4-10-2012 12:00 AM

Investigation of Bio-hydrogen and Bio-methane Production From Thin Stillage

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A thesis submitted in partial fulfillment of the requirements for the Master of Engineering Science degree in Civil and Environmental Engineering

INVESTIGATION OF BIO-HYDROGEN AND BIO-METHANE PRODUCTION FROM THIN STILLAGE

(Spine title: Bio-H_2 and Bio-CH_4 Production from Thin Stillage)

(Thesis format: Integrated Article)

by

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Graduate Program in Engineering Science

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Engineering Science

The School of Graduate and Postdoctoral Studies
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London, Ontario, Canada

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entitled:

INVESTIGATION OF BIO-HYDROGEN and BIO-METHANE PRODUCTION FROM THIN STILLAGE

is accepted in partial fulfillment of the requirements for the degree of

Master of Engineering Science

Date

Chair of the Thesis Examination Board

Abstract

An evaluation of single-stage and two-stage anaerobic digestion processes for biomethane and biohydrogen production using thin stillage was performed to assess the viability of biohydrogen production from thin stillage and the impact of separating the acidogenic and methanogenic stages on anaerobic digestion with hydrogen production in the first stage. A comparative evaluation of anaerobic digester sludge (ADS) and acclimatized anaerobic digester sludge (AADS) for biohydrogen production was performed at various S°/X° ratios. The optimum range of S°/X° ratio for hydrogen production was found to be 1 to 2 gCOD/gVSS using conventional ADS and 3 to 6 gCOD/gVSS using AADS. Maximum methane yields of 0.33 L CH_4/$gCOD_{added}$ and 0.26 L CH_4/$gCOD_{added}$ were achieved in the two-stage and the single-stage processes, respectively. An artificial neural network model was developed to estimate the hydrogen production profile with time in batch studies and successfully predicted it with a correlation coefficient of 0.965.

Keywords

Hydrogen, Dark fermentation, Substrate-to-Biomass ratio, Anaerobic digestion, Methane, Two-stage anaerobic digestion, Thin stillage, Artificial neural network

Co-Authorship Statement

Chapter 3: Bio-Hydrogen Production from Thin Stillage using Conventional and Acclimatized Anaerobic Digester Sludge

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Published in the International Journal of Hydrogen Energy. 2011; 36: 12761-12769

Chapter 4: Comparative Assessment of Single-Stage and Two-Stage Anaerobic Digestion for the Treatment of Thin Stillage

Nasr, N., Elbeshbishy, E., Hafez, H., Nakhla, G., and El Naggar, M.H.

Bioresource Technology, In Press, Corrected Proof, Available online 14 February 2012.

Chapter 5: Application of Artificial Neural Networks for Modeling of Bio-Hydrogen Production

Nasr, N., Hafez, H., Nakhla, G., and El Naggar, M.H.

To be submitted to Bioresource Technology.

To My Father, Mother and Family for their Love and Support

To my Beloved Husband, Ahmed, for his Love and Patience

To my Lovely Kids, Malak and Youssef, for their Smile

To my Dear Friends for their Support

Acknowledgments

The author would like to express her sincere gratitude to her advisors, Prof. George Nakhla and Prof. M. Hesham El Naggar for their guidance, advice and encouragement throughout the course of this work. Their support and patience are highly appreciated. The author is indebted to Western University for supporting her masters studies through the Western Engineering Scholarship (WES).

Special thanks are due to Dr. Hisham Hafez from GreenField Ethanol Inc. for his support, guidance, and encouragement. Many thanks to Dr. Elsayed Elbeshbishy from Western University for his advice and encouragement. The author would also like to thank the Technicians and Staff at the Department of Civil and Environmental Engineering, who contributed directly or indirectly to the accomplishment of this thesis.

Finally, the author would like to express her deep gratitude and appreciation to her father, mother, brother, husband, beloved daughter and son for their continuous support and encouragement throughout the course of this work. In particular, without my husband's support, help and patience, this work would not have been possible.

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Nomenclatures

AADS	Acclimatized anaerobic digester sludge
ADM1	Anaerobic digestion model no. 1
ADS	Anaerobic digester sludge
ANN	Artificial neural network
APE	Average percentage error
B	Methane production
B_o	Methane potential
BOD	Biological oxygen demand
BPNN	Back propagation neural network
CDS	Condensed distillers' solubles
COD	Chemical oxygen demand
CPR	Continuous pressure release
CSTR	Continuous stirred tank reactor
DDG	Distillers' dried grains
DDGS	Distillers' dried grains with solubles
DG	Distillers' grains
DGGE	Denaturing gradient gel electrophoresis
F/M	Food to microorganisms ratio
GHG	Greenhouse gas

H	Cumulative hydrogen value
H_{max}	Maximum cumulative hydrogen value
HP	Hydrogen production
HPR	Hydrogen production rate
HRT	Hydraulic retention time
HY	Hydrogen yield
IBR	Integrative biological reactor
IPR	Intermittent pressure release
k	Maximum rate of substrate utilization/First order kinetic constant
k_d	Biomass decay coefficient
MAE	Mean absolute error
MBR	Membrane bioreactor
MSE	Mean square error
n	Number of experimental data points
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
ORP	Oxidation-reduction potential
P	Ultimate/Cumulative hydrogen production
P_{max}	Maximum cumulative hydrogen production
PBR	Packed-bed reactor

pH_i	initial pH
PFSS	Preserved fruits soaking solution
R^2	Correlation coefficient
R_m	Rate of hydrogen production
R_{max}	Maximum hydrogen production rate
SBHR	Sonicated biological hydrogen reactor
SBR	Sequencing batch reactor
SBOD	Soluble biological oxygen demand
SCOD	Soluble chemical oxygen demand
SCRD	Semi-continuous rotating drum
SHPR	Specific hydrogen production rate
SRT	Solid retention time
SS	Suspended solids
STP	Standard temperature and pressure
S_o	Initial substrate concentration
S_o/X_o	Substrate to biomass ratio
T	Temperature
t	Fermentation/Digestion time
TBOD	Total biological oxygen demand
$Tcarb_i$	Initial total carbohydrates

$Tcarb_f$	Final total carbohydrates
TCD	Thermal conductivity detector
TCOD	Total chemical oxygen demand
TKN	Total Kjehldahl nitrogen
TOC	Total organic carbons
TOC_{eff}	Effluent total organic carbons
TPAD	Two-phase anaerobic digestion
TS	Total solids
TSS	Total suspended solids
TVFAs	Total volatile fatty acids
$TVFAs_i$	Initial total volatile fatty acids
$TVFAs_f$	Final total volatile fatty acids
UASB	Up-flow anaerobic sludge blanket
UASBAF	Up-flow anaerobic sludge blanket-anaerobic filter
$V_{G,i}$	Total biogas volume
$V_{H,i}$	Current cumulative hydrogen gas volume
$V_{H,i-1}$	Previous cumulative hydrogen gas volume
V_s	Volume of sludge
V_t	Volume of thin stillage
$V_{X,i}$	Current cumulative hydrogen or methane gas volume

$V_{X,i-1}$	Previous cumulative hydrogen or methane gas volume
VFAs	Volatile fatty acids
VLR	Volume loading rate
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
X_o	Initial biomass concentration
$Y_{i,e}$	Experimental data
$Y_{i,p}$	Predicted data
λ	Lag phase duration

CHAPTER 1

Introduction

1.1. Introduction

Some processes employed in the production of renewable biofuels, such as, bioethanol can result in significant amounts of wastewater with high chemical oxygen demand (COD). Disposal of this wastewater can represent significant pollution problems. One of such wastewater streams is thin stillage, the main by-product of the fermentation process in a conventional ethanol plant, which can be a strong candidate for biological hydrogen production as well as anaerobic digestion. Usually, less than 50% of thin stillage is recycled as fermentation broth (called backset in the corn-to-ethanol industry) [Egg et al., 1985; Shojaosadati et al., 1996; Julian et al., 1990]. The main concern with thin stillage recirculation without any treatment is the accumulation of fermentation inhibitors (acetate, lactate, glycerol and ethanol) in the fermentation tank [Pejin et al., 2009; Julian et al., 1990]. The recirculation of thin stillage reduces water intake and subsequently waste disposal, increases corn processing capacity, and reduces nutrient and buffer requirements [Ahn et al., 2011]. Therefore, using thin stillage in anaerobic digestion could facilitate maximizing recirculation rates by improving its characteristics.

Anaerobic dark fermentation is an attractive biological process for hydrogen production because of its higher rate of hydrogen production relative to photo-fermentative processes as well as its potential for using waste streams [Levin et al., 2004; Wang and Wan, 2009]. A major problem in the process of biological hydrogen production is the existence of hydrogen consuming bacteria such as methanogens and hemoacetogens in mixed cultures [Adams and Stiefel, 1998]. To suppress the hydrogen consuming bacteria, different types of

pretreatment were investigated such as heat treatment [Chang et al., 2002; Baghchehsaraee et al., 2008], acid treatment [Chen et al., 2002], base treatment [Cai et al., 2004; Chen et al, 2002], and chemical inhibition [Park et al., 2004; Sparling et al., 1997].

In a single-stage anaerobic digestion process, a variety of higher organic acids, such as propionic, butyric, and lactic, as well as alcohols and ketones, are formed during the breakdown of the organic substrates by acidogens. However, in a well operated process, these products are mostly converted to acetic acid and hydrogen, which, in turn, are converted to methane gas [Cooney et al., 2007]. On the other hand, in a two-stage anaerobic digestion process, the acidogenic and the methanogenic steps are separated. This provides enhanced stability to the different groups of microorganisms that are responsible for both steps and better process control [Demirel and Yenigun, 2002]. The end products of volatile fatty acids breakdown from the acidification stage are ideal for anaerobic treatment and methane production [Pavan et al., 2000]. The purpose of a two-stage anaerobic digestion system is not only to further degrade waste, but also to extract more net energy [Thompson, 2008].

1.2. Problem Statement

The impact of microbial cultures on biohydrogen production from soluble substrates as glucose is well documented in the literature [Ling et al., 2009; Zhu and Beland, 2006; Wang and Wan, 2008]. In addition, many studies used conventional anaerobic digester sludge in order to assess biohydrogen production from different wastes. For example, Chen et al. [2006] and Yu et al. [2002] used it to process food wastes. Most of these studies used different sludge treatment methods to enrich hydrogen producers [Elbeshbishy et al., 2010]. Other studies used pure cultures for biohydrogen production [Lin et al., 2007; Chen et al.,

2005; Ahn et al., 2011]. However, hydrogen production using mixed cultures is more practical since they are simpler to operate, easier to control, and applicable for a broader range of feedstocks [Li and Fang, 2007]. Due to lack of data on specific populations, hydrogen yields vary considerably even for a specific substrate which results in a misleading assessment of the potential of hydrogen production from different wastes.

Separating the acidogenic and methanogenic stages in a two-stage anaerobic digestion process has been usually investigated in order to maximize the acidification process, regardless of the acidification pathways and the hydrogen produced in the first stage [Vinas et al., 1993; Pavan et al., 2000; Demirel and Yenigun, 2002]. A few studies investigated the effect of hydrogen production in the first stage on the methane production in the second stage. Chu et al. [2008] investigated two-stage process comprising thermophilic hydrogen production and mesophilic methane production for the treatment of organic fraction of municipal solid waste (OFMSW), and achieved stable performance for simultaneous hydrogen and methane production for over 150 days with average hydrogen and methane yields of 0.25 $m^3/KgVS_{added}$ and 0.464 $m^3/KgVS_{added}$, respectively. Han and Shin [2004] treated food waste in a leaching-bed reactor for hydrogen production and an up-flow anaerobic sludge blanket (UASB) reactor for methane production under mesophilic conditions, and achieved hydrogen and methane yields of 0.31 $m^3/KgVS_{added}$ and 0.21 $m^3/KgVS_{added}$.

The complexity of modeling fermentative biohydrogen production process is due to the numerous interdependent factors that affect the process such as temperature, pH, type and concentration of wastes and cultures, and bioreactor configuration [Wang and Wan, 2009]. Many studies investigated these factors using the conventional “one factor at a time” method with models such as Gompertz and the Logistic models and some of them studied the

combined effect of two or three factors only on the biohydrogen production process [Ginkel and Sung, 2001; Li et al., 2008; Hwang et al., 2009]. These methods are ineffective, since they do not take into consideration the interaction between the various factors.

1.3. Research Objectives

In the present research, hydrogen and methane production using thin stillage is investigated. In addition, modeling the fermentative hydrogen production process using artificial neural network method is undertaken. The specific objectives of this study are:

1. Assessment of the viability of biohydrogen production from thin stillage in batch studies, and determination of the optimal substrate to biomass (S_o/X_o) ratio and the maximum hydrogen production potential
2. Comparative evaluation of anaerobic digester sludge and acclimatized anaerobic digester sludge for biohydrogen production
3. Comparative evaluation of single and two-stage anaerobic digestion processes using thin stillage
4. Development of an Artificial Neural Network model for the prediction of biological hydrogen production in batch tests using glucose

1.4. Research Contributions

Hydrogen production potentials of different waste streams have been investigated in the literature using conventional anaerobic digester sludge [Wang and Wan, 2009]. In addition, a two-stage anaerobic digestion process was proven to be more stable than single-

stage digestion with higher methane production rates and yields in the second stage [Demirel and Yenigun, 2002]. The main contributions of this research are:

1. Demonstrating for the first time the advantages of two-stage anaerobic digestion over single-stage for thin stillage treatment from bioethanol plants i.e. increased biogas production and enhanced biosolids destruction efficiency, as a result of improved acidification
2. Emphasization of the need to conduct batch biohydrogen studies using enriched cultures of hydrogen producers derived from short-term continuous-flow systems as opposed to simply useing pre-treated anaerobic digester sludges from existing methanogenic digesters

1.5. Thesis Organization

This thesis includes six 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 Western University. A literature review including background on dark fermentative hydrogen production and its modeling, and two-stage anaerobic digestion process is presented in Chapter 2.

Chapter 3 introduces the idea of using acclimatized anaerobic digester sludge instead of conventional anaerobic digester sludge in biohydrogen production assessment of new wastes. Chapter 4 presents a comparative assessment of single and two-stage anaerobic digestion of thin stillage. Chapter 5 presents an Artificial Neural Network model developed for the analysis of fermentative biohydrogen production in batch studies. Chapter 6

summarizes the major conclusions of this research and provides future work recommendations based on the findings of this study.

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CHAPTER 2

Literature Review

2.1. Introduction

Even though hydrogen is not commercialized as an energy source till now, it is widely used as a chemical reactant in fertilizers production, for diesel refinement, and in ammonia synthesis [Guo et al., 2010]. Hydrogen usage as an energy source has been limited due to high production costs, technical storage requirements, and distribution systems [Dunn 2002]. Biological hydrogen production has the potential to alleviate some of these limitations, since it requires much less energy. Bio-hydrogen can be produced in direct water biophotolysis by green algae, indirect water biophotolysis by cyanobacteria, photofermentation by photosynthetic bacteria, and dark fermentation by strict or facultative anaerobic bacteria [Levin et al., 2004]. Considering that many types of wastes are made up of complex substrates that can be degraded biologically by complex microbial ecosystems, dark fermentation is a key process for the production of hydrogen from food wastes, crop residues, and agricultural wastes [Guo et al., 2010].

2.2. Ethanol Production

Ethanol is a renewable fuel source that can be obtained from a variety of biomass sources. It has been produced from three major groups of feedstocks: sugary feedstocks, such as sugar cane, sugar beet, and sweet sorghum; starchy materials such as corn, wheat, cassava, and sweet potatoes; and lignocellulosic biomass such as wood, straw, and grasses [Balat and Balat, 2009]. Ethanol production via the fermentation route using sugars or starch involves microorganisms such as *Saccharomyces cerevisiae* that ferments the C_6 sugars into ethanol

and other by-products, such as acetic acid [Miller 2010]. Theoretically, 1 kg of glucose produces approximately 514 g (650 mL) of ethanol and 488 g of carbon dioxide, and a bushel of corn (25.3 kg at 15% moisture) can produce from 9.4 to 10.9 L (2.5 to 2.9 gallons) of ethanol [Badger 2002]. From an environmental perspective, ethanol from corn starch biomass presents numerous advantages over petroleum. Corn starch ethanol has high renewable energy content, displacing fossil fuel consumption by almost 26% and reducing greenhouse gas (GHG) emissions by 13% [Vincent 2010].

Ethanol derived from biomass has the potential to be a sustainable transportation fuel, as well as a fuel oxygenate that can replace gasoline [Wu et al., 2006]. Among the different types of feedstock, corn grain is the main feedstock for ethanol production in North America [Kim and Dale, 2004]. Figure 2.1 shows a simplified diagram for a conventional bioethanol process. Milled corn first enters a slurry tank where it is mixed with process water to produce corn slurry. The slurry is then gelatinized in a jet cooker in a process called liquefaction. During liquefaction, the resulting corn mash is typically diluted with addition of thin stillage (backset) prior to fermentation. Fresh water and process water streams such as hot condensate from the evaporator and thin stillage are added to the corn slurry tank or to the mash in the liquefaction to give approximately 80% moisture content [Dale and Tyner, 2006]. The gelatinized mash from the liquefaction process is further hydrolyzed to glucose in a saccharification tank. The glucose-rich stream is then transferred to a fermentation vessel for ethanol fermentation by yeast. Beer from the fermentation tank is distilled and further dehydrated into a fuel grade ethanol.

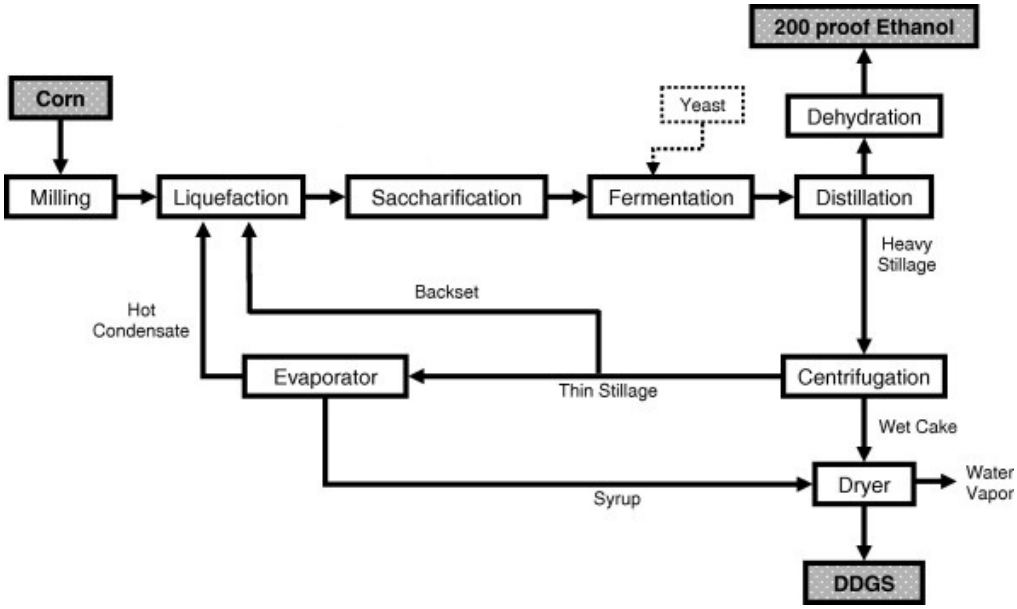

Figure 2.1 – Schematic diagram for a conventional ethanol plant [Kim et al., 2008]

The fermentation process produces highly nutritional co-products which are composed of unhydrolyzed and unfermented components as well as yeasts [Kim et al., 2008]. After fermentation and removal of the ethanol with fractional distillation, the remaining slurry, called whole stillage, is centrifuged to separate solid and liquid streams. The solid part is called wet cake or distillers' grains (DG), while thin stillage which is the liquid stream, is concentrated in evaporators to make condensed distillers' solubles (CDS), commonly known as syrup. The centrifuged solids can be dried alone in rotary drums to produce distillers dried grains (DDG), but are typically added back to the CDS and this mixture is then dried to make distillers' dried grains with solubles (DDGS) [Cassidy et al., 2008]. The DG and DDGS are composed mainly of seed hull, germ, proteins, and oil, and are marketed as animal feed due to their high nutritional value [Mustafa et al., 2000].

