrane bioreactor (Figure 2.7). In side-stream AnMBR, membrane modules are placed outside the bioreactors. For this configuration, a pump is required to push the digestate through the membrane. In submerged AnMBR, the membrane is placed inside the bioreactor submerged in the liquid phase. Compared to submerged AnMBR, side-stream AnMBR is widely used for anaerobic digestion applications [82]. However, side-stream AnMBR technology is more expensive due to the operational cost of pumping. The major drawbacks limiting the use of membrane in anaerobic bioreactor are cake formation, membrane fouling, and

cost of membrane. The cost of AnMBR is higher than the UASB technology. Although AnMBR has been used for anaerobic treatment of various wastewaters, commercial application of AnMBR is still very limited.

COD removal efficiency achieved using AnMBR is 56%-99% depending on the characteristics of the waste [82]. For practical application, more research is needed to assess the feasibility of AnMBR treatment of different wastewater types (low and high strength), assess in greater depth the use of immersed membranes, strategies for membrane fouling control, the combination of membranes with anaerobic fixed film technologies, evaluate the impact of membranes on biological activity, and determine the conditions under which AnMBR systems will be economically feasible [82].

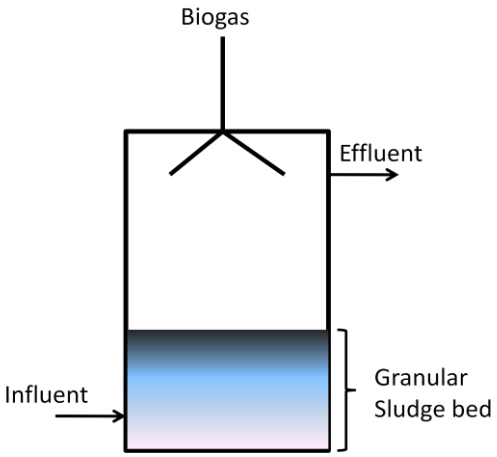

Figure 2.6 Upflow anaerobic sludge bed (UASB).

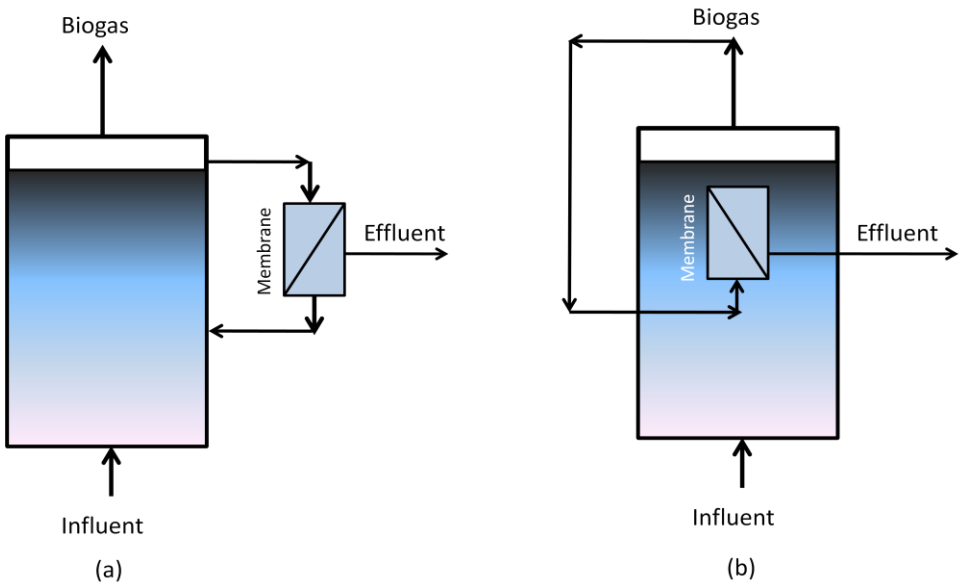

Figure 2.7 Configurations for Anaerobic Membrane Bioreactors (a) Side-stream, (b) Submerged

Although the use of electrolysis for hydrogen production is an emerging field of research now, use of electrolysis in anaerobic digestion to enhance bio-methane production is a very new idea. Recently, water electrolysis has been incorporated with anaerobic digestion in order to enhance the bio-methane production from waste. The idea of electrolysis-enhanced anaerobic digestion (eAD) has been developed by Biotechnology Research Institute, National Research Council, Canada. Inspired by the several studies reporting on the positive impact of micro-aerobic conditions at very low dissolved oxygen (DO) concentrations created by limited aeration or feeding small amount of hydrogen peroxide (H_2O_2) during anaerobic digestion, the researchers have successfully applied the water electrolysis at a low current density to laboratory scale upflow anaerobic sludge bed (UASB) reactors fed with low strength synthetic wastewater at two OLRs of 1.7 and 15.5 g COD/$L_{reactor}$.d [83]. The electrodes were placed at the bottom of the UASB reactor. Incorporation of water electrolysis with anaerobic digestion has solved several limitations of anaerobic digestion such as slow hydrolysis, generation of hydrogen

sulfide (H_2S) in biogas and poor biogas yield. The oxidation of H_2S by oxygen produced through the water electrolysis resulted in a significant removal of H_2S from biogas (<1ppm). The microareobic condition through water electrolysis also increased the hydrolysis of organic matters as well as improved the COD removal and methane production at both low and high organic loading rates. Although the presence of oxygen was anticipated to exert a negative impact on anaerobic microbial populations, no deterioration of methane production was noticed. The authors suggested that the amount of oxygen formed at low current density did not prevent the methane formation. Besides, the typical diameter of biomass granules (>500μm) in the UASB reactor is much greater than the oxygen penetration depth of 50μm at an ambient DO concentration of 2-4 mg/L. However, detailed techno-economic evaluation should be conducted using comprehensive studies on real wastes to determine the viability of electrolysis combined with anaerobic digestion.

2.2.9 Benefits of Bio-Methane Production

Typically biogas contains 40%-70% methane by volume, with a heating value of 5-7.5 kWh/m^3 [26]. Anaerobic digestion of waste produces stabilized solid and liquid residues (known as digestate) that can be used as a supplement to chemical fertilizers, as anaerobic digestion provides complete retention of fertilizer nutrients such as nitrogen, phosphate, and potassium [85] in the digested sludge. Anaerobic digestion for bio-methane production can provide several benefits by capturing methane [86]. Over a 100 years period, CH_4 is 20 times more effective in trapping heat in the atmosphere than CO_2 [87]; methane is the major greenhouse gas emitted from agricultural sources. Use of biogas can decrease CO_2 emission by 1 lb/kWh energy generation as heat [88] compared to traditional fossil fuel.

2.3. Bio-hydrogen

Hydrogen gas has been deemed the fuel carrier of the future, and it is believed that a hydrogen based economy would be less polluting than a fossil fuel based economy [89]. Hydrogen as an energy carrier has been proven to be one of the best fuels for transportation, the most versatile, the most efficient and also one of the safest fuels [90]. The combustion of hydrogen produces only water vapour without CO, CO_2, hydrocarbons or fine particles, and since it can be produced without causing any environmental problems, hydrogen as a future fuel has been drawing more and more attention [91].

2.3.1 Mechanisms of Bio-hydrogen production

There are four basic mechanisms for biohydrogen production: direct biophotolysis, indirect biophotolysis, photofermentation, and fermentation. Table 2.4 shows a comparison of the four biological hydrogen production processes.

Direct biophotolysis: Biological hydrogen can be generated from plants by biophotolysis of water using microalgae (green algae and Cyanobacteria), fermentation of organic compounds, and photodecomposition of organic compounds by photosynthetic bacteria [92]. Photosynthetic production of hydrogen from water is a biological process that can convert sunlight into useful, stored chemical energy by the following general reaction [89]:

$$2H_2O + \text{solar energy} \rightarrow 2H_2 + O_2 \qquad (2.7)$$

Indirect biophotolysis: Cyanobacteria can also synthesize and evolve H_2 through photosynthesis via the following processes [89]:

$$12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (2.8)$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \quad (2.9)$$

Indirect biophotolysis, therefore, consists of two stages in series: photosynthesis for carbohydrate accumulation and dark fermentation of the carbon reserve for hydrogen production [93]. In the first stage, acidogenic bacteria naturally present in the environment-derived energy and produce some hydrogen by degrading waste carbohydrate matter into simple organic acids and alcohols. In the second stage, organic acids are harvested and fed as a substrate to photoheterotrophic bacteria for additional hydrogen production [94].

Table 2.4 *Comparison of important biological hydrogen production processes.*

Process	Microorganisms	Advantages	Disadvantages
Direct biophotolysis	Green algae	Can produce H_2 directly from water and Sunlight Solar conversion energy increased by ten folds as compared to trees, crops	Requires high intensity of light O_2 can be dangerous for the system
Indirect biophotolysis	Cyanobacteria	Can produce H_2 from water Has the ability to fix N_2 from atmosphere	Lower photochemical efficiency Uptake hydrogenase enzymes are to be removed to stop degradation of H_2 About 30% O_2 present in gas mixture O_2 has an inhibitory effect on nitrogenise
Photofermentation	Photosynthetic bacteria	A wide spectral light energy can be used by these bacteria Can use different waste materials like distillery effluents, waste etc.	Light conversion efficiency is very low, only 1–5% O_2 is a strong inhibitor of hydrogenase
Dark fermentation	Fermentative bacteria	It can produce H_2 all day long without light A variety of carbon sources can be used as Substrates It produces valuable metabolites such as butyric, lactic and acetic acids as by products It is anaerobic process, so there is no O_2 limitation problem	Relatively lower achievable yields of H_2 As yields increase H_2 fermentation becomes thermodynamically unfavorable Product gas mixture contains CO_2 which has to be separated

Photofermentation: H_2 production by purple non-sulfur bacteria is mainly due to the presence of nitrogenase under oxygen-deficient conditions using light energy and reduced compounds (organic acids). The reaction is as follows:

$$C_6H_{12}O_6 + 12H_2O + \text{Light energy} \rightarrow 12H_2 + 6CO_2 \quad (2.10)$$

Dark fermentation: Dark fermentation is a ubiquitous phenomenon under anoxic or anaerobic conditions. The oxidation of the substrate by bacteria generates electrons which need to be disposed off in order to maintain the electrical neutrality. Under the aerobic conditions O_2 serves as the electron acceptor while under the anaerobic or anoxic conditions, other compounds, such as protons, act as the electron acceptor and are reduced to molecular H_2 [89, 95]**.** Carbohydrates, mainly glucose are the preferred carbon sources for this process, which predominantly give rise to acetic and butyric acids production together with H_2 evolution [96]**.**

2.3.2 Biochemical reactions for dark fermentation

Dark hydrogen fermentation processes produce a mixed gas which mainly contains hydrogen and carbon dioxide, but may also contain methane, carbon monoxide, and hydrogen sulfide depending on the different systems and feedstocks [97, 98, 99, 100, 101]. The complete oxidation of glucose to hydrogen and carbon dioxide yields a maximum of 12 moles hydrogen per mole of glucose (see equation 2.11). However, there is no metabolic energy is obtained in this case implying that bacterial growth is severely hampered.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \qquad \Delta G_o = +3.2\ \text{kJ} \quad (2.11)$$

The most common products in the fermentation of carbohydrates are acetate and butyrate. This acidification process may be expressed by the two following reactions, using glucose as the model carbohydrate [102]:

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ $\Delta G_o = -206$ kJ (2.12)

$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ $\Delta G_o = -254$ kJ (2.13)

Thus, the stoichiometric yields are 4 moles of hydrogen for each mole of glucose (i.e., 544 ml H_2/g hexose at 25^{o}C) in the production of acetic acid, according to reaction (2.12), and 2 moles of hydrogen (i.e., 272 ml H_2/g hexose at 25^{o}C) in the production of butyric acid, according to reaction (2.13). In addition to these acids, ethanol may also be produced, as shown in the following reaction [103]:

$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2H_2 + 2CO_2$ (2.14)

The corresponding stoichiometric yield is 2 moles of hydrogen for each mole of glucose. However, the actual hydrogen yield may be substantially lower than these stoichiometric values for at least four reasons. First, glucose may be degraded through other pathways without producing hydrogen. Second, a fraction of glucose is consumed, instead, for biomass production. Third, a stoichiometric yield is achievable only under near equilibrium condition, which implies a slow production rate and a low hydrogen partial pressure [104, 105]. Lastly, some hydrogen produced may be consumed for the production of other by-products, such as propionate [106], as shown in the following reaction:

$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (2.15)

About 40 hydrogenase genes have been sequenced so far, all of them contain Fe, and some contain Ni and Se as well [107]. Those hydrogenases containing Ni and Se facilitate the uptake of hydrogen, whereas those containing Fe alone (Fe hydrogenases) catalyze the

production of hydrogen [108]. Several hydrogenases have been sequenced and characterized from *Clostridium* species, including *C. pasteurianum* [109], *C. acetobutylicum* [110, 111], *C. perfringens* [112], and *C. paraputrificum* [113]. However, there is no information so far on Fe hydrogenase in the mixed hydrogen-producing sludge [114].

2.3.3 Biohydrogen producing microorganisms

There are numerous types of microorganisms that are found to produce hydrogen during anaerobic conditions. Strictly anaerobic bacteria are the most common class of bacteria that produce hydrogen, mesophilically or thermophilically within pH 4–7 [115]. However, a few facultative bacteria have been identified as hydrogen producers when the hydrogenase enzyme was found in these bacteria, even though the production rate of hydrogen was lower than in strictly anaerobic bacteria. Recently, hydrogen production was found to be possible by aerobic bacteria [115].

Anaerobic bacteria: *Clostridium* sp. is a typical acid and hydrogen producer which ferments carbohydrate to acetate, butyrate, hydrogen, carbon dioxide and organic solvent. *Clostridium butyricum* [115], *Clostridium acetobutyricum* and *Clostridium beijerinckii* [116], *C. thermolacticum* [117], *C. saccharoperbutylacetonicum* [118], *Clostridium tyrobutyricum* [119], *C. thermocellum* [120] and *Clostridium paraputrificum* [121] are examples of anaerobic and spore forming hydrogen producers. Clostridia species produce hydrogen gas during the exponential growth phase. When reaching stationary phase, metabolism shifts from hydrogen/acid production to solvent production [122].

Facultative anaerobic bacteria: Facultative anaerobes produce ATP by aerobic respiration if oxygen is present and are capable of switching to anaerobic fermentation, and thus have an advantage compared to anaerobic bacteria which is sensitive to the presence of oxygen. Facultative bacteria can consume oxygen by aerobic respiration, leaving anaerobic conditions that favour hydrogen production. *Enterobacter* sp. is the most common gram negative and facultative anaerobe with the ability to produce hydrogen. Oh et al. [123] isolated *Citrobacter* sp. Y19 from anaerobic sludge digester which could produce hydrogen from CO and water. This bacterium could also produce hydrogen from glucose at wide range of pH (5–9) and temperature (25–40^{o}C).

Thermophilic bacteria: Hydrogen production at high temperatures (40-65 ^{o}C) using mixed thermophilic bacteria has been identified as a potential process that favourable to reaction kinetics, avoiding contamination by hydrogen consuming bacteria. *Thermoanaerobacterium* sp. has been identified as effective hydrogen producing bacteria [124]. *Thermotoga maritima, Thermotoga neapolitana and Thermotoga elfii* are the commonly reported as thermophilic hydrogen producers [125, 126].

Co- and mixed-cultures: It is widely known that hydrogen production by obligate anaerobic bacteria is about 2 mol H_2/mol glucose by *Clostridium* sp. compared to 1 mol H_2/mol glucose by *Enterobacter* sp. [127, 128]. However, cultivation of anaerobic bacteria was rather difficult as trace amounts of oxygen inhibited their growth. Yokoi et al. [129] suggested a co-culture of *C. butyricum* and *E. aerogenes*, where *E. aerogenes* will first consume dissolved oxygen in the liquid, leaving anaerobic conditions that are favorable to *C. butyricum*. A hydrogen yield of 2

mol H_2/mol glucose without addition of reducing agent was achieved in the aforementioned study. Co-immobilization of both strains on porous glass beads gave a yield at 2.6 mol H_2/mol glucose. Experimental results supported the hypothesis that co-culturing increases hydrogen yield. Co-cultures of *C. thermocellum* and *T. thermosaccharolyticum* showed the same effect where hydrogen production increase about 2-fold and hydrogen yield increased to 1.8 mol H_2/mol glucose [130].

Mixed and pure cultures: In general, for a full-scale application the selection of mixed cultures is considered to be favorable, at least from an engineering standpoint. This is due to the fact that the control and operation of the process is facilitated when no medium sterilization is required, reducing thus the overall cost, while it also allowing for a broader choice of feedstocks [131]. The mixed consortia can be derived from a variety of different natural sources, such as sewage sludge [132], anaerobically digested sludge [133], acclimated sludge [134], compost [135], animal manure [136] and soil [137] or even from the indigenous microorganisms found in certain wastes [138]. Alternatively, many researchers have focused on the use of pure cultures of selected hydrogen producing species. The main arguments for their advantageous use are the selectivity of substrates, the ease of metabolism manipulation by altering growth conditions, the higher observed hydrogen yields due to the reduction of undesired by-products, as well as the repeatability of the process. On the other side of the coin, pure cultures can be quite sensitive to contamination and thus their use demands, in most cases, the presence of aseptic conditions, which significantly increases the overall cost of the process [139].

Studies on microbial hydrogen production have been conducted mostly by pure cultures [140, 141, 142]. Processes using mixed cultures are more practical than those using pure

cultures, because the former are simpler to operate and easier to control, and may sustain a broader spectrum of feedstock; thus preferable for wastewater treatment [114]. However, in a mixed culture system, under anaerobic conditions, hydrogen produced by hydrogen-producing bacteria, such as *Clostridium* and *Enterobacter*, is often readily consumed by hydrogen-consuming microorganisms, such as methanogens and homoacetogens [114]. Therefore, in order to harness hydrogen from a mixed culture system, the seed sludge needs pretreatment to suppress as much hydrogen-consuming microbial activity as possible while still preserving the activity of the hydrogen-producing bacteria [143]. Methods for pretreating sludge include mechanical pretreatment [144], ultrasonic disintegration [145], alkali pretreatment [146], heat pretreatment [147] and thermo-chemical pretreatment [148].

2.3.4 Feedstocks for dark hydrogen fermentation

Theoretically any organic substrate rich in carbohydrates, fats, and proteins could be considered as possible substrate for biohydrogen production. However, as reported by numerous studies, carbohydrates are the main source of hydrogen during fermentation processes and therefore wastes and biomass rich in sugars and/or complex carbohydrates turn out to be the most suitable feedstocks for biohydrogen generation [149]. According to a comparative study by Lay et al. [150], using substrates of different chemical composition treated with the same mixed consortium, it was shown that the hydrogen-producing potential of carbohydrate- rich waste (rice and potato) was approximately 20 times higher than that of fat-rich waste (fat meat and chicken skin) and of protein-rich waste (egg and lean meat). The major criteria that have to be met for the selection of substrates suitable for fermentative bio-hydrogen production are availability, cost, carbohydrate content and biodegradability [151]. Simple sugars such as glucose, sucrose and

lactose are readily biodegradable and thus preferred as model substrates for hydrogen production [152, 153, 154].

2.3.5 Reactors for dark hydrogen fermentation

Possible improvements to biohydrogen production have been sought through specialized bioreactor configurations (see Table 2.5, Ref. 155-166). Biohydrogen fermentation, as most other fermentations, can be carried out in either batch or continuous-flow modes. Batch fermentation has been shown to be more suitable for initial optimization studies [149, 167], but any industrially feasible process would most likely have to be performed on a continuous-flow or at least semi-continuous (fed or sequencing batch) basis. Many studies have employed continuously stirred tank reactors (CSTRs) with either purified strains or microbial mixtures [149, 167, 168].

In the CSTRs, hydraulic retention time (HRT) controls the microbial growth rate and therefore dilution rate (1/HRT) must be greater than the increase of the maximum growth rate of the organism(s), because faster dilution rates cause washout. Overcomes this problem and offers several advantages for a practical bioprocess. Because microbial growth and biomass concentration are rendered independent of HRT, high cell concentrations can be achieved, fostering high volumetric biohydrogen production rates, and high throughput, allowing the use (and treatment) of dilute waste streams with relatively small reactor volumes. Indeed, many recent studies have shown that high volumetric hydrogen production rates can be achieved in these reactors, as exemplified in Table 2.5.

Table 2.5 *Available dark fermentation reactors.*

Microorganisms	Substrate	Type of reactor	H_2 rate ($L\ H_2/L.h$)	Ref.
Sludge (wastewater treatment plant)	Molasses	Continuous stirred-tank reactor (CSTR)	0.20	[155]
Sludge (wastewater treatment plant)	Glucose	Anaerobic sequencing batch reactor (ASBR)	0.23	[156]
Sludge (wastewater treatment plant)	Sucrose	Fixed bed bioreactor with activated carbon (FBBAC)	1.2	[157]
Activated sludge and digested sludge	Glucose	Anaerobic fluidized bed reactor (AFBR)	2.4	[158]
Sludge (wastewater treatment plant)	Sucrose	Upflow anaerobic sludge blanket reactor (UASB)	0.27	[159]
Anaerobic sludge	Sucrose	Polymethymethacrylate (PMMA) immobilized cells	1.8	[160]
Sludge (wastewater treatment plant)	Sucrose	Carrier-induced granular sludge bed (CIGSB)	9.3	[161]
Sludge (wastewater treatment plant)	Sucrose	Fluidized bed reactor (FBR)	1.4	[162]
Sludge (wastewater treatment plant)	Glucose	Anaerobic fluidized bed reactor (AFBR)	7.6 biofilm; 6.6 granules	[163]
Sludge (wastewater treatment plant)	Sucrose	Continuously stirred anaerobic bioreactor (CSABR)	15.0	[164]
Heat-treated soil	Glucose	Membrane bioreactor (MBR)	0.38	[165]
Anaerobic sludge	Glucose	integrated biohydrogen reactor clarifier systems (IBRCSs)	1.48	[166]

2.3.6 Hybrid two-stage systems

The basic principle of a two-stage process is as follows: (i) in the first stage, the fermentation of the substrate to hydrogen and organic acids takes place; (ii) then, in the second stage, additional gaseous energy, either methane or (more preferably) hydrogen, is extracted from the effluent of the first stage reactor. Three different two-stage systems that are theoretically capable of complete energy extraction have been proposed (Figure 2.8). The first approach is to use a different reactor for the second stage that is operated under different conditions, such as higher pH and longer HRT, than the first reactor, thus favouring methanogenesis. Despite the disadvantage of generating two different gas streams, hydrogen and methane, in practical terms this might be useful because hydrogen-methane mixtures are cleaner fuels for internal combustion engines than methane alone in that they produce less NO_X [169]. This hybrid two-stage system, producing both hydrogen and methane using a mixture of pulverized garbage and shredded paper wastes as substrate, is nearly ready to be put into practice and has already been scaled up to pilot scale [170]. Such a two-stage system might offer several advantages over traditional simple methane fermentation, including an effective solubilisation of substrates such as organic solid wastes and increased tolerance to high OLR. Several recent studies have reported the successful operation of such two-stage systems using actual wastes [171, 172, 173]. The efficiency of this process is demonstrated by the fact that methane yields were twofold higher than a comparable single-stage process [170]. The second possible process for increasing the overall energy extraction is the use of photofermentation in the second stage, with the aim of recovering additional hydrogen from the products of a dark hydrogen-generating fermentation. The third approach employs microbial electrohydrogenesis cells (MECs), in which

electricity applied to a microbial fuel cell provides the necessary energy to convert organic acids, which are typical side products of a hydrogen fermentation, to hydrogen [174].

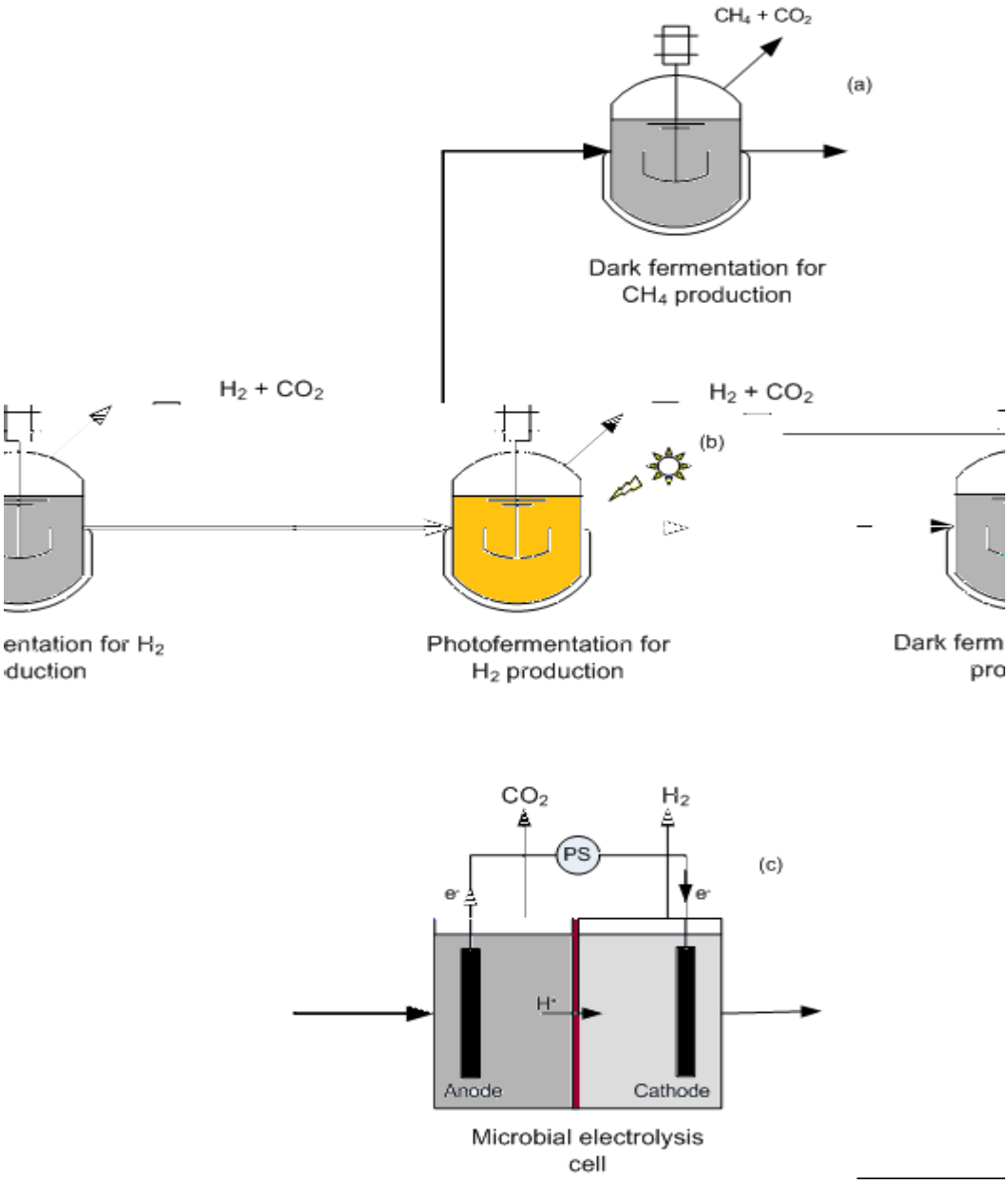

Figure 2.8 Hybrid two-stage systems: dark fermentation for H_2 production followed by (a) dark fermentation for CH_4 production (b) photo-fermentation for H_2 production (c) Microbial electrolysis cell for H_2 production.

2.3.7 Parameters affecting dark hydrogen fermentation

Hydrogen fermentation has been extensively studied because it has the potential for providing sustainable and renewable energy for the future. It has been reported that the temperature, pH, HRT, hydrogen/carbon dioxide partial pressure, volatile fatty acids and inorganic content are the main parameters that affect the anaerobic hydrogen fermentation process [175].

pH: Bacteria respond to change in internal and external pH by adjusting their activity and synthesis of proteins associated with many different processes, including proton translocation, amino acid degradation, adaptation to acidic or basic conditions and virulence [176]. pH plays a critical role in governing metabolic pathways of organism where activity of H_2 producing bacteria is considered to be crucial [177, 178]. It is necessary to avoid the presence of organisms utilizing H_2, particularly methanogens, and this has been achieved in laboratory studies by operating at low pH and/or short retention times, since methanogens are more affected by lower pH and grow slower than fermentative organisms [151]. Optimum pH range for H_2 uptake bacteria (methanogens) is between 6 to 7.5, while H_2 producing bacteria function well below a pH of 6 [179, 180, 181]. The pH range of 5.5–6.0 is ideal to avoid methanogenesis and solventogenesis [180, 182]. Initial pH values of 5.5-7.5 represent optimum and acceptable pH ranges for H_2 production in batch studies, where H_2 yield sharply drops at pH lower than 5.5 or higher than 7.5 [183].

Temperature: Temperature affects the hydrogen producing bacteria activities and hydrogen production rate [184, 185]. Dark hydrogen fermentation reactions can be operated at different temperatures: mesophilic (25-40°C), thermophilic (40-65°C), extreme thermophilic (65-80°C) or hyperthermophilic (>80°C) [89].

Most of dark fermentation experiments are conducted at 35-55^{o}C. The extreme thermophilic process provides a number of advantages compared with the mesophilic and thermophilic. First, the hydrogen production rate is much higher at extreme-thermophilic conditions than at mesophilic and thermophilic conditions. It has been reported that extreme-thermophilic anaerobic hydrogen fermentation can achieve more hydrogen production and higher hydrogen production rates than mesophilic hydrogen fermentation [185]. Second, extreme-thermophilic digestion achieves higher pathogen destruction efficiency than both mesophilic and thermophilic digestion [196]. Third, it minimizes the contamination by hydrogen consumers such as methanogens, solventogens. Hallenbeck [187] reported that a high fermentation temperature (60-90^{o}C) it was thermodynamically favorable for a hydrogen-producing reaction as the high temperature resulted in the increase in the entropy term, and made dark hydrogen fermentation more energetic while the hydrogen utilization processes were negatively affected with the temperature increase [188, 189].

Hydraulic Retention Time (HRT): HRT is also an important parameter for dark fermentation process. In a CSTR system, short HRTs are used to wash out the slow growing methanogens and select for the acid producing bacteria [190], while too high dilution rates (low HRTs) could lead to poor hydrolysis of organic wastes [122]. In a CSTR system, Kim et al. [191] reported that short HRT (< 3 days) would favour hydrogen production as methanogens require more than

approx. 3 days HRT before they were washed out from a CSTR. Both pH and HRT have been demonstrated as effective ways to separate hydrogen producing bacteria and hydrogen consuming archaea at mesophilic and thermophilic conditions [192]. The reported optimal HRTs for biohydrogen production from glucose and sucrose were mostly in the range of 3–8 h, with the lowest being 1 h [157] and the highest 13.7 h [193].

Hydrogen partial pressure: The hydrogen concentration in the liquid phase, which is related to hydrogen partial pressure, is one of the key factors affecting the hydrogen production [151]. The partial pressure of H_2 (pH_2) is an extremely important factor especially for continuous H_2 synthesis [194]. Hydrogen synthesis pathways are sensitive to H_2 concentrations and are subject to end-product inhibition. As H_2 concentrations increase, H_2 synthesis decreases and metabolic pathways shift to the production of more reduced substrates such as lactate, ethanol, acetone, butanol, or alanine [175]. Continuous H_2 synthesis requires pH_2 of 50 kPa at 60^{o}C [195], 20 kPa at 70^{o}C [126], and 2 kPa at 98^{o}C under standard conditions [89, 196]. Various techniques have been used to remove metabolic gases (H_2, CO_2) from the liquid phase [197]. Gas sparging has been the most common method used to decrease the concentrations of dissolved gases in fermentative H_2-producing reactors. Various gases have been used to decrease the dissolved hydrogen concentration in the liquid such as nitrogen [98], CO_2, methane [171], biogas [198], argon [199], argon and H_2 sparging [200]. Other techniques to decrease concentrations of dissolved gases include increased stirring [201], decreasing the reactor headspace pressure i.e. applying a vacuum [202], using an immersed membrane to directly remove dissolved gases [203], and using ultrasonication to remove dissolved gases [204].

Organic acids concentration: It has been reported that high concentrations of organic acids result in a collapse of the pH gradient across the membrane and cause complete inhibition of all metabolic functions in the cell [205]. It has been claimed that both the total acetate or butyrate acid concentration and the undissociated form of these acids can inhibit dark hydrogen fermentation process [205, 206, 207]. A near-complete H_2 production inhibition was observed by Van Ginkel and Logan [206] at a pH of 5.5 with the addition of 165 mM resulted in an undissociated acid concentration in the reactor of 63 mM. The aforementioned authors reported that the fermentation pathway changed from organic acid and hydrogen to solvent was not detected.

2.4 Pretreatment Technologies for Digestion

2.4.1 Principle of Sludge Pretreatment

Since the organic excess municipal sludge from wastewater treatment plants is relatively large in volume and is rich in organic content, anaerobic digestion as further stabilization is used commonly. Pretreatment has been developed to enhance anaerobic digestion and reduce ultimate solids disposal [208]. Sludge pretreatment enhances the performance of anaerobic digestion in many ways. Due to the particulate nature of waste sludge as a substrate, subsequent microbial degradation is not favored [209]. Since the first step in anaerobic digestion of hydrolysis is also the rate-limiting step, the anaerobic digestion is a very slow process [210].

Sludge pretreatment principally aims to overcome the slow rate of hydrolysis by converting the particulate substrate into bioavailable substrate. Therefore, sludge pretreatment breaks up cell walls and produces bioavailable substrate for anaerobic digestion [209, 211]. There are various pretreatment methods. Generally pretreatment can be classified as mechanical, chemical, thermal

or biological pretreatment or combination of these methods such as thermo-chemical pretreatment.

Mechanical Pretreatment: The underlying principle of mechanical pretreatment is to mechanically stress the sludge using stirred ball mills, high pressure homogenizers, ultrasonic homogenizers, mechanical jet, high performance pulses technique and lysat-centrifugal-technique [208].

Chemical Pretreatment: Chemical pretreatment involves the application of chemicals to the sludge for cell wall dissolution. Common chemical methods include acid or alkali pretreatment, ozonation, and hydrogen peroxide addition. HCl, H_2SO_4, NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$ are chemical agents used to alter the pH for acid or alkali pretreatment [212, 213].

Thermal Pretreatment: Thermal pretreatment releases intracellular bound water and generally involves heating in the range of 150 – 200 ^{0}C [209]. Combined with chemical pretreatment, thermal pretreatment can also be applied; the process is called thermo-chemical pretreatment [214].

Biological Pretreatment: Biological pretreatment disintegrates the sludge with or without enzymes, generally biological pretreatment uses external enzymes, enzyme catalyzed reactions and autolytic processes for cracking the compounds of cell wall [208].

2.4.2 Ultrasound pretreatment

Ultrasound is a cyclic sound pressure with a frequency greater than the upper limit of human hearing. The lower and upper limits of ultrasonic frequencies inaudible for human are 20 kHz and 10 MHz, respectively [215]. There are numerous application areas of ultrasonication in various branches of science such as biology, biochemistry, engineering, dentistry, geography, geology and medicine, and ultrasonication can also be used as a pretreatment for disintegration of excess sludge prior to anaerobic digestion [216]. The chemistry of sonication is complex and is a combination of shearing, chemical reactions with radicals, pyrolysis, and combustion [217].

2.4.2.1 Mechanisms of Ultrasound Disintegration

When the ultrasound wave propagates in a medium such as sludge, it generates a repeating pattern of compressions and rarefactions in the medium. The rarefactions are regions of low pressure (excessively large negative pressure) in which the liquid or slurry is torn apart [218]. As a result of reduced pressure, microbubbles are formed in the rarefaction regions (Figure 2.9). These microbubbles, also known as cavitation bubbles, essentially contain vaporized liquid and gas that was previously dissolved in the liquid [218]. As the wave fronts propagate, microbubbles oscillate under the influence of positive pressure, thereby growing to an unstable size before violently collapsing. Cavitation is the phenomenon where microbubbles are formed in the aqueous phase and expand to unstable size, and then rapidly collapse (Figure 2.10). The collapsing of the bubbles often results in localized temperatures up to 5000 K and pressures up to 180 MPa [219, 220]. The sudden and violent collapse of huge numbers of microbubbles generates powerful hydro-mechanical shear forces in the bulk liquid surrounding the bubbles [221]. The collapsing bubbles disrupt adjacent bacterial cells by extreme shear forces, rupturing

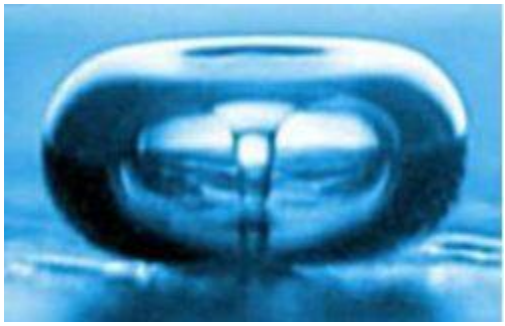

Figure 2.9 Cavitation Bubble.

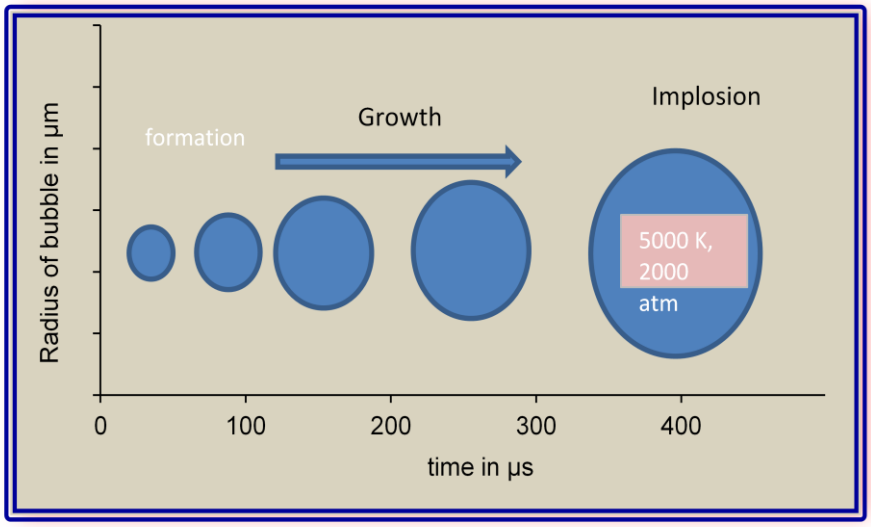

Figure 2.10 The illustration shows how a cavity builds up successively until it implodes [215].

the cell wall and membranes. The localized high temperature and pressure could also assist in sludge disintegration. At high temperatures, lipids in the cytoplasmic membrane are decomposed, resulting in holes within the membrane, through which intracellular materials leak to the aqueous phase [222]. In addition, sonochemical reactions that result in the formation of highly reactive radicals (e.g., $OH^{\bullet}$, $HO^{\bullet}_2$, $H^{\bullet}$) and hydrogen peroxide have also been reported to contribute to the ultrasonic disintegration of sludge [223]. The shear effect of ultrasonication

becomes more efficient when the acoustic frequency is below 100 kHz. On the other hand sonochemical reactions dominate the liquid when the acoustic frequency is higher than 100 kHz [223]. Tiehm et al. [223] studied ultrasonication at different frequencies in the range of 41 kHz and 3217 kHz and showed that disintegration of WAS is most effective when the frequency is set to 41 kHz which was the lowest frequency studied, showing that microbubbles radius were inversely proportional to frequency i.e. lower frequencies created larger cavitation bubbles which released more shear stress into liquid upon explosion.

2.4.2.2 Delivery of Ultrasound Energy

An ultrasound system has three major components: the converter (or transducer), booster, and horn. A converter basically converts electrical energy into ultrasound energy (or vibration). The booster is a mechanical amplifier that helps to increase the amplitude (vibration) generated by the converter. The horn is a specially designed tool that delivers the ultrasonic energy to the sludge [218].

2.4.2.3 Merits and Demerits of Ultrasound Pretreatment

Ultrasound disintegration is essentially a physical process and therefore it neither generates secondary toxic compounds nor contributes additional chemical compounds [218]. In addition to physical sludge disintegration, many toxic and recalcitrant organic pollutants, such as aromatic compounds, chlorinated aliphatic compounds, surfactants, organic dyes, etc., are also broken down into simpler forms. This is due to the generation of the highly oxidative/reactive radicals-hydroxyl ($OH^{\bullet}$), hydrogen ($H^{\bullet}$), and hydroperoxyl ($HO^{\bullet}_2$) and hydrogen peroxide (H_2O_2)

during ultrasound pretreatment, which lead to the oxidative breakdown of recalcitrant organic compounds [224].

Some other merits of ultrasound pretreatment reported in the literature [223, 225, 226, 227, 228] include:

- Compact design and easy retrofit within existing systems.
- Efficient operation compared to several other pretreatments.
- Production of an in situ carbon source for denitrification plants.
- Complete process automation.
- Potential to control filamentous bulking and foaming in digesters.
- Better digester stability.
- Improved VS destruction and biogas production.
- Better sludge dewaterability.
- Improved biosolids quality (i.e., biosolids with low residual biodegradable organics, low pathogen counts, etc.).

The ultrasound pretreatment also faces several challenges. One of the major issues is the high capital and operating costs of ultrasound units. The cost may go down as the technology matures. Similarly, long-term performance data of full-scale ultrasound systems are still limited. This discourages design engineers from recommending ultrasound systems for full-scale applications [218].

2.4.2.5 Expressions for sludge disintegration

The applied power/energy supplied for sludge disintegration is expressed in many ways, (a) specific energy input, (b) ultrasonic dose, (c) ultrasonic density and (d) ultrasonic intensity and the expressions are given in Table 2.6.

2.4.2.6 Factors influencing cavitation

The sludge disintegration efficiency is essentially based on cavitation and the factors influencing the cavitation are shown in Table 2.7. As shown in Table 2.7, different parameters affect the cavitation, some has negative effect and others have positive effect. Presence of gas and particulate matter, high solvent vapour pressure, higher frequency, and high temperature are negatively affect the cavitation, while external applied pressure, high viscosity of liquid, high solvent surface tension, Increase in sonication density are positively affect the cavitation.

Table 2.6 *Expressions for sludge disintegration* [229].

Parameter	Expression	Unit	Reference
Specific energy input	$Es = \frac{P * t}{V * TS}$	kJ/kg TS or kW s/kg TS	[239]
Ultrasound dose	$UDo = \frac{P * t}{V}$	J/L	[223]
Ultrasound density	$UD = \frac{P}{V}$	W/L	[223]
Ultrasound intensity	$UI = \frac{P}{A}$	W/cm^2	[240]

Es: specific energy in kW s/kg TS (kJ/kg TS); *P*: power input (kW); *T*: sonication time (s); *V*: volume of sludge (L); *TS*: total solids concentration (kg/L); A: surface area of the probe in cm^2.

Table 2.7 *Factors influencing the cavitation phenomena* [229].

No.	Factors	Influence on cavitation phenomena
1	Gas and particulate matter	Presence of gas/air in the liquid will lower the cavitational threshold and reduces the intensity of the shock wave released, as much of the shock wave will be utilized to collapse the gas bubbles. Particulate matters, especially like trapped vapour gas nuclei in their crevices and recesses, will reduce the cavitation effect [216]
2	External applied pressure	Increasing the external pressure raises the rarefaction pressure, which increases the cavitation collapse intensity [230, 231]
3	Solvent viscosity	If the natural cohesive forces acting in the liquid are lower, then they will suppress the negative pressure in the expansion or rarefaction cycle [232]. Therefore to increase the cavitation threshold the natural cohesive forces need to be increased by increasing the viscosity of liquid
4	Solvent surface tension	The addition of surfactant to an aqueous solution certainly facilitates the cavitation. Increase in solvent viscosity and surface tension, reduces the rate of microbubbles formation but increases the intensity of bubble collapse. With addition of surfactants will reduces the solvent surface tension and facilities bubble nucleation (i.e., fewer microbubbles are formed) [230,233]
5	Solvent vapour pressure	If the vapour pressure of the liquid is low, then it is difficult to induce cavitation in the liquid. Because, low vapour will enter into the bubble and results in low cavitation [232]
6	Applied frequency	The rarefaction phase is shortened by increasing the frequency of irradiation, but to maintain an equivalent amount of cavitational energy into the system the power should be increased. That is at higher frequency more power is required to maintain same cavitational effect [230,234, 235]
7	Temperature	The cavitation threshold increases with decrease in temperature of bulk solution. With increase in temperature, the solvent reaches the solvent boiling point and produces larger number of cavitation bubbles concurrently, which acts as barrier to sound transmission and nullify the effectivity of ultrasound energy [233]
8	Sonication density	Increase in sonication density increases the sonication effects on the sludge as given by the equation, $P_A = \sqrt{2I\rho C}$, [236]; where P_A = acoustic pressure, I = intensity, ρ = density, C = velocity of sound in the medium
9	Acoustic intensity	Increasing the sonication intensity increases the sonication effects, and it is directly proportional to the square root of the amplitude (P_A) of the acoustic wave divided by the density of the liquid (ρ) and the speed of sound in the liquid (c). $I = \frac{P_A^2}{2\rho c}$ [231,237]
10	Types of ultrasound cavitation	The collapse of the cavitation bubbles produces high velocity waves and temperature, causing inter-particle collision and the rupture of cell wall. Depending on bubble types, the ultrasound cavitation is classified as transient or stable (non-inertial cavitation). Transient is believed to occur at 10 W/cm2 and the later at 1–3 W/cm^2 [232]; the stable bubbles bound to have significant long term effect. The transient and stable bubble growth is explained by bubble growth time by Abramov [238]; $\tau_g = 0.75T + (i - 1)T$; $T = 1/f$, where τ_g is the bubble growth time, 'f' is the ultrasound frequency, 'T' is the period of ultrasound wave and 'i' is number of acoustic cycles the bubble experienced
11	Attenuation	The intensity of the ultrasound is attenuated as it progress through the medium. The attenuation is inversely proportional to the frequency of the ultrasound (i.e., energy is dissipated in form of heat which is not considered in the bulk medium). High power and high frequency is required to have the same intensity at the lower depth for a given sample
12	Field type	The standing wave field is pronounced with more acoustic cavitation than a progressive field [232]

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CHAPTER 3

Impact of Ultrasonication of Hog Manure on Anaerobic Digestability[2]

3.1 Introduction

Ultrasonication has been widely tested to improve the hydrolysis rate in anaerobic digestion of biosolids [1, 2]. Ultrasonication disrupts biosolids flocs and bacterial cells, releasing intracellular components, subsequently improving the rate of anaerobic degradation due to the solubilisation of the particulate matter, decreasing solid retention time (SRT) and improving the overall performance of anaerobic digestion [3]. The use of ultrasonication in the pretreatment of waste activated sludge (WAS) improved the operational reliability of anaerobic digesters, decreased odor generation and clogging problems, and enhanced sludge dewatering [4]. However, economical feasibility and durability due to erosion of the sonotrode as well as high energy inputs are major challenges that need to be resolved for the technology to spread [4]. Sludge characteristics such as type of sludge (primary solids, waste activated sludge or animal manure, etc.), total solids (TS) content and particle size could highly impact the disintegration efficiency and improve the overall economy of the process. Ultrasonication pretreatment studies found in the literature have focused mainly on WAS. While anaerobic digestion of hog manure is widely practiced, there has been sparse research on enhancing its hydrolysis. The main differences between hog manure and municipal biosolids, i.e. primary and waste activated sludge are: solids concentration, composition and heterogeneity. In general, the limiting step for the anaerobic digestion is the first step, hydrolysis, wherein the cell wall is broken and particulate

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substrates are enzymatically hydrolyzed allowing the organic matter inside the cell to be available for biodegradation.

Hydrolysis is well documented to be a function of specific surface area among other variables [5]. Since hydrolysis is also a function of the ratio of biomass to particulate concentration (both of which are combined as volatile suspended solids), the rate of solubilisation depends on the nature and concentration of the particulates. Fibrous substrates such as hog manure will likely hydrolyze slower than WAS and primary sludges due to differences in particle size and the ratio of biomass to particulate substrates. Thus, pretreatment is required in order to achieve the release of lignocellulosic material and thus accelerate the degradation process by means of waste solubilisation. In the literature, there is a contradiction about the effect of TS content on disintegration efficiency. Akin et al. [6] studied WAS disintegration efficiency at various TS contents (2, 4 and 6%), specific energy (SE) inputs (up to 40000 kJ/kgTS) and ultrasonic densities (from 0.44 to 3.22 W/mL), and found that at constant TS content, the soluble chemical oxygen demand (SCOD) release showed an increasing trend with the increase in both specific energy input and ultrasonic density at all TS contents. However, at constant specific energy, the SCOD release decreased with the increase in initial TS content. This finding contradicts other studies that reported significant improvement in SCOD release with WAS for TS concentration in the 0.8 to 2.5% range [7, 8].

