Hydrogen Production By Anaerobic Fermentation Using Agricultural and Food Processing Wastes Utilizing a Two-Stage Digestion System

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HYDROGEN PRODUCTION BY ANAEROBIC FERMENTATION USING AGRICULTURAL AND FOOD PROCESSING WASTES UTILIZING A TWO-STAGE DIGESTION SYSTEM

by

Reese S. Thompson

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

MASTER OF SCIENCE

in

Biological Engineering

Approved:

Dr. Conly L. Hansen Dr. Carl S. Hansen
Major Professor Committee Member

Dr. Sridhar Viamajala Dr. Byron Burnhan
Committee Member Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2008
Hydrogen production by means of anaerobic fermentation was researched utilizing three different substrates. Synthetic wastewater, dairy manure, and cheese whey were combined together at different concentrations under batch anaerobic conditions to determine the optimal hydrogen producing potential and waste treatment of each. Cheese whey at a concentration of 55% was combined with dairy manure at a concentration of 45% to produce 1.53 liters of hydrogen per liter of substrate. These results are significant because the control, synthetic wastewater, which was a glucose-based substrate, produced less hydrogen, 1.34 liters per liter of substrate, than the mixture of cheese whey and dairy manure. These findings indicate that cheese whey and dairy manure, which are of little value, have potential to produce clean combusting hydrogen fuel.

The effluent from the anaerobic hydrogen fermentations was then placed into a second continuous-fed reactor as part of a two-phase anaerobic digestion system. This system was designed to produce hydrogen and methane for a mixture of approximately
10% hydrogen. The two-stage process also further treated the synthetic wastewater, dairy manure, and cheese whey. The two-phase anaerobic methanogenic reactor was shown to produce more methane in the second phase (56 L IBR anaerobic digester), 1.36 mL per minute per liter substrate, as compared to the single-phase anaerobic reactor (56 L IBR), which produced 1.22 mL per minute per liter substrate.

In general, this research has suggested that agricultural and food processing wastes provide the needed nutrients for hydrogen production and that a two-phase anaerobic digestion system is ideally set up to produce hydrogen-methane mixtures while treating wastes for discharge into the environment.

(88 pages)
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I would like to thank the United State Department of Agriculture for funding this project. Appreciation is expressed to the Blaine Wade Dairy, Ogden, UT; Utah State Dairy Plant, Logan, UT; and Gossner Foods, Inc., Logan, UT, for the accommodation and help in gathering test samples.

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I want to also thank my wife and family for all their love and support they have offered during this process. None of this would have been possible without them.

Reese Thompson
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<td>USDA</td>
<td>United States Department of Agriculture</td>
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<td>VFA</td>
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CHAPTER 1
INTRODUCTION AND OBJECTIVES

INTRODUCTION

As energy consumption continues to grow throughout the world, fossil fuels are still one of the biggest energy contributors. It is estimated that the global power supply is still based on 84.8% fossil energy (Zurawski et al. 2005). World energy consumption is expected to climb steadily over the next thirty years as a result of economic growth from developing nations and population growth throughout the world. In 2006, the rate of oil consumption globally was 30.6 billion barrels per year (Lattin and Utgikar 2007). The U.S. Geological Survey estimates the total worldwide oil reserves to be 2.6 trillion barrels, 1.7 trillion barrels in proven reserves and 900 trillion in undiscovered reserves (Hallenbeck and Benemann 2002). As these oil reserves continue to be depleted, it is therefore a necessity to find alternative, sustainable energy sources that compensate for growing energy demands.

Along with finding an alternative fuel that supplies the growing energy demands these alternative fuels must also curb the environmental effects of burning fossil fuels. When combusted, fossil fuels release byproducts which have been recognized as causing global pollution and possible climate changes (Das and Veziroglu 2001). In the search for an alternative fuel, special consideration has been put on a fuel that not only supplies the world’s energy demands, but is also a cleaner option to the fossil fuels used today. One source of energy which has received special attention for meeting these requirements is hydrogen.
Hydrogen is considered to be an alternative fuel of great potential use. In 1976, the first World Hydrogen Conference identified hydrogen as a clean energy carrier for the future (Lattin and Utgikar 2007). Instead of greenhouse gases, water with trace amounts of nitric oxide is produced when hydrogen is combusted. It also has a high energy yield of 122 kJ/g, which is 2.75 times greater than gasoline (Antonopoulou et al. 2006). Hydrogen has the potential to lessen the world’s dependency on fossil fuels, but further research and technology is needed before a sustainable hydrogen economy can be established.

Biological production of hydrogen by anaerobic fermentation is one such area of research which shows great potential, but requires further study. The biological production of hydrogen provides a pollution-free and energy-saving process. Biological methods produce hydrogen that is less energy intensive than chemical or electrochemical methods because biological methods are normally carried out at ambient temperature and pressure (Jo et al. 2007). Hydrogen production by fermentative bacteria is technically a simpler process over other biological processes because it proceeds at higher rates and does not require light sources (Han and Shin 2003).

Anaerobic fermentation is also considered a simpler option because it allows the production of hydrogen by relatively straightforward procedures and can utilize substrates from many different sources (Nath and Das 2004). Current research has studied many different types of substrates for the use of hydrogen production. The major criteria for substrate selection are the availability, cost, carbohydrate content, and biodegradability (Kapdan and Kargi 2006). Commercially produced food products, such as corn and sugar, are not yet economical for hydrogen production. Alternatively,
wastewaters with organic waste such as food processing and animal waste have great potential as substrate sources (Benemann 1996). Utilizing wastewaters from agricultural and food processing industries, which are generally high in carbohydrates, can provide the essential nutrients required for hydrogen production and reduce treatment and disposal costs currently needed for these particular waste streams. Treating these waste streams to protect public health and the environment while producing a clean energy source makes biological hydrogen production an attractive alternative to fossil fuels (Kapdan and Kargi 2006).

Several obstacles must be overcome before hydrogen from biological processes can be produced economically. In the anaerobic process there are several stages that occur simultaneously. The last stage, methanogenesis, utilizes the intermediate products from the preceding stages and converts them into methane, carbon dioxide, and water (Parawira 2004). Under normal anaerobic conditions the majority of hydrogen produced is consumed by methanogens. Therefore, to extract hydrogen from this process the methanogenic bacteria must be inhibited to prevent the hydrogen from being used to form methane. A procedure must be established that inhibits the methanogenic bacteria in a continuous process over time while remaining economical and efficient. Once accomplished, the hydrogen formed by the process can be collected and utilized as an energy source.

Another major issue concerning hydrogen production by anaerobic fermentation is controlling the pH of the system. The pH was found to have a profound effect on both hydrogen production potential and other byproducts (Khanal et al. 2004). The chemicals used in laboratory experiments to control the pH are expensive and cause safety issues.
A more economical way to control the pH must be found before large scale production can successfully take place. Along with pH control, proper knowledge of substrate composition and correct concentrations for fermentation must be gathered on each substrate to determine the hydrogen potential and treatment efficiency. Investigation of these issues will be beneficial to understand system requirements and the measures needed to control them. This research will provide essential data and information on producing hydrogen economically and efficiently.

Although this research will provide valuable information regarding anaerobic hydrogen production, this process is not an immediate solution to our current fuel crisis. In order to utilize hydrogen as a fuel the infrastructure must be present for production, storage, distribution, and utilization. The transition into a hydrogen economy has been slowed by both technological challenges and overall economics. In the past hydrogen and hydrogen utilizing technology (i.e. fuel cells) have not been economically competitive with gasoline and internal combustion engines. The demand for hydrogen energy has therefore been limited. This in turn has caused the hydrogen infrastructure to evolve at a very slow rate.

A solution to utilizing hydrogen in the short to medium term until the infrastructure can be established is through hydrogen-methane mixtures. Methane produces less atmospheric pollutants and carbon dioxide per unit energy than other hydrocarbon fuels and already has a distribution network in place (Bauer and Forest 2001). When combined with hydrogen it has been shown to improve engine performance, extend operability ranges, and reduce pollutant emissions (Sarli and
Hydrogen-methane mixtures are a potential immediate solution to a cleaner fuel supply. Another aim of this research will be to develop a two-stage anaerobic digestion system to produce hydrogen and methane quantities necessary for these mixtures. The two-stage process is ideally set up to produce both hydrogen and methane while further degrading waste streams. Although promising in theory, two-stage anaerobic digestion has not been widely accepted because of increased complexity and higher investment and operational costs. The aim of this research will be to determine if the two-stage system can be successfully operated while producing hydrogen and methane and establish if there is a significant difference in potential energy yields between a single-phase system and a two-phase system.

Therefore, the scope of this research will be to investigate a system to produce hydrogen using anaerobic fermentation of inexpensive substrates and develop a two-stage anaerobic digestion system to produce hydrogen-methane mixtures. The overall objective of the first aim will be to investigate methods of producing hydrogen from agricultural and foods wastes in a more cost effective and efficient manner. Optimal operating conditions will be investigated such as pH and substrate concentration. The overall objective of the second aim is to determine the feasibility of a two-stage anaerobic digestion system to produce hydrogen and methane. The results of the experiments will be analyzed and further recommendations specified. This research will give further knowledge and understanding for continued development of anaerobic technology to produce hydrogen.
The overall objective of this research is to develop technology for economical production of hydrogen from agricultural and food production and processing wastes.

1. Perform anaerobic fermentations to produce hydrogen from synthetic wastewater and cheese processing wastes with a mixed bacterial culture that has undergone stress enrichment.

2. Demonstrate quantity and quality of hydrogen that can be produced from these materials.

3. Conduct hydrogen production fermentations using dairy manure and cheese whey as the substrate.

4. Investigate using a two-stage anaerobic fermentation system to treat dairy manure and cheese whey by combining the effluent from the hydrogen production with synthetic wastewater in a pilot scale IBR digester. This determines if more energy and further waste degradation can be accomplished from the substrates.

5. Analyze the success of the experiments by comparing system performance and making recommendations for further research and scale up.
CHAPTER 2
LITERATURE REVIEW

Hydrogen Background

Research into alternative fuels has been an area of great interest throughout the world within the past decades. Reasons for this research include limited fossil fuel supplies and the concerns over global warming. It is estimated that an increase of 35% in the world oil demand will occur over the next 30 years because of growth in the world’s population (Nandi and Sengupta 1998). From this increase, 62% will be from population growth and rapid economic expansion from developing countries (Lattin and Utgikar 2007).

Despite being a clean and high energy fuel, currently only 50 million tons of hydrogen are traded every year with a growth rate of about 10% (Winter 2005). The majority of this hydrogen is used to produce ammonia fertilizer, as feedstock for chemical and petroleum refining areas, plastics, solvents and other commodities (Dunn 2002). Approximately 95% of hydrogen produced is consumed at the site of production with 1.5 million tons being sold for industrial and chemical uses (Lattin and Utgikar 2007). The technology currently used to make hydrogen has been well established, but the majority of hydrogen produced uses fossil fuels in the production process. Approximately 50% of hydrogen production globally comes from natural gas, 30% oil, and 20% coal, see Figure 2-1 (Romm 2005).
There are many different ways of producing hydrogen. Hydrogen production can be divided into physical, chemical and biological methods. The most common and least expensive method used currently is steam methane reforming (Crabtree et al. 2004). Producing hydrogen from steam methane reforming does not reduce fossil fuel use and also generates greenhouse gas emissions (Lattin and Utgikar 2007). Another method of producing hydrogen which may be the cleanest technology is through electrolysis of water. Although clean, the process requires large amounts of electricity and is only seen as practical in areas of the world with relatively cheap electricity rates. At present this method only produces 4% of the world’s hydrogen (Dunn 2002).

A third method, which has only recently started to be explored, is biological hydrogen production. There are several different methods to produce hydrogen biologically. Biological hydrogen processes differ based on the microorganisms involved, the substrates, and the light dependence (Zurawski et al. 2005). The most prominent difference occurs between light dependence. Two routes are possible, the anaerobic fermentative process and the photosynthetic process.

Photosynthetic hydrogen production is accomplished by either biophotolysis or photofermentation. Sunlight is the main driving force for both of these processes. Biophotolysis involves different microalgae and cyanobacteria which are able to split
water into hydrogen and oxygen with use of absorbed light energy. Photo-fermentation involves organic compounds which are converted into hydrogen and carbon dioxide bacteria which utilize sunlight (Reith et al. 2003). Photosynthetic hydrogen production is typically a more complicated process which is easily upset if operational parameters are not strictly followed.