2.2.1. Thin Stillage

The production and characteristics of stillage are highly variable and dependent on feedstocks and different aspects of the ethanol production process. However, while the volume and COD concentration of stillage may vary considerably, the total amount of COD produced can be expected to be more consistent with the amounts of feedstock processed and ethanol produced [Wilkie et al., 2000]. Up to 20 litres of stillage may be generated for every litre of ethanol produced, thus necessitating effective solutions for stillage management [Wilkie et al., 2000]. Thin stillage characteristics are influenced by the type of cereal grain that is used in the fermentation process [Mustafa et al., 2000]. Table 2.1 shows the characteristics of corn thin stillage with chemical oxygen demand (COD) that can range from 64,500 mg/L [Ganapathi 1984] up to 100,000 mg/L [Schaefer and Sung, 2008]. The high variance in thin stillage characteristics depends on the efficiency of starch conversion to alcohol in the fermentation process. In the context of biohydrogen, the high COD and carbohydrates concentrations of thin stillage, makes it a strong candidate for biological hydrogen production.

Thin stillage from centrifugation of whole stillage is partially recycled as backset to produce slurry in the liquefaction and makes up 20%-40% of the total water input in the liquefaction [Dale and Tyner, 2006]. Some plants recycle up to 25% of thin stillage to reduce the waste load, conserve energy and water, and ferment residual sugars [Egg et al., 1985]. Therefore, the recirculation of thin stillage reduces water intake and subsequently waste disposal, increases corn processing capacity, and reduces nutrient and buffer requirements [Ahn et al., 2011]. Also, in ethanol plants where stillage must be evaporated before disposal, recycling is employed to reduce evaporation costs [Shojaosadati et al., 1996].

Table 2.1 – Corn thin stillage characteristics

Parameter	Thin Stillage Quality (mg/L)	Reference
TS	90300	Schaefer and Sung, 2008
VS	83500	Schaefer and Sung, 2008
TSS	34200	Schaefer and Sung, 2008
VSS	32900	Schaefer and Sung, 2008
TCOD	64500	Ganapathi 1984
SCOD	30800	Ganapathi 1984
TBOD	26900	Ganapathi 1984
SBOD	19000	Ganapathi 1984
TVFAs as HAc	1310	Khanal et al., 2005
Acetic acid	1000	Ahn et al., 2011
2,3 Butanediol	400	Ahn et al., 2011
Ethanol	300	Ahn et al., 2011
Glycerol	5100	Ahn et al., 2011
Lactic acid	5700	Ahn et al., 2011
Glucose	750	Ganapathi 1984
S-Carb. As glucose	13600	Khanal et al., 2005
Total Protein	4590	Ganapathi 1984
TOC	9850	Ganapathi 1984
TKN as N	755	Ganapathi 1984
NH_3-N	130	Ganapathi 1984
Total P	1170	Wilkie et al., 2000
Total S as SO_4	299	Wilkie et al., 2000
pH	3.7	Ahn et al., 2011

TS: Total solids, VS: Volatile solids, TSS: Total suspended solids, VSS: Volatile suspended solids, TCOD: Total chemical oxygen demand, SCOD: Soluble chemical oxygen demand, TBOD: Total biological oxygen demand, SBOD: Soluble biological oxygen demand, TVFAs: Total volatile fatty acids, S-Carb.: Soluble carbohydrates, TOC: Total organic carbons, TKN: Total Kjehldahl nitrogen

The main concern with thin stillage recirculation without any treatment is the accumulation of fermentation inhibitors such as acetate, lactate, glycerol, and ethanol in the fermentation tank [Julian et al., 1990]. Shojaosadati et al. [1996] studied the effect of stillage recycling on ethanol yields in batches. They observed that the use of up to 50% (v/v) stillage in fermentation media did not greatly affect the alcohol yield. On the other hand, when the volume of stillage used was greater than 50% (v/v), alcohol yield was adversely affected after the third cycle.

2.3. Value of Hydrogen

Bio-hydrogen offers a clean renewable energy source. It does not evolve green house gases, is easily converted to electricity by fuel cells [St-Pierre and Wilkinson, 2001; Cheng et al., 2007], and upon combustion it produces only water [Ginkel and Sung, 2001]. It has a high energy yield of 142.35 kJ/g, which is triple that of any hydrocarbon fuel [Das and Veziroglu, 2001]. However, there are major challenges that hinder the commercialization of biohydrogen production processes including lower hydrogen yields and rates of hydrogen production.

To date, hydrogen is not commercialized as an energy source but it is widely used as a chemical reactant in the production of fertilizers, for refining diesel and for the industrial synthesis of ammonia [Guo et al., 2010].

2.4. Hydrogen Production

Hydrogen production can be classified into chemical-physical and biological methods [Cai et al., 2004]. The chemical-physical methods (e.g., through fossil fuel processing, water electrolysis using solar power) are energy-intensive and expensive [Mizuno et al., 2000]. On the other hand, biological hydrogen production are environmentally favourable and consume less energy.

2.4.1. Bio-Hydrogen Production Processes

Bio-hydrogen can produced following a number of processes including:

- Direct Biophotolysis
- Indirect Biophotolysis
- Photofermentation

- Dark Fermentation

In the following sections, the general description of these methods is provided with their main advantages and disadvantages.

2.4.1.1. Direct BioPhotolysis

Certain green algae can produce hydrogen gas using solar energy to convert water [Ghirardi et al., 2000], which is a readily available substrate into oxygen and hydrogen by the following reaction:

$$2H_2O + \text{light energy} \rightarrow 2H_2 + O_2 \qquad (2.1)$$

The main advantage of this process is its carbon-free nature, where water is split by solar energy producing hydrogen and oxygen [Resnick 2004]. On the other hand, providing solar energy itself is a disadvantage for the process [Das and Veziroglu, 2001] and the main challenge with direct biophotolysis is the need for separation of hydrogen and oxygen which makes the process impractical. Simultaneous hydrogen and oxygen production with this process has achieved very low concentrations of hydrogen due to the need for an inert sparger gas [Hallenbeck and Benemann, 2002]. Maximum hydrogen production rate of 0.07 mmol/L-h [Levin et al., 2004] and solar conversion efficiency of 10% [Melis et al., 2000] were reported using this process.

2.4.1.2. Indirect Biophotolysis

In an indirect biophotolysis process, a certain class of autotrophic microalgae known as cyanobacteria synthesise hydrogen by splitting water in a two step process [Resnick 2004]:

$$6H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (2.2)$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \quad (2.3)$$

In the first step, cyanobacteria convert water and carbon dioxide into glucose and oxygen through a complex process of photosynthesis. In the second step, glucose is broken down into hydrogen and carbon dioxide. The advantage of the indirect biophotolysis over the direct biophotolysis process is that cyanobacteria can utilize nitrogen from the atmosphere to meet its nutritional requirements. One of the disadvantages for this process is the presence of carbon dioxide in the produced gas mixture with oxygen and hydrogen [Das and Veziroglu, 2001]. Maximum hydrogen production rate of 0.36 mmol/L-h was reported using this process which is five times that reported for direct biophotolysis [Kotay and Das, 2008]. Solar efficiency of 10% has been reported using indirect biophotolysis in open ponds [Benemann 1998].

2.4.1.3. Photofermentation

A class of purple non-sulfur bacteria can produce hydrogen in the absence of nitrogen [Levin et al., 2004] by directing the flow of electrons to the reduction of hydrogen instead of fixing nitrogen when growing on poor nitrogen source [Brentner et al., 2010]. They convert glucose and water into hydrogen and carbon dioxide under the following chemical Equation:

$$C_6H_{12}O_6 + 6H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \quad (2.4)$$

Several microalgae have been tested for hydrogen production by photofermentation such as *Rhodopseuodomonas capsulate* [Jouanneau et al., 1984, Levin et al., 2004], *Rhodobacter spheroids* [Resnick 2004], and *Rhodospirillum rubrum* [Resnick 2004]. Different types of wastes such as whey and distillery effluents can be used as a source of glucose in photofermentation. The main disadvantages are the presence of carbon dioxide in the gas mixture and the water pollution caused by the fermented broth that should be wasted after fermentation [Das and Veziroglu, 2001]. A maximum hydrogen production rate of 0.16 mmol/L-h using *Rhodobacter spheroids* was reported by Kotay and Das [2008], and a substrate conversion efficiency of up to 91% using *Rhodopseudomonas palustris* [Brentner et al., 2010].

2.4.1.4. Anaerobic Dark Fermentation

Dark fermentation offers a huge potential for hydrogen production, involving a wide variety of anaerobic bacteria species such as *Clostridium* [Lin et al., 2007], *Enterobacter* [Yokoi et al., 2001], or *Bacillus* [Kalia et al., 1994], activated at different reaction temperatures. It can be divided into mesophilic (25-40°C), thermophilic (40-65°C), extreme thermophilic (65-80°C), or hyperthermophilic (>80°C) [Levin et al., 2004]. Dark fermentative hydrogen production also depends on the type of carbohydrates source, such as glucose, hexose, starch, or cellulose [Guo et al., 2010] and on the process conditions such as the pH [Ginkel and Sung, 2001]. Furthermore, the end products can vary widely, including acetate, butyrate, propionate, lactic acid, and ethanol [Guo et al., 2010].

Among the large range of end products generated by the various microbial metabolisms, acetate and butyrate are the only end products with theoretical yields of four and two moles of hydrogen per each mole of glucose as shown [Batstone et al., 2002]:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2.5)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2.6)$$

However, the accumulation of acetate in the medium does not necessarily imply higher biohydrogen production since several microbial species can convert hydrogen and carbon dioxide to acetate in a hydrogen consuming pathway [Guo et al., 2010]:

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (2.7)$$

The by-products of the fermentation process include propionate, ethanol, and lactic acid. Propionate is a metabolite of a hydrogen-consuming pathway (Equation 2.8), while ethanol and lactic acid are involved in a zero-hydrogen balance pathway (Equations 2.9 - 2.10) [Batstone et al., 2002]:

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (2.8)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (2.9)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH + 2CO_2 \quad (2.10)$$

Nandi and Sengupta [1998] classified the major hydrogen producing and consuming bacteria into: anaerobes (*Clostridia, Methylotrophs, Methanogenic bacteria, Rumen Bacteria,*

Archaea) and facultative anaerobes (*Escherichia coli, Enterobacter*). In a mixed culture, both facultative and anaerobic hydrogen-producing and hydrogen-consuming microorganisms can exist.

Operational conditions highly affect the bacterial metabolism and consequently hydrogen yields. Low hydrogen yields have been achieved in fermentation processes, optimized for biomass instead of hydrogen production [Hallenbeck and Benemann, 2002]. In order to maximize the hydrogen yield, substrate metabolism should be directed towards the production of volatile fatty acids (VFAs) instead of alcohols or lactic acid. The following sections will review the main parameters that affect fermentative biohydrogen production.

2.4.2. Factors Affecting Dark Fermentative Bio-Hydrogen Production

2.4.2.1. pH

It is important to regulate pH during a biohydrogen production process, because it affects the hydrogen production yields and the by-products and microbial community structure [Ye et al., 2007; Temudo et al., 2007; Ginkel and Sung, 2001]. Table 2.2 shows the optimum initial pH values for various substrates.

Generally, batch and continuous-flow experiment studies have shown that the initial pH has a significant effect on hydrogen yields, hydrogen production rates, and VFAs concentrations. However, the trends are not consistent. Optimal hydrogen production was achieved at a pH range of 5.0-6.0 for food wastes [Shin and Youn, 2005; Kim et al., 2004], while a neutral pH was recommended for crop residues and animal manure [Li and Chen, 2007; Yokoyama et al., 2007].

Li and Chen [2007] investigated a wide range of initial pH values varying from 4 to 8 in batch tests, where optimum conversion of corn straw to biohydrogen with maximum

hydrogen production yields occurred at pH of 7.0-7.5. In continuous-flow reactors, pH is usually controlled. Shin and Youn [2005] tested pH values in the range of 5.0 to 6.0 using food waste in a continuous stirred tank reactor (CSTR) operated at an organic loading rate (OLR) of 8 gVS/L-d, HRT of 5 days, and under thermophilic conditions of 55°C and found that a pH of 5.5 was optimum for hydrogen production. A similar value was proposed in another study using brewery waste in a CSTR operated at an OLR of 70 gCOD/L-d, HRT of 18 hours, and under mesophilic conditions of 37°C with a pH ranging from 5.0 to 6.5 [Fan et al., 2006a]. Using synthetic waste as glucose, sucrose, and starch, most experiments found an optimum range for pH of 5.0-6.0 [Lay 2000; Masset et al., 2010; Hwang et al., 2009; Jun et al., 2008; Wang et al., 2005], while Lee et al. [2008] found an optimum pH of 7.0 using starch in batch experiments. The disagreement on the optimal initial pH is due to differences in the inoculums used, substrate type and concentration, and operational temperature.

Table 2.2 – Optimal pH for Biohydrogen production

Substrate	Inoculum	Reactor (S_o/X_o)[1]	Temp. °C	H_2 Yield[2]	pH range	Optimum pH_i	Ref.
Cattle wastewater	Sewage sludge	Batch (1.24)	45	12.3 $mmol/gCOD_{consumed}$	4.5-7.5	5.5	Tang et al., 2008
Food waste	ADS[4]	CSTR (8)[3]	55	1.83 mol/mol_{hexose}	5-6	5.5	Shin and Youn, 2005
Corn straw	Clostridium butyricum	Batch	35	2.55 $mmol/g_{substrate}$	4-8	7-7.5	Li and Chen, 2007
Vegetable kitchen waste	Compost	Batch (10)	55	0.4 mmol/gCOD	5.5-7	6-7	Lee et al., 2008
Glucose	ADS	Batch (1.5)	25	0.89 $mol/mol_{glucose}$	6.2-7.5	6.2	Oh et al., 2003
Glucose	Clostridium butyricum	Batch	30	1.53 $mol/mol_{glucose}$	4.5-7.5	5.2	Masset et al., 2010
Glucose	ADS	Chemostat (15)[5]	35	1.51 $mol/mol_{glucose}$	5.5-6.2	5.8	Hwang et al., 2009
Glucose	River sludge	Batch (8)[5]	37	1.63 $mol/mol_{glucose}$	5-7	7	Li et al., 2008
Starch	Clostridium butyricum	Batch	30	1.8 mol/mol_{hexose}	4.5-7.5	5.6	Masset et al., 2010
Sucrose	Sewage sludge	Batch (10.7)[5]	30	4.73 mmol/gCOD	3-10	5	Jun et al., 2008
Sucrose	ADS	Batch (25.9)[5]	35	3.19 $mol/mol_{sucrose}$	4.7-6.0	5.5	Wang et al., 2005

[1] substrate to biomass ratio (gCOD/gVSS) for batches

[2] H_2 Yield*: at standard temperature and pressure (STP) conditions of 0°C and 1 atm

[3] OLR (gVS/L-d)

[4] ADS: Anaerobic digester sludge

[5] Substrate concentration (gCOD/L)

In addition, the concentrations of different VFAs vary with pH. Butyrate and acetate are the two main by-products of biohydrogen production, and they are favourably produced at pH ranging between 4.5 and 6.0 and found that the lower the pH, the greater is the butyrate/acetate ratio [Guo et al., 2010]. At neutral or higher pH conditions, ethanol and propionate, both of which are not conducive to hydrogen production, were found to accumulate [Kim et al., 2004]. Fan et al. [2006a] also found that acetate and butyrate were predominant at pH lower than 6.0, while other by-products as propionate and ethanol were found at higher pH using brewery as the substrate. This was confirmed by Fang et al. [2006] in a study investigating the effect of pH from 4.0 to 7.0 on by-product formation. At low pH, butyrate and acetate were dominant products while ethanol, lactate, and propionate appeared at higher pHs. In the aforementioned study, the optimal pH was found to be 5.5 with a hydrogen yield of 346 $mL/g_{carbohydrates}$ using rice waste as the substrate. Temudo et al. [2008] studied the impact of the pH on metabolic activity and microbial diversity in fermentation processes with glucose, xylose, and glycerol at 30°C. The experiments showed that at pH less than 6, the by-products consisted mainly of butyrate and acetate while at higher pH above 6, the products shifted to acetate and ethanol. It was also noticed in the DGGE analysis that under both high and low pH conditions, the fermentation pattern was clearly associated with the dominance of Clostridium species, whereas at intermediate pHs, metabolic shifts involved higher microbial diversity [Temudo et al., 2008]. Thus, pH not only affects the metabolic pathway but also the microbial community.

2.4.2.2. Temperature

Temperature is one of the most important factors that affect both biohydrogen production yields and microbial metabolism [Guo et al., 2010]. Fermentation reactions can be operated at mesophilic [Wang and Wan, 2008a], thermophilic [Shin and Youn, 2005], extreme thermophilic [van Niel et al., 2002], or hyper-thermophilic conditions [Nakashimada and Nishio, 1999]. Within the optimum temperature ranges, hydrogen production increases as the temperature increases, but the activity of hydrogen producing bacteria rapidly decrease outside the optimum range [Wang and Wan, 2008a].

Table 2.3 summarizes several studies that investigated the optimum temperature for biohydrogen production. No specific optimum temperature has been determined for biohydrogen production because of the complexity of the wastes as well as the variable operating conditions, though most fermentative hydrogen production studies have been operated at mesophilic conditions [Guo et al., 2010].

Table 2.3 – Optimal temperature for Biohydrogen production

Substrate	Inoculum	Reactor (S_o/X_o)[1]	pH_i	H_2 Yield[2]	Temperature range (°C)	Optimum (°C)	Ref.
Cow waste slurry	Cow waste slurry	Batch	-	14.4 $mmol/L_{slurry}$	37-85	60	Yokoyama et al., 2007
Cattle wastewater	Sewage sludge	Batch (1.24)	5.5	12.3 $mmol/gCOD_{consumed}$	30-55	45	Tang et al., 2008
Rice slurry	ADS[4]	Batch	4.5	13.7 $mmol/g_{carbohydrates}$	37-55	37	Fang et al., 2006
Organic waste	ADS	Semi-continuous (11)[3]	6.4	13.5 mmol/gVS	37-55	55	Valdez-Vazquez et al., 2005
Glucose	ADS	Batch (0.91)	7.0	10.8 $mmol/g_{glucose}$	20-55	40	Wang and Wan, 2008a
Glucose	ADS	Batch (10.7)[5]	5.5	1.45 $mol/mol_{glucose}$	33-41	41	Mu et al., 2006a
Sucrose	Sewage sludge	Granular sludge bed reactor (19)[5]	6.7	3.38 $mol/mol_{sucrose}$	30-45	40	Lee et al., 2006
Sucrose	ADS	Batch (25.9)[5]	5.5	3.19 $mol/mol_{sucrose}$	25-45	35.1	Wang et al., 2005
Xylose	Sewage sludge	Chemostat (40)[5]	7.1	1.18 mol/mol_{xylose}	30-55	50	Lin et al., 2008
Starch	Sewage sludge	Batch (10)	6.0	8.34 $mmol/g_{starch}$	37-55	37	Lee et al., 2008

[1] substrate- to-biomass ratio (gCOD/gVSS) for batches

[2] H_2 Yield*: at standard temperature and pressure (STP) conditions

[3] gVS/Kg-d

[4] ADS: Anaerobic dugester sludge; pH_i: Initial pH

[5] Substrate concentration (gCOD/L)

As shown in Table 2.3, although different optimum temperatures were investigated for different substrates, most of them were in the range of mesophilic and thermophilic conditions between 35 and 60°C [Wang et al., 2005; Yokoyama et al., 2007]. Wang and Wan [2008a] investigated a wide range of temperature (20-55°C) for batch glucose fermentation using ADS and observed an increase in the volumetric hydrogen production and rate, as well as a decrease in the lag phase with the increase in temperature from 20 to 40°C. In the same study, the authors reported an increase in the acetate concentration with increasing the temperature from 20 to 35°C, and then a decrease with further increase in the temperature till 55°C. Tang et al. [2008] reported that the optimum temperature for biohydrogen production using cattle wastewater to be 45°C, at which they observed higher butyrate and acetate concentrations and minimum propionate and ethanol concentrations. These findings were consistent with Mu et al. [2006a] who observed the lowest propionate and ethanol concentrations with highest acetate and butyrate concentrations at the reported optimum temperature of 41°C using glucose as the substrate.

Agricultural wastes usually achieve higher yields at thermophilic conditions due to the better hydrolysis for the lignocellulosic compounds. Pakarinen et al. [2008] used grass as the substrate and achieved maximum hydrogen yield of 16 mL/gVS at 70°C. A wide temperature range from 37 to 85°C was investigated by Yokoyama et al. [2007] using cow waste slurry as both substrate and inoculum. A maximum hydrogen yield of 392 mL/L_{slurry} was achieved at a temperature of 60°C. DGGE analysis showed that the predominant bacteria at 60°C were *Clostridium stercorarium* and *Clostridium thermocellum* [Yokoyama et al., 2007]. The main disadvantage of thermophilic fermentative hydrogen production processes is the energy requirement for heating and maintenance [Guo et al., 2010].