It is well known that sludge viscosity increases with solids concentration, with the critical concentration around 25 g/L or 2.5% TS content [9]. Ultrasonication efficiency is expected to decline with increasing viscosity due to resistance to energy flow, and theoretically increased TS concentrations are detrimental to ultrasonication, despite the lack of consensus on the critical solids concentrations.

Odor generation from biosolids is a significant global problem as it negatively affects natural environments. Laboratory tests have indicated that protein degradation, especially the bound protein, i.e. proteins that are physically adsorbed on the outer cell wall which can detach during high speed centrifugation, a very popular sludge dewatering technology, is the main precursor for the odor production in biosolids [10]. Proteins are hydrolysed by extracellular enzymes (proteases) into their constituent polypeptides and amino acids. Hydrogen sulfide (H_2S) can be formed from the degradation of the sulfur containing amino acid such as cysteine, leucine, tyrosine and methionine. The pathways for production of methyl mercaptan and hydrogen sulfide from protein are described by Higgins et al. [10]. Based on an extensive literature search, it can be concluded that the effect of ultrasonication on odor compounds precursors, especially bound protein needs more research since the very limited studies on protein solubilisation focused primarily on total and soluble protein measurements with no information on the critical bound proteins from an odor perspective. For instance, Wang et al. [11] examined protein release using WAS (TS content of 3%) at different ultrasonication densities (from 0.528 to 1.44 W/mL) and different ultrasonication times (from 5 to 30 min). The aforementioned authors investigated the protein in EPS, total protein and cell protein (difference between total protein and protein in EPS). Akin et al. [6] studied the effect of ultrasonication on protein release at different TS content.

The evaluation of ultrasonication efficiency in the literature is mostly based on the degree of disintegration (DD), which is the ratio between SCOD releases by ultrasonication divided by SCOD releases by chemical disintegration. It appears from the literature that there is no unique method for determining chemical disintegration. For instance, Kunz and Wagner [12] used 1 M NaOH in the ratio of 1:3.5 by volume at 20°C for 22 h, while Muller and Pelletier [13] used 1 M

NaOH at a ratio of 1:2 by volume at 90°C for 10 min, and Bougrier et al. [14] used 1 M NaOH at room temperature for 24 h. Additionally, the used techniques are time consuming and expensive [15].

The extensive literature reviewed above highlighted the challenges of applying ultrasonication to hog manure vis-a-vis WAS and primary sludges due to its characteristics such as fibrous versus excess biomass, particulate to biomass ratios, total solids concentrations well above the 2% - 3% for WAS and primary sludge leading to increase viscosity, and heterogeneity. Furthermore, it is apparent that despite the few studies on protein solubilization, the bound protein fraction implicated in odor generation has not been investigated.

Therefore, the overall objective of this study is to evaluate the impact of ultrasonication on solubilisation and anaerobic biodegradability of hog manure with high solid content and wide ranges of particle sizes, with particular emphasis on the effect of ultrasonication on proteins solubilisation, especially bound protein. Additionally, in this work, correlations between standardized and easy to measure solubilisation parameters and the laborious and expensive method of degree of disintegration will be presented.

3.2 Material and methods

3.2.1 Analytical methods

Samples were analyzed for total solids (TS), volatile solids (VS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), and soluble total Kjeldahl nitrogen (STKN) using standard methods [16]. Total and soluble chemical oxygen demand (TCOD, SCOD) and ammonia (NH_4-N) were measured using HACH methods and test kits (HACH Odyssey DR/2500). Soluble parameters were determined after filtering the samples through 0.45 µm filter

paper. Particle size distribution was determined by Malvern Mastersizer 2000 (version 5.22) laser beam diffraction granulometer. The total gas volume was measured by releasing the gas pressure in the vials using appropriately sized glass syringes (Perfektum; Popper & Sons Inc., NY, USA) in the 5–100 mL range to equilibrate with the ambient pressure as recommended by Owen et al. [17]. Biogas composition was determined by a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 6 ft × 1/8 in). The temperatures of the column and the TCD detector were 90 and 105˚C, respectively. Argon was used as carrier gas at a flow rate of 30 mL/min. The concentrations of volatile fatty acids (VFAs) were analyzed after filtering the sample through 0.45 μm using a gas chromatograph (Varian 8500, Varian Inc., Toronto, Canada) with a flame ionization detector (FID) equipped with a fused silica column (30 m × 0.32 mm). Helium was used as the carrier gas at a flow rate of 5 mL/min. The temperatures of the column and detector were 110 and 250 °C, respectively. Carbohydrate was determined by the colorimetric method of Dubois et al. [18] with UV wavelength of 490 nm using glucose as standard.

3.2.2 Protein measurement

Protein was determined by micro-bicinchoninic acid protein assay (Pierce, Rockford, USA) which was modified from Lowry et al. [19] using a standard solution of bovine serum albumin. Cell protein was calculated as the difference between particulate and bound protein. In order to measure proteins, 50 mL samples were centrifuged at 10000 rpm for 15 minutes at 5˚C to separate the liquid and solids in the sample. The supernatant was filtered through a 1.5 μm glass microfiber filter and the filtrate was analysed for the soluble protein fraction. Bound

protein was extracted from the suspended solids by a mild pH 8 phosphate buffer (50 mM), while particulate protein representing both the bound protein adsorbed on biomass and the protein within the biomass was extracted by an alkaline 1 N Na OH solution [19]. The solids were resuspended to a total volume of 50 mL with pH 8 phosphate buffer (50 mM) for measuring bound protein and 1 N NaOH for particulate protein. The solution was mixed using a magnetic stirrer at 1500 rpm for 10 minutes, and centrifuged at 10000 rpm for 15 minutes at 5˚C, with the centrate filtered through a 1.5 μm glass microfiber filter, prior to protein analysis.

3.2.3 Experimental set-up

A lab scale ultrasonic probe was used to treat hog manure obtained from local hog farm in Southwestern, Ontario, Canada. The average characteristics of the hog manure used in this study in (mg/L); TCOD: 144900, SCOD: 55800, TS: 93180, VS: 66980, particulate protein: 22862, bound protein: 15938, soluble protein: 9134, TKN: 16580, STKN: 96820 and ammonia: 7020. The ultrasonic probe was supplied by Sonic and Materials, Newtown, USA (model VC-500, 500 W, and 20 kHz). 200 mL of hog manure was sonicated for different sonication times corresponding to different specific energy inputs, with sonication pulses set to 2 seconds on and 2 seconds off. To control the temperature rise of the sludge, a cooling water bath was used, and the sludge temperature during the experiments did not exceed 30˚C.

3.2.4 Batch anaerobic digestion

Anaerobic batch reactors were used to study the anaerobic biodegradability, and determine the ultimate methane potential and methane production rate for sonicated and unsonicated manure. The 250 mL serum flasks sealed with rubber septa on a screw-cap was

placed on the shaker- incubator (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) at 37˚C and rpm of 180. Eighteen (18) flasks were used in this study, two of them were used as blank and the rest were used for sonicated and non-sonicated samples for different specific energy inputs, as described later. The volumes of substrate (hog manure) and seed (anaerobic digester sludge from St Marys plant, St Marys, Ontario, Canada) calculated based on food to microorganisms (F/M) ratio of 4 on COD to VSS basis. For the blank, the substrate volume was replaced by distilled water.

3.2.5 Specific energy input

The specific energy input is a function of ultrasonic power, ultrasonic duration, and volume of sonicated sludge and TS concentration, and can be calculated using the following equation [14]:

$$SE = \frac{P \times t}{V \times TS} \qquad (3.1)$$

Where *SE* is the specific energy input in kWs/kg TS (kJ/kg TS), P is the ultrasonic power in kW, t is the ultrasonic duration in seconds, V is the volume of sonicated sludge in litres, and TS is the total solids concentration in kg/L.

3.2.6 Degree of disintegration (DD)

In this study, the degree of disintegration was determined based on the equation of Muller and Pelletier [13]:

$$DD = \left[\frac{COD_{Ultrsound} - COD_{Original}}{COD_{NaOH} - COD_{Original}} \right] \times 100\% \qquad (3.2)$$

Where $COD_{ultrasound}$ is the COD of supernatant of ultrasound treated sample (mg/L), $COD_{original}$ is the COD of supernatant of original (untreated) sample (mg/L), and COD_{NaOH} (mg/L) is the COD in the supernatant after addition of 1M NaOH for 24 h at room temperature.

3.2.7 $COD_{solubilisation}$

$COD_{solubilisation}$ was calculated using the SCOD released, which is the difference between SCOD at any time after ultrasonication ($SCOD_t$) and the initial SCOD ($SCOD_0$) divided by the initial particulate COD ($TCOD_i - SCOD_0$):

$$COD_{solubilisation} = \left[\frac{SCOD_t - SCOD_0}{TCOD_i - SCOD_0} \right] \times 100\% \qquad (3.3)$$

Where $TCOD_i$ is the initial TCOD concentration.

3.2.8 $TKN_{solubilisation}$

$TKN_{solubilisation}$ was calculated using the STKN released which is the difference between STKN at any time after ultrasonication ($STKN_t$) and the initial STKN ($STKN_0$) divided by the initial particulate TKN (TKN_i-$STKN_0$):

$$TKN_{solubilisation} = \left[\frac{STKN_t - STKN_0}{TKN_i - STKN_0} \right] \times 100\% \qquad (3.4)$$

Where TKN_i is the initial TKN concentration.

3.3 Results and Discussion

3.3.1 Comparison of solubilisation and degree of disintegration

Using $COD_{solubilisation}$ and plotting the results with respect to DD, $TKN_{solubilisation}$, % increase in soluble protein, and % decrease in particulate protein (Figure 3.1), a perfect linear

relationship with an R^2 = 1.0 was obtained for the correlation between $COD_{solubilisation}$ and DD (Figure 3.1a). The linear relationship between $COD_{solubilisation}$ and $TKN_{solubilisation}$ emphasizes that the solubilisation of nitrogenous compounds followed the similar trend of COD solubilisation (Figure 3.1b). Figures 3.1c and 3.1d illustrating the relationship between $COD_{solubilisation}$ on one hand and % increase in soluble protein, and % decrease in particulate protein on the other hand emphasize that $COD_{solubilisation}$ is more strongly linearly related with % decrease of particulate protein than % increase in soluble protein. Thus, $COD_{solubilisation}$ from now on can be used to evaluate the solubilisation degree in lieu of the DD procedure, as it proved to be an accurate and easy measure.

3.3.2 Particle size distribution

Particle size distribution is widely used as qualitative measure for sludge disintegration. Anaerobic digestion of waste is governed by hydrolysis (solubilisation of particulates) that is highly affected by the particle size. Smaller particle sizes and the lower concentration of particulates, measured as VSS lead to higher degradation efficiency. As shown in Figure 3.2, the hog manure is characterized by a wide range of particle size ranging from 0.6 µm to 2500 µm, compared to a range of 0.4 µm to 1000 µm reported for WAS [14, 1]. As shown in Figure 3.2, the particle size distribution for the hog manure shows a bi-modal distribution, with two peaks, the first at 60 µm and the second at 1200 µm, respectively. Interestingly, the disintegration effect was more pronounced for the particles in the range of 0.6 µm to 60 µm; while a minor effect was observed for particles > 200 µm. The mean particle size diameter (d_{50}) decreased from 59 µm in the raw hog manure to 21.9 µm with the specific surface area (SSA) increasing from 0.523 to 1.2 $\mu m^2/g$ at a specific energy of 30000 kJ/kgTS (Table 3.1). Using WAS, Gonze et al. [1], Bougrier

et al. [14] achieved decrease in mean particle size diameters from 320 to 18.1 µm and from 32 to 12.7µm, at TS content of 1.2 to 3.2 gDS/L and 18.5 g/L, respectively. In another study, Akin et al. [6] achieved decrease in mean diameters from 209 to 18.1, from 217 to 38.2 and from 225 to 33.4 µm, at TS content of 2, 4 and 6% of WAS, respectively.

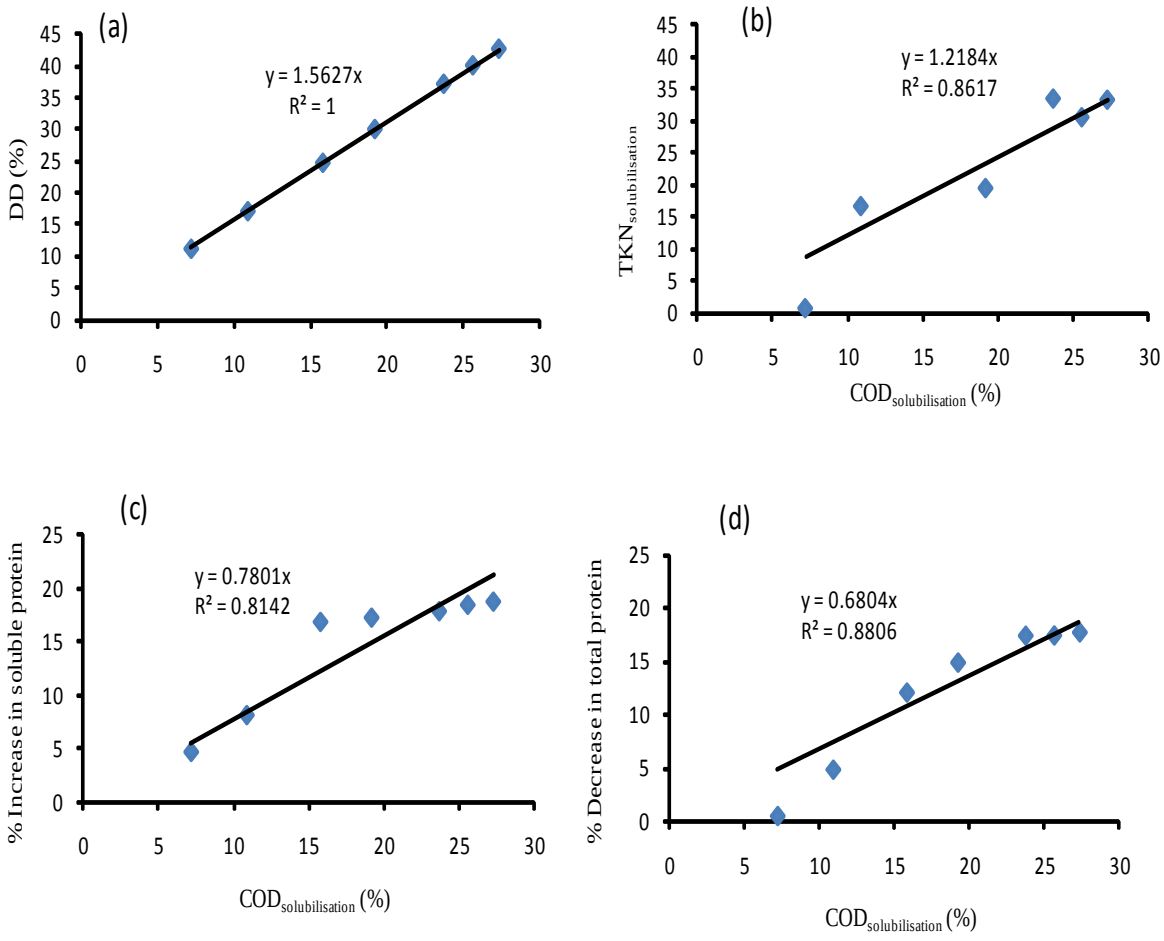

Figure 3.1 Relationships between $COD_{solubilisation}$ and: (a) DD_{SCOD} (%), (b) $TKN_{solubilisation}$, (c) % Increase in soluble protein, (d) % Decrease in total protein.

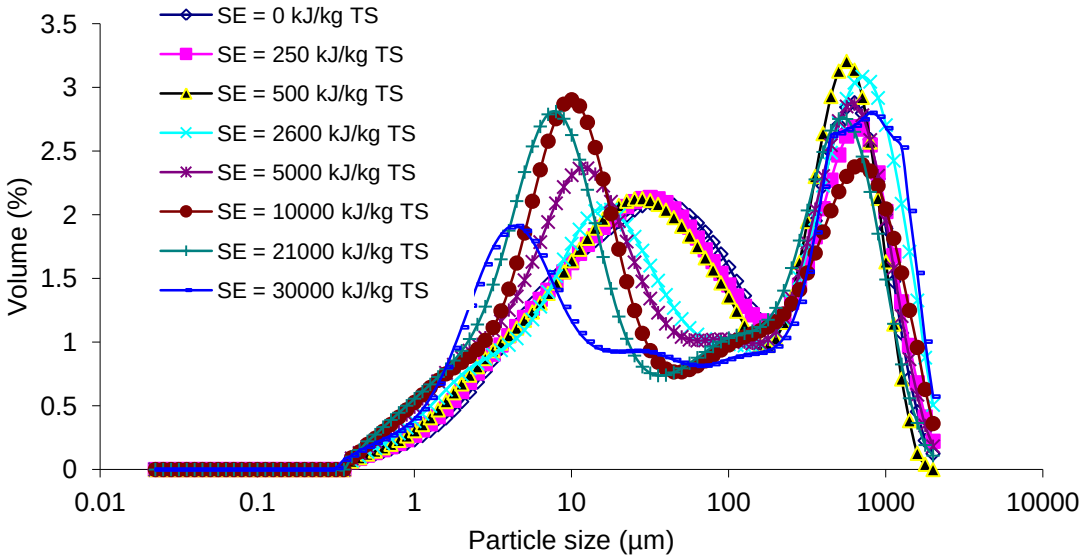

Figure 3.2 Particle size distributions for different specific energy inputs.

Table 3.1 *Particle size and* $COD_{solubilisation}$ *at different specific energy inputs.*

SE (kJ/kg TS)	0	250	500	2500	5000	10000	21000	30000
d_{50} (µm)	59.0	56.0	53.9	47.3	39.7	33.3	27.4	21.9
SSA ($(\mu m^2/g)$	0.52	0.56	0.59	0.63	0.78	0.8	0.91	1.2
% Reduction in VS	-	5	20	24	24	30	31	32
DD (%)	-	11	17	25	30	37	40	43
$COD_{solubilisation}$ (%)	-	7	11	16	19	24	26	27

d_{50}: 50% of particles volume having a diameter lower than or equal to d_{50}.

Thus, it is evident that the effect of ultrasonication on particle size depends on the nature of the biomass and the TS content. For WAS smallest particle size (18.1 µm) has been achieved at lower TS content of 2% [6]. While for manure, the smallest particle size 21.9 µm was achieved at higher TS content of 9.3%.

3.3.3 Solubilisation of hog manure

Ultrasonic pretreatment solubilises extracellular matter and extracellular polymeric substances (EPS), increasing the SCOD. Thus, SCOD is mostly used to measure the sludge disintegration efficiency. The specific energies for various TS contents and DD from this study and two other studies are plotted in Figure 3.3. A sharp decline in the required specific energy from 65000 kJ/kg TS to 10000 kJ/kg TS was observed when the TS increased from 0.5% to 2%. The slope of the curve then decreased drastically and the required specific energy to achieve a certain DD was almost constant regardless of the increase in TS. For hog manure with a TS content of 9.3%, only 3000 kJ/kg TS was required to increase the DD by 15% (from 10% to 25%), while for WAS, a specific energy of 20000 and 25000 kJ/kg TS is required to achieve the same increase in DD for WAS with TS content of 2% and 0.5%, respectively.

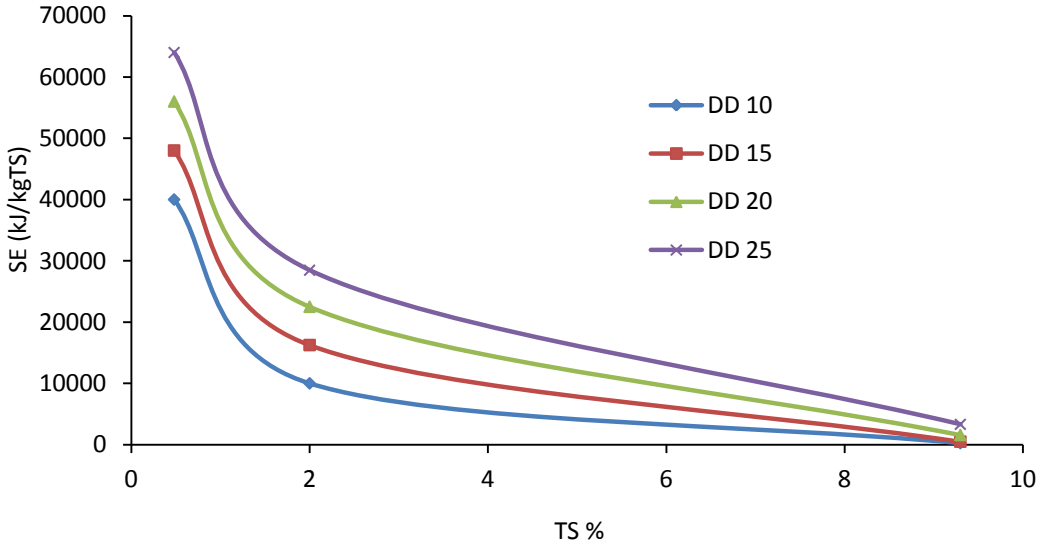

Figure 3.3 Specific energy input for different TS at different degree of disintegrations.

*Data in this graph from this study, Tiehm et al. (2001); Rai et al. 2004.

Two other studies have been conducted on WAS with different TS content but they did not report the SE input, and therefore can not be compared. Gronroos et al. [7] studied WAS with dry solids (DS) content (0.8, 1.6 and 2.5%), different ultrasonic densities (50, 175 and 300 W/L), different frequencies (22 and 40 kHz) and treatment time (5, 17.5 and 30 min). The aforementioned authors observed that the largest SCOD increase was obtained with the highest power, highest DS and longest sonication time. Wang et al. [8], using WAS, at two TS content (0.5% and 1%) studied different disintegration times (10, 20 and 30 min), different intensities (from 30 to 230 W/cm^2) and different densities (0.25, 0.5, 1.0 and 1.5 W/mL), and found that the highest power, highest DS and longest treatment time resulted in highest SCOD increase consistent with Gronroos et al., [7]. Thus, the high solids content of hog manure of 9.3% versus the 0.5% to 2.5% for WAS in this case did not adversely impact solubilization. Comparing the 3000 kJ/kg TS required to achieve a 15% increase in DD for hog manure with the 20000 and 25000 kJ/kg TS for WAS implies that hog manure is about 6-8 times more amenable to ultrasonication than WAS.

The maximum solubilisation of hog manure measured as $COD_{solubilisation}$ was 27.3% at 30000 kJ/kg TS, whereas Khanal et al. [20] and Bougrier et al. [14] using WAS, achieved 16.2% and 41.6% at specific energies of 66800 kJ/kg TS and 14547 kJ/kg TS, respectively. Applying ultrasonication of hog manure at different specific energy inputs achieved an increase of 1.35 mg SCOD/(kJ/kg TS) compared to 0.15, 0.12, 0.45 and 0.9 mg SCOD/(kJ/kg TS) calculated from data reported by Khanal et al. [20]; Gronroos et al. [7]; Navaneethan [2]; and Bunrith [21], respectively indicating greater pretreatment potential of hog manure by ultrasonication compared to WAS. On the other hand the average reduction in VS for hog manure was 22.5 ± 2% for the specific energy in the range of 500 to 5000 kJ/kg TS. While increasing the specific energy to

10000 kJ/kg TS raised the VS reduction percentage to 29.6%. Increasing the specific energy beyond 10000 kJ/kg TS did not improve the VS reduction significantly.

The TKN remained constant throughout the experiments, and thus no nitrogen mineralisation or volatilisation was observed. As shown in Table 3.2, ultrasonication of hog manure increased the STKN from 9682 mg/L to 11994 mg/L corresponding to a $TKN_{solubilisation}$ about 34% at a specific energy input of 10000 kJ/kg TS, after which the STKN remained constant, comparable to the nitrogen solubilisation of 40% at specific energy input of 10000 kJ/kg TS observed by Bougrier et al. [14] for WAS. The ammonia-nitrogen concentration increased from 7020 mg/L in the raw hog manure to 8380 mg/L after sonication, with increase in the ratio of NH_4-N/TKN of only 10% at 10000 kJ/kg TS (Table 3.2). The increase in ammonia concentration also indicates the hydrolysis of organic nitrogen due to ultrasonication.

3.3.4 Proteins (particulate, bound and cell) solubilisation

Proteins are usually divided into three types; particulate protein, bound protein, and soluble protein [22]. The particulate protein was considered as the tightly bound protein in flocs and is composed of particles in the bacterial cell mass. Bound protein is the labile fraction loosely attached on biomass, while the soluble protein represents protein in solution. Bound protein is considered to be one of the main causes for odor in anaerobic digestion; and the effect of ultrasonication on the proteins needs to be characterized. The effect of ultrasonication on proteins is summarized in Table 3.2. While approximately a 17% decrease in the particulate proteins was achieved at a specific energy of 10000 kJ/kg TS, the soluble protein increased by 18%. It was observed that at specific energy inputs less than 500 kJ/kg TS, the reduction in particulate protein of up to 5% was attributed to the decrease in bound protein, while a 17.7%

reduction in cell protein was observed for specific energy of 10000 kJ/kg TS, after which the solubilisation efficiency remained constant. In another study by Akin et al. [6] on ultrasonication of WAS, the protein release was significantly reduced at higher TS content. The maximum protein released was 73 mg/g TS at a TS content of 2% and SE of 10000 kJ/kg TS, but decreased to 40 and 22 mg/g TS at SE of 5000 kJ/kg TS for TS content of 4% and 6%, respectively. The soluble protein released in this work is about 17 mg/g TS at SE of 2600 kJ/kg TS in fact follows the same trend of decreasing protein solubilisation with the simultaneous decrease of SE respectively. Comparing the protein per unit energy for hog manure with the WAS results of Akin et al. [6] reveals that for hog manure protein solubilisation of 17 mg/g TS at ultrasonication density of 234 MJ/m^3 is identical to the 22 mg/g TS at ultrasonication density of 300 MJ/m^3 since the 29% difference in protein released is commensurate with the 28% difference in ultrasonication density.

Upon comparing the results of this study with Akin et al. [6] with respect to the impact of TS content, it is readily discerned that for WAS, solubilisation of proteins decreased with increasing TS content in the 2-6% range, while for hog manure even a 9.3% TS content did not negatively impact protein solubilization, reflecting the difference in the nature of hog manure.

It is interesting to note that a minimum of 500 kJ/kg TS specific energy input was required in order to rupture the cell wall and to release the cell protein, and it is more than an order of magnitude lower than 7700 kJ/kg TS required by Wang et al. [11] for WAS.

Table 3.2 *$TKN_{solubilisation}$, ammonia and protein solubilisation at different specific energy inputs.*

SE (kJ/kg TS)	STKN (mg/L)	$TKN_{solubilisation}$ (%)	NH_4-N/TKN (%)	% Decrease in P-P	% Decrease in B-P	% Increase in S-P	% Decrease in Cell-P
0	9682	-	42	-	-	-	-
250	9731	0.7	48	0.4	8.0	4.8	0
500	10832	16.7	48	4.8	9.2	8.3	4.5
2600	10518	12.1	51	12.0	12.7	17	12.0
5000	11026	19.5	52	14.9	13.4	17.4	15.0
10000	11994	33.5	52	17.4	13.0	18.0	17.7
21000	11792	30.6	53	17.7	12.8	18.6	17.7
30000	11981	33.3	53	18.1	13.5	18.9	18.0

- % Decrease = [(initial value – value after ultrasonication)/ initial value]*100
- % Increase = [(value after ultrasonication - initial value)/ initial value]*100
- P-P = Particulate protein, B-P = Bound protein, S-P = Soluble protein, and Cell-P = cell protein

Data in Table 3.2 emphasizes that at low specific energy inputs (less than or equal to 2600 kJ/kg TS), up to 12.7% reduction in bound protein is achievable. The data for bound protein in Table 3.2 emphatically shows that ultrasonication has reduced bound protein by 8% to 13.5%, with the rate change diminishing rapidly at a specific energy higher than 2600 kJ/kg TS, at which a 12.5% reduction was achieved. Thus, it is evident that pretreatment by ultrasonication does significantly abate the potential for odor generation caused by bound proteins.

3.3.5 Methane production and economics

The biochemical methane potential (BMP) test was used to evaluate anaerobic biodegradability in batch reactors. Figure 3.4 shows the cumulative methane production over time at different sonication energy inputs, with the data summarized in Table 3.3. As shown in Figure 3.4, no lag phase was observed due to the sufficiency of soluble substrates. With respect to the results in Table 3.3, it is clearly observed that ultrasonication of hog manure enhanced the biogas production at low energy inputs compared to unsonicated hog manure. Methane potential increased by 28% relative to the unsonicated hog manure for a specific energy input of 500 kJ/kg TS, while the increase at high energy inputs (30000 kJ/kg TS) was only 20.7%. While the % increase in methane production rate increased by increasing the energy input, maximum increase in methane production rate was 80.6% compared to unsonicated hog manure at a specific energy input of 30000 kJ/kg TS.

The increase in methane production rate for specific energy input of 500 kJ/kg TS (high methane potential) was about 61.3%, and decreased for SE of 500 to 10000 kJ/kg TS before increasing again. Therefore, since ultrasonic pretreatment of hog manure with SE of 500 kJ/kg TS gave a comparable methane production enhancement in both rate and potential with SE of 21000-30000

kJ/kg TS, the 500 kJ/kg TS can be considered to be the optimum energy input for the pretreatment of ultrasonicated hog manure prior to anaerobic digestion. On the other hand, the reported optimum specific energy for ultrasonic pre-treatment of WAS in the literature was significantly higher at 11000 kJ/kg TS [11] and 12000 kJ/kg TS [2].

The COD mass balance for all the batches was computed considering the initial and final TCOD, and the equivalent COD of methane (0.395 LCH_4/gTCOD), which indicated a closure at 90–95%, thus emphasizing data reliability.

The maximum difference between the final VSS concentration in the sonicated and unsonicated hog manure after digestion was 14% of the unsonicated VSS at a SE of 10000 kJ/kg TS.

An economic analysis (the results are summarized in Table 3.3) was conducted based on power and natural gas costs of \$0.07/kWh and \$0.28/m^3, respectively. As apparent from Table 3.3, the specific energy of 500 kJ/kg TS can be considered to be the optimum energy input for anaerobic digestion of ultrasonic pretreated hog manure to be economically viable, as the value of the energy output exceeds that of the energy input by \$ 4.1/ton of dry solids.

Table 3.3 *Ultrasonication and Methane Energy per ton of TS.*

	Methane		Power input		Methane out		
SE (kJ/kg TS)	% Increase in methane potential	% Increase in maximum methane production rate	kWh/ton TS_{in}	Price \$/ton TS_{in}	Increase of CH_4 (mL)	CH_4 m^3/ton TS_{in}	Price \$/ton TS_{in}
0	-	-	0	-	-	-	-
250	11.7	33.7	69	4.9	67	17.2	4.8
500	28.0	61.3	139	9.7	160	50.4	14.1
2600	10.9	43.5	722	50.6	62	201.2	5.6
5000	16.3	35.5	1389	97.2	93	29.3	8.2
10000	19.9	46.6	2778	194.4	114	37.9	10.6
21000	18.7	75.4	5833	408.3	107	36.3	10.2
30000	20.7	80.6	8333	583.3	118	40.0	11.2

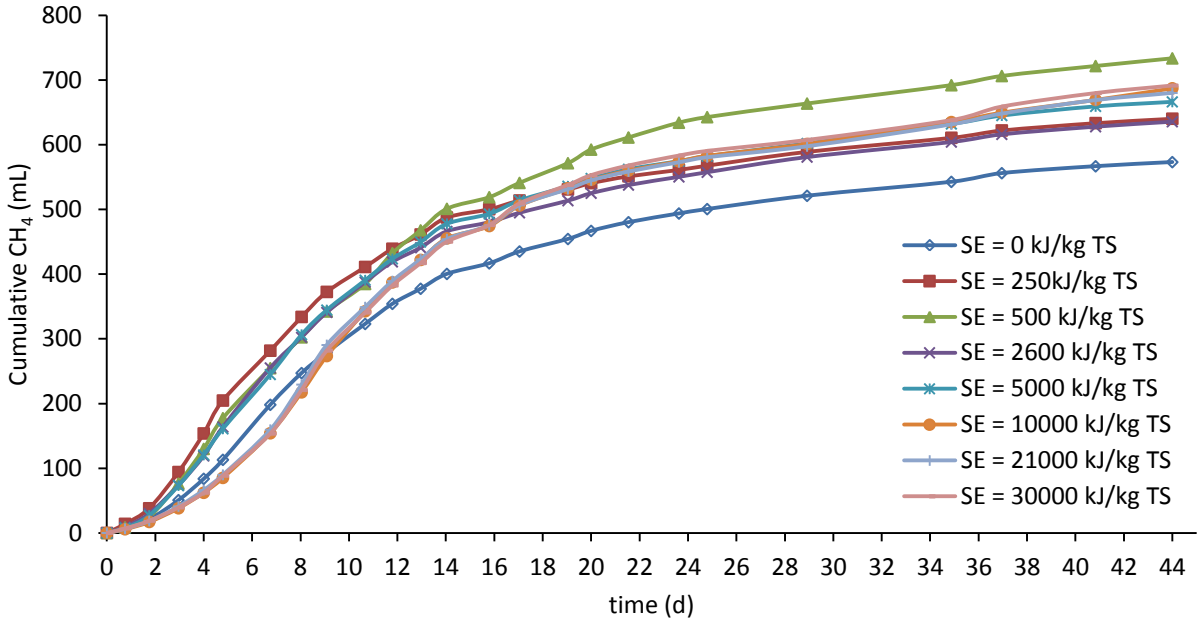

Figure 3.4 cumulative methane productions at different specific energy inputs.

3.4 Conclusions

Based on the finding of this study, the following conclusions can be drawn:

- The $COD_{solubilisation}$ correlated very well with the DD, the $TKN_{solubilisation}$ and the % decrease in particulate protein. Thus, $COD_{solubilisation}$ can be used to evaluate the degree of solubilisation in lieu of the labour and time intensive DD procedure, as it proved to be an accurate and easy to measure method.
- For hog manure, the disintegration of particles by ultrasonication was more pronounced for the smaller sizes, i.e., in the 0.6 to 60 µm range, as well as the reduction of VS by ultrasonication increased with increasing specific energy input in the 500-5000 kJ/kg TS and reached a plateau at 10000 kJ/kg TS.
- At solids content of 2%, the specific energy input increased from 10000 to about 30000 kJ/kg TS for an additional 15% increase in degree of disintegration, whereas at TS of about 9%, the specific energy input increased from 250 to about 3,300 kJ/kg TS to achieve the same increase in DD. Therefore, ultrasonication is more effective

pretreatment process for hog manure with higher TS content than WAS and primary sludges.

- Upon comparing the results of this study with Akin et al. [6] with respect to the impact of TS content, it is readily discerned that for WAS, solubilisation of proteins decreased with increasing TS content in the 2-6% range, while for hog manure even a 9.3% TS content did not negatively impact protein solubilization, reflecting the effect of difference in the nature of sludge on the efficiency of pretreatment.
- Bound proteins decreased by 13.5% at specific energy of 5000 kJ/kg TS. Thus, the impact of ultrasonication on odor precursors such as bound proteins appears to be significant.
- The cell wall appeared to be ruptured at a minimum specific energy input of 500 kJ/kg TS, whereas the optimum specific energy was 10000 kJ/kg TS, affecting a 17.7% reduction in cell protein.
- The optimum specific energy input for methane production was 500 kJ/kg TS, and resulted in a 28% increase in methane production, and subsequently about $ 4.1/ton of dry solids excess energy output.

3.5 References

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CHAPTER 4

Simulation of the Impact of SRT on Anaerobic Digestability of Ultrasonicated Hog Manure[3]

4.1 Introduction

Although swine wastewater is widely used as fertilizer because of its high organic, nitrogen and phosphorus content, many countries are paying attention to the pollution resulting from livestock farms, and have tightened legislation and discharge standards recently. As far as swine waste treatment is concerned, anaerobic digestion (AD) is an important alternative to land application, because it reduces pollution and recovers methane. A number of studies have been reported for anaerobic digestion of swine waste [1–4] in the literature.

In general, the limiting step of anaerobic digestion of solid waste is the first step of hydrolysis or solubilization, where the cell wall is broken down allowing the organic matter inside the cell to be available for biological degradation [5–8]. Particularly, in the case of livestock residues, the hydrolysis step is restricted by the presence of fibres [9]. The anaerobic digestion process may therefore be improved if hydrolysis can be enhanced. Thus, pretreatment is often required in order to achieve the release of lignocellulosic material and thus accelerate the degradation process by means of waste solubilisation and consequently enhance the biogas production during anaerobic digestion [9]. Various pretreatment methods such as thermal, chemical, ultrasonic, and biological have been studied by many researchers [10–13]. Since the hydrolysis rate is directly related to the surface area of the sludge particles

[3] A version of this chapter has been published in *Energies*, 2010

[14], increasing particles surface area will also increase the hydrolysis rate [15]. The use of ultrasonication in the pretreatment of sludge improved the operational reliability of anaerobic digesters, decreased odor generation and clogging problems and enhanced sludge dewatering [16].

It must be noted that while H_2S has been accepted as the main odorous contaminant in biogas, recently bound proteins *i.e.*, proteins loosely attached to the cell wall, have been determined as a major odor precursor downstream of anaerobic digestion, specifically during dewatering. Despite the numerous advantages of ultrasonic pretreatment of municipal biosolids, operational reliability, ease of implementation, elimination of odors and clogging, and good sludge dewaterability, the rapid wear on the sonotrode and negative energy balance [17] hindered widespread use of the technology.

The presence of high sulfate concentration in wastewater restricts the application of the anaerobic digestion treatment technology due to the production of the toxic and odorous hydrogen sulfide (H_2S) by sulfate-reducing bacteria [18]. The extensive ultrasonication research available in the open literature focused primarily on improving hydrolysis of municipal biosolids, with little or sparse data on applications to other wastes and impact on odor. Thus, the aim of this study is to investigate the effect of ultrasonication of hog manure on the performance of anaerobic digestion and its effect in odor reduction, specifically the removal of bound protein and hydrogen sulfide in the headspace.

4.2 Experimental Section

4.2.1 Analytical methods

The produced biogas was collected by wet tip (Gas meters for laboratories, Nashville, TN). The gas meter consists of a volumetric cell for gas-liquid displacement, a sensor device for liquid level detection, and an electronic control circuit for data processing and display

[19]. H_2S was measured using the Odalog (model odalog type I, App-Tek International Pty Ltd, Brendale 4500, Australia), which has a detection range of 0–1000 ppm with an accuracy of 2 ppm. SO_4^{2-} was measured using an ion chromatography (IC) system (Dionex 600, USA) equipped with CS16-HC and AS9- HC columns, respectively. All other liquid parameters and gas compositions were analyzed as described in chapter 3 (section 3.2.1 Analytical methods).

4.2.2 Ultrasonication and anaerobic digestion set-up

A lab scale ultrasonic probe was used to treat hog manure obtained from local hog farm in Southwestern, Ontario, Canada. The ultrasonic probe was supplied by Sonic and Materials (model VC-500, 500 W, and 20 kHz). Hog manure was sonicated with specific energy inputs of 500 kJ/kgTS, with sonication pulses set to 2 seconds on and 2 seconds off to control the temperature rise of the sludge. Digestion of hog manure was carried out using anaerobic digester (10 L), with a working volume of 7.5 L and a solids retention time (SRT) of 15 days, operated in completely mixed continuous flow mode and maintained at constant temperature of 37 °C. Table 4.1 lists the feed characteristics used for the unsonicated and sonicated runs. The digester was operated at steady-state, as reflected by constant specific biogas production rate and digester sludge biomass concentration (was reached after more than three turnovers of the mean SRT).

4.2.3 Specific energy input:

The specific energy input (SE) is a function of ultrasonic power, ultrasonic duration, and volume of sonicated sludge and TS concentration, and can be calculated using the following equation Bougrier *et al.* [20]:

$$SE = \frac{P \times t}{V \times TS} \qquad (4.1)$$

where *SE* is the specific energy input in kWs/kgTS (kJ/kgTS), P is the ultrasonic power in kW, t is the ultrasonic duration in seconds, V is the volume of sonicated sludge in litres, and TS is the total solids concentration in kg/L.

Table 4.1 *Feed characteristics used for the unsonicated and sonicated manure.*

Parameter (mg/L)	Unsonicated manure (influent to the control digester)	Sonicated manure	
		Manure before sonication	Manure after sonication (influent to the digester)
TSS	15,100 ± 550	15,800 ± 680	13,900 ± 780
VSS	11,000 ± 530	11,500 ± 510	8800 ± 400
TCOD	26,600 ± 1800	28,000 ± 1540	28,300 ± 1500
SCOD	12,700 ± 1200	13,100 ± 1260	15,900 ± 1300
Ammonia	750 ± 30	820 ± 90	460 ± 70
P-Protein	2700 ± 90	2850 ± 210	2570 ± 180
B-Protein	680 ± 70	710 ± 60	620 ± 80
S-Protein	2600 ± 200	2900 ± 360	3400 ± 210
TKN	1800 ± 90	1900 ± 100	1800 ± 110
STKN	940 ± 110	940 ± 70	1100 ± 40
VFA*	1650 ± 190	1680 ± 310	1800 ± 260

*VFA in mgCOD/L

4.3 Results and Discussion

4.3.1 Ultrasonication of hog manure

Ultrasonication causes a localized pressure drop to below the evaporating pressure in the aqueous phase, resulting in the formation of micro bubbles by evaporation. The micro bubbles oscillate in sound field, grow by rectified diffusion and collapse in a non-linear manner. The combination of bubble oscillation and the resulting vacuum created by the collapse of the bubble leads to strong mechanical forces that can erode solid particles [21]. The hog manure was sonicated at a specific energy input of 500 kJ/kgTS. The characteristics

of hog manure before and after ultrasonication are shown in Table 4.1. While there was no significant change in TCOD and TKN after ultrasonication, TSS, VSS, particulate protein and bound protein decreased by 17%, 21%, 10% and 12%, respectively, after sonication. Furthermore, as expected, SCOD, VFA, ammonia, soluble protein and STKN increased by 29%, 12%, 17%, 17% and 12%, respectively, after sonication. A paired t-test was conducted to evaluate the statistical significance of the observed differences as elaborated upon later.

4.3.2 Solids destruction

Figure 4.1 shows the steady-state average reductions of TSS, VSS, TCOD, and SCOD during AD for the unsonicated and sonicated manure. As shown in Figure 4.1, anaerobic VSS degradation efficiency of sonicated manure is higher than the unsonicated manure by 13% (51% for sonicated versus 45% for unsonicated). However, considering the overall VSS removal efficiency of sonicated manure both during ultrasonication and digestion into consideration, there was a 36% increase in VSS removal efficiency due to sonication, with ultrasonication/AD achieving 61% versus 45% reduction for AD alone. This increase of VSS removal is consistent with the findings of Nickel and Neis [22], who observed an increase in VSS degradation of sonicated waste activated sludge (WAS) by 30% at an SRT of 16 days compared to the conventional digestion. In another study, Braguglia et al. [23] applied ultrasonication as a pretreatment for WAS at a specific energy of 5000 kJ/kgTS, and found that the VS removal increased only from 36% to 39% at SRT of 20 days, while at SRT of 10 days, the VS removal efficiency of untreated sludge declined from 36% to 31% and for sonicated sludge from 39% to 33% *i.e.*, at both SRTs sonication affected a marginal 6–8% increase in VS destruction efficiency.

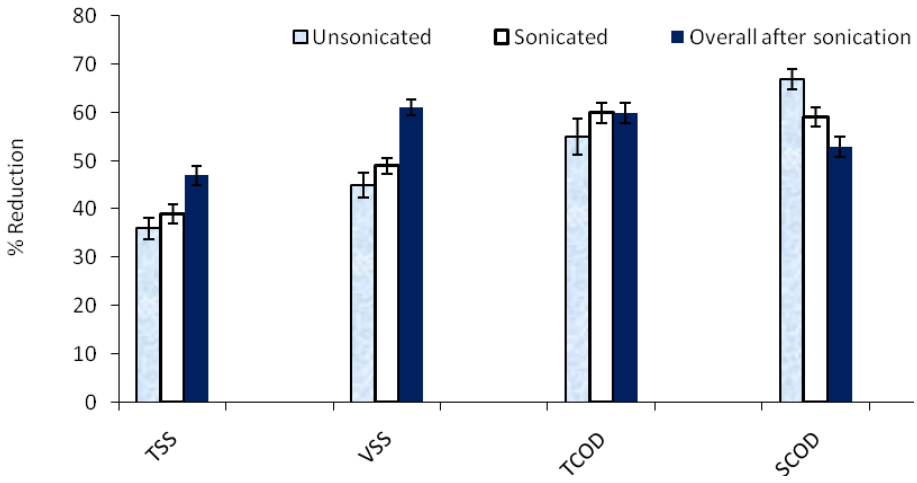

Figure 4.1 Degradation efficiency of unsonicated and sonicated manure.

Tiehm *et al.* [10] applied ultrasonication in a pilot plant using a high performance ultrasound reactor (3.6 kW, 31 kHz) for 64 sec on a mixture of primary sludge and WAS (53% primary sludge and 47% WAS) with average VSS of 25 g/kg, and observed a 10% increase in VS removal efficiency of sonicated waste over the conventional AD process at an SRT of 22 days, although no enhancement in VS reduction was observed at an SRT of 8 days. On the other hand, TSS removal efficiency in the digester increased from 36% to 43% with sonication, while the overall removal efficiency of TSS for sonicated manure was 47%.

4.3.3 COD destruction

As expected, unsonicated and sonicated manure have approximately the same influent TCOD (less than 10% difference) while the SCOD for sonicated manure was higher than of the unsonicated manure by 34% (Table 4.1). After digestion, there was no significant difference in TCOD removal efficiency for the sonicated and unsonicated manure. TCOD removal efficiency was 55% and 60% for unsonicated and sonicated manure, respectively (Figure 4.1) due to a higher soluble fraction of COD in the influent. The relatively higher TCOD removal efficiency agrees with McDermott et al. [24], who applied ultrasonication on

aquaculture waste (consisting predominantly of fecal material and waste fish food pellets) as a pretreatment to AD and reported COD removal efficiencies of 85% and 77% for sonicated and unsonicated waste, respectively.

In our case, the SCOD concentrations deceased from 13,100 to 4188 and from 15,900 to 6147 mg /L for unsonicated and sonicated hog manure during the anaerobic digestion. The SCOD removal efficiency in the digester receiving sonicated manure was 60% versus 67% for unsonicated manure, attributable to the high initial SCOD resulting from ultrasonication of manure, consistent with the observation of McDermott et al. [24] who reported no appreciable difference in reactor effluent SCOD values between the sonicated and unsonicated waste.

4.3.4 Nitrogen compounds and odorous contaminants

As depicted in Figure 4.2, the TKN after digestion decreased by 19% and 11% for sonicated and unsonicated manure, respectively to 1450 and 1580 mg/L. STKN increased by 34% in the unsonicated manure after digestion to 1260 mg/L, while STKN in the digested sonicated manure remained constant, potentially due to higher influent STKN due to ultrasonication. Ammonia exhibited the same trend of STKN in the reactor although it was below the inhibition level (1500 mg/L) in both cases. Digested manure ammonia concentration for unsonicated manure of 1200 mg/L was higher than the 980 mg/L for digested sonicated manure.

Proteins in sludge are usually divided into three types; particulate protein, bound protein, and soluble protein [25]. The particulate protein was considered as the tightly bound protein in flocs and is composed of particles in the bacterial cell mass, and the bound protein is the labile fraction loosely attached to biomass, while the soluble protein represents protein

in the solution. Bound protein is considered to be one of the main causes for odor in anaerobic digestion [26].

