A modular technology for fermentative hydrogen production and capture from wastewater

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Dedication

This thesis is dedicated to my parents, Walter and Marlien Sigtermans.

Abstract

Despite the inherent chemical energy in wastewater, current wastewater treatment practices expend a considerable amount of energy to aerobically remove organic pollutants. Anaerobic fermentation of these dissolved organics to produce hydrogen could instead provide a positive energy output while delivering the ancillary benefit of lessening aeration demands for downstream treatment processes. A scalable and modular technology, based on the membrane-encapsulation of hydrogen-producing mixed consortia onto hollow fiber membranes for efficient hydrogen collection, was developed to produce and capture hydrogen from dissolved phase organics in wastewater. The membranes were tested in a continuously stirred tank reactor (CSTR) and monitored for hydrogen production and capture. The results showed that two different membrane polymer chemistries were successful in producing and capturing hydrogen from high-strength synthetic wastewater, with maximum captured yields of 25-50 mL/g hexose. Low available carbohydrate content, pH conditions, and leakage of microorganisms into and out of the membranes may have contributed to the failure of hydrogen production in trials using municipal wastewater. Batch tests of dairy manufacturing waste demonstrated the potential for future application of this technology for producing hydrogen from a real industrial wastewater.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction and Literature Review

1.1 Introduction

The need to convert the global energy market from fossil fuels to renewable sources has commanded significant scientific, political, and societal attention over the past decade because of increasing alarm over greenhouse gas emissions and their climate altering effects. Despite these concerns, fossil fuels still accounted for greater than 81% of US energy consumption by energy source in 2013 (US Energy Information Administration, 2014). New renewable energy options are needed to move the US away from fossil fuel use. Among the various alternative energy options, hydrogen gas is regarded as an attractive future clean energy technology by virtue of its high energy density (142 MJ/kg heat of combustion), efficient conversion to electricity by fuel cells, and the fact that water is the only byproduct when combusted. Nevertheless, fossil fuels are currently the dominant source of industrial hydrogen. The leading technologies for direct hydrogen production include steam reforming of hydrocarbons such as natural gas, coal gasification, and partial oxidation of heavier hydrocarbons (US Department of Energy, 2013). Renewable sources of hydrogen must be considered and developed to realize its full potential as a clean energy source.

Certain anaerobic bacteria are able to consume dissolved carbohydrates and produce hydrogen as a byproduct. Hydrogen produced in this way is also known as biohydrogen. For this process to be energetically and economically profitable, however, a low-cost, renewable feedstock is needed to supply these carbohydrates. Agricultural,

manufacturing, and municipal wastewaters are feedstocks that meet these criteria and have been studied for biohydrogen production (Okamoto et al., 2000; Lay et al., 2012; Van Ginkel et al., 2005).

Despite the energy potential of wastewater, current wastewater treatment technologies are net energy consumers. A typical municipal wastewater treatment plant, for example, allots more than 50% of its total energy use to aeration (Curtis et al., 2010), converting the reduced chemical energy within wastewater into biomass growth. Using anaerobic fermentation of the dissolved organics in wastewater to produce hydrogen could instead provide a positive energy output while delivering the ancillary benefit of reducing the concentration of oxygen-demanding organic waste and consequently lessening aeration demands for downstream treatment processes. This chapter discusses advancements in biological hydrogen production and the technical challenges that must be overcome before the technology is implemented for municipal and industrial wastewater treatment.

1.2 Biological hydrogen production pathways

Hydrogen is produced via several distinct biochemical mechanisms, two of which are driven by light energy: biophotolysis and photofermentation. Biophotolysis is performed by autotrophic algae and cyanobacteria. When light is absorbed by these microorganisms, water molecules are split into protons (H^+), electrons, and O_2. Under anaerobic or excessive energy conditions, the protons and electrons are recombined by chloroplast hydrogenase, via absorption of light, to form molecular hydrogen gas (Hankamer et al., 2007). Photofermentation is performed by heterotrophic

photosynthetic bacteria under anaerobic conditions. Electrons extracted from simple organic acids are used by ATP synthase within the chloroplasts to reduce protons into hydrogen gas (Su et al., 2009). While similar to dark fermentation, photofermentation proceeds only in the presence of light.