2.4.2.3. Inoculum

Many studies investigated the use of mixed cultures for fermentative hydrogen production. Mixed cultures (can be obtained from many sources such as anaerobic sludge digesters [Morimoto et al., 2004; Zhu and Beland, 2006], natural microflora [Ling et al., 2009; Li et al., 2008] and composts [Ginkel and Sung, 2001; Fan et al., 2004]) for the degradation of either simple sugars as glucose and sucrose [Mu et al., 2006a; Zhang et al., 2005], or complex substrates such as food wastes and brewery mixtures [Chen et al., 2006a; Fan and Chen, 2004]. On the other hand, many studies have explored the use of known pure cultures for hydrogen production [Lin et al., 2007]. The main advantage of using pure cultures is preventing microbial shifts which are problematic in mixed cultures.

Many pure cultures have been tested for hydrogen production from different substrates. Table 2.4 summarizes selected experiments that used pure cultures for fermentative hydrogen production. It was found that Clostridium and Enterobacter genus were most widely used than any other genus. Species of genus *Clostridium* such as *C. beijerinckii, C. butyricum, C. acetobutylicum, C. pasteurianum* are gram-positive, rod-shaped, strict anaerobes and endospore formers, while *Enterobacter* species as *E. Cloacae* and *E. Aerogenes* are gram-negative, rod-shaped, and facultative anaerobes [Li and Fang, 2007]. Most studies use *Clostridium* bacteria for its high hydrogen yields [Lin et al., 2007; Yokoi et al., 2001]. It is noteworthy that in a DGGE analysis of a mixed culture producing hydrogen yield of 1.22 mol/mol $hexose_{consumed}$ from sucrose at mesophilic conditions in a CSTR revealed the predominance of *Clostridium* bacteria [Ogino et al., 2005].

Enterobacter cloacae and *aerogenes* are facultative bacteria that can produce hydrogen anaerobically with high hydrogen yields of 2.2 $mol/mol_{glucose}$ [Kumar and Das, 1999] but usually lower than that produced by *Clostridium* species of 2.81 $mol/mol_{glucose}$ [Lin

et al., 2007]. The main disadvantage of using pure cultures is the strict sterilization and anaerobic media that should be maintained during the process which is impractical on a large industrial scale [Hawkes et al., 2002]. Also, to avoid microbial contamination from real wastes, most of the studies were done on synthetic wastewater.

Table 2.4 – Pure cultures for fermentative hydrogen production

Inoculum	Substrate	Reactor	H2 Yield $mol/mol_{substrate}$	Temperature (°C)	Ref.
Clostridium beijerinckii	Glucose	Batch	2.81	35	Lin et al., 2007
Clostridium beijerinckii	Starch	Batch	1.80	36	Taguchi et al., 1992
Clostridium butyricum	Glucose	Batch	2.29	35	Lin et al., 2007
Clostridium butyricum	Starch	Batch	2.40	37	Yokoi et al., 2001
Clostridium butyricum	Glucose	Continuous	2.22	37	Heyndrickx et al., 1990
Clostridium butyricum	Sucrose	Batch	2.91	37	Chen et al., 2005
Clostridium butyricum	Xylose	Batch	0.73		Lo et al., 2008
Clostridium acetobutylicum	Glucose	Batch	1.80	35	Lin et al., 2007
Clostridium tyrobutyricum	Glucose	Batch	1.47	35	Lin et al., 2007
Clostridium pasteurianum	Glucose	Continuous	2.16	37	Heyndrickx et al., 1990
Clostridium thermocellum	Lactose	Continuous	3.00		Collet et al., 2004
Clostridium thermocellum	Cellulose	Batch	0.80	60	Liu et al., 2008
Clostridium sp. No. 2	Glucose	Batch	2.00	36	Taguchi et al., 1993
Clostridium sp. No. 2	Glucose	Continuous	2.36	36	Taguchi et al., 1995
Clostridium sp. No. 2	Arabinose	Batch	2.20	36	Taguchi et al., 1993
Clostridium sp. No. 2	Xylose	Batch	2.10	36	Taguchi et al., 1993
Clostridium sp. No. 2	Xylose	Continuous	2.06	36	Taguchi et al., 1995
Enterobacter cloacae	Glucose	Batch	2.20	36	Kumar and Das, 1999
Enterobacter cloacae	Sucrose	Batch	6.00	36	Kumar and Das, 1999
Enterobacter cloacae	Cellobiose	Batch	5.40	36	Kumar and Das, 1999
Enterbacter aerogenes	Glucose	Batch	1.00	35	Yokoi et al., 1995
Enterbacter aerogenes	Sucrose	Batch	1.89	35	Yokoi et al., 1995
Enterbacter aerogenes	Glycerol	Batch	0.60		Nakashimada et al., 2002

Table 2.4 (cont.) – Pure cultures for fermentative hydrogen production

Inoculum	Substrate	Reactor	H2 Yield $mol/mol_{substrate}$	Temperature (°C)	Ref.
Enterbacter aerogenes	Starch	Batch	1.09		Fabiano and Perego, 2002
Enterbacter aerogenes	Glycerol	Batch	0.60		Nakashimada et al., 2002
Enterbacter aerogenes	Starch	Batch	1.09		Fabiano and Perego, 2002
Escherichia coli	Glucose	Batch	2.00		Bisaillon et al., 2006
Escherichia coli	Glucose	Continuous	2.00		Turcot et al., 2008
Thermotoga elfii	Glucose	Batch	2.80	65	van Niel et al., 2002
Thermoanaerobacterium thermosaccharolyticum	Glucose	Batch	2.43	60	O-Thong et al., 2008
Thermoanaerobacterium thermosaccharolyticum	Sucrose	Batch	5.06	60	O-Thong et al., 2008
Thermoanaerobacterium thermosaccharolyticum	Starch	Batch	2.80	60	O-Thong et al., 2008
Enterobacter aerogenes + *Clostridium butyricum*	Starch	Batch	1.7	37	Yokoi et al., 2001
Enterobacter aerogenes + *Clostridium butyricum*	Starch	Batch	540*	36	Yokoi et al., 1998
Enterobacter aerogenes + *Clostridium butyricum*	Sweet potato	Batch			Yokoi et al., 2002
Clostridium thermocellum + *Thermoanaerobacterium thermosaccharolyticum*	Cellulose	Batch	1.8	60	Liu et al., 2008
Clostridium acetobutylicum + *Ethanoigenes harbinense*	Cellulose	Batch	16.2**	37	Wang et al., 2008

* Volumetric hydrogen (mL)

** $mmol/g_{cellulose}$

Mixed cultures have been widely used for biohydrogen production experiments since they are simpler to operate, easier to control, and can utilize more varieties of real wastes, which makes them more practical [Li and Fang, 2007]. A wide range of microbial sources has been used as inocula for biohydrogen production, including anaerobic sludge from municipal wastewater plants and cow dung composts [Chu et al., 2008; O-Thong et al., 2008; Tang et al., 2008], cattle or dairy residue composts [Fan et al., 2006a; Fan et al., 2004], sludge from palm oil mill effluent [Vijayaraghavan and Ahmad, 2006; Chong et al., 2009a], soil, rice straw compost, and fermented soy bean meal [Noike and Mizuno, 2000]. Biohydrogen production is impacted by the inoculums origin [Akutsu et al., 2008]. Tang et al., [2008] compared four different natural mixed microflora of sludge from sewage treatment, cow dung compost, chicken manure compost, and river sludge for fermentative hydrogen production from cattle wastewater, and concluded that sewage sludge achieved the highest hydrogen production.

In order to increase the hydrogen yield, some studies used mixed pure cultures. Yokoi et al., [1998, 2001, 2002] used a mixture of *Clostridium* and *Enterobacter* species to avoid using L-cysteine, which is an expensive reducing agent used to assure completely anaerobic conditions for *Clostridium* bacteria. Liu et al., 2008] used two thermophilic anaerobic bacteria to produce hydrogen from cellulose. *Clostridium thermocellum* cannot completely utilize the cellobiose and glucose produced by the degradation of cellulose with a hydrogen yield of 0.8 mol/$mol_{glucose}$ in a monoculture batch, with lactate as the main by-product. However, when *Clostridium thermocellum* was co-cultured with *Thermoanaerobacterium thermosaccharolyticum*, hydrogen yield increased to 1.8 mol/$mol_{glucose}$ and butyrate was the main by-product while lactate was not detected. Wang et al., [2008] observed no lag phase in hydrogen production batches when using a co-culture of *Clostridium acetobutylicum and*

Ethanoigenes harbinense. *Ethanoigenes harbinense* rapidly removed the reduced sugar produced by cellulose hydrolysis by *Clostridium acetobutylicum, hence improved cellulose hydrolysis and hydrogen production rates.*

2.5. Laboratory Bioreactors Used for Bio-Hydrogen Production

In laboratory scale, most studies for biohydrogen production are conducted in batch reactors Pakarinen et al., 2008, Fan et al., 2006b], since they are easily operated and efficiently controled. However, from an industrial perspective, continuous-flow bioreactors should be more investigated for practical and economic considerations. Continuous-flow hydrogen production reactors include completely mixed, packed-bed, fluidized-bed, sequencing batch reactor, trickling biofilter, and membrane bioreactors. Table 2.5 shows different bioreactors configurations for biohydrogen production using various substrates.

Table 2.5 – Reactors configuration for Biohydrogen production

Reactor	Substrate	Inoculum	OLR (gCOD/L-d)	HRT (h)	H_2 Yield*	Temperature (°C)	Ref.
Batch	Molasses	Soil	-	-	4.18 mmol/gCOD	26	Logan et al., 2002
SBR	Sucrose	WAS	88		1.15 mol/mol_{hexose}	35	Lin and Jo, 2003
ASBR	Food waste	ADS	27	24	2.51 mmol/gVS	35	Kim and Shin, 2008
CSTR	Sugar factory wastewater	Compost	-	12	11.8 mmol/gCOD	60	Ueno et al., 1996
CSTR	Noodle wastewater	ADS	-	18	7.44 mmol/gCOD	35	Noike 2002
CSTR	Sugar beet wastewater	ADS	-	15	8.68 mmol/gCOD	32	Hussy et al., 2005
PBR	Sugar & ethyl alcohol wastewater	ADS	-	8	-	37	Kim 2002
UASB	Sucrose	WAS	52		1.95 mol/mol_{hexose}		Fang et al., 2002
SCRD	Food waste	Anaerobic granular sludge	-	-	2.54 mmol/gVS	40	Wang and Zhao, 2009
Biohydrogenator	Corn syrup	ADS	81	8	17 mmol/gCOD	37	Hafez et al., 2009b
SBHR	Glucose	ADS	46	12	1.85 mol/mol	37	Elbeshbishy and Nakhla, 2011

CSTR: continuous stirred tank reactor; PBR: packed-bed reactor; ADS: anaerobic digester sludge; SBR: sequencing batch reactor; UASB: up-flow anaerobic sludge blanket reactor; WAS: waste activated sludge; SCRD: semi-continuous rotating drum; SBHR: sonicated biological hydrogen reactor

*H_2 Yield at STP conditions

Guo et al. [2010] indicated that no biohydrogen industrial scale reactor has been set up, but expected to be similar in design and system configuration to methane plants bioreactors. However, biohydrogen production reactors will differ in the operational conditions. CSTRs are the most common design for anaerobic hydrogen production studies [Kotsopoulos et al., 2009; Lay 2001]. Other studies reported successful hydrogen production in anaerobic sequencing batch reactors [Lin and Jo, 2003; Kim and Shin, 2008]. Jayalakshmi et al. [2009] set up a 0.15 m^3 inclined plug-flow pilot scale bioreactor fed kitchen waste at 7 Kg/day using heat treated biogas-plant slurry as inoculum. The plant achieved a 40% VS destruction efficiency and a hydrogen yield of 72 mL/gVS_{added}.

In a conventional CSTR, biomass is well suspended in the liquid and therefore the solid retention time (SRT) is the same as the hydraulic retention time (HRT). At short HRTs of 3-8 hours, biomass washout can occur due to high dilution rates [Hafez et al., 2009a]. To overcome this problem, decoupling of SRT from HRT has been achieved by using biofilms on different media such as activated carbon, glass beeds [Zhang et al., 2006], and by using membranes [Vallero et al., 2005]. Fang et al. [2002] achieved a hydrogen yield of 2.2 mol/mol_{hexose} in an up-flow anaerobic sludge blanket reactor (UASB), using sucrose as the substrate at an HRT of 6 hours. The problem with UASBs is its long start-up time, as well as problems with particle granulation. Hafez et al. [2009b] introduced a novel system for biohydrogen production that included a gravity settler with a completely-mixed biohydrogen reactor for decoupling of SRT from HRT. Using corn syrup as the substrate, the aforementioned authors achieved a maximum hydrogen yield of 430 mL/gCOD at a loading rate of 81 gCOD/L.d and HRT of 8 days. Another novel system was introduced by Elbeshbishy and Nakhla [2011] by integrating an ultrasonic probe in a CSTR and was called sonicated biological hydrogen reactor (SBHR). The authors compared biohydrogen

production from glucose at a loading rate of 46 gCOD/L.d and HRT of 2 days using a conventional CSTR with the SBHR and found that hydrogen yield was enhanced from 1.2 to 2.1 mol/$mol_{glucose}$ in the CSTR and the SBHR, respectively.

2.6. Bio-Hydrogen Production Challenges

Biological hydrogen production processes are increasing in popularity because they can utilize renewable energy resources, and can usually be operated at ambient temperature and atmospheric pressure [Cai et al., 2004]. However, the reported biohydrogen production rates, stabilities and efficiency of these processes are still insufficient to make them commercially viable. Major challenges need to be overcome so as to transfer hydrogen production process from laboratory to industrial scale [Kotay and Das, 2008; Das et al., 2008]. These challenges are:

- Insufficient knowledge on the metabolism of hydrogen producing bacteria
- Low yields obtained using renewable biomass
- Sensitivity of hydrogenase to oxygen and hydrogen partial pressure that leads to low hydrogen yields
- High cost of suitable feedstock (glucose) or processing biomass feed stocks
- Hydrogen separation, purification, and storage
- A lack of understanding on the improvement of economics of the process by integration of hydrogen production with other processes

2.7. Two-Stage Anaerobic Digestion Process

Separating the acidogenic and methanogenic steps in the anaerobic digestion process, provides enhanced stability to the different groups of microorganisms and better process control [Demirel and Yenigun, 2002]. The purpose of a two-stage anaerobic digestion system is not only to further degrade waste, but also to extract more net energy from the system [Thompson 2008]. In a single-stage anaerobic digestion process, a variety of higher organic acids, such as propionic, butyric, and lactic, as well as alcohols and ketones, are formed during the breakdown of the organic substrates by acidogens. However, in a well operated process, these products are mostly converted to acetic acid and hydrogen, which, in turn, are converted to methane gas [Cooney et al., 2007]. On the other hand, in a two-stage anaerobic digestion process, the end products from acidification stage are usually ideal for anaerobic treatment with high VFAs concentrations [Pavan et al., 2000].

Vinas et al. [1993] used a two-stage process and achieved an increase in the methane production yield of 13% over the single-stage process using a cellulosic material as the substrate. Similarly, Rincon et al. [2009] achieved an increase of 10% by employing a two-stage process in methane yield using olive mill solid residue as the substrate over the single-stage process. Although acidification stage was used in many studies as a pretreatment for anaerobic digestion, biohydrogen production was not considered in the first stage.

Despite their higher loading rates, improved process stability and flexibility, there are relatively few commercial two-stage anaerobic digestion units. The added complexity and expense of building and operating commercial two-stage systems have so far counteracted the yield and rate enhancements [Rapport et al., 2008]. The theoretical higher biogas yields have also been questioned since the acidogenic phase separation prevents the hydrogen to methane pathway [Reith et al., 2003].

In the acidification stage, a variety of VFAs by-products are produced. In a biohydrogen production process, the larger the acetate to butyrate ratio the higher the hydrogen yield [Hafez et al., 2010a], which indicates that the hydrogen-producing acetate and butyrate pathways were favoured rather hydrogen consuming pathways. It is well known that in a methane reactor, 67% of the methane is produced by acetate-utilizing methanogens and 33% is produced by hydrogenophilic methanogens [Kotsyurbenko et al., 2004]. Many studies investigated the effect of pH and HRT on hydrogen production and concluded that the optimum pH is 5.5 and optimal HRT is in the range of 3-8 hours [Hafez et al., 2009b]. In addition, the by-products were primarily acetic, which is favourable for acetate-utilizing methanogens [Kotsyurbenko et al., 2004], and butyric acids [Hafez et al., 2010b], On the other hand, a wide range of pH (4.5-7) and HRT (2-5 days) was reported for the acidification stage with negligible hydrogen production and presence of by-products such as lactic acid, propionic acid, or ethanol that are not as favourable as acetate for methane production [Elbeshbishy and Nakhla, 2011; Takashima and Tanaka, 2010]. Therefore, the more acetate produced in the first stage (i.e. more hydrogen produced), the more methane produced in the second stage, which emphasises the importance of maximizing the first stage for hydrogen production and not for acidification only, which will subsequently maximize the methane production in the second stage.

In a two-stage anaerobic digestion, Elbeshbishy and Nakhla [2011] studied the impact of food waste treatment by sonication in the first stage of hydrogen production on the second stage methane production. For the first stage of hydrogen production, the aforementioned authors observed an increase in the hydrogen yield by 27% for the sonicated feed over the unsonicated one, accompanied with an increase of 28% and 53% in the acetate and butyrate concentrations, respectively. In the second stage, they observed an increase of 17% in the

methane yield as a result of sonication. The aforementioned authors also compared the performance of a conventional single and two-stage anaerobic digestion processes with unsonicated substrate. They observed a 39% increase in the methane production rate in the two-stage over the single-stage process.

Cooney et al. [2007] also studied the effect of different dilution rates in the first stage of hydrogen production on both hydrogen and methane yields in the first and second stage respectively. The authors used glucose as the substrate and conventional anaerobic digester sludge as the inoculums. By increasing the dilution rate from 2 to 2.5 d^{-1}, they observed an increase in the hydrogen production rate by 53% in the first stage, followed by an increase in the methane production rate by 60% in the second stage. A further increase in the dilution rate to 3 d^{-1} lead to a sharp decrease in both hydrogen and methane production rates by 29% and 11% , respectively, which emphasizes the impact of the first stage on the second stage in a two-stage anaerobic digestion process.

Anaerobic hydrogen production achieves low COD removal efficiencies [Mohan 2009; Chong et al., 2009b], however when followed by a second stage methane production, the overall COD reduction efficiency increases over that in a single stage anaerobic digestion process [Park et al., 2010]. Elbeshbishy and Nakhla [2011] achieved an increase of 16% in the overall COD reduction efficiency from food wastes using a two-stage anaerobic digestion process over a single-stage operating at an HRT of 2 days for a hydrogen production CSTR and 7 days for the methane digester.

2.8. Bio-Hydrogen Production Modeling

Mathematical models are very important to provide information such as the type and concentration of substrate and VFAs, headspace pressure release methods, pH, and

temperature; i.e, how the different factors affecting biohydrogen production processes impact system performance. For the design and optimization of bioreactors the conventional "one factor at-a-time" experimental optimization method is ineffective, since it does not take into consideration the interaction between these factors. Some studies investigated the combined effect of two variables such as pH and substrate concentrations [Ginkel and Sung, 2001; Li et al., 2008], temperature and pressure release methods [Gadhamshetty et al., 2009], and pH and sulphate concentration [Hwang et al., 2009] on the biohydrogen production process. However, it is very difficult to conduct studies with more than three variables [Gadhamshetty et al., 2010].

Most studies on biohydrogen production modeling used modified Gompertz equation for batch experiments (Equation 2.11) [Elbeshbishy et al., 2010; Wang and Wan, 2009a; Gadhamshetty et al., 2010]. The modified Gompertz equation is an empirical formula, which includes three parameters that are used to fit the equation: lag time, hydrogen production potential, and hydrogen production rate as shown below:

$$P = P_{max} \exp\left\{-\exp\left[\frac{R_{max} e}{P_{max}}(\lambda - t) + 1\right]\right\} \quad (2.11)$$

where P is the cumulative hydrogen production, P_{max} is the maximum cumulative hydrogen production, R_{max} is the maximum hydrogen production rate, λ is the lag time, and t is the fermentation time.

Although high correlation coefficients are obtained between observed and predicted data [Ginkel and Sung, 2001], the model has limited predictive ability. In addition, due to the empirical nature of the model, it does not take into consideration the effect of many

important parameters such as the substrate concentration, pH, and temperature. Some studies used the modified Gompertz model to describe the progress of the biomass growth, VFAs concentration and substrate degradation, where P denoted the cumulative degraded substrate, cumulative biomass growth value, or cumulative VFA concentration, and P_{max} denoted the maximum cumulative degraded substrate, maximum cumulative HPB growth value, or maximum cumulative VFA concentration [Mu et al., 2006b].