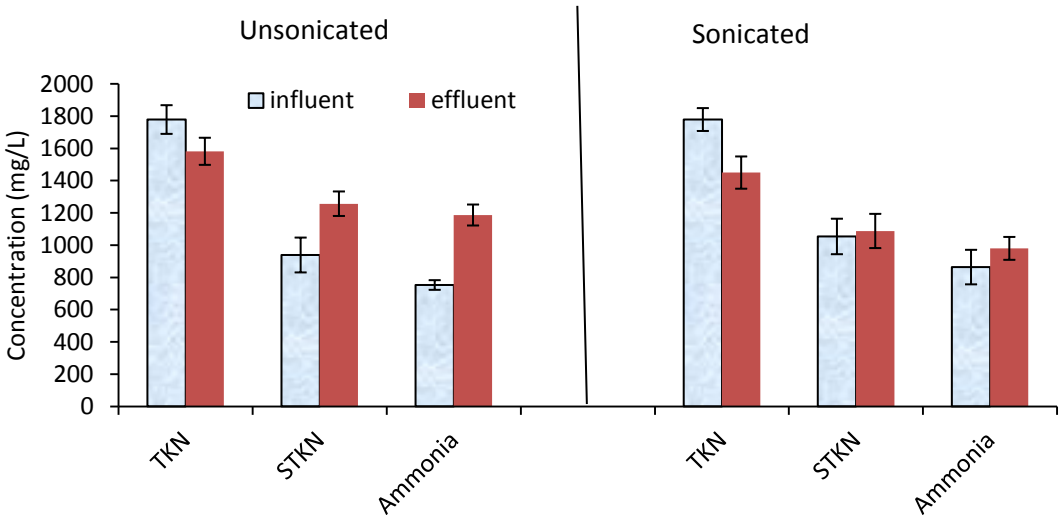

Figure 4.2 Nitrogen compounds (TKN, STKN and ammonia) concentrations for sonicated and unsonicated manure.

Figure 4.3 shows the removal efficiency of the three different types of proteins (particulate, bound and soluble) along with the sulphate reduction efficiency for the sonicated and unsonicated manure. During digestion, particulate protein removal efficiency averaged 58% and 60% for the unsonicated and sonicated manure, respectively, while the overall removal efficiency of particulate protein for the sonicated manure was 64%. The digester removal efficiency of soluble protein for unsonicated manure of 75% was higher than the 65% for sonicated manure, and the overall efficiency of soluble protein for combined sonication and digestion was 59%. This is due to the higher soluble protein concentration in the sonicated sludge due to the solubilisation of the particulates. The enhancement of bound protein removal efficiency was highly discernible; a 13% increase in bound protein removal efficiency for sonicated manure during digestion relative to the unsonicated sludge, while the

overall removal efficiency of bound protein for sonicated manure was higher than the unsonicated by 17.5% (Figure 4.3) which reflects the effect of ultrasonication on odor reduction caused by bound protein. In addition to the enhancement in bound protein reduction there was a decline in H_2S production in the digester headspace due to ultrasonication prior to digestion. The average concentration of H_2S in the headspace of the bioreactor deceased from 988 to 566 ppm for unsonicated and sonicated manure, respectively. The aforementioned reduction may reflect the effect of ultrasonication on sulfate reducing bacteria. Furthermore, SO_4^{2-} reduction during anaerobic digestion was 59% and 38% for unsonicated and sonicated manure, respectively (Figure 4.3).

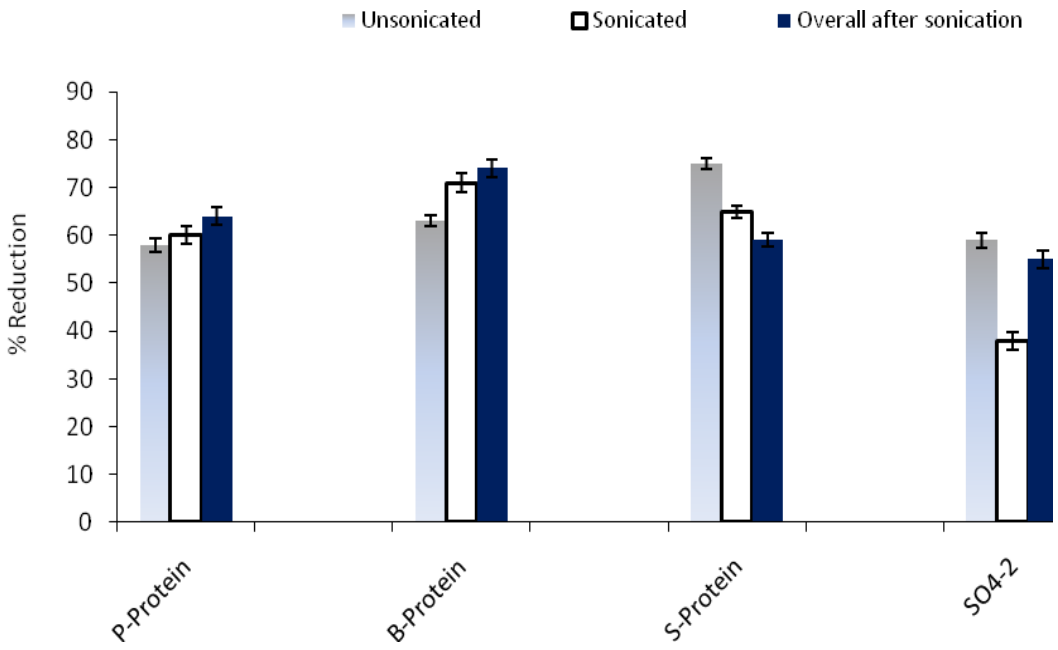

Figure 4.3 Degradation efficiency of particulate protein, bound protein, soluble protein and sulfate.

A theoretical estimation of the headspace H_2S concentration in the biogas was conducted using observed sulfate reduction of 11.4 and 25.4 mg/L, for the sonicated and unsonicated manure with the measured values using the equation of Lens and Kuenen [27]:

$$H_2 + SO_4^{2-} \rightarrow H_2S + HS^- + 5H_2O + 3OH^- \quad (4.2)$$

Henry's constant for H_2S of 9.8 atm L/mol K at 25 °C [28] was corrected for the operating temperature of 37 °C. The calculated H_2S concentrations in both the sonicated and unsonicated manures of 490 and 950 ppm, respectively, are 13% and 4% lower than the observed 566 and 998 ppm, indicated a good mass balance in the system.

A statistical-paired t-test used to evaluate the observed differences in parameter reduction during anaerobic digestion between the sonicated and unsonicated manures, revealed that TSS, VSS, TCOD, bound protein, soluble protein and H_2S efficiencies were statistically different at the 95% confidence level with only SCOD and particulate protein insignificant at the 95% confidence level. Thus, it is evident that ultrasonication has achieved significant improvement of odor compounds (particularly bound protein and H_2S in the headspace).

4.3.5 Biogas production

One of the most evident differences between sonicated and unsonicated manure was biogas production. Figure 4.4a shows the measured and theoretical methane (calculated as 0.4 L/g COD consumed) for the unsonicated and sonicated hog manure. As can be seen from the Figure, the methane production rate for the digester at an SRT of 15 days increased from 2.5 L/d in the unsonicated manure to 3.0 L/d for the sonicated manure, concomitant with a marginal increase in methane content from 53% to 56%. Figure 4.4b shows the cumulative methane production for sonicated and unsonicated manure, the maximum volumetric methane production rate increasing from 0.34 $L_{CH4}/L_r.d$ in the unsonicated manure to 0.39 $L_{CH4}/L_r.d$ for the sonicated manure.

4.3.6 BioWin model

BioWin (EnviroSim Associates, Flamborough, Ontario, Canada) was used to study the performance of anaerobic digestion of sonicated and unsonicated manures at different SRTs. The experimental data for the two runs (sonicated and unsonicated) were used to calibrate the model. Table 4.2 summarizes the model output for the calibration runs. As depicted in Table 4.2, the effluent characteristics were mostly in the range of measured average and standard deviations for both manures.

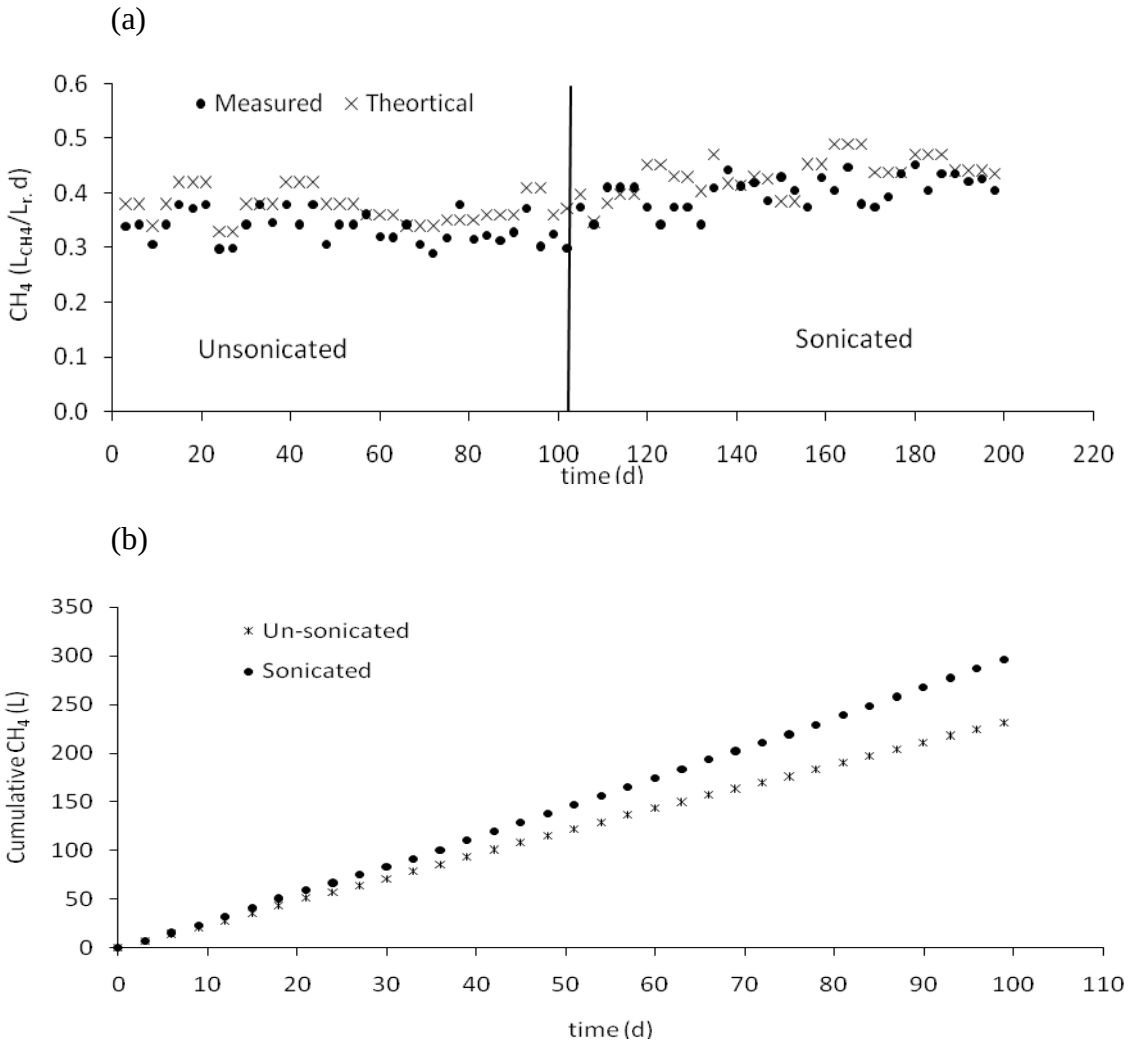

Figure 4.4 (a) Measured and theoretical methane production for unsonicated and sonicated hog manure. (b) Cumulative methane productions for unsonicated and sonicated hog manure.

Based on the comparison of the simulated and measured digested sludge characteristics listed in Table 4.2, the deviations for the unsonicated manure TSS, VSS, TCOD, SCOD, ammonia, TKN, STKN, acetic acid plus propionic acid, and daily methane production rate are 0.6%, 7.6%, 10.9%, 3.6%, 4.9%, 7.2%, 6.9%, 0.7 and 2.8%, respectively. The corresponding values for the sonicated manure are 7.9%, 6.1%, 9%, 3.5%, 22.6%, 0.2%, 14%, 19.8% and 3.3%. It is thus evident that the model default kinetic coefficients and stoichiometric parameters fit the data very well, and the effect of ultrasonication pretreatment did not change the main biochemical reactions in the anaerobic digestion significantly. Following the successful model calibration, the same influent characteristics of both raw manure and sonicated manure were used to study the effect of SRT on VSS destruction efficiency and biogas production rate. Table 4.3 clearly indicates that at shorter SRTs, VSS destruction efficiencies for sonicated manure were less than the unsonicated manure despite higher methane production. However, interestingly the improvement in VSS destruction efficiencies during anaerobic digestion by sonication becomes apparent at longer SRTs. At an SRT of 3 days, while the model predicts 30% more methane in digestion of sonicated manure relative to unsonicated, VSS destruction efficiencies for sonicated manure is only 60% of that for unsonicated manure. However at SRT of 30 days, a 20 % increase in methane production was projected for anaerobic digestion of sonicated manure relative to the unsonicated manure, in close agreement with the 22% increase in VSS destruction efficiencies.

4.3.7 Economic analysis

Table 4.4 shows the economic evaluation of ultrasonication pretreatment. Unit costs for dewatering and transportation, methane, and electrical energy used in the economic evaluation are \$ 250/ton dry solids, \$ 0.28/m^3CH_4, and \$ 0.07/kWh. Using the specific sonication energy of 500 kJ/kgTS, the cost of sonication translates to \$ 9.7/ton dry solids. The

net benefit was calculated as the difference between the costs of methane price minus dewatering minus pretreatment (*i.e.*, sonication) for the manure. It is interesting to note that the net benefit increases sharply initially and stabilizes at $ 42–49/ton dry solids for SRTs of 15 to 30 days. The net benefit was most sensitive to methane production. The aforementioned discernible observation appears to be counter intuitive since logically the impact of pretreatment should have been more pronounced on heavily loaded digesters.

4.4 Conclusions

Based on the finding of this study, the following conclusions can be drawn:

- The overall TSS and VSS removal efficiencies of sonicated manure were higher than the unsonicated manure by 36% and 31%, respectively.
- There was no significant difference in TCOD removal efficiency for the sonicated and unsonicated manure during anaerobic digestion, while the SCOD removal efficiency in the digester receiving sonicated manure was lower than that receiving the unsonicated manure.
- There was no significant difference in particulate protein removal efficiency for the sonicated and unsonicated manure in the anaerobic digester, whereas the overall removal efficiency was slightly increased (by 10%) for sonicated manure.
- The overall removal efficiency of bound protein for sonicated manure was higher than the unsonicated manure by 17.5%.
- The concentration of H_2S in the headspace of the bioreactor decreased from 988 ppm in the unsonicated manure digester to 566 ppm for sonicated manure digester, respectively.
- The effluent ammonia for digested unsonicated manure (1200 mg/L) was higher than that of sonicated manure (980 mg/L).

- The methane production rate increased from 0.34 L_{CH4}/L_r.d for the unsonicated manure to 0.39 L_{CH4}/L_r.d for the sonicated one.
- BioWin simulations indicated that at shorter SRTs, VSS destruction efficiencies for sonicated manure were less than the unsonicated manure despite higher methane production. However, interestingly the improvement in VSS destruction efficiencies during anaerobic digestion by sonication becomes apparent at SRTs around 15–30 days, which are commonly used SRTs for anaerobic digestion of biosolids in full scale.
- The net cost benefit of ultrasonication, calculated as the difference between the cost of methane output minus cost of energy input (only for ultrasonication) minus the cost of biosolids dewatering and disposal for the sonicated and unsonicated manure, increases sharply initially and stabilizes at $ 42–49/ton dry solids for SRTs of 15 to 30 days.

Table 4.2 *Measured and simulated data using BioWin software.*

Parameter (mg/L)	Unsonicated				Sonicated			
	Measured		Actual model influent	Simulated effluent	Measured		Actual model influent	Simulated effluent
	Influent	effluent			influent	effluent		
TSS	15,119 ± 552	9618 ± 687	14,642	9556	15,792 ± 680	7432 ± 409	14,402	8019
VSS	11,000 ± 526	6050 ± 414	11,640	6510	11,496 ± 510	4489 ± 768	9360	4762
TCOD	26,638 ± 1829	11,890 ± 998	26,600	13,188	28,000 ± 1540	11,284 ± 978	27,600	12,301
SCOD	12,645 ± 1238	4188 ± 507	12,396	4340	13,050 ± 1260	6147 ± 462	16,250	6360
Ammonia	753 ± 30	1187 ± 65	846	1129	824 ± 88	980 ± 100	846	1201
TKN	1779 ± 89	1582 ± 184	1779	1468	1879 ± 98	1450 ± 164	1779	1453
STKN	939 ± 108	1257 ± 176	1404	1170	939 ± 66	1088 ± 71	1517	1240
Acetic and propionic acids*	1187 ± 123	140 ± 8	1187	139	843 ± 162	172 ± 14	843	138
VSS_{dest} (%)	45 ± 2.5		44.1		51 ± 1.6		50.8	
CH_4**	2.53 ± 0.21		2.6		3.0 ± 0.26		3.1	

*Acetic and propionic acids in (mgCOD/L)

**CH_4 in (L/d)

Table 4.3 *VSS destruction and methane production at different SRTs using BioWin software.*

SRT (d)	Unsonicated manure		Sonicated manure	
	VSS destruction (%)	CH_4 (L/d)	VSS destruction (%)	CH_4 (L/d)
3	21	3.0	13.2	3.9
5	26	6.0	22	7.0
7.5	32.9	4.5	33.2	5.4
10	37.8	3.6	40.9	4.3
15	44.1	2.6	50.8	3.1
20	48	2.0	56.9	2.5
25	50.6	1.7	61.1	2.0
30	52.5	1.4	64.1	1.7

Table 4.4 *Economical study calculation based on ton dry solids influent.*

SRT (d)	Unsonicated manure				Sonicated manure				Net* $
	Energy in		Energy out		Energy in		Energy out		
	Dewatering		Gas		dewatering		Gas		
	wt of sludge after treatment (ton)	$ for dewatering and transportation	CH_4 (m^3)	$ from CH_4	wt of sludge after treatment (ton)	$ for dewatering and transportation	CH_4 (m^3)	$ from CH4	
3	0.87	218	80	22	0.80	199	112	31	18
5	0.83	207	267	75	0.74	186	335	94	31
7.5	0.76	191	300	84	0.68	169	383	107	36
10	0.72	180	320	90	0.63	157	411	115	39
15	0.66	165	347	97	0.57	141	445	125	42
20	0.63	156	356	100	0.53	132	467	131	46
25	0.60	150	378	106	0.50	125	482	135	44
30	0.58	146	373	105	0.48	121	492	138	49

*Net $ = [$ from CH_4 – $ for ultrasonication – $ for dewatering and transportation]$_{\text{sonicated manure}}$ – [$ from CH_4 – $ for dewatering and transportation]$_{\text{unsonicated manure}}$

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CHAPTER 5

Viability of Ultrasonication of Food Waste for Hydrogen Production [4]

5.1 Introduction

Research on biological waste-to-energy including hydrogen has gained renewed interest, due to global awareness of accumulated carbon dioxide in the atmosphere as a potential cause of climate change [1]. However, the rate and efficiency of biological H_2 production is low and the technology needs further development [2]. Commercially produced food products, such as corn and sugar, are not yet economical for hydrogen production. Alternatively, wastewaters with high organic content such as food processing and animal waste have great potential for conversion to energy [3]. Food wastes constitute a major fraction of the municipal solid wastes. High carbohydrate content in the form of simple sugars, starch and cellulose renders food wastes a viable feedstock for biological hydrogen production. Therefore, food wastes meet all the abovementioned criteria, which can make them ideal candidates for hydrogen production via microbial processes [4].

Ultrasonication has been increasingly used recently as a pre-treatment method for anaerobic digestion due to its ability to enhance solubilisation of organic matter. Although ultrasonication is widely used as a pretreatment method to solubilise organic matter and enhance methane production, few studies addressed its applicability for enhancement of biohydrogen production. Based on an extensive search, there are only few studies in the literature on the application of ultrasonication on the waste activated sludge (WAS) for biohydrogen production. Wang et al. [5] studied the effects of five pre-treatments (ultrasonication, acidification, sterilization, freezing/thawing and adding

[4] A version of this chapter has been published in *Int J Hydrogen Energy*, 2011

methanogenic inhibitor) on the production of hydrogen in a batch reactor from wastewater sludge using a *clostridium* strain isolated from the sludge as inoculum. A lab scale probe ultrasonication with a frequency of 20 kHz was used to sonicate 300 mL of the sludge for 20 min. The aforementioned authors found that ultrasonication marginally improved the ultimate hydrogen production from 0.6 to 0.7 mmol H_2/g $COD_{initial}$. Another report by Guo et al. [6] studied the effect of sterilization, microwave, and ultrasonication pretreatment of waste activated sludge for biohydrogen production in a batch reactor, applied the sonication on 200 mL of sludge for 5 min with an intensity of 2 w/mL, and observed a lag phase of only 3 hr and a hydrogen yield of 4.68 mL/g TCOD. Xiao and Liu [7] evaluated the effect of four pretreatment methods, acid pretreatment, alkaline pretreatment, thermal pretreatment and ultrasonic pretreatment on biohydrogen production from sewage sludge without extra-seeds (the sewage sludge was used as substrate and seed at the same time). The ultrasonication was applied on 250 mL of sludge for 30 min with sonication power of 200 W. They found that the hydrogen yield increased from 1.21 (without pretreatment) to 3.83 mL H_2/g VS. On the other hand, some other studies applied the ultrasonication on the seed to eliminate the methanogenesis and enrich the hydrogen producers [8, 9, 10]. As apparent from the aforementioned literature, all the previous studies applied the ultrasonication on WAS. There is no previous study addressing the impact of sonication on anaerobic digestion of food waste despite its potential solubilisation of carbohydrates and proteins, which are conducive for hydrogen production. Thus, the primary objective of this work was to study the effect of ultrasonication on food waste solubilisation and therefore enhancement of hydrogen production.

5.2 Materials and methods

5.2.1 Experimental set-up

Pulp waste obtained the Dufferin Organics Processing Facility (DOPF) in Toronto, Ontario, Canada was used as substrate; the average characteristics of this food waste in (mg/L) were: TCOD: 91900, SCOD: 49900, TS: 65500, VS: 46100, particulate carbohydrate: 26500, soluble carbohydrate: 20000, particulate protein: 6250, and soluble protein: 8710. The VFAs was 1990 mg COD/L. The aforementioned characteristics of the food waste are the average of three samples and the standard deviations of all parameters were less than 10%. 100 mL of food waste was sonicated for different sonication times (0.5, 2.5, 5, 10, 20, and 30 min) corresponding to specific energy inputs of 0.35, 1.2, 3, 5.5, 15, and 23 kJ/g TS, with sonication pulses set to 2 seconds on and 2 seconds off. To control the temperature increase of the food waste during ultrasonication, a cooling water bath was used, and the food waste temperature during the experiments did not exceed 30 ^{0}C. Batch anaerobic studies were conducted as described in our previous work [11] using a pre-heated (70^{o}C for 30 min) anaerobic digested sludge as seed.

5.2.2 Analytical methods

All liquid and gas parameters were analyzed as described in chapter 3 (section 3.2.1 Analytical methods).

5.3 Results and discussion

5.3.1. Ultrasonication and food waste solubilization

Due to ultrasonication pretreatment, the TS, VS, particulate carbohydrates, and particulate protein decreased, while the SCOD, soluble carbohydrate, soluble protein, and VFAs were increased. As shown in Table 5.1, the SCOD increased with increasing sonication time up to 10 min with an 18.6% increase compared with the unsonicated one. After 10 min sonication, a small increase in SCOD was observed (only 3.5 % after 30 min sonication time, from 18.6% to 22.1%). The same trend of a rapid initial increase followed by a modest increase was observed for soluble carbohydrate and soluble protein with soluble carbohydrate increasing by 29.1% and 30.3% at 10 and at 30 min sonication times, respectively. Soluble protein however did not exhibit the high increase as the SCOD and the carbohydrate, increasing only by 11% and 13.6% at 10 and 30 min sonication times, respectively. VFAs increased by about 38% after 5 min sonication and after that there was no significant increase in the VFAs. The aforementioned results concur with the finding of Xiao and Liu [7], who observed that the SCOD, soluble carbohydrate, and soluble protein increased after ultrasonication of waste sludge from 114, 24, and 27 to 1484, 135, and 569 mg/L, respectively. Moreover, Guo et al [6] observed an increase of SCOD, soluble carbohydrate, and soluble protein from 80, zero, and zero, to 1200, 102, and 72 mg/L, respectively. On the other hand, all the particulate components decreased with increasing sonication time up to 20 min, and remained steady thereafter. The TS, VS, particulate carbohydrate, and particulate protein decreased by 12%, 14%, 19%, and 12% after 20 min sonication time, respectively. Xiao and Liu [7] observed a reduction in dry solids (DS) by about 11%, while the reduction in volatile solids (VS) was only 6%, which is lower than what was observed in this study, but did not report the particulate carbohydrate or particulate protein. Based on the aforementioned results, it is evident that the

ultrasonication pretreatment can enhance the solubilisation of carbohydrates and proteins, thus increasing hydrogen production.

5.3.2 Hydrogen production

Batch experiments were conducted to study the effect of ultrasonication at different sonication times of food waste on the hydrogen production. The unsonicated food waste was examined as well. The batch experiment showed that the biogas production contained only hydrogen and carbon dioxide, without detection of methane. The cumulative hydrogen productions from the unsonicated food waste and the sonicated food waste at different sonication times are shown in Figure 5.1 (all the experiments were conducted in triplicates and the error bars are not shown as error was less than 12%). As shown in Figure 5.1, the ultimate hydrogen production for the sonicated food waste was higher than that of the unsonicated food waste (185 mL), with the difference increasing with increasing sonication time.

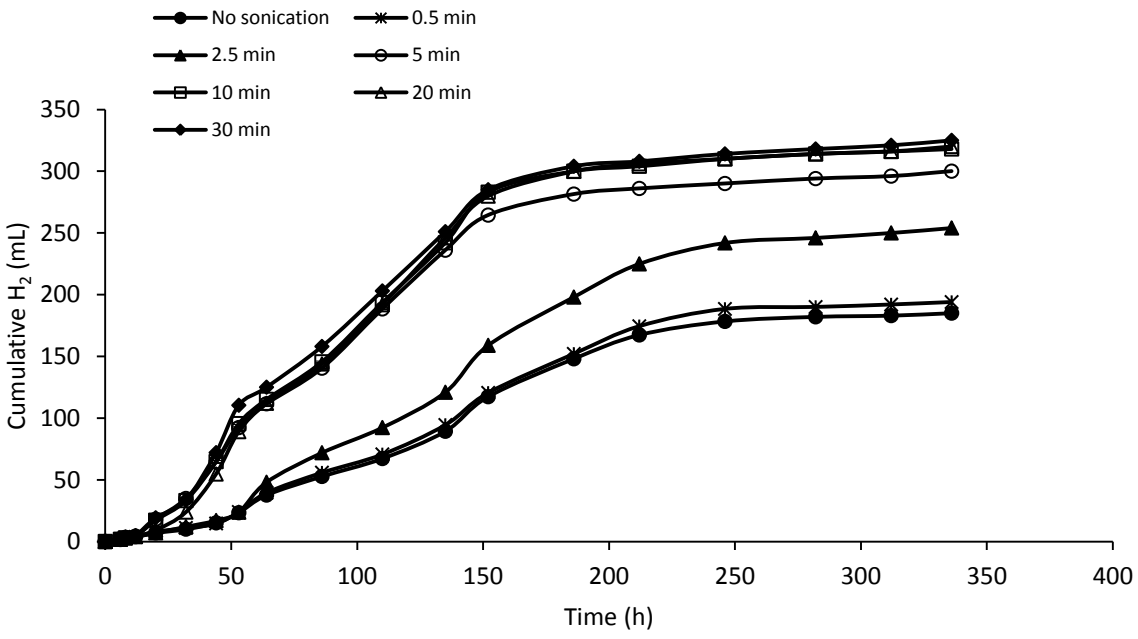

Figure 5.1 cumulative hydrogen productions for sonicated and unsonicated food waste.

Table 5.1 *Percentage increase and decrease in different components.*

sonication time	SE (kJ/kg TS)	Percentage decrease				Percentage increase			
		TS	VS	Particulate Carbohydrate	Particulate Protein	SCOD	Soluble Carbohydrate	Soluble Protein	VFA
No sonication	0	0	0	0	0	0	0	0	0
0.5 min	350	1.7	1.2	10.7	0.8	0.2	7.6	2.4	8.0
2.5 min	1200	4.6	5.4	19.6	2.0	4.3	12.1	7.6	12.3
5 min	3000	6.4	7.2	14.4	5.3	6.9	23.8	8.5	38.2
10 min	5500	9.5	10.4	17.2	8.4	18.6	29.1	11.1	37.4
20 min	15000	12.0	14.1	18.8	11.9	20.4	29.7	11.1	40.2
30 min	23000	11.5	13.8	21.9	12.5	22.1	30.3	13.6	40.3

The highest ultimate hydrogen production of 325 mL, representing a 77% increase over the control, was achieved at sonication time of 30 min. At 0.5 min sonication, there was no significant increase (only about 5% from 185 to 194 mL) in hydrogen production relative to the control. For sonication times of 2.5, 5, 10, and 20 min, ultimate hydrogen production was 254, 300, 318, and 320 mL, respectively.

Figure 5.2 illustrates the hydrogen yield for the unsonicated food waste and the sonicated one at different sonication times. The lowest hydrogen yield of 80 mL/g VS_{added} was observed for the unsonicated food waste, while the highest hydrogen yield of 141 mL/g VS_{added} was achieved at 30 min sonication time. Hydrogen yields of 110, 130, 138, and 139 mL/g VS_{added} were observed for the 2.5, 5, 10, and 20 min sonication times, respectively. The maximum increase in hydrogen yield relative to the control was about 77% at 30 min sonication time.
The hydrogen yield after 0.5 min sonication was about the same as the unsonicated food waste (84 mL/g VS_{added}). Based on the abovementioned results, it is evident that with increasing the sonication time, the hydrogen yield increases up to 5 min after which there was no significant effect of the ultrasonication (less than 10%).

Figure 5.3 shows the final VFAs after fermentation for the unsonicated and sonicated food wastes at different sonication times. For the unsonicated and sonicated food waste at 0.5 and 2.5 min, the VFAs ranged from 1835 to 2158 mg COD/L.

The VFAs of the sonicated food waste at sonication times of 5, 10, and 20 min ranged from 2985 to 3065 mg COD/L. The highest final VFAs after fermentation of 3125 mg COD/L, corresponding to 70% increase over the control, was achieved at a sonication time of 30 min. As apparent in Figure 5.4, there were no significant differences between the acetate to butyrate

ratios (HAc/HBu) for all the samples, the average value of the HAc/HBu was 1.89 with a variation of about 6% only.

Table 5.2 shows the COD mass balances for all the batches computed considering the initial and final TCOD, and the equivalent COD of hydrogen (8 g COD/g H_2). As shown in the Table, the COD mass balance indicated a closure of 90%–97%, thus emphasizing data reliability.

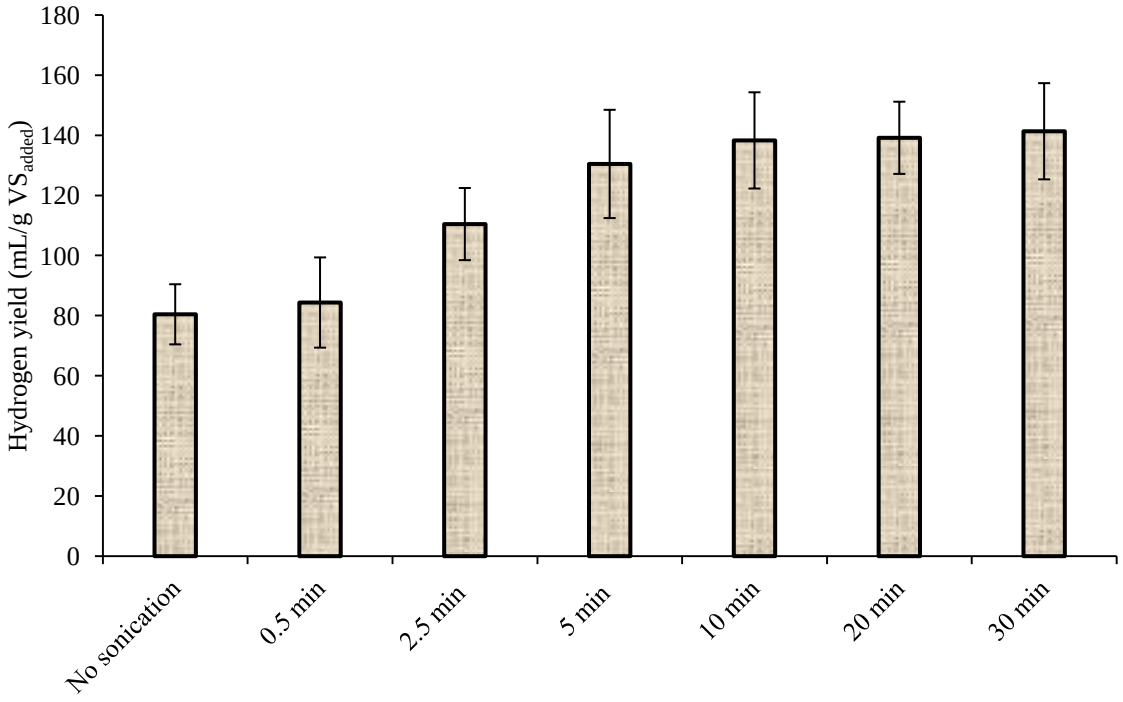

Figure 5.2 Hydrogen yield for sonicated and unsonicated food waste.

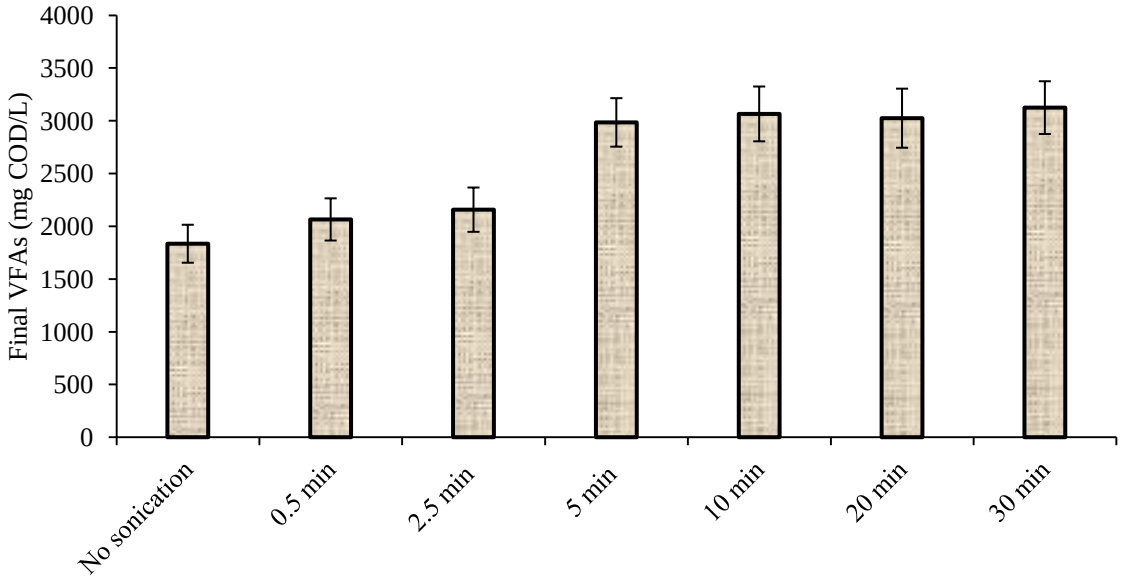

Figure 5.3 Final VFAs after fermentation for sonicated and unsonicated food waste.

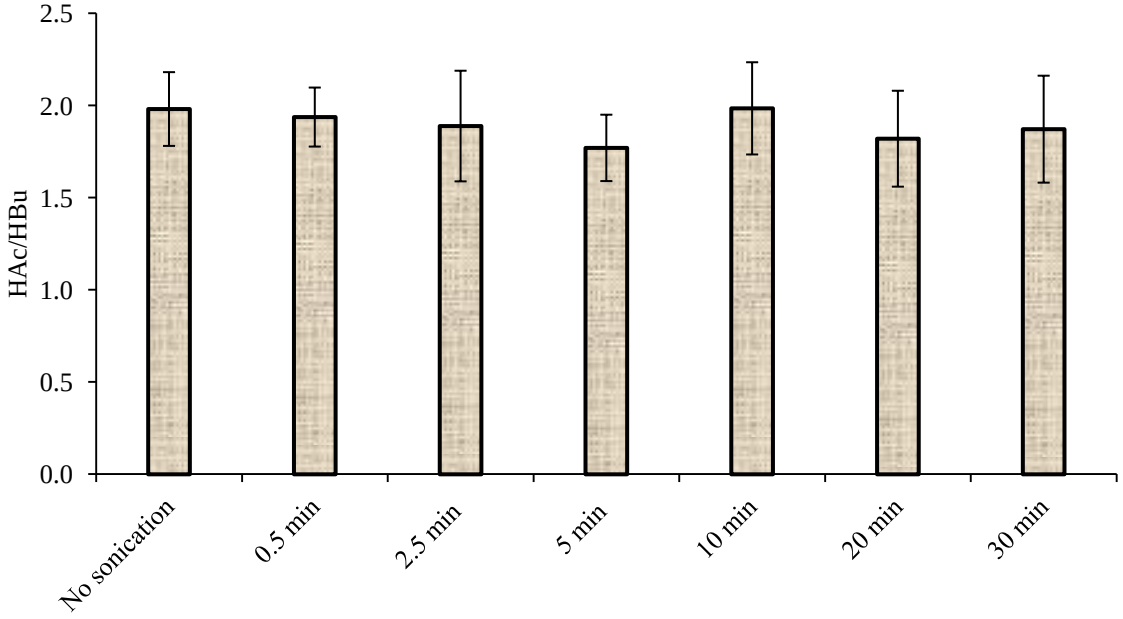

Figure 5.4 Molar acetate/butyrate ratios for sonicated and unsonicated food waste.

5.3.3 Kinetic analysis

The cumulative hydrogen data were fitted with Gompertz equation using the Newton-Raphson method for non-linear numerical estimation as described in [10]. Table 5.3 summarizes the results of the kinetic analysis. The determination coefficient (R^2) of over 0.99 for all the regressions confirms the applicability of the modified Gompertz model. The maximum hydrogen production potentials were 207, 267, 299, 320, 320, and 323 mL for sonication food waste at 0.5, 2.5, 5, 10, 20, and 30 min sonication times, respectively, while the maximum hydrogen production potential was 197 mL for the unsonicated food waste. The highest hydrogen production rate of about 2.5 mL/h was observed for the sonicated samples at sonication times of 10, 20, and 30 min, followed by 2.3 and 1.5 mL/h for sonicated samples at sonication times of 5 and 2.5 min, respectively. The hydrogen production rate for the unsonicated sample and for the 0.5 min sonication times were the lowest one (1.1 mL/h). The lag phase of the sonicated food waste of about 20 hrs was observed for the sonication times of 5, 10, 20, and 30 min, while for the unsonicated food waste and for sonicated waste at sonication times of 0.5 and 2.5 min was about 40 hrs. The reason for the short lag phase might be due to the interior structure influences the penetration of enzymatic substances and the release of metabolic products from the flocs; the structure of a floc governs the resistance of its interior to mass transfer [13]. It is evident that the ultrasonication has a positive effect on all kinetic parameters, with the ultimate hydrogen production increasing by 77%, the hydrogen production rate increased by 127%, and the lag phase decreased by 50% relative to the control.

Table 5.2 *COD mass balances for sonicated and unsonicated food waste.*

Sonication time	Initial TCOD	Final TCOD	$TCOD_{consumed}$	$TCOD_{consumed}$	Hydrogen		COD balance
	mg/L	mg/L	mg/L	mg	(mL)	mg COD	%
No sonication	91902	90460	1442	144	185	133	92
0.5 min	92259	90760	1499	150	194	140	93
2.5 min	92412	90480	1932	193	254	183	95
5 min	91443	89100	2343	234	300	216	92
10 min	92055	89690	2295	230	318	223	97
20 min	91851	89320	2531	253	320	230	91
30 min	91698	89120	2778	278	325	251	91

Table 5.3 *Kinetic coefficients for sonicated and unsonicated food waste.*

Sonication time	P	R_m	λ	R^2
No sonication	197	1.1	39	0.998
0.5 min	207	1.1	39	0.998
2.5 min	267	1.5	42	0.998
5 min	299	2.3	20	0.999
10 min	320	2.4	21	0.999
20 min	320	2.5	22	0.999
30 min	323	2.5	18	0.999

5.4 Summary and conclusions

The outcome of this study emphatically revealed the positive effect of sonication on food waste solubilisation and biological hydrogen production. Based on the findings of this study, the following conclusions can be drawn:

- The ultrasonication pretreatment promoted the release of carbohydrate and protein into the liquid phase, which enhanced hydrogen production.
- There was no significant effect of the ultrasonication on hydrogen production or waste solubilisation after 5 minutes of sonication.
- The lowest hydrogen yield of 80 mL/g VS_{added} was observed for the unsonicated food waste, while the highest hydrogen yield was 141 mL/g VS_{added} at a sonication time of 30 min.

- Ultrasonication has a positive effect on all kinetic parameters; the ultimate hydrogen production increased by 77%, hydrogen production rate increased by 127%, and the lag phase decreased by 50%.
- The highest final VFAs after fermentation was achieved at a sonication time of 30 min, which reflects a 70% increase compared to the unsonicated food waste.
- There was no significant difference between the acetate to butyrate ratios (HAc/HBu) for the all samples.

It is thus concluded that ultrasonication of food wastes can not only enhance hydrogen production but also improve anaerobic digestion efficiency due to increased solubilisation of organic matter, coupled with an increase in VFAs.

5.5 References

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CHAPTER 6

Enhancement of Biohydrogen Production Using Ultrasonication[5]

6.1 Introduction

Research on alternative energy sources has gained renewed interest, due to global awareness of accumulated carbon dioxide in the atmosphere as a potential cause of climate change [1]. Combustion of H_2 produces no greenhouse gases, and has a high-energy yield of 122 kJ/g, which is 2.75-fold greater than that of hydrocarbon fuels [2]. However, the improvement of bio-hydrogen producing efficiency is an urgent requirement for its industrialization [3]

There are many methods by which hydrogen can be generated, such as, water electrolysis, thermo-chemical processing, photo-chemical processing, photo-catalytic processing, and photo-electro-chemical processing [4]. The two methods for hydrogen production from microorganisms are photosynthetic and dark hydrogen fermentation. The most promising method for hydrogen production seems to be dark hydrogen fermentation [5]. Studies on microbial hydrogen production have been conducted mostly by pure cultures [6, 7, 8]. However, these pure cultures normally have special growth requirements. For an example, the cultures from deep-sea volcanoes need high NaCl concentrations and cultures from hot springs require high sulfur concentrations for growth [9, 10]. Thus, processes using mixed cultures are more practical than those using pure cultures, because the former are simpler to operate, easier to control, and may be applicable to a broader range of feedstocks [11]. However, in a mixed culture system, under

[5] A version of this chapter has been published in *Int J Hydrogen Energy*, 2010

anaerobic conditions the hydrogen produced by hydrogen-producing bacteria, such as *Clostridium* and *Enterobacter*, is often readily consumed by hydrogen-consuming bacteria, such as methanogens and homoacetogens [12, 13]. Thus, in order to harness hydrogen from a mixed culture system, the seed sludge needs pretreatment to suppress hydrogen-consuming bacterial activity while still preserving the activity of the hydrogen-producing bacteria. Several methods for preparing hydrogen producing seeds have been reported in the literature (Table 6.1). Heat-shock treatment has been widely used [14-26]. Thermal pretreatment is based on the inactivation of temperature-sensitive hydrogenotrophic bacteria and harvesting anaerobic spore-forming bacteria such as *Clostridium*. Heat-shock treatment parameters reported in the literature vary depending on the bacterial source, with temperatures ranging from 70 to 104 $^{\circ}$C and exposure times ranging between 15 and 120 min.

The pH control method is based on inhibiting/inactivating the methanogens in a low pH environment or high pH environment. Successful preparation of hydrogen producing seeds by acid treatment or base treatment [15, 17, 21, 27] has been reported. Methanogens are also obligate anaerobic archaeobacteria i.e. when they are exposed to an aerobic environment; the oxygen lowers their adenylate charge and causes them to die [7]. A few researchers have reported the preparation of hydrogen producing seeds by pre-aeration [17,21,28] with purging times ranging between 30 min to 24 h. Methanogenic inhibitors such as 2-bromoethanesulfonic acid (BESA), chloroform and iodopropane have also been used [17,21]. Table 6.1 summarizes selected studies using different pretreatment methods and glucose or sucrose as a substrate in batch reactors. As depicted from Table 6.1, the H_2 yield varied widely from 0.48 to 2.3 mol-H_2/mol-substrate and from 0.61 to 6.12 mol-H_2/mol-substrate in the case of glucose and sucrose respectively.

Table 6.1 *Different pretreatment methods in batch studies*

Seed	Pretreatment method	Pretreatment conditions	Substrate	Max. H_2 yield mol-H_2/mol-substrate	Ref.
Soil	Heat	100 ^oC for 2 h	Glucose	2.1	14
Anaerobic sludge	Heat	100 ^oC for 90 min	Glucose	2.0	15
Cracked cereals	Heat	2 h baked and 30 min boil	Sucrose	2.73	16
Digested sludge	Heat	Boiled at 100 ^oC for 30 min	Glucose	1.78	17
Anaerobic sludge	Heat	Baked at 104 ^oC for 2 h	Glucose	0.97	18
Anaerobic sludge	Heat	Heated in boiling water bath for 30 min	Glucose	1.1	19
Soil	Heat	Dried at 104 ^oC for 2 h	Glucose	0.92	20
Digested sludge	Heat	Boiled for 20 min	Sucrose	3.18	21
Cow dung compost	Heat	Baked at 100-105 ^oC for 2 h	Sucrose	2.24	22
Anaerobic sludge	Heat	102 ^oC for 90 min	Sucrose	4	23
Anaerobic sludge	Heat	85 ^oC for 1 h	Glucose	1.67	24
Soil	Heat	104 ^oC for 2 h	Sucrose	1.8	25
Anaerobic sludge	Heat	65 ^oC for 30 min	Glucose	1.64	26
WAS	Heat	65 ^oC for 30 min	Glucose	2.3	26
Anaerobic sludge	Acid	pH = 3-4 for 24 h	Glucose	1.3	15
Digested sludge	Acid	pH = 3 for 24 h	Glucose	0.8	17
Digested sludge	Acid	pH = 3 for 24 h	Sucrose	3.1	21
Sewage sludge	Acid	pH = 3 for 24 h	Glucose	1.0	27
Anaerobic sludge	Base	pH = 12 for 24 h	Glucose	0.48	15
Digested sludge	Base	pH = 10 for 24 h	Glucose	1.09	17
Digested sludge	Base	pH = 10 for 30 min	Sucrose	6.12	21
Sewage sludge	Base	pH = 10 for 24 h	Glucose	0.58	27
Digested sludge	Aeration	Aerated with air for 24 h	Glucose	0.86	17
Digested sludge	Aeration	Aerated with air for 30 min	Sucrose	4.84	21
Digested sludge	Aeration	Aerated with air for 24 h	Glucose	2.1	28
Digested sludge	MI	10 mmol/L BESA (30 min)	Sucrose	5.28	21
Digested sludge	MI	2% chloroform	Glucose	0.69	17
Digested sludge	MI	10 mmol/L iodopropane (30 min)	Sucrose	5.64	21

BESA is the abbreviation of 2-bromoethanesulfonic acid, MI: Methanogenic inhibitors, WAS: waste activated sludge

As shown in Table 6.1, the pretreatment methods are predominantly chemical (acid, base, and methanogenic inhibitors) and thermal (heat-shock). It is evident from the literature that hydrogen producing bacteria are spore-formers and hence their resistance to heat-shock and chemical attack is very strong.