From an engineering point of view, biophotolysis and photofermentation suffer from several disadvantages that do not favor light-based biological hydrogen production in wastewater treatment processes. First, light penetration and its even distribution are difficult process parameters to control. Second, light-based hydrogen production technologies are likely not cost-effective unless a free source of light (i.e. sunlight) is available. The use of artificial light would subtract from the net energy balance of the process. Third, biophotolysis is performed by autotrophic organisms, which do not use complex organic matter. Heterotrophic hydrogen-producers, such as fermentative bacteria, are of greater interest because biohydrogen production is coupled to the degradation of the organic pollutant load in the wastewater, leading to an environmental benefit. Because of the disadvantages of the light-based processes, dark fermentation has been the major focus of research regarding biological hydrogen production in wastewater treatment.

1.2.1 Anaerobic digestion and dark fermentation

Fermentative hydrogen production is but one process within a larger number of processes known as anaerobic digestion, whereby microorganisms break down biodegradable material in the absence of oxygen. There are four key biological and chemical stages of

anaerobic digestion: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1-1; adapted from Metcalf and Eddy, 2003). In the first stage, large organic polymers such as fats, proteins, and carbohydrates are broken down into their constituent monomers, dissolving them and making them readily accessible to other bacteria. Acidogenesis results in the breakdown of simple sugars, amino acids, and fatty acids by fermentative bacteria into volatile fatty acids, alcohols, ammonia, carbon dioxide, and hydrogen, among other byproducts. The third stage, acetogenesis, results in the further breakdown of volatile fatty acids and other products of acidogenesis into acetic acid, carbon dioxide, and hydrogen gas. The final stage of the process, methanogenesis, converts acetate and hydrogen gas into methane, carbon dioxide, and water. Thus, the simplified chemical equation for the overall anaerobic decomposition of the model substrate glucose is as follows (Madigan and Martinko, 2006):

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \quad (1)$$

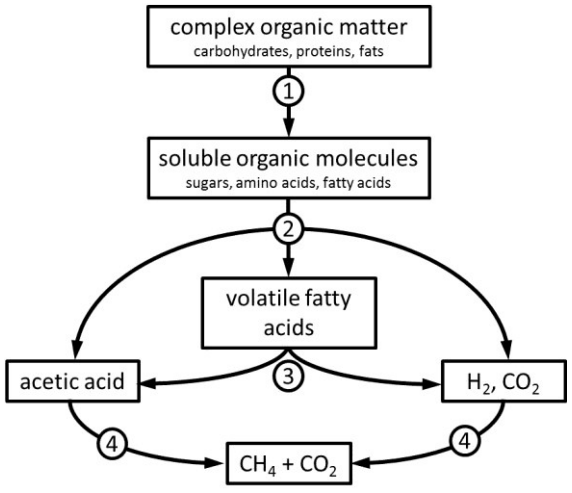

Figure 1-1. Overview of the anaerobic digestion process (Adapted from Metcalf and Eddy, 2003). (1) hydrolysis; (2) acidogenesis; (3) acetogenesis; (4) methanogenesis.

Dark fermentation encompasses the first three stages of anaerobic digestion, whereby organic substrate is converted to biohydrogen, carbon dioxide, and a mixture of lower molecular weight organic acids and alcohols. From the cellular point of view, dark fermentation is the oxidation of bacterial substrate, generating excess electrons that are transferred to an electron acceptor. Under anaerobic conditions, protons serve as the electron acceptor and are reduced to molecular hydrogen through the activity of hydrogenase. Bacteria that possess the capability of hydrogen production include strict anaerobes (*Clostridia*, methylotrophs, rumen bacteria, *Archaea*), facultative anaerobes (*Escherichia coli*, *Enterobacter*, *Citrobacter*), and even aerobes (*Alcaligenes*, *Bacillus*) (Nandi and Sengupta, 1998). Among the hydrogen-producing bacteria, *Clostridium* sp. and *Enterobacter* sp. are the most widely studied (Wang and Wan, 2009).