Some studies used the modified Logistic model (Equation 2.12), which has a very similar curve to that of Gompertz model to describe hydrogen production in batch tests [Wang and Wan, 2008b; Nath et al., 2008]. Mu et al. [2007a] compared the ability of the modified Gompertz model, modified Logistic model, and modified Richards to describe the biomass growth in batch tests and concluded that the modified Gompertz was the most suitable model. Other studies used the conventional Monod kinetics to describe the biohydrogen production rates [Lee et al., 2008; Zheng and Yu, 2005] or the biomass growth [Kumar et al., 2000; Nath et al., 2008].

$$H = \frac{H_{max}}{1+\exp[4R_{max}(\lambda-t)/H_{max}+2]} \qquad (2.12)$$

where H is the cumulative hydrogen value, H_{max} is the maximum cumulative hydrogen value, R_{max} is the maximum rate of hydrogen production, λ is the lag time, and t is the fermentation time.

The Anaerobic Digestion Model 1 (ADM1) is a mechanistic model that integrates biokinetics with association-dissociation, gas-liquid transfer, and cellular processes involving hydrolysis, acidogenesis, acetogenesis, and methanogenesis [Batstone et al., 2002]. ADM1 was

successfully used for describing methane production in many studies [Jeong et al., 2005; Antonopoulou et al., 2012]. Peiris et al. [2006] modified the ADM1 to describe biohydrogen production by adding two intermediate products (lactate and ethanol) that were excluded from the model due to their low impact on the methanogenic process. The modified model was able to predict the bioreactor pH well but failed to predict the hydrogen and biomass yields accurately. The problems with the aforementioned empirical models include:

- Inability to predict the process with various input parameters
- Limited number of parameters taken into consideration when studying the interactive effects among them

Furthermore, the main criticism of the complex mechanistic ADM model is its extensive input of kinetic and stoichiometric parameters.

2.8.1. Artificial Neural Network for Bio-Hydrogen Production Modeling

Artificial Neural Network (ANN) is a mathematical representation of the neurological functioning of a brain. It simulates the brain's learning process by mathematically modeling the network structure of interconnected nerve cells [Nagata and Chu, 2003]. ANN is a powerful modeling tool for problems where the parameters that govern the results are either not defined properly or too complex [Flood and Kartam, 1994]. It is able to describe the interactive effects among these different parameters in a complicated bioprocess [Wang and Wan, 2009b]. ANN is capable of modeling these complex relationships between input and output parameters without requiring a detailed mechanistic description of the phenomena that is governing the process [Shi et al., 2010].

A typical neural network has an input layer, one or more hidden layer, and an output layer. The neurons in the hidden layer, which are linked to the neurons in the input and

output layers by adjustable weights, enable the network to compute complex associations between the input and output variables [Nagata and Chu, 2003]. Training the model is the process of determining the adjustable weights and it is similar to the process of determining the coefficients of a polynomial by regression. The weights are initially selected in random and an iterative algorithm is then used to find the weights that minimize the differences between the model-calculated and the actual outputs.

The most commonly used algorithm in ANN is the back propagation [Nagata and Chu, 2003]. In this training algorithm, the error between the model results of the output neurons and the actual outputs is calculated and propagated backward through the network. The algorithm adjusts the weights in each successive layer to reduce the error. This procedure is repeated until the error between the actual and network-calculated outputs satisfies a pre-specified error criterion [Nagata and Chu, 2003].

ANN has gained an increasing consideration in wastewater treatment and biogas production [Cinar et al., 2006; Choi and Park, 2001; Chen et al., 2008; Lemoine et al., 2003; Wang et al., 2009]. Hamed et al., [2004] used ANNs to model the effluent biochemical oxygen demand (BOD) and suspended solids (SS) concentration at a major wastewater treatment plant. Another use for the ANN was to predict the effluent wastewater quality parameters such as effluent COD or total Kjehldahl nitrogen (TKN) concentrations [Aguado et al., 2006].

A few studies in the literature investigated the modeling of biohydrogen production in batch studies using ANN. Wang and Wan [2009b] studied the effects of temperature, initial pH, and glucose concentration on fermentative hydrogen production by mixed cultures in batch tests. The ANN model successfully described the effects of these parameters on the substrate degradation efficiency, hydrogen yield, and average hydrogen production rate.

Shi et al. [2010] presented a back propagation neural network (BPNN) that accurately predicted the steady-state performance of bioreactors for biohydrogen production using sugar refinery wastewater in an integrative biological reactor (IBR), which is the integration of a CSTR and a UASB reactor. The model consisted of 4 neurons in the input layer of volume loading rate (VLR), oxidation-reduction potential (ORP), alkalinity, and pH, three neurons in a single hidden layer, and hydrogen production rate as the output of the model.

Another continuous flow system performance was simulated using ANN by Mu and Yu 2007b]. A model was designed, trained and validated to predict the steady-state performance of a granular-based hydrogen-producing UASB reactor. OLR, HRT, and influent bicarbonate alkalinity were the model inputs, while the output variable was either hydrogen concentration, hydrogen production rate, hydrogen yield, effluent total organic carbon, or effluent aqueous products including acetate, propionate, butyrate, valerate, and caporate. The model effectively described the daily variations of the UASB reactor performance and predicted the steady-state performance at various substrate concentrations and HRTs.

2.9. References

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CHAPTER 3

Bio-Hydrogen Production from Thin Stillage using Conventional and Acclimatized Anaerobic Digester Sludge

3.1. Introduction

Hydrogen production from renewable substrates is rapidly emerging as an alternative to fossil fuels, since it has triple the energy yield of hydrocarbon fuels [Rifkin 2002] and produces only water with no CO, CO_2, hydrocarbons, or fine particles when combusted [Liu 2008]. Hydrogen can be produced in many ways: electrolysis, photolysis, bio-photolysis, photo-fermentation, or dark fermentation. Fermentative technology is well established, and the co-products in dark fermentative hydrogen production are valuable (e.g. organic acids). Hence, dark fermentation is the most commonly used method in biological hydrogen production, especially when combined with waste treatment [Mizuno et al., 2000].

Thin stillage, the main by-product of the fermentation process in a conventional ethanol plant, is a strong candidate for biological hydrogen production. It is characterized by high chemical oxygen demand (COD) of up to 100 g/L, volatile solids (VS) of 60 g/L [Schaefer and Sung, 2008], volatile suspended solids (VSS) of 21 g/L, volatile fatty acids (VFAs) of 1.31 g/L [Khanal et al., 2005], and total carbohydrates of 65% (based on dry mass) [Mustafa et al., 2000]. In a conventional ethanol plant, a portion of the thin stillage is re-circulated back to fermentation tanks in order to minimize waste discharge. The recirculation of thin stillage reduces water intake and subsequently waste disposal, increases corn processing capacity, and reduces nutrient and buffer requirements [Ahn et al., 2011]. The main concern with thin stillage recirculation without any treatment is the accumulation of fermentation inhibitors (acetate, lactate, glycerol and ethanol) in the fermentation tank

[Julian et al., 1990]. Therefore, treating thin stillage could facilitate the maximization of recirculation rates by improving its characteristics.

In the context of biohydrogen, the high suspended solids concentration of thin stillage is problematic, as it may necessitate long contact times to hydrolyze particulate carbohydrates. The optimum hydraulic retention time (HRT) for biohydrogen production ranges from 4 to 8 hours [Wu et al., 2008; Hafez et al., 2010a]. Furthermore, the food-to-microorganisms (F/M) ratio is a critical parameter that affects hydrogen production with hydrogen yield increasing linearly at F/M ratios of 4 to 6.6 gCOD/gVSS.d [Hafez et al., 2010a]. For particulate wastes, the computation of F/M ratio is complicated as the VSS impacts both the food and microorganisms calculations. It is thus not surprising that given the challenges of biohydrogen production from thin stillage, searches on Google Scholar, Scifinder, and Engineering Village data bases with keywords "thin stillage, biohydrogen production, and particulate waste" revealed that no previous work has been conducted on hydrogen production from thin stillage. Furthermore, as apparent from Table 3.1 there are only a handful of studies on biohydrogen production from particulate wastes [Pan et al., 2008; Chen et al., 2006a; Yu et al., 2002; Lay et al., 2010].

For batch experiments, the initial substrate concentration (S_o) represents the carbon and energy source for biosynthesis requirements and other energy purposes, while the initial biomass concentration ($X°$) is the microorganisms responsible for substrate utilization [Liu 1996]. The S_o/X_o ratio reflects the initial energy level of batch cultivation. There is strong evidence that this ratio directly affects the growth patterns of microorganisms [Speece et al., 1973]. As apparent from Table 3.1, the extensive work by Pan et al. [2008] indicated that as the value of S_o/X_o ratio increases from 1 to 6 $gVS_{substrate}/gVS_{seed}$, hydrogen production potential increases then decreases beyond an S_o/X_o ratio of 6.

Table 3.1 - Hydrogen production potentials and yields for different S_o/X_o ratios using different substrates and biomass in batch experiments

Substrate	Seed	S_o/X_o[a]	H_2 Production Potential (mL)	Max. Hydrogen Yield			Ref.
				mol/mol_{subst}	$L/L_{substrate}$	$mL/gCOD_{added}$	
Food waste	ADS[b]	1[c]	10				Pan et al., 2008
		2	25				
		3	55				
		4	163				
		5	250				
		6	360				
		7	175				
		8	30				
		9	10				
		10	5				
Food waste	ADS	7.8	70			101	Chen et al., 2006a
Rice Winery	ADS			2.14			Yu et al., 2002
PFSS[d]	ADS	0.09		2.64			Lay et al., 2010
Brewery mixture	Grass compost	0.62				10.2	Morimoto et al., 2004
		1.08				12.8	
		2.12				19.3	
		4				24.9	
		6.4				19.8	

[a] S_o/X_o ratio calculated based on $gTCOD_{substrate}/gVSS_{sludge}$
[b] ADS: Anaerobic digester sludge
[c] S_o/X_o ratio was calculated based on $gVS_{substrate}/gVS_{sludge}$ in Pan et al. [2008]
[d] PFSS: Preserved fruits soaking solution

Table 3.1 (cont.) - Hydrogen production potentials and yields for different S_o/X_o ratios using different substrates and biomass in batch experiments

Substrate	Seed	S_o/X_o [a]	H_2 Production Potential (mL)	Max. Hydrogen Yield			Ref.
				mol/mol$_{subst}$	L/L$_{substrate}$	mL/gCOD$_{added}$	
Sucrose	ADS[b]				1.23		Wang & Wan, 2008
	ADS			3.18			Kumar & Das, 2000
	ADS			2.59			Kumar & Das, 2000
	ADS			2.73			Oh et al., 2003
	Compost						Ueno et al., 2001
Glucose	ADS	1		3.09			Zhang et al., 2005
	Sludge			1.6			Zhu & Beland, 2006
	Sludge compost			2.1			Zhu & Beland, 2006
	Clostridium sp.			2.8			Liu 1996
	Enterobacter cloacae IIT-BT 08			2.2			Speece et al., 1973
	Actinomyces spp.			1.21			Elbeshbishy et al., 2010
	Clostridium st.			1.17			Elbeshbishy et al., 2010
	Porphyromonas sp.			1.08			Elbeshbishy et al., 2010
Arabinose	Clostridium sp. Strain			2.3			Liu 1996
Xylose	Clostridium sp. Strain			2.3			Liu 1996
Cellobiose	Enterobacter cloacae IIT-BT 08			5.4			Speece et al., 1973
Fructose	Enterobacter cloacae IIT-BT 08			1.6			Speece et al., 1973
Cellulose	Sludge compost			2[c]			Ozkan et al., 2010

[a] S_o/X_o ratio calculated based on gTCOD$_{substrate}$/gVSS$_{sludge}$
[b] ADS: Anaerobic digester sludge
[c] mol/mol hexose

The impact of microbial cultures on biohydrogen production from soluble substrates is well documented in the literature is evidenced in Table 3.1. For example, biohydrogen production from glucose varied from 1.08 mol H_2/mol glucose [Oh et al., 2003] to 3.09 mol H_2/mol glucose [Wang and Wan, 2008]. As expected, and due to lack of data on specific populations, hydrogen yields varied considerably even for a specific substrate/microorganism system, as demonstrated in Table 3.1. The hydrogen yields from glucose using *Clostridium* species varied from 1.17 mol H_2/mol glucose [Oh et al., 2003] to 2.8 mol H_2/mol glucose [Taguchi et al., 2000].

Typically, the design of biological treatment systems is predicated on batch and continuous flow studies. For biohydrogen processes, the focus has been predominantly on batch studies due to concerns with long-term stability of continuous-flow systems associated with contamination due to methanogens in the feed. In such cases, batch studies are biased because they are conducted on pre-treated seed biomass as opposed to the enriched cultures that prevail in sustained continuous-flow systems. Pretreatment of anaerobic digester sludge is required primarily to restrain the hydrogen consuming bacteria and enrich the hydrogen producing bacteria, and this can be done by several methods such as heat, acid, base, aeration, or ultrasonication pretreatment [Elbeshbishy et al., 2010]. Acclimatization of anaerobic digester sludge to enrich the hydrogen producers in a hydrogen bioreactor, where methanogens are washed out and hydrogen producers become the predominant community in the sludge in continuous-flow systems [Hafez et al., 2010a; Ozkan et al., 2010], is the most representative microbial culture for assessment of biohydrogen production potential from various substrates. An extensive search in Google Scholar, Scifinder, and Engineering Village data bases using keywords “biohydrogen production, acclimated sludge, acclimatized sludge, anaerobic digester sludge, fermentative hydrogen batches” revealed that no previous

work has been conducted on hydrogen production in batch experiments using acclimatized anaerobic digester sludge from a continuous-flow biohydrogen system.

The main objectives of this study are threefold: assessment of the viability of biohydrogen production from thin stillage, comparative evaluation of anaerobic digester sludge (ADS) and acclimatized anaerobic digester sludge (AADS) for biohydrogen production, and determination of the optimal S_o/X_o ratio and maximum hydrogen production potential.

3.2. Materials and methods

3.2.1. Seed sludge

ADS was collected from the primary anaerobic methane digester at Guelph's wastewater treatment plants (Guelph, Ontario, Canada) and used as seed sludge for the first run (sludge from methane reactor). The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the ADS were 22.9 and 13.2 g/L respectively. Heat pretreatment for the ADS was conducted by heating the sludge at 70˚C for 30 minutes [Hafez et al., 2010a]. AADS was collected from a continuous flow biohydrogen system with aforementioned ADS seed. The continuous system ran for 10 days with a flow of 15 L/d, using glucose as a substrate with a concentration of 30 g/L and anaerobic digester sludge as a seed at hydraulic retention time (HRT) of 8 hrs and solids retention time (SRT) of 42 hrs. The TSS and VSS concentrations of the AADS were 10.9 and 9.4 g/L respectively.

Microbial community analysis

Biomass samples for the AADS were collected from the continuous flow system at the end of the acclimatization period for microbial community analysis. The total genomic

community DNA was extracted using UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) and after PCR amplification were analyzed by denaturing gradient gel electrophoresis (DGGE). For further details refer to Hafez et al. [2010a].

3.2.2. Raw thin stillage (substrate)

Raw thin stillage was used as the substrate to assess the hydrogen production rates. Table 3.2 lists the different characteristics of the raw thin stillage measured in quadruplicates.

Table 3.2 - Raw thin stillage characteristics

Parameter (mg/L)	**Raw Thin Stillage Quality (Av. ± SD)**
TS	71500 ± 724
VS	64800 ± 595
TSS	36900 ± 486
VSS	35300 ± 437
TCOD	122000 ± 1400
SCOD	60600 ± 450
TBOD	68600 ± 800
SBOD	20800 ± 3300
TVFAs	12320 ± 860
Glucose	285 ± 10
Soluble Carbohydrates	35200 ± 1200
Total Carbohydrates	41200 ± 1600
NH_3-N	202 ± 6.7
NO_3 -N	16 ± 1.5
pH	3.46
Alkalinity ($CaCO_3$)	Not measured (pH < 4.3)

3.2.3. Batch experiments

Batch anaerobic studies were conducted in serum bottles with a liquid volume of 250 mL and head space volume of 60 mL. Experiments were conducted in triplicates for initial

substrate-to-biomass (S_o/X_o) ratios of 0.5, 1, 2, 4, 6 and 8 $gCOD_{substrate}/gVSS_{seed}$. Volumes of thin stillage and sludge used in batches were calculated using the following Equation:

$$S_o/X_o = \frac{V_t(L) * \text{Thin Stillage TCOD } (\frac{g}{L})}{V_s(L) * \text{Sludge VSS } (\frac{g}{L})} \quad (3.1)$$

where V_t is the volume of thin stillage and V_s is the volume of sludge, and Table 3.3 shows the volumes used in bottles for each S_o/X_o ratio. The initial pH value for the mixed solution in each bottle was adjusted using HCl and measured to be 5.47±0.04 for both runs. A 5 g/L buffer solution ($NaHCO_3$) was also added for pH control.

Table 3.3 - Volumes of seed and substrate used in bottles

S_o/X_o	ADS		AADS	
(gCOD/gVSS)	V_t (mL)	V_s (mL)	V_t (mL)	V_s (mL)
0.5	15	235	9	241
1	30	220	16	234
2	50	200	30	220
4	80	170	54	196
6	100	150	73	177
8	120	130	89	161

Ten milliliter samples of the mixtures were collected initially. The head space was flushed with oxygen-free nitrogen gas for a period of 2 min and capped tightly with rubber stoppers. The bottles were then placed in a swirling-action shaker (Max Q4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) operating at 180 rpm and maintained at a temperature of 37°C. Two control bottles were prepared using ADS and AADS without thin stillage for both runs respectively. Final samples were taken at the end of the batch experiment. The final pHs for the mixed solution in each bottle were measured to be 5.05±0.15 for both runs.

3.2.4. Analytical methods

The biogas production was measured using suitable sized glass syringes in the range of 5-100 mL where the gas was released from headspace of the serum bottles to equilibrate with the ambient pressure [Owen et al., 1979]. The biogas composition including hydrogen, methane, and nitrogen was determined by a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Mole sieve 5A, mesh 80/100, 6 ft × 1/8 in). . Argon was used as the carrier gas at a flow rate of 30 mL/min and the temperatures of the column and the TCD detector were 90°C and 105°C, respectively. Total volatile fatty acids (TVFAs), as well as total and soluble chemical oxygen demand (TCOD, SCOD) were measured using HACH methods and test kits (HACH Odyssey DR/2500 spectrophotometer manual) [Hafez et al., 2010b]. TSS and VSS concentrations were analyzed using standard methods [APHA 1995]. Soluble parameters were determined after filtering the samples through 0.45 μm filter paper.

3.2.5. Data analysis

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

$$V_{H,i} = V_{H,i-1} + C_{H,i} * V_{G,i} \quad (3.2)$$

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

3.3. Results and Discussion

3.3.1. Hydrogen Production

Figures 3.1 and 3.2 show the cumulative hydrogen production at different S_o/X_o ratios for both runs using ADS and AADS, respectively. Standard deviation values were not shown on the curve since the coefficients of variation (calculated as standard deviation divided by the average) in both runs were approximately less that 10%. In the ADS batches as the S_o/X_o ratio increased from 0.5 to 2 gCOD/gVSS, hydrogen production rapidly increased from 49 mL at S_o/X_o ratio of 0.5 gCOD/gVSS to a maximum of 386 mL at S_o/X_o ratio of 2 gCOD/gVSS after which it decreased to 163 mL with further increase in S_o/X_o ratio. This behavior is consistent with another study [Pan et al., 2008] that used food waste as a substrate and anerobic digester sludge as the seed, where a wide range of S_o/X_o ratios from 1 to 10 gVS_{feed}/gVS_{seed} was studied in mesophilic batch fermentation tests. In the aforementioned study, hydrogen production initially increased at high S_o/X_o ratios and reached a maximum of 357 mL at an S_o/X_o ratio of 6 gVS_{feed}/gVS_{seed}, then decreased at S_o/X_o ratios greater than 6 gVS_{feed}/gVS_{seed}. In the AADS batches, the same behavior was observed and a maximum hydrogen production of 1974 mL (5 times the ADS batches) was achieved at an S_o/X_o ratio of 6 gCOD/gVSS. The type of sludge also affected the biogas composition, with the maximum hydrogen content of the headspace in batches using ADS and AADS reaching 54% and 69%, respectively.