An extensive literature search indicated lack of mechanical disintegration pretreatment methods for biohydrogen production, the most prominent of which is ultrasonication. Ultrasonication causes a localised pressure drop to below the evaporating pressure in the aqueous phase, resulting in the formation of micro bubbles by evaporation. The micro bubbles oscillate in sound field, grow by rectified diffusion and collapse in a non-linear manner. The combination of bubble oscillation and the resulting vacuum created by the collapse of the bubble leads to strong mechanical forces that can erode solid particles [31]. Guo et al. [29] who studied the impact of ultrasonic pretreatment on hydrogen production from boiled anaerobically digested sludge at 90 ^{o}C for 15 minutes with sucrose as substrate, found that the optimal ultrasonication time of 10 s and intensity of 130 W/l, increased hydrogen production rate by 1.30 fold with direct ultrasonication of digested sludge and by 1.48 fold when ultrasound was applied to the solution. In another study, More and Ghangrekar [30] evaluated the effect of ultrasonication pretreatment on mixed anaerobic sludge to inoculate the microbial fuel cells, and reported that the ultrasonication pretreatment of 5 minutes affected maximum power density of 2.5 times higher than the untreated sludge.

The hypothesis of this research is that ultrasonication will not adversely impact spore-forming hydrogen producing bacteria, while inactivating methanogenic bacteria. Thus, the primary objective of this work was to explore the impact of ultrasonication on biohydrogen

producers and compare it with most common pretreatment methods (heat-shock, acid, and base) reported in the literature.

6.2 Materials and methods

6.2.1 Seed sludge and pretreatment

Anaerobic sludge was collected from the primary anaerobic digester at St Mary's wastewater treatment plant (St Mary's, Ontario) and used as seed sludge. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the sludge were 11 and 8 g/L, respectively. Five different pretreatment methods (sonication with temperature control, sonication without temperature control, heat-shock, acid, and base) were used in this study. A lab scale ultrasonic probe was used for sonication pretreatment. The ultrasonic probe was supplied by Sonic and Materials (model VC-500, 500 W, and 20 kHz). Two hundred mL of anaerobic digester sludge were sonicated with sonication pulses set to 2 seconds on and 2 seconds off. To control the temperature rise of the sludge, a cooling water bath was used, and the sludge temperature during the experiments did not exceed 30˚C. Initially, different sonication times were used to optimize the sonication time, after which the optimum sonication time was then employed for the comparative study.

The sonication pretreatment was conducted by sonicating a 200 mL of sludge for the optimum time with and without temperature control. The heat-shock pretreatment was conducted by heating the sludge at 70 ^{0}C for 30 min. Acid pretreatment was conducted by adjusting the pH of the sludge to 3.0 with 1N HCl and maintaining it for 24 h in the cold room (4 ^{o}C). Base pretreatment was conducted by adjusting the pH of the sludge to 10.0 with 1N NaOH and

maintaining it for 24 h in the cold room (4 ^{o}C). For acid and base pretreatment, the pH was readjusted to 6.5 before starting the experiment.

6.2.2 Specific Methanogenic Activity (SMA) and Batch Experiments

Batch anaerobic studies were conducted using acetate and glucose as substrates to respectively assess methane and hydrogen production rates. SMA experiments were conducted in triplicates in a series of serum bottles (liquid volume of 250 ml and headspace volume of 60 ml). To each bottle, 50 mL of seed, 200 mL of deionized water with the required amount of substrate (0.75 mL acetic acid for SMA, and 2 g of glucose for hydrogen batch experiment), and 1 mL of nutrient stock solution were added. Each liter of nutrient stock solution contained 1000 g $NaHCO_3$, 280 g NH_4Cl, 250 g of K_2HPO_4, 100 g of $MgSO_4.7H_2O$, 10 g of $CaCl_2.2H_2O$, 2 g of $FeCl_2.4H_2O$, 0.05 g of H_3BO_3, 0.05 g of $ZnCl_2$, 0.03 g of $CuCl_2$, 0.5 g of $MnCl_2.4H_2O$, 0.05 g of $(NH_4)_6Mo_7O_{24}$, 0.05 g of $AlCl_3$, 0.05 g of $CoCl_2.6H_2O$, and 0.05 g of $NiCl_2$. The initial pH value for the mixed solution in each bottle was adjusted to 7.0 and 6.5 using 1N NaOH and HCl for SMA and hydrogen production batch experiments, respectively. The initial concentration of VSS in each vial was 1.6 g/L. A 10 mL sample of the mixture was collected as the initial samples. The headspace was flushed with oxygen-free nitrogen gas for a period of 3 minutes and capped tightly with rubber stoppers. The bottles were then placed in a swirling-action shaker (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) operating at 180 rpm and maintained at a temperature of 37 ^{0}C. Control bottles were prepared using the sludge without pretreatment with addition of the nutrient stock solution only.

6.2.3 Analytical methods

All liquid and gas parameters were analyzed as described in chapter 3 (section 3.2.1 Analytical methods).

6.2.4 Data analysis

Hydrogen gas production was calculated from headspace measurements of gas composition and the total volume of biogas produced, at each time interval, using the following mass balance equation:

$$V_{H,i} = V_{H,i-1} + C_{H,i}\left(V_{G,i} - V_{G,i-1}\right) + V_H\left(C_{H,i} - C_{H,i-1}\right) \quad (6.1)$$

where $V_{H,i}$ and $V_{H,i-1}$ are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ are the total biogas volumes in the current and previous time intervals, $C_{H,i}$ and $C_{H,i-1}$ are the fractions of hydrogen gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals, and V_H is the total volume of headspace in the reactor [32].

Methane gas production was calculated by multiplying the total gas volume in the head space by the methane content, as after 48 hours the methane content in the headspace was approximately constant (within 10% variation).

6.3 Results and discussion

6.3.1 Optimization of sonication time

SMA tests were conducted to study the effect of sonication at different sonication times to optimize the time required for elimination of methanogenesis. The seed without pretreatment (as control) and heat-shock pretreatment were examined as well. Figure 6.1 shows the methane

production for the sonication pretreatment, heat-shock pretreatment, and no pretreatment (error bars are not shown as error was less than 10%). As depicted in the figure, there was no methanogenic activity in the case of heat-shock pretreatment, while for sonication, an inverse relationship between sonication time (from 0.5 min to 30 min) and methanogenesis was observed, wherein the methanogenic activity decreased proportionally with increasing the sonication time. As expected, the bottle with no pretreated seed produced higher ultimate methane production of 106 mL, as compared to 80, 64, 35, and 22 mL for sonication times of 0.5, 1, 2.5, and 5 minutes. After 5 minutes sonication, the potential methane production was less than 10 mL in all bottles which is less than 10% of the control bottle. Based on the aforementioned results, the sonication pretreatment time should be higher than 5 minutes to eliminate more than 90% of methanogenic activity. The subsequent step of optimizing the sonication pretreatment time involved studying the hydrogen production for different sonication times. Based on the SMA results, sonication times of 5, 10, 20, 30, and 40 minutes were used for the hydrogen production experiment together with untreated sludge (as a control) and heat-shock pretreatment.

Figure 6.2 shows the cumulative hydrogen production during the batch experiment for untreated sludge, heat-shock pretreated and sonicated sludges. As shown in Figure 6.2, the cumulative hydrogen production from the sonication pretreatment were 196, 281, 349, 356, and 362 mL for sonication times of 5, 10, 20, 30, and 40 minutes respectively. It must be asserted that the incremental increases in hydrogen production for sonication times of 30 and 40 minutes over the 20 minutes were less than 5%, and accordingly the optimum sonication time is 20 minutes, corresponding to a specific energy of 79 kJ/g TSS.

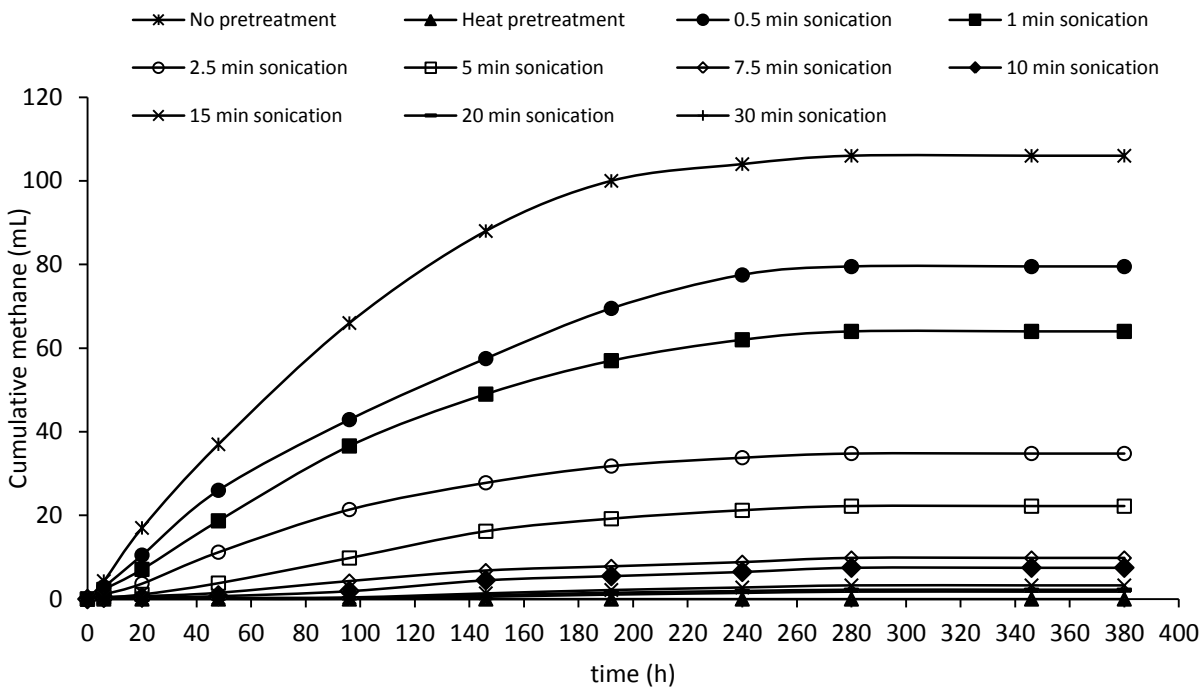

Figure 6.1 cumulative methane productions for optimizing the sonication time.

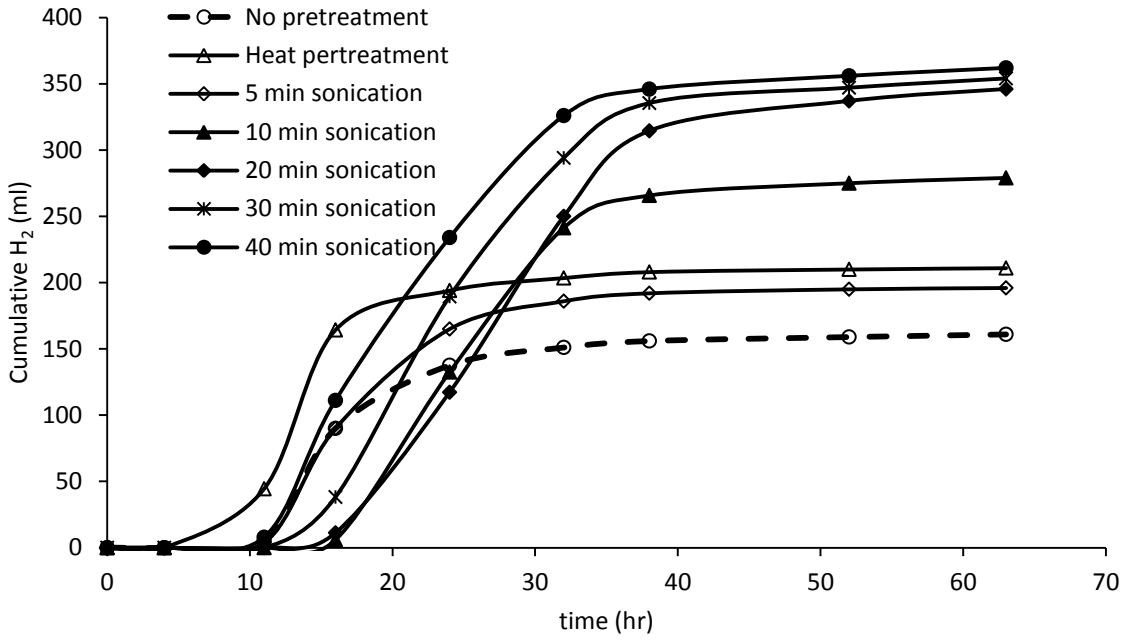

Figure 6.2 cumulative hydrogen productions for optimizing the sonication time.

6.3.2 Hydrogen production

Five different pretreatment methods were used in this study; sonication with temperature control, sonication without temperature controls, heat-shock, acid, and base. The untreated seed served as a control. The initial temperature of the sludge was 4 ^{o}C as the sludge was stored in the cold room prior to use and the temperature reached 68 ^{o}C after 10 minutes sonication while the maximum temperature at the end of sonication was 92 ^{o}C. Thus during the last 10 minutes of sonication, the temperature of the sludge was higher than 60 ^{o}C with an average of 77 ^{o}C.

The batch study of the five different pretreatment methods showed that the biogas production contained only hydrogen and carbon dioxide, with no of methane for heat-shock, acid, base, and sonication without temperature control. Traces of methane have been detected in the first 16 hours for the sonicated sludge with temperature control, and control sludge. The cumulative methane detected during the experiment in the aforementioned was less than 2 mL during the first 16 hours, after which no methane was detected. Detection of methane at the beginning may be due to the relatively high initial pH of 6.5, but after the pH dropped to below 5.5, methanogenic activity was completely eliminated.

Figure 6.3 illustrates the cumulative hydrogen production for the five various pretreatment methods and the untreated sludge. As apparent from Figure 6.3, the ultimate hydrogen production of the pretreated sludges was higher than that of the untreated sludge (176 mL). Only the base pretreatment exhibited the same hydrogen production as the control. The ultimate hydrogen production of 382 mL was achieved from sonicated sample with temperature control, followed by the acid (272 mL), heat-shock (258 mL), sonicated without temperature control (251). Of the five different pretreatment methods used in this study, the base pretreatment showed the lowest ultimate hydrogen production of 164 mL.

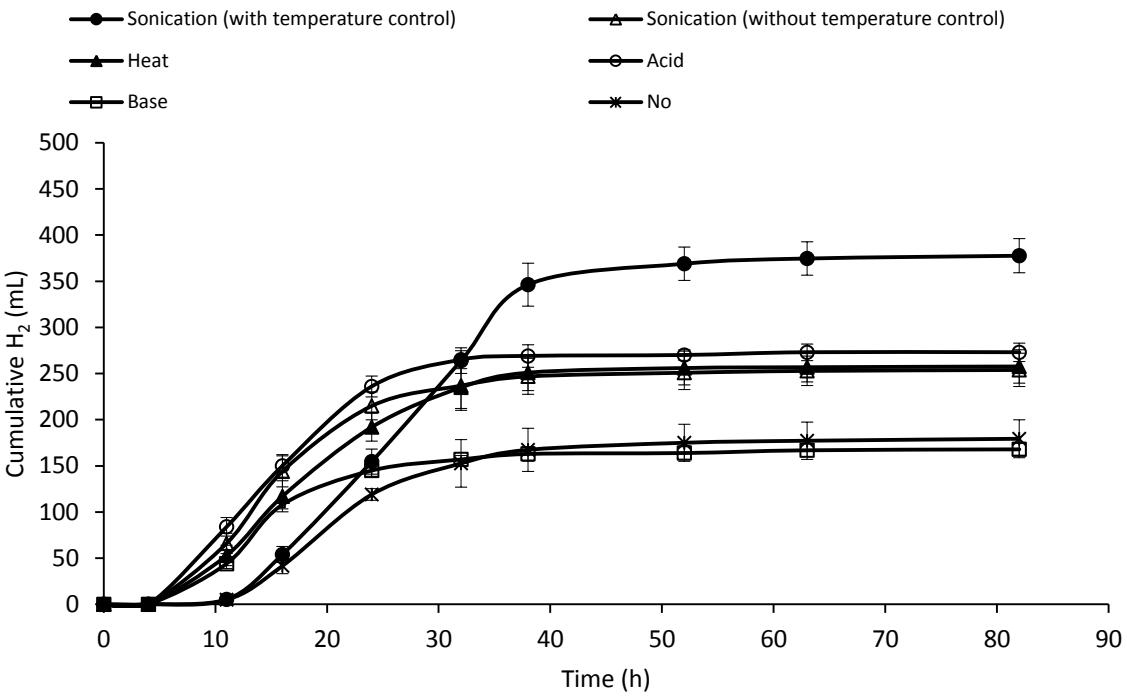

Figure 6.3 cumulative hydrogen productions for different pretreatment methods.

Figure 6.4 shows the hydrogen yield for the different pretreatment methods used in this study. The hydrogen yield of sonication with temperature control was higher than all other pretreatment methods. The hydrogen yield was 1.55, 1.11, 1.04, 1.03, and 0.68 mol H_2/mol glucose for sonication with temperature control, acid, heat-shock, sonication without temperature control, and base pretreatment, respectively. The hydrogen yield of the untreated sludge was 0.7 mol H_2/mol glucose. The percentage increase in hydrogen yield due to the pretreatment compared with the control sludge were 121%, 59%, 49% and 47% for sonication with temperature control, acid, heat-shock, and sonication without temperature control, respectively, with no increase in the hydrogen yield for base pretreatment.

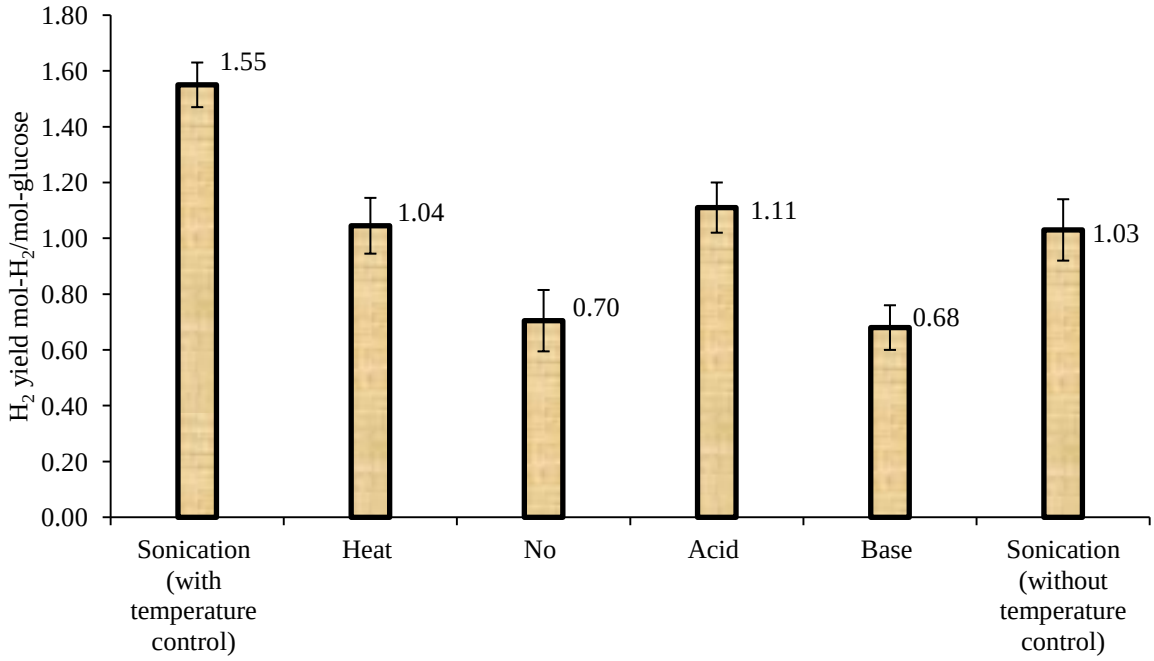

Figure 6.4 Hydrogen yield for different pretreatment methods.

As depicted in Table 6.1, the hydrogen yield for the heat-shock pretreatment, using anaerobic sludge as seed and glucose as substrate, is in range of 0.97 to 2.0 mol H_2/mol glucose.

Since the variation in the yield is attributable to the pretreatment conditions (temperature and time) and/or the seed characteristics (TSS and VSS), the best way to compare different pretreatment methods is by using the same seed. Three studies using acid pretreatment on the anaerobic sludge as seed and glucose as substrate are reported in Table 6.1, with hydrogen yields of 0.8 - 1.3 mol H_2/mol glucose, which matches with the 1.11 mol H_2/mol glucose obtained in this study. Furthermore, the two studies using base pretreatment on anaerobic sludge as seed and glucose as substrate reported in Table 6.1, showed hydrogen yields in the range of 0.48 - 1.09 mol H_2/mol glucose, which is consistent with the 0.68 mol H_2/mol glucose obtained in this study.

Statistical analysis using the paired t-test was conducted to evaluate the significance of the difference in hydrogen yield at the 95% confidence interval. Paired test involving the five pretreatment methods revealed that a- the base pretreatment was ineffective (i.e. no different than the control), b- differences between sonication (with and without temperature control), heat-shock, acid on one hand and base and control on the other are significant, c- differences between heat, acid, and sonication without temperature control are insignificant, d- differences between sonication with temperature control on one hand and heat-shock, acid, and sonication without temperature control on the other hand are significant. Thus, the statistical analysis corroborated the superiority of sonication with temperature control over the conventional heat-shock and acid pretreatment methods with hydrogen yields increasing by about 45%.

As apparent from Figure 6.4, sonication without temperature control essentially achieved the same hydrogen yield as heat-shock pretreatment (1.03 vs 1.04). This finding might be due to the high temperature during sonication. Since in the last 10 minutes the impact of both sonication and heat-shock can not be discerned individually, it can be deduced that from 10 to 20 minutes, the negative impact of sonication without temperature control relative to sonication with temperature control is reflective of the adverse impact of the temperature on hydrogen production bacteria, decreasing the molar hydrogen yield by 30% relative to sonication with temperature control.

The hydrogen content in the headspace reached maxima of 61%, 60%, 55%, 52%, 46%, and 45% after 32, 16, 24, 24, and 16 h for sonication with temperature control, acid, heat-shock, sonication without temperature control, and base pretreatment, respectively. The hydrogen content in the headspace reached maximum of 45% after 24 h for the control sludge. Fan et al.

[33] reported maximum hydrogen content of 60% (in both studies when they used heat-shock pretreatment on anaerobes obtained from cow dung composter).

At the end of the experiment, glucose was completely consumed in all bottles, with maximum conversion efficiency of glucose to hydrogen (based on a theoretical yield of 4 mol-H_2/mol-glucose) of 39% for sonication with temperature control followed by 28% for acid pretreatment methods. Both the heat-shock and sonication without temperature control achieved the same conversion efficiency of 26%, while 18 % was achieved for base pretreatment and the control sludge (Figure 6.5). The final pH in all experiments ranged from 4.4 to 5.5.

6.3.3 Volatile fatty acids (VFAs)

The formation of hydrogen is accompanied with VFAs or solvent production during the anaerobic digestion process. Thus, the VFAs concentrations are a useful indicator for monitoring hydrogen production. The major VFAs detected in this study were acetate (HAc), butyrate (HBu) and propionate (HPr). The degree of acidification can be expressed based on the ratio of the COD equivalent of the acidogenic products (organic acids, and hydrogen) to the initial SCOD. As evident from the degree of acidification depicted in Table 6.2, the main products of glucose utilization were acetate (6-21%), butyrate (16-31%), propionate (5-13%), ethanol (15-26%), and hydrogen (2-4%). Sonication with temperature control achieved the highest degree of acidification to acetate (21%), followed by 15% and 13% for acid and sonication without temperature control pretreatments, respectively. The highest degrees of acidification to butyrate of 31% and 28% were observed for acid pretreatment and sonication with temperature control, respectively, while sonication without temperature control and the untreated sludge exhibited the same degree of acidification to butyrate (~0.24), with base pretreatment showing the least degree

of acidification to both acetate and butyrate of 6% and 16%. The highest degree of acidification to propionate was observed for acid pretreatment (13%), followed by base pretreatment (11%), and the lowest was for sonication with temperature control (5%). Base pretreatment and the untreated sludge both achieved approximately the same degree of ethanol formation of 25% and 26%, respectively.

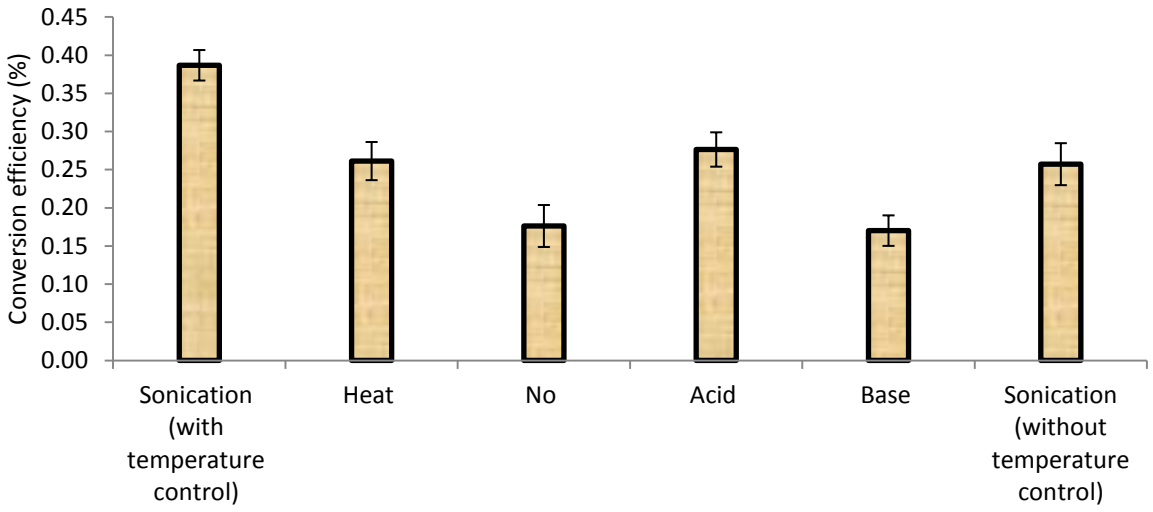

Figure 6.5 Conversion efficiency of glucose to hydrogen for different pretreatment methods.

Table 6.2 *Degree of acidification for different pretreatment methods.*

Pretreatment method	Degree of acidification				
	Acetic acid	Butyric acid	Propionic acid	Ethanol	Hydrogen
Sonication (with temperature control)	0.21	0.28	0.05	0.15	0.04
Heat	0.10	0.18	0.07	0.17	0.02
Acid	0.15	0.31	0.13	0.14	0.03
Base	0.06	0.16	0.11	0.25	0.02
Sonication (without temperature control)	0.13	0.24	0.09	0.19	0.02
No	0.07	0.23	0.08	0.26	0.02

It is evident that the molar yield of acidification to acetate is 4 moles of hydrogen versus 2 moles for butyrate. Thus, the molar ratio of acetate to butyrate (HAc/HBu) significantly impacts the hydrogen yield [34]. Accordingly the HAc/HBu ratio has been examined in this study. Figure 6.6 shows the HAc/HBu ratio formed for the different pretreatment methods. Sonication with temperature control has the highest ratio of acetate to butyrate of 1.9. The HAc/HBu ratio for heat-pretreatment, acid pretreatment, and sonication without temperature control varied narrowly from 1.2 to 1.4, while the base pretreatment and the control sludge had the lowest ratio of 0.9 and 0.8, respectively. Figure 6.7 depicting the relationship between hydrogen yield and the corresponding values of HAc/HBu ratio for the different pretreatment methods and the control sludge clearly emphasizes that the hydrogen yield increased linearly with the increase in HAc/HBu ratio consistent with the literature studies [21, 35, 36]. These results show that the pretreatment method impacts the metabolic pathways. It is thus evident for all pretreatments excluding base pretreatment acetate pathway was more favourable than the butyrate pathway, while control sludge and base pretreatment both showed an inverse trend where the butyrate pathway was dominant.

6.3.4 Biomass yield

Biomass yield (as mg VSS/mg COD) was calculated based on the increase in biomass (final minus initial) and the COD destroyed (initial total minus final total). Figure 6.8 illustrates the biomass yield for the five different pretreatment methods and the untreated sludge. Biomass yield of the untreated sludge of 0.24 mg VSS/mg COD was the highest of other pretreatment sludges. The highest biomass yield for the pretreated sludges was observed for base pretreatment (0.22 mg VSS/mg COD), followed by heat-shock and sonication without temperature control

(0.18 mg VSS/ mg COD), acid pretreatment (0.15 mg VSS/ mg COD), and sonication with temperature control (0.13 mg VSS/ mg COD).

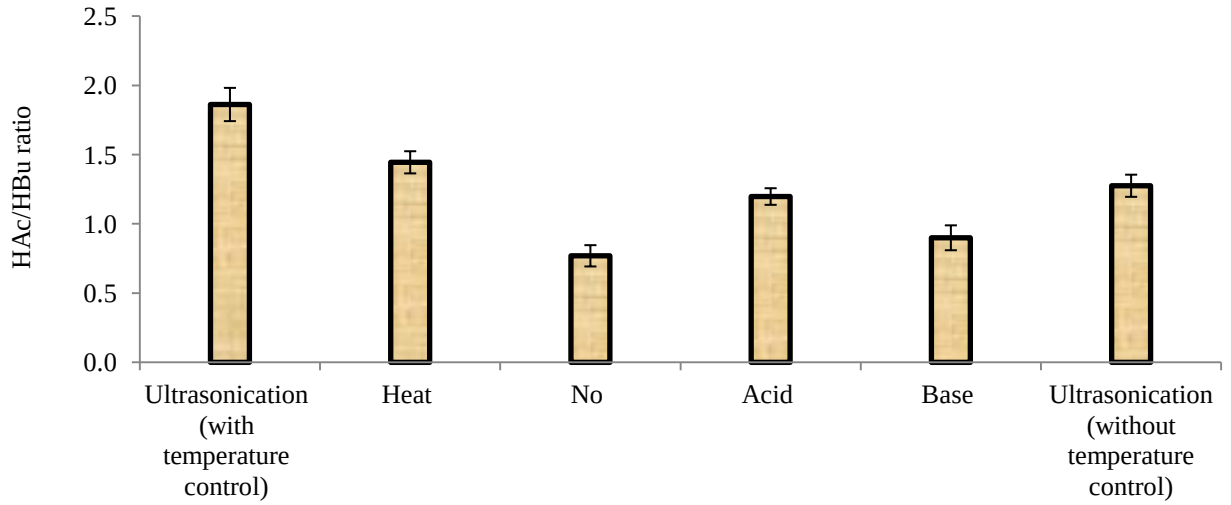

Figure 6.6 molar acetate/butyrate ratios for different pretreatment methods.

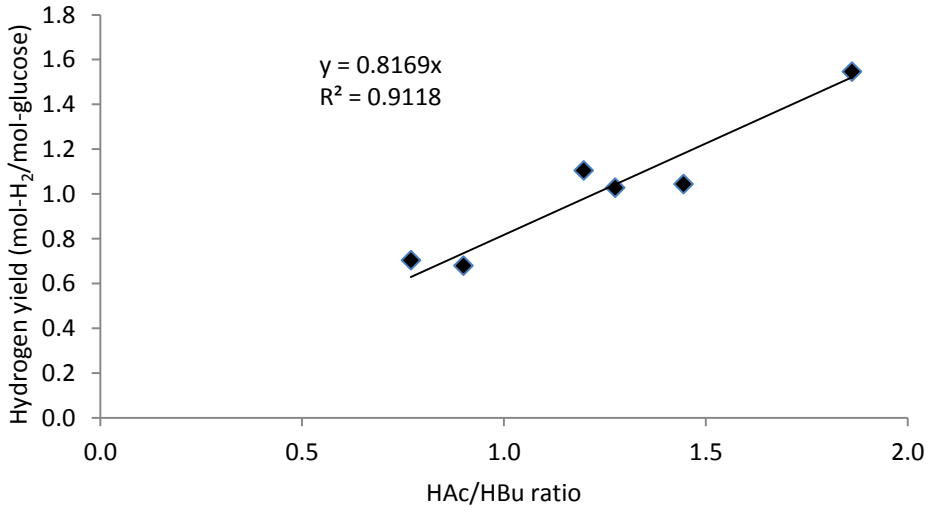

Figure 6.7 Correlation between molar acetate/butyrate ratio and hydrogen yield.

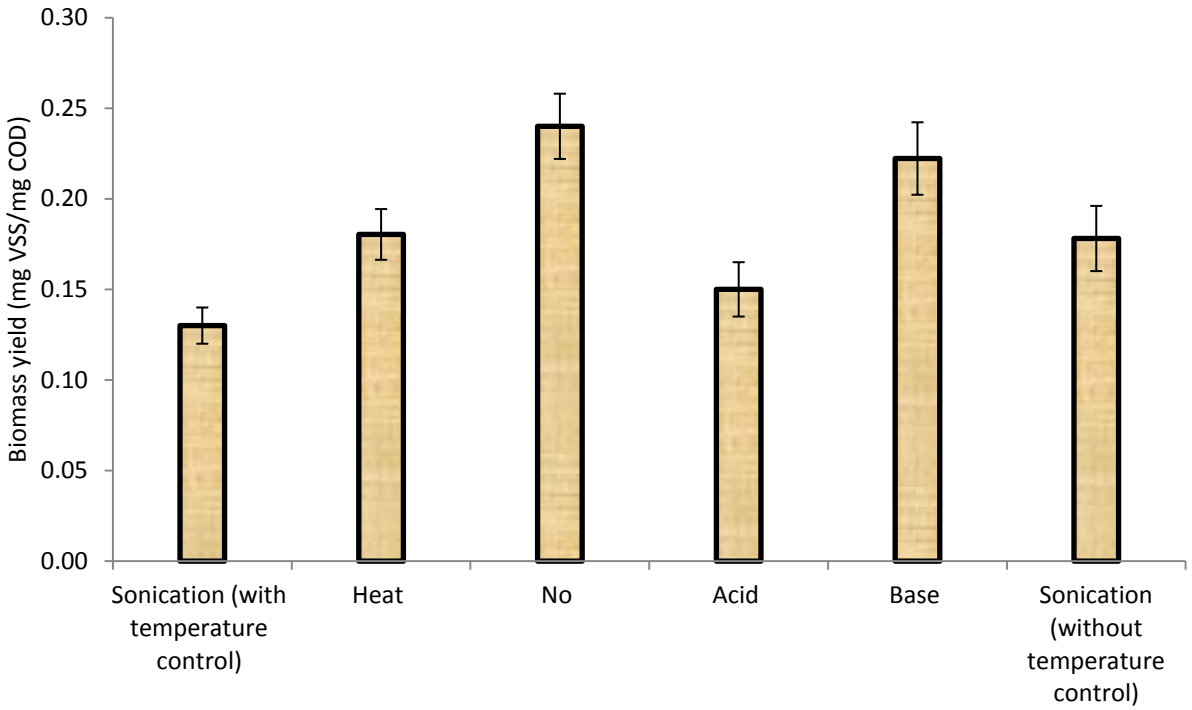

Figure 6.8 Biomass yield for different pretreatment methods.

The inverse relationship between the biomass yield and hydrogen yields observed here depicted in Figure 6.9 is consistent with the finding of Hafez et al. [37] who observed the same trends, using data from continuous stirred tank reactor (CSTR) and literature results. COD mass balances for all the batches computed considering the initial and final TCOD, and the equivalent COD of hydrogen (8 g COD/g H_2), indicated a closure of 89%–95%, thus emphasizing data reliability.

6.3.5 Kinetic analysis

The following modified Gompertz model has been successfully used to describe the progression of cumulative hydrogen production in the batch tests [38]:

$$H = P.\exp\left\{-\exp\left[\frac{R_m\, e}{P}(\lambda - t) + 1\right]\right\} \qquad (6.2)$$

where H is the cumulative hydrogen production (mL), P is the maximum hydrogen production (mL), R_m is the maximum hydrogen production rate (mL/h), λ is the lag phase time (h), t is the incubation time (h), and e = exp (1) = 2.718.

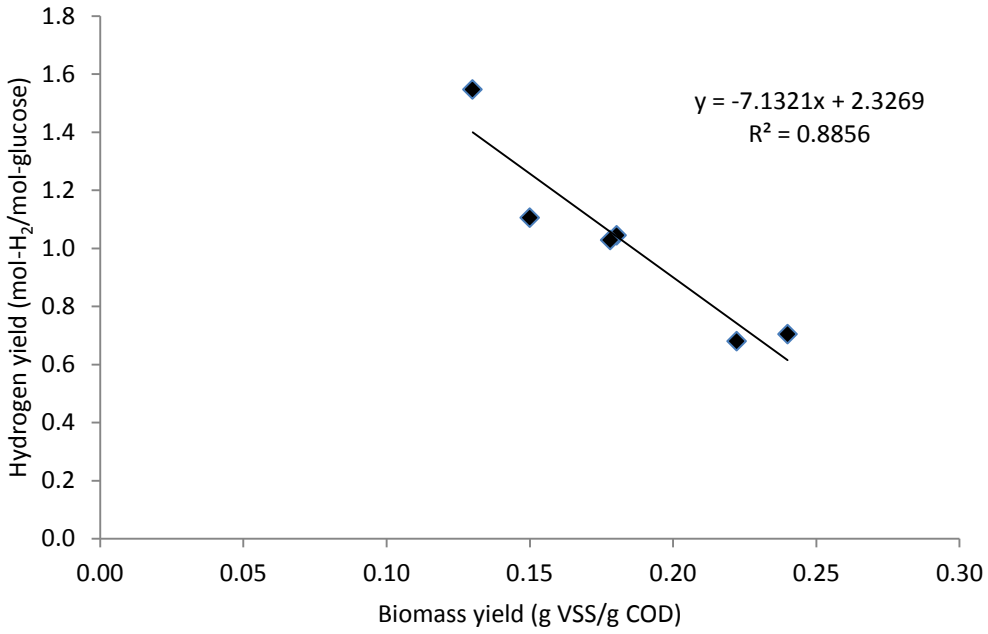

Figure 6.9 Correlation between biomass yield and hydrogen yield.

The cumulative hydrogen data were fitted with Gompertz equation using the Newton-Raphson method for non-linear numerical estimation. The Newton's method was programmed using Visual Basic application language available in Excel 2003. Table 6.3 summarizes the results of the kinetic analysis. The determination coefficient (R^2) of over 0.99 for all the regressions confirms the applicability of the modified Gompertz model. The maximum hydrogen production potentials were 382, 273, 258, 251, and 164 mL for sonication with temperature control, acid, heat-shock, sonication without temperature control, and base pretreatment methods, respectively, while the maximum hydrogen production potential was 176 mL for the

control sludge. The sludge pretreated by sonication with temperature control achieved the highest hydrogen production rate of 16.6 mL/h, followed by 15.4, 14.6, 12.8, and 12.2 mL/h for sonication without temperature control, acid, heat-shock and base pretreatment methods, respectively. The hydrogen production rate for the control sludge was the lowest one (10.1 mL/h). It is evident that although sonication without temperature control significantly increased hydrogen production, the maximum hydrogen production rate was marginally (less than 10%) higher than sonication without temperature control.

Table 6.3 *Kinetic coefficient for different pretreatment methods.*

Pretreatment method	λ	R_m	P	R^2
Sonication (with temperature control)	14.2	16.6	382	0.99999
Sonication (without temperature control)	6.7	15.4	251	0.9999
Heat	7	12.8	258	0.99999
Acid	5.6	14.6	272	0.9998
Base	7.2	12.2	164	0.9998
No	11.9	10.1	176	0.99999

6.4 Conclusions

The outcome of this study emphatically revealed the superiority of sonication with temperature control over conventional pretreatment methods to biological hydrogen production such as heat-shock, acid, and base pretreatment.

Based on the findings of this study, the following conclusions can be drawn:

- The optimum specific energy of sonication for inactivation of methanogenesis observed in this study was 79 kJ/g TSS (20 min, 200 mL).
- Sonication pretreatment with temperature control showed promising results, as reflected by 120% increase in volumetric hydrogen production over untreated sludge, as well as 40% over pretreated sludge (acid pretreatment).
- Based on the results on this study, it is apparent that temperature adversely impacts hydrogen producing bacteria resulting in 30% lower hydrogen yield.
- Hydrogen yields of 1.55, 1.11, 1.04, 1.03, 0.68, and 0.7 mol H_2/mol glucose were observed for sonication with temperature control, acid, heat-shock, sonication without temperature control, base pretreatment, and untreated sludge, respectively.
- Hydrogen yield correlated linearly with HAc/HBu molar ratio, and inversely with biomass yield.

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CHAPTER 7

Single and Combined Effect of Various Pretreatment Methods for Biohydrogen Production from Food Waste[6]

7.1 Introduction

Considering global environmental impacts, such as greenhouse effect and resource recovery, there is a pressing need to develop non-polluting and renewable energy sources [1]. Compared with fossil fuels as traditional energy sources, hydrogen is a promising candidate as a clean energy carrier in the future because of its high-energy yield (122 kJ/g) and production of only water instead of greenhouse gases on burning [2]. There are many methods by which hydrogen can be generated, such as, water electrolysis, thermo-chemical processing, photo-chemical processing, photo-catalytic processing, and photo-electro-chemical processing [3]. The two methods for hydrogen production from microorganisms are photosynthetic and dark hydrogen fermentation. Among the various biological hydrogen production methods such as biophotolysis of water, photo-fermentation, and dark fermentation of organic matter, the most promising method for hydrogen production seems to be dark hydrogen fermentation [4]. Studies on microbial hydrogen production have been conducted mostly by pure cultures [5,6,7]. Processes using mixed cultures are more practical than those using pure cultures, because the former are simpler to operate and easier to control, and may sustain a broader sources of feedstock; thus preferable for wastewater treatment [8]. However, in a mixed culture system, under anaerobic conditions, hydrogen produced by hydrogen-producing bacteria, such as *Clostridium* and *Enterobacter*, is often readily consumed by hydrogen-consuming

[6] A version of this chapter has been published in *Int J Hydrogen Energy*, 2011

microorganisms, such as methanogens and homoacetogens [8]. Therefore, in order to harness hydrogen from a mixed culture system, the seed sludge needs a pretreatment to suppress as much hydrogen-consuming microbial activity as possible while still preserving the activity of the hydrogen-producing bacteria [9]. Methods for pretreating sludge include mechanical pretreatment [10], ultrasonic disintegration [11], alkali pretreatment [12], heat pretreatment [13] and thermo-chemical pretreatment [14]. This latter treatment usually involves heating and alkali pretreatment prior to hydrogen production from sludge [15]. Heat shock pretreatment, efficient to remove H_2 consuming microorganisms while protecting spore forming bacteria, is reported to repress methanogenic activity completely [16]. Acid pretreatment is efficient in removing H_2 consumer microorganisms and also protects spore forming bacteria by repressing methanogenic activity [17]. Alkaline pretreatment (pH 8.5 – 12, exposure period, 24 h) suppresses growth of hydrogen consuming microorganisms and enhances H_2 production [18]. Integration of multiple pretreatment procedures (chemical, heat shock, and acid) showed positive influence on H_2 production with distillery wastewater-based parent inoculum [19].

On the other hand, the hydrolysis step is considered as the rate limiting step in the overall anaerobic solids digestion process [20]. Various pretreatment methods of substrate have been used to disrupt the microbial cells and release the organics to liquid so as to enhance the anaerobic digestion of sludge [21]. Thermal pretreatment, alkaline pretreatment, acidification, ultrasonic pretreatment, etc., are a few pretreatment methods employed to enhance H_2 production [22, 23, 24, 25]. However, the impact of the four pretreatments (ultrasonic, heat, acid, and base) on biohydrogen production from various substrates and with different sludges were different, because their modes of action are distinctively different: acid pretreatment depends on the free H+, alkaline pretreatment depends on the free OH^-, thermal pretreatment utilizes on the high

temperature and ultrasonic pretreatment depends on the shear produced in the sonication [26, 27]. Moreover, in the literature, different pretreatment methods have different degrees of success i.e. an optimum method in one study is the least in other study. This discrepancy in the literature is due to the use of different substrates or seed. Mu et al. [28] reported the highest hydrogen yield with heat pretreatment compared to acid and base pretreatment, while Elbeshbishy et al. [11] reported that the ultrasonication is superior to heat, acid, and base pretreatment in biohydrogen production from glucose. In another study, Xing et al [29] reported that the acid pretreatment produced the highest hydrogen yield compared to base, and infrared radiation.

In our previous study [11], ultrasonication with temperature control showed superiority over heat shock, acid, and base pretreatment methods individually. In this study, while the primary objective was to explore the impact of four individual pretreatment methods (ultrasonic, heat shock, acid, and base) and three combined pretreatment methods (ultrasonic with heat shock, ultrasonic with acid, and ultrasonic with base), the study focus on two aspects i.e. the solubilisation of food waste and biohydrogen production without using extra-seed.

7.2 Materials and methods

7.2.1 Experimental set-up

Food waste obtained from the Dufferin Organics Processing Facility (DOPF) in Toronto, Ontario, Canada was used in this study; the average characteristics of this food waste in (mg/L) were: Total chemical oxygen demand (TCOD): 91900, soluble chemical oxygen demand (SCOD): 49900, total solids (TS): 65500, volatile solids (VS): 46100, particulate carbohydrate: 26500, soluble carbohydrate: 20000, particulate protein: 6250, and soluble protein: 8710. The volatile fatty acids (VFAs) concentration was 1990 mg COD/L. The aforementioned

characteristics of the food waste are the average of three samples and the standard deviations of all parameters were less than 10%. The lab scale ultrasonic probe used in this study was supplied by Sonic and Materials, Newtown, USA (model VC-500, 500 W, and 20 kHz). To control the temperature increase of the food waste during ultrasonication, a cooling water bath was used, and the food waste temperature during the experiments did not exceed 30 ^{0}C. Food waste was subjected to various pretreatment procedures, with experimental conditions employed for each pretreatment procedure described in Table 7.1.

Table 7.1 *Description of pretreatment procedure used in this study*.

Pretreatment method	Symbol	Pretreatment conditions adapted
No pretreatment	C	No pretreatment was applied
Ultrasonic	U	Food waste was sonicated at specific energy inputs of 79 kJ/g TS
Heat shock	H	Heating the food waste at 70 ^{o}C for 30 min
Acid	A	Adjusting the pH of 300 mL food waste to 3.0 with 1 N HCl and maintaining it for 24 h in the cold room (4 ^{o}C).
Base	B	Adjusting the pH of 300 mL food waste to 11.0 with 1 N NaOH and maintaining it for 24 h in the cold room (4 ^{o}C).
Ultrasonic with heat	UH	Ultrasonic pretreatment then heat shock pretreatment
Ultrasonic with acid	UA	Ultrasonic pretreatment then acid pretreatment

Ultrasonic with base	UB	Ultrasonic pretreatment then base pretreatment

Batch anaerobic studies were conducted using the pretreated food waste as substrates and seed at the same time (no extra seed was added). The batch experiments were conducted in triplicates in a series of serum bottles (liquid volume of 200 ml). Control bottles were also prepared using the food waste without any pretreatment. The initial pH value of all bottles was adjusted to 5.5 using 1N NaOH and 1N HCl before starting the experiment. The headspace was flushed with oxygen-free nitrogen gas for a period of 3 minutes and capped tightly with rubber stoppers. The bottles were then placed in a swirling-action shaker (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) operating at 180 rpm and maintained at a temperature of 37 ^{0}C.

7.2.2 Analytical methods

All liquid and gas parameters were analyzed as described in chapter 3 (section 3.2.1 Analytical methods).