The stoichiometric yield of hydrogen is dependent on the fermentation byproducts produced, which are determined by the identity of the microorganisms performing the fermentation reaction and the environmental conditions. Carbohydrates, mainly glucose, are the preferred carbon sources for this process, of which the most common fermentation products are acetate and butyrate (Madigan and Martinko, 2006):

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

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2\ H_2 \quad (3)$$

According to reactions (2) and (3), the stoichiometric yields are 4 moles of hydrogen for each mole of glucose (i.e. 544 mL hydrogen/g hexose at 25 °C) in the production of acetic acid, and 2 moles of hydrogen (i.e. 272 mL hydrogen/g hexose at 25 °C) in the

production of butyric acid. In addition to organic acids, alcohols such as ethanol may be produced from the fermentation of glucose (Madigan and Martinko, 2006):

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2H_2 + 2CO_2 \quad (4)$$

In the production of ethanol, the stoichiometric yield of hydrogen is 2 moles for every mole of glucose. Observed hydrogen yields, however, are often substantially lower than the stoichiometric yields for at least four reasons. First, some glucose will be consumed for biomass production. Second, glucose may be degraded through other pathways without producing hydrogen. Third, hydrogenase activity is inhibited at high hydrogen partial pressures, making stoichiometric yields only achievable under low hydrogen partial pressures. Finally, hydrogen may be consumed for the production of other byproducts.

1.3 Biological hydrogen production theory

When engineering a process to biologically produce hydrogen gas, the aim is to direct the anaerobic decomposition of organic compounds towards the end products of hydrogen gas and acetic acid and, at the same time, to inhibit the consumption of hydrogen via processes such as methanogenesis, thereby maximizing biohydrogen yields. Because hydrogen is consumed by methanogens to produce methane during anaerobic digestion, the first engineering challenge is to inhibit methanogens. If such inhibition is achieved, a major sink for hydrogen is eliminated. Hydrogen will build up unless it is efficiently extracted, and the buildup makes further hydrogen production thermodynamically unfavorable. The second major engineering challenge, therefore, is to ensure the

hydrogen partial pressure is kept low enough to allow favorable thermodynamics for hydrogen production.

1.4 Dark fermentation bioreactor configurations

Studies on batch, semi-continuous, and continuous hydrogen-producing bioreactors at laboratory scales have been conducted (Lay et al., 2012; Bhaskar et al., 2008; Ding et al., 2010). In general, batch fermentation results in lower hydrogen production rates than continuous or semi-continuous operation, but are easy to perform from an experimental methods standpoint and are often used for exploratory experimentation. From an engineering perspective, however, batch studies do not scale up well and continuous hydrogen production is preferred. This section gives an overview of different reactor configurations used for biological hydrogen production.

The continuous stirred tank reactor (CSTR) is an effective and common mode of continuous biological hydrogen production. Complete mixing provides intimate contact between the substrate and biomass, allowing for efficient mass transfer. Effective pH and temperature control are also accomplished. Because the biomass is suspended in the reactor liquor, however, the solid retention time and the hydraulic retention time (HRT) are the same, meaning biomass concentrations and thus the hydrogen production rates are restricted. Depending on the HRT, biomass concentrations normally range between 1 and 4 g VSS/L (Show at al., 2007, 2010; Zhang et al., 2006). Some studies have demonstrated that hydrogen-producing biomass in a CSTR can be flocculated under proper conditions, allowing biomass retention time to be decoupled from HRT. In a

study using sucrose-based wastewater, the formation of this granular sludge drastically increased biomass concentrations to 20 g/L while concurrently increasing the hydrogen production rate to 540 mL/h/L (H.H.P. Fang et al., 2002). In another granular sludge study, a glucose wastewater was fermented with a maximum yield of 1.81 mol hydrogen/mol glucose at an extremely short HRT of 0.5 hours (Zhang et al. 2007a).