In fermentative anaerobic hydrogen production, microorganisms only need chemical energy which is obtained from the substrate for metabolism (Zurawski et al. 2005). This process, also commonly called dark fermentation, takes place day and night without the need of sunlight. It is considered to have several advantages over photosynthetic hydrogen production such as continuous hydrogen production, a variety of carbon sources which can be used as substrate, production of useful metabolites, and elimination of aeration (Hwang et al. 2004). Also, the bioreactors used in this process require much less space and the effluent produced does not require special waste treatment (Zaborsky 1998).

**Waste Management**

Although the eventual depletion of fossil fuels is a long-term incentive for development of sustainable energy forms, more urgent incentives for renewable energy are related to concerns about global environmental quality. The first concern openly recognized was the release of toxic compounds and oxides of nitrogen and sulfur resulting from combustion of fossil fuels. These air pollutants contribute globally to health and environmental problems the most common of which is referred to as acid rain. The second and greatest concern, however, is the threat of global warming related to
increasing concentrations of carbon dioxide. Use of renewable biomass (including energy crops and organic wastes) as an energy resource is not only "greener" with respect to most pollutants, but its use represents a closed balanced carbon cycle regarding atmospheric carbon dioxide (Spencer 1991). A third concern is the recognized need for effective methods for treatment and disposal of large quantities of municipal, industrial, and agricultural organic wastes. These wastes not only are a major threat to environmental quality, but also represent a significant renewable energy resource.

Millions of tons of solid waste are generated each year from municipal, industrial, and agricultural sources. Large portions of this waste are unmanaged and decompose in the environment. When untreated waste accumulates and is allowed to go septic, the decomposition of the organic matter it contains will lead to problematic conditions which include the production of foul smelling gases and numerous pathogenic microorganisms. This decomposition contaminates huge amounts of land, water, and air and is also a risk to public health and the environment (Parawira 2004). These wastes cost large amounts of money to manage and represent many problems to environmental quality. Although these issues are unfavorable, the waste streams also have potential energy and nutrient values that are not being utilized. Agricultural and food industry wastes are increasingly being examined for alternative uses because of more and more regulatory actions from governmental agencies concerning waste disposal and the volume of which they are being produced (Kargi and Kapdan 2005). The following sections give a brief overview of the agricultural and food processing wastes being produced along with the current treatment options being utilized to treat them.
Agricultural Waste

There are approximately 238,000 farms in the United States where animals are kept and raised in confinement such as the dairy shown above, Figure 2-2. These farms which are known as animal feeding operations produce more than 500 million tons of waste annually (Bryant et al. 1977). In 2002, the Environmental Protection Agency (EPA) revised the Clean Water Act regulation for Concentrated Animal Feeding Operations, or CAFOs. If the animal feeding operation falls within the CAFO regulations a National Pollutant Discharge Elimination System (NPDES) permit must be acquired. As part of this permit each CAFO must plan and begin to execute a comprehensive nutrient management plan (CNMP) (US EPA 2007). The purpose of this plan is to ensure that transport of excess nutrients to groundwater and surface water does not take place. Redistribution of these nutrients can be achieved by various ways, but often include disease risks, high transportation costs, and lack of accessible areas for disposal. For these reasons, disposal using alternative methods has been proposed.
including the use of anaerobic digestion. Anaerobic digestion produces beneficial byproducts which may offset part of the cost of waste management practices.

**Food Industrial Waste**

The food processing industry in the United States is composed of more than 20,000 companies (Elitzak 2000). It is estimated that the average large food processing industry annually produces about 1.4 billion liters of wastewater (Van Ginkel et al. 2005). Wastes from these industries are usually high in organic matter and normally contain sufficient nitrogen, phosphorus, and trace elements for biological growth (Gray 2004). These waste streams usually require treatment practices before being discharged into local sewer districts. The cost of treatment and monitoring is the responsibility of the discharging facility and may be subject to criminal charges and/or fines if done incorrectly. If utilized correctly, these wastes could contain high energy values capable of heating, electrical power generation, and/or fuel for equipment which would return part of the cost of disposal.

**Wastewater Treatment**

Wastewater treatment is essentially a mixture of settlement and biological and/or chemical unit processes (Gray 2004). Unit treatment processes can be classified into five stages: preliminary, primary, secondary, tertiary, and sludge treatment (Rae 1998). Preliminary treatment removes grit, gross solids, and will separate storm water. Other substances such as oil or grease can be removed in this step if high concentrations are present. Primary treatment is sometimes referred to as the sedimentation step. It is the first major stage of treatment and will remove solids that settle or float which are
separated as sludge. The secondary treatment step is also known as the biological step. The dissolved and colloidal organics are treated either aerobically or anaerobically in the presence of microorganisms. The tertiary step involves further biologically treatment if necessary to remove bacteria, available oxygen, suspended solids, toxic compounds, or nutrients. This is done so that the discharge complies with set limits. The sludge treatment will dewater, stabilize, and dispose of sludge. Many different processes are employed in each step to treat the waste according to the quality of the effluent desired (Droste 1997; Gray 2004).

**Biological Wastewater Treatment**

Biological wastewater treatment is primarily used to remove dissolved and colloidal organic substances in a wastewater stream. Organic substances in water naturally decay due to the presence of microorganisms in receiving bodies of water (Droste 1997). Two processes are available, aerobic and anaerobic treatment. Aerobic treatment is accomplished by microorganisms using oxygen supplied through aeration to break down and assimilate wastewater. Aeration of wastewater requires large amounts of energy and mixing to ensure proper treatment. Anaerobic processes, which are operated in the absence or oxygen, are typically used to treat strong organic wastewaters.

For industrial wastewaters with much higher biodegradable chemical oxygen demand (COD) concentrations and elevated temperature, anaerobic processes are typically more economical. Strong organic wastes generated by the agricultural and food industries, often in large quantities, provide a particularly difficult wastewater treatment problem (Gray 2004). Anaerobic treatment, which usually proceeds at a slower rate,
offers a number of attractive advantages in the treatment of strong organic wastes.
This treatment includes a high degree of purification, the ability to treat high organic loads, production of a small quantity of excess sludge, and the production of an inert combustible gas (methane) as an end product (Steritt and Lester 1988). Anaerobic processes also have a low consumption of energy, smaller space requirements, and lower overall costs (Demirel and Yenigun 2002). Although anaerobic processes have several advantages over aerobic processes, they also require stricter operating parameters, are easily upset causing reduced waste treatment, and may produce odors and corrosive gases. Anaerobic treatment can be an effective option for dealing with strong organic wastes, but must be monitored and controlled for optimal waste treatment.

**Biochemical and Microbiological Knowledge of the Anaerobic Process**

The anaerobic process is the degrading of organic substrates in the absence of oxygen to carbon dioxide and methane with only a small amount of bacterial growth (Gray 2004). The digestion process consists of several interdependent, complex sequential and parallel biological reactions. During these reactions the products from one group of microorganisms serve as the substrates for the next (Noykova et al. 2002). The overall conversion process is often described as a three stage process which occurs simultaneously within the anaerobic digester (Parawira 2004). The first is the hydrolysis of insoluble biodegradable organic matter, the second is the production of acid from smaller soluble organic molecules, and the third is methane generation. The three stage scheme involving various microbial species can be described as follows: (1) hydrolysis and liquefaction; (2) acidogenesis, and (3) methane fermentation, Figure 2-3.
Figure 2-3. Anaerobic decomposition of organic matter (Zehnder et al. 1982).

**Hydrolysis and Liquefaction**

Hydrolysis and liquefaction are the breakdown of large, complex and insoluble organics into small molecules that can be transported into the microbial cells and metabolized (Droste 1997). Hydrolysis of the complex molecules such as proteins, carbohydrates, and lipids is catalyzed by extracellular enzymes. Some of the enzymes present include cellulase, amylase, protease, and lipase (Parawira 2004). Essentially, organic waste stabilization does not occur during hydrolysis, and the organic matter is simply converted into a soluble form that can be utilized by the bacteria (McCarty and Smith 1986; Parkin and Owen 1986).

**Acidogenesis**

The acidogenesis stage is a complex phase involving acid forming fermentation, hydrogen production, and an acetogenic step. Sugars, long chain fatty acids, and amino
acids from hydrolysis are used as substrates. Microorganisms produce organic acids (acetic, propionic, butyric and others), alcohols, hydrogen, and carbon dioxide (Parawira 2004). The products formed vary with the types of bacteria as well as environmental conditions. Bacteria responsible for acid production include facultative anaerobic bacteria, strict anaerobic bacteria, or both (i.e. *Bacteroides, Bifidobacterium, Clostridium, Lactobacillus*, and *Streptococcus*) (Cheong 2005). Hydrogen is produced by the acidogenic bacteria and hydrogen-producing acetogenic bacteria.

Organisms that produce fermentation products, such as propionate, butyrate, lactate, and ethanol, generally exhibit obligate proton-reducing metabolism (i.e. they produce hydrogen as a fermentation product). This mechanism is commonly referred to as inter-species hydrogen transfer. The organisms are referred to as syntrophs and may be obligate as is the case of S organisms, *Syntrophomonas wolfei*, and *Syntrophobacter wolinii* or facultative as with many other syntrophs (Zinder 1993). Acetogenic microorganisms can also tolerate a wide range of environmental conditions (Novaes 1986; Parkin and Owen 1986).

The main pathway of acidogenesis is through acetate, carbon dioxide, and hydrogen (Parawira 2004). The accumulation of lactate, ethanol, propionate, butyrate, and higher volatile fatty acids is the response of the bacteria to increased hydrogen concentration in the medium (Schink 1997). In the absence of methanogens to utilize these substrates, hydrogen backs up the overall degradative process and organic acids accumulate causing a decrease in pH which ultimately inhibits and stops the fermentation unless controlled. The overall performance of the anaerobic digestion system is affected by the concentration and proportion of individual volatile fatty acids formed in the
acidogenic stage because acetic and butyric acids are the preferred precursors for methane production (Hwang et al. 2001). These reactions are shown below with glucose as the substrate, Figure 2-4. A theoretical maximum of 4 moles of hydrogen is obtained from acetic acid and 2 moles of hydrogen from butyric acid.

\[
C_6H_{12}O_6 + 2 \text{H}_2\text{O} \rightarrow 2 \text{CH}_3\text{COOH} + 4 \text{H}_2 + 2 \text{CO}_2
\]

\[
C_6H_{12}O_6 + 2 \text{H}_2\text{O} \rightarrow \text{CH}_2\text{CH}_2\text{CH}_2\text{COOH} + 2 \text{H}_2 + 2 \text{CO}_2
\]

Figure 2-4. Glucose conversion during acidogenesis of acetic acid and butyric acid (Nath and Das 2004).

In the acetogenic stage of acidogenesis bacteria will degrade organic acids such as propionic, butyric, and valeric acids to acetate, carbon dioxide, and hydrogen. This intermediate conversion is important for proper anaerobic digestion and methane production because methanogens do not utilize these volatile fatty acids directly (Parawira 2004). During acidogenesis, a clear distinction between acetogenic and acidogenic reactions is not always present (Fox and Pohland 1994).

**Methanogenesis**

The third and final stage is methane fermentation, which is the ultimate product of anaerobic treatment. Formic acid, acetic acid, methanol, and hydrogen can be used as energy sources by the various methanogens. The methane bacteria are such a unique group of organisms that they have been placed into a new evolutionary domain (separate from eukaryotic plants and animals and prokaryotic bacteria) referred to as Archaea (Woese et al. 1990). The majority of methanogenic bacteria belong to the genera *Methanobacterium, Methanosarcina, Methanospirillum, and Methanococcus* (Gray
Methanogens are unique because of the very different cell morphologies found between the species. Most have simple nutritional requirements, carbon dioxide, ammonia, and sulfide. The primary route of methanogenesis is the fermentation of acetic acid to methane and carbon dioxide. The bacteria which utilize acetic acid are classified as acetoclastic bacteria, or acetate splitting bacteria (Cheong 2005). About two thirds of methane gas is derived from acetate conversion by acetoclastic methanogens, see Figure 2-5. Some methanogens are also able to use hydrogen to reduce carbon dioxide to methane (hydrogenophilic methanogens) with an overall reaction as shown in Figure 2-5.