7.3 Results and discussion

7.3.1 Effect of various pretreatment methods on food waste solubilization

Figure 7.1 shows the percentage increase in soluble compounds for the different pretreatment methods. As shown in Figure 7.1, UB pretreatment showed the highest increase in SCOD and soluble protein of 33% and 40%, respectively. The highest increase in soluble carbohydrate of 31% was observed for UA pretreatment. Heat pretreatment had the lowest impact on all the soluble components, which is reflected by 20%, 8%, and 10% increase in

SCOD, soluble carbohydrate, and soluble protein, respectively. On the other hand, ultrasonic pretreatment increased the SCOD, soluble carbohydrate, and soluble protein by 25%, 16%, and 17%, respectively. Combined ultrasonic with heat pretreatments (UH) did not significantly affect the increase in neither SCOD (27% for UH versus 25% for ultrasonic pretreatment) nor soluble carbohydrate (18% for UH versus 16% for ultrasonic pretreatment). This finding agrees with Kim et al [21] who reported that the SCOD of waste activated sludge (WAS) increased by 19.1% for combined ultrasonic with thermal pretreatment compared to 17.6% for thermal (121 ^{o}C for 1.5 hr) and 18.4% for ultrasonic (42 kHz for 120 min with temperature control), respectively. Furthermore, UB pretreatment had a significant impact on all soluble components reflected by highest increase in SCOD (33%) and highest increase in soluble protein (40%) and the second highest soluble carbohydrate (27%). Yiying et al. [30] who studied different combinations of ultrasonic and alkaline pretreatments of WAS, reported that the SCOD increased from 275 mg/L for the untreated sludge to 4529, 5976, 6408, and 6797 mg/L for alkaline, ultrasonic followed by alkaline, alkaline followed by ultrasonic, and simultaneous ultrasonic and alkaline pretreatment, respectively. Moreover, among the four individual pretreatment methods, acid and base pretreatment showed the uppermost increase in SCOD of 28%, followed by 25% and 20% for ultrasonic and heat pretreatments, respectively. Xiao and Lui [31] applied four different pretreatment methods to WAS, found that the base pretreatment resulted in the highest increase in SCOD compared to acid, thermal, and ultrasonic. They reported that the SCOD of sewage sludge increased from about 114 mg/L for untreated sludge to about 3049, 2442, 1485, and 766mg/L for base (NaOH, 6N, pH of 12), thermal (121 ^{o}C, 30 min), ultrasonic (250 mL, 30 min, 200 W, no temperature control), and acid (HCl, 6N, pH of 2) pretreatment, respectively. In another study, Lopez Torres and Espinosa Llorens [32] evaluated the effect of alkaline (Ca

$(OH)_2$) on the organic fraction of municipal solid waste (OFMSW), and reported that the SCOD increased from 13675 to 20101 mg/L at a lime concentration of 70 meq $Ca(OH)_2$/L.

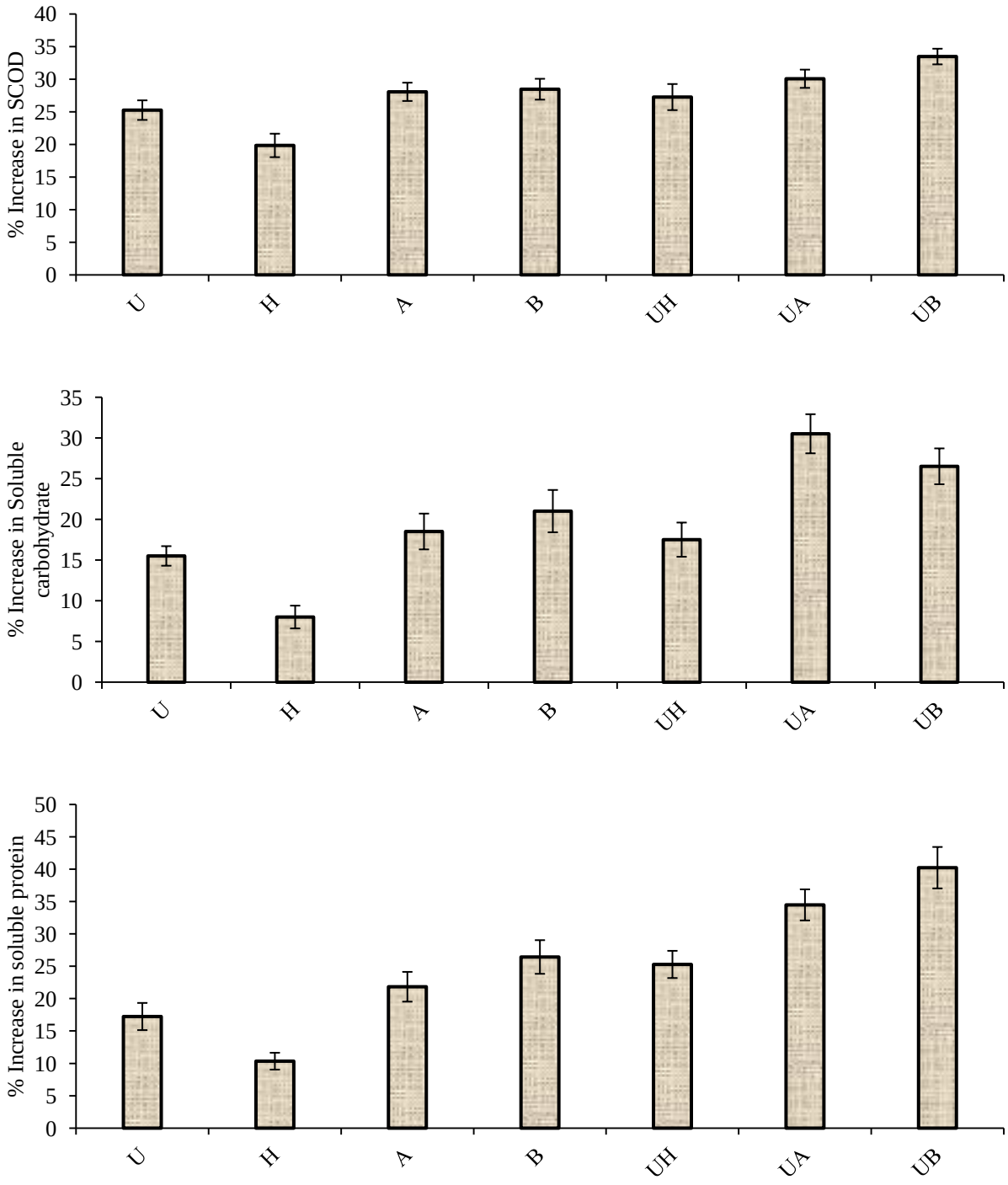

Figure 7.1 % Increase in soluble compounds for different pretreatment methods.

As apparent in Figure 7.1, it is obvious that the acid, base, and UH pretreatments had almost the same solubilisation effect reflected by about 28% increase in SCOD, 18% - 21% increase in soluble carbohydrate, and 23% - 26% increase in soluble protein. Based on the aforementioned results, the order of solubilisation (based on increase in SCOD) of the four individual pretreatment methods was: base and acid > ultrasonic > heat, while the order of solubilisation of the three combined pretreatment methods was: UB > UA > UH. The order of solubilization of the seven pretreatment methods used in this study was: UB > UA > base and acid > UH > ultrasonic > heat.

Figure 7.2 shows the percentage decrease in particulate components after pretreatment. As depicted in Figure 7.2, UA exhibited the highest decrease in all particulate matter: 20% of VS, 41% of particulate protein, and 20% of particulate carbohydrate. Heat pretreatment showed the lowest decrease in VS, particulate carbohydrate, and particulate protein of 12%, 10%, and 18%, respectively. Among the four individual pretreatment methods, base pretreatment showed the highest decrease in all particulate components of 17%, 15%, and 31% in VS, particulate carbohydrate, and particulate protein, respectively, consistent with the Solubilisation data discussed above.

7.3.2 Effect of various pretreatment methods on biohydrogen production

Batch study of the seven pretreatment methods and untreated food waste showed that the biogas production contained only hydrogen and carbon dioxide, with no methane detection in the head space. Figure 7.3 illustrates the cumulative hydrogen production for the seven various pretreatment methods and the untreated food waste (error bars are not shown as errors were less than 10%).

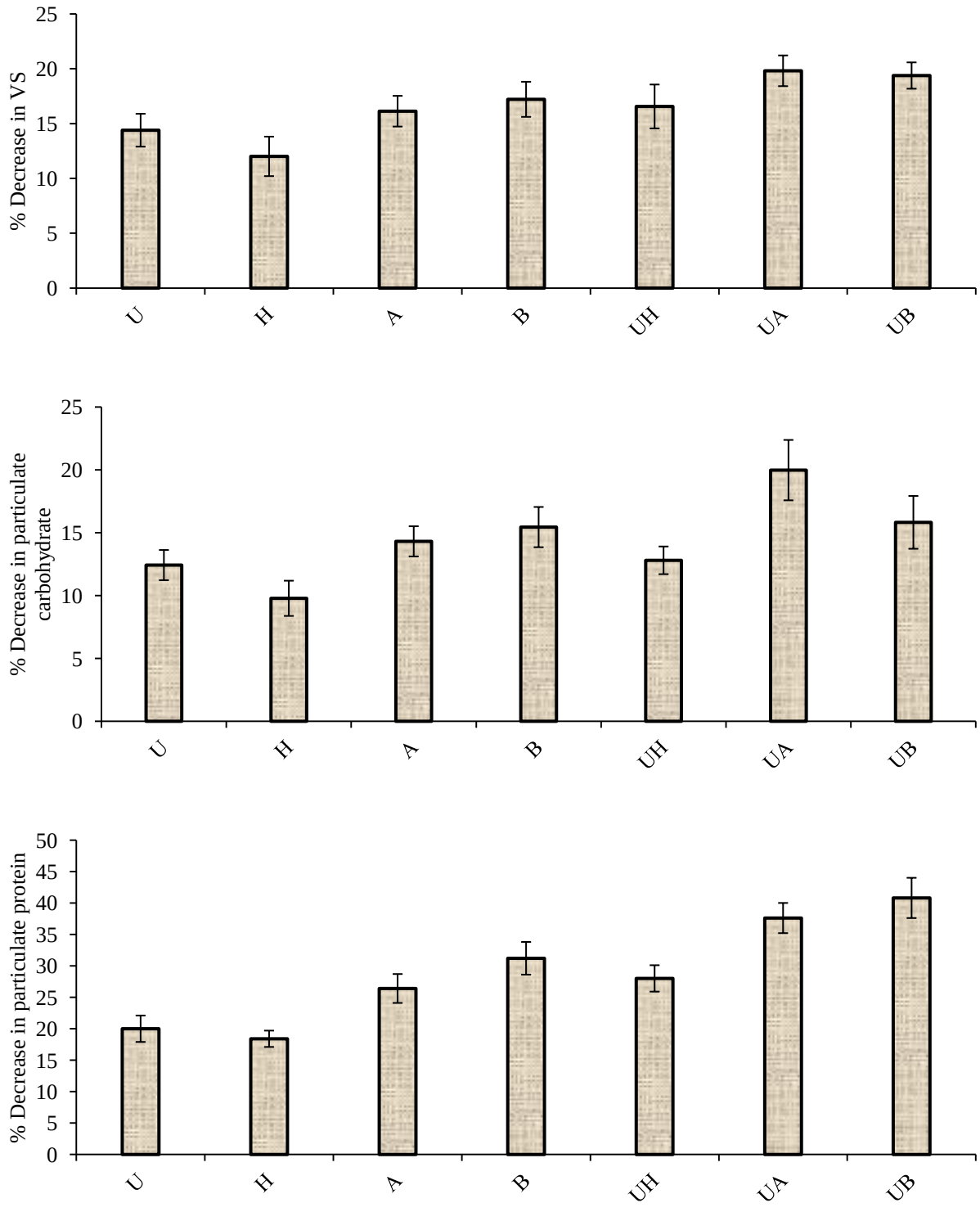

Figure 7.2 % Decrease in particulate compounds for different pretreatment methods.

As apparent in Figure 7.3, the ultimate hydrogen production of the pretreated sludges was higher than that of the untreated sludge (388 mL). Of the seven different pretreatment methods used in this study, base pretreatment showed the lowest ultimate hydrogen production of 420 mL, while the highest ultimate hydrogen production of 1090 mL was observed for UA pretreatment. Among the four individual pretreatment methods, the highest ultimate hydrogen production of 894 mL was achieved for the ultrasonic pretreatment followed by 640, 510, and 420 mL for heat, acid, and base pretreatment, respectively. On the other hand, and although UA pretreatment had a positive effect on hydrogen production reflected by highest ultimate hydrogen production, UH and UB pretreatments had a negative impact on the ultimate hydrogen production compared to the ultimate hydrogen production of the ultrasonic pretreatment. The ultimate hydrogen production decreased from 894 mL for ultrasonic only to 720 mL for ultrasonic combined with heat pretreatment (UH) and to 620 mL for ultrasonic combined with base pretreatment (UB).

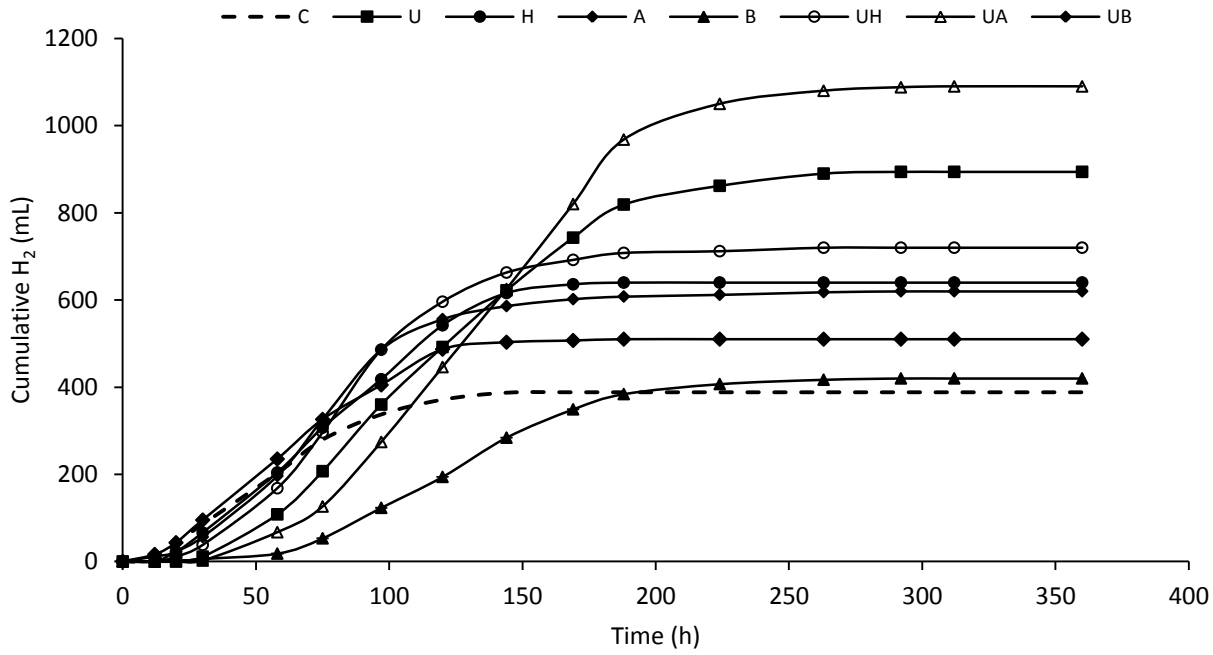

Figure 7.3 Cumulative hydrogen productions for different pretreatment methods.

Figure 7.4 shows the hydrogen yield as mL/ g $VS_{initial}$ for the seven pretreatment methods and the untreated food waste. As revealed in the Figure, among the four individual pretreatments, ultrasonic showed the highest hydrogen yield of 97 mL/ g $VS_{initial}$, followed by 70, 55, and 46 mL/ g $VS_{initial}$ for heat, acid and base, respectively. This finding is contrary to the finding of Xiao and Lui [31], who found that the highest hydrogen yield from WAS of 11.68 mL H_2/g VS was observed for alkaline pretreatment followed by 8.62 and 3.25 mL H_2/g VS for thermal and acid pretreatments, respectively. In another study, Xing et al [29] who investigated the enhancement of hydrogen production from dairy manure reported that the acid pretreatment produced the highest hydrogen yield compared to base, and infrared radiation. The aforementioned authors reported hydrogen yields of 18.1, 14.2, and 13.9 mL H_2/g VS for acid, base, and infrared radiation pretreatments, respectively, compared to 13.3 mL H_2/g VS for the untreated one.

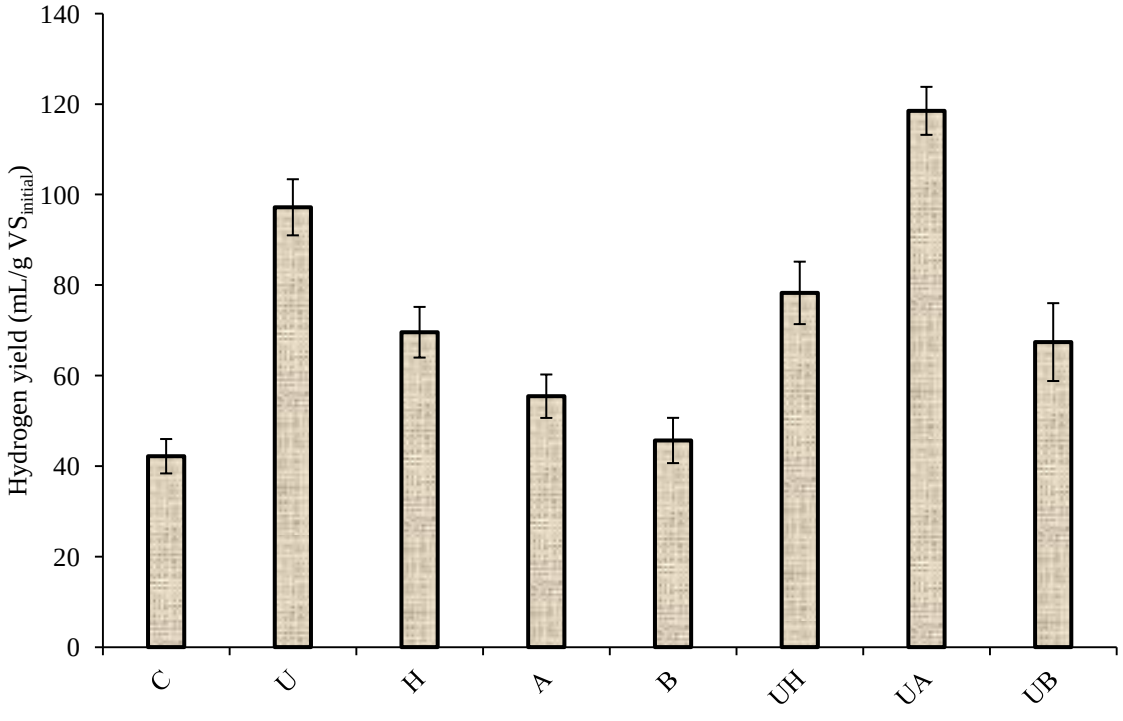

Figure 7.4 Hydrogen yield for different pretreatment methods.

As apparent in Figure 7.4, among the three combined pretreatment methods, UA pretreatment produced the highest hydrogen yield of 118 mL/ g $VS_{initial}$, while hydrogen yield of 78 and 67 mL/ g $VS_{initial}$ were observed for UH and UB, respectively. Based on the aforementioned results, the highest hydrogen yield was achieved for combined ultrasonic with acid pretreatment. This finding is contrary to the study by Mohan and Sarma [33] who reported that the highest hydrogen yield was achieved for chemical pretreatment. Mohan and Sarma [33] studied the effect of three pretreatment methods; acid (pH of 3, adjusted with ortho-phosphoric acid; 24 h), chemical (2-bromoethane sulphonic acid sodium salt (0.2 g/l); 24 h), heat (100 ^{o}C, 1 h) and their four possible combinations (acid and chemical, acid and heat, heat and chemical, and acid, heat and chemical) on the anaerobic inoculum using dairy wastewater as substrate. The highest hydrogen yield of 0.78 mL/g COD was observed for the chemical pretreatment, while the lowest hydrogen yield of 0.19 mL/g COD was observed for the acid pretreatment.

Statistical analysis using the paired t-test was conducted to evaluate the significance of the difference in hydrogen yield at the 95% confidence interval, Table 7.2 summaries the *P*-values. Paired test involving the seven pretreatment methods and the control revealed that differences between all different pretreatment methods and/or control are significant except the differences between base pretreatment and control are significant and differences between heat pretreatment and sonication with base pretreatment are insignificant.

Based on the results of our study, the order of cumulative hydrogen production of the four individual pretreatment methods was: ultrasonic > heat > acid > base, while the order of cumulative hydrogen production of the three combinations was: UA > UH > UB. The order of cumulative hydrogen production of the seven pretreatment methods used in this study is: UA > ultrasonic > UH > heat > UB > acid > base. It is noteworthy that ranking of the seven

pretreatment based on hydrogen production differs from that based on Solubilisation, clearly emphasizing the role of other mechanisms in biohydrogen production.

Table 7.2 *P values from the t-test of the different groups (pretreatment and/or control).*

	C	U	H	A	B	UH	UB	UA
C		< 0.001	< 0.001	< 0.001	0.088	< 0.001	< 0.001	< 0.001
U			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.012
H				0.004	< 0.001	0.011	0.353	< 0.001
A					0.01	< 0.001	0.002	< 0.001
B						< 0.001	< 0.001	< 0.001
UH							< 0.001	< 0.001
UB								< 0.001
UA								

7.3.3 Production of VFAs

The hydrogen yield depends on the fermentation pathway and end-products [34]. When acetic acid is the end-product, a theoretical maximum of 4 moles hydrogen per mole glucose is obtained:

$$C_6H_{12}O_6+2H_2O \longrightarrow 4H_2+2CH_3COOH+2CO_2 \quad (7.1)$$

When butyrate is the end-product, a theoretical maximum of 2 moles hydrogen per mole glucose is produced:

$$C_6H_{12}O_6+2H_2O \longrightarrow 2H_2+CH_3CH_2CH_2COOH+2CO_2 \quad (7.2)$$

Figure 7.5 shows the final VFAs after fermentation for the seven pretreatment methods and the untreated food waste. Among the four individual pretreatment methods, ultrasonic produced the highest final VFAs of 13100 mg COD/L, followed by 12400, 11800, and 9670 mg COD/L for heat, acid, and base, respectively, while the final VFAs of 8950 mg COD/L was observed for the untreated food waste. Based on the aforementioned results, it is obvious that the

final VFAs of the four individual pretreatment methods are directly and positively correlated with the ultimate hydrogen production. Xiao and Lui [31] who studied four different pretreatment methods (acid, base, thermal, and ultrasonic) of WAS, reported that the highest final VFAs of 1166 mg COD/L was for base pretreatment followed by 1142, 820, and 798 mg COD/L for heat, ultrasonic, and acid pretreatment, respectively.

On the other hand, among the three combined pretreatments method, the highest final VFAs of 16900 mg COD/L was observed for UA pretreatment which also produced the highest ultimate hydrogen production. UB pretreatment resulted in about 10% increase in the final VFAs compared to the final VFAs of ultrasonic pretreatment, while UH decreased the final VFAs by about 8% compared to the final VFAs of ultrasonic pretreatment. Moreover, although the final VFAs of UB were higher than the final VFAs of UH pretreatment, the ultimate hydrogen production of UH was higher than of UB pretreatment.

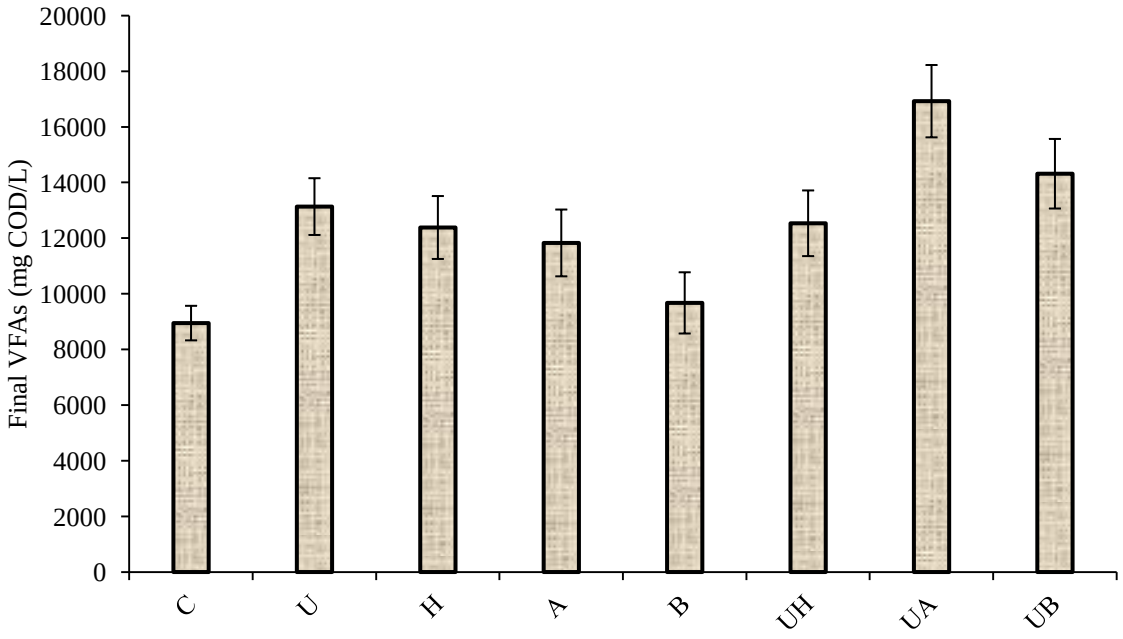

Figure 7.5 Final VFAs for different pretreatment methods.

Based on Equations (1) and (2), it is evident that the molar yield of acidification to acetate is 4 moles of hydrogen versus 2 moles for butyrate. Thus, the molar ratio of acetate to butyrate (HAc/HBu) significantly impacts the hydrogen yield [35]. Accordingly, the HAc/HBu ratio has been examined in this study. Figure 7.6 shows the HAc/HBu ratio formed for the different seven pretreatment methods and the untreated one. As illustrated in Figure 7.6, UA pretreatment has the highest ratio of acetate to butyrate of 1.87, while base pretreatment has the lowest ratio of HAc/HBu of 0.61. The HAc/HBu ratio for ultrasonic pretreatment, heat pretreatment and acid pretreatment varied narrowly from 1.22 to 1.29, while UB and UH pretreatment had almost the same ratio of 1.11 and 1.14, respectively. Table 7.3 shows the COD mass balances for all the batches computed considering the initial and final TCOD, and the equivalent COD of hydrogen (8 g COD/g H_2). As shown in the Table, the COD mass balance indicated a closure of 86%–93%, thus emphasizing data reliability.

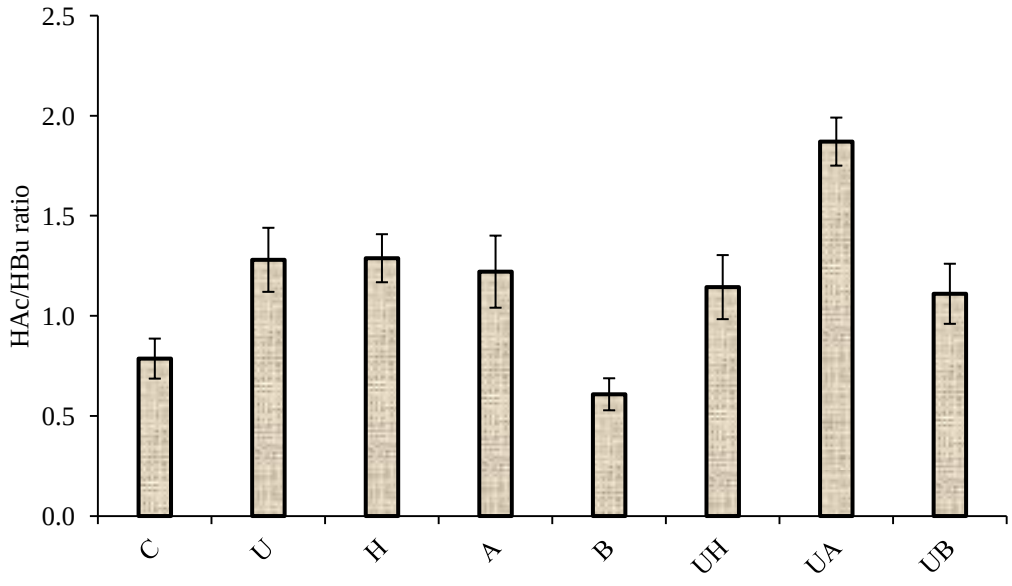

Figure 7.6 Molar acetate/butyrate ratios for different pretreatment methods.

Table 7.3 *COD mass balances for different pretreatment methods.*

Pretreatment method	Initial TCOD	Final TCOD	$TCOD_{consumed}$	$TCOD_{consumed}$	Hydrogen		COD balance
	mg/L	mg/L	mg/L	mg	(mL)	mg COD	%
C	91900	88760	3140	314	388	279	89
U	91400	84500	6900	690	894	644	93
H	92100	86750	5350	535	640	461	86
A	91300	87100	4200	420	510	367	87
B	91800	88300	3500	350	420	302	86
UH	92200	86400	5800	580	720	518	89
UA	91100	82600	8500	850	1090	785	92
UB	91600	86700	4900	490	620	446	91

7.3.4 Kinetic analysis

The cumulative hydrogen data were fitted with Gompertz equation using the Newton-Raphson method for non-linear numerical estimation as described in [11]. Table 7.4 summarizes the results of the kinetic analysis. The determination coefficient (R^2) of over 0.96 for all the regressions confirms the applicability of the modified Gompertz model.

The maximum hydrogen production potentials of the four individual pretreatment methods were 910, 650, 515, and 426 mL for ultrasonic, heat, acid, and base pretreatment, respectively, while the maximum hydrogen production potential was 391 mL for the untreated food waste. The maximum hydrogen production potentials of the combined pretreatments were 1119, 721, and 620 mL for UA, UH, and UB, respectively. The highest hydrogen production rate of UH and UA pretreatment varied narrowly from 8.6 to 8.9 mL/h, followed by 7.9 mL/h for UB pretreatment. Hydrogen production rates of 7.1, 6.8, and 6.0 mL/h were observed for the

ultrasonic, heat, and acid pretreatment, respectively. The lowest hydrogen production rate of 3.9 ml/h was observed for the base pretreatment. The hydrogen production rate of the untreated food waste was 5.1 ml/h. It is evident that UA pretreatment had a positive effect on ultimate hydrogen production and hydrogen production rate as reflected by about 200% increase in maximum hydrogen production potential, and 75% increase in the hydrogen production rate relative to the untreated food waste. The maximum lag phase of about 67 hrs was observed for the base and UB pretreatment, while the minimum lag phase of about 17 hrs was observed for untreated food waste and acid pretreatment. The lag phase of about 29, 32, 39, and 47 hrs were observed for heat, UA, UH, and ultrasonic pretreatment, respectively.

Table 7.4 *Kinetic coefficients for different pretreatment methods.*

Pretreatment method	P	R_m	λ	R^2
C	391 ± 6	5.1 ± 0.4	16.3 ± 1.6	0.998 ± .001
U	910 ± 9	7.1 ± 0.2	47.4 ± 0.4	0.97 ± 0.01
H	650 ± 30	6.8 ± 0.7	28.5 ±2.4	0.984 ± 0.01
A	515 ± 17	6.0 ± 0.7	17.7 ± 2.3	0.985 ± 0.008
B	426 ± 28	3.9 ± 0.2	66.5 ± 4.0	0.968 ± .011
UH	721 ± 13	8.6 ± 0.7	38.8 ± 2.6	0.989 ± 0.006
UA	1119 ± 99	8.9 ± 0.8	67.6 ± 2.9	0.966 ± 0.011
UB	620 ± 10	7.9 ± 0.8	31.7 ± 2.5	0.992 ± 0.005

Note: Values represents average ± STD

7.4 Summary and conclusions

The outcome of this study emphatically revealed the positive effect of combined ultrasonic and acid pretreatment on biohydrogen production without extra seed, while combined ultrasonic with heat or base pretreatment had negative impact on hydrogen production. Based on the findings of this study, the following conclusions can be drawn:

- The highest increase in SCOD and soluble protein of 33% and 40% were achieved for UB pretreatment, respectively, while the highest increase in soluble carbohydrate of 31% was observed for UA pretreatment.
- Of the seven pretreatment methods, the highest hydrogen yield of 118 mL/ g $VS_{initial}$ was observed for UA pretreatment, while the lowest hydrogen yield of 46 mL/ g $VS_{initial}$ was observed for base pretreatment.
- Hydrogen yield decreased from 97 mL/ g $VS_{initial}$ for ultrasonic only to 78 mL/ g $VS_{initial}$ when ultrasonic combined with heat pretreatment (UH) and to 67 mL/ g $VS_{initial}$ when ultrasonic combined with base pretreatment (UB).
- UA exhibited the highest final VFAs of 16900 mg COD/L as well as the highest HAc/ HBu ratio of 1.87, while base pretreatment had the lowest final VFAs of 9700 mg COD/L and the lowest HAc/ HBu ratio of 0.61.
- The highest hydrogen production rate of UA and UH pretreatment varied narrowly from 8.6 to 8.9 mL/h and the lowest hydrogen production rate of 3.9 mL/h was observed for base pretreatment.

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CHAPTER 8

Hydrogen Production Using Sono-Biohydrogenator [7]

8.1 Introduction

Hydrogen, as an energy carrier, offers numerous advantages over other conventional energy carriers. Hydrogen combustion provides energy based on mass basis with lower heating value (LHV), which is 2.4, 2.8, and 4 times higher than methane, gasoline and coal respectively [1]. In addition, hydrogen gas has the potential to be a useful energy carrier in a wide range of applications through the use of fuel cells, and is expected to become more important in the future [2,3]. The major advantage of energy from hydrogen is the absence of polluting emissions since the utilization of hydrogen, either via combustion or via fuel cells, results in pure water [4].

At present, hydrogen is produced mainly from fossil fuels, biomass, and water using chemical or biological processes. Anaerobic (or dark) fermentation and photosynthetic degradation are the two most widely studied biohydrogen production techniques [5]. Anaerobic fermentation is promising for sustainable hydrogen production since organic matter, including waste products, can be used as a feedstock for the process [6]. However, the rate of biological H_2 production is low and the technology needs further development [7]. Current H_2 yields reported in the literature are usually in the range of 1–2 mol H_2/mol glucose converted [8], much less than the theoretical maximum of 4 mol H_2/mol glucose converted. Therefore, improving the H_2 yield from dark fermentation of organics is an active area of research [9].

Hydrogen partial pressure and the resulting H_2 concentration in the liquid phase are key factors affecting fermentative H_2 production [10]. Generally, high H_2 partial pressure has a

[7] A version of this chapter has been published in *Int J Hydrogen Energy*, 2011

negative effect on H_2 production by decreasing the activity of *hydrogenase* and making the H_2 production reaction thermodynamically unfavourable [11]. Various techniques have been used to remove metabolic gases (H_2, CO_2) from the liquid phase [12]. Gas sparging has been the most common method used to decrease the concentrations dissolved gases in fermentative H_2-producing reactors. Various gases have been used to decrease the dissolved hydrogen concentration in the liquid such as nitrogen [13, 17], CO_2, methane [18], biogas [16], argon [19], argon and H_2 sparging [20]. Other techniques to decrease concentrations of dissolved gases include increased stirring [21], decreasing the reactor headspace pressure i.e. applying a vacuum [10], and using an immersed membrane to directly remove dissolved gases [22]. Table 8.1 summarizes some studies which used gas sparging to enhance the hydrogen production. As shown in the table, the maximum increases in hydrogen yield were 66%, 88% and 118% using the N_2, CO_2, and methane, respectively.

Table 8.1 *Different gas sparging in CSTR, adapted from Kraemer and Bagley* [12].

Sparge gas	H_2 yield mol H_2/mol hexose		Yield increase (%)	Ref.
	No sparging	With sparging		
N_2	0.85	1.43	68	[11]
N_2	1.26	1.87	48	[13]
N_2	0.9	1.5	66	[14]
N_2	1.23	1.65	34	[15]
N_2	0.77	0.95	23	[16]
N_2	1.3	1.8	38	[17]
CO_2	0.77	1.68	118	[16]
CH_4	Not reported	Not reported	88	[18]
Biogas	0.77	0.86	12	[16]

Ultrasonication causes a localised pressure drop to below the evaporating pressure in the aqueous phase, resulting in the formation of micro bubbles or cavitation bubbles [23]. During cavitation, microbubbles form at various nucleation sites in the fluid and grow during the rarefaction phase of the sound wave [24]. Subsequently, in the compression phase, the bubbles implode and the collapsing bubbles release a violent shock wave that propagates through the medium [25].

Based on an extensive search, there are only a limited numbers of studies (six studies) where the impact of ultrasonication on biological hydrogen production has been investigated. Table 8.2 summarizes the six studies which applied ultrasonication either on substrate or on the seed to enhance hydrogen production. Three studies applied ultrasonication on sewage sludge as a substrate [26,27,28], and the other three applied the ultrasonication on the seed biomass [29,30,31]. Guo et al. [29] studied the impact of ultrasonic pretreatment on hydrogen production from boiled anaerobically digested sludge at 90 ^{0}C for 15 min with sucrose as substrate. In another study, More and Ghangrekar [30] evaluated the effect of ultrasonication pretreatment on mixed anaerobic sludge to inoculate the microbial fuel cells, and reported that the ultrasonication pretreatment of 5 min affected a maximum power density 2.5 times higher than the untreated sludge. Moreover, in our previous study, using batches, we examined the effect of ultrasonication on eliminating methanogenesis and therefore enhancing the biohydrogen production [31]. The optimized sonication energy for hydrogen production using anaerobically digested sludge was 79 kJ/g TS and the hydrogen yield increased by 45% compared with the untreated sludge.

Table 8.2 *Different applications of ultrasonication on biological hydrogen production.*

Reactor	Seed	Substrate	Ultrasonication application	Main finding	Ref.
Batch	*Clostridium bifermentans*	Wastewater sludge, solids content of 16 500 mg/L	Sonicated the substrate. Frequency of 20 kHz, sludge 300 ml, 20 min.	Ultrasonication reduced the bio-hydrogen yield (mmol H_2/g COD_i): No pretreatment ≈ 0.6, Sterilization ≈ 1.5, Ultrasonication ≈ 0.7, Acidification ≈ 0.8, Methanogenic inhibitor ≈ 0.3, Freezing and thawing ≈ 2.1.	[26]
Batch	*Pseudomonas sp.*	Wastewater sludge, 8.29 g/L VSS	Sonicated the substrate. sonication density of 2 w/ml. sludge 200 ml, sonication time of 5 min	Ultrasonication resulted the lowest hydrogen yield. Sterilization pretreated sludge 15.02 ml H_2/g COD Microwave sludge 11.44 ml H_2/g COD Ultrasonication sludge 4.68 ml H_2/g COD	[27]
Batch	Sewage sludge	The sewage sludge was used as seed and substrate	Sonicated the seed and substrate. sonication power 200 W. 250 mL, 30 min, no temperature control (16 ^{0}C to 41^{0}C)	Hydrogen yield (mL H_2/g VS) at different pH: No pretreatment; 0 (2.5)[a], 1.21 (7), 7.57 (11.5) Acid; 0 (2.5), 3.25 (7), Base; 1.46 (7), 11.68 (11.5), Sterilization; 8.62 (6.8), Ultrasonication; 3.83 (6.9)	[28]
Batch	Anaerobic digester sludge	sucrose	Sonicated the seed and substrate. Applied different sonication times and different amplitudes.	Ratio of hydrogen production rate compared with control[b]: Seed was boiled and sucrose was sonicated; 1.17 Seed was boiled and sonicated; 1.3, Seed was boiled and mixture of seed and sucrose was sonicated; 1.48	[29]
Microbial fuel cell (MFC), continuous	anaerobic sewage sludge	sucrose	Sonicated the seed before inoculated. Sonication times 2.5, 5, 7.5, 15 min, specific energies 1050, 2075, 3130, 6235 kJ/kg TS	Maximum power density during polarization in a MFC inoculated with ultrasonication pre-treatment to the sludge for 5 min (40 kHz, 120 W) was 2.5 times higher than that obtained without any pre-treatment to the inoculum sludge.	[30]
Batch	Anaerobic digester sludge	Glucose	Sonicated the seed. 200 mL of sludge, different sonication times from 0.5 to 30 min, with and without temperature control.	* Optimum specific energy of sonication for inactivation of methanogenesis was 79 kJ/g TSS. Hydrogen yields of 1.55, 1.11, 1.04, 1.03, 0.68, and 0.7 mol H_2/mol glucose for sonication with temperature control, acid, heat-shock, sonication without temperature control, base, and untreated sludge, respectively;	[31]

[a] pH in the practice.
[b] Experiments' set up: The seed was boiled and then used as inoculums, and no sonication on seed or sucrose (control)

It is indeed intriguing that despite the well established enhancement of biohydrogen production by degassing alluded to above, and the positive influence of ultrasonication on mass transfer, no single study attempted to explore the use of ultrasonication inside continuous biohydrogen systems. Thus, the primary objective of this study was to explore the impact of ultrasonication on biohydrogen production in a new sonicated biological hydrogen reactor (SBHR) and compare it with the most common bioreactor, the continuous stirred tank reactor (CSTR).

8.2 Material and methods

8.2.1 Systems setup and operation

Two continuous-flow completely mixed reactors (10 cm diameter, 30 cm height) with a working volume of 2 L each were used in this study (Figure 8.1). One is a conventional continuous stirred tank reactor and the other one is the sonicated biological hydrogen reactor (SBHR) which comprised a conventional continuous stirred tank reactor connected with a lab scale 2.5-inch diameter ultrasonic probe at the bottom of the reactor (1 cm above the bottom of the reactor). The sonication pulses (inside the reactor) were set to 1 second on and 59 seconds off. The ultrasonic probe was supplied by Sonic and Materials (model VC-500, 500 W, and 20 kHz). These two systems (CSTR and SBHR) were operated on synthetic glucose-based feed for 90 days. The two reactors were seeded with 2 L of anaerobically digested sludge and maintained at a constant temperature of 37 ^{o}C. After seeding, the two reactors were first operated in a batch mode for 24 h, after which the reactor was shifted to the continuous-flow mode with a hydraulic retention time (HRT) of 12 h. A summary of the operational conditions is shown in Table 8.3. The two systems were operated at two organic loading rates (OLRs): OLR-1 of 21.4 g COD/L.d

with an influent glucose concentration of 10 g/L and OLR-2 of 32.1 g COD/L.d with an influent glucose concentration of 15 g/L.

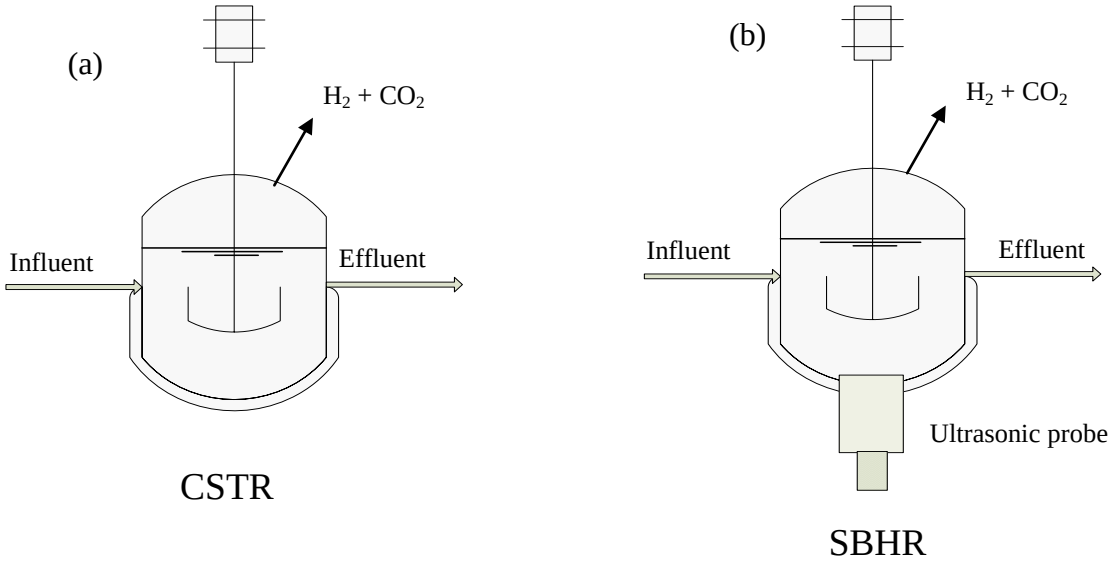

Figure 8.1 Experimental set up for the biohydrogen production systems.

Table 8.3 *Operational conditions of the hydrogen production systems.*

Parameter	Units	Phase 1		Phase 2	
		CSTR	SBHR	CSTR	SBHR
HRT	h	12	12	12	12
Glucose concentration	g/L	10	10	15	15
OLR	g COD/L.d	21.4	21.4	32.1	32.1
pH		5-6	5-6	5-6	5-6

8.2.2 Inocula and media compositions

Anaerobic sludge was collected from the primary anaerobic digester at St Mary's wastewater treatment plant (St Mary's, Ontario) and used as seed sludge after sonication. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the sludge were 11 and 9 g/L, respectively. In order to enrich hydrogen producing bacteria, the sludges were sonicated using a lab scale sonication device at specific energy of 20 kJ/g TS with temperature control as described in Elbeshbishy et al. [31]. The feed containing glucose at two different concentrations of 10 g/L (Phase 1) and 15 g/L (Phase 2), was supplied by 5 mL/L of a nutrient stock solution with the following composition per liter of stock: 1000 g $NaHCO_3$, 280 g NH_4Cl, 250 g of K_2HPO_4, 100 g of $MgSO_4.7H_2O$, 10 g of $CaCl_2.2H_2O$, 2 g of $FeCl_2.4H_2O$, 0.05 g of H_3BO_3, 0.05 g of $ZnCl_2$, 0.03 g of $CuCl_2$, 0.5 g of $MnCl_2.4H_2O$, 0.05 g of $(NH_4)_6Mo_7O_{24}$, 0.05 g of $AlCl_3$, 0.05 g of $CoCl_2.6H_2O$, and 0.05 g of $NiCl_2$.

8.2.3 Analytical methods

Biogas production was collected by wet tip gas meters (Gas Meters for Laboratories, Nashville, TN). The gas meter consists of a volumetric cell for gas–liquid displacement, a sensor device for liquid level detection, and an electronic control circuit for data processing and display. All other liquid parameters and gas compositions were analyzed as described in chapter 3 (section 3.2.1 Analytical methods).

8.2.4 Microbial community analysis

Under all four reactor conditions, at the end of each phase, the total genomic community DNA was extracted using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories,

Carlsbad, CA, USA) and after PCR amplification were analyzed by denaturing gradient gel electrophoresis (DGGE). The primer set of 357FGC (50-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGC AG- 30) and 518R (50-ATTACCGCGGCTGCT GG-30) at the annealing temperature of 53^{O}C was used for PCR amplification of the variable V3 region of 16S rDNA from the purified genomic DNA. Denaturing gradient gel electrophoresis (DGGE) of PCR products was performed with a DCode universal mutation system (Bio-Rad Laboratories, Hercules, CA, USA). The PCR products were applied directly to 8% (w/v) polyacrylamide gel with 15–55% denaturant gradients. Electrophoresis was performed at a constant voltage of 130 V at 58 °C for 5 h. The DNA templates of the bands of interest were reamplified and the PCR products were purified using QIAquick PCR Purification Kit (Qiagen Sciences, MD, USA) in accordance with the manufacturer's protocol. The sequences of the re-amplified DNA fragments were determined by dideoxy chain termination (Sequencing Facility, John P. Robarts Research Institute, London, Ontario) and compared with available sequences in the GenBank database using the BLAST program [32].

8.3 Results

8.3.1 Hydrogen production

Figure 8.2 illustrates the hydrogen production rates for the conventional CSTR and the SBHR at the two different OLRs of 21.4 (Phase 1) and 32.1 g COD/ L.d (Phase 2). As apparent from Figure 8.2, after the 10-days start-up period, stable hydrogen production rates were observed in both the conventional CSTR and SBHR. The hydrogen production rates in the SBHR were significantly higher than those in the conventional CSTR at both OLRs.