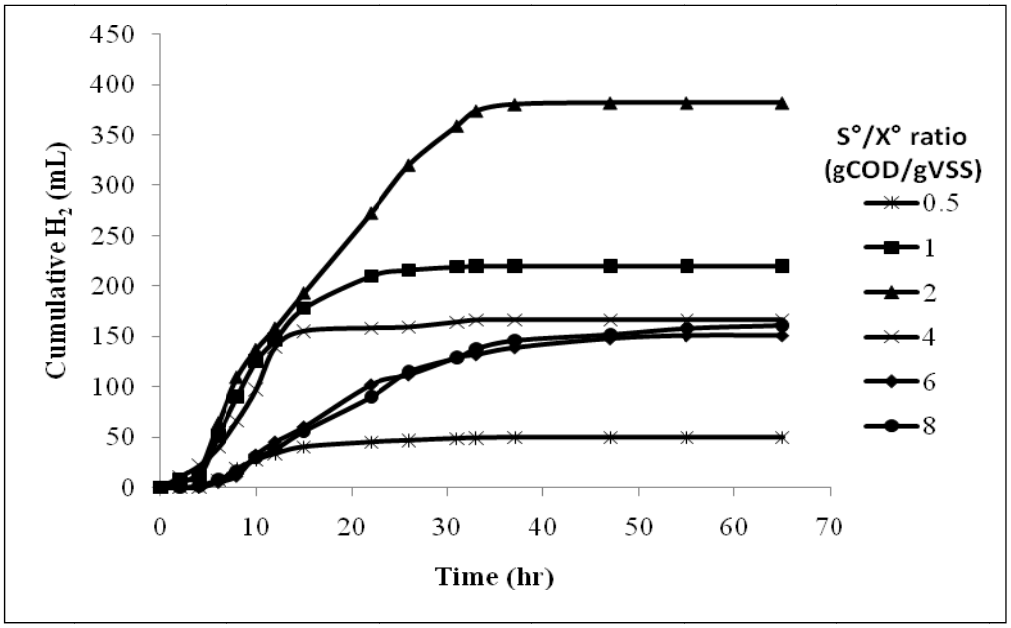

Figure 3.1 - Cumulative hydrogen production using ADS

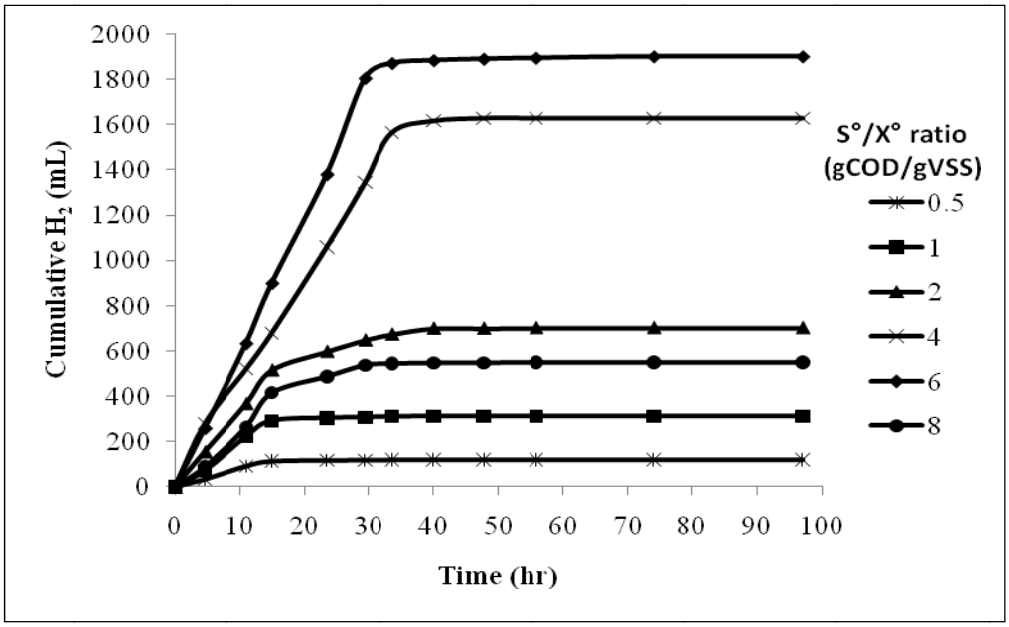

Figure 3.2 - Cumulative hydrogen production using AADS

3.3.2. Hydrogen Yields

Figure 3.3 shows the hydrogen yield based on the total carbohydrates converted for batches using both ADS and AADS. As depicted in Figure 3.3, for the ADS batches, a low hydrogen yield of 130 mL H_2/gT-carb.$_{converted}$ was obtained at S_o/X_o ratio of 0.5 gCOD/gVSS which is due to insufficient feed, after which hydrogen yield stabilized at an average of 248 mL H_2/gT-carb.$_{converted}$ within the S_o/X_o ratio of 1 - 2 gCOD/gVSS before declining to an average of 90 mL H_2/ gT-carb.$_{converted}$ at S_o/X_o ratios of 4 - 8 gCOD/gVSS. On the other hand, the hydrogen yields for the AADS batches followed the same aforementioned trend but the optimum range of S_o/X_o ratio was 3 - 6 gCOD/gVSS and a maximum yield of 470 mL H_2/gT-carb.$_{converted}$ was achieved. However, considering the 5% standard deviation of hydrogen gas production, it is likely that the optimum S_o/X_o range is between 3 - 6 gCOD/gVSS. This trend is similar to that observed by Pan et al. [2008] who used food waste as the substrate and anaerobic digester sludge as the seed, where the hydrogen yield increased slowly to a maximum of 39 mL H_2/gVS at S_o/X_o ratio of 6 gVS_{feed}/gVS_{seed} prior to decreasing to almost zero at S_o/X_o ratio of 8 gVS_{feed}/gVS_{seed} and higher. In addition, in another study [Chen et al., 2006a], the same trend was observed in batches using seed sludge from a local anaerobic digester and food waste as the substrate, with a maximum yield of 101 mL H_2/gCOD at S_o/X_o ratio of 7.68 gCOD/gVSS. The differences in the optimum S_o/X_o ratios in the literature can be attributed to the differences in the waste type and characteristics as well as the anaerobic digester sludges.

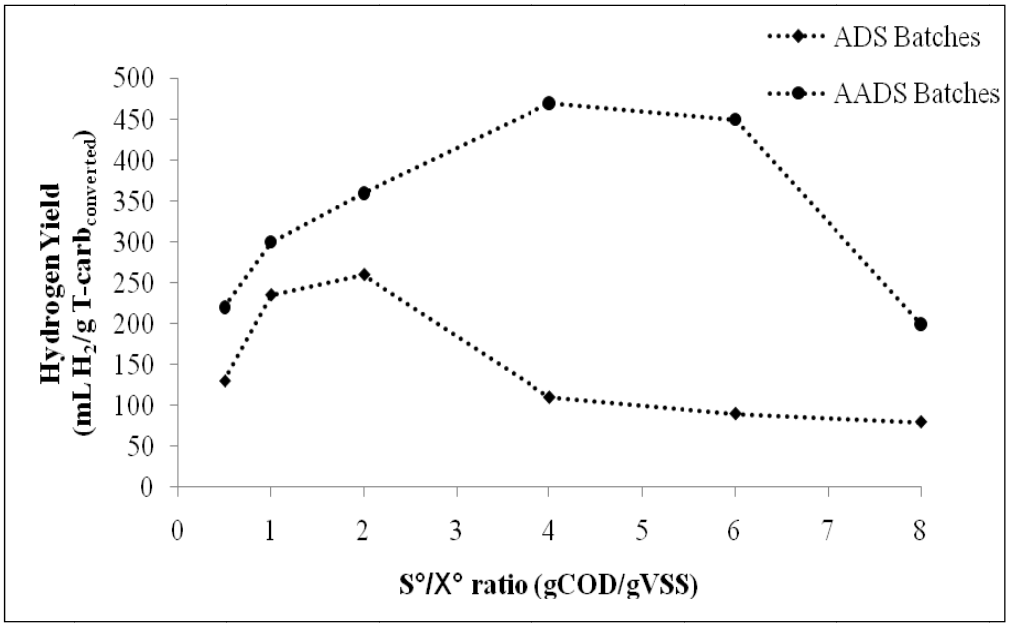

Figure 3.3 - Hydrogen Yield

Carbohydrates, which represent 30% of thin stillage (Table 3.2), play the main role in H_2 production [Lay et al., 2003]. It is obvious that with the low percentage of hydrogen producers in the ADS, only a part of the carbohydrates in thin stillage was converted to hydrogen with a maximum conversion efficiency of 74% at S_o/X_o ratio of 1 gCOD/gVSS, while in batches using AADS the carbohydrates conversion efficiency reached 90% at S_o/X_o ratio of 4 gCOD/gVSS as illustrated in Table 3.4.

Table 3.4 - Summary of initial and final batches data

	S_o/X_o (gCOD/gVSS)	$Tcarb_i$[a] (g/L)	$Tcarb_f$[b] (g/L)	Carbohydrates removal (%)	$Tcarb_{converted}$ (g)	$TVFAs_i$[c] (gCOD/L)	$TVFAs_f$[d] (gCOD/L)	Final TVFAs/TCOD (%)	H_2 yield (mL/g$Tcarb_{converted}$)	H_2 yield (L H_2/$L_{thin\ stillage}$)
ADS	0.5	2.5	1.0	60	0.38	0.7	2.7	8.6	130	3.3
	1	5.0	1.3	74	0.94	1.5	6.4	20.3	235	7.3
	2	8.4	2.5	70	1.47	2.5	11.8	31.6	260	7.6
	4	13.4	7.4	45	1.51	3.9	12.9	26.8	110	2.1
	6	16.8	10.1	40	1.68	4.9	16.2	27.9	90	1.5
	8	20.2	12.1	40	2.02	5.9	14.5	23.3	80	1.3
AADS	0.5	4.4	2.2	50	0.55	0.4	3.6	9.8	220	8.1
	1	6.4	2.2	65	1.04	0.8	6.8	17.5	300	11.3
	2	10.0	2.2	78	1.95	1.5	11.8	27.6	360	14.0
	4	15.4	1.5	90	3.47	2.7	24.0	47.1	470	19.5
	6	19.2	2.3	88	4.22	3.6	29.5	53.6	450	17.7
	8	22.0	11.0	50	2.75	4.4	21.4	31.5	200	4.4

[a] Initial total carbohydrates
[b] Final total carbohydrates
[c] Initial total volatile fatty acids
[d] Final total volatile fatty acids

To assess the acidification efficiency, total volatile fatty acids (TVFAs) were measured for both sets of batches. The maximum final TVFAs concentrations were 16.2 gCOD/L and 29.5 gCOD/L for the ADS and the AADS, respectively corresponding to the maximum hydrogen yield and carbohydrates conversion efficiency at an S_o/X_o ratio of 6 gCOD/gVSS. On the other hand, TVFAs constituted 10% of the TCOD of the raw thin stillage, (Table 3.2). However, the percentage of TVFAs increased to 27.9% and 53.6% of the TCOD at the end of the batches for ADS and AADS, respectively, at S_o/X_o ratio of 6 gCOD/gVSS (Table 3.4).

Figure 3.4 shows the relationship between the S_o/X_o ratio and the ultimate hydrogen yield per liter of thin stillage for batches using both ADS and AADS. As illustrated in Figure 3.4, the hydrogen yield per liter of thin stillage followed the same trend as in Figure 3.3 with a maximum yield of 7.6 and 19.5 L H_2/L thin stillage at S_o/X_o ratio of 2 and 4 gCOD/gVSS in batches using ADS and AADS respectively. The much higher observed hydrogen yields, both in terms of per unit waste volume or carbohydrates converted in the AADS relative to the ADS clearly highlighted the limitation of the most widely used approach for assessing biohydrogen production, i.e. using unacclimatized and unenriched ADS in batch studies and emphasize the need for continuous-flow studies.

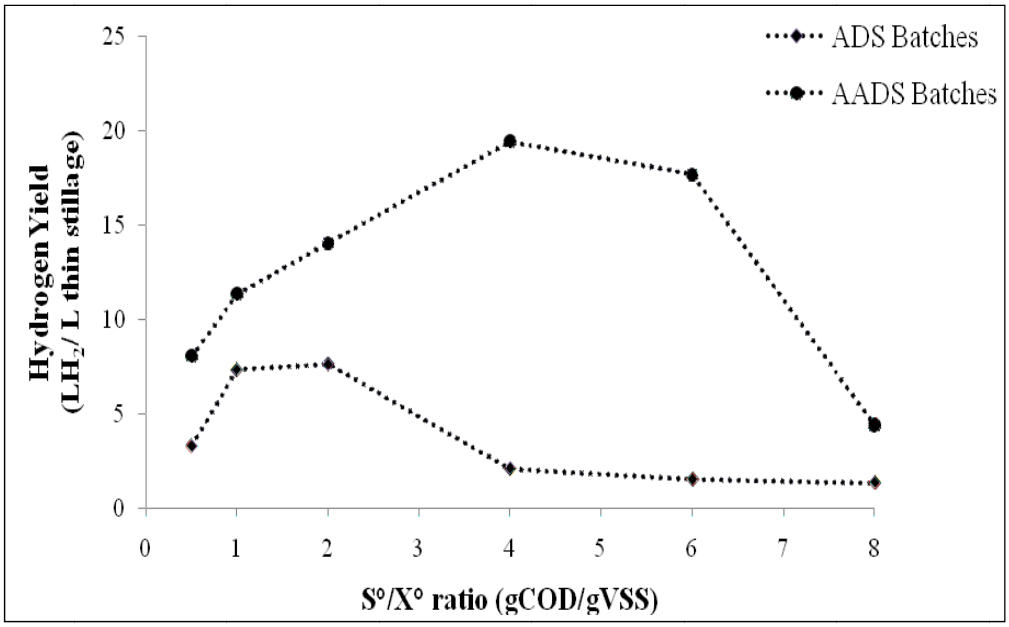

Figure 3.4 - Specific Hydrogen Yield

3.3.3. Specific Hydrogen Production Rate

Figure 3.5 shows the specific hydrogen production rate (SHPR) for the six different S_o/X_o ratios. The maximum SHPR for batches using AADS was 585 mL H_2/gVSS.d at an

S_o/X_o ratio of 6 gCOD/gVSS while in batches using ADS the maximum only reached 181 mL H_2/gVSS.d at S_o/X_o ratio of 4 gCOD/gVSS. Hafez et al. [2010a] observed the same pattern of a maximum SHPR followed by a sharp decline at high S_o/X_o ratio using the authors' data as well as seven other literature studies.

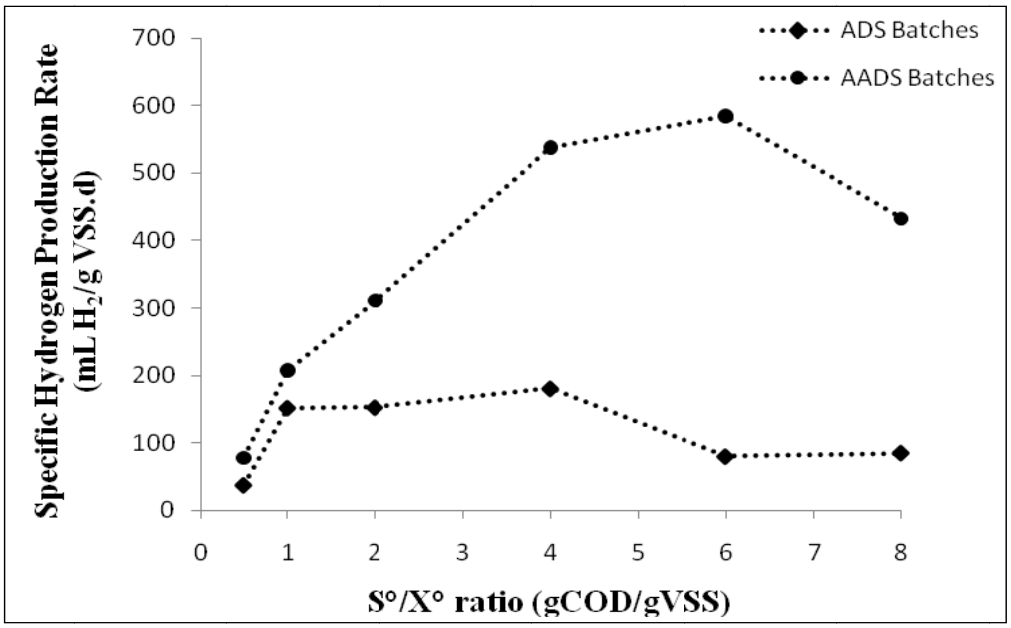

Figure 3.5 - Biomass Specific Hydrogen Production Rate

The findings of this study contradicts the observations of Lay et al. [1999] who studied hydrogen production using organic fraction of municipal solid waste (OFMSW) as the substrate and two types of sludges at different mixing ratios; pretreated anaerobic digester sludge and acclimatized sludge from a hydrogen producing chemostat reactor with an HRT of 10 hrs and sucrose as the substrate, and reported no trend for the SHPR with increasing the acclimated sludge percentage.

Table 3.5 shows the kinetics from the Gompertz model [Lay et al., 1999] for both batches using ADS and AADS. The coefficient of determination R^2 was 0.999 for all Gompertz data. It is apparent that the lag phase in the AADS batches with an average of 2.3 hours is much lower than that in the ADS batches with an average of 4.4 hours and this also can be related to the increase in the percentage of hydrogen producers in the AADS relative to the ADS. The maximum hydrogen production rate in batches using ADS was 18.4 mL/hr at S_o/X_o ratio of 1 gCOD/gVSS which is one third the 57.9 mL/hr in batches using AADS at S_o/X_o ratio of 4 gCOD/gVSS. The trend of an increase to the maximum followed by a decline at higher S_o/X_o ratio is consistent with the findings of Pan et al. [2008] who observed an increase in the hydrogen production rate with the increase of S_o/X_o ratio to a maximum of 19.5 mL/hr at an S_o/X_o ratio of 5 gVS_{feed}/gVS_{seed}, followed by a decrease with further increase in the S_o/X_o ratio. A correlation (not shown) of the biomass specific production rate for ADS and AADS (R^2 of 0.72) revealed that over the range of S_o/X_o ratios that was studied, the active biomass (hydrogen producers) in the AADS is 3.5 times than that of the ADS calculated based on the specific hydrogen production rates for both sludges.

Table 3.5 - Gompertz data for both ADS and AADS batches

	ADS				AADS			
S_o/X_o (gCOD/gVSS)	**P[a] (mL)**	**R_m[b] (mL/hr)**	**λ[c] (hr)**	**SHPR[d] (mL/gVSS.d)**	**P (mL)**	**Rm (mL/hr)**	**λ (hr)**	**SHPR (mL/gVSS.d)**
0.5	49	4.8	4.5	37	121	11.5	1.7	78
1	220	18.4	3.3	152	311	28.9	2.2	208
2	386	16.8	2.8	153	704	38.9	2.3	311
4	159	16.9	3.6	181	1676	57.9	2.4	538
6	150	6.6	6.1	80	1974	52.1	2.5	585
8	163	6.1	6.1	85	550	33.8	2.6	433

[a] P: Ultimate hydrogen production
[b] R_m: Rate of hydrogen production
[c] λ: Lag phase duration
[d] SHPR: Specific hydrogen production rate

3.3.4. COD Balance

COD mass balance data is presented in Table 3.6. The closure of COD balances at 88±4 % verifies the reliability of the data. The percentage average COD reduction was 12±4 % for the ADS batches and 16±7 % for the AADS batches. COD reduction increased at S_o/X_o ratios from 0.5 to 2 gCOD/gVSS and reached a maximum of 16 % at S_o/X_o ratio of 2-4 gCOD/gVSS in batches using ADS, and 24 % at S_o/X_o ratio of 4 gCOD/gVSS in batches using AADS after which it decreased at higher S_o/X_o ratios. As apparent from Table 3.6, in batches using ADS, although at an S_o/X_o ratio of 8 gCOD/gVSS, the COD removed was 10.5 g/L (14%), at an S_o/X_o ratio of 4 gCOD/gVSS the COD removed was 9.2 g/L (16 %).