Anaerobic sequencing batch reactors (ASBRs) result in longer sludge ages because reaction and solids-liquid separation occur in the same vessel (Metcalf and Eddy, 2003). The advantage to longer sludge age provided by ASBRs is that less of the chemical energy potential of the feed is used for biomass growth, and thus, hydrogen yield is increased. The disadvantage of ASBRs is that they are more complicated to operate and depend on the development of a good settling granulated sludge (Metcalf and Eddy, 2003). In a study of hydrogen production from tequila vinasse using a sequencing batch reactor, it was found that at a HRT of 12 hours and an operating temperature of 25 °C an insignificant amount of biogas was produced (Buitron and Carvajal, 2010). When the temperature was increased to 35 °C, a maximum hydrogen production rate of 50.5 mL/h/L was observed.

Membrane bioreactors (MBRs) have become increasingly common in anaerobic wastewater treatment (Seung and Krishna, 2006; Martin-Garcia et al., 2011). The MBR relies on membrane separation of solids in the effluent to retain sludge within the reactor liquor so that high biomass concentrations can be achieved while also increasing sludge

age. MBRs used for hydrogen production experience the same drawback that most MBRs do, namely, membrane fouling and high maintenance costs required to manage the symptoms of fouling (Metcalf and Eddy, 2003). In a study using 10 g/L glucose as a feedstock with an HRT of 3.3 hours, Oh et al. (2004) reported an increase in biomass concentration from 2.2 g/L in a control reactor with no membrane to 5.8 g/L in an MBR with a sludge retention time of 12 hours. The hydrogen production rate also increased from 500 mL/h/L to 640 mL/h/L. Further increases in sludge retention time were observed to increase the biomass concentrations even higher, yet the hydrogen production rates decreased. Another study by Lee et al. (2007) described an MBR system that operated stably at a very short HRT of 1 hour with a maximum hydrogen production rate of 2750 mL/h/L and a yield of 1.36 mol hydrogen/mol hexose using fructose at a concentration of 20 g COD/L as the carbon source. Under CSTR operation, cell washout occurred at an HRT of 2-4 hours.

In a packed-bed reactor, biomass is immobilized within a packed media, and the feedstock flows through the interstitial spaces. The flow pattern within a packed-bed reactor is plug flow with little mixing. Two flow schemes are common: flow entering at the bottom and exiting from the top is commonly known as an upflow packed-bed reactor, whereas trickling biofilter reactors use a downflow mode. Generally, higher biomass concentrations are maintained due to biomass immobilization; solids accumulation and the plugging of packing are potential disadvantages (Metcalf and Eddy, 2003). Chang et al. (2002) entrapped hydrogen-producing biomass in three porous

media: loofah sponge, expanded clay, and activated carbon. The loofah sponge was inefficient, but the reactor with activated carbon had a production rate of 1.21 L hydrogen/h/L and demonstrated increased stability over the alternative media types at a HRT of 1 hr and a sucrose concentration of 20 g/L. Wu et al. (2003) produced hydrogen from a sucrose-rich wastewater using biomass immobilized in alginate beads with acrylic latex and silicone inside a three-phase fluidized-bed reactor. A maximal yield of 182 mL hydrogen/g hexose and a production rate of 929 mL hydrogen/h/L were reported.

The upflow anaerobic sludge blanket (UASB) bioreactor has been used in biohydrogen research because of its good treatment efficiency and ability to retain high biomass concentrations (Metcalf and Eddy, 2003). The key feature of the UASB process is the development of a dense granulated sludge. Granulation is very successful with high carbohydrate or sugar wastewaters, while feedstock high in protein results in fluffier floc (Thaveesri et al., 1994). The UASB, therefore, may not be the optimal reactor configuration for treating more complex waste streams such as municipal wastewater. Mu and Yu (2004) reported a maximum yield of 186 mL hydrogen/g hexose and a production rate of 200 mL hydrogen/h/L from a sucrose feedstock using self-granulated biomass. Chang and Lin (2004) also fed a self-granulated biomass a sucrose-rich synthetic feed. They observed a maximum yield of 102 mL hydrogen/g hexose and a hydrogen production rate of 275 mL hydrogen/h/L.