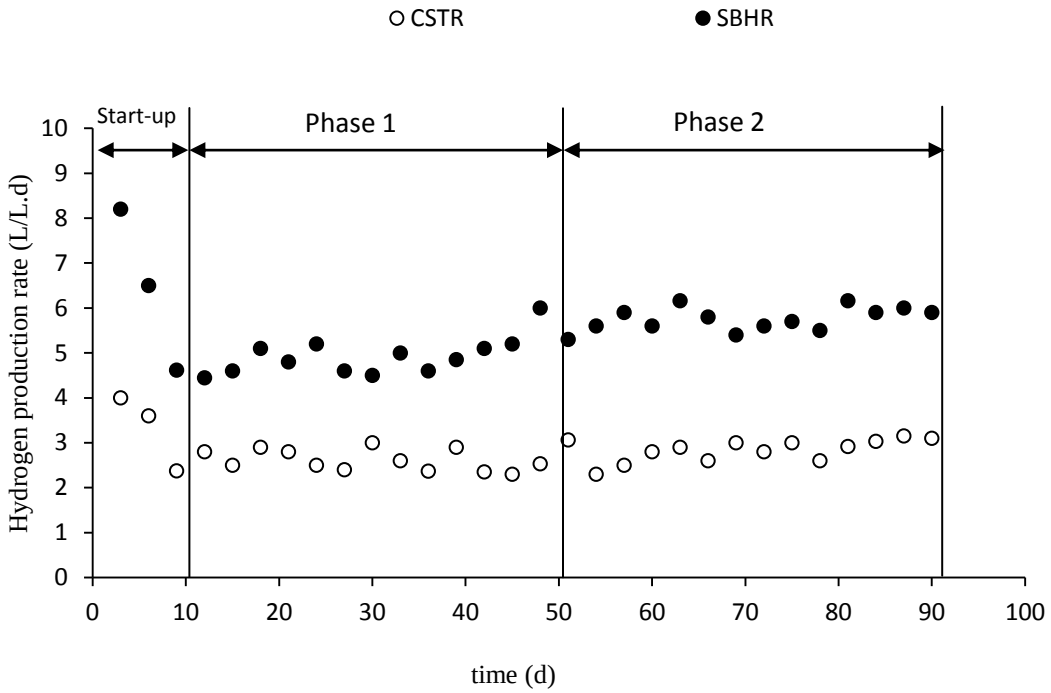

Figure 8.2 Diurnal variations in hydrogen production rate.

The average hydrogen production rates per unit reactor volume for the conventional CSTR were 2.6 and 2.8 L/L.d, as compared with 4.8 and 5.6 L/L.d for SBHR, in Phases 1 and 2, respectively. Figure 8.3 shows the hydrogen yields for the conventional CSTR and the SBHR in the two phases. As depicted in Figure 8.3, hydrogen yields of 1.2 and 1.0 mol H_2/mol glucose converted were observed for the CSTR in Phases 1 and 2, respectively, while for the SBHR, the hydrogen yields in Phases 1 and 2 were 2.1 and 1.9 mol H_2/mol glucose, respectively.

Table 8.4 summarizes the steady state data for the two systems during the two phases. Generally in biological treatment systems, steady-state data is collected after a minimum of 3 turnovers of the mean solids retention time (SRT). In addition to the aforementioned criteria, steady-state in this case also entailed less than 10% variation in biogas quantity, and reactor water quality parameters.

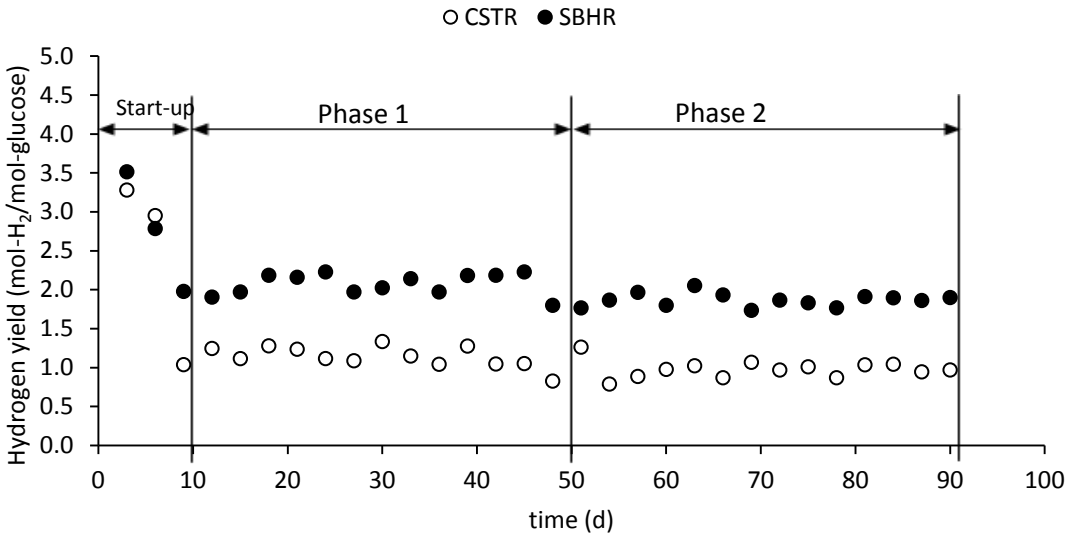

Figure 8.3 Diurnal variations in hydrogen yield.

The stability of both systems is evident from the very low coefficient of variation (CV), calculated as the standard deviation divided by the average of the steady state data based on 12 samples. Glucose conversion efficiencies of 92% and 94% were achieved in Phase 1 for the conventional CSTR and SBHR, respectively. In Phase 2, glucose conversion efficiencies decreased to 76% and 84% in the CSTR and SBHR. The conversion efficiency of glucose to hydrogen (based on the theoretical yield of 4 mol-H_2/mol-glucose) for the CSTR and SBHR were 23% and 51% in Phase 1, and 25% and 46% in Phase 2, respectively. Based on the aforementioned glucose conversion efficiencies, it is evident that by increasing the OLR, the glucose conversion decreased in the two systems. Furthermore in both phases, glucose conversion efficiencies in the SBHR were higher than that in the conventional CSTR. As shown in Table 8.4, the average hydrogen concentrations in the headspace of the conventional CSTR were 38% and 35% for the Phases 1 and 2, respectively, as compared with 42% and 46% in the SBHR, respectively.

Table 8.4 *Summary of steady state data in the hydrogen production systems.*

Measured parameter	Units	Phase 1		Phase 2	
		CSTR	SBHR	CSTR	SBHR
Hydrogen production rate	(L/L.d)	2.6 ± 0.25	4.8 ± 0.3	2.8 ± 0.38	5.6 ± 0.51
Percentage Hydrogen	%	38 ± 6	42 ± 3	35 ± 5	46 ± 2
Hydrogen yield	mol H_2/mol glucose	1.2 ± 0.15	2.1 ± 0.23	1.0 ± 0.13	1.9 ± 0.21
Glucose conversion	%	92 ± 4	94 ± 2	76 ± 4	84 ± 4
Biomass concentration	mg/L	1186 ± 69	1017 ± 81	1100 ± 64	939 ± 42
Biomass yield[a]	(mg VSS/mg $COD_{consumed}$)	0.30	0.24	0.34	0.23
Specific H_2 production rate	L/g VSS.d	2.2 ±0.3	4.7 ± 0.5	2.5 ± 0.3	6.2 ± 0.3
Acetate/Butyrate		0.63 ± 0.19	1.13 ± 0.12	0.75 ± 0.17	1.20 ± 0.16

* Values represent averages ± standard deviations based on 12 steady-state samples.

[a] Calculated based on the slope of the cumulative biomass produced versus the cumulative SCOD consumed.

8.3.2 Volatile Fatty Acids (VFAs)

Hydrogen yield depends on the fermentation pathway and end-products [7]. The available hydrogen production from glucose is determined by the butyrate/acetate ratio [33]. When acetic acid is the end-product, a theoretical maximum of 4 moles hydrogen per mole glucose is obtained:

$$C_6H_{12}O_6+2H_2O \longrightarrow 4H_2+2CH_3COOH+2CO_2 \quad (8.1)$$

When butyrate is the end-product, a theoretical maximum of 2 moles hydrogen per mole glucose is produced:

$$C_6H_{12}O_6+2H_2O \longrightarrow 2H_2+CH_3CH_2CH_2COOH+2CO_2 \quad (8.2)$$

The major VFAs detected in this study were acetate (HAc), butyrate (HBu) and propionate (HPr). The HAc/HBu ratio has been examined in this study. As presented in Table 8.4, the HAc/HBu ratio in the SBHR was higher than in the conventional CSTR in Phases 1 and 2. During Phase 1, HAc/HBu ratios of 0.63 and 1.13 were observed for the conventional CSTR and the SBHR, respectively, increasing to 0.75 and 1.20 in Phase 2 in both systems, respectively. The relationship between hydrogen yield and the corresponding values of HAc/HBu ratio for the two systems (data not shown) during the two phases clearly emphasizes that the hydrogen yield increased linearly with the increase in HAc/HBu ratio consistent with the literature studies [34]. As shown in Table 8.5, the VFAs in the CSTR were higher that in the SBHR in both phases. The VFAs accounted for 92% of the effluent soluble COD for both CSTR and SBHR in Phase 1, as compared to 71% and 67% in the CSTR and SBHR in phase 2, respectively. Using the stoichiometric yields of 4 and 2 mol H_2/mol glucose from Eq. 1 and 2, and according to the measured average concentrations of acetate and butyrate, the contribution of the two pathways was estimated. For the CSTR, the steady-state acetate concentrations ranged from 8154 mg/L to

10221 mg/L while the butyrate varied from 17308 mg/L to 20163 mg/L, with acetate and butyrate pathways contributing 41% and 59% of the hydrogen produced in Phase 1, and 43% and 57% in Phase 2, respectively. In the SBHR, the steady-state acetate concentrations ranged from 9317 mg/L to 12426 mg/L while the butyrate varied from 12360 mg/L to 15101 mg/L, with acetate and butyrate pathways contributing 53%, 47% of the hydrogen production in Phase 1 and 55%, 45% in Phase 2, respectively.

8.3.3 Biomass yield

The initial biomass concentration in the two reactors was 9 g VSS/L and it decreased sharply during the start up period (first 10 days). After the start up period, the biomass concentration in both the conventional CSTR and SBHR stabilized at average concentrations of 1.2 and 1.0 g VSS/L, respectively during Phase 1. In Phase 2, as shown in Table 8.4, the biomass concentration in the two systems did not change significantly from Phase 1 (1.1 and 0.9 g VSS/L for the conventional CSTR and SBHR, respectively). The biomass yield (as g VSS/g SCOD) was calculated based on the slope of the cumulative biomass produced versus the cumulative SCOD consumed (Figure 8.4). As depicted in Figure 8.4, for the conventional CSTR, the biomass yield increased from 0.30 to 0.34 g VSS/g SCOD when the OLR increased from 21.4 g COD/ L.d to 32.1 g COD/ L.d. Moreover, the biomass yield of the SBHR remained constant at about 0.23 g VSS/g SCOD throughout the two phases. The biomass-specific hydrogen production rates were 2.2 and 2.5 L/g VSS.d in the CSTR in Phases 1 and 2, respectively, while in the SBHR, the specific hydrogen production rates were 4.7 and 6.2 L/g VSS.d in Phases 1 and 2, respectively.

Table 8.5 *Summary of products and COD mass balance.*

Measured Parameter	Units	Phase 1		Phase 2	
		CSTR	SBHR	CSTR	SBHR
VSS_{out}	(mg COD/d)[a]	6739 ± 389	5775 ± 460	6248 ± 362	5335 ± 236
$SCOD_{out}$	(mg COD/d)	28791 ± 1154	25420 ± 1097	49063 ± 1149	48520 ± 2100
$Glucose_{out}$	(mg COD/d)[b]	3833 ± 467	2833 ± 392	14490 ± 2572	10251 ± 1883
Acetic acid	(mg COD/d)	8154 ± 1234	9317 ± 748	10221 ± 823	12426 ± 1798
Propionic	(mg COD/L)	811 ± 46	898 ± 105	3111 ± 193	2956 ± 152
Isobutyric	(mg COD/d)	42 ± 12	106 ± 19	337 ± 39	397 ± 34
Butyric	(mg COD/d)	17308 ± 929	12360 ± 1140	20163 ± 1725	15101 ± 2097
Isovaleric	(mg COD/d)	17 ± 6	355 ± 76	496 ± 51	559 ± 48
Valeric	(mg COD/d)	104 ± 18	242 ± 37	556 ± 82	824 ± 71
VFAs	(mg COD/d)	26436 ± 1771	23279 ± 1664	34885 ± 1926	32263 ± 3158
Ethanol	(mg COD/d)	259 ± 33	339 ± 56	2297 ± 313	2920 ± 86
Hydrogen gas	(L/d)	5.2 ± 0.5	9.6 ± 0.6	5.6 ± 0.6	11.2 ± 1
Hydrogen gas	(mg COD/d)[c]	3744 ± 360	6912 ± 432	4032 ± 432	8064 ± 720
COD balance	(%)[d]	92 ± 3	89 ± 4	92 ± 5	96 ± 7

[a] Based on 1.42 gCOD/g VSS
[b] Based on 1.07 gCOD/g Glucose
[c] Based on 8 gCOD/g H_2
[d] COD balance (%) = (VSS out (gCOD/d) + H_2 (gCOD/d) + SCOD out (gCOD/d))/(TCOD in (gCOD/d)).

* Values represent averages ± standard deviations based on 12 steady-state samples.

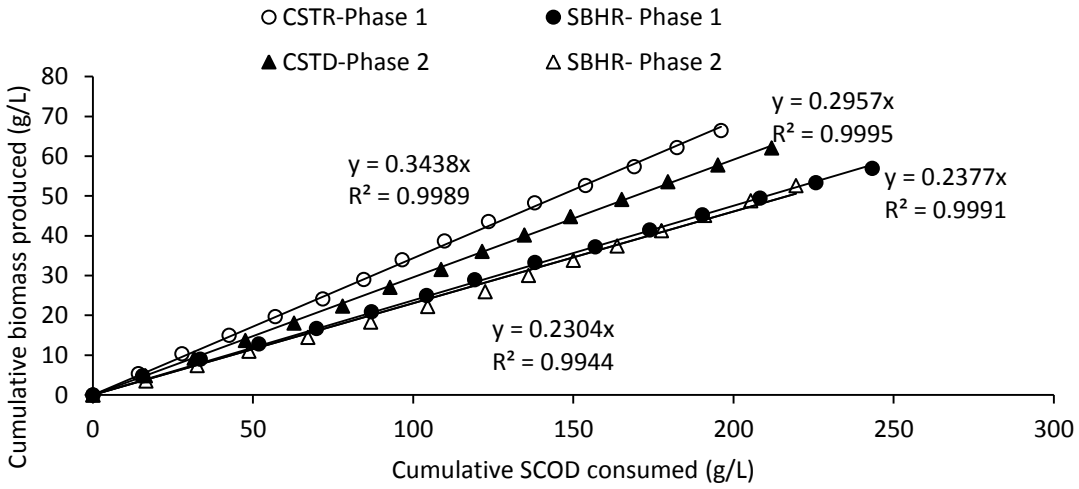

Figure 8.4 Biomass yield estimation for the two systems in the two phases.

The COD mass balances for the two systems in the two phases, computed considering the measured influent and effluent CODs, and the equivalent CODs for both gas and biomass are shown in Table 8.5. The closure of COD balances at 89%–96% confirms the data reliability.

8.3.4 Microbial community analysis

The microbial community structure was evaluated by extraction of total DNA from samples taken from the CSTR and SBHR, followed by PCR-DGGE. The DGGE profiles of the 16S rDNA gene fragment at each treatment condition are illustrated in Figure 8.5. Table 8.6 shows the results of the sequence affiliation. In total, 14 bands and 11 species were identified. The number of the bands detected in SBHR (9 and 10 bands in Phases 1, and 2 respectively) was more than those detected in the CSTR (7 bands in each phase), indicating that ultrasonication increases microbial diversity. By excluding the uncultured bacterium, 6 and 5 species were identified for the CSTR in Phases 1 and 2, respectively, compared to 8 and 7 species for the SBHR.

Lactococcus sp. (band 1), *Clostridium butyricum* (band 7), and *Clostridium butyricum* (band 13) were detected in both reactors in Phases 1 and 2. *Clostridium butyricum* species is one of the most frequently reported species in hydrogen-producing mixed cultures [16, 35]. *Lactococcus sp*. (band 1) observed in the two reactors in the two phases is known as lactic acid producing bacteria [36]. *Bacillus circulans* (band 4) and *Enterobacter cloacae* (band 9) were detected in both systems in Phase 1 only, while *Leuconostoc pseudomesenteroides* (band 2) was detected in Phase 2 only. *Clostridium acetobutyricum* (band 10) was detected in the CSTR in Phase 2 only. *C. acetobutyricum* ferments carbohydrates to hydrogen and carbon dioxide with acetate and butyrate as the main soluble metabolites [37]. *Enterobacter cloacae* is reported in the literature as one of the dominant populations in hydrogen producing biomass when molasses wastewater from a sugarbeet or glucose refinery was used as a substrate [38]. On the other hand, oxidation reduction potential (ORP) decreased rapidly in the presence of *Bacillus circulans*, and an anaerobic environment suitable for the growth of anaerobic and hydrogen-producing bacteria was established [39]. *Clostridium* sp. (band 6) and *Citrobacter freundii* (band 11) were detected in the SBHR and not detected in the CSTR either in Phase 1 or Phase 2. Moreover the diversity of the species appears to have a positive effect on biohydrogen production. On the other hand, it appears that ultrasonication did not affect the lactic acid producing bacteria.

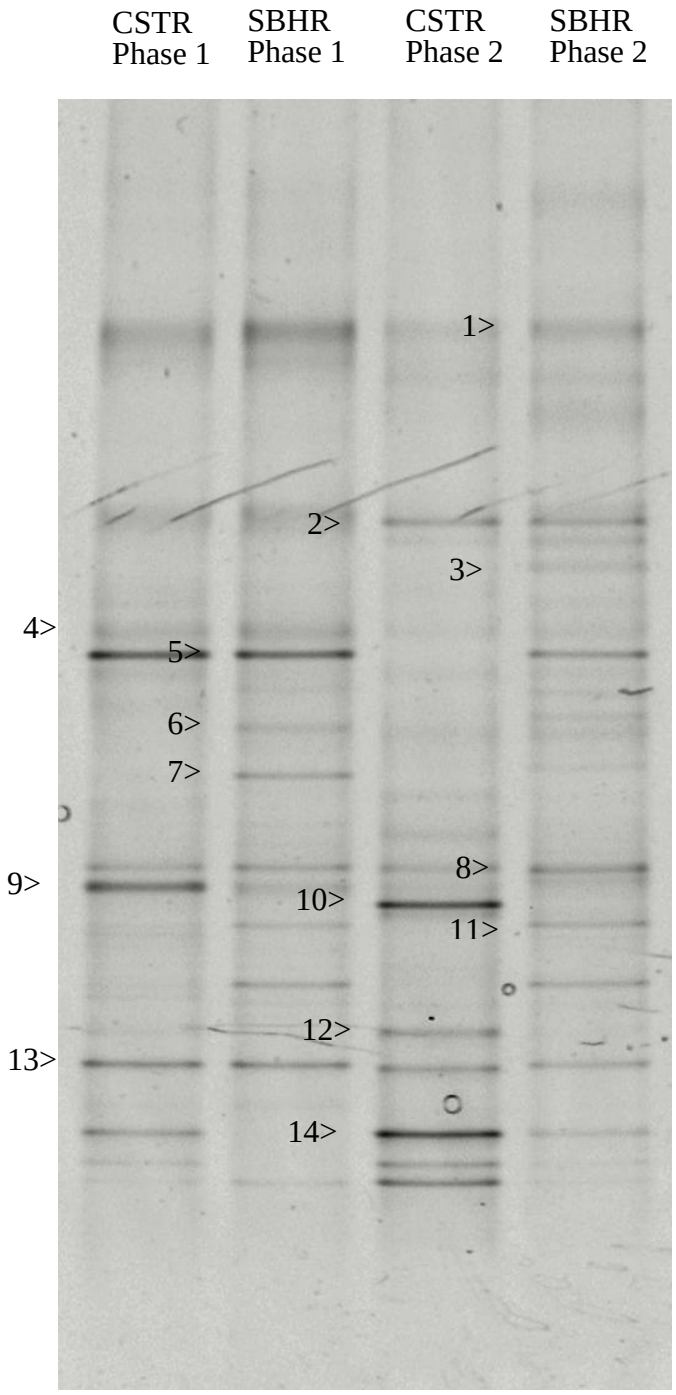

Figure 8.5 DGGE profile of the 16S rDNA gene fragment.

Table 8.6 *Affiliation of denaturing gradient gel electrophoresis (DGGE) fragments determined by their 16S rDNA sequence*

Band	Affiliation (accession no.)	Similarity (%)	Phase 1		Phase 2	
			CSTR	SBHR	CSTR	SBHR
1	*Lactococcus* sp. (EU689105.1)	99	×	×	×	×
2	*Leuconostoc pseudomesenteroides* (AB494729.1)	96			×	×
3	Uncultured bacterium (FJ982841)	95				×
4	*Bacillus circulans* (GQ478244.1)	95	×	×		
5	*Streptococcus gallolyticus* (FN597254.1)	100	×	×		×
6	*Clostridium* sp. (DQ986224.1)	99		×		×
7	Uncultured bacterium (FJ370100.1)	100		×		×
8	*Clostridium butyricum* (DQ831124.1)	98	×	×	×	×
9	*Enterobacter cloacae* (FP929040.1)	100	×	×		
10	*Clostridium acetobutyricum* (FM994940.1)	100			×	
11	*Citrobacter freundii* (AB548829.1)	100		×		×
12	Uncultured bacterium (EF515734.1)	98			×	
13	*Clostridium butyricum* (AY458857.1)	97	×	×	×	×
14	Uncultured bacterium (EF515734.1)	97	×		×	×

8.4 Discussion

Based on the outcome from this study, upon comparing the SBHR with the CSTR the percentage increases in hydrogen production rates due to the ultrasonication were 85% and 100% in Phases 1 and 2, respectively. Similarly, the percentage increases in the hydrogen yield were 75 % and 90% in Phases 1 and 2, respectively. It is interesting that for both the conventional CSTR and the SBHR, the hydrogen production rate increased with increasing OLR, while the hydrogen yield decreased with increasing the OLR from 21.4 to 32.1 g COD/ L.d. The decrease in hydrogen yield with the increase of OLR might be due to the incomplete conversion of glucose. The hydrogen content in the SBHR headspace was higher than that in the CSTR by 10% and 31% in Phases 1 and 2, respectively. As evident from the aforementioned values, the hydrogen content in the head space did exhibit a significant improvement, which is potentially attributable to the effect of the ultrasonication on removing the dissolved CO_2 and H_2 from the liquid. Although Kim et al. [18] achieved a maximum hydrogen yield of 1.68 mol H_2/mol $hexose_{consumed}$ when they used CO_2 sparging at flow rate of 60 mL/min.$L_{reactor}$, with a 118% increase compared with the control reactor at 0.77 mol H_2/mol $hexose_{consumed}$, they observed only a 25% increase in hydrogen yield using N_2 sparging at the same flow rate. In another study, Kraemer et at. [19] reported that the hydrogen yield increased from 1.0 to 2.0 mol H_2/mol glucose when they used N_2 sparging at flow rate of 12 mL/min.$L_{reactor}$. Therefore the use of ultrasonication to enhance the hydrogen production achieved higher hydrogen yields compared with the aforementioned studies. Moreover, the challenge with the gas sparging is that the sparging gas should be free of CO_2 so as not to inhibit *hydrogenase* [11]. In addition, too much sparger gas dilutes the H_2 content in the headspace and creates problems in the separation and utilization of the biogas [40].

Figure 8.6 shows the relationship between the food to microorganisms (F/M) ratio and the hydrogen yield using the results from this study and seven literature studies, three of them used gas sparging to enhance the hydrogen production from a CSTR [11,14,16] and the others for conventional CSTR [41,42,43] . As depicted in Figure 8.6, for the CSTR systems (two in this study and seven from the literature), at an F/M below 5 g COD/g VSS.d, the hydrogen yield decreased sharply with increasing the F/M ratio, while after that a smooth decline in the hydrogen yield is observed upon increasing the F/M. The hydrogen yield in the CSTR for F/M ratios higher than 20 g COD/g VSS.d seems to be constant at average value of about 0.8 mol H_2/ mol hexose, while for CSTRs with gas sparging, the hydrogen yields are higher than in the CSTR. As depicted in the Figure 8.6, it is evident that the effect of gas sparging in the enhancement of hydrogen yield is significant (about 60% increase) at F/M ratios below 26 g COD/g VSS.d, while at F/M ratios above 26 g COD/g VSS.d, the enhancement in hydrogen production is not significant at about 20%. Although the hydrogen yields of the two CSTR systems in this study (hollow triangles) match literature values as shown in the Figure 8.6, the hydrogen yields of the SBHR (solid triangle) are higher than both the CSTR alone and CSTR with gas sparging even at high F/M ratio. The data presented in Figure 8.6 emphasizes the beneficial impact of ultrasonication inside the reactor at all ranges of F/M ratios. The hydrogen yield from the SBHR is higher than that of the CSTRs with gas sparging by about 40% and 60% at OLR of 24.1 and 32.1 g COD/ L.d, respectively.

As depicted in Table 8.5, the acetic acid in the SBHR was generally higher than in the CSTR in both phases, in contrast with the butyric acid which was higher in the CSTR. The contribution of the acetate pathway to hydrogen production in the SBHR was on average 28% higher than in the CSTR. The propionic acid concentrations in both reactors were comparable in

both phases, although the propionic acid increased sharply in Phase 2 in both reactors. The same trend has been observed for ethanol concentration; it was very low in Phase 1 and increased sharply in Phase 2, which might be due to the microbial shift as emphasized by the DGGE analysis (Table 8.6). *Leuconostoc pseudomesenteroides*, which is known as a lactic acid producer [36] has been observed in Phase 2 only. This microbial shift might explain the decrease in hydrogen production rate, hydrogen yield, and glucose conversion in Phase 2 compared with Phase 1. On the other hand, as *Clostridium* is one of the most widely reported species in high hydrogen production systems and *Citrobacter freundii* is also a hydrogen producing bacteria [44], the DGGE results substantiate that the observed higher hydrogen yield in the SBHR compared with the CSTR may be due to the microbial shift as two different hydrogen producers (*Clostridium* sp. and *Citrobacter freundii*) were detected in the SBHR and not in the CSTR.

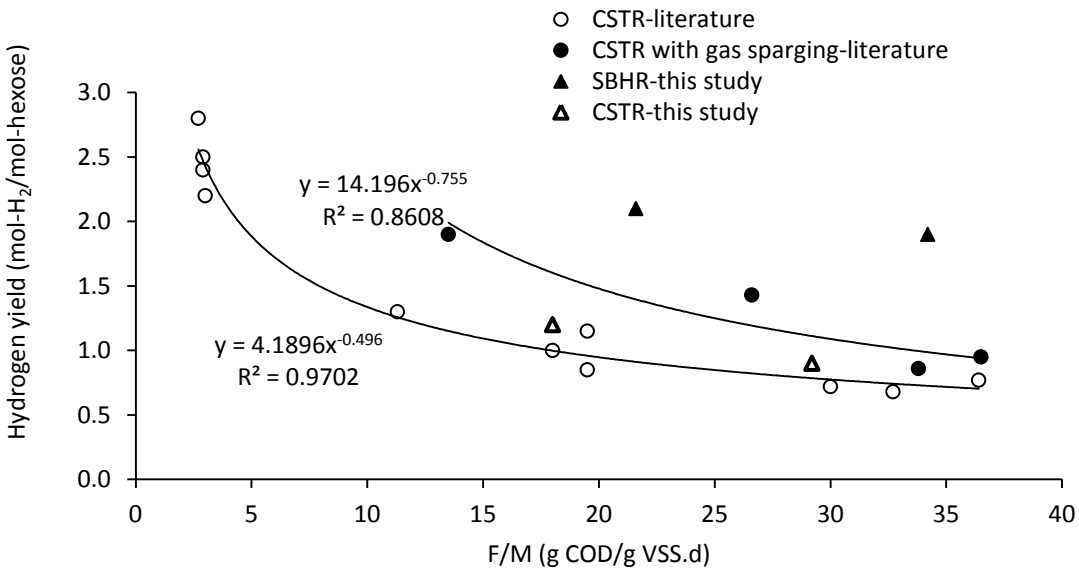

Figure 8.6 Correlation between food to microorganisms (F/M) ratio and hydrogen yield.

The biomass yield in the SBHR was lower than that of conventional CSTR by 18% and 32% in Phases 1 and 2, respectively. The inverse relationship between the biomass yield and hydrogen yields observed here is consistent with the findings of Hafez et al. [45] who observed the same trends, using data from their CSTR and literature studies.

The mechanisms for enhancement of hydrogen production due to ultrasonication inside the CSTR might be one or more of the following: (1) decreasing the dissolved hydrogen concentration, (2) enhancement of the mass transfer, (3) increasing the microorganisms' growth rate and/or (4) Solubilisation. Decreasing the dissolved H_2 concentration is known to increase the H_2 production via one of two possible scenarios: (i) increase the H_2 production, or (ii) decrease the H_2 consumption. H_2 generation is mediated by *hydrogenase* using electrons from ferreodoxin (Fd) to reduce protons. On the other hand, higher H_2 yields during N_2 sparging may be caused by decreased H_2 consumption. H_2 consumption may be via homoacetogenesis or methanogenesis and as in most cases there were no detection of methane production in the hydrogen production reactors due to the high dilution rate and the low pH. Therefore, the main mechanism responsible for the consumption of H_2 is the homoacetogenesis, which reduces dissolved CO_2 using the dissolved H_2 to produce acetate [46]. Mizuno et al. [11] and Kim et al. [16] reported that the increase in H_2 production using gas sparging is due to the decrease of dissolved H_2 concentration and hence enhancement of the activity of the relevant H_2 producing enzymes. Kraemer and Bagley [17] who observed an increase in H_2 production at a dissolved H_2 concentration of 485 μM, much greater than the threshold concentration of 0.5 μM below which H_2 production increased, attributed the increase to a decrease in the rate of dissolved H_2 consumption.

On the other hand, ultrasound is known to enhance some multiphase chemical reactions, by affecting the yield of the reaction and/or its selectivity [47]. Chisti [25] attributed part of the

beneficial effects of ultrasound in biotechnology to mass transfer improvements, not only increased mass transfer around the cells (improving the exchanges of nutrients and products), but also inside the cells [48,49]. Kumar et al. [50] investigated gas–liquid mass transfer with a 20 kHz ultrasonic horn, and concluded that low frequency (20 kHz) appeared more favourable than high frequency (500 kHz). The aforementioned researchers attributed the observed enhancement of mass transfer to a reduction in gas bubble size. Moreover, intermittent-power low frequency ultrasound of short duration can enhance a productivity of live microbial systems [25]. It was found that low-frequency ultrasound (70 kHz) of low acoustic intensity (<2 W/cm^2) increased the growth rate of cells compared to growth without ultrasound [51]. Moreover, Guo et al. [27] who reported an increase in hydrogen production when they applied ultrasonication on the substrate and/or on the seed, attributed the increase to the Solubilisation and increase of SCOD. The specific ultrasonication energy required for cell lysis is sparsely reported in the literature, and is primarily derived from the Solubilisation of cell protein data.

Elbeshbishy et al [52] reported that a minimum specific ultrasonication energy of 500 kJ/kg TS is required for initiation of cell protein solubilisation from hog manure while Wang et al [53] reported that cell protein solubilisation from waste activated sludge was maximum at a specific energy of 7700 kJ/kg TS. It should be noted that a significant variability in ultrasonication energy requirement for cell lysis is observed due to biomass nature, source, and characteristics. On the other hand, our previous work on batch systems [31] clearly indicated that ultrasonication energy of 20000 kJ/kg TS only inhibited methanogenic bacteria and did not adversely impact biohydrogen producers.

Based on the aforementioned discussion, the beneficial effects of ultrasonication in our study might be due to degassing, mass transfer, increasing the microorganisms' growth rate,

and/or solubilisation, although the impact of solubilisation may not be significant in light of using a soluble substrate. While the delineation of the mechanisms contributing to H_2 enhancement is beyond the scope of this study, further research is definitely needed in this emerging field.

8.5 Conclusions

The outcome of this study emphatically revealed the benefits of using the SBHR compared with the CSTR for biological hydrogen production. Based on the findings of this study, the following conclusions can be drawn:

- Applying ultrasonication inside the reactor has a positive effect on both hydrogen production rate and hydrogen yield. Both hydrogen production rate and hydrogen yield increased by about 93% and 83% in the SBHR compared with the CSTR, respectively.
- The glucose conversion efficiency in the SBHR was higher that in the conventional CSTR at both OLRs. The HAc/HBu ratio in the SBHR was higher than what was observed in the CSTR at both OLRs.
- The hydrogen content in the SBHR headspace was higher than that in CSTR by 10% and 31% at OLRs of 21.4 and 32.1 g COD/L.d, respectively.
- The inverse relationship between the biomass yield and hydrogen yields observed, in addition to the higher biomass yield of about 0.32 g VSS/g COD observed in the CSTR relative to the 0.23 g VSS/g COD in the SBHR substantiate the higher H_2 yield in the SBHR.
- There were two different hydrogen producers (*Clostridium* sp. and *Citrobacter freundii*) detected in the SBHR and not detected in the CSTR.

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CHAPTER 9

Ultrasonication for Biohydrogen Production from Food waste[8]

9.1 Introduction

Hydrogen gas has been deemed the fuel of the future, and it is believed that a hydrogen-based economy would be less polluted than a fossil fuel based economy [1]. Hydrogen as an energy carrier has been proven to be one of the best fuels for transportation, the most versatile, the most efficient and also one of the safest fuels [2]. Among the various biological hydrogen production methods such as biophotolysis of water, photofermentation, and dark fermentation of organic matter [3], dark fermentation is the simplest technology with the highest rate. Carbohydrate- and/or, starch-rich wastes/wastewaters as well as cellulose-rich biomass are considered the most suitable feedstock [4, 5]. Theoretically any organic substrate rich in carbohydrates, fats, and proteins is a viable substrate for biohydrogen production. However, as reported by numerous studies, carbohydrates are the main source of hydrogen during fermentative processes and therefore wastes and biomass rich in sugars and/or complex carbohydrates turn out to be most suitable feedstocks for biohydrogen generation [3]. According to a comparative study by Lay et al. [6], using substrates of different chemical composition treated with the same mixed culture, it was shown that the hydrogen-producing potential of carbohydrate- rich waste (rice and potato) was approximately 20 times higher than that of fat-rich waste (meat fat and chicken skin) and of protein-rich waste (egg and lean meat). The major criteria that have to be met for the selection of substrates suitable for fermentative bio-hydrogen production are availability, cost, carbohydrate content and biodegradability [7]. Simple sugars

[8] A version of this chapter has been published in *Int J Hydrogen Energy*, 2011

such as glucose, sucrose and lactose are readily biodegradable and thus preferred as model substrates for hydrogen production [8]. However, pure carbohydrate sources are expensive raw materials for large-scale hydrogen production [9]. High carbohydrate content in the form of simple sugars, starch, and cellulose makes organic fraction of municipal solid wastes (OFMSW) a potential feedstock for biological hydrogen production [10]. Hydraulic retention time (HRT) is also an important parameter for dark fermentation processes. In a continuous stirred tank reactor (CSTR) system, short HRTs are used to wash out the slow growing methanogens and select for the acid producing bacteria [11], while too high dilution rate, corresponding to long HRTs could lead to inefficient hydrolysis of organic wastes [12]. In a CSTR system, Kim et al. [13] reported that short HRT (< 3 days) would favour hydrogen production as methanogens which consume hydrogen require more than approx. 3 days HRT before they are washed out from a CSTR reactor.

Table 9.1 summarizes the studies which used food wastes in different bioreactor systems (continuous, semi-continuous, packed-bed reactor, anaerobic sequencing batch reactor (ASBR), and batch). As shown in the Table, the hydrogen yields ranged from 65 to 205 mL H_2/g VS_{added} in continuous and semi-continuous reactors, from 65 to 97 mL H_2/g VS_{added} in ASBR, from 57 to 250 mL H_2/g VS_{added} in batch reactors. Valdez-Vazquez et al [16] reported hydrogen yield of 360 mL H_2/g $VS_{rem.}$ The data of Table 9.1 clearly emphasizes the wide disparity of hydrogen yields among processes and between various researchers.

In our previous study [32], a significant improvement in hydrogen production rate and hydrogen yield was observed when a continuous-flow sonicated biological hydrogen reactor (SBHR) involving ultrasonication inside the reactor was used compared with CSTR using glucose as substrate at two different organic loading rates.

Based on an extensive search, there are only a limited number of studies (three studies) where the impact of ultrasonication pretreatment of the substrate on biological hydrogen production has been investigated, all of which were in batch reactors. Wang et al. [33] applied ultrasonication for 20 min to a 300 mL of waste activated sludge (WAS), and found that there was no improvement in the hydrogen production due to the sonication pretreatment, reporting a hydrogen yield of 0.7 mmol H_2/g $COD_{initial}$ for the sonication pretreatment versus 0.6 mmol H_2/g $COD_{initial}$ for the non-pretreated sludge. In another study by Guo et al [34] applied sonication for 5 min on 200 mL WAS, and reported hydrogen yield of 4.68 mL H_2/g COD. Xiao and Lui [35] applied sonication pretreatment for 30 min in 250 mL of raw sludge obtained from the aeration tank of a municipal wastewater treatment plant, and placed the sludge in a batch reactor without using additional seed. They observed a hydrogen yield of 3.83 mL/ g VS for the sonication pretreatment. There was no single study in the literature that addressed the effect of sonication pretreatment on food waste for hydrogen production in a continuous flow system. Unlike waste activated sludge which comprises predominant microorganisms, food wastes contain predominantly particulate organic substrates rich in carbohydrates, proteins, and fats. Thus, the primary objective of this study was to explore the applicability of ultrasonication to food wastes and compare the hydrogen production from three different systems employing various approaches for ultrasonication denoted henceforth as , A, B, and C. System A is a conventional continuous stirred tank reactor fed by raw food waste, system B is conventional continuous stirred tank reactor fed by sonicated food waste (the sonication was applied outside the reactor), and system C is the sonicated biological hydrogen reactor (SBHR). The study focuses not only on biohydrogen production but also on the characteristics of the process effluents

Table 9.1 *Hydrogen yield from food waste.*

Substrate	Microorganisms	Reactor	SRT	Temp.	H_2 yield (mL/g VS_{added})	Ref.
Food waste	Anaerobic digester sludge	CSTR	1.3 d	55 ^{0}C	205	14
Food waste	Anaerobic digester sludge	CSTR	5 d	55 ^{0}C	2.2[a]	15
Food waste	Anaerobic digested sludge	Semi-continuous	N.A.	35, 55 ^{0}C	360[b]	16
Food waste	Food waste	SCRD	96 h to 240 h	40 ^{0}C	65	17
Food waste	Anaerobic digester sludge	Packed-bed reactor	N.A.	35 ^{0}C	157	12
Food waste	Anaerobic digester sludge	ASBR	N.A.	35 ^{0}C	65	18
Food waste	Anaerobic digested sludge	ASBR	N.A.	37 ^{0}C	97.3	19
Food waste	Anaerobic digested sludge	ASBR	N.A.	37 ^{0}C	80.5	19
Food waste	Anaerobic digested sludge	Packed-bed reactor	N.A.	37 ^{0}C	249	19
Food waste	Sewage sludge	Batch	N.A.	36 ^{0}C	193.85	20
Food waste	Anaerobic digester sludge	Batch	N.A.	35 ^{0}C	122.9[c]	21
Food waste	*Clostridium*-rich composts	Batch	N.A.	35 ^{0}C	77	22
Food waste	Anaerobic digester sludge	Batch	N.A.	35, 55 ^{0}C	92	23
Food waste	Anaerobic digester sludge	Batch	N.A.	35, 50 ^{0}C	57	24
Food waste	POME	Batch	N.A.	35 to 60 ^{0}C	593[d]	25
Food waste	Anaerobic digester sludge	Batch	N.A.	35 ^{0}C	120	26
Food waste	POME[e]	Batch	N.A.	55.7 ^{0}C	120[d]	27
Food waste	*Clostridium beijerinckii* KCTC1875	Batch	N.A.	30 to 45 ^{0}C	128[f]	28
Food waste	WAS	Batch	N.A.	35 ^{0}C	109.2	29
Food waste	Anaerobic digested sludge	Batch	N.A.	35 ^{0}C	250	30
Food waste	Anaerobic digested sludge	Batch	N.A.	35 ^{0}C	59.2	31

[a] mol H_2/mol $hexose_{consumed}$ [b] mL/g $VS_{removed}$ [c] mL H_2/g carbohydrate-COD [d] mL H_2/g carbohydrate
[e] from Settling tank in Palm Oil Mill wastewater treatment plant [f] mL H_2/g $COD_{degraded}$ SCRD: Semi-continuous rotating drum
POME: Palm Oil Mill Effluent ASBR: Anaerobic sequencing batch reactor

9.2 Materials and methods

9.2.1 Systems setup and operation

Three continuous-flow completely-mixed reactors (10 cm diameter, 30 cm height) with a working volume of 2 L each were used in this study (Figure 9.1). One is a conventional continuous stirred tank reactor fed with unsonicated food waste (system A), the second one is a conventional continuous stirred tank reactor fed with sonicated food waste (system B), and the third one is sonicated biological hydrogen reactor (SHBR) which comprised a conventional continuous stirred tank reactor connected with a lab scale 2.5-inch diameter ultrasonic probe at the bottom of the reactor (1 cm above the bottom of the reactor) fed with unsonicated food waste (system C). The sonication pulses were set to 1 second on and 59 seconds off (in total the sonication time is 24 min/day, which is equivalent to 24 min sonication per liter feed as the HRT = 2 days and the reactor volume = 2 L). The ultrasonic probe was supplied by Sonic and Materials (model VC-500, 500 W, and 20 kHz). These three reactors were seeded with 2 L of anaerobically digested sludge and maintained at a constant temperature of 37 ^{o}C. After seeding, the three reactors were first operated in batch mode for 24 h, after which the reactor was shifted to the continuous flow mode with hydraulic retention time (HRT) of 2 days. A summary of the operational conditions is shown in Table 9.2.

9.2.2 Inocula and feed

Anaerobic sludge was collected from the primary anaerobic digester at St Mary's wastewater treatment plant (St Mary's, Ontario) and used as seed sludge. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the sludge were 11 and 9 g/L, respectively. In our pervious paper [36], we have proven that the ultrasonication pretreatment is

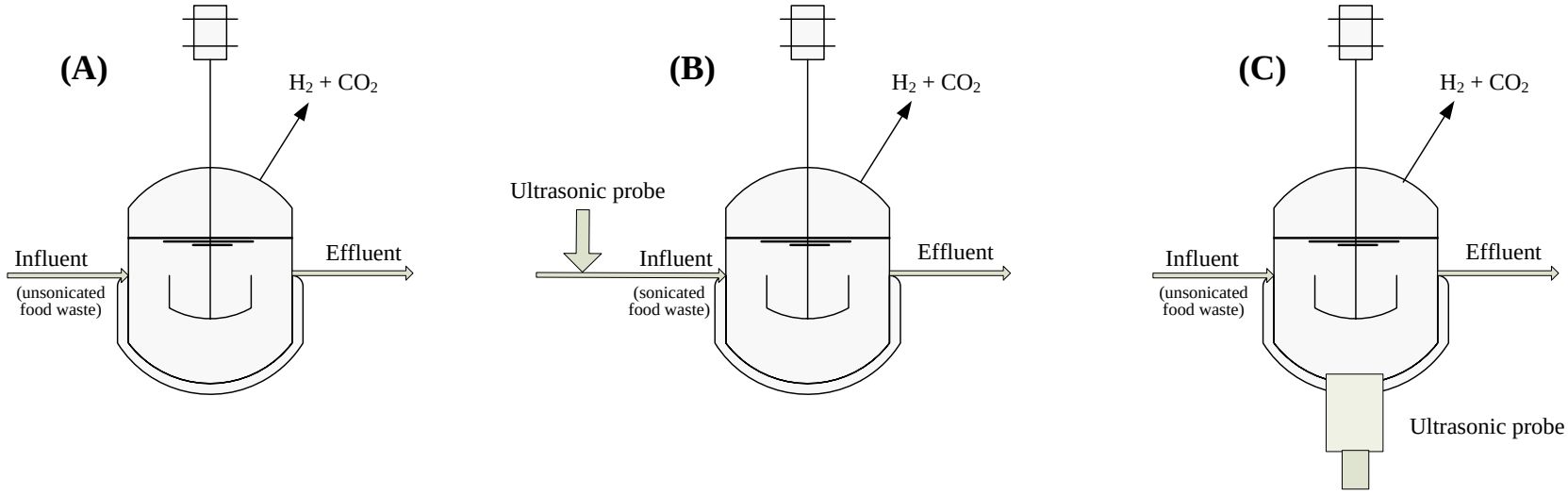

Figure 9.1 Experimental set up for the biohydrogen production systems.

Table 9.2 *Operation conditions.*

Parameter	Unit	Reactors		
		A	B	C
HRT	d	2	2	2
OLR	g COD/L.d	45.9	45.7	45.9
OLR	g VSS/L.d	14.5	13.4	14.5
pH	pH	5 to 6	5 to 6	5 to 6
Feed	Sonicated feed	No	Yes	No
Feed	Unsonicated feed	Yes	No	Yes

superior to other pretreatment methods including heat pretreatment and accordingly we adopted sonication in this study. Therefore in order to enrich hydrogen producing bacteria, the sludges were sonicated using a lab scale sonication device at specific energy of 79 kJ/g TS with temperature control at room temperature as described in Elbeshbishy et al. [36].

The food waste was obtained from Dufferin Organics Processing Facility (DOPF) in Toronto, Ontario, Canada after conversion to slurry, denoted henceforth as "pulp waste", prior feeding into an anaerobic digester. For the sonicated food waste, 1 L of food waste was sonicated for 24 min (the same sonication time per liter feed of the SBHR) using a lab scale ultrasonic probe, with sonication pulses set to 2 seconds on and 2 seconds, To control the temperature rise of the sludge, a cooling water bath was used, and the sludge temperature during the experiments did not exceed 30 ^{0}C. The specific energy input was about 5000 kJ/kgTSS. Systems A and C were fed with unsonicated food waste, while system B was fed with sonicated food waste for 45 days. Table 9.3 lists the feed characteristics used for the unsonicated and sonicated food waste. Both total and soluble (filtered) carbohydrates were measured as well as total, soluble, and bound proteins as described below.

9.2.3 Analytical methods

The gas meter consisted of a volumetric cell for gas–liquid displacement, a sensor device for liquid level detection, and an electronic control circuit for data processing and display. All other liquid parameters and gas compositions were analyzed as described in chapter 3 (section 3.2.1 Analytical methods).

9.2.4 Specific energy input:

The specific energy input (SE) is a function of ultrasonic power, ultrasonic duration, and volume of sonicated sludge and TS concentration, and can be calculated using the following equation, Bougrier et al. [37]:

$$SE = \frac{P \times t}{V \times TS} \qquad (9.1)$$

where *SE* is the specific energy input in kWs/kg TS (kJ/kg TS), P is the ultrasonic power in kW, t is the ultrasonic duration in seconds, V is the volume of sonicated sludge in litres, and TS is the total solids concentration in kg/L.

9.3 Results and discussion

9.3.1 Ultrasonication of pulp waste

Ultrasonication causes a localized pressure drop to below the evaporating pressure in the aqueous phase, resulting in the formation of micro bubbles by evaporation [38]. The micro bubbles oscillate in sound field, grow by rectified diffusion and collapse in a non-linear manner [38]. The combination of bubble oscillation and the resulting vacuum created by the collapse of the bubbles leads to strong mechanical forces that can erode solid particles [38]. The feed pulp waste was sonicated at a specific energy input of 5000 kJ/kg TS. The characteristics of the feed pulp waste before and after sonication are shown in Table 9.3. As depicted in Table 9.3, there was no significant change in TCOD and total carbohydrate after sonication. Figures 9.2 (a) and (b) show the percentage increase in the soluble parameters (SCOD, soluble carbohydrate, soluble protein, and VFAs) and the percentage decrease in the particulate parameters (TSS, VSS, particulate carbohydrate, and particulate protein) of the sonicated feed. SCOD, soluble carbohydrate, soluble protein, and VFAs increased by 9%, 17%, 20%, and 29%, respectively,

after sonication. Furthermore, TSS, VSS, particulate carbohydrate, and particulate protein decreased by 9%, 7%, 6% and 12%, respectively, after sonication.