The microbial ecology of biomethanogenesis is difficult to study. The organisms are fastidious, slow-growing anaerobes and many species will not even grow in pure culture (Chynoweth 1987). When grown in pure culture, isolates may produce fermentation products different than those produced in the presence of hydrogen and acetate metabolizing bacteria which are present in their natural environment (Wolin and Miller 1982). Each anaerobic environment may differ in the types of bacteria involved in methanogenesis, depending on differing factors such as substrate, retention time, temperature, pH, and fluctuations in other environmental parameters. Although certain general properties are common from one environment to another, each environment may have its own unique population of bacteria and associated microbial activities.
Hydrogen: \[ 4 \text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \]

Acetate: \[ \text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2 \]

Formate: \[ 4 \text{HCOOH} \rightarrow \text{CH}_4 + 3 \text{CO}_2 + 2 \text{H}_2\text{O} \]

Methanol: \[ 4 \text{CH}_3\text{OH} \rightarrow 3 \text{CH}_4 + \text{CO}_2 + 2 \text{H}_2\text{O} \]

Carbon monoxide: \[ 4 \text{CO} + 2 \text{H}_2\text{O} \rightarrow \text{CH}_4 + 3 \text{CO}_2 \]

Trimethylamine: \[ 4(\text{CH}_3)_3\text{N} + 6 \text{H}_2\text{O} \rightarrow 9 \text{CH}_4 + 3 \text{CO}_2 + 4 \text{NH}_3 \]

Dimethylamine: \[ 2(\text{CH}_3)_2\text{NH} + 2 \text{H}_2\text{O} \rightarrow 3 \text{CH}_4 + \text{CO}_2 + 2 \text{NH}_3 \]

Monomethylamine: \[ 4(\text{CH}_3)\text{NH}_2 + 2 \text{H}_2\text{O} \rightarrow 3 \text{CH}_4 + \text{CO}_2 + 4 \text{NH}_3 \]

Methyl mercaptans: \[ 2(\text{CH}_3)_2\text{S} + 3 \text{H}_2\text{O} \rightarrow 3 \text{CH}_4 + \text{CO}_2 + \text{H}_2\text{S} \]

Metals: \[ 4 \text{Me}^0 + 8 \text{H}^+ + \text{CO}_2 \rightarrow 4 \text{Me}^{++} + \text{CH}_4 + 2 \text{H}_2\text{O} \]

Figure 2-5. Principal Methanogenic reactions (Novaes 1986; Morgan et al. 1991; Chynoweth 1995)

The microbial ecology of biomethanogenesis is difficult to study. The organisms are fastidious, slow-growing anaerobes and many species will not even grow in pure culture (Chynoweth and Isaacson 1987). When grown in pure culture, isolates may produce fermentation products different than those produced in the presence of hydrogen and acetate metabolizing bacteria which are present in their natural environment (Wolin and Miller 1982). Each anaerobic environment may differ in the types of bacteria involved in methanogenesis, depending on differing factors such as substrate, retention time, temperature, pH, and fluctuations in other environmental parameters. Although certain general properties are common from one environment to another, each
environment may have its own unique population of bacteria and associated microbial activities.

Methane producing microorganisms are very sensitive to environmental changes (Rozzi et al. 1994). The hydrogenophilic methanogens are more resistant to environmental changes than acetoclastic methanogens (Parawira 2004). Research has shown the metabolic rates of acetoclastic methanogens, which are responsible for the majority of methane production, are lower than those of acid forming bacteria (Mosey and Fernandes 1989). Therefore, methane production is generally the rate-limiting step in anaerobic digestion (Speece 1996).

**Process Fundamentals of Anaerobic Treatment**

There are many environmental and operational variables associated with anaerobic treatment. The important factors currently known include temperature, pH, alkalinity, and nutrient requirements (Demirel and Yenigun 2002).

**Temperature**

Temperature plays an important role in the anaerobic degradation. Stable and uniform temperature is essential for consistent and efficient reactor operation. It also results in the best treatment of the substrate. Temperature fluctuation has a net adverse effect on digester performance and contributes to instability of anaerobic treatment (Droste 1997).

Microorganisms used in this degradation are divided into several categories depending on their optimal temperature. Psychrophilic organisms grow best in temperatures (0 - 20 °C), mesophilic (20 - 42 °C), and thermophilic (42 – 75 °C)
Anaerobic reactors most often operate at mesophilic and thermophilic ranges (van Lier et al. 1996). Methanogenesis is possible under psychrophilic conditions but occurs at lower rates. Bacterial activity and growth decrease by one half for every 10 °C decrease in temperature below 35 °C (Hulshoff-Pol 1998). In municipal wastewater plants, anaerobic treatment is carried out in the mesophilic ranges from 25 to 40 °C with an optimum temperature of approximately 35 °C (Parkin and Owen 1986).

Thermophilic anaerobic digestion has several advantages including higher rates of degradation resulting in a smaller digester size at less capital cost, faster solid-liquid separation, and better control of bacterial and viral pathogens (Mackie and Bryant 1995). In thermophilic temperature ranges, reaction rates precede at much higher rates than mesophilic ranges. Loading potentials of anaerobic bioreactors are significantly higher (Dugba and Zhang 1999). Even with these advantages, thermophilic wastewater treatments are not as commonly applied. Reasons for this can be attributed to the conflicting and sometimes disappointing results. In comparison to mesophilic operational systems, thermophilic reactors require more energy for heating, produce poorer quality supernatant which contains larger quantities of dissolved solids, and have less process stability (Parkin and Owen 1986; van Lier et al. 1996).

**pH and Alkalinity**

The pH is perhaps the most important anaerobic process control parameter. Each microbial group involved in anaerobic degradation has a specific pH range for optimal growth. The optimum pH range for specific hydrogen production rate is 5.5 – 5.7 (Van
Ginkel et al. 2001; Khanal et al. 2004). For methanogenic microbes the range is 6.5 – 7.5. Growth below this pH deceases sharply (Moosbrugger et al. 1993).

Experiments on pH levels from 6.0 – 8.0 reported that the dominant microbial population was affected at different values within that range (Demirel and Yenigun 2002). Acidogenic bacteria produce organic acid, which tend to lower the pH of the anaerobic reactor. Under normal conditions, this pH reduction is buffered by the bicarbonate produced by methanogens (Cheong 2005). To prevent accumulation of surplus volatile acids, excess alkalinity or pH control must be used. Anaerobic processes can operate over a wide range of volatile acid concentrations if proper control is maintained. Constant pH provides stability to this process (Parawira 2004). The common materials used to increase alkalinity are lime, soda ash, ammonia, ammonium bicarbonate, sodium hydroxide, or sodium bicarbonate. Generally lime, sodium hydroxide, and ammonia are the least expensive of these chemicals (Parkin and Owen 1986; Anderson and Yang 1992).

**Nutrient Requirements**

All organisms need essential nutrients for growth. A lack of nutrients, therefore, will negatively affect their growth (Lettinga 1995). Nutrients that are needed in the highest concentrations include nitrogen and phosphorous. One advantage of anaerobic digestion is the lower growth yields of bacteria compared to aerobic digestion. This means that fewer nutrients are required for growth and that more substrate can be broken down into by-products (Cheong 2005). The optimum carbon: nitrogen: phosphorus (C: N: P) ratio for a high methane yield was found to be 100: 3: 1 (Rajeshwari et al. 2000).
Trace elements such as sulfur, potassium, calcium, magnesium, iron, nickel, cobalt, zinc, manganese, and copper are required for efficient anaerobic degradation. These nutrients are usually found in sufficient amounts in most wastes that are treated through anaerobic digestion (Rajeshwari et al. 2000).

Sulfide precursors may also be needed in addition to the nitrogen and phosphorous requirements for anaerobic microbial systems. Biomass found in anaerobic systems has significantly higher sulfur content than biomass found in aerobic systems. An empirical cell formulation of anaerobic cells can be considered as $C_3H_7O_2P^{0.06}S^{0.1}$ (Speece 1996). Zehnder et al. (1980) recommended a sulfur content of approximately 0.001 to 1.0 mg/L for optimal growth and methane production in anaerobic systems.

**Hydrogen Production from Anaerobic Fermentation**

Several factors have been studied in the research to develop a sustainable anaerobic fermentation system to produce hydrogen. The motivation for this research has been the potential economic and environmental benefits that hydrogen could deliver. The bacterial culture utilized and number of anaerobic stages used to produce hydrogen have received renewed attention from researchers. Before large scale quantities of hydrogen can be produced these factors and others must be evaluated.

**Bacterial Culture**

One problem with using organic waste from agricultural processes such as manure is the naturally occurring bacteria within the manure. Overall performance of anaerobic treatment systems is totally dependent on the composition of microbial populations in the anaerobic reactors (Ince and Ince 2000). An anaerobic reactor fed with
non-sterile material has in the past created a bacterial culture of methanogenic or sulfate-reducing bacteria that consumes hydrogen generated by acidogenic bacteria (Cheong 2005). Absent from intervention, hydrogen-consuming bacteria will grow until most or all the hydrogen being produced is simultaneously consumed.

Several systems have been developed to allow hydrogen to be produced in an anaerobic digester (Zajic et al. 1978; Minton and Clarke 1989). These systems typically require growing and maintaining pure strains of hydrogen-producing bacteria and sterilizing the material to be digested. These systems are not commercially viable because maintaining a pure strain of bacteria in a digester is difficult and sterilizing the material to be digested is very expensive (Oh et al. 2003).

Recently, an improved method was developed for obtaining quantities of hydrogen-producing bacteria (Noike et al. 2003). In this method, a mixed culture of bacteria was heat treated to destroy the hydrogen-consuming bacteria. The hydrogen-producing bacteria survive the heat treatment by creating spores. Thus, the treated culture is enriched with hydrogen-producing bacteria as compared to hydrogen-consuming bacteria. The enriched culture is then used to seed an anaerobic digester. The problem with forming a seed culture in this manner is that it requires an expensive heat treatment step. The research by Noike et al. (2003) also sterilized the material to be digested. This would prove to be impossible for large scale operations because of the expenses involved. If the substrate did not require sterilization it would make the process much simpler. Research by Cheong et al. (2006) continued to explore bacterial cultures for hydrogen production by investigating different bacterial stress enrichment treatments. Their research discovered that chemical acidification as a pretreatment step gave the best
hydrogen production potential and formed a healthy acidogenic bacterial culture. Another benefit of their research was that the process worked without sterilizing the substrate. Utilization of the research from Cheong et al. (2006) in the current research is expected to make the process more economical and simplify the process.

**Two-Stage Anaerobic System**

Anaerobic digestion converts organic matter in wastewater into biogas. In order to produce hydrogen in an anaerobic system, the methanogens must be inhibited. The chemical oxygen demand removal seen by Van Ginkel et al. (2005) when producing hydrogen from food processing and domestic wastewaters was between 5-11%. With removal efficiency that low, further treatment of the waste will be required before being discharged (Gray 2004). The purpose of a two-stage anaerobic digestion system is to further degrade waste and extract more net energy from the system. A two-stage system has been shown to profoundly enhance substrate conversion and produce a lower chemical oxygen demand effluent using continuous stirred tank reactors (CSTR) (Azbar and Speece 2001).

In the two-phase or multiple phase system, the microbial phases are separated to increase process stability (Ghosh and Klass 1978; Van den Berg 1984). The acidogenic phase is operated at short retention times. This results in washout of methanogens leading to formation of acids. The effluent from this phase is transferred to a methanogenic phase digester where acids are converted to methane. There are three major advantages to a two-phase design. The first involves improved stability. In a single-phase digester, overloading and inhibitors can result in the accumulation of
volatile organic acids. The populations of organisms are not available to metabolize all of these volatile organic acids causing reactor upset. A proper bacterial community can take months to develop causing an extended period of under treatment. In a two-phase system, acid formation is promoted during the acid phase. Therefore the methane phase is constantly receiving acids to encourage maintenance of high populations of these organisms. The acid-phase is maintained as an imbalanced digester which is resistant to further imbalances resulting from overloading or inhibitors. The second advantage is that the slow-growing populations of microorganisms, acidogens and methanogens, can be maintained at each of their optimal growth conditions. They can also be concentrated onto biofilms which allow short retention times for each reactor. By separating the phases the overall reactor volume requirements also decrease. The third advantage is higher methane content in the methanogenic phase reactor. This is caused by release of carbon dioxide during the acidogenic phase allowing for less carbon dioxide within the methane. This advantage allows for decreased gas conditioning requirements of the methane (Azbar and Speece 2001)

**Induced Blanket Reactors**

The conventional continuous-flow stirred-tank reactor (CSTR) design which has been the standard for anaerobic digestion is being replaced by more innovative designs. These designs are selected primarily on the basis of feed suspended solids content (Fannin and Biljetina 1987). The purpose of most of the advanced reactor designs is to increase solids and microorganism retention, decrease reactor size, and reduce process energy requirements
One such patented design is the Induced Blanket Reactor (IBR) digestion system, Figure 2-6. This system is designed to treat waste at a high rate while still retaining the slow growing anaerobic bacteria. The reactor design causes a sludge blanket or bed to form in the lower part of the bioreactor vessel when operated under correct conditions. Treatment of pig and dairy farm wastes demonstrated a 3-6 time faster treatment period and has been shown to remove up to 80% of the volatile suspended solids within the waste (Hansen and Hansen 2002).