Table 3.6 - Summary of COD balance

	S_o/X_o (gCOD/gVSS)	$COD_{initial}$ g/L	COD_{final} g/L	COD removed g/L	cumulative H_2 mL	H_2 gCOD/L	COD balance[a] %
ADS	0.5	32.7	30.7	2.0	49	0.14	94
	1	38.4	31.5	6.9	220	0.63	84
	2	46.0	37.3	8.7	386	1.10	84
	4	57.4	48.2	9.2	159	0.48	85
	6	65.0	58.1	6.9	150	0.43	90
	8	72.6	62.1	10.5	163	0.46	86
AADS	0.5	39.0	36.6	2.4	121	0.35	95
	1	43.3	38.8	4.4	311	0.90	92
	2	51.0	42.7	8.3	704	2.02	88
	4	62.7	51.0	11.7	1676	4.68	89
	6	70.9	55.0	14.1	1974	5.46	85
	8	76.9	68.0	8.9	550	1.58	90

[a] COD balance (%) = [H_2 (gCOD) + COD_{final} (gCOD)] / [$COD_{initial}$ (gCOD)]

3.3.5. Microbial Community

Hafez et al. [2010a] conducted DGGE analysis for the AADS and the profiles of the 16S rDNA gene fragments are demonstrated in Figure 3.6. Table 3.7 shows the results of the sequence affiliation. The results revealed that *Clostridium acetobutyricum* (band A), *Klebsiella pneumonia* (band B), uncultured bacteria (DQ464539.1) and (DQ414811.1) for bands F and G, respectively, were the main identified bands for the AADS. *Clostridium acetobutyricum and Klebsiella pneumonia* are frequently reported as candidates for hydrogen production [Hafez et al., 2010a; Liu and Fang, 2007; Kim et al., 2006a,b; Chen et al., 2006b]. In addition, another hydrogen producers including *Clostridium butyricum* (band C), a *Clostridium acetobutyricum* affiliated strain (band D) and *Clostridium pasteurianum* (band E) were detected. In a continuous system for biohydrogen production, Hafez et al. [2010a] have shown that high hydrogen yields can be achieved using *Clostridium butyricum* and *Clostridium pasteurianum*.

Figure 3.6 - DGGE profile of the 16S rDNA gene fragments for the AADS [Hafez et al., 2010a]

Table 3.7 - Affiliation of denaturing gradient gel electrophoresis (DGGE) fragments determined by their 16S rDNA sequence

Affiliation (accession no.)	Bands	Similarity (%)	AADS
Clostridium acetobutyricum (FM994940.1)	A	99	×
Klebsiella pneumonia (GQ214541.1)	B	100	×
Clostridium butyricum (DQ831124.1)	C	99	×
Clostridium acetobutyricum (FM994940.1)	D	95	×
Clostridium pasteurianum (GQ214541.1)	E	99	×
Uncultured bacterium (DQ464539.1)	F	96	×
Uncultured bacterium (DQ414811.1)	G	97	×

3.4. Conclusions

The outcome of this study revealed the importance of using AADS over the conventional ADS in hydrogen batches. It is highly recommended to use acclimatized sludges from a continuous-flow system to assess biohydrogen production from a particular waste as opposed to the most widely used technique of batch studies with pretreated anaerobic digester sludge. Based on the findings of this study, the following conclusions can be drawn:

- Thin stillage has a potential for hydrogen production with a yield of 19.5 L H_2/L thin stillage with AADS while tests with ADS only revealed a maximum potential of 7.5 L H_2/L thin stillage.
- The optimum experimental range of S_o/X_o ratio for hydrogen production is 1 to 2 $gCOD_{substrate}/gVSS_{seed}$ using conventional ADS.
- The optimum experimental range of S_o/X_o ratio for hydrogen production within the investigated range is 3 to 6 $gCOD_{substrate}/gVSS_{seed}$ using AADS.

- The biomass specific hydrogen production rate for the AADS was 3.5 times higher than that of the ADS throughout the range of S_o/X_o ratio that was studied.
- The DGGE profiles of the 16S rDNA gene fragments for the AADS confirmed its superior performance over the ADS where, hydrogen producers such as *Clostridium acetobutyricum*, *Klebsiella pneumonia, Clostridium butyricum* and *Clostridium pasteurianum* were the predominant species that were detected.

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CHAPTER 4

Comparative Assessment of Single-Stage and Two-Stage Anaerobic Digestion for the Treatment of Thin Stillage

4.1. Introduction

Some processes employed in the production of renewable biofuels, such as, bioethanol can result in significant pollution problems. In a typical bioethanol plant process, up to 20 liters of stillage can be generated during fermentation for each liter of ethanol produced [van Haandel and Catunda, 1994]. Thin stillage is characterized by high total chemical oxygen demand (TCOD) of up to 122 g/L, biological oxygen demand (BOD) of up to 70 g/L, volatile solids (VS) of 60 g/L [Schaefer and Sung, 2008; Nasr et al., 2011] and total carbohydrates of 65% (based on dry mass) [Mustafa et al., 2000]. Therefore, it is a strong candidate for anaerobic digestion. Usually, due to solids build up and toxicity to yeast by lactic acid, acetic acid, glycerol and sodium, less than 50% of thin stillage is recycled as fermentation broth (called backset in the corn-to-ethanol industry) [Egg et al., 1985; Shojaosadati et al., 1996; Julian et al., 1990; Pejin et al., 2009].

In a single-stage anaerobic digestion, Stover et al. [1984] observed promising performances from mesophilic digestion of thin corn stillage (64.5 gTCOD/L; 32.2 gTS/L) in both suspended growth and fixed-film systems with a methane yield ranging from 0.22 to 0.33 m^3/kg $TCOD_{removed}$ (STP) that could replace 60% of the daily energy requirement of the bioethanol plant. One pilot scale upflow anaerobic sludge blanket (UASB) reactor achieved 76% TCOD removal with 0.33 m^3 CH_4/kgTCOD removed. It was also used for a corn ethanol plant as a stillage pretreatment step before aerobic trickling filters; however influent wastewater TCOD was only 3.6 g/L [Lanting and Gross, 1985].

Separating the acidogenic and methanogenic steps in the anaerobic digestion process, provides enhanced stability to the different groups of microorganisms and better process control [Demirel and Yenigun, 2002]. The purpose of a two-stage anaerobic digestion system is not only to further degrade waste, but also to extract more net energy from the system [Thompson, 2008]. In a single-stage anaerobic digestion process, a variety of higher organic acids, such as propionic, butyric, and lactic, as well as alcohols and ketones, are also formed during the breakdown of the organic substrates by acidogens. However, in a well operated process, these products are mostly converted to acetic acid and hydrogen, which, in turn, are converted to methane gas [Cooney et al., 2007]. On the other hand, in a two-stage anaerobic digestion process, the end products from acidification stage using thin stillage are ideal for anaerobic treatment with total volatile fatty acids (TVFAs) that can reach 29.5 gCOD/L [Pavan et al., 2000; Nasr et al., 2011].

Vinas et al. [1993] achieved a methane production yield of 0.31 L/$gCOD_{removed}$ (STP) in a two-stage process with an increase of 13% over the single-stage process using a cellulosic material as the substrate. Also, Rincon et al. [2009] achieved an increase of 10% using olive mill solid residue as the substrate. Although both studies used acidification stage as a pretreatment for anaerobic digestion, they did not consider biohydrogen production.

Despite of their higher loading rates, improved process stability and flexibility, there are relatively few commercial two-stage anaerobic digestion units. The added complexity and expense of building and operating commercial two-stage systems have so far counteracted the yield and rate enhancements [Rapport et al., 2008]. The theoretical higher biogas yields have also been questioned since the acidogenic phase separation prevents the hydrogen to methane pathway [Reith et al., 2003].

The main objective of this research is to compare and evaluate the methane production from thin stillage in single-stage and two-stage anaerobic digestion processes, by investigating the effect of the acidogenic stage with hydrogen production on the methane production in batch studies under mesophilic conditions, and to determine if there is a significant difference in potential energy yields between single-stage and two-stage anaerobic digestion systems.

4.2. Materials and methods

4.2.1. Seed sludge

Anaerobic digester sludge (ADS) was collected from the primary methane digester at Guelph's wastewater treatment plant (Guelph, Ontario, Canada) and was used as seed sludge for the single-stage anaerobic digestion and the second stage of the two-stage anaerobic digestion for methane production. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the ADS were 22.9 and 13.2 g/L, respectively. Acclimatized anaerobic digester sludge (AADS) was collected from a continuous-flow biohydrogen system [Nasr et al., 2011]. The 15 L/d continuous-flow system was run for 10 days, using 30 g/L glucose as a substrate and heat pretreated ADS as a seed at a hydraulic retention time (HRT) of 8 h and solids retention time (SRT) of 42 h. The TSS and VSS concentrations of the AADS were 10.9 and 9.4 g/L, respectively.

4.2.2. Feed (substrate)

Raw thin stillage was used as the substrate to assess its hydrogen and methane production potentials. For the single-stage methane production and the first stage hydrogen production, raw thin stillage was used as the substrate with TCOD, TVFAs, TSS, and VSS of

122, 12.3, 36.9, and 35.3 g/L, respectively. Detailed characteristics of the raw thin stillage have been reported elsewhere [Nasr et al., 2011]. Hydrogen batch tests were tested at an initial substrate-to-biomass ratio (S_o/X_o) of 4, 6, and 8 gCOD/gVSS based on the TCOD of the thin stillage and seed sludge VSS concentration [Nasr et al., 2011]. After the hydrogen production stage, the bottles of the three different S_o/X_o ratios were left for three hours to settle and the supernatant was then used as substrate for the second stage methane production. TCOD of the supernatants from S_o/X_o ratios of 4, 6, and 8 gCOD/gVSS described below were 49.6, 51.5, and 53.3 g/L, respectively.

4.2.3. Batch experiments

Hydrogen and methane batch anaerobic experiments were conducted in serum bottles with a liquid volume of 250 mL and head space volume of 60 mL. Table 4.1 shows the volumes of substrates and sludges used in bottles and initial pH for each stage. For hydrogen production as a first stage, the experiments were conducted in triplicates for initial (S_o/X_o) ratios of 4, 6 and 8 $gTCOD_{substrate}/gVSS_{seed}$ using AADS as the seed and raw thin stillage as the substrate [Nasr et al., 2011]. For methane production, the experiments were conducted in triplicates for an initial S_o/X_o ratio of 2 gCOD/gVSS using ADS as the seed and the supernatant from the hydrogen production stage as the substrate. The volumes of thin stillage and supernatant as substrates and ADS and AADS as seeds used in batches were calculated using the following Equation:

$$S_o/X_o = [V_{substrate}\ (L) * TCOD_{substrate}\ (g/L)] / [\ V_{sludge}\ (L) * VSS_{sludge}\ (g/L)] \qquad (4.1)$$

where $V_{substrate}$ is the volume of substrate and V_{sludge} is the volume of sludge. Buffer ($NaHCO_3$) with concentrations of 5 g/L and 12 g/L were added for pH control in both hydrogen and methane batches, respectively. The initial pH for the mixed solution in each bottle was subsequently adjusted using HCl or NaOH and measured to be 5.47±0.04 for hydrogen batches and 7.17±0.07 for methane batches.

Initially, 10 mL samples of the mixtures were collected. The head space was flushed with oxygen-free nitrogen gas for a period of 2 min and capped tightly with rubber stoppers. The bottles were then placed in a swirling-action shaker (Max Q4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) operating at 180 rpm and maintained at a temperature of 37°C. Two control bottles of seed material only, without substrate, were prepared using ADS for methane production runs and one control bottle using AADS for hydrogen production run. Final samples were taken at the end of the batch experiment.

Table 4.1 - Volumes of substrate and sludge used in batches

			S_o/X_o (gCOD/gVSS)	$V_{substrate}$ (mL)	V_{sludge} (mL)	$pH_{initial}$
Two-stage CH_4 production	Single-stage CH_4 production (using ADS)		2	45	205	7.17±0.05
	1^{st} stage H_2 production (Run A) (using AADS)	A1	4	54	196	5.47±0.05
		A2	6	73	177	5.48±0.02
		A3	8	89	161	5.50±0.01
	2^{nd} stage CH_4 production (Run B) (using ADS)	B1	2 from 4	67	183	7.18±0.06
		B2	2 from 6	65	185	7.16±0.08
		B3	2 from 8	64	186	7.18±0.05

4.2.4. Analytical methods

The biogas production 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 [Owen et al., 1979]. The composition of biogas including hydrogen, methane, and nitrogen was determined by employing a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Mole sieve 5A, mesh 80/100, 6 ft × 1/8 in). The temperatures of the column and the TCD detector were 90°C and 105°C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min. TVFAs, TCOD, and SCOD were measured using HACH methods. TSS and VSS concentrations were analyzed using standard methods [APHA, 1995]. Soluble parameters were determined after filtering the samples through 0.45 μm filter paper.

4.2.5. Data analysis

Hydrogen and methane gas productions were calculated from head space measurements of gas composition and the total volume of biogas produced at each time interval, using the mass balance Equation:

$$V_{X,i} = V_{X,i-1} + C_{X,i} * V_{G,i} \quad (4.2)$$

where $V_{X,i}$ and $V_{X,i-1}$ are cumulative hydrogen or methane gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ is the total biogas volume in the current and previous time intervals, $C_{X,i}$ is the fraction of hydrogen or methane gas in the headspace of the bottle measured using gas chromatography in the current time interval.

4.3. Results and Discussion

4.3.1. Biogas production

The first stage (i.e. acidogenic stage) was carried out with three different S_o/X_o ratios of 4, 6, and 8 gCOD/gVSS (runs: A1, A2, and A3) as described in detail by Nasr et al. [2011]. Figure 4.1 shows the hydrogen production rates achieved for runs A1, A2, and A3 with ultimate hydrogen production potentials of 1676, 1974, and 550 mL, respectively. It can be inferred from the Figure that as the S_o/X_o ratio increased from 4 to 6 gCOD/gVSS, hydrogen production rate increased from 47 mL/hr to 62 mL/hr, respectively, after which it decreased significantly to 28 mL/hr at S_o/X_o ratio of 8 gCOD/gVSS. This trend is consistent with another study that observed the same pattern of maximum hydrogen production at food to microorganism (F/M) ratio of 6 gCOD/gVSS-d followed by a sharp decline at higher F/M ratios [Hafez et al., 2010a].

It is noteworthy that in the single-stage anaerobic digestion process, there was no hydrogen gas detected with methane gas production. The COD degradation was 80% complete in the single-stage experiments (A runs) after 28 days while in the two-stage experiments (B runs), it took only 17.5, 17.8, and 16.7 days to reach 80% degradation for the three runs B1, B2, and B3, respectively. Therefore, a shorter SRT can be attained in the two-stage anaerobic digestion process leading to improvement in the overall performance of the anaerobic digestion. The final pHs for the mixed solution in each bottle were measured and found to be 7.56±0.01 for methane runs and 5.05±0.15 for the hydrogen runs.

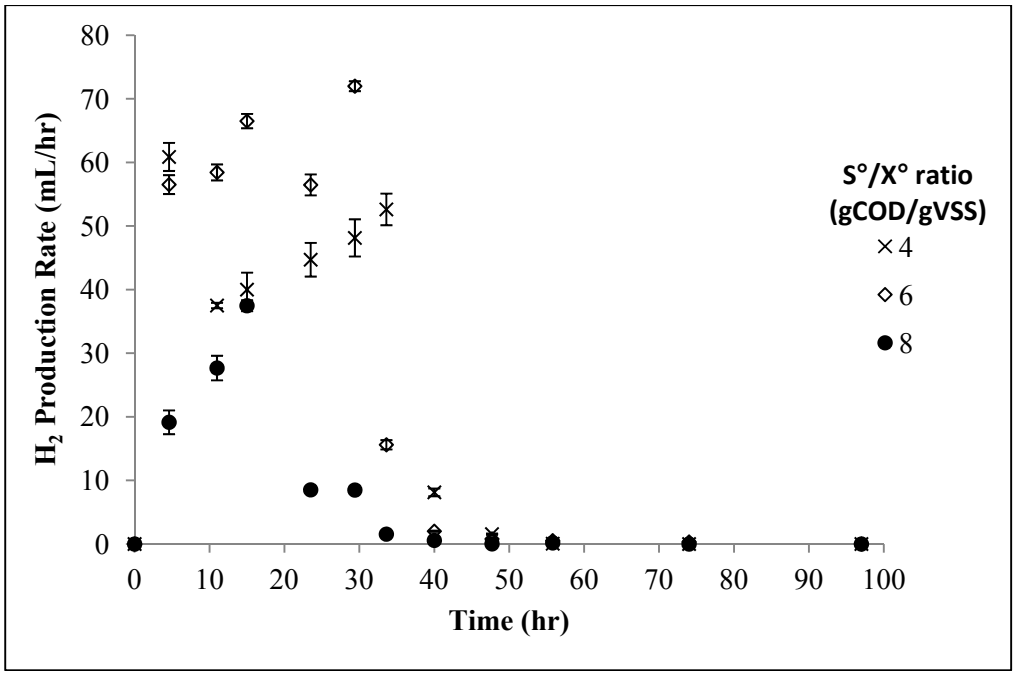

Figure 4.1 - H_2 production rates for the acidogenic step in the two-stage batches

4.3.2. Hydrogen and methane yields

Tables 4.2 and 4.3 show the summary for initial and final batches data in both single-stage and two-stage anaerobic digestion experiments. Figure 4.2 shows the methane yield during the single-stage and two-stage anaerobic digestion of thin stillage. Standard deviation values were less than 10% for all experimental data. In the two-stage anaerobic digestion, the methane yields based on COD removed were 321, 333, and 317 mL $CH_4/gCOD_{removed}$ (STP) for the methanogenic batches of runs B1, B2, and B3, respectively. On the other hand, a methane yield of only 268 mL $CH_4/gCOD_{removed}$ (STP) was achieved in the single-stage experiment. The maximum methane yield of 333 mL/$gCOD_{removed}$ (STP) was 24% higher than the yield achieved in the single-stage experiment compared to an increase of 9.8% achieved by Rincon et al. [2009] and 13.3% by Vinas et al. [1993].

Table 4.2 - Samples characteristics for the single-stage anaerobic digestion batches at S_o/X_o ratio of 2 gCOD/gVSS

CH_4 (mL)	pH_{final}	$TCOD_i$ (mg/L)	CH_4 Yield ($L_{CH4}/gCOD_{substrate\text{-}initial}$)	($L_{CH4}/L_{thin\ stillage}$)
1299	5.05±0.15	35483	0.3	29

Table 4.3 - Samples characteristics for the two-stage anaerobic digestion batches (methanogenic step) at S_o/X_o ratio of 2 gCOD/gVSS

From S_o/X_o [a]	pH_{final}	CH_4 (mL)	$TCOD_i$ (mg/L)	CH_4 Yield ($L_{CH4}/gCOD_{substrate\text{-}initial}$)
4	7.57±0.01	1020	27060	0.37
6	7.55±0.01	1073	27780	0.38
8	7.54±0.02	1035	26853	0.36

[a] from the acidogenic stage (hydrogen production)

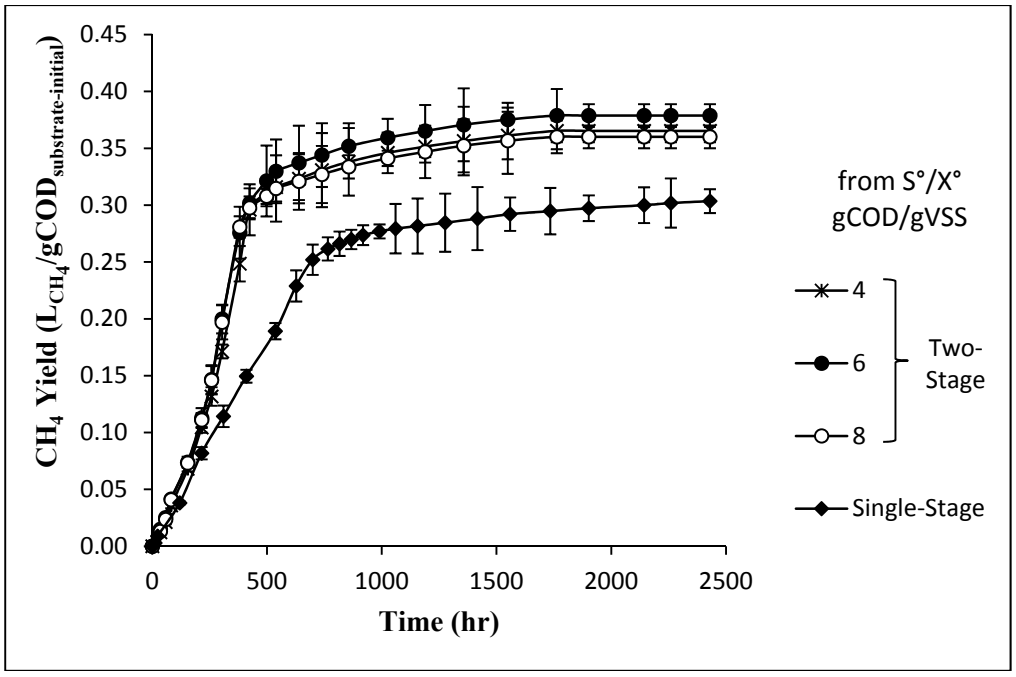

Figure 4.2 - CH_4 yield for single and two-stage batches

Figure 4.3 shows the maximum methane production rates for the single and two-stage anaerobic digestion processes. The methane production rate in the two-stage anaerobic digestion was higher than that in the single-stage process. Maximum methane production

rates of 3.67, 3.88, and 3.78 mL CH_4/hr were achieved in the three runs B1, B2, and B3, respectively, which were 38% higher than the 2.82 mL CH_4/hr in the single-stage experiment.