Table 9.3 *Feed characteristics.*

Parameter	Unit	Influent	
		Unsonicated feed	Sonicated feed
TCOD	mg/L	91700 ± 4750*	91400 ± 3130
SCOD	mg/L	44200 ± 860	48200 ± 1120
TSS	mg/L	42500 ± 2670	38700 ± 2220
VSS	mg/L	28900 ± 2100	26800 ± 1910
Total Carbohydrate	mg/L	47800 ± 5830	46900 ± 3770
Soluble Carbohydrate	mg/L	8200 ± 630	9630 ± 790
Particulate Protein	mg/L	6260 ± 400	5520 ± 670
Bound Protein	mg/L	1150 ± 300	1030 ± 110
Soluble Protein	mg/L	8650 ± 330	10400 ± 420
Acetic acid	mg COD/L	550 ± 63	610 ± 80
Propionic	mg COD/L	540 ± 120	500 ± 90
Isobutyric	mg COD/L	120 ± 13	200 ± 21
Butyric	mg COD/L	370 ± 40	520 ± 120
Isovaleric	mg COD/L	220 ± 48	450 ± 68
Valeric	mg COD/L	100 ± 35	170 ± 42
VFAs	mg COD/L	1890 ± 220	2440 ± 240
Ethanol	mg COD/L	400 ± 56	440± 45

*Values represent averages ± standard deviations based on 12 steady-state samples.

9.3.2 Biogas production

Figure 9.3 illustrates the hydrogen production rates normalized per unit reactor volume for the conventional CSTR with unsonicated feed, CSTR with sonicated feed, and SBHR. As depicted in Figure 9.3, after the 10-days (5 turnovers of SRT) start-up period, stable hydrogen production rates were observed in all three reactors. The average hydrogen production rates per unit reactor volume were 2.6, 3.3, and 4.8 L/L.d for the CSTR, CSTR with sonicated feed, and SBHR, respectively.

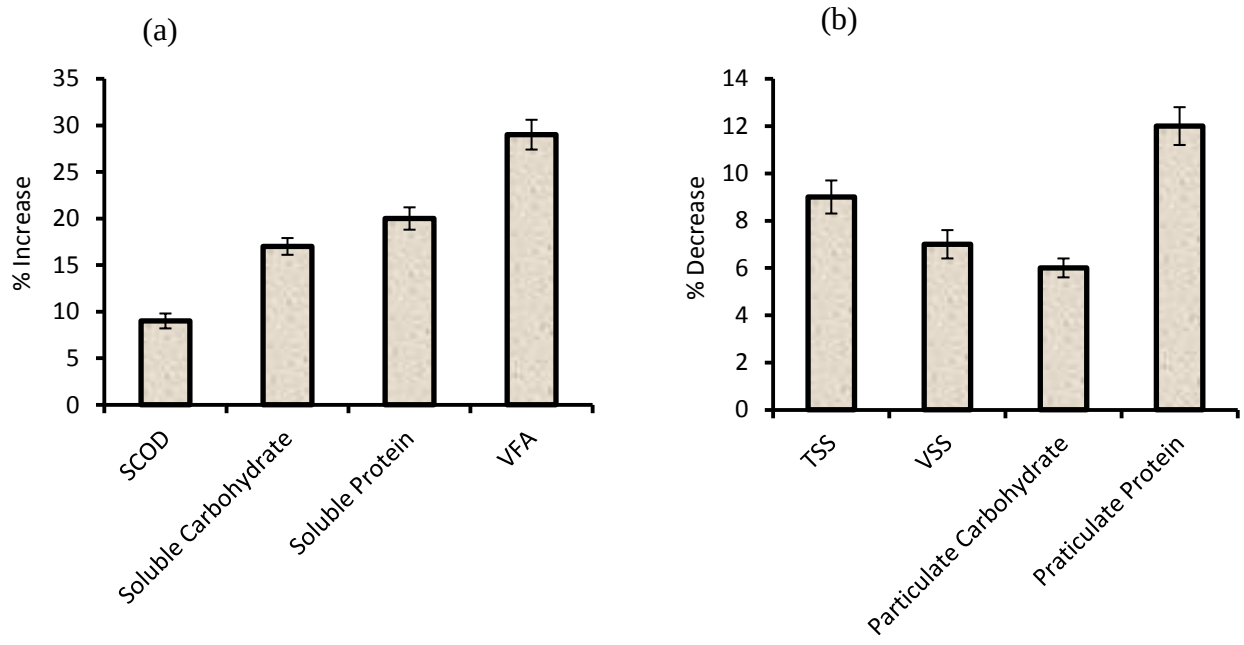

Figure 9.2 Percentage increase/decrease due to ultrasonication for (a) soluble components (b) particulate components.

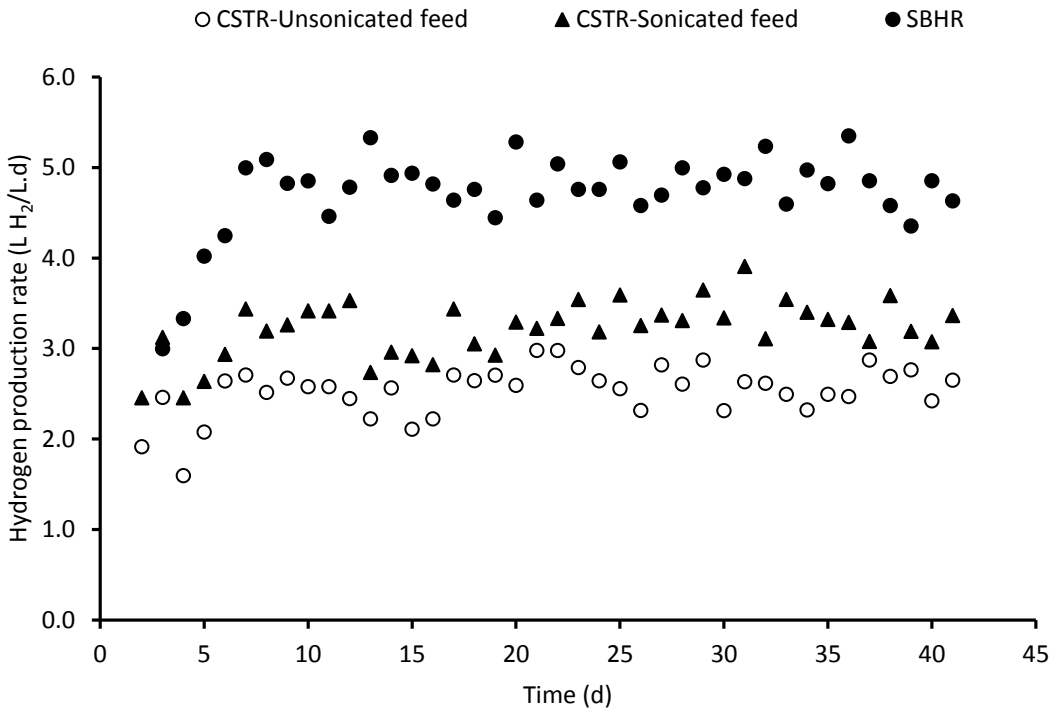

Figure 9.3 Diurnal variations in hydrogen production rate.

The hydrogen production rate per unit volume of the reactor in the SBHR was higher than those in the CSTR and CSTR with sonicated feed by 85% and 45%, respectively, while the hydrogen production rate in the CSTR with sonicated feed was greater than that in CSTR by 27%. Figure 9.4 shows the hydrogen yields as mL H_2/g VSS_{added} for the three reactors. As depicted in Figure 9.4, hydrogen yields of 180, 247, and 332 mL H_2/g VSS_{added} were observed for CSTR without sonicated feed, CSTR with sonicated feed, and SBHR, respectively. The CSTR with sonicated feed showed a 23% increase in hydrogen yield as mol H_2/mol $hexose_{consumed}$ compared to a 62% increase in the SBHR, relative to CSTR.

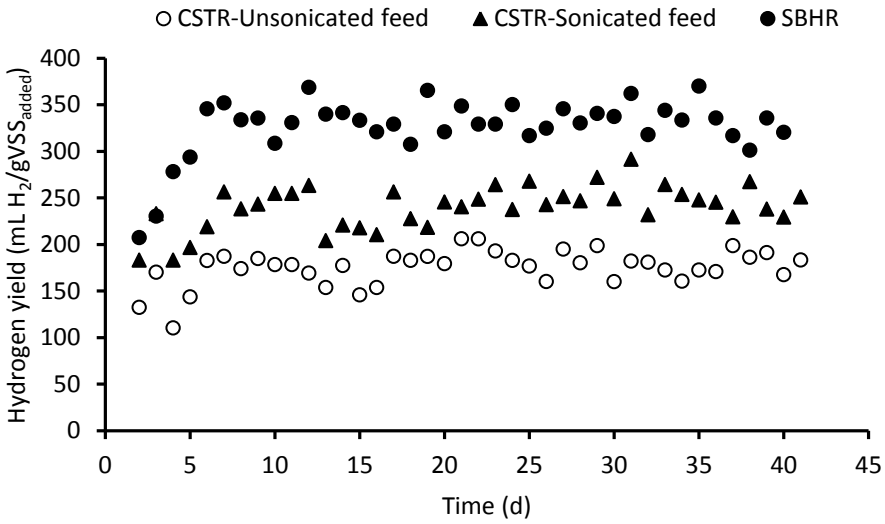

Figure 9.4 Diurnal variations in hydrogen yield.

Figure 9.5 shows the methane production rate for all three reactors. As shown in Figure 9.5, during the first 5 days, there was no detection of methane production in the headspace of the three reactors, and during the following 5 days, there was methane production in biogas in the CSTRs with and without sonicated feed, with only traces in the headspace of the SBHR. After 10 days of operation, methane disappeared completely from the headspace of the SBHR reactor, although methanogenesis persisted in the CSTRs with and without sonicated feed. The average methane production rates in the CSTRs with and without sonicated feed were 0.25 and 0.45 L/L.d, respectively. The average methane concentrations in the headspace of only 6% and 3% were observed for the CSTR and CSTR with sonicated feed, respectively, and there was no methane in the headspace of the SBHR after the first 5 days. Therefore, it is evident that applying the ultrasonication inside the reactor had the positive impact of eliminating the microbial contaminations due to the incoming feed, as reflected by the absence of methane production in the headspace of the SBHR. Furthermore, applying sonication outside the reactor

at the same specific energy did not completely eliminate the methanogenesis in the headspace, although the methanogenic activity decreased by about 45% compared to the CSTR with unsonicated feed.

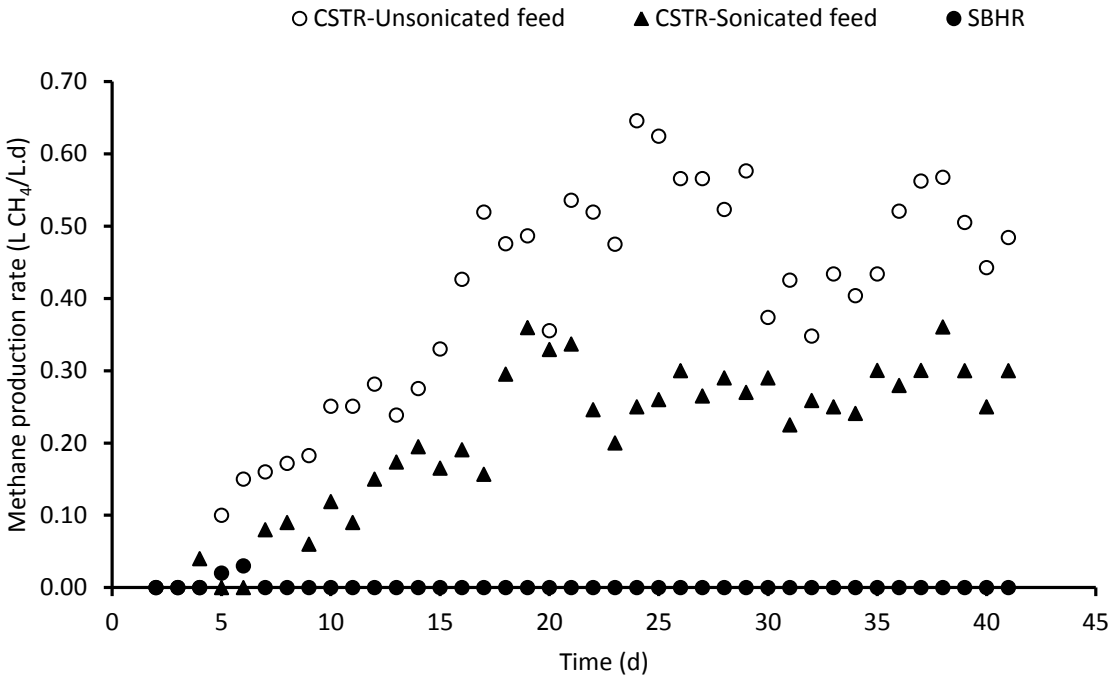

Figure 9.5 Diurnal variations in methane production rate.

Table 9.4 summarizes the steady-state data for the three reactors. Generally in biological treatment systems, steady-state data is collected after a minimum of 3 turnovers of the mean solids retention time (SRT). In addition to the aforementioned criteria, steady-state in this case also entailed less than 10% variation in biogas quantity. The stability of the three systems is evident from the very low coefficient of variation (CV), calculated as the standard deviation divided by the average of the steady-state data based on 12 samples. As shown in Table 9.4 the average hydrogen concentrations in the headspace of 39%, 38%, and 44% were observed for the

CSTR without sonicated feed, CSTR with sonicated feed, and SBHR, respectively. The hydrogen concentration in the headspace in this study of 38-44% were comparable to the 29.4% to 30.9% reported by Wang and Zhao [Table 9.1- Ref. 17] and lower than of 52 to 56% reported by Chu et al [Table 9.1- Ref. 14].

Table 9.4 *Summary of steady state data*

Parameter	Units	CSTR		SBHR
		Unsonicated feed	Sonicated feed	
H_2 conversion efficiency**	%	4.1 ± 0.32	5.2 ± 0.41	7.5 ± 0.62
H_2 content	%	39 ± 4	38 ± 5	44 ± 5
CH_4 content	%	6 ± 2	3 ± 1	0
H_2 Yield	mL/g $hexose_{consumed}$	157 ± 15	193 ± 16	258 ± 23
H_2 Yield	mol H_2/mol $hexose_{consumed}$	1.3 ± 0.12	1.6 ± 0.13	2.1 ± 0.19
Acetate/Butyrate	mol/mol	1.45 ± 0.14	1.21 ± 0.1	2.04 ± 0.16
(HAc + HBu)/VFAs	%	60 ± 4	78 ± 5	86 ± 5

* Values represent averages ± standard deviations based on 12 steady-state samples
** H_2 conversion efficiency was calculated based on the equivalent COD of the hydrogen produced per day divided by the TCOD entered the reactor per day.

The hydrogen yield of 1.3, 1.6, and 2.1 mol H_2/mol $hesxose_{consumed}$ were achieved for the CSTR, CSTR with sonicated feed, and SBHR, respectively. The hydrogen yield in the SBHR as mol H_2/mol $hesxose_{consumed}$ was higher than those of CSTR and CSTR with sonicated feed by 62% and 31%, respectively, while the hydrogen yield of CSTR with sonicated feed was higher

than that of CSTR by 23%. The hydrogen conversion efficiency of 4.1%, 5.2%, and 7.5% were observed for the CSTR, CSTR with sonicated feed, and SBHR, respectively. The H_2 conversion efficiency in the SBHR was higher than those in CSTR alone and CSTR with sonicated feed by 83% and 44%, respectively. The H_2 conversion efficiencies during this study of 4.1%-7.5% were comparable to the 4.2% to 9.7% reported by Shin and Young [Table 9.1- Ref. 15] and 5.78% which reported by Wang and Zhao [Table 9.1- Ref. 17] and lower than 9.3% reported by Chu et al [Table 9.1- Ref. 14].

9.3.3 Volatile fatty acids (VFAs)

Hydrogen yield depends on the fermentation pathway and end-products [39]. When acetic acid is the end-product, a theoretical maximum of 4 moles hydrogen per mole glucose is obtained:

$$C_6H_{12}O_6+2H_2O \longrightarrow 4H_2+2CH_3COOH+2CO_2 \quad (9.2)$$

And when butyrate is the end-product, a theoretical maximum of 2 moles hydrogen per mole glucose is produced:

$$C_6H_{12}O_6+2H_2O \longrightarrow 2H_2+CH_3CH_2CH_2COOH+2CO_2 \quad (9.3)$$

As depicted in Table 9.5, steady-state VFAs concentrations of 15250, 16420, and 18090 mg COD/L were observed for the CSTR, CSTR with sonicated feed, and SBHR, respectively, which correspond to 36%, 37%, and 39% of the SCOD in the effluent, respectively. The main VFAs in the CSTR with sonicated feed and SBHR were acetic acid and butyric acid constituting 78%, 86% of the residual VFAs on a COD basis, respectively, as compared to only 60% in CSTR with unsonicated feed. The abovementioned fractions of acetic and butyric acids observed for the CSTR with sonicated feed and SBHR in this study (78% and 86%) were slightly higher

than those reported in the literature; Wang and Zhao [Table 9.1- Ref. 17] reported 71% of the VFAs in the effluent were acetate, butyrate, and ethanol, while Chu et al [Table 9.1- Ref. 14] reported that 71% of the residual VFAs were acetic and butyric acids.

The acetic acid in the SBHR was significantly higher than those in the CSTR and CSTR with sonicated feed, almost double that in the CSTR with unsonicated feed, and about one and half times in the CSTR with sonicated feed. On the other hand, the butyric acid in the CSTR was lower than those in the CSTR with sonicated feed and SBHR by 54% and 42%, respectively, while there was no significant difference of the butyric acid (the difference is about 8%) in the CSTR with sonicated feed and the SBHR. The propionic acid concentration in the CSTR was 3688 mg COD/L compared to 1318 mg COD/L in the CSTR with sonicated and 1480 mg COD/L in SBHR. The valeric acid in the SBHR was the smallest concentration (180 mg COD/L) compared with the CSTR (1290 mg COD/L) and CSTR with sonicated feed (924 mg COD/L). The ethanol concentrations in all three reactors were less than 5% of the SCOD in the effluent. The available hydrogen production from glucose is determined by the butyrate/acetate ratio (HAc/HBu) [34]. As depicted in Table 9.4, HAc/HBu in the SBHR was the highest one at 2.04 compared to 1.45 and 1.21for the CSTR, CSTR with sonicated feed, respectively.

9.3.4 Solids destruction

Figure 9.6 illustrates the average steady-state percentage reductions of the liquid parameters in all three reactors. The percentage reduction in the CSTR with sonicated feed reported here represents the percentage reduction in the digester. As depicted in Figure 9.6, average TSS removal efficiencies of 13%, 11%, and 19% were observed for CSTR, CSTR with

sonicated feed, and SBHR, respectively. VSS removal efficiencies of 16%, 15%, and 24% were achieved in the CSTR, CSTR with sonicated feed, and SBHR, respectively.

VSS removal efficiencies reported in this study are comparable to the 16.9% to 25.6% reported by Wang and Zhao [Table 9.1- Ref. 17]. VSS destruction in the SBHR was at 50% and 60% higher than those in the CSTR and CSTR with sonicated feed, respectively. Moreover, the removal efficiencies of total carbohydrate were 38%, 46%, and 56% in CSTR, CSTR with sonicated feed, and SBHR, respectively. Soluble carbohydrates, removal efficiencies of 67%, 59%, and 64%, were observed for SBHR, CSTR, and CSTR with sonicated feed, respectively. On the other hand, the removal efficiencies of particulate protein were 21%, 23%, and 35% in CSTR, CSTR with sonicated feed, and SBHR, respectively. Although the removal efficiency of particulate protein in the SBHR was higher than those in the CSTR and CSTR with sonicated feed, the removal efficiency of soluble protein in the SBHR was lower than those in the CSTR and CSTR with sonicated feed by 36% and 23%, respectively, and that might be due to the Solubilisation of protein.

The COD mass balances for the three systems, computed considering the measured influent and effluent CODs, and the equivalent CODs for both gas and biomass are shown in Table 9.5. The closure of COD balances at 90% - 93% confirms the data reliability.

Table 9.5 *Summary of products and COD mass balance.*

Effluent parameter	Units	Reactors		
		A	B	C
SCOD	mg COD/L	42400 ± 3260	44740 ± 3860	46100 ± 3760
VSS	(mg COD/L)[a]	34400 ± 2300	32300 ± 2650	31300 ± 2640
Acetic acid	mg COD/L	4530 ± 520	5800 ± 460	9000 ± 640
Propionic acid	mg COD/L	3690 ± 280	1320 ± 130	1480 ± 110
Isobutyric acid	mg COD/L	560 ± 80	530 ± 64	480 ± 52
Butyric acid	mg COD/L	4580 ± 320	7000 ± 420	6500 ± 560
Isovaleric acid	mg COD/L	600 ± 70	810 ± 72	430 ± 36
Valeric acid	mg COD/L	1290 ± 65	900 ± 76	180 ± 46
VFAs	mg COD/L	15300 ± 860	16400 ± 1430	18100 ± 1100
ethanol	mg COD/L	1870 ± 120	1070 ± 90	1430 ± 120
Soluble carbohydrates	mg /L	7970 ± 670	8250 ± 590	6430 ± 420
Soluble proteins	mg /L	6050 ± 630	7550 ± 540	6750 ± 650
Hydrogen gas	L/d	5.2 ± 0.44	6.6 ± 0.5	9.6 ± 0.6
Hydrogen gas	(mg COD/d)[b]	3740 ± 300	4750 ± 360	6910 ± 430
Methane gas	L/d	0.9 ± 0.06	0.5 ± 0.03	0
Methane gas	(mg COD/d)[c]	2270 ± 150	1260 ± 80	0
COD balance	(%)[d]	92 ± 3	91 ± 4	93 ± 4

[a] Based on 1.42 gCOD/g VSS

[b] Based on 8 gCOD/g H_2

[c] Based on 4 gCOD/g CH_4

[d] COD balance (%) = (VSS out (gCOD/d) + H_2 (gCOD/d) + CH_4 (gCOD/d) + SCOD out (gCOD/d))/(TCOD in (gCOD/d)).

* Values represent averages ± standard deviations based on 12 steady-state samples.

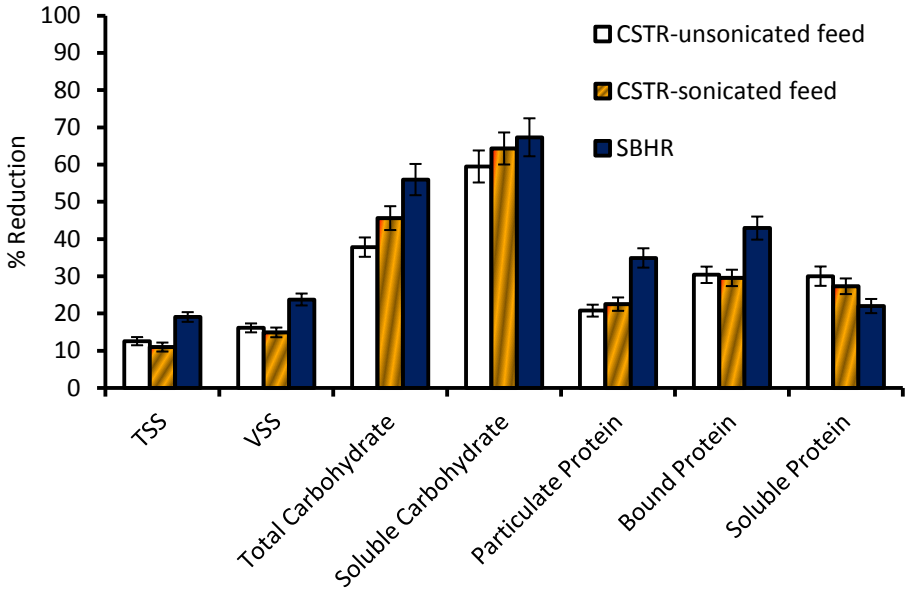

Figure 9.6 Percentage reductions in liquid components.

Based on the aforementioned discussion and also the enhancement of the Solubilisation of the feed (CSTR with sonicated feed) due to sonication which was reflected by an increase in the soluble parameters especially SCOD and soluble carbohydrate, it is evident that applying sonication inside the reactor (SBHR) showed superior results to sonication of the feed outside the reactor at the same specific energy of 5000 kJ/kg TS. The significant difference (46%) in hydrogen production and in hydrogen yield (31%) between the SBHR and the CSTR with sonicated feed emphasizes the numerous advantages of ultrasonication inside the reactor i.e. Solubilisation the particulate organics, removed of the dissolved gaseous, improvement of the mass transfer, and increase of the microbial growth rate [36].

9.4 Conclusions

The outcome of this study emphatically revealed the superior effect of applying sonication inside the reactor on biological hydrogen production and solids destruction. It is evident that the methanogenic activity decreased when sonication was applied outside the reactor and ceased completely with sonication inside the reactor. The observed hydrogen yield of 332 mL H_2 /g VSS_{added} in the SBHR is at the higher end of the range reported in the literature if not the highest. VFAs in the CSTR with sonicated feed and SBHR were mainly acetic acid and butyric acid constituting 78%, 86% of the residual VFAs on a COD basis, respectively, as compared to only 60% in CSTR with unsonicated feed. Moreover, after 10 days of start-up, methane disappeared completely from the headspace of the SBHR reactor, while there was still methanogenesis in the CSTR and CSTR with sonicated feed.

9.5 References

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CHAPTER 10

Comparative Study of the Effect of Ultrasonication on the Anaerobic Biodegradability of Food Waste in Single and Two-Stage Systems[9]

10.1 Introduction

Food waste is the third-largest component of municipal solid waste generated in the United States. According to a report by U.S. Environmental Protection Agency [1], approximately 32 million tons of food wastes are generated annually. Due to increasing demand for renewable energy and diversion of organic residuals from landfills to reduce greenhouse gas emissions among other environmental impacts, treatment of food waste using anaerobic digestion technologies has become a more attractive method for food waste management [2]. Anaerobic digestion processes have been widely applied to various complex feedstocks including municipal wastewater sludges, chemical and agricultural industry wastewaters [3, 4, 5], but its use to process source separate organics (SSO) from the municipal solid waste (MSW) stream is relatively new, especially in North America [6]. However, in general, the limiting step of anaerobic digestion of solid waste is the first step of hydrolysis or Solubilisation, where the cell wall is broken down allowing the organic matter inside the cell to be available for biological degradation [7]. Therefore, many studies have been conducted to enhance the hydrolysis either using two-stage process [8] or feed pretreatment [9].

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In conventional anaerobic digestion, the acid-forming and methane-forming microorganisms are kept together in a single reactor and there is a delicate balance between these two groups of organisms, because both groups differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions [10]. Pohland and Ghosh [10] firstly proposed the physical separation of acid-formers and methane-formers in two separate reactors, where optimum environmental conditions for each group of organisms would be provided to enhance the overall process stability and control. This kind of two-stage process has been reported to achieve enhanced stability and higher loading capacities for the methanogenesis process compared with the traditional one stage process [10]. Furthermore, two-stage process achieved greater process efficiencies overall [11]. The two-stage anaerobic digestion process for sequential hydrogen and methane production has been operated with various types of organic substrates such as glucose [12], sucrose [13], food waste [14], olive pulp [15] and cheese whey [16].

On the other hand, the use of ultrasonication in the pretreatment of sludge not only enhanced the hydrolysis step but also improved the operational reliability of anaerobic digesters, decreased odor generation, and enhanced sludge dewatering [17]. Ultrasonication causes a localised pressure drop to below the evaporating pressure in the aqueous phase, resulting in the formation of micro bubbles or cavitation bubbles [18]. During cavitation, micro bubbles form at various nucleation sites in the fluid and grow during the rarefaction phase of the sound wave [19]. Subsequently, in the compression phase, the bubbles implode and the collapsing bubbles release a violent shock wave that propagates through the medium [18]. Ultrasonication pretreatment studies found in the literature have focused mainly on Solubilisation of waste activated sludge (WAS) and enhancement of the methane production [20]. There are only a

limited number of studies where the impact of ultrasonication pretreatment of WAS on biological hydrogen production has been investigated, all of which were in batch reactors [21]. Based on an extensive search, there was no single study in the literature that addressed the effect of sonication pretreatment on food waste for hydrogen production in a continuous flow system.

In our previous study [21], a significant improvement in hydrogen production rate and hydrogen yield was observed when a continuous-flow sonicated biological hydrogen reactor (SBHR) involving ultrasonication inside the reactor was used compared with conventional continuously-stirred tank reactor (CSTR) using glucose as a substrate at two different organic loading rates. The novelty of this work lies primarily in the application of ultrasonication to food wastes including in-reactor sonication that is discussed here. The paper also advances our understanding of comparative Solubilisation of food waste using acidification and ultrasonication. Despite the sparse handful of papers that explored single and two-stage anaerobic food waste treatment, this work offers a comprehensive comparison of single and two-stage process utilizing ultrasonication. Therefore, the primary objective of this study was to evaluate the impact of ultrasonication on solubilisation, biogas (hydrogen and methane) production, and anaerobic biodegradability of food waste in single stage and two-stage anaerobic digestion using five different systems A, B, C, D and E (Figure 10.1).

10.2 Materials and methods

10.2.1 Systems setup and operation

Five systems A, B, C, D, and E were used in this study; systems A and B are one stage mesophilic for methane production, while systems B, C, and E are two-stage mesophilic systems for hydrogen and methane production. System A is a conventional one stage CSTR fed with

unsonicated food waste for methane production. System B is a conventional one stage CSTR fed with sonicated food waste for methane production. System C is a conventional two-stage process

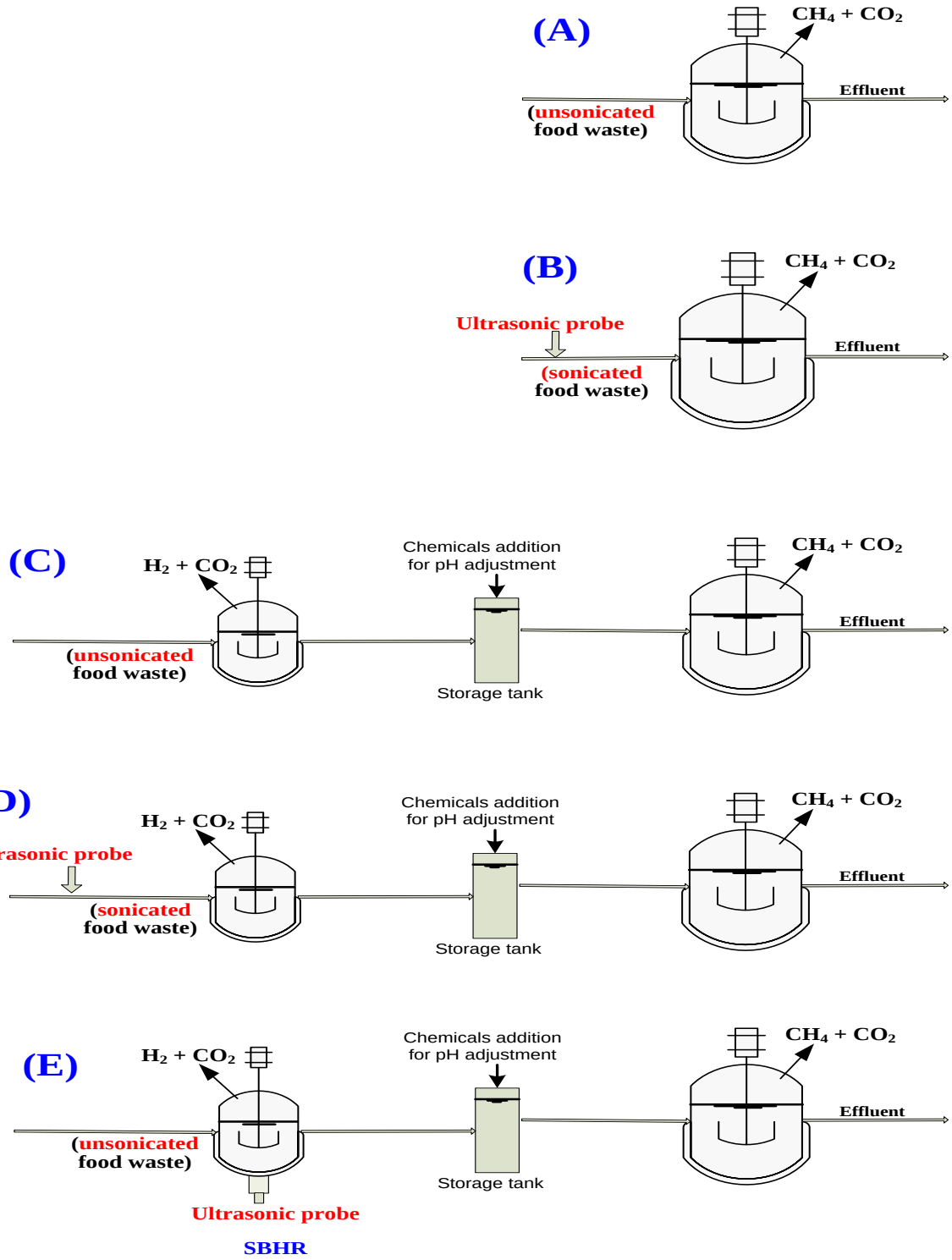

Figure 10.1 Experimental set up for the five systems.

fed by unsonicated food waste, with the first stage for hydrogen production and the second stage for methane production. System D is a conventional two-stage process fed by sonicated food waste. System E comprises the SBHR as a first stage for hydrogen production followed by CSTR for methane production as second stage. The five systems (A, B, C, D and E) used in this study were operated in completely-mixed continuous-flow mode at solids retention times (SRTs) of 2 days and 7 days for the first and second stage, respectively. All the digesters were maintained at a constant temperature of 37 ^{o}C. A summary of the operational conditions is shown in Table 10.1.

10.2.2 Inocula and feed

Anaerobic sludge was collected from the primary anaerobic digester at St Mary's wastewater treatment plant (St Mary's, Ontario) and used as seed sludge for all reactors used in this study. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the sludge were 11 and 9 g/L, respectively. In order to enrich hydrogen producing bacteria before seeding the first stage hydrogen reactors in systems C, D, and E, the sludges were sonicated using a lab scale sonication device at a specific energy of 79 kJ/g TS with temperature control at room temperature as described in [22]. In our pervious paper [22], we have proven that the ultrasonication pretreatment is superior to other pretreatment methods including heat pretreatment and accordingly we adopted sonication in this study. Moreover, before starting the hydrogen reactors, the anaerobically digested sludge was acclimatized for two weeks using glucose as substrate at OLR of 21.4 g COD/L.d. In our previous study [21] using the same

conditions, the microbial community analysis by denaturing gradient gel electrophoresis (DGGE) showed different hydrogen-producing microorganisms such as *Clostridium sp.* and *Citrobacter freundii*.

The food waste was obtained from Dufferin Organics Processing Facility (DOPF) in Toronto, Ontario, Canada. The city of Toronto's DOPF receives approximately 25,000 metric tons/year of source separated organics (SSO) material from Toronto's residual Green Bin and the commercial Yellow Bag collection programs. The purpose of the DOPF is to separate the film plastic bin finer and contaminant materials fractions of the SSO from the organic material and convert the organic fraction into a material that is a suitable feedstock for the anaerobic digester [23]. For the sonicated food waste (systems B and D), 1 L of food waste was sonicated for 24 min (the same sonication time per liter feed of the SBHR in system E) using a lab scale ultrasonic probe, with sonication pulses set to 2 seconds on and 2 seconds, To control the temperature rise of the sludge, a cooling water bath was used, and the sludge temperature during the experiments did not exceed 30 ^{0}C. The specific energy input was about 5000 kJ/kg TS. This sonication condition is the optimal condition for Solubilisation of food waste based on preliminary work which revealed that the optimum sonication condition within the range of specific energies 500 to 20000 kJ/kg TS was 5000 kJ/kg TS. Table 9.3 in chapter 9 lists the characteristics for the unsonicated and sonicated food waste used in this experiment.

Table 10.1 *Operation conditions*

Parameter	Unit	System A		System B		System C		System D		System E	
		First stage	Second stage	First stage	Second stage	First stage	Second stage	First stage	Second stage	First stage	Second stage
HRT	d	NA	7	NA	7	2	7	2	7	2	7
OLR	g COD/L.d	NA	12.8	NA	12.8	45.9	12	45.7	12	45.9	11.7
pH	pH	NA	6.9 - 7.2	NA	6.9 - 7.2	5 - 6	6.9 - 7.2	5 - 6	6.9 - 7.2	5 - 6	6.9 - 7.2
Feed	Sonicated/ unsonicated	NA	unsonicated	NA	sonicated	unsonicated		Sonicated before first stage		Sonicated inside the first stage (SBHR)	

10.2.3 Analytical methods

Biogas production was collected by wet tip gas meters (Gas meters for Laboratories, Nashville, TN). The gas meter consisted of a volumetric cell for gas–liquid displacement, a sensor device for liquid level detection, and an electronic control circuit for data processing and display. All other liquid parameters and gas compositions were analyzed as described in chapter 3 (section 3.2.1 Analytical methods).

10.3 Results and discussion

10.3.1 Hydrogen reactors

The Solubilisation of food waste by ultrasonication and the first-stage reactors' performance (hydrogen production as well as solids destructions) were discussed in chapter 9.

10.3.3 Methane reactors

10.3.3.1 Methane and overall energy production

Figure 10.2 illustrates the steady-state data for methane production rates normalized per unit reactor volume for the second stage of the five systems. However, the highest methane production rate of 3.2 L $CH_4/L_{reactor}$.d was achieved in system E, while the lowest methane production rate of 1.6 L $CH_4/L_{reactor}$.d was observed for system A. Average methane production rates of 2.1, 2.3, and 2.6 L $CH_4/L_{reactor}$.d were observed for systems B, C, and D, respectively. Systems D and E achieved the same methane contents of 66% in the headspace, while methane content of 56%, 59%, 62% were observed in the headspace of systems A, B, and C, respectively. Based on an energy content of 286 kJ/mol for hydrogen and 891 kJ/mol for methane, overall steady-state energy production rates of 288, 365, 462, 531 and 670 kJ/d were observed for

systems A to E, respectively. Moreover, based on the TSS of the feed (42.5 g/L), feed flow (0.7 L/d), and SE input of 5000 kJ/kg TSS, the energy applied to the feed was 91 kJ/d. Therefore it is obvious that sonicating the feed was uneconomical either prior to single stage (91 kJ input versus 77 kJ gain as determined from comparison of systems A and B) or prior to two-stage (91 kJ input versus 69 kJ gain as derived from comparative assessment of systems C and D). On the other hand, the only economical scenario was using the SBHR in the first stage followed by methane reactor (system E) compared to two-stage with unsonicated feed, system C, (91 kJ input versus 139 kJ gain).

In order to assess the influence of feed sonication on the mesophilic completely-mixed digesters studied in this work; a comprehensive performance assessment of systems A and B is warranted. It is apparent that feed sonication affected a 27% increase in volumetric methane production to 2.1 L $CH_4/L_{reactor}$.d (Figure 10.2). Moreover, it is evident that the overall performance of system D was superior to system C as reflected by 13% increase in volumetric methane production and 15% increase in overall energy production. Additionally, the total influent VFAs and acetic acid concentrations to the mesophilic methane reactor of systems C and D increased marginally from 15300 to 16400 mg COD/L and from 4530 to 5800 mg COD/L, respectively.

Upon comparing the performance of system A (single stage) and system C (two-stage), both receiving unsonicated feed, the superiority of the two-stage is evidenced by 39% increase in volumetric methane production and 60% increase in overall energy production. As expected, acidification significantly increased the concentration of VFAs in the feed of the anaerobic digester by almost an order of magnitude from 1890 mg COD/L in system A to 15300 mg COD/L in system C, more specifically the concentration of acetic acid increased from 550 to

4530 mg COD/L for systems A and C, respectively. On the other hand, a comparison of systems B and D indicates that volumetric methane production in system D (two-stage with sonicated feed) was approximately 24% higher than system B (single stage with sonicated feed). Overall energy production increased from 365 kJ/d in system B to 531 kJ/d in system D, translating to an additional 45% energy over the single stage. Furthermore, the performance of the patent-pending SBHR utilizing sonication inside the reactor was superior to the other systems as the methane production in system E was 94%, 53%, 39%, and 23% higher than systems A , B, C, and D, respectively, with the corresponding differential enhancement of overall energy production of 133%, 83%, 45%, and 26%, respectively.

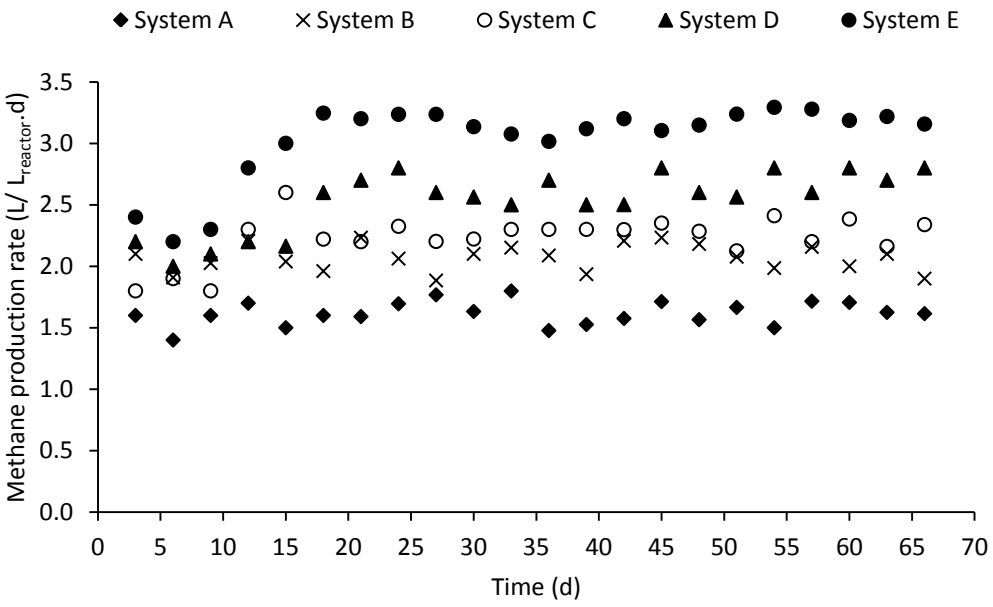

Figure 10.2 Diurnal variations in methane production rate in the methane reactors.

The typical control strategy in methanogenic anaerobic reactors is to maintain a relatively low concentration of volatile fatty acids (VFA) and a pH range of 6.6 < pH < 7.4. Normally in such reactors the carbonate system forms the main weak-acid system responsible for maintaining

the pH around neutrality, while the VFA systems (acetic, propionic, and butyric acids) are the major cause for pH decline [24]. Under stable operating conditions, the H_2 and acetic acid formed by acidogenic and acetogenic bacterial activity are utilized immediately by the methanogens and converted to methane [24]. The VFA/alkalinity ratio can be used as a measure of process stability. When this ratio is less than 0.3 to 0.4 the process is considered to be operating favourably without acidification risk [25]. As summarized in Table 10.2, the averages VFA to alkalinity ratios in the second stage methanogenic digesters were lower than the suggested limits value in all methane reactors (0.25 to 0.36) which confirms the digesters stability. Based on the aforementioned discussion and also the enhancement of the Solubilisation of the feed (systems B and D) due to sonication, as reflected by an increase in the soluble parameters especially SCOD and soluble carbohydrate, it is evident that applying sonication inside the reactor (system E) showed superior results to sonication of the feed outside the reactor at the same specific energy of 5000 kJ/ kg TS. The significant increase in overall energy production in system E of 53% and 23% relative to systems B and D, respectively, emphasizes the numerous advantages of ultrasonication inside the reactor, i.e. Solubilisation of the particulate organics, removal of the dissolved gaseous, improvement of the mass transfer, and increase of the microbial growth rate [22].

10.3.3.2 Solids reduction in methane reactors

Figure 10.3a shows the percentage reduction of liquid parameter in the second stage only of the five systems based on the steady-state data. As depicted in Figure 10.3a, the highest TCOD reduction efficiency of 70% was observed in system E, followed by 59% in system D, while the lowest TCOD removal efficiency of only 43% was observed in system A. Systems B

and C realized TCOD removal efficiencies of 46% and 50%, respectively. The highest TSS removal efficiency of 51% was observed for system E followed by 42% for system D, while the lowest TSS removal efficiency of 30% was observed in system A. Systems B and C achieved the same TSS reduction efficiency of 33%. VSS removal efficiencies in systems A, B, and C were within 10% variation and were in the range of 36% to 40%, while VSS removal efficiencies of 48% and 57% were observed for systems D and E, respectively.

On the other hand, Figure 10.3b shows the overall percentage reduction (based on raw food waste and final effluent after the second stage) of liquid parameter of the five systems. The overall TCOD removal efficiencies of 43%, 46%, 54%, 62%, and 73% were observed in systems A to E, respectively. The overall TSS removal efficiencies of systems B and C were very close in the range of 39% to 42%, while the highest overall TSS removal efficiency of 60% was observed for system E followed by 54% for system D. Furthermore, the overall VSS removal efficiencies of 36%, 44%, 51%, 59%, and 67% were observed for systems A, B, C, D, and E, respectively. Based on the aforementioned results, it is evident that the overall TSS removal efficiency in system E was higher than systems A, B, C, and D by 101%, 55%, 45%, and 12%, respectively, with corresponding VSS removal efficiency improvements of 86%, 52%, 31%, and 13%. Upon examination of the impact of sonication on two-stage systems and one-stage systems discussed above, it is evident that sonication of feed more strongly influences performance in one stage than two-stage systems in term of overall TSS reduction efficiencies. This due to the TSS destruction and Solubilisation of organic matter in the acidification stage which tends to partially offset some of the benefits of sonication. Furthermore, when the influent to the digestion system, irrespective of single or two-stage, is sonicated, the impact of the acidification stage is marginally less pronounced than with unsonicated feed.

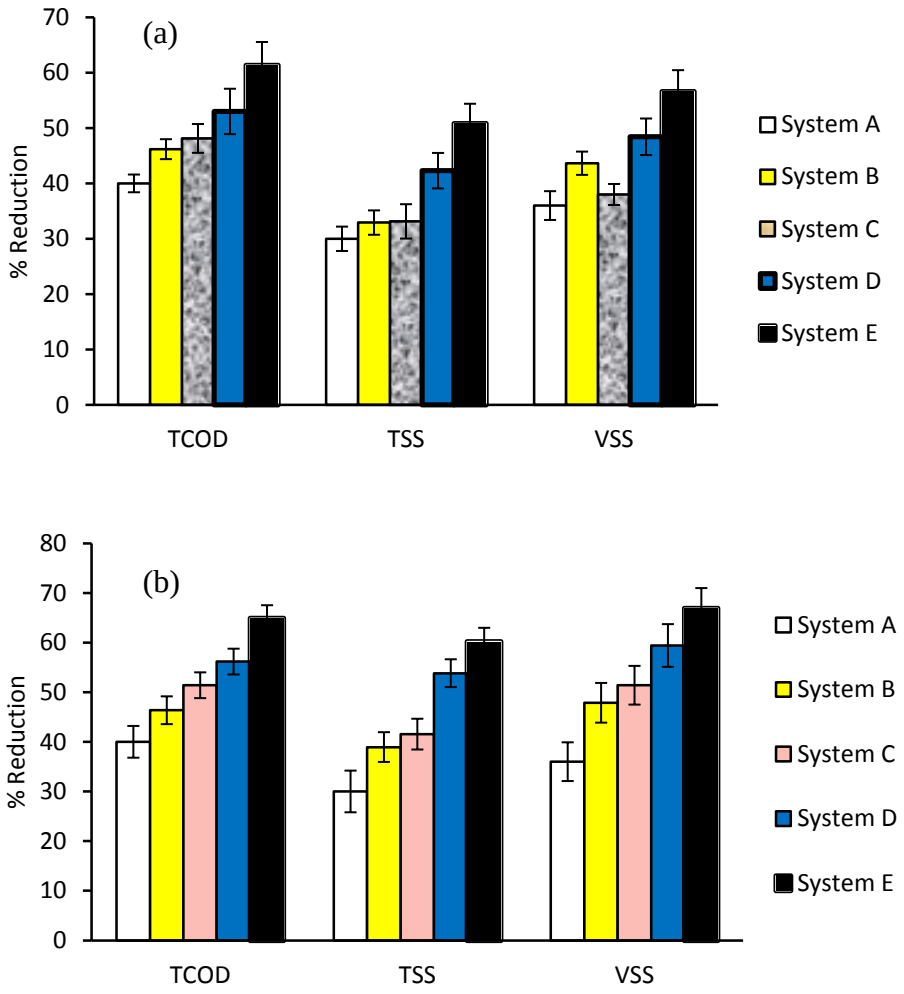

Figure 10.3 Percentage reductions of TCOD, TSS, and VSS; (a) second stage only, (b) overall.