Figure 2-6. Induced Blanket Reactors (IBR) under construction.
CHAPTER 3
ANAEROBIC HYDROGEN PRODUCTION USING AGRICULTURAL AND
FOOD PROCESSING WASTES

Abstract

Despite its clean and green nature when utilized in fuel cells and other devices, most hydrogen is currently produced from non-renewable sources such as natural gas, oil, and coal. Anaerobic digestion provides a better alternative to manufacturing hydrogen than fossil fuels. Anaerobic digesters can produce hydrogen from inexpensive and renewable energy sources such as organic wastes. The objective of this research was to perform anaerobic fermentations to produce hydrogen from substrates such as cheese processing wastes and dairy manure. The quality and quantity of the hydrogen produced was determined and the chemical oxygen demand and solids information analyzed. Three anaerobic batch reactors were constructed for the experiments to monitor pH, temperature, and agitation. Cheese whey and dairy manure proved to be excellent substrates for hydrogen production producing as much as 63.16 mmol hydrogen per liter substrate. COD and total solids removal were also observed for each of the trials performed. Anaerobic hydrogen production utilizing food processing and animal wastes supplies a clean, inexpensive energy and also treats environmentally harmful wastes. Although these results are promising, further research is necessary to develop a continuous anaerobic digestion process to produce a constant hydrogen supply.
Introduction

The global power supply is currently 84.8% fossil energy (Zurawski et al. 2005). World energy consumption is expected to climb steadily over the next thirty years as a result of economic growth, particularly in developing nations and population growth throughout the world. The rate of oil consumption globally in 2006 was 30.6 billion barrels (Lattin and Utgikar 2007). The U.S. Geological Survey estimates the total worldwide oil reserves to be 2.6 trillion barrels, 1.7 trillion barrels in proven reserves and 900 trillion in undiscovered reserves (Hallenbeck and Benemann 2002). In the search for an alternative fuel to supply this energy demand, special consideration has been put on a fuel that not only supplies the world’s energy demands, but is also a cleaner option to the fossil fuels used today. One source of energy which has received special attention for meeting these requirements is hydrogen fuel.

Hydrogen is considered to be an alternative fuel of great potential use. In 1976, the first World Hydrogen Conference identified hydrogen as a clean energy carrier for the future (Lattin and Utgikar 2007). Hydrogen gas is termed as a clean fuel because water instead of greenhouse gases is produced when combusted. It has a high energy yield of 122 kJ/g, which is 2.75 times greater than gasoline (Antonopoulou et al. 2006). Hydrogen has the potential to lessen the world’s dependency on fossil fuels, but further research and technology is needed before a sustainable hydrogen economy can be established.

Despite its clean and green nature, most hydrogen is currently produced from fossil fuels, such as natural gas, oil, and coal as seen from Figure 3-1. An alternative
hydrogen production source is anaerobic fermentation. Anaerobic fermentation produces hydrogen that is less energy intensive than chemical or electrochemical methods because it is normally carried out at ambient temperature and pressure (Jo et al. 2007). Anaerobic production of hydrogen is an exciting new area of technology development that offers the potential to produce usable hydrogen from a variety of renewable resources such as organic wastes (i.e. food processing waste and animal waste) (Nath and Das 2004).

![Pie chart showing worldwide hydrogen production sources]

Figure 3-1. Overall world wide hydrogen production sources by percent (Romm 2005).

Researchers have studied different types of substrates for biological production of hydrogen. The major criteria for substrate selection are availability, cost, carbohydrate content, and biodegradability (Kapdan and Kargi 2006). Commercially produced food products, such as corn and sugar, are not yet economical for hydrogen production. Alternatively, wastewaters with organic waste such as food processing and animal waste have great potential as substrate sources (Benemann 1996). Utilizing wastewaters from agricultural and food processing industries, which are generally high in carbohydrates, can provide the essential nutrients required for hydrogen production and reduce treatment and disposal costs currently needed for these particular waste streams. Removing and/or
degrading potentially toxic material from waste materials and producing clean energy makes anaerobic hydrogen production an attractive alternative to fossil fuels (Kapdan and Kargi 2006).

Several obstacles must be overcome before hydrogen from biological processes can be produced economically. In the anaerobic process, bacteria such as methanogens consume hydrogen for growth in a symbiotic process (Parawira 2004). Under normal anaerobic conditions the majority of hydrogen produced is consumed by such bacteria. These bacteria must be inhibited so that hydrogen producing bacteria can flourish and produce only hydrogen. In doing this, hydrogen can be extracted and used as an energy source. Another major issue is controlling the pH of the system. The pH was found to have a profound effect on both hydrogen production potential and other byproducts (Khanal et al. 2004). The chemicals used in laboratory experiments to control the pH are expensive and cause safety issues. A more economical way to control the pH must be found before large scale production can successfully take place. These issues along with substrate composition and concentration are important issues that need to be understood and controlled.

Therefore, the scope of this research was to investigate a more economical system to produce hydrogen using anaerobic fermentation. The overall objective was to investigate methods of producing hydrogen from agricultural and foods wastes in a more cost effective and efficient manner.
Materials and Methods

Seed Preparation

The anaerobic hydrogen producing mixed microbial communities were enriched at the start of this study. The seed sludge for the experiments was obtained from the bottom portion of an anaerobic induced blanket reactor at a local cattle manure treatment plant (Wade Dairy Farm, Ogden, UT). Using a patent pending process, raw seed sludge was filtered through a screen (pore size: 2 mm) to remove fiber-like undigested materials before using. Before seeding, the filtered raw sludge was preacidified in suspension at a pH 3.0, at 37 °C for 48 hours. After 48 hours, hydrochloric acid (HCl) was mixed with the preacidified seed sludge for 10 min to alter the pH of the suspension to 2.0. Then the sample was stored at 4 °C for 2 – 4 hours. The treatments were intended to inhibit the bioactivity of hydrogenotrophic non-spore formers present in the natural anaerobic food chain (Chen et al. 2002; Cheong 2005). The enriched seed sludge was cultivated at 37 °C, using a separate completely mixed batch reactor (working volume, 1.9 L) with an inoculation ratio of 30:70 for seed and substrate mixture.

Medium Composition and Collection

The main substrate solution consisted of organic and inorganic nutrients. The synthetic wastewater solution was published by Cheong et al. (2006) and consisted of organic and inorganic nutrients. It had a chemical oxygen demand (COD) of 25,000 mg/L, which was derived mainly from glucose. The solution was composed of approximately 21,300 mg/L glucose and the following nutrients: 2,000 mg/L meat extract; 2,125 mg/L NH₄Cl; 420 mg/L K₂HPO₄; 180 mg/L FeCl₂·4H₂O; 375 mg/L
CaCl$_2$·2H$_2$O; 312.5 mg/L MgSO$_4$·7H$_2$O; 250 mg/L KCl. To prevent deficiency of microbial trace elements, a trace nutrients solution (50 mg/L H$_3$BO$_3$, 50 mg/L ZnCl$_2$, 30 mg/L CuCl$_2$, 500 mg/L MnSO$_4$·H$_2$O, 50 mg/L (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O, 50 mg/L AlCl$_3$, 50 mg/L CoCl$_2$·6H$_2$O, 50 mg/L NiCl$_2$, and 1 mL HCl (36%) was added by 0.1% (v/v). The components were similar to those used by Zehnder et al. (1980) for cultivating anaerobic bacteria. 8,000 mg/L of NaHCO$_3$ was added to maintain initial buffering capacity. Tap water was used as diluting water (City of Logan, UT).

Dairy manure was collected from a local dairy (Wade Dairy, Ogden, UT). Cheese whey was gathered at two cheese production plants (Gossner Cheese, Logan, UT; Utah State Dairy Plant, Logan, UT).

**Anaerobic Batch Reactor Setup**

Three anaerobic batch reactors (total volume of 2.0, 2.5, and 2.5 L) were setup (Wheaton M-100, Wheaton Instruments, Millville, NJ) equipped with temperature controllers and magnetic agitation controls, Figure 3-2. Peristaltic pumps (Cole-Parmer, Inc., Vernon Hills, IL) were used to transfer the influent and effluent of each reactor. During the experiments, the anaerobic batch reactors were controlled at 37.0 ± 0.5 °, and pH controllers (Cole-Parmer, Inc.) controlled the pH. The mixed liquor’s pH was maintained above pH 5.5 unless otherwise stated by automatically feeding a 5 N mixed solution of NaOH via peristaltic pumps. The head space was flushed with nitrogen gas prior to each trial. A volumetric gas meter measured gas production, and gas samples were collected using Tedlar gas bags (CEL Scientific, Santa Fe Springs, CA).
The total COD was measured by the closed reflux colorimetric method (APHA et al. 1992). The total suspended solids (TSS) and volatile suspended solids (VSS) for biomass determination were analyzed and calculated from influent and effluent samples, according to standard methods (APHA et al. 1992).

The hydrogen, methane, oxygen, and nitrogen contents in the biogas were analyzed by gas chromatography (HP 6890 series, Hewlett-Packard, Wilmington, DE) using an RT-Msieve 5A Plot capillary column (Restek) with dimensions of 30.0 m * 320 μm * 30.0 μm. The column temperature was 35 °C, while the inlet port and thermal conductivity detector temperatures were 43 °C and 200 °C, respectively. Argon was used as the carrier gas at a flow rate of 3.3 mL / min. Gas standards were obtained from Scott Specialty Gases (Plumsteadville, PA). Samples of methane (99.0%), nitrogen (80.0%), and hydrogen (10.0%) were used in calibrating the gas chromatograph.
Figure 3-3. Process flow with batch digester setup using manure and cheese whey.

**Experiment Setup**

Anaerobic digestion for hydrogen production was carried out utilizing synthetic wastewater, cheese whey, and manure. The enriched seed sludge was added to the anaerobic batch reactors at an inoculation ratio of 10:90 for seed and substrate mixture for trial one. The subsequent trials used effluent from the previous trial to seed the next trial at a ratio of 10:90 as was done by Cheong et al. (2006).

The project began by using synthetic wastewater as a substrate for the hydrogen fermentations. The trials were to determine the optimal pH within a range of 5.0 - 6.0 and determine the quality and quantity of biogas production (Khanal et al. 2004; Van Ginkel et al. 2001). Hydrogen producing fermentations were conducted with and without pH control to understand the importance of pH control within the process.

The manure, cheese whey, and cheese whey and manure trials were all setup using four different concentrations. The manure and cheese whey trials were mixed with synthetic wastewater substrate at different concentrations. Concentrations of 0, 15, 30, and 45% were all tested for each of the substrates with three trials per concentration.
being tested. The manure trials contained an extra trial of 100% manure. The cheese whey and manure trials were setup similarly to the previous trials with cheese whey being mixed with manure at concentrations of 0, 15, 30, and 45% cheese whey, Figure 3-3.

The pH of the system was adjusted to the designated pH for the synthetic wastewater trials using hydrochloric acid (Cheong et al. 2006). The pH was not adjusted in any of the other trials except in the 100% manure tests.

**Results and Discussion**

The batch fermentations were conducted for approximately 48 hours. A lag phase was noticed at the beginning of the trials ranging between 2-4 hours. The majority of the biogas production was produced within 24 hours after inoculation with the stress enriched bacterial culture.

**Synthetic Wastewater Trials**

Preliminary tests without pH control showed the system becoming increasingly acidic within 4-6 hours. The pH dropped to values ranging between 3 - 4. The system produced normal amounts of biogas until dropping below pH 5.0. At this point biogas production decreased rapidly. Composition of the biogas produced in this trial showed hydrogen contents between 0 - 10% with the remainder being carbon dioxide.