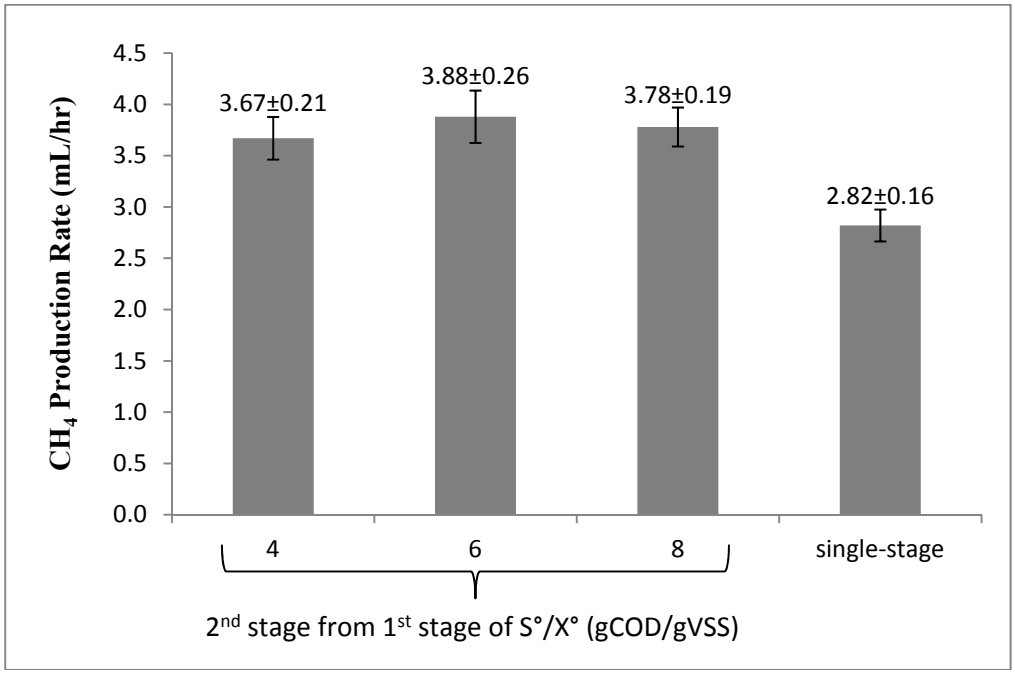

Figure 4.3 - CH_4 production rates for single and two-stage batches

In the single-stage anaerobic digestion, the methane yield based on the thin stillage COD added was 0.26 L/$gCOD_{added}$ (STP) as compared to 0.33 L/$gCOD_{added}$ (STP) in the two-stage anaerobic digestion process. Lee et al. [2011] reported a methane yield of 0.22 L/$gCOD_{added}$ (STP) using corn thin stillage of TCOD 131 g/L in a single-stage anaerobic digestion process. After correcting for the methane produced from the blank (inoculum only), the volumetric yield of thin stillage used was 26 L $CH_4/L_{thin\ stillage}$ (STP) in the single-stage experiment. Yields based on thin stillage used were not calculated for the two-stage anaerobic digestion since the substrate used was the supernatant from the acidogenic step and not raw thin stillage.

In the first step of the two-stage anaerobic digestion process, hydrogen yields of 557, 478, and 247 mL/$gCOD_{removed}$ were achieved in the acidogenic step for runs A1, A2, and A3, respectively [Nasr et al., 2011].

4.3.3. Volatile fatty acids

After the hydrolysis stage, the acid forming bacteria ferment glucose to produce a mixture of VFAs of acetic, butyric, and propionic acids [Batstone et al., 2002] according to the reactions:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH \text{ (acetic)} + 4H_2 + 2CO_2 \quad (4.3)$$

$$C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COOH \text{ (butyric)} + 2H_2 + 2CO_2 \quad (4.4)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH \text{ (propionic)} + 2H_2O \quad (4.5)$$

$$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH \text{ (propionic)} + 2CH_3COOH \text{ (acetic)} + 2CO_2 + + 2H_2O \quad (4.6)$$

The TVFAs measured for the final samples after the acidogenic step were 24, 29.5, and 21.4 gCOD/L for S_o/X_o ratios of 4, 6, and 8 gCOD/gVSS, respectively. The hydrogen potential from S_o/X_o ratio of 8 gCOD/gVSS (run A3) was around one third for the other two S_o/X_o ratios (runs A1 and A2), and the hydrogen yield based on COD removed was less than half the hydrogen yields in runs A1 and A2. However, the final TVFAs for run A3 were 87.5% and 72.4% of the final TVFAs for runs A1 and A2, respectively.

It is noteworthy that in the methanogenic phase of the two-stage anaerobic digestion process, the concentration of TVFAs in the influent accounted for 53.6% of the TCOD, while TVFAs in the single-stage anaerobic digestion influent was only 10% of the TCOD. Since it

is widely known that in methanogenesis 67% of the methane is produced by acetate-utilizing methanogens and 33% is by hydrogenophilic methanogens [Kotsyurbenko et al., 2004], the importance of separating the acidification phase in a two-stage anaerobic digestion process, is emphasized.

4.3.4. Anaerobic biodegradability

The extent of anaerobic biodegradability (BD_{CH4}) of thin stillage can be calculated from the experimental methane yield, taking into consideration the theoretical methane yield of 0.35 L/gCOD (STP) [Raposo et al., 2011], i.e.:

$$BD_{CH4}\,(\%) = (B_{o\,exp} / B_{o\,th}) * 100 \qquad (4.7)$$

where $B_{o\ exp}$ is the experimental methane potential (L) and $B_{o\ th}$ is the theoretical methane potential (L) based on the initial TCOD of thin stillage. The anaerobic biodegradability of thin stillage was 88.2% in the single-stage anaerobic digestion and 99% in case of the two-stage anaerobic digestion. This emphasizes that indeed the acidogenic step enhanced the anaerobic biodegradability of thin stillage.

Anaerobic digestion is commonly described as a first-order reaction, and can be expressed as:

$$\ln [(B_o - B)/B_o] = - k\, t \qquad (4.8)$$

where t is the digestion time (d), k is the first order kinetic constant (d^{-1}), B_o is the methane potential at the end of the experiment, and B is the methane production at time t [Chen and

Hashimoto, 1978]. In the single-stage anaerobic digestion, the value of the kinetic constant *k* was 0.05 d^{-1}, while in the two-stage anaerobic digestion a kinetic constant of 0.07 d^{-1} was achieved.

4.3.5. Hydrogen and methane energy yields

COD destruction efficiencies during the methanogenic stage were relatively low at 43-53% due to the high initial S_o/X_o value of 2 gCOD/gVSS, and accordingly are not representative of continuous flow digestion which operates at SRTs of 15 days and loadings of 0.15-0.30 gCOD/gVSS-d. To compare the performance of single-stage vs. two-stage digestion, energy outcome from both systems was calculated using the following assumptions: theoretical methane yield of 0.35 L $CH_4/gCOD_{consumed}$ (STP), energy content of hydrogen and methane of 142 $kJ/g_{hydrogen}$ (equivalent to 12.8 $kJ/L_{hydrogen}$) [Cai et al., 2004] and 50 $kJ/g_{methane}$ (equivalent to 35.8 $kJ/L_{methane}$) [Ogden, 2002], respectively, and COD destruction efficiency of 80% in the single stage anaerobic digestion process [Elbeshbishy and Nakhla, 2011] and an overall COD destruction efficiency of 90% in the two-stage process [Blonskaja et al., 2003; Vinas et al., 1993; Hafez et al., 2010b]. One liter of thin stillage in a single-stage continuous-flow anaerobic digestion process generates 38.5 liters of methane which is equivalent to 1380 kJ. On the other hand, one liter of thin stillage in a two-stage continuous-flow anaerobic digestion process generates 19.5 liters of hydrogen in the first stage and 38.7 liters of methane in the second stage which is equivalent to a total of 1635 kJ with an 18.5% increase in the energy yield. Similarly, Luo et al. [2011] observed an 11% increase in overall energy yield in a thermophilic two-stage hydrogenic and methanogenic digestion of thin stillage as compared to a single-stage thermophilic system.

The advantages of two-stage over single-stage mesophilic digestion of waste activated sludge (i.e. higher organic stabilization and gasification rates and efficiencies, enhanced net energy production, and greater pathogen kills) have been known for decades [Ghosh et al., 1995]. The fundamental difference between the conventional two-stage anaerobic digestion process with acidification as a first stage and a two-stage process with hydrogen production in the first stage is the optimization of hydrogen production with respect to environmental and operational conditions in the latter one. Many studies investigated the pH and HRT effect on hydrogen production and concluded that the optimal pH is 5.5 and optimal HRT is in the range of 3-8 hours [Li and Fang, 2007]. Recently, Kvesitadze et al. [2012] has confirmed that thermophilic hydrogen production from the organic fraction of municipal solid wastes in batches at pH 5.5 peaked at 8 hours. On the other hand, A wide range of HRT (2-5 days) was reported for the acidification stage with negligible hydrogen production [Elbeshbishy and Nakhla, 2011; Takashima and Tanaka, 2010].

4.4. Conclusions

The use of two-stage digestion for the treatment of thin stillage led to an increase in the TVFAs to TCOD ratio from 10% to 56.8% due to the acidification process during hydrogen production in the first stage. The methane yield in the anaerobic digestion stage increased from 0.26 L CH_4 / g COD_{added} to 0.33 L CH_4 / g COD_{added}. Comparison of energy outcome from both digestion scenarios revealed that an overall increase of 18.5% in energy yield can be achieved in the two-stage digestion due to the enhancement in methane yield and the additional energy produced from hydrogen gas.

4.5. References

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CHAPTER 5

Application of Artificial Neural Networks for Modeling of Bio-Hydrogen Production

5.1. Introduction

Dark fermentative hydrogen production is a promising method for biohydrogen production since it has higher production rates than other processes, and utilizes a wide range of renewable feedstock [Mizuno et al., 2000]. Many factors can influence the fermentative process such as the inoculum type and concentration, substrate type and concentration, reactor configuration, temperature, and pH because they affect the activity and type of the hydrogen producing bacteria [Wang and Wan, 2009a].

To date, hydrogen is not commercialized as an energy source but it is widely used as a chemical reactant in the production of fertilizers, diesel refining, and industrial synthesis of ammonia [Guo et al., 2010]. It has been well documented that modeling fermentative hydrogen production process is one of the most critical requirement for improving our ability to predict the biohydrogen yield [Prakasham et al., 2011]. Modeling the biohydrogen process is very important so as to provide information on the different factors affecting biohydrogen production processes.

Experimental optimization methods such as the "One-factor-at-a-time" are ineffective, time and materials consuming and they do not take into consideration the interaction between these factors. Some studies investigated the combined effect of two variables such as pH and substrate concentrations [Ginkel and Sung, 2001; Li et al., 2008], temperature and pressure release methods [Gadhamshetty et al., 2009], and pH and sulphate concentration [Hwang et al., 2009] on the biohydrogen production process. Ginkel and Sung [2001] tested the effect of varying pH (4.5 – 7.5) and substrate concentration (1.5 – 44.8

gCOD/L) and their interaction on hydrogen production in batch tests using compost as the seed microflora and sucrose as the substrate. The aforementioned authors achieved maximum hydrogen production of 74.7 mL/L-h at pH 5.5 and substrate concentration of 7.5 gCOD/L. These findings were consistent with Li et al. [2008] who observed optimum conditions of pH 6.0 and substrate concentration of 8 gCOD/L to achieve a hydrogen yield of 1.83 $mol/mol_{glucose}$ using seed sludge from a river bed and glucose as the substrate. Gadhamshetty et al. [2009] investigated two different pressure release methods for hydrogen batches, intermittent pressure release (IPR) and continuous pressure release (CPR), each at temperatures of 22°C and 37°C. The IPR method at 22°C gave the maximum hydrogen yield of 4.3 $mol/mol_{sucrose}$. The effect of varying sulphate concentration (0-20 g/L) with pH (5.5-6.2) on continuous fermentative hydrogen production were investigated using anaerobic digester sludge (ADS) growing on glucose in a chemostat reactor [Hwang et al., 2009]. The aforementioned authors found optimum conditions of pH 5.5 and sulphate concentration of 3 g/L to produce maximum hydrogen production rate of 2.8 L/d.

Mathematical models can be empirical as the modified Gompertz equation, which has been widely used for batch fermentative biohydrogen production [Elbeshbishy et al., 2010; Wang and Wan, 2009b; Gadhamshetty et al., 2010]. The modified Gompertz equation includes three parameters that are used to fit the equation; lag time, hydrogen production potential, and hydrogen production rate. Due to the empirical nature of the model, it does not take into consideration the effect of many important parameters such as the substrate concentration, pH, and temperature. Other mathematical models were derived from the conventional kinetic equations of Monod to describe the biohydrogen production rates [Lee et al., 2008; Zheng and Yu, 2005] or the biomass growth [Kumar et al., 2000; Nath et al., 2008].

Artificial Neural Network (ANN) is a mathematical representation of the neurological functioning of a brain. It simulates the brain's learning process by mathematically modeling the network structure of interconnected nerve cells [Nagata and Chu, 2003]. ANN is a powerful modeling tool for problems where the parameters that govern the results are either not defined properly or too complex [Flood and Kartam, 1994]. It is able to describe the interactive effects among these different parameters in a complicated bioprocess [Wang and Wan, 2009c]. ANN is capable of modeling these complex relationships between input and output parameters without requiring a detailed mechanistic description of the phenomena that is governing the process [Shi et al., 2010].

A typical neural network has an input layer, one or more hidden layers, and an output layer. The neurons in the hidden layer, which are linked to the neurons in the input and output layers by adjustable weights, enable the network to compute complex associations between the input and output variables [Nagata and Chu, 2003]. Training the model is the process of determining the adjustable weights and it is similar to the process of determining the coefficients of a polynomial by regression. The weights are initially selected in random and an iterative algorithm is then used to find the weights that minimize the differences between the model-calculated and the actual outputs.

The most commonly used algorithm in ANN is the back propagation (BPNN) [Nagata and Chu, 2003]. In this training algorithm, the error between the model results of the output neurons and the actual outputs is calculated and propagated backward through the network. The algorithm adjusts the weights in each successive layer to reduce the error. This procedure is repeated until the error between the actual experimental and network-calculated outputs satisfies a pre-specified error criterion [Nagata and Chu, 2003].

ANN has gained an increasing interest in wastewater treatment and biogas production applications due to the complex microbial and physiochemical processes [Cinar et al., 2006; Chen et al., 2008]. Cinar et al. [2006] succeeded in developing an ANN model for the modeling of a submerged membrane bioreactor processing cheese whey wastewater. They used SRT, HRT, flux, influent COD, influent ammonia, influent nitrate, influent phosphate, and pressure in membrane as the input parameters and the effluent concentration of COD, ammonia, nitrate, and phosphate as the output parameters. In another study, Chen et al. [2008] used the ANN in simulating a two-phase anaerobic digestion (TPAD) system comprised of a continuous stirred tank reactor (CSTR) for acidogenic phase and an up-flow anaerobic sludge blanket-anaerobic filter (UASBAF) for methanogenic phase followed by a subsequential membrane bioreactor (MBR). The TPAD-MBR system treated chemical synthesis-based pharmaceutical wastewater and the ANN model was able to simulate the removal of COD. Hamed et al., [2004] used ANNs to model the effluent biochemical oxygen demand (BOD) and suspended solids (SS) concentration at a major wastewater treatment plant. Aguado et al. [2006] used the ANN to estimate the effluent wastewater quality parameters such as effluent chemical oxygen demand (COD) or total Kjehldahl nitrogen (TKN) concentrations.

Few studies in the literature investigated the modeling of biohydrogen production in batch studies using ANN. Table 5.1 shows a summary for different biohydrogen production studies that used ANN as a modeling tool. Wang and Wan [2009c] studied the effects of temperature, initial pH and glucose concentration on fermentative hydrogen production by mixed cultures in batch tests. The ANN model successfully described the effects of these parameters on the substrate degradation efficiency, hydrogen yield, and average hydrogen production rate.

Shi et al. [2010] presented a BPNN model that accurately predicted the steady-state performance of bioreactors for the biohydrogen production process using sugar refinery wastewater in an integrative biological reactor (IBR) which is the integration of a CSTR and a UASB reactor. The model consisted of 4 neurons in the input layer of volume loading rate (VLR), oxidation-reduction potential (ORP), alkalinity, and pH, three neurons in a single hidden layer, and hydrogen production rate as the output of the model.

Another continuous flow system performance was simulated using ANN by Mu and Yu [2007]. A model was designed, trained and validated to predict the steady-state performance of a granular-based hydrogen-producing upflow anaerobic sludge blanket (UASB) reactor. Organic loading rate (OLR), hydraulic retention time (HRT), and influent bicarbonate alkalinity were the inputs of the model, while the output variable was either hydrogen concentration, hydrogen production rate, hydrogen yield, effluent total organic carbon, or effluent aqueous products including acetate, propionate, butyrate, valerate, and caporate. The model effectively described the daily variations of the UASB reactor performance and predicted the steady-state performance at various substrate concentrations and HRTs.

Table 5.1 – Experimental data used for BPNN model

Input	Output	Reactor	Substrate	Inoculum	ANN structure	Number of data points	Ref.
ORP, pH, dissolved CO_2	HP with time	Batch	Cheese whey	E.coli	-	102	Rosales-Colunga et al., 2010
HRT, S_o, X_o, ethanol, organic acids conc., ORP, pH, recycle ratio, alkalinity	HPR	CSTR	Sucrose	Sewage Sludge	12-20-1	-	Nikhil et al., 2008
OLR, ORP, pH, alkalinity	HP	CSTR	Kitchen wastes	Anaerobic Activated Sludgs	4-3-1	-	Shi et al., 2010
OLR, HRT, influent alkalinity	H_2%, HPR, HY, TOC_{eff}, products conc.	UASB	Sucrose	ADS	-	140	Mu and Yu, 2007
pH, Glucose:Xylose, Inoculum size, Inoculum age	Cumulative H_2	Batch	Glucose + Xylose	Compost	4-10-1	16	Prakasham et al., 2011
T°C, pH_i, S_o	HY	Batch	Glucose	ADS	3-4-1	20	Wang and Wan, 2009a
T°C, pH_i, S_o	Substrate degradation efficiency %, HPR, HY	Batch	Glucose	ADS	3-5-1	29	Wang and Wan, 2009c

ORP: Oxidation reduction potential, HP: Hydrogen production, HRT: Hydraulic retention time, S_o: initial substrate concentration, X_o: initial biomass concentration, HPR: Hydrogen production rate, CSTR: Continuous stirred tank reactor, OLR: Organic loading rate, HY: Hydrogen yield, TOC_{eff}: Effluent total organic carbons, UASB: Up-flow anaerobic sequencing batch reactor.

ANN models may be successfully applied in biohydrogen production systems and can capture effectively the nonlinear relationships existing between variables in complex systems like fermentative biohydrogen production. However, one of the main limitations of ANN is the uncertainty of outputs prediction outside the data range, used in establishing the model [Chai et al., 2010; Cunge, 2003]. In addition, the network functions known as the "Black boxes" with largely unkown rules of operation, do not provide direct equations relating input and output parameters or any kinetic coefficients such as the maximum rate of substrate utilization (k) or the biomass decay coefficient (k_d) [Cunge 2003].

The few studies that investigated hydrogen production modeling using ANN not only varied widely in terms of input parameters and there was no explicit agreement on the most crucial input parameters, but also focussed on the maximum hydrogen production rates and yields. The aim of this study is to use the capabilities of ANN to predict hydrogen production profile with time in a batch system.

5.2. Methodology

5.2.1. Experimental data

Data was collected from the literature in order to establish the BPNN model. Table 5.2 shows the experimental data sources, as well as the minimum and maximum values for the input and output parameters. Initial pH ranged from 5.5 to 7.5, initial substrate (glucose or sucrose) concentration ranged from 0.3 to 58.56 gCOD/L, initial biomass concentration ranged from 0.86 to 17.62 gCOD/L, temperature ranges from 20 to 55 °C (mesophilic and thermophilic conditions), maximum fermentation time for batches was 97 hours, and maximum volumetric hydrogen production was 382 mL. All experiments were in batch studies and were using glucose or sucrose as the substrate and mixed cultures as the seed

microflora. Three hundred and thirteen data points from 26 different batch experiments were collected from 7 different studies as shown in Table 5.2. Ranges for the input and output data used in establishing the BPNN model are shown in Table 5.3. Input variables were normalized in the range of (-1, 1) to avoid any numerical overflow prior to training, as well as reducing the errors and decreasing the training time [Sola and Sevilla, 1997]. The ANN divided the data set randomly for training (60%), validation (20%), and testing (20%) the model.