The removal efficiencies in the digester of particulate proteins of 39%, 42%, 31%, 32%, and 35%was observed for systems A, B, C, D and E, respectively. Bound protein has been implicated in odour generation during dewatering of municipal biosolids using high speed centrifuges. The average removal efficiencies of bound proteins in systems A, B, C, D, and E observed in this study were 56%, 54%, 56%, 58%, and 64%, respectively. Thus, it can be concluded based on the aforementioned bound proteins data that acidification, sonication of feed, and even sonication inside the reactor were not advantageous for reduction of odor precursors.

Figures 10.4 a, b, c, and d show the proteins and carbohydrates removal efficiencies for the second stage, and overall removal efficiency. Although the overall removal efficiency of particulate protein in system B (single stage with sonicated feed) was 26% higher than that of system A (single stage with unsonicated feed), the removal efficiency in the two digesters A and B were only 8% different (42% VS. 39%), which reveals that the enhancement of particulate protein removal was due to applying ultrasonic to the feed. Moreover, the highest removal efficiency of particulate protein in the digester of 42% was observed in system B compared to on average of 33% in systems C, D, and E, while the highest overall removal efficiency of particulate protein of 58% was achieved in system E, followed by 54%, 49%, 45% and 39% in systems D, B, C, and A, respectively. Similarly, the highest removal efficiency of bound protein in the digester of 56% was observed in system A, but the highest overall removal efficiency of bound protein of 64% was observed in system E. Based on the abovementioned data, it is evident that the effect of the acidification stage had more significant impact than the effect of ultrasonication, reflected by only 12% reduction in particulate protein due to sonicated the feed (Solubilisation) versus a minimum of 21% reduction in the first stage in system C (CSTR with unsonicated feed). On the other hand, an overall soluble protein removal efficiency of 76% was achieved in system E compared to about 70% in systems D and C and about 62% in systems A and B. Achieving almost the same overall efficiency of soluble protein in either single stage with and without sonicated feed (systems A and B) or two-stage with and without sonicated feed (systems C and D) emphasizes that applying ultrasonication outside the reactor did not have any improvement on soluble protein overall removal efficiency. Moreover, the first-stage acidification process showed significant effect on soluble protein removal efficiency as reflected by the 16% increase in soluble protein when comparing single stage with two-stage with

unsonicated feed (systems A and C) and 11% increase when comparing single stage with two-stage with sonicated feed (systems B and D).

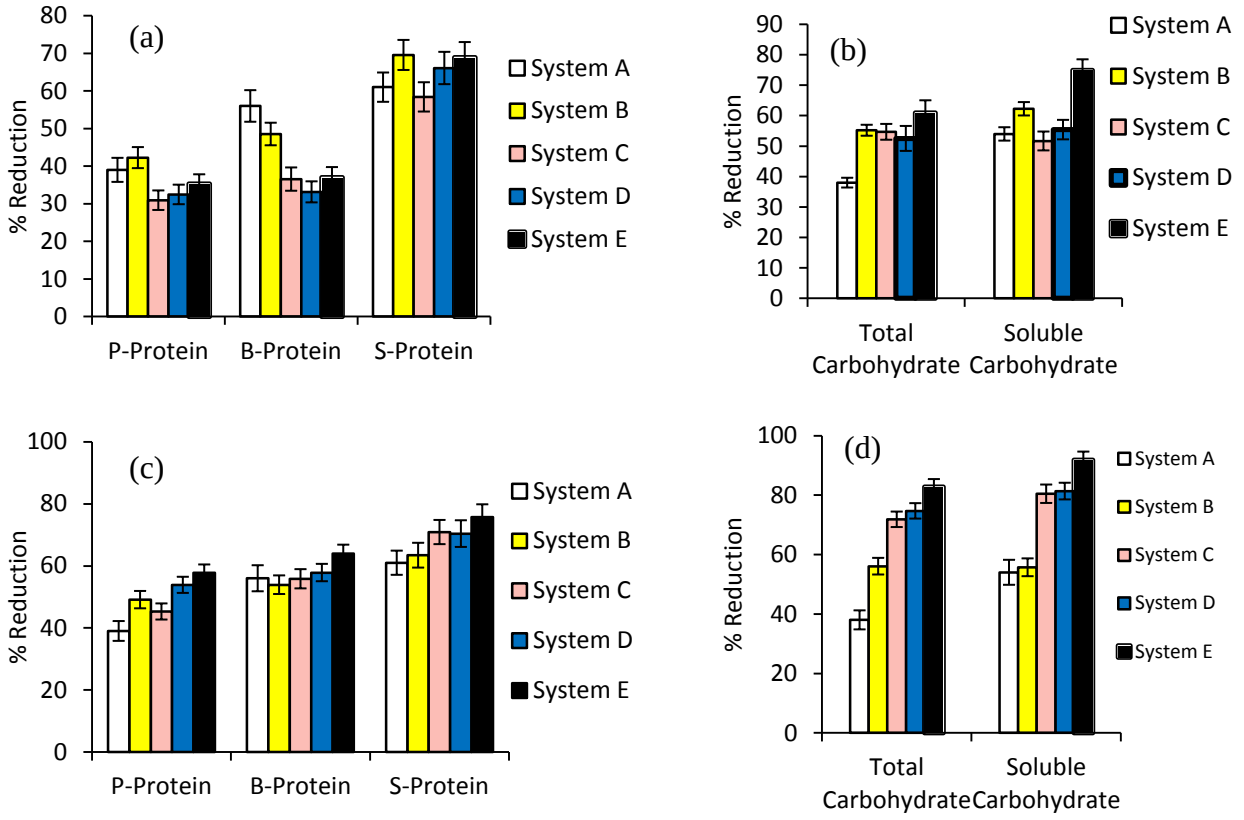

Figure 10.4 Percentage reductions of proteins and carbohydrates; (a) and (b) second stage only, (c) and (d) overall.

The removal efficiency of total carbohydrates in system E was 83%, as compared to 38%, 56%, 72%, and 75% in systems A, B, C, and D, respectively. The highest soluble carbohydrate removal efficiency of 92% was achieved in system E followed by about 80% removal efficiency in both systems C and D, and about 55% removal efficiency in systems A and B. The abovementioned soluble carbohydrate removal efficiencies emphasize that the effect of ultrasonication outside the reactor was insignificant i.e. 54% versus 56% in systems A and B,

respectively, and 80% versus 81% in systems C and D. Interestingly, however ultrasonication inside the reactor in system E enhanced soluble carbohydrate removal by additional 14%, 64%, 15%, and 70% relative to systems A, B, C, and D, respectively.

The COD balance was calculated based on 4 g COD/g CH_4 and considering the TCOD of the influent and effluent of the five methane reactors, and as shown in Table 10.2, the COD mass balance closures of 92% – 97% for the five methane reactors confirm data reliability.

10.4. Conclusions

Based on the aforementioned discussion, it is evident that applying sonication inside the reactor in the first stage (system E) showed superior results compared to all other treatment scenarios. The hydrogen production rate in SBHR was higher than those in the CSTR with unsonicated and sonicated feed by 85% and 45%, respectively. The methane production rate in system E was higher than in systems D, C, B, and A by 23%, 39%, 53%, and 94%, respectively. The overall solids reduction in system E was higher than all other scenarios.

Table 10.2 *Summary of products and COD mass balance in the methanogenic stage in systems A, B, C, D, and E.*

Effluent parameter	Units	Systems				
		A	B	C	D	E
SCOD	mg COD/L	26000 ± 1850	22900 ± 1620	20700 ± 1960	16500 ± 1530	11500 ± 980
VSS	(mg COD/L)[a]	26400 ± 1640	22900 ± 1350	20000 ± 1300	16700 ± 1120	13300 ± 1060
T-Carbohydrate	mg/L	29600 ± 1460	21000 ± 1120	13410 ± 1230	12100 ± 960	8230 ± 580
S-Carbohydrate	mg/L	9150 ± 650	8720 ± 620	3850 ± 320	3680 ± 260	1610 ± 110
P-Protein	mg/L	3810 ± 360	3190 ± 320	3430 ± 260	2890 ± 270	2641 ± 240
B-Protein	mg/L	500 ± 70	530 ± 76	510 ± 53	490 ± 46	410 ± 36
S-Protein	mg/L	3360 ± 290	3160 ± 240	2520 ± 190	2560 ± 210	2100 ± 170
VFAs	mg COD/L	1280 ± 90	1480 ± 110	1660 ± 130	2260 ± 160	1780 ± 120
Alkalinity	mg $CaCO_3$/L	5120 ± 460	5480 ± 490	5930 ± 390	6300 ± 460	5560 ± 420
VFA/Alkalinity ratio		0.25	0.27	0.28	0.36	0.32
Methane gas	L/d	8.2	10	11.4	13	16
Methane gas	(mg COD/d)[b]	20765	26208	28728	33516	39816
COD balance	(%)[c]	90	91	95	95	98

[a] Based on 1.42 gCOD/g VSS

[b] Based on 4 gCOD/g CH_4

[c] COD balance (%) = (VSS out (gCOD/d) + CH_4 (gCOD/d) + SCOD out (gCOD/d))/(TCOD in (gCOD/d)).

Values represent averages ± standard deviations based on 12 steady-state samples.

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CHAPTER 11

Conclusions and Recommendations

11.1 Conclusions

The outcome of this study emphatically revealed the positive effects of sonication on biosolids waste (hog manure and food waste) Solubilisation and biogas (hydrogen and methane) production. Based on the findings of this study, the following conclusions can be drawn:

11.1.1 Effect of ultrasonication on Solubilisation and anaerobic digestability of hog manure and food waste in batch and continuous systems

a. Effect of ultrasonication on hog manure (batch reactor)

- The $COD_{solubilisation}$ correlated very well with the degree of disintegration, the $TKN_{solubilisation}$ and the % decrease in particulate protein. Thus, $COD_{solubilisation}$ can be used to evaluate the degree of solubilisation in lieu of the labor and time intensive procedure, as it proved to be an accurate and easy to measure method.
- The disintegration of particles by ultrasonication was more pronounced for the smaller sizes, i.e., in the 0.6 to 60 µm range, as well as the reduction of VS by ultrasonication increased with increasing specific energy input in the 500-5000 kJ/kg TS and reached a plateau at 10000 kJ/kg TS. Moreover, at solids content of 2%, the specific energy input increased from 10000 to about 30000 kJ/kg TS for an additional 15% increase in degree of disintegration, whereas at TS of about 9%, the specific energy input increased from 250 to about 3,300 kJ/kg TS to achieve the same increase in degree of disintegration. Therefore,

ultrasonication is more effective pretreatment process for hog manure with higher TS content than WAS and primary sludges.

- The bound proteins decreased by 13.5% at specific energy of 5000 kJ/kg TS. Thus, the impact of ultrasonication on odor precursors such as bound proteins appears to be significant. Furthermore, the cell wall appeared to be ruptured at a minimum specific energy input of 500 kJ/kg TS, whereas the optimum specific energy was 10000 kJ/kg TS, affecting a 17.7% reduction in cell protein. Additionally, the optimum specific energy input for methane production in batch reactors was 500 kJ/kg TS, and resulted in a 28% increase in methane production, and subsequently about $ 4.1/ton of dry solids excess energy output.

b. Effect of ultrasonication on hog manure (continuous reactor)

- The overall TSS and VSS removal efficiencies of sonicated manure were higher than the unsonicated manure by 36% and 31% respectively.
- There was no significant difference in TCOD removal efficiency for the sonicated and unsonicated manure, while the SCOD removal efficiency in the digester receiving sonicated manure lower than that receiving the unsonicated manure.
- There was no significant difference in particulate protein removal efficiency for the sonicated and unsonicated manure in the anaerobic digester, whereas the overall removal efficiency was slightly increased (by 10%) for sonicated manure.
- The overall removal efficiency of bound protein for sonicated manure was higher than the unsonicated by 17.5%.
- The concentration of H_2S in the headspace of the bioreactor reduced from 988 to 562 ppm_v for unsonicated and sonicated manure respectively.

- The effluent ammonia for unsonicated manure (1200 mg/L) was higher than that of sonicated manure (980 mg/L).
- The methane production rate increased from 0.34 $L_{CH4}/L_{reactor}$.d in the unsonicated manure to 0.39 $L_{CH4}/L_{reactor}$.d for the sonicated.
- BioWin simulated results indicated that at shorter SRTs, VSS destruction efficiencies for sonicated manure were less than the unsonicated manure despite higher methane production. However, interestingly the improvement in VSS destruction efficiencies during anaerobic digestion by sonication becomes apparent at long SRTs.
- The net benefit increases sharply initially and stabilizes at $ 42-49/ton for SRTs of 15 to 30 days.

c. Effect of ultrasonication on food waste (batch reactor)

- The ultrasonication pretreatment promoted the release of carbohydrate and protein into the liquid phase, which enhanced hydrogen production.
- There was no significant effect of the ultrasonication on hydrogen production or waste Solubilisation after 5 minutes of sonication.
- The lowest hydrogen yield of 80 mL/g VS_{added} was observed for the unsonicated food waste, while the highest hydrogen yield was 141 mL/g VS_{added} at a sonication time of 30 min.
- Ultrasonication has a positive effect on all kinetic parameters; the ultimate hydrogen production increased by 77%, hydrogen production rate increased by 127%, and the lag phase decreased by 50%.
- The highest final VFAs after fermentation was achieved at a sonication time of 30 min, which reflects a 70% increase compared to the unsonicated food waste.

- There was no significant difference between the acetate to butyrate ratios (HAc/HBu) for the all samples.

11.1.2 The applicability of using ultrasonication as a pretreatment method for anaerobically digested sludge to enhance biohydrogen production from glucose

a. Applicability of ultrasonication as a pretreatment method

- The optimum specific energy of sonication for inactivation of methanogenesis observed in this study was 79 kJ/g TSS.
- Sonication pretreatment with temperature control showed promising results, as reflected by 120% increase in volumetric hydrogen production over untreated sludge, as well as 40% over pretreated sludge (acid pretreatment).
- Based on the results of this study, it is apparent that temperature adversely impacts hydrogen producing bacteria resulting in 30% lower hydrogen yield.
- Hydrogen yields of 1.55, 1.11, 1.04, 1.03, 0.68, and 0.7 mol H_2/mol glucose were observed for sonication with temperature control, acid, heat-shock, sonication without temperature control, base pretreatment, and untreated sludge, respectively.
- Hydrogen yield correlated linearly with HAc/HBu molar ratio, and inversely with biomass yield.

b. Single and combined pretreatment methods of food waste without extra seed (batch reactor)

- The highest increase in SCOD and soluble protein of 33% and 40% were achieved for ultrasonic with base pretreatment, respectively, while the highest increase in soluble carbohydrate of 31% was observed for ultrasonic with acid pretreatment.

- Of the different pretreatment methods, the highest hydrogen yield of 118 mL/g $VS_{initial}$ was observed for ultrasonic with acid pretreatment, while the lowest hydrogen yield of 46 mL/g $VS_{initial}$ was observed for base pretreatment.
- Hydrogen yield decreased from 97 mL/g $VS_{initial}$ for ultrasonic only to 78 mL/g $VS_{initial}$ when ultrasonic combined with heat pretreatment and to 67 mL/g $VS_{initial}$ when ultrasonic combined with base pretreatment.
- Ultrasonic with acid pretreatment exhibited the highest final VFAs of 16900 mg COD/L as well as the highest HAc/ HBu ratio of 1.87, while base pretreatment had the lowest final VFAs of 9700 mg COD/L and the lowest HAc/ HBu ratio of 0.61.
- The highest hydrogen production rate of ultrasonic with acid and ultrasonic with heat pretreatment varied narrowly from 8.6 to 8.9 mL/h and the lowest hydrogen production rate of 3.9 mL/h was observed for base pretreatment.

11.1.3 Development of a novel US patent-pending sonicated biological hydrogen reactor (SBHR)

a. Hydrogen production from glucose

- Ultrasonication has a positive effect on both hydrogen production rate and hydrogen yield. Both hydrogen production rate and hydrogen yield increased by about 93% and 83% in the SBHR compared with the CSTR, respectively.
- The glucose conversion efficiency in the SBHR was higher that in the conventional CSTR at both OLRs. The HAc/HBu ratio in the SBHR was higher than what was observed in the CSTR at both OLRs.

- The hydrogen content in the SBHR headspace was higher than that in CSTR by 10% and 31% at OLRs of 21.4 and 32.1 g COD/ $L_{reactor}$.d, respectively.
- The inverse relationship between the biomass yield and hydrogen yields observed, in addition to the higher biomass yield of about 0.32 g VSS/g COD observed in the CSTR relative to the 0.23 g VSS/g COD in the SBHR substantiate the higher H_2 yield in the SBHR.
- There were two different hydrogen producers (*Clostridium* sp. and *Citrobacter freundii*) detected in the SBHR and not detected in the CSTR.

b. Hydrogen production from food waste

- It is evident that the methanogenic activity decreased when sonication was applied outside the reactor and ceased completely with sonication inside the reactor.
- The volumetric hydrogen production rates of 4.8, 3.3, and 2.6 L $H_2/L_{reactor}$.d were achieved in the SBHR, CSTR with and without sonicated feed, respectively
- The observed hydrogen yield of 332 mL H_2 /g VSS_{added} in the SBHR is at the higher end of the range reported in the literature if not the highest.
- VFAs in the CSTR with sonicated feed and SBHR were mainly acetic acid and butyric acid constituting 78%, 86% of the residual VFAs on a COD basis, respectively, as compared to only 60% in CSTR with unsonicated feed. Moreover, after 10 days of start-up, methane disappeared completely from the headspace of the SBHR reactor, while there was still methanogenesis in the CSTR and CSTR with sonicated feed.
- The highest TCOD reduction efficiency of 9.3% was achieved in SBHR, while about the same TCOD reduction efficiency of 6.4% was observed in both CSTR with unsonicated

or sonicated feed. Similarly, the highest VSS reduction efficiency of 24% was achieved in SBHR and about 16% was observed in both CSTRs with and without sonicated feed.

- The removal efficiencies of total carbohydrate were 38%, 46%, and 56% in CSTR, CSTR with sonicated feed, and SBHR, respectively.
- Although the removal efficiency of particulate protein in the SBHR was higher than those in the CSTR and CSTR with sonicated feed, the removal efficiency of soluble protein in the SBHR was lower than those in the CSTR with and without sonicated feed.

c. Comparative study for hydrogen and methane production from food waste

An extensive comparison study of five different mesophilic systems was done using food waste. Systems A and B were one stage methane with unsonicated and sonicated feeds, respectively, while, systems C and D were two-stage hydrogen and methane with unsonicated and sonicated feeds, respectively. System E comprised SBHR followed by methane reactor. The findings of this study are as follows:

- It is evident that applying sonication inside the reactor in the first stage (system E) showed superior results compared to all other treatment scenarios.
- The hydrogen production rate in SBHR was higher than those in the CSTR with unsonicated and sonicated feed by 85% and 45%, respectively.
- The highest methane production rate of 3.2 L $CH_4/L_{reactor}$.d was achieved in system E, while the lowest methane production rate of 1.6 L $CH_4/L_{reactor}$.d was observed for system A. Average methane production rates of 2.1, 2.3, and 2.6 L $CH_4/L_{reactor}$.d were observed for systems B, C, and D, respectively. Therefore, the methane production rate in system E was higher than in systems D, C, B, and A by 23%, 39%, 53%, and 94%, respectively.

- Systems D and E achieved the same methane contents of 66% in the headspace, while methane content of 56%, 59%, 62% were observed in the headspace of systems A, B, and C, respectively.
- Based on an energy content of 286 kJ/mol for hydrogen and 891 kJ/mol for methane, overall steady-state energy production rates of 288, 365, 462, 531 and 670 kJ/d were observed for systems A to E, respectively.
- The highest TCOD reduction efficiency of 70% was observed in system E, followed by 59% in system D, while the lowest TCOD removal efficiency of only 43% was observed in system A. Systems B and C realized TCOD removal efficiencies of 46% and 50%, respectively.
- The overall VSS removal efficiencies of 67%, 59%, 51%, 44%, and 36% were achieved in systems E, D, C, B, and A, respectively.
- The highest overall removal efficiency of particulate protein of 58% was achieved in system E, followed by 54%, 49%, 45% and 39% in systems D, B, C, and A, respectively.
- The removal efficiency of total carbohydrates in system E was 83%, as compared to 38%, 56%, 72%, and 75% in systems A, B, C, and D, respectively.

11.2 Main Finding

- $COD_{solubilisation}$ can be used to evaluate the degree of solubilisation in lieu of the labor and time intensive DD procedure, as it proved to be an accurate and easy to measure method.
- The optimum specific energy input for methane production in batch reactors was 500 kJ/kg TS, and resulted in a 28% increase in methane production, and subsequently about $ 4.1/ton of dry solids excess energy output.

- BioWin simulated results indicated the improvement in VSS destruction efficiencies during anaerobic digestion by sonication becomes apparent at long SRTs.
- Ultrasonication can be used as a new effective pretreatment method for enrichment of H_2 producers in anaerobically digested sludge at a specific energy of 79 kJ/g TS.
- Combining ultrasonication with other pretreatment methods (heat, acid, and base) showed that ultrasonication with acid pretreatment at a pH of 3 had a positive effect on hydrogen production, while ultrasonication with heat pretreatment and ultrasonication with base pretreatment had a negative impact on hydrogen yield.
- The outcome of this study emphatically revealed the superior impact of applying sonication inside the reactor on biological hydrogen production and solids destruction. It is evident that the methanogenic activity in the biohydrogen reactor decreased when sonication was applied outside the reactor and ceased completely with sonication inside the reactor. Moreover, the novel SBHR followed by a conventional anaerobic digester showed superior results with respect to hydrogen production, methane production, and/or solids destruction compared to all other single and two-stage digestion processes.

11.3 Limitation of ultrasonic applications on the anaerobic digestion

Ultrasound pretreatment has several challenges. One of the major issues is the high capital and operating costs of ultrasound units due to both power consumption and probe wear and tear. The cost may go down as the technology becomes mature. Furthermore, the mechanisms contributing to degassing and enhanced mass transfer reportedly associated with ultrasonication are very poorly understood, thus necessitating studies targeting elucidation. Similarly, since availability of long-term performance data of full-scale ultrasound systems is

limited, design engineers are discouraged from recommend ultrasound systems for full-scale application.

11.4 Future work

Based on the findings of this research, further research is required:

- Optimize the operational conductions of the SBHR: Specific energy input, HRT, and SRT.
- Integrate the sonicated biological hydrogen reactor with a solid/liquid separator for decoupling SRT from HRT in the first stage to enhance the treatment of carbohydrate-rich wastewaters.
- Integrate the sonicated biological hydrogen reactor with two solid/liquid separators in series wherein first one has low HRT to selectively separate the hydrogen producers for recirculation to the SBHR and the second one with long HRT to enhance hydrogen and methane production from particulate wastes.
- Widen the scope of application of SBHR by testing various types of wastes (i.e. municipal wastewater treatment plant sludges, thin stillage from bioethanol plants, food waste, and corn syrup).
- Investigate the effect of ultrasonication on various microbial cultures: hydrogen producers, ethanol producers, and lactic acid producers, hydrogenotrophic methanogens, and sulfate reducing bacteria.
- Investigate the effect of adding hydrogen peroxide (H_2O_2) to the SBHR.
- Explore SBHR followed by microbial electrolysis cell (MEC) for hydrogen production.

APPENDICES

APPENDIX A

t-test results of the different pretreatment methods used in chapter 7

Control and ultrasonic pretreatment

Normality Test: Passed (P = 0.171)

Equal Variance Test: Passed (P = 0.804)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	387.333	5.686	3.283
Col 2	3	0	890.333	6.658	3.844

Difference -503.000

t = -99.501 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -517.036 to -488.964

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Control and heat pretreatment

Normality Test: Passed (P = 0.628)

Equal Variance Test: Passed (P = 0.128)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	387.333	5.686	3.283
Col 2	3	0	640.000	29.462	17.010

Difference -252.667

t = -14.585 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -300.765 to -204.568

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Control and acid pretreatment

Normality Test: Passed (P = 0.745)

Equal Variance Test: Passed (P = 0.172)

Group Name	N	Missing	Mean	Std Dev	SEM

Col 1	3	0	387.333	5.686	3.283
Col 2	3	0	510.333	23.459	13.544

Difference -123.000

t = -8.826 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -161.694 to -84.306

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Control and base pretreatment

Normality Test: Passed (P = 0.666)

Equal Variance Test: Passed (P = 0.243)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	387.333	5.686	3.283
Col 2	3	0	420.333	24.826	14.333

Difference -33.000

t = -2.244 with 4 degrees of freedom. (P = 0.088)

95 percent confidence interval for difference of means: -73.826 to 7.826

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.088).

Power of performed test with alpha = 0.050: 0.334

The power of the performed test (0.334) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Control and ultrasonic with heat pretreatment

Normality Test: Passed (P = 0.839)

Equal Variance Test: Passed (P = 0.360)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	387.333	5.686	3.283
Col 2	3	0	720.333	8.505	4.910

Difference -333.000

t = -56.377 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -349.400 to -316.600

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Control and ultrasonic with acid pretreatment

Normality Test: Passed (P = 0.169)

Equal Variance Test: Passed (P = 0.432)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	387.333	5.686	3.283
Col 2	3	0	1089.333	78.545	45.348

Difference -702.000

t = -15.440 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -828.236 to -575.764

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Control and ultrasonic with base pretreatment

Normality Test: Passed (P = 0.857)

Equal Variance Test: Passed (P = 0.152)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	387.333	5.686	3.283
Col 2	3	0	620.333	13.577	7.839

Difference -233.000

t = -27.417 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -256.595 to -209.405

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic and heat pretreatment

Normality Test: Passed (P = 0.734)

Equal Variance Test: Passed (P = 0.138)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	890.333	6.658	3.844
Col 2	3	0	640.000	29.462	17.010

Difference 250.333

t = 14.355 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 201.916 to 298.751

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic and acid pretreatment

Normality Test: Passed (P = 0.843)

Equal Variance Test: Passed (P = 0.188)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	890.333	6.658	3.844
Col 2	3	0	510.333	23.459	13.544

Difference 380.000

t = 26.990 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 340.910 to 419.090

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic and base pretreatment

Normality Test: Passed (P = 0.687)

Equal Variance Test: Passed (P = 0.261)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	890.333	6.658	3.844
Col 2	3	0	420.333	24.826	14.333

Difference 470.000

t = 31.671 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 428.798 to 511.202

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic and ultrasonic with heat pretreatment

Normality Test: Passed (P = 0.605)

Equal Variance Test: Passed (P = 0.515)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	890.333	6.658	3.844
Col 2	3	0	720.333	8.505	4.910

Difference 170.000

t = 27.261 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 152.686 to 187.314

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic and ultrasonic with acid pretreatment

Normality Test: Passed (P = 0.183)

Equal Variance Test: Passed (P = 0.438)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	890.333	6.658	3.844
Col 2	3	0	1089.333	78.545	45.348

Difference -199.000

t = -4.373 with 4 degrees of freedom. (P = 0.012)

95 percent confidence interval for difference of means: -325.358 to -72.642

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.012).

Power of performed test with alpha = 0.050: 0.888

Ultrasonic and ultrasonic with base pretreatment

Normality Test: Passed (P = 0.858)

Equal Variance Test: Passed (P = 0.186)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	890.333	6.658	3.844
Col 2	3	0	620.333	13.577	7.839

Difference 270.000

t = 30.926 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 245.760 to 294.240

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Heat and acid pretreatment

Normality Test: Passed (P = 0.322)

Equal Variance Test: Passed (P = 0.668)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	640.000	29.462	17.010
Col 2	3	0	510.333	23.459	13.544

Difference 129.667

t = 5.963 with 4 degrees of freedom. (P = 0.004)

95 percent confidence interval for difference of means: 69.297 to 190.036

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.004).

Power of performed test with alpha = 0.050: 0.990

Heat and base pretreatment

Normality Test: Passed (P = 0.235)

Equal Variance Test: Passed (P = 0.759)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	640.000	29.462	17.010
Col 2	3	0	420.333	24.826	14.333

Difference 219.667

t = 9.875 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 157.909 to 281.425

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Heat and ultrasonic with heat pretreatment

Normality Test: Passed (P = 0.845)

Equal Variance Test: Passed (P = 0.151)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	640.000	29.462	17.010
Col 2	3	0	720.333	8.505	4.910

Difference -80.333

t = -4.537 with 4 degrees of freedom. (P = 0.011)

95 percent confidence interval for difference of means: -129.489 to -31.178

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.011).

Power of performed test with alpha = 0.050: 0.909

Heat and ultrasonic with acid pretreatment

Normality Test: Passed (P = 0.296)

Equal Variance Test: Passed (P = 0.604)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	640.000	29.462	17.010
Col 2	3	0	1089.333	78.545	45.348

Difference -449.333

t = -9.277 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -583.806 to -314.861

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Heat and ultrasonic with base pretreatment

Normality Test: Passed (P = 0.976)

Equal Variance Test: Passed (P = 0.239)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	640.000	29.462	17.010
Col 2	3	0	620.333	13.577	7.839

Difference 19.667

t = 1.050 with 4 degrees of freedom. (P = 0.353)

95 percent confidence interval for difference of means: -32.334 to 71.667

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.353).

Power of performed test with alpha = 0.050: 0.057

The power of the performed test (0.057) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Acid and base pretreatment

Normality Test: Passed (P = 0.103)

Equal Variance Test: Passed (P = 0.948)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	510.333	23.459	13.544
Col 2	3	0	420.333	24.826	14.333

Difference 90.000

t = 4.564 with 4 degrees of freedom. (P = 0.010)

95 percent confidence interval for difference of means: 35.248 to 144.752

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.010).

Power of performed test with alpha = 0.050: 0.912

Acid and ultrasonic with heat pretreatment

Normality Test: Passed (P = 0.905)

Equal Variance Test: Passed (P = 0.212)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	510.333	23.459	13.544
Col 2	3	0	720.333	8.505	4.910

Difference -210.000

t = -14.576 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -250.000 to -170.000

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Acid and ultrasonic with acid pretreatment

Normality Test: Passed (P = 0.323)

Equal Variance Test: Passed (P = 0.555)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	510.333	23.459	13.544
Col 2	3	0	1089.333	78.545	45.348

Difference -579.000

t = -12.234 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -710.402 to -447.598

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Acid and ultrasonic with base pretreatment

Normality Test: Passed (P = 0.900)

Equal Variance Test: Passed (P = 0.375)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	510.333	23.459	13.544
Col 2	3	0	620.333	13.577	7.839

Difference -110.000

t = -7.029 with 4 degrees of freedom. (P = 0.002)

95 percent confidence interval for difference of means: -153.448 to -66.552

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.002).

Power of performed test with alpha = 0.050: 0.999

Base and ultrasonic with heat pretreatment

Normality Test: Passed (P = 0.774)

Equal Variance Test: Passed (P = 0.295)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	420.333	24.826	14.333
Col 2	3	0	720.333	8.505	4.910

Difference -300.000

t = -19.801 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -342.066 to -257.934

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Base and ultrasonic with acid pretreatment

Normality Test: Passed (P = 0.310)

Equal Variance Test: Passed (P = 0.565)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	420.333	24.826	14.333
Col 2	3	0	1089.333	78.545	45.348

Difference -669.000

t = -14.067 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -801.046 to -536.954

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Base and ultrasonic with acid pretreatment

Equal Variance Test: Passed (P = 0.450)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	420.333	24.826	14.333
Col 2	3	0	620.333	13.577	7.839

Difference -200.000

t = -12.242 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: -245.358 to -154.642

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic with heat and ultrasonic with base pretreatment

Normality Test: Passed (P = 0.214)

Equal Variance Test: Passed (P = 0.450)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	720.333	8.505	4.910
Col 2	3	0	1089.333	78.545	45.348

Difference -369.000

t = -8.090 with 4 degrees of freedom. (P = 0.001)

95 percent confidence interval for difference of means: -495.642 to -242.358

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic with heat and ultrasonic with base pretreatment

Normality Test: Passed (P = 0.889)

Equal Variance Test: Passed (P = 0.189)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	720.333	8.505	4.910
Col 2	3	0	620.333	13.577	7.839

Difference 100.000

t = 10.811 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 74.319 to 125.681

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Ultrasonic with acid and ultrasonic with base pretreatment

Normality Test: Passed (P = 0.278)

Equal Variance Test: Passed (P = 0.483)

Group Name	N	Missing	Mean	Std Dev	SEM
Col 1	3	0	1089.333	78.545	45.348
Col 2	3	0	620.333	13.577	7.839

Difference 469.000

t = 10.191 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 341.227 to 596.773

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

APPENDIX B

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CV

Elsayed Elbeshbishy

PROFILE

- Experience in anaerobic treatment of wastes, biohydrogen, and biomethane production
- Experience in water treatment, potable water compact units
- Experience in pretreatment of solid wastes
- Experience in ultrasound application for solids reduction and biogas (hydrogen and methane) production
- Experience in municipal and industrial wastewater treatment process engineering
- Experience in wastewater process simulation software Biowin
- Experience in writing technical reports
- Well-developed planning, problem solving, analytical, and communication skills
- Excellent presentations skills

EDUCATION

- Ph.D. Civil and Environmental Engineering, University of Western Ontario, Canada, 2011
- M.Sc., Civil Engineering, Tanta University, Egypt, 2002
- B.Sc., Civil Engineering, Tanta University, Egypt, 1995

PATENT APPLICATIONS

- **Elsayed Elbeshbishy**, George Nakhla. Sonicated Biological Hydrogen Reactor (SBHR) and Methane Generator from Wastes, US patent application, W-11-006, 2010.

REFEREED BOOK CHAPTERS

- B.R. Dhar. **E. Elbeshbishy**, G. Nakhla, M.B Ray. 2011. State of the Art of Biogas Production from Solid Waste and Wastewater. 1st edition, Edited by: Nadya Gotsiridze-Columbus. Nova Science Publishers, Inc. 2011.

PUBLICATIONS

1. **E. Elbeshbishy**, A. Saad, H. Hafez, G Nakhla, M.B Ray. 2011. Impact of Ultrasonication of Hog Manure on Anaerobic Digestability. *Ultrasonics Sonochemistry*. 1 (18), 164-171.

2. **E. Elbeshbishy**, H. Hafez, G. Nakhla. 2010. Enhancement of biohydrogen producing using ultrasonication. *Int. J. Hydrogen Energy*. 35(12), 6184-6193.

3. **E. Elbeshbishy**, A. Nakevski, H. Hafez, M.B. Ray, G. Nakhla. 2010. Simulation of the impact of SRT on anaerobic digestability of ultrasonicated hog manure. *Energies*. 3(5), 974-988.

4. **E. Elbeshbishy**, H. Hafez, G. Nakhla. 2011. Hydrogen Production Using Sono-Biohydrogenator. *Int. J. Hydrogen Energy* 36 (2), 1456-1465.

5. **E. Elbeshbishy**, H. Hafez, G. Nakhla. 2010. Viability of Ultrasonication of Food Waste for Hydrogen Production, *Int. J. Hydrogen Energy*, doi:10.1016/j.ijhydene.2011.01.008.

6. **E. Elbeshbishy**, H. Hafez, G. Nakhla. 2011. Ultrasonication for Biohydrogen Production from Food waste, *Int. J. Hydrogen Energy* 36 (4), 2896-2903.

7. **E. Elbeshbishy**, H. Hafez, B. Dahr, G. Nakhla. 2011. Single and Combined Effect of Various Pretreatment Methods for Biohydrogen Production from Food waste, *Int. J. Hydrogen Energy* 36(7), 11379-11387.

8. **E. Elbeshbishy**, G. Nakhla. 2011. Comparative Study of ultrasonic Effect on the Anaerobic Biodegradability of Food Waste. *Bioresour. Technol*. 102 (11), 6449-6457.

9. H. Hafez, **E. Elbeshbishy**, N. Chowdhury, G.Nakhla, J. Fitzgerald, A.Van Rossum, G. Gauld. 2010. Pushing the Hydraulic Retention Time Envelope in MLE Systems. *Chem. Eng. J*. 3 (163); 202-211.

10. A.E. Youssef, **E. Elbeshbishy,** H. Hafez, G. Nakhla, P. Charpentier. 2010. Sequential Supercritical Water Gasification and Partial Oxidation of Hog Manure for Hydrogen Production. *Int. J. Hydrogen Energy* 35(21), 11756-11767.

11. S. Aldin, **E. Elbeshbishy**, G. Nakhla, M. Ray. 2010. Modeling the Effect of Sonication on the Anaerobic Digestion of Biosolids, *Energy Fuels*, *24* (9), 4703–4711.

12. H. Hafez, G. Nakhla, H. El Naggar, **E. Elbeshbishy**, B. Baghchehsaraee. 2010. Effect of Organic Loading on a Novel Hydrogen Bioreactor. *Int J Hydrogen Energy*. 35(1), 81-92.

13. H. Hafez, **E. Elbeshbishy**, G. Nakhla, H. El Naggar 2011. Simulating the impact of suppression of methanogenesis in continuous flow biohydrogen reactors. *Int J Hydrogen Energy* 36 (10), 5885-5894.

14. B.R. Dhar. **E. Elbeshbishy**, H. Hafez, G. Nakhla, M. Ray. 2011. Thermo-Oxidative Pretreatment of Municipal Waste Activated Sludge for Volatile Sulfur Compounds Removal and Enhanced Anaerobic Digestion. *Chemical Engineering Journal* 174(1), 166-174.

15. N. Nasr, **E. Elbeshbishy**, H. Hafez, B. Dahr, G. Nakhla. 2011. Bio-Hydrogen Production from Thin Stillage using Conventional and Acclimatized Anaerobic Digester Sludge, *Int J Hydrogen Energy* 36 (20), 12761-12769.

REFEREED CONFERENCE PROCEEDINGS

1. **E. Elbeshbishy**, B.R. Dhar, H. Hafez, G. Nakhla. Application of Ultrasound with Various Pretreatments to Improve Biological Hydrogen Production from Food Waste. AIChE 2011 Annual Meeting, October 16-21, 2011, Minneapolis, MN.

2. **E. Elbeshbishy**, H. Hafez, A. Eldyasti, G. Nakhla. Novel Application of Ultrasonic for Enhancement of Biohydrogen and Biomethane Production from Food Waste. WEFTEC conference Oct. 15-19, 2011 in Los Angeles, California.

3. **E. Elbeshbishy**, B.R. Dhar, H. Hafez, G. Nakhla. Combination of Various Pretreatments with Ultrasonication for Enhanced Biohydrogen Production from Food Waste. 61st Canadian Chemical Engineering Conference, October 23-26, 2011, London, ON, Canada.

4. **E. Elbeshbishy**, B.R. Dhar, H. Hafez, G. Nakhla. Sonicated Biological Hydrogen Reactor for Biohydrogen Production. 61st Canadian Chemical Engineering Conference, October 23-26, 2011, London, ON, Canada.

5. **E. Elbeshbishy**, H. Hafez, G. Nakhla. Enhancement of Hydrogen and Methane Production from Pulp Waste Using Ultrasonication. 46th Central Canadian Symposium on Water Quality Research, Feb. 22 & 23, 2011, Burlington, Ontario.

6. **E. Elbeshbishy**, Hafez, H., Nakhla, G. Effect of Ultrasonication on Pulp Waste Solubilization and Enhancement of Hydrogen Production in Batch Study. Proceedings of the AIChE 2010 Annual Meeting. November 7-12, 2010, Salt Lake City, UT, USA.

7. **E. Elbeshbishy**, H. Hafez, A. Nakevski, M. Ray, G. Nakhla. Effect of Ultraonication on hog manure anaerobic digestion. WEFTEC Conference October 2-6, 2010 in New Orleans, Louisiana U.S.A.

8. **E. Elbeshbishy**, H. Hafez, G. Nakhla. Application of Ultrasonication for Biohydrogen Production. Proceedings of ICH2P 2010. June 16-18, Istanbul, Turkey.

9. **E. Elbeshbishy**, S. Aldin, G. Nakhla, M. B. Ray. Pretreatment of Hog Manure Prior to Anaerobic Digestion. AIChE 2009 Annual Meeting. Nov. 8-13, 2009, Nashville, USA.

10. B.R. Dhar. **E. Elbeshbishy**, G. Nakhla, M. Ray. Sono-Thermal Pretreatment of Waste Activated Sludge for Enhanced Anaerobic Digestion and Volatile Sulfur Compounds Control in Biogas. WEFTEC conference, October 15-18, 2011, Los Angeles.

11. B.R. Dhar. **E. Elbeshbishy**, H. Hafez, G. Nakhla, M. Ray. BioWin Modeling to Simulate the Impact of Thermo-Chemical Pretreatment of Sludge on Enhanced Anaerobic Digestion. 61st Canadian Chemical Engineering Conference, October 23-26, 2011, London, ON, Canada.

12. N. Nasr, **E. Elbeshbishy**, G. Nakhla, H. El Naggar, Hafez, H. Impact of Sludge Acclimatization on Biological Hydrogen Production from Thin Stillage. 61st Canadian Chemical Engineering Conference, October 23-26, 2011, London, ON, Canada.

13. Youssef, A.E., Nakhla, G., Charpentier, P., **Elbeshbishy, E**., Hafez, H. A Novel Technique for Hydrogen Production from Hog-Manure in Supercritical Partial Oxidation (SCWPO). Proceedings of the World Hydrogen Energy Conference 2010, May 16-21, 2010, Essen, Germany.

14. H. Hafez, **E. Elbeshbishy**, H. El Naggar, G. Nakhla. Modeling of Biohydrogen Production Using BioWin. Proceedings of the AIChE 2010 Annual Meeting. November 7-12, 2010, Salt Lake City, UT, USA.

15. S. Aldin, **E. Elbeshbishy**, G. Nakhla, M. B. Ray. Viability of Ultrasonication for Pre-Treatment of Biosolids. WEFTEC conference Oct. 10-14, 2009 in Orlando, FL.

16. S. Aldin, **E. Elbeshbishy**, G. Nakhla, M. B. Ray. The Effect of Ultrasonic on Primary and Secondary Sludge Prior to Anaerobic Digestion. WCCE Conference Aug. 23-27, 2009 in Montréal, Quebec, Canada.

17. S. Aldin, **E. Elbeshbishy**, F. Tu, G. Nakhla, M. B. Ray. Pretreatment of Primary Sludge Prior to Anaerobic Digestion. AIChE Annual Meeting Nov. 16-21, 2008 in Philadelphia.

POSTERS

1. **E. Elbeshbishy**, H. Hafez, G. Nakhla. Ultrasonication for Biohydrogen Production from Food waste. HFC2011, May 15-18, 2011, Vancouver, BC, Canada.

2. **E. Elbeshbishy**, H. Hafez, G. Nakhla. A Novel Application of Ultrasonication for Enhancement of Hydrogen Production. Third Annual Research Day in Engineering, Great Hall, Somerville house, London, Ontario, January 2010.

3. E. Youssef, **E. Elbeshbishy,** H. Hafez, G. Nakhla, P. Charpentier. A Novel Technique for Hydrogen Production from Hog-Manure in Supercritical Partial Oxidation (SCWPO). Accepted for a poster presentation in the session "HP.4a Reforming and Gasification - Fossil". WHEC 2010, on Tuesday, May 18, 2010 and Wednesday, May 19, 2010.

4. H. Hafez, **E. Elbeshbishy**, H. El Naggar, G. Nakhla. Modeling the impact of suppression of methanogenesis in continuous flow biohydrogen reactors. HFC2011, May 15-18, 2011, Vancouver, BC, Canada.

WORKSHOPS

- Symposium on solving problems of industrial wastewater at Gharbia Governorate, Egypt, 2006.
- A regional workshop on wastewater and biosolids treatments and reuse, Alexandria, Egypt, 2005.
- Workshop on developing irrigation & hydraulics courses (one of the organizers), 2004.

AWARDS AND SCHOLARSHIPS

- Ross and Clarke scholarship, University of Western Ontario, 2011
- Thesis research award, University of Western Ontario, 2011
- Western Graduate Research Scholarship Engineering, University of Western Ontario, 2007-2011
- Egyptian Government Ph.D. Scholarship, Ministry of higher education, Egypt, 2007-2011
- Ministry of High Education Leadership Group Meeting Award in Culture activity, Egypt 1993
- Egyptian Engineering Syndicate Award "Honourship of Distinction", Egypt, 1996
- Elshoban Elmoslmeen committee Award in excellence in education, Egypt, 1996
- Excellence Award for Top Academic Performance, Tanta University, Egypt, 1990-1995
- Ideal Student Honourship, Tanta University, Egypt, 1992-1995
- Engineering Award in Social activity, Tanta University, Egypt, 1992-1994
- Engineering Award in Culture activity, Tanta University, Egypt, 1993, 1994

PROFESSIONAL AFFILIATIONS

- Member of Water Environment Federation (WEF) and Water Environment Association of Ontario (WEAO)

- Member of Western Canada Water (WCW)
- Member of American Institute of Chemical Engineers (AIChE)
- The Canadian Society of Civil Engineering (CSCE)
- UWO Ontario Water Works Association and Water Environment Association of Ontario Joint Student Chapter
- Member of Egyptian Engineering Syndicate.

WORK EXPERIENCE

- Designed, operated and evaluated a novel patent-pending sonicated biological hydrogen reactor (SBHR) system utilizing source separated organics City of Toronto, Municipal Solid wastes Division, April 2010
- Ultimate biomethanation tests for source separated organics, City of Toronto, Municipal Solid wastes Division. January 1st to April 30th, 2010
- Treatability Studies and Kinetics of Landfill Leachate for the North Bay Pilot Treatment System, including resperometric, specific methananogenic activity, and batch studies, modeling using GPSX software. March 25th to May 5th, 2010
- Designed, performed and evaluated studies on physio-chemical treatment of high strength landfill leachate including coagulation/flocculation, sedimentation and pre-aeration tests at the Green Lane landfill. Ontario. Canada, March 2009
- Operation of a 24 m^3/d pilot Modified Ludzack Ettinger (MLE) plant at the Greenway Pollution Control Plant. London, Ontario, Canada, April 2009
- Laboratory determination of nitrification and denitrification kinetics for the conventional activated sludge system and pilot MLE process at Greenway Pollution Control Plant. London, Ontario, Canada, July 2009
- Respirometric testing and detailed wastewater characterization as well as delineation of biokinetics in support of computer modeling for the primary effluent at the Greenway Pollution Control Plant. London, Ontario, Canada, April 2009
- Long-term assessment of full-scale anaerobic digesters at Guelph Wastewater Treatment Plant by batch digestion studies and biomethanation potential tests. Guelph, Ontario, Canada, October 2009

- Development of steady-state and dynamic models for two full-scale anaerobic digesters treating combined primary and secondary sludges as well as alkaline-hydrolyzed dewatered sludges. Guelph, Ontario, Canada, November 2009
- Trained fellow graduate students on water/wastewater analysis and data interpretation
- Reviewed, analyzed and compiled research results and prepared industrial project proposals
- Environmental, concrete design engineer and record of completion of small and large construction projects Tanta University Privet consultancy Center. Tanta, Egypt, 1997-2006.
- Worked on the design of R.C structures and Steel structures in Dr. Elbahrawy Engineering Consultancy Office, including a complete design, detailed preparation of shop drawings for various industrial facilities. Cairo, Egypt, 1996-1997.

TEACHING EXPERIENCE

- Teaching assistant, Civil and Environmental Engineering 2008-2011
  University of Western Ontario, London, Ontario, Canada
- Lab supervisor, Civil and Environmental Engineering 2008-2011
  University of Western Ontario, London, Ontario, Canada
- Teaching assistant, Public Works Engineering 2005-2007
  Tanta University, Tanta, Egypt
- Teaching assistant, Water Engineering 2003-2004
  Tanta University, Tanta, Egypt
- Demonstrator, Water Engineering 1999-2002
  Tanta University, Tanta, Egypt
- Demonstrator, Engineering physics and mathematics 1996-1998
  Tanta University, Tanta, Egypt

TEACHING SUPPORT ACTIVITIES

- *Workshops in Future Professor Series, University of Western Ontario,* 2007 - 2010
- *Teaching Assistant Training Program (TATP)* January 2010
- *Graduate Career Day* Winter 2010