Three trials were successfully performed using the synthetic wastewater and controlling the pH at 5.0, 5.5, and 6.0. Synthetic wastewater trials produced 55.88 mmol of hydrogen per liter of substrate at a pH of 5.5, Figure 3. This was found to be the optimal pH for a constant volume of hydrogen was produced and it maintained a low
enough pH to inhibit methanogenic bacteria. The average hydrogen percentage within the biogas at pH 5.5 was 39.91%. The chemical oxygen demand tests showed a COD removal of 18.77 % ± 3.74 % at pH 5.5, Table 3-1.

**Manure Trials**

Tests using straight dairy manure and the hydrogen producing mixed bacterial culture did not produce hydrogen. An increased digestion time was allowed to analyze if a longer time period was needed. Only minimal amounts of biogas were produced when utilizing longer periods of digestion. The biogas composition contained trace amounts of methane and carbon dioxide. The manure was then diluted two fold and four fold with deionized water. No biogas was formed with diluted manure. Similar results were found when attempting to control the pH at exactly 5.5. Finally, glucose was added to the manure. The digestion was then accomplished as stated in the methods section. Biogas containing hydrogen was produced in these trials. It was then determined to mix the synthetic wastewater, which was mainly composed of glucose, and the manure.

Three trials were successfully performed mixing the synthetic wastewater and animal manure. Three concentrations of animal manure were tested, 15%, 30%, and 45%. The hydrogen yields decreased as the percent of manure increased. The 45% manure concentration resulted in the lowest hydrogen yield of these trials and was 24.04 mmols of hydrogen produced per liter substrate, while the 15% manure produced 40.00 mmols of hydrogen per liter substrate, Figure 3-4. The chemical oxygen demand removal was lower for the manure trials compared to the synthetic wastewater trials. At a manure concentration of 45% the COD removal was 9.74%. The 15% manure
concentration only had a COD removal of 2.79%, Table 3-1. The results of the solids tests indicated that there was a total solids removal in each of the trials for each concentration. The most noticeable was the 30% manure concentration which saw a removal of 4.16 gram per liter or about 21.84% of the total solids.

![Graph showing hydrogen production with error bars for manure and synthetic wastewater concentrations.](image)

Figure 3-4. Hydrogen gas production of manure mixed with synthetic wastewater. Reported as volume of hydrogen per liter of substrate.

Cheese Whey Trials

Trial one gave very promising data showing high biogas volumes. The 45% cheese whey concentration produced 83.03 mmols of hydrogen per liter substrate. The hydrogen concentration within the biogas ranged between 27.9 - 39.02%. The second trial using 15% and 30% cheese whey concentrations with synthetic wastewater produced significantly less biogas with no biogas formation in the 45% concentration. The third trial with cheese whey concentrations of 15, 30, and 45% did not have any biogas production, Figure 3-5. Although the biogas production dropped of significantly, the
chemical oxygen demand removal stayed consistently higher than other trials using different substrates. The 45% cheese whey concentration had an average removal of 13.59 grams per liter or 33.16% total COD removal. Similar results were reported by Cooney et al. (2007) in which an increase of lactic acid producing bacteria within the digestion was blamed for lower biogas yields. Results from the current research and conclusions from Cooney et al. (2007) suggest that the use of cheese whey and poor mixing in the reactor favored lactic acid bacteria growth, which out competed the hydrogen producing bacteria causing a decrease in hydrogen production.

The average pH of the cheese whey used in the digestions was 6.4. The cheese whey was collected from local cheese manufacturing plants (Gossner Foods, Logan, UT; Utah State Dairy Plant, Logan, UT) and stored at -20 ° C until used for the trials. After further investigation, it was decided to submit the cheese whey to a proprietary process that made it more suitable for hydrogen manufacture. The pretreated cheese whey was then utilized in the cheese whey and manure trials.

![Graph of hydrogen production](image_url)

Figure 3-5. Results of hydrogen production for each of the cheese whey mixed with synthetic wastewater trials. Trial three showed no biogas formation and trial two showed reduced biogas production. Results reported as volume of hydrogen per liter substrate.
Table 3-1. Chemical oxygen demand measurements made from the manure, cheese whey, and cheese whey and manure trials.

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>COD Start (mg/L)</th>
<th>COD Finish (mg/L)</th>
<th>COD Removed (mg/L)</th>
<th>Percent Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure and S.W.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Man. 100% S.W.</td>
<td>25,000</td>
<td>20,307 ± 936</td>
<td>4,693</td>
<td>18.77%</td>
</tr>
<tr>
<td>15% Man. 85% S.W.</td>
<td>20,320 ± 755</td>
<td>19,753 ± 1,070</td>
<td>567</td>
<td>2.79%</td>
</tr>
<tr>
<td>30% Man. 70% S.W.</td>
<td>19,780 ± 607</td>
<td>17,987 ± 912</td>
<td>1,793</td>
<td>9.06%</td>
</tr>
<tr>
<td>45% Man. 55% S.W.</td>
<td>18,860 ± 837</td>
<td>16,860 ± 546</td>
<td>2,000</td>
<td>9.74%</td>
</tr>
<tr>
<td>Cheese Whey and S.W.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% C.W. 100% S.W.</td>
<td>25,000</td>
<td>20,307 ± 936</td>
<td>4,693</td>
<td>18.77%</td>
</tr>
<tr>
<td>15% C.W. 85% S.W.</td>
<td>28,387 ± 1,078</td>
<td>26,233 ± 2,196</td>
<td>2,153</td>
<td>8.41%</td>
</tr>
<tr>
<td>30% C.W. 70% S.W.</td>
<td>33,233 ± 1,797</td>
<td>27,240 ± 3,010</td>
<td>5,993</td>
<td>19.1%</td>
</tr>
<tr>
<td>45% C.W. 55% S.W.</td>
<td>39,040 ± 2,311</td>
<td>23,453 ± 1,148</td>
<td>13,587</td>
<td>33.16%</td>
</tr>
<tr>
<td>Cheese Whey and Manure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% C.W. 100% Man.</td>
<td>17,290 ± 910</td>
<td>15,520 ± 740</td>
<td>1770</td>
<td>10.24%</td>
</tr>
<tr>
<td>15% C.W. 85% Man.</td>
<td>30,917 ± 2,863</td>
<td>29,570 ± 2,031</td>
<td>1,347</td>
<td>4.36%</td>
</tr>
<tr>
<td>30% C.W. 70% Man.</td>
<td>40,650 ± 1,227</td>
<td>37,467 ± 1,938</td>
<td>3,183</td>
<td>7.83%</td>
</tr>
<tr>
<td>45% C.W. 55% Man.</td>
<td>47,226 ± 589</td>
<td>41,343 ± 1,439</td>
<td>5,883</td>
<td>12.46%</td>
</tr>
</tbody>
</table>

**Cheese Whey and Manure Trials**

Three successful trials were completed using pretreated cheese whey and manure. It was observed that the higher the pretreated cheese whey concentration, the more hydrogen produced. The 45% pretreated cheese whey mixed with manure produced 63.16 mmols of hydrogen per liter substrate. The average hydrogen content of the biogas at this concentration was 35.88 %, Figure 3-6 and Table 3-3. The pretreated cheese whey
had several advantages when utilized as a substrate. The pretreatment process caused a more stable digestion process by consistently producing a significant and constant amount of hydrogen at every concentration tested. Another major advantage was that the low pH of the cheese whey caused the pH of the total substrate to drop within an inhibitory pH range for methanogenic bacteria, while still providing nutrients for the hydrogen producing bacteria. By utilizing the low pH of the cheese whey no acidic pH control was necessary to promote hydrogen production.

![Figure 3-6](image)

Figure 3-6. Hydrogen gas production using cheese whey mixed with manure at different concentrations. Reported as volume of hydrogen produced per liter substrate.

Two other important findings from these trials are the COD and total solids removal. At higher concentrations of cheese whey, there was greater COD removal with less total solids removal. This indicates that the solids in the cheese whey that were removed were relatively high in COD. At the lower cheese whey concentrations, lower
COD removal was seen, but higher total solids removal took place, see Table 3-1 and 3-2; Figure 3-7 and 3-8. One explanation of this was the higher solids content within the manure and higher COD found within the cheese whey. By increasing the cheese whey concentration, the COD was also increased, but the solids decreased due to less manure.

Table 3-2. Solids data collected on the cheese whey mixed with manure trials

<table>
<thead>
<tr>
<th>Cheese Whey</th>
<th>Run Period</th>
<th>Total Solids (g/L)</th>
<th>Volatile Solids (g/L)</th>
<th>Suspended Solids (g/L)</th>
<th>Volatile Suspended Solids (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45%</td>
<td>Start</td>
<td>27.75 ± 2.24</td>
<td>16.40 ± 1.64</td>
<td>6.49 ± 0.73</td>
<td>5.21 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>26.03 ± 0.55</td>
<td>15.77 ± 0.56</td>
<td>8.30 ± 0.68</td>
<td>5.46 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>Solids Difference</td>
<td>1.72</td>
<td>0.63</td>
<td>1.81</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Percent Difference</td>
<td>6.20%</td>
<td>3.84%</td>
<td>27.89%</td>
<td>4.80%</td>
</tr>
<tr>
<td>30%</td>
<td>Start</td>
<td>29.51 ± 0.234</td>
<td>17.54 ± 1.47</td>
<td>9.48 ± 1.63</td>
<td>7.21 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>25.78 ± 2.84</td>
<td>15.50 ± 1.59</td>
<td>10.64 ± 1.82</td>
<td>8.17 ± 1.30</td>
</tr>
<tr>
<td></td>
<td>Solids Removed</td>
<td>3.73</td>
<td>2.04</td>
<td>1.16</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Percent Removed</td>
<td>12.64%</td>
<td>11.63%</td>
<td>12.24%</td>
<td>13.31%</td>
</tr>
<tr>
<td>15%</td>
<td>Start</td>
<td>27.26 ± 2.41</td>
<td>19.28 ± 2.84</td>
<td>7.63 ± 1.95</td>
<td>5.77 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>Solids Removed</td>
<td>5.64</td>
<td>5.79</td>
<td>1.56</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>Percent Removed</td>
<td>20.69%</td>
<td>30.03%</td>
<td>20.45%</td>
<td>23.74%</td>
</tr>
</tbody>
</table>

Figures 3-7 and 3-8. Figure 3-7 shows the chemical oxygen demand (COD) removal for the cheese whey mixed with manure trials. Figure 3-8 show the total solid removal for the cheese whey mixed with manure trials.
Table 3-3. Hydrogen production of all three trials. Manure and cheese whey (CW) trials were mixed with synthetic wastewater (SW). The cheese whey and manure trial used cheese whey mixed with manure. The hydrogen yield was determined by liter hydrogen produced per gram COD utilized. The energy yield used a density of 8.32E-05 g/cm$^3$ and 122 kJ/g (Antonopoulou et al. 2006).