1.5 Factors affecting biohydrogen production

The efficiency of biological hydrogen production is determined by various factors, including environmental conditions (type of feedstock, temperature, hydrogen partial pressure), operational and process parameters (hydraulic retention time, seed culture), and chemical conditions (pH). Each parameter is detailed in this section.

Apart from pure cultures, various mixed consortia and co-cultures have also been explored for hydrogen production. Mixed cultures of bacteria have been developed from anaerobic sludge, municipal sewage sludge, compost and soil (Li and Fang, 2007). Co-cultures of specific hydrogen-producing bacteria have also been studied (Chou et al., 2011; Masset et al., 2012). Processes using mixed cultures are in many ways more practical than those using pure cultures. They are easier to operate and control and are generally able to utilize a broader range of feedstock types (Li and Fang, 2007). Non-sterile, complex, but readily available feedstocks such as municipal or industrial wastewater present a challenge for pure culture fermentations, especially with limited pretreatment. Mixed consortia derived from sludge are able to address these issues because they are robust and have been selected for under similar conditions (Zhang et al., 2007b). Lastly, mixed cultures are intrinsically more resilient to changes in environmental conditions, such as periods of nutrient limitation where metabolite exchange between different bacteria occurs (Brenner et al., 2008).

Simple sugars such as glucose, sucrose and lactose are preferred as model substrates for hydrogen production. The costs of pure carbohydrate sources, however, are too high to be

practical at commercial scales. Waste streams rich in sugars or complex carbohydrates are thus well suited for biological hydrogen production. Many studies have used synthetic wastewater comprised of a single substrate, often a simple sugar, cellulose, or starch (Horiuchi et al., 2002; Logan et al., 2002; Khanal et al., 2004). Hydrogen yields in continuous reactors using synthetic wastewater have ranged from 64 mL hydrogen/g hexose (Chang et al., 2002) to 308 mL hydrogen/g hexose (Chen and Lin, 2003). A few studies utilizing real industrial wastewater as feedstock have shown promise. Ueno et al. (1996) reported a yield of 343 mL hydrogen/g hexose in a CSTR treating sugar factory wastewater. A study using winery wastewater yielded 291 mL hydrogen/g hexose (Yu et al., 2002), and another study using sugarbeet wastewater yielded 231 mL hydrogen/g hexose (Hussy et al., 2005). Some complex substrates are not ideal for fermentative hydrogen production. Low yields have indicated that it might be uneconomical to recover hydrogen from municipal wastewater sludge (Okamoto et al., 2000; Van Ginkel et al., 2005). Another study using restaurant food waste reported a maximum hydrogen yield of 3.48 mL/g COD (Wongthanate et al., 2014). Municipal and food waste streams contain fewer simple carbohydrates but more complex organics.

Biological hydrogen production is strongly influenced by system pH. At the cellular scale, the environmental pH plays a crucial role in governing the metabolic pathways of microorganisms. System pH influences the efficiency of substrate metabolism, protein synthesis, hydrogenase activity, and the release of metabolic by-product. In addition, pH is also a crucial factor in the suppression of hydrogen-consuming methanogenic activities

(Chen et al., 2002). In a study of fermentative hydrogen production from food and beverage processing wastewater, an optimum initial pH of 6.5 was reported (Wongthanate et al., 2014). In a CSTR study using a glucose feedstock, Fang and Liu (2002) investigated the volatile fatty acid product profiles between pH 4.0 and 7.0. Butyrate was found to be the predominant product (up to 45.6%) at pH 6.0 or below. Conversely, acetate became predominant (up to 34.1%) at pH 6.5 or above. It was concluded that the optimum hydrogen yield was obtained at pH 5.5. Kim et al. (2004) also reported that butyrate was the main product at pH 5.5, but butanol became predominant at pH 4.3.