Table 5.2 – Data base sources and experimental conditions

	Source	No. of batches	No. of data points	pH_i	T°C	S_o gCOD/L	X_o gCOD/L
1	Wang and Wan 2008b	8	72	7	20-55	10.7	1.68
2	Zheng and Yu 2005	1	6	6	37	10.7	3.12
3	Baghchehsaraee et al. 2008	1	6	6.7	37	10.7	2.84
4	Elbeshbishy et al. 2010	1	9	6.5	37	8.6	2.27
5	Chen et al. 2006	6	56	5.5	36	0.3-9.0	1.15-0.87
6	Oh et al. 2003	2	10	5.5	25	3.0	2.84
7	Nasr et al. 2011	7	154	5.5	37	4.4-58.6	9.74-17.62

Table 5.3 – Range for input and output parameters used in BPNN model

Parameter	Minimum	Maximum	Unit
pH_i	5.5	7.5	-
S_o	0.3	58.56	gCOD/L
X_o	0.86	17.62	gCOD/L
T	20	55	°C
t	0	97	hr
H_2	0	382	mL

X_o: biomass initial concentration,T: temperature, t: time
pH_i: initial pH, S_o: substrate initial concentration
H_2: volumetric hydrogen production

5.2.2. ANN structure

To predict hydrogen production with time, a BPNN was considered and the chosen input parameters were initial pH, initial substrate concentration (S_o), initial biomass concentration (X_o), temperature (T), and time (t). The input layer consisted of five neurons (pH, S_o, X_o, T, t), while the output layer had one neuron which is the hydrogen production with time. A one layer configuration with different numbers of neurons was tested but showed high errors. Therefore, a double layer configuration was selected for the hidden layer. In order to determine the number of neurons in the hidden layers, different trials were investigated. Figure 5.1 shows the mean square error (MSE) between the experimental and predicted data calculated by the following Equation for different number of neurons in both hidden layers.

$$\mathrm{MSE} = \frac{\sum_{i=1}^{n}(Y_{i,e} - Y_{i,p})^2}{n} \qquad (5.1)$$

where $Y_{i,e}$ is the experimental data, $Y_{i,p}$ is the corresponding predicted data, and n is the number of experimental data points.

Figure 5.1 indicates that the minimum MSE occurred at 6 neurons and 4 neurons in the first and second hidden layers, respectively. It has been reported that when the number of neurons in the hidden layer is higher than the optimum, the neural network becomes very complex and will take longer time to train [Wang and Wan, 2009c].

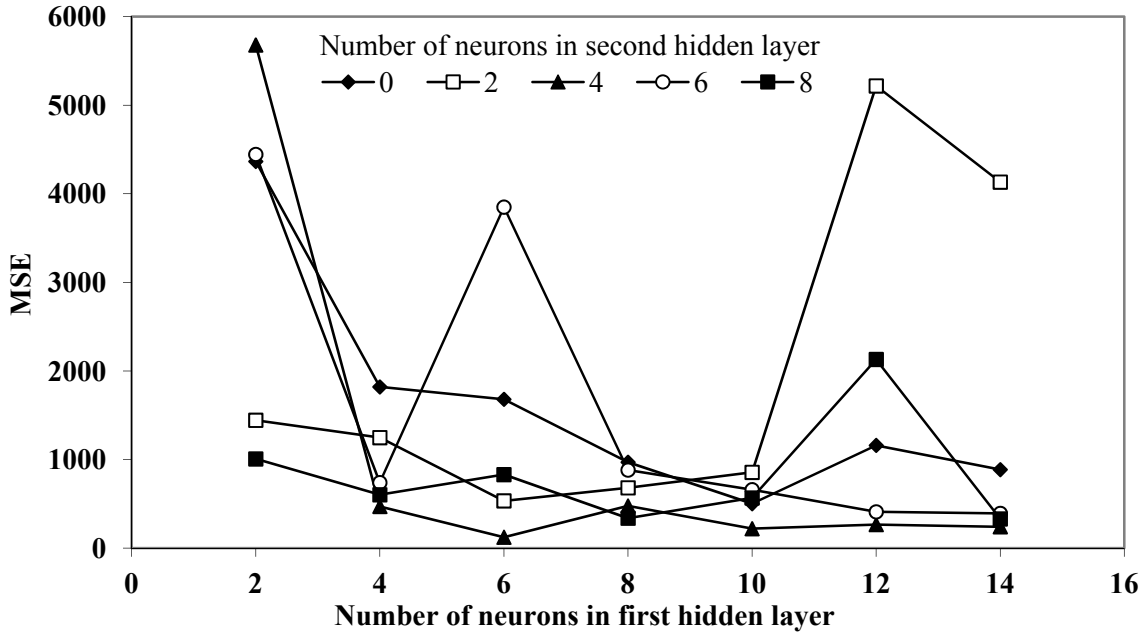

Figure 5.1 – Error calculated at different number of neurons in first and second hidden layers

5.2.3. BPNN training

All the neurons in the hidden layer were non-linear with sigmoid transfer function. Figure 5.2 shows the structure of the BPNN and the type of transfer functions between the input and hidden layer 1, hidden layer 1 and hidden layer 2, and that between hidden layer 2 and the output layer. The BPNN was trained on a Matlab platform R2009 (MathWorks, Inc.).

A feed forward neural network with back propagation algorithm was used in this study. In the BPNN training process, the calculated error between the experimental data and the corresponding predicted data MSE was calculated and then propagated backward through the network in each cycle. The algorithm adjusts the weights between the input, hidden layer, and output neurons in order to reduce the error and the procedure is repeated until the error between the experimental and predicted data satisfies certain error criterion.

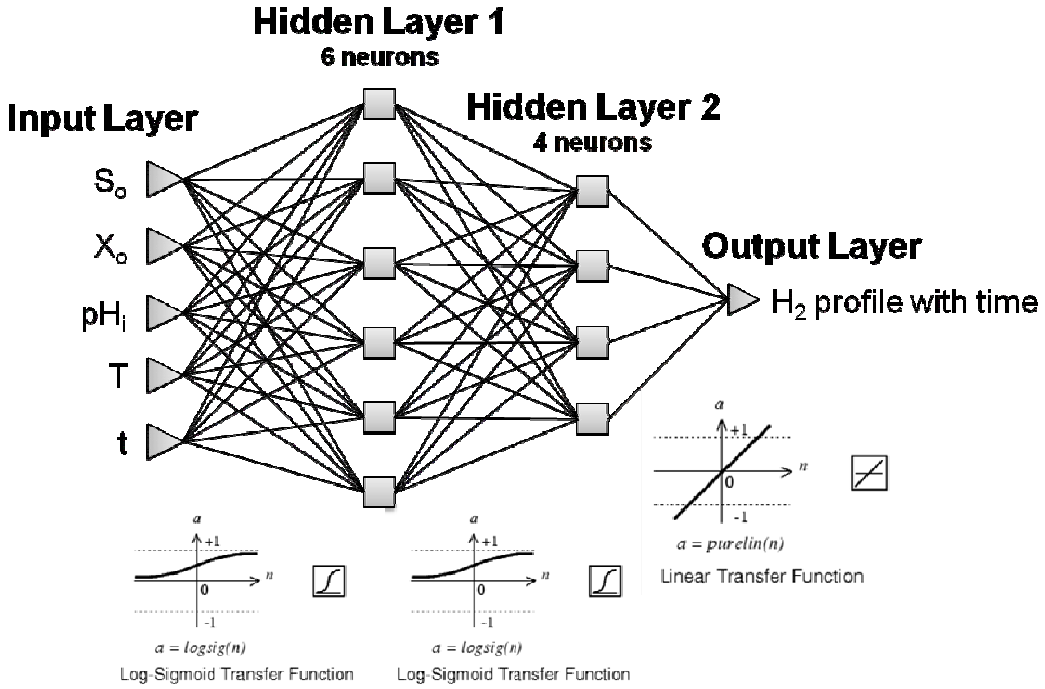

Figure 5.2 – ANN configuration

5.3. Results and discussion

5.3.1. Hydrogen production prediction using BPNN

In order to evaluate the BPNN modeling ability, experimental data were plotted against the predicted data, where the closer the points to the line of perfect prediction, the higher the modeling prediction ability. The correlation coefficient was calculated to assess the model performance as well as the mean absolute error (MAE) that is used to measure how close predictions are to the actual data values, and is calculated as follows:

– (5.2)

where $Y_{i,p}$ is the predicted value, $Y_{i,e}$ is the corresponding experimental value, and n is the number of experimental data points.

Figure 5.3 shows the correlation between the experimental hydrogen production data and the hydrogen production predicted by the BPNN for data points used for training, validating, and testing the model (Table 5.2). Correlation coefficients of 0.988, 0.987, and 0.996 and MAE of 1.89 mL, 6.16 mL, and 4.89 mL were achieved for the training, validating, and testing data points, respectively.

The BPNN model was then used to estimate the hydrogen evolution with time for three new data sets adopted from Chen et al. [2006], Nasr et al. [2011], and Wang and Wan [2008a] that were not used in the training process. Chen et al. [2006] investigated biohydrogen production from sucrose in batch studies using ADS at 36°C and initial pH of 5.5. Nasr et al. [2011] investigated biohydrogen production from thin stillage as the substrate using ADS as the seed microflora at 37°C and initial pH of 5.5. Wang and Wan [2008a] investigated biohydrogen production from glucose in batch studies at 35°C using preheated anaerobic digester sludge at an initial pH of 7. Figure 5.4 shows the correlation between the predicted and experimental data points from the aforementioned sets of data, where a correlation coefficient of 0.965 and an MAE of 11.2 mL were obtained. Average percentage error (APE), defined as the summation of the absolute difference between the experimental and predicted values divided by the experimental values, averaged over the number of data points were 1.4% and 9.6% for the data sets adopted from Nasr et al. [2011] and Chen et al. [2006], respectively. Figure 5.5 shows the experimental and predicted hydrogen production profile using the two sets of data. Although Nasr et al. [2011] used a real waste as a substrate as opposed to glucose or sucrose that were mostly used in establishing the model, the model

was able to predict the hydrogen production profile accurately. The reason is that the substrate concentration was expressed in gCOD/L for all data points.

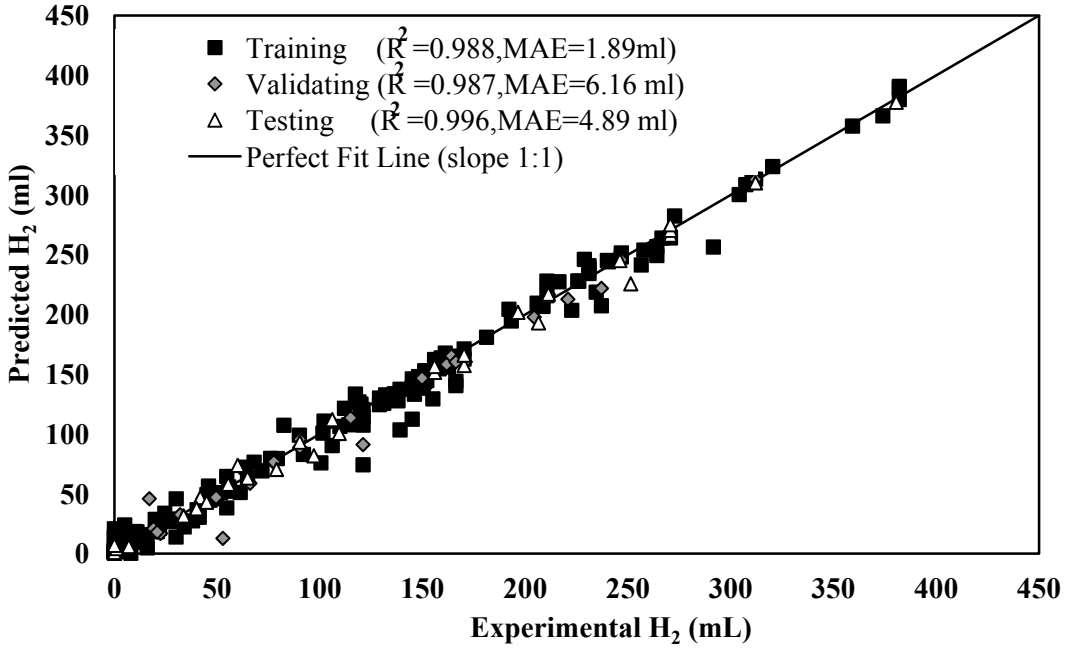

Figure 5.3 – Correlation between experimental and predicted data used in BPNN model

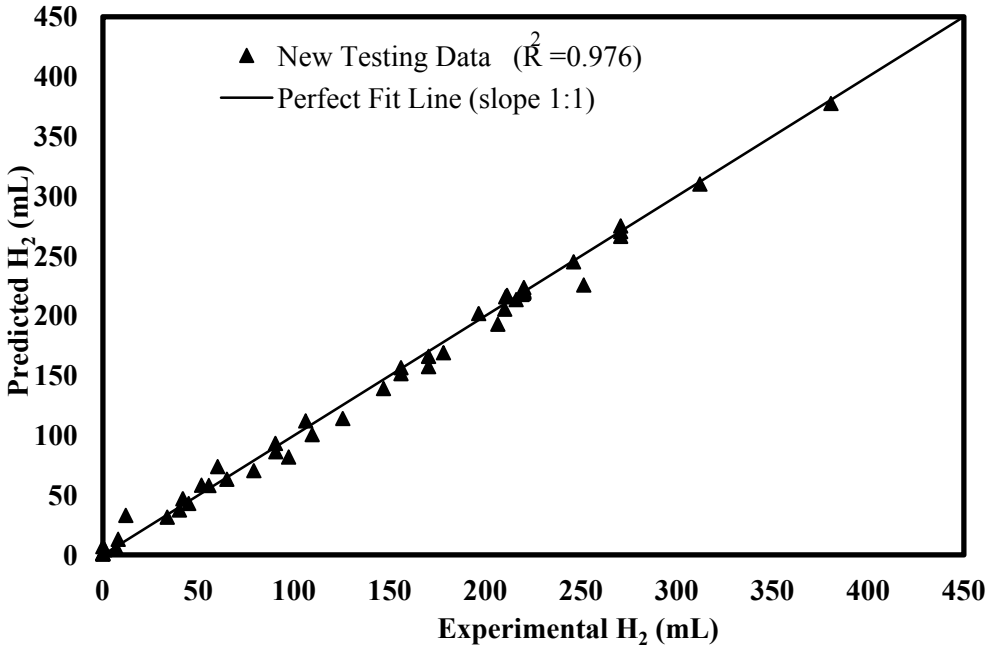

Figure 5.4 – Correlation between experimental and predicted data adopted from Chen et al. [2006], Nasr et al. [2011], and Wang and Wan [2008a]

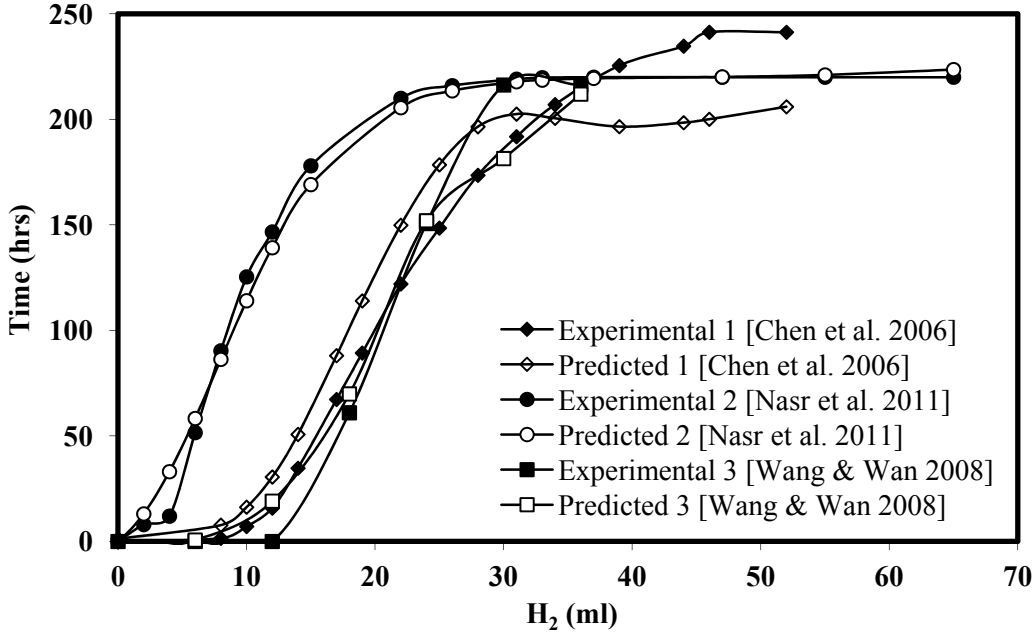

Figure 5.5 – Experimental and predicted hydrogen production profile using data from Chen et al. [2006], Nasr et al. [2011], and Wang and Wan [2008a]

5.4. Conclusion

Dark fermentative hydrogen production is a highly complex process that is difficult to model. This study is aimed at demonstrating the possibility of adapting artificial neural networks to predict the hydrogen production profile with time as a function of initial pH, initial substrate and biomass concentrations, temperature and time in batch experiments. A database for the hydrogen production tests was adopted from the literature and used for training, validating and testing the ANN model. The results support the following conclusions:

- The developed ANN model is a viable method for predicating hydrogen production profile with time. It showed an excellent ability to capture the interrelationships between the process parameters
- Correlation coefficients of 0.988, 0.987, and 0.996 and MAE of 1.89 mL, 6.16 mL, and 4.89 mL were achieved for the training, validating, and testing data points, respectively
- A correlation coefficient of 0.965 and an MAE of 11.2 mL were obtained when testing the proposed model using a new data set

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CHAPTER 6

Conclusions and Recommendations

6.1. Conclusions

The following findings summarize the major outcomes of this research according to the major objectives as follows:

- *Biohydrogen Production:*
  1. Thin stillage has a potential for hydrogen production with a yield of 19.5 L $H_2/L_{thin\ stillage}$ with acclimatized anaerobic digester sludge (AADS) while tests with anaerobic digester sludge (ADS) only revealed a maximum potential of 7.5 L $H_2/L_{thin\ stillage}$.
  2. The optimum experimental range of S_o/X_o ratio for hydrogen production is 1-2 $gCOD_{substrate}/gVSS_{seed}$ using conventional ADS.
  3. The optimum experimental range of S_o/X_o ratio for hydrogen production within the investigated range is 3-6 $gCOD_{substrate}/gVSS_{seed}$ using AADS.
  4. The biomass specific hydrogen production rate for the AADS was 3.5 times higher than that of the ADS throughout the range of S_o/X_o ratio that was studied.
  5. The DGGE profiles of the 16S rDNA gene fragments for the AADS confirmed its superior performance over the ADS due to the predominance of high hydrogen producers such as *C. acetobutyricum, K. pneumonia, C. butyricum* and *C. pasteurianum*.

- *Two-Stage Anaerobic Digestion:*

  1. The use of two-stage digestion for thin stillage led to an increase in the TVFAs to TCOD ratio from 10% to 56.8% due to the acidification process during hydrogen production in the first stage.
  2. The methane yield in the anaerobic digestion stage increased from 0.26 L CH_4 / g COD_{added} in the single-stage process to 0.33 L CH_4 / g COD_{added} in the two-stage process.
  3. Comparison of energy outcome from both digestion scenarios revealed that an overall increase of 18.5% in energy yield can be achieved in the two-stage digestion due to the enhancement in methane yield and the additional energy produced from hydrogen gas.

- *Artificial Neural Network Model:*

  1. The ANN model developed is a viable method for predicting fermentative biohydrogen production in batch studies
  2. At a given initial pH, substrate and biomass initial concentrations, temperature and time, hydrogen production potential can be predicted
  3. The proposed model is not capable of predicting beyond the range of the data used which is
     a. initial pH (5.5-7.5)
     b. initial substrate concentration (0.30-58.56 gCOD/L)
     c. initial biomass concentration (0.86-17.62 gCOD/L)
     d. temperature (20-55 °C)
     e. time (0-97 hr)

6.2. Recommendations

Based on the findings of this research, the recommended future research should include:

1. Assessment of different waste streams such as food wastes, brewery wastes, kitchen wastes, and starch for biohydrogen production using acclimatized anaerobic digester sludge
2. Investigation of the impact of optimizing the operational conditions for biohydrogen production in the first stage such as the HRT, SRT, and OLR on methane production in the second stage of an anaerobic digestion process in a continuous flow system
3. Extension of the proposed Artificial Neural Network model beyond the current data range as well as including more parameters as inputs to the model such as the reactor volume, the substrate to biomass ratio, and the buffer concentration.

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