<table>
<thead>
<tr>
<th>Trial Description</th>
<th>Hydrogen (mmol/L Substrate)</th>
<th>Biogas a (L/L Substrate)</th>
<th>Hydrogen within Biogas (%)</th>
<th>Hydrogen yield (L/g-COD)</th>
<th>Energy Produced (kJ/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure and S.W.</td>
<td>55.88</td>
<td>3.36 ± 0.27</td>
<td>39.91 ± 2.19</td>
<td>0.29</td>
<td>13.62</td>
</tr>
<tr>
<td>15% Man. 85% S.W.</td>
<td>40.00</td>
<td>2.38 ± 0.21</td>
<td>34.49 ± 0.16</td>
<td>1.45</td>
<td>8.33</td>
</tr>
<tr>
<td>30% Man. 70% S.W.</td>
<td>30.35</td>
<td>2.31 ± 0.21</td>
<td>31.59 ± 1.11</td>
<td>0.41</td>
<td>7.42</td>
</tr>
<tr>
<td>45% Man. 55% S.W.</td>
<td>24.04</td>
<td>2.02 ± 0.08</td>
<td>28.72 ± 1.53</td>
<td>0.29</td>
<td>5.89</td>
</tr>
<tr>
<td>Cheese Whey and S.W.</td>
<td>55.88</td>
<td>3.36 ± 0.27</td>
<td>39.91 ± 2.19</td>
<td>0.29</td>
<td>13.62</td>
</tr>
<tr>
<td>15% C.W. 85% S.W.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30% C.W. 70% S.W.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45% C.W. 55% S.W.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cheese Whey and Manure</td>
<td>0.41</td>
<td>0.26 ± 0.16</td>
<td>5.31 ± 2.09</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>15% C.W. 85% Man.</td>
<td>25.77</td>
<td>2.18 ± 0.12</td>
<td>28.36 ± 2.24</td>
<td>0.40</td>
<td>6.30</td>
</tr>
<tr>
<td>30% C.W. 70% Man.</td>
<td>52.49</td>
<td>3.95 ± 0.61</td>
<td>32.04 ± 1.27</td>
<td>0.40</td>
<td>12.90</td>
</tr>
<tr>
<td>45% C.W. 55% Man.</td>
<td>63.16</td>
<td>4.37 ± 0.66</td>
<td>35.88 ± 6.97</td>
<td>0.27</td>
<td>15.55</td>
</tr>
</tbody>
</table>

a- Biogas consisted of carbon dioxide, hydrogen, and nitrogen. No methane was produced during the regular fermentation time.
The fermentation of cheese whey and dairy manure produced large quantities of hydrogen gas. Table 3-3 shows that hydrogen production using pretreated cheese whey and manure as substrate can produce more hydrogen than using a synthetic glucose based substrate. Also important to note from Table 3-3, is the energy production per liter of substrate. The 45% pretreated cheese whey mixed with manure produced 15.55 kilojoules per liter substrate of energy compared to the synthetic wastewater of 13.62 kilojoules per liter substrate. These results not only indicated that hydrogen production from cheese whey and dairy manure is possible, but that a considerable amount of energy in the form of hydrogen can be produced from these substrates.

**Conclusion**

Over the course of the study it was shown that synthetic wastewater successfully produced hydrogen as a byproduct of anaerobic fermentation. During these fermentations it was determined that an optimal pH of 5.5 successfully inhibited methanogenic bacteria while consistently producing biogas containing hydrogen gas at concentrations between 28-40%. Without pH control the pH of the system dropped to levels which inhibited hydrogen production.

Trials attempting to produce hydrogen using dairy manure were unsuccessful until combined with either synthetic wastewater containing glucose or cheese whey containing lactose. Increased concentrations of either the synthetic wastewater or cheese whey showed an increase in hydrogen production. It was also shown that trials utilizing fresh cheese whey did not produce consistent amounts of hydrogen. An aging process was required before the cheese whey could continually produce hydrogen during each trial.
Combining both cheese whey and dairy manure produced significant amounts of hydrogen as reported in the results above. Along with hydrogen production, the fermentations also demonstrated significant removal of COD and solids.

Anaerobic hydrogen production utilizing synthetic wastewater, manure, and cheese whey provides a treatment management plan for these wastes. It also provides clean energy which could save money and possibly turn a wastewater treatment plant into a power plant.
CHAPTER 4

TWO-PHASE ANAEROBIC DIGESTION SYSTEM FOR HYDROGEN AND METHANE PRODUCTION

Abstract

Treatment of industrial and agricultural wastes is becoming increasingly important because of the large quantities produced every year and the risks this waste represents to public health and the environment. In an effort to treat this waste and produce valuable byproducts as hydrogen and methane, a two-stage anaerobic digestion system was designed. The two-stage system was investigated for both hydrogen and methane production and was compared to a single-stage anaerobic digestion system to determine overall energy production and waste treatment. From trials performed it was shown that the two-stage anaerobic digestion system produced significant amounts of hydrogen, and produced more methane, $81.07 \pm 12.76$ mmol per day per liter substrate than the single-stage system, $72.72 \pm 11.93$ mmol per day per liter substrate at a 4 day hydraulic retention time. The two-stage anaerobic digestion system was shown to be uniquely setup to produce hydrogen-methane mixtures and treat nutrient rich agricultural and food processing waste.

Introduction

Due to rapid industrialization throughout the world, large quantities of wastes from municipal, industrial, and agricultural sources are generated each year. These effluents contain high organic content. Unmanaged, the organic waste fractions
decompose in the environment causing large scale contamination of land, water, and air. Besides being a threat to environmental quality, the wastes also possess a potential energy value that is seldom utilized despite being in large quantities throughout the world.

Extensive research into treating this waste by biological methods has been conducted in recent years. Anaerobic treatment of wastes has been of particular interest because of several advantages during the process. A few of these advantages include: production of less sludge, production of high energy and valuable gases, lower energy consumption, lower space requirements, and decreased costs (Demirel and Yenigun 2002). Besides the advantages of waste treatment, anaerobic digestion has also been the subject of interest for production of alternative renewable energy such as methane and hydrogen.

Anaerobic digestion involves two main groups of bacteria, acidogenic and methanogenic bacteria. The acidogenic bacteria break down substrates into hydrogen, volatile organic acids (mainly in the form of acetic acid), and carbon dioxide. The methanogenic bacteria convert the volatile organic acids, hydrogen, and carbon dioxide into methane gas. Typical anaerobic digesters operate as a single-phase process, where these two groups of bacteria are combined resulting in a high yield conversion of fermentable substrates to methane with trace amounts of hydrogen within the biogas (Ferris 1993).

Operation of this single-phase anaerobic digestion results in a fragile balance between the two groups of bacteria. Both groups differ extensively in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental
conditions. Problems are often encountered with system stability and control during operation because of these differences. In previous research it has been proposed to separate the two groups of bacteria into two physically separate phases (Pohland and Ghosh 1971). This is accomplished by operating the anaerobic digestion in two separate reactors instead of one, where the optimum environmental conditions for each group of organisms can be controlled to stabilize the overall reactions (Demirel and Yenigun 2002). The two-stage process has been traditionally used for methane production to cause higher reaction rates and biogas yields, but the current interest of this research is to produce both hydrogen and methane separately because both are promising renewable fuels (Vollmer and Scholz 1985; Blonskaja et al. 2003).

Hydrogen has been proposed as the fuel of the future because it is a clean and environmentally friendly fuel. When hydrogen is combusted, water with trace amounts of nitric acid is produced instead of greenhouse gases (Antonopoulou et al. 2006). Hydrogen has a high energy yield of 122 kJ/g and can be used to produce electricity directly through fuel cells (Benemann 1996). Hydrogen can be produced during the acidogenic phase of anaerobic digestion, commonly called dark fermentation. Much research has been carried out in recent years on this method of hydrogen production. In all such studies, the overall hydrogen yields have been relatively low with only 10-20% of the substrate energy being converted to hydrogen fuel with the remainder converted to organic acids, and other products (Cooney et al. 2007). This corresponds to a mean hydrogen production of 2.5 mol/mol glucose (Antonopoulou et al. 2006). With lower substrate conversions, the need for further treatment of substrates is required of the hydrogen fermentation effluent before discharge into the environment takes place. The
lower energy yields and possibility of further treatment of substrates are two problems that must be investigated for large scale hydrogen production from dark fermentation.

Although hydrogen produced by biological methods is an alternative to fossil fuels, currently hydrogen is a rare commodity when compared to hydrocarbon fuels. The transition from a fossil fuel economy to a hydrogen energy based economy has many technical challenges. This is due in part to the lack of a distribution infrastructure and sufficient production, storage, and transmission quantities. This puts large scale use of hydrogen as a fuel into a long term prospective (Bauer and Forest 2001).

Methane, the main product of anaerobic digestion, is another attractive source of energy because it can be produced close to consumption points and is therefore ideal for decentralized power generation in remote rural areas. It can also be produced on a large scale from urban waste material and be used to generate electricity for local communities. Methane as compared to other hydrocarbon fuels produces less atmospheric pollutants and carbon dioxide per unit energy and as a result is being used more and more for appliances, vehicles, and power generation (Bauer and Forest 2001). Another advantage of methane is that the distribution network is already in place.

Research into combining hydrogen and methane into what is referred to as hydrogen-methane mixtures for use as an alternative fuel is currently being studied (Porpatham et al. 2006). These mixtures are being investigated for two main reasons. The first is to improve performance, extend operability ranges, and reduce pollutant emissions in stationary and mobile systems utilizing methane alone. The second stems from concerns about global warming and a push to reduce greenhouse emissions (Sarli and Benedetto 2006). Porpatham et al. (2006) reported that the addition of 10%
hydrogen to biogas (methane and carbon dioxide) enhanced engine performance and reduced emissions. Hydrogen-methane mixtures are a potential immediate solution to a cleaner fuel supply.

The two-stage anaerobic digestion system is ideally set up to produce the hydrogen and methane necessary for these mixtures. This two-stage process, although promising in theory, has not been widely accepted because of increased complexity and higher investment and operational costs. The theoretical higher biogas yields have also been questioned since the acidogenic phase separation prevents hydrogen transfer to methanogens (Reith et al. 2003). The aim of this research was to produce hydrogen and methane in a two-stage digestion system and determine if there is a significant difference in potential energy yields between a single-phase anaerobic digestion system and a two-phase anaerobic digestion system.

**Materials and Methods**

In order to accomplish this research, hydrogen fermentations utilized several different substrates in an acidogenic batch reactor. Synthetic wastewater, dairy manure, and cheese whey were the three substrates tested within this study. The effluent from the acidogenic phase reactions was collected and placed into a methanogenic phase continuous induced blanket reactor (IBR), used commonly in single-phase anaerobic digestion, Figure 4-1. The system was monitored and compared against a single-phase anaerobic digester to evaluate if a two-phase separation affected the overall energy yield and digestion properties of the process.
Hydrogen producing batch reactors were inoculated with seed sludge at the beginning of the experiment for trial one at a ratio of 10:90 for seed and substrate mixtures. The subsequent trials used effluent from the previous trial to seed the following trials at a ratio of 10:90 (Cheong et al. 2006). All reactors were sparged with nitrogen gas previous to operation to model anaerobic growth conditions. The IBR process was inoculated at a ratio of 15:85 seed and substrate mixture. The reactors were fed at an HRT of 4 days. The IBR reactors were allowed to build up bacterial cultures prior to the study. Once constant biogas production was recorded in each reactor a period of 14 days was allowed to pass to help stabilize the system. Data collection began after stable bacterial cultures were able to form and methane production within the biogas exceeded 50% as reported in similar research (Cooney et al. 2007). The pH of the hydrogen producing phase was not allowed to drop below 5.5 during fermentation (Liu et al. 2006). The pH of the IBR system was controlled during the inoculation period. During the trial period the pH of these systems was monitored but did not require control. The pH of this system varied between a pH of 6.5 – 8 within the normal operating ranges for these reactors (Mann et al. 2004).

Figure 4-1. Flow diagram of two-phase system.
Seed Preparation

The seed sludge for the experiments was obtained from the bottom portion of an anaerobic induced blanket reactor at a local cattle manure treatment plant (Wade Dairy Farm, Ogden, UT). The anaerobic hydrogen producing mixed microbial communities were enriched at the start of this study (Cheong et al. 2006).

Medium Composition and Collection

The synthetic wastewater solution was published by Cheong et al. (2006) and consisted of organic and inorganic nutrients. It had a chemical oxygen demand (COD) of 25,000 mg/L, which was derived mainly from glucose. The solution was composed of approximately 21,300 mg/L glucose and the following nutrients: 2,000 mg/L meat extract; 2,125 mg/L NH₄Cl; 420 mg/L K₂HPO₄; 180 mg/L FeCl₂·4H₂O; 375 mg/L CaCl₂·2H₂O; 312.5 mg/L MgSO₄·7H₂O; 250 mg/L KCl. To prevent deficiency of microbial trace elements, a trace nutrients solution (50 mg/L H₃BO₃, 50 mg/L ZnCl₂, 30 mg/L CuCl₂, 500 mg/L MnSO₄·H₂O, 50 mg/L (NH₄)₆Mo₇O₂₄·4H₂O, 50 mg/L AlCl₃, 50 mg/L CoCl₂·6H₂O, 50 mg/L NiCl₂, and 1 mL HCl (36%) was added by 0.1% (v/v). The components were similar to those used by Zehnder et al. (1980) for cultivating anaerobic bacteria. 8,000 mg/L of NaHCO₃ was added to maintain initial buffering capacity. Tap water was used as diluting water (City of Logan, UT).