Dissolved hydrogen concentration in the liquid phase, which contributes to the hydrogen partial pressure, is one of the key parameters that influence the metabolic pathways in fermentative bacteria. Hydrogen production is less favorable as the hydrogen partial pressure rises. It is therefore imperative that excess hydrogen is removed from the system to support efficient hydrogen production. Strategies for removing excess hydrogen gas have been developed to avoid these negative thermodynamic effects; many of these methods, however, are energy intensive. Mizuno et al. (2000) found that hydrogen yields increased from 115 to 194 mL/g hexose when the reactor was sparged with nitrogen at 15 times the hydrogen production rate. Hussy et al. (2003) also reported an enhanced hydrogen yield from 177 to 258 mL/g hexose by sparging the reactor with nitrogen to reduce hydrogen in the off-gas from 50% to 7%. Lay (2000) reported that

increasing the mixing in a CSTR from 100 rpm to 700 rpm to avoid supersaturation of dissolved hydrogen enhanced the hydrogen production rate by 130%.

Biological hydrogen production has been demonstrated at a range of temperatures between 15 and 85 °C (Kanai et al., 2005). Generally, hydrogen production studies are conducted within three temperature ranges: ambient (15-30 °C), mesophilic (32-39 °C), and thermophilic (50-64 °C). A review of over 100 laboratory-scale studies suggested that hydrogen yields and production rates were comparable at mesophilic and thermophilic temperatures, but lower at ambient temperatures (Li and Fang, 2007). The highest reported yields were 266 mL hydrogen/g hexose for studies at 15-30 °C (H.H.P. Fang et al., 2002); 333 mL hydrogen/g hexose (Van Ginkel et al., 2001) for 32-39 °C, and 327 mL hydrogen/g hexose for 50-64 °C (Ueno et al., 1995). Studies using mesophilic cultures (Minnan et al., 2005) seemed to indicate that increasing operational temperatures from ambient to mesophilic improved hydrogen production, but further increasing the temperature beyond the mesophilic range was detrimental to hydrogen production (Lee et al., 2006). This is likely because of the physiological characteristics of the mesophilic cultures used.

Hydraulic retention time is one of the most important control parameters affecting continuous hydrogen production. HRT is a tool for selecting microbial populations whose growth rates are able to keep pace with the mechanical dilution of suspended biomass in a continuous flow system. In particular, methane-producing bacteria have a

growth rate of about 0.0167-0.02 h^{-1}, which is much lower than that of hydrogen-producing bacteria, which demonstrate a growth rate of about 0.172 h^{-1} (Lo et al., 2009a). Selection of hydrogen-producing bacteria within the reactor volume is thus achieved while washing out methanogens, reducing the impact of interspecies hydrogen transfer on process yields. Li and Fang (2007) reviewed available literature on biological hydrogen production and found that the most commonly reported optimal HRT values for systems fed glucose or sucrose were in the range of 3 to 8 hours, with the lowest being 1 hour (Chang et al., 2002) and the highest 13.7 hours (Fang and Liu, 2004). Longer HRTs may be necessary for more complex feedstocks, however, because hydrolysis of the complex organic matter is required. For example, Hussy et al. (2003) and Lay (2000) reported much higher optimal HRTs, 15 and 17 hours respectively, for systems using starch feedstocks.

1.6 Engineering advances applicable to biohydrogen production

The efficiency of gas–liquid–microbe mass transfer is often limiting in multiphase bioreactors, which has spurred research and advancements in the areas of microbial immobilization and gas transfer membranes. Even when a reactor is categorized as being well-mixed, Grasso et al. (1995) demonstrated that significant mass transfer resistance occurs in the liquid-microbe boundary layer using methanogens. Additionally, dissolved gaseous substrates such as biologically produced hydrogen encounter mass transfer resistance at the stagnant interface between the liquid phase and the gaseous phase of the headspace of a bioreactor. The surface area of this liquid-gas interface is constrained by the reactor dimensions, compounding the mass transfer limitations. This section

discusses advances that have relevance on improving these transfer processes, thereby increasing the efficiency of hydrogen-producing bioreactors.