Dairy manure was collected from a local dairy (Wade Dairy, Ogden, UT). Cheese whey was gathered at two cheese production plants (Gossner Cheese, Logan, UT; Utah State Dairy Plant, Logan, UT).
Anaerobic Reactor Setup

Three anaerobic batch reactors (total volume of 2.0, 2.5, and 2.5 L) were setup (Wheaton M-100, Wheaton Instruments, Millville, NJ) equipped with temperature controllers and magnetic agitation controls. Peristaltic pumps (Cole-Parmer, Inc., Vernon Hills, IL) were used to transfer the influent and effluent of each reactor. During the experiments, the anaerobic batch reactors were controlled at 37.0 ± 0.5 °, and pH controllers (Cole-Parmer, Inc.) controlled the pH. The mixed liquor’s pH was maintained above pH 5.5 unless otherwise stated by automatically feeding a 5 N mixed solution of NaOH via peristaltic pumps. The head space was flushed with nitrogen gas prior to each trial. A volumetric gas meter measured gas production, and gas samples were collected using Tedlar gas bags (CEL Scientific, Santa Fe Springs, CA).

Two Induced Blanket Reactors were constructed with a working volume of 56 liters each. Each reactor’s temperature was controlled using temperature controllers, thermocouples, and heaters (Delta Electronics, Fremont, CA; Cole-Parmer, Inc., Vernon Hills, IL). The pH was monitored using pH controllers (Eutech Instruments, Vernon Hills, IL). Dosing pumps were used for continuous flow through the reactors with a hydraulic retention time of 4 days (LMI Milton Roy, Action, MA). Previous research determined the hydraulic retention time of four days to be the optimal digestion range (Mann et al. 2004). Water traps were used to provide a constant pressure and to establish anaerobic conditions within the reactors.

The IBR digesters were fed with synthetic wastewater and effluent from the hydrogen fermentations, Figure 4-2. Effluent from hydrogen fermentations, which consisted of digested cheese whey, manure, and synthetic wastewater, was stored at 0 ° C
before being added to the digesters. The feed tank and pumps were refrigerated at 2 °C when in operation to avoid degradation of the substrate before entering the reactors.

![Image](image.jpg)

Figure 4-2. Pilot scale IBR systems (56 L each) contain temperature control, pH control, variable hydraulic retention times, and ports for easy sampling and maintenance.

The total COD was measured by the closed reflux colorimetric method (APHA et al. 1992). The total suspended solids (TSS) and volatile suspended solids (VSS) for biomass determination were analyzed and calculated from influent and effluent samples, according to the procedures described in the standard methods reported in previous work (APHA et al. 1992).

Hydrogen, methane, oxygen, and nitrogen contents in the biogas were analyzed by gas chromatography (HP 6890 series, Hewlett-Packard, Wilmington, DE) using an RT-Msieve 5A Plot capillary column (Restek) with dimensions of 30.0 m * 320 μm *
30.0 µm. The column temperature was 35 °C, while the inlet port and thermal conductivity detector temperatures were 43 °C and 200 °C, respectively. Argon was used as the carrier gas at a flow rate of 3.3 mL / min. Gas standards were obtained from Scott Specialty Gases (Plumsteadville, PA). Samples of methane (99.0%), nitrogen (80.0%), and hydrogen (10.0%) were used in calibrating the gas chromatograph.

**Results and Discussion**

**Hydrogen Experiment**

Three different substrates were added to batch reactors for the hydrogen producing phase of the two-phase system. These were synthetic wastewater (SW), dairy manure mixed with different concentrations of synthetic wastewater (MSW), and cheese whey mixed at different concentrations with dairy manure (CWM). Each of these substrates underwent a minimum of nine trials at a run period of 48 hours. Table 4.1 shows the quantity, and methane and hydrogen content for each trial. Within all hydrogen producing phase trials, there was no methane detected in any of the gas collected. The average hydrogen content sampled in the SW trials was 42.58 ± 6.60% hydrogen. The MSW trials produced an average of 31.31 ± 3.21% hydrogen, while the CWM produced 32.09 ± 7.21% hydrogen. The remainder of most of the gas sampled in all three experiments was likely carbon dioxide. There was only a trace amount of nitrogen detected.
Table 4-1. Results of the biogas quality from the hydrogen fermentations, single-phase, and two-phase-methanogenic phase trials per liter substrate. Energy yields were calculated according to a pressure of 1 atmosphere and 22 °C. The heating values were calculated as 120 kJ/g for hydrogen and 50 kJ/g for methane (Ogden 2002).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Biogas (L/day)</th>
<th>Hydrogen (mmol/day)</th>
<th>Methane (mmol/day)</th>
<th>Energy Potential (kJ/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>1.05 ± 0.11</td>
<td>48.07 ± 19.52</td>
<td>0</td>
<td>5.79</td>
</tr>
<tr>
<td>MSW</td>
<td>1.11 ± 0.15</td>
<td>28.92 ± 6.39</td>
<td>0</td>
<td>3.55</td>
</tr>
<tr>
<td>CWM</td>
<td>1.75 ± 0.63</td>
<td>54.20 ± 17.63</td>
<td>0</td>
<td>6.29</td>
</tr>
<tr>
<td>Methane Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAD</td>
<td>2.95 ± 0.42</td>
<td>0.81 ± 0.60</td>
<td>72.72 ± 11.93</td>
<td>58.51</td>
</tr>
<tr>
<td>TPAD-MP</td>
<td>2.84 ± 0.47</td>
<td>0.23 ± 0.32</td>
<td>81.07 ± 12.76</td>
<td>64.99</td>
</tr>
</tbody>
</table>

Figure 4-3. Volume of hydrogen per liter substrate produced from the synthetic wastewater trials, manure trials, and cheese whey and manure trials.

The overall hydrogen production for SW averaged 48.07 ± 19.51 mmol per day per liter substrate. The MSW trials produced an average of 28.92 ± 6.39 mmol per day per liter substrate. The highest yielding trials were the CWM trials. These experiments produced on average 54.20 ± 17.63 mmol per day per liter substrate, Table 4-1 and
Figure 4-3. The majority of the gas production was noted to take place during the first twenty four hour period.

The maximum total energy was calculated for each of the substrates utilized, see Table 4-1. The density of hydrogen was calculated at 1 atmosphere and 22 °C. The energy of hydrogen was assumed to be 122 kJ/g (Antonopoulou et al. 2006). The MSW trials produced approximately 3.55 kJ/day of energy per liter substrate while the CWM trials produced 6.29 kJ/day per liter substrate.

**Methane Experiment**

To determine if there was a difference in methane output from a single-phase anaerobic digestion (SPAD) process or the methanogenic phase of a two-phase anaerobic digestion (TPAD-MP) process trials were conducted utilizing an IBR system following the hydrogen production phase. The induced blanket reactors used for these trials ran on a continuous 4-day hydraulic retention time. Synthetic wastewater was utilized as substrate for the single-phase IBR anaerobic reactions. The second phase reactions were performed with a concentration of 50% effluent from the first phase hydrogen production and 50% synthetic wastewater. Each trial was operated and maintained over a 25-day period. Data from these trials was collected and analyzed to prove or disprove the hypothesis that utilizing effluent from the first phase of a two-stage system would negatively affect the methane yields of a two-stage system over a single-stage system.

Biogas production for the SPAD produced an average of 115.91 ± 17.33 mL/min. The TPAD-MP produced an average of 110.76 ± 18.12 mL/min, Table 4-1. From this data it can be concluded that the two trials were not statistically significantly different
indicating there was no difference in biogas production between the SPAD and TPAD-MP.

Figure 4-4. Methane production for the two-phase methanogenic reactor and the single-phase anaerobic digestion system.

The biogas produced from the trials was analyzed for the composition. The biogas was shown to be a majority of methane with the remainder being carbon dioxide and trace amounts of hydrogen. A statistical analysis of the methane contents between the SPAD and TPAD-MP trials confirmed that there was a statistically significant difference between the two. The average methane content within the SPAD trials was measured to be 61.49 ± 5.06%. The TPAD-MP tests had an average methane content of 69.15 ± 5.75%. This data indicates TPAD-MP on average produced more energy as methane combined with the hydrogen produced in the first stage than the single-stage.

The SPAD reactor average methane production rate was calculated to be 72.72 ± 11.93 mmol per day per liter substrate. The TPAD-MP reactor averaged 81.07 ±12.76
mmol per day per liter substrate of methane, Table 4-1 and Figure 4-4. An average methane production rate per liter of substrate was about 1.22 mL/min for the SPAD and 1.36 mL/min for the TPAD-MP. From these results it can be concluded that there was not a decrease in methane production when digesting effluent from the hydrogen producing phase and synthetic wastewater. In fact, there may have been an increase in methane production when utilizing this effluent commingled with synthetic wastewater.

The total maximum energy calculations for the SPAD and TPAD-MP trials were computed using a pressure of 1 atmosphere and a temperature of 22 °C. The energy in methane is 50 kJ/g (Ogden 2002). The SPAD trials produced about 59 kJ/day per liter substrate while the TPAD-MP trials produced about 65 kJ/day per liter substrate.

**Chemical Oxygen Demand**

A measure of the chemically oxidizable organic compounds present in the substrates was completed to analyze the amount of COD removed during each process. COD removal is an important factor in determining the efficiency of waste stream treatment. The COD was measured for the influent and effluent of each of the experiments performed. The results are shown in Table 4-2 and Figure 4-5. The removal percentage was computed to understand the overall removal of COD within the trials. The hydrogen production trials reported varying degrees of removal for each of the substrates analyzed. The SW trials had the greatest removal out of the three substrates with 18.56% of the total COD removed during hydrogen production fermentation, see Table 4-2. Since the synthetic wastewater was a glucose based substrate and almost completely soluble it was expected to have the highest removal efficiency. The CWM
trials had the highest reported COD within the influent at 41,594 ± 3,315 mg/L. The CWM trials removed about 12.34% of the total COD within the trials performed. The MSW trials had a COD removal of 7.33%. This was the lowest removal of the three substrates. The lower COD removal coincided with the lower hydrogen production for this substrate as reported above, see Table 4-2.

The chemical oxygen demand measured for the influent of the SPAD and TPAD-MP was approximately 24,000 mg/L, see Figure 4-5 and Table 4-2. Both trials exhibited good COD removal by having an average COD in the effluent of 13,398 ± 4,522 for the SPAD trials and 9,855 ± 4,063 for the TPAD-MP trials. These results indicate that approximately 50% of the COD was removed during the anaerobic reactions. This is a significant removal of the chemically oxidizable organic compounds and shows promising results in the treatment of these wastes. The COD removal for methane production (Figure 4.5) was much higher than COD removal for hydrogen production alone as would be expected since the effluent from the hydrogen removal process provided energy for methane production.

Table 4-2. Chemical oxygen demand measurements of the hydrogen and methane tests.

<table>
<thead>
<tr>
<th>Trial</th>
<th>COD Start (mg/L)</th>
<th>COD Finish (mg/L)</th>
<th>COD Removed (mg/L)</th>
<th>Percent Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydrogen Tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>25,440 ± 1,759</td>
<td>20,718 ± 2,438</td>
<td>4,722</td>
<td>18.56%</td>
</tr>
<tr>
<td>MSW</td>
<td>19,653 ± 1,478</td>
<td>18,213 ± 2,061</td>
<td>1,440</td>
<td>7.33%</td>
</tr>
<tr>
<td>CWM</td>
<td>41,594 ± 3,315</td>
<td>36,460 ± 3,093</td>
<td>5,134</td>
<td>12.34%</td>
</tr>
<tr>
<td><strong>Methane Tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAD</td>
<td>23,948 ± 5,625</td>
<td>13,398 ± 4,523</td>
<td>10,550</td>
<td>44.05%</td>
</tr>
<tr>
<td>TPAD-MP</td>
<td>24,300 ± 5,289</td>
<td>9,855 ± 4,063</td>
<td>14,445</td>
<td>59.44%</td>
</tr>
</tbody>
</table>
Solids Tests

Solids tests were performed to determine the amount of solids destruction during all the sets of trials performed. The total solids and volatile solids data is shown in Tables 4-3 and 4-4. The CWM trials reported a total solids removal of 3.69 grams per liter or 13.11% of the total solids. The volatile solids removal was 2.82 grams per liter or 15.90% of the total volatile solids. The experiments using the IBR systems had high removal rates for both trials. The SPAD had a total solids removal of 38.72% and a volatile solids removal of 53.15%. The TPAD-MP trials reported less removal of volatile solids than the SPAD system, but were still very significant. The TPAD-MP trials removed 24.50% of the total solids and 44.16% of the volatile solids. Although these experiments are investigating the use of a two-phase anaerobic digestion system for energy production, the high removal rates reported for the COD, total solids, and volatile
solids is a significant additional benefit for the treatment of these waste streams using anaerobic digestion.

Table 4-3. Total solid influent and effluent numbers for the hydrogen and methane tests.

<table>
<thead>
<tr>
<th>Trial</th>
<th>TS Influent (g/L)</th>
<th>TS Effluent (g/L)</th>
<th>TS Removal (g/L)</th>
<th>Percent TS Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>21.99 ± 0.26</td>
<td>20.05 ± 5.01</td>
<td>1.94</td>
<td>8.80%</td>
</tr>
<tr>
<td>MSW</td>
<td>15.41 ± 2.20</td>
<td>15.25 ± 2.02</td>
<td>0.16</td>
<td>1.04%</td>
</tr>
<tr>
<td>CWM</td>
<td>28.17 ± 5.46</td>
<td>24.48 ± 5.51</td>
<td>3.69</td>
<td>13.11%</td>
</tr>
<tr>
<td>Methane Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAD</td>
<td>19.50 ± 3.86</td>
<td>11.95 ± 1.27</td>
<td>7.55</td>
<td>38.72%</td>
</tr>
<tr>
<td>TPAD-MP</td>
<td>16.41 ± 3.98</td>
<td>12.39 ± 2.37</td>
<td>4.02</td>
<td>24.50%</td>
</tr>
</tbody>
</table>

Table 4-4. Volatile solid influent and effluent numbers for the hydrogen and methane tests.

<table>
<thead>
<tr>
<th>Trial</th>
<th>VS Influent (g/L)</th>
<th>VS Effluent (g/L)</th>
<th>VS Removal (g/L)</th>
<th>Percent VS Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>20.63 ± 0.21</td>
<td>7.55 ± 1.97</td>
<td>13.08</td>
<td>63.40%</td>
</tr>
<tr>
<td>MSW</td>
<td>11.94 ± 2.08</td>
<td>8.55 ± 1.21</td>
<td>3.39</td>
<td>28.39%</td>
</tr>
<tr>
<td>CWM</td>
<td>17.74 ± 4.93</td>
<td>14.92 ± 3.07</td>
<td>2.82</td>
<td>15.90%</td>
</tr>
<tr>
<td>Methane Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAD</td>
<td>9.67 ± 2.33</td>
<td>4.53 ± 0.97</td>
<td>5.14</td>
<td>53.15%</td>
</tr>
<tr>
<td>TPAD-MP</td>
<td>8.74 ± 2.89</td>
<td>4.88 ± 1.77</td>
<td>3.86</td>
<td>44.16%</td>
</tr>
</tbody>
</table>

**Conclusion**

Demonstration of a two-phase anaerobic digestion system compared against a single-phase process was successfully shown during this study. Bacterial seed preparation and pH control successfully separated the anaerobic digestion process into an acidogenic and methanogenic phases. Methane production was not detected during the fermentations of the acidogenic phase. Hydrogen production was most successful using
cheese whey and manure producing an average of 54.20 ± 17.63 mmol of hydrogen per day per liter substrate. The energy in the hydrogen produced from the cheese whey and manure was 6.29 kJ per day per liter of substrate.

The methanogenic phase utilizing 50% effluent from the acidogenic phase operated stably under optimal operating conditions over the course of the study. An average of 81.07 ± 12.76 mmol per day of methane per liter substrate was produced in the methanogenic phase reactor. This was compared against the single-phase reactor production rate of 72.72 ± 11.93 mmol per day of methane per liter substrate. The TPAD-MP trials produced an average of 11% more energy than the single-phase trials. It was demonstrated that the overall potential energy was not affected by preventing interspecies hydrogen transfer between acidogenic bacteria and methanogenic bacteria. These results from the methanogenic phase are significant for the fact that energy was already extracted from this substrate in the form of hydrogen during the acidogenic phase. Additional potential energy was converted during the acidogenic phase ranging between 3.55 – 6.29 kJ per day per liter substrate, Table 4-1. This study found that the addition of 50% effluent from the acidogenic phase combined with synthetic wastewater produced more energy on average in the form of methane than the single-phase anaerobic digestion system. In addition to the higher methane yields, there was also potential energy in the form of hydrogen which increased the overall energy yield of the two-stage system.

In order to produce a hydrogen-methane mixture it was reported that the addition of hydrogen up to 10% on an energy basis enhanced performance of engines running on biogas and reduced emissions (Porpatham et al. 2006). The energy yields for each of the
substrates compared to the methane produced in the second phase methanogenic reactor were 8.91% for synthetic wastewater, 5.47% for manure mixed with synthetic wastewater, and 9.69% for the cheese whey and manure mixture. These values come very close to the 10% limit for hydrogen addition, specifically the cheese whey and manure mixture trials. Running the current setup described in this study would supply the hydrogen and methane required for the optimal hydrogen-methane mixture as reported by Porpatham et al. (2006). Further gas conditioning in the form of carbon dioxide removal would be required before such a mixture could be produced, but the two-phase system is ideally setup to produce the required gas quantities.

Another advantage observed during the methanogenic phase trials was the amount of chemically oxidizable organic matter removed. Removal rates ranged between 7.33 – 18.56% in the acidogenic phase and 59.44% during the methanogenic phase. Compared to the single-phase removal of 44.05%, the two-phase digestion removed much more of the COD which is a major process parameter that must be reduced in wastewater treatment. The amount of COD removal gages the amount of additional chemical or biological treatment required for proper discharge.

The total solids and volatile solids removal was significant for both the single-stage digestion and the two-phase digestion. The single-stage process removed a substantial 38.72% of the total solids and 53.15% of the volatile solids. The second phase of the two-phase digestion removed 24.50% of the total solids and 44.16% of the volatile solids. The total solids removal for the acidogenic phase ranged between 1.04 – 13.11% and would warrant further investigation before a specific solids removal range could be established for the two-phase system.
Although these results demonstrate a substantial argument for the use of a two-phase anaerobic digestion system, it is expected that further energy in the form of hydrogen can be extracted from the system by optimizing the batch reaction times during fermentations. As noted, the majority of the hydrogen was produced during the first 24 hour period of the acidogenic phase. By lowering the fermentation time several advantages could be possible; more substrate degradation, smaller reactor size, and higher energy yields.

The two-phase anaerobic digestion system described in this paper is uniquely setup to treat possibly environmentally harmful waste streams which are of negative value while simultaneously producing a ratio of hydrogen and methane. With further study and research the treatment of certain agricultural and food processing wastes could have a unique wastewater treatment step which produces two valuable byproducts making the treatment process much more economical.
CHAPTER 5
GENERAL CONCLUSION

The overall objective from the current research was to investigate the use of anaerobic fermentation technology for the production of hydrogen. The research was divided into two main sections. The first was to determine if hydrogen could be produced through anaerobic fermentation using dairy manure, synthetic wastewater, and cheese whey. The second section was to determine if the effluent from the hydrogen fermentations could be further utilized through a methanogenic phase reactor to produce methane. The following conclusions summarize the major findings of this research:

1. Bacterial seed preparation and pH control successfully separated the acidogenic phase from the other phases of anaerobic digestion. Bacterial seed preparation was accomplished through a patent pending process where raw seed sludge was filtered, acidified, and held at different temperatures for given periods of time. Trials to determine a pH which promoted acidogenic bacteria while inhibiting methanogenic bacteria were conducted. Results indicated a pH of 5.5 to fulfill these requirements which was used throughout the remainder of the study. Acidogenic phase separation was successfully maintained by monitoring of the biogas produced during fermentation. Varying amounts of hydrogen were detected during each batch anaerobic test while no methane was detected within the defined time limit for each trial.

2. Trials attempting to produce hydrogen using only dairy manure were not successful. No hydrogen was produced from this substrate until mixed with another substrate.
Dairy manure mixed at different concentrations with synthetic wastewater produced between 24.04 - 40.00 mmol of hydrogen per liter substrate. Higher hydrogen yields were associated with higher concentrations of synthetic wastewater.

3. Fresh cheese whey was shown not to be suitable for hydrogen production. Initial cheese whey trials, which were cheese whey mixed with synthetic wastewater, produced large quantities of hydrogen during the first run of each trial. Subsequent runs produced significantly less hydrogen resulting in no hydrogen production during the third and final run regardless of the cheese whey to synthetic wastewater concentration. A possible reason for occurrence is the lactic acid bacteria found naturally within cheese whey. The lactic acid bacteria likely out competed the hydrogen forming bacteria causing no hydrogen to be formed. This explanation is supported by the fact the COD was still removed, between 8.4 – 33.2%, while no biogas was formed.

4. An aging step was required for the cheese whey in order to use it as a substrate. Due to the results of the initial cheese whey trials, an aging step was developed for the process. It was shown that once the fresh cheese whey had undergone this process, a continuous, stable amount of hydrogen could be produced without competition from non-hydrogen forming bacteria.

5. Cheese whey and manure produced significant amounts of hydrogen. Cheese whey and manure mixtures of different concentrations were examined once the cheese whey aging process showed potential. These trials demonstrated that significant amounts of hydrogen can be produced from such mixtures. At a mixture of 15% cheese whey and 85% manure, 25.77 mmol of hydrogen per liter substrate were
produced. At 45% cheese whey and 55% manure, 63.16 mmol of hydrogen per liter substrate were produced on average. Hydrogen content within the biogas was 28.36 ± 2.24% for the lower concentration of cheese and 35.88 ± 6.97% for the higher concentration of cheese whey. Potential energy was estimated to be 6.30 kJ per liter substrate for the 15% cheese whey, 85% manure mixture and 15.55 kJ per liter substrate for the 45% cheese whey, 55% manure.

6. Along with the successful production of hydrogen additional benefits from a waste management perspective were the solids and COD removal seen in each trial. The manure and synthetic wastewater trials observed COD removal percentages for the different concentrations between 2.79 – 9.74%. The cheese whey and manure trials had COD removal percentages between 4.36 – 12.46%. The volatile solids removal for these runs was between 3.84% for the higher concentration of cheese whey and 30.03% for the lower concentration of cheese whey.

7. Additional energy in the form of methane was produced continually by combining effluent from the hydrogen fermentations with synthetic wastewater. Hydrogen fermentation effluent was combined at a mixture of 1:1. Biogas collected from the methanogenic reactor was successfully analyzed for quality and quantity of methane produced. An average biogas production rate of 2.84 ± 0.47 liters per day was obtained from these trials.

8. The two-phase anaerobic digestion- methanogenic phase (TPAD-MP) produced more methane on average than the single-phase anaerobic digestion (SPAD). TPAD-MP which combined hydrogen fermentation and synthetic wastewater produced 81.07 ± 12.76 mmol per day of methane per liter substrate. The SPAD fed with synthetic
wastewater produced 72.72 ± 11.93 mmol per day of methane per liter substrate.

The potential energy for TPAD-MP was 64.99 kJ per day per liter substrate and 58.51 kJ per day per liter substrate for SPAD.

9. TPAD-MP and SPAD removed significant amounts of COD and solids. TPAD-MP removed 59.44% of the total COD and 44.16% of the total volatile solids. SPAD removed 44.05% of the total COD and 53.15% of the total volatile solids.

In summary, when the results of this research are considered together, hydrogen production from agricultural and food processing industries can be successfully produced and further energy and waste treatment can occur with the use of a two-phase anaerobic digestion system. Cheese whey and manure are excellent choices of substrate with the use of a pretreatment step to produce hydrogen. Once the hydrogen was successfully produced a second stage process was shown to produce additional energy in the form of methane and further treat the waste stream. This two-stage process shows great potential to treat waste streams from agricultural and food processing industries while being able to extract valuable by products which can be used for fuel.

Recommendation for further study are as follows:

1. Further research is needed to develop a process that operates continuous hydrogen fermentation on substrates such as dairy manure and cheese whey.

2. Investigation of the use of a two-stage system utilizing a continuous effluent flow from the hydrogen fermentation is needed along with studies into the use of different substrate concentrations used within in the methanogenic reactor.

3. Detailed analysis for the reason dairy manure did not produce hydrogen will allow a better idea of what type of wastes can be used to produce hydrogen and what
concentrations of additional wastes need to be added to promote hydrogen production.

4. An economic analysis of the entire two-stage process utilizing agricultural and food processing wastes will give greater understanding of the overall efficiency of the process and the payback possible.
REFERENCES