1.6.1 Membrane immobilization of microorganisms

Cell immobilization on membranes or porous supports concentrates and intensifies biological reactivity and stabilizes microbes. Encapsulation of microorganisms allows localized control of a microbial community within a polymer matrix. The entrapped organisms are prevented from escaping, and unwanted organisms cannot penetrate the membrane as long as its physical integrity is maintained (Wessel et al., 2013). The selection of membrane chemistries with high mechanical strength is important to prevent leaking out of encapsulated cells. Conversely, the membranes must be semi-permeable to allow for the diffusion of dissolved nutrients required for cell metabolism and the byproducts released by them (Hannoun and Stephanopoulos, 1986). If diffusion is limited, biological activity may be limited or even prevented.

Casting is a common process for immobilizing microorganisms within a polymer or silica matrix. The culture of interest is homogenized within a solution of polymer precursor or silica solution and then cast into a mold. Drying of the solvent and some sort of sintering or cross-linking process enhances the structural durability of the final product. One disadvantage to cast polymer/silica membranes is their thickness, which is often several orders of magnitude larger than that of thin films. Because of this, diffusion of substrates is often limited.

Karube et al. (1976) immobilized a culture of a strict anaerobe species *Clostridium butyricum* in a polyacrylamide gel. Hydrogen production by the immobilized cells from glucose was achieved in a batch system under aerobic conditions, whereas no hydrogen production occurred in the control reactors, demonstrating active metabolism by the encapsulated bacteria and the protective nature of the polymer matrix. The pH stability of hydrogen production was also increased with immobilization. Another study (Reategui et al., 2012) investigated the encapsulation of *Escherichia coli* cells expressing an atrazine-dechlorinating enzyme in a silica nano particle/polyethylene glycol polymer porous gel. Viability of the encapsulated cells was confirmed by cell viability assay and visually by electron microscopy. Atrazine biodegradation by the encapsulated cells was also observed. It was found that increasing the surface area of the gel matrix by shaping into microbeads increased atrazine degradation rates close to those values obtained with free cells. Gosse et al. (2012) developed a hydrated latex coating containing embedded bacterial cells that demonstrated absorption and evolution of several gases including hydrogen. Among four different species of bacteria successfully encapsulated, *Rhodopseudomonas palustris* was demonstrated to consume acetate and produce hydrogen.

Microorganisms can also be immobilized via electrospinning, a method of producing sub-micron diameter fibers by extruding a polymer solution out of a spinneret while applying an electrical field to the droplet (Li and Xia, 2004). The electrical field stretches the droplet into a jet, which exhibits electrically-induced bending instability, causing

stretching of the bent sections of the jet. This stretching determines the final thickness of the fiber. Massive evaporation of the solvent takes place and the jet dries extremely quickly, resulting in nanofibers that are deposited on the collector electrode after a few milliseconds. Encapsulation of bacteria into electrospun fibers is technically more challenging than casting because of the environmental stresses cells experience during the process. Exposure to toxic organic solvents and rapid desiccation are among the challenges to protecting cell viability. Because of their incredibly large surface areas, the diffusion of substrate is greatly improved for electrospun fibers compared to cast membranes. The incorporation of microorganisms within electrospun fibers is accomplished by mixing cell cultures into the polymer solution prior to spinning.

Microbial encapsulation using such techniques has been demonstrated in several studies. Salalha et al. (2006) encapsulated *Escherichia coli* and *Staphylococcus albus* into polyvinyl alcohol nanofibers while maintaining 19% and 100% viability, respectively. When glycerol was added to the spinning solution to prevent desiccation of cells, the viability of encapsulated *E. coli* was improved to 48%. Liu et al. (2009) developed a polyethylene oxide-polypropylene oxide-polyethylene oxide triblock polymer electrospun fiber and encapsulated three different microbial species including *Zymomonas mobilis*, which is used industrially to produce ethanol from glucose. A low toxicity redox system that allowed the microbes to survive was selected to cross-link the fibers to prevent dissolution of the fibers in aqueous solution. Ethanol production of the encapsulated bacteria verified that their metabolic activity was not inhibited. In another study,

Pseudomonas ADP cells were encapsulated within microtubules created by spinning a core solution of aqueous polyethylene oxide containing cells and a shell solution of polycaprolactone and polyethylene glycol in organic solvent (Klein et al., 2009). The two solutions were spun in a coaxial configuration, resulting in microtubules with porous walls. The entrapped cells were demonstrated to retain their denitrification activity, confirming cell viability.

1.6.2 Efficient gas transfer and capture

Efficient hydrogen transfer is critical to improving the efficiency of hydrogen production. Hydrogenase activity is inhibited at high hydrogen partial pressures. If hydrogen partial pressures are kept low, process rates and yields will be maximized and may approach stoichiometric ideals. The restricted ability of passive off-gassing techniques to control hydrogen partial pressures, however, is a barrier to optimizing hydrogen production efficiencies (Show et al., 2011). An effective technology that allows for greater control of this parameter is therefore needed.

Hollow fiber membranes are microporous tubes several hundred microns in diameter that create a semi-permeable barrier of defined molecular weight cut-off. This allows for the selective control of mass transfer of particular substrates, in this case gas. The shape and size of the hollow fibers provide a high specific surface area to maximize mass transfer. Often, a sweep gas is used in the permeation side of the membrane to keep the permeation biogas partial pressure as low as possible, increasing the rate of diffusion. Mass transfer rates far superior to those of conventional devices are possible with hollow

fibers at lower operational and maintenance costs (Yang and Cussler, 1986). As an added benefit, the self-contained and modular nature of this biogas collection mechanism forgoes traditional system requirements (such as covers for tanks) that have substantially higher capital costs.

A large body of work exists on the use of hollow fiber membranes for gas transfer applications in aqueous environments. Hollow fibers have been used to supply substrates including hydrogen to reactors or groundwater to support biodegradation of pollutants (Agarwal et al., 2005; Schnobrich et al., 2007; Chaplin et al., 2009; Y. Fang et al., 2002; Clapp et al., 2004). The same general principles apply for the use of hollow fiber membranes for the removal of gaseous products from reactors. Voolapalli and Stuckey (1998) used a submerged silicone membrane to remove dissolved CO_2 and hydrogen from an anaerobic digester to improve process stability at high organic loading rates. While hydrogen removal was only partially successful due to biofilm buildup of hydrogen consuming microorganisms, the presence of the membrane enhanced volatile fatty acid degradation, stabilized pH, and improved reactor stability in comparison to a control. Another study used hollow fiber membranes submerged within the reactor liquor of an anaerobic fermenter to reduce the partial pressure of hydrogen, resulting in a 10% increase in the rate of hydrogen production and a 15% increase in the hydrogen yield (Liang at al., 2002).

1.7 Challenges

Major challenges must be overcome before biohydrogen production is considered a viable renewable energy source. First, the yield of hydrogen from biological processes is too low for commercial application and remains energetically unfavorable when the cost of growing, harvesting, transporting, and pretreating feedstock is accounted for. To circumvent some feedstock processing costs, industrial and municipal waste streams have received serious consideration as renewable feedstock. Yields, however, are not as highly optimized. Second, the need to prevent interspecies hydrogen transfer in non-sterile conditions results in process strategies such as (1) feedstock pretreatment (e.g. heating) which lowers the net process energy balance or (2) operating at low pH or short hydraulic retention times to inhibit methanogen growth. These strategies, however, result in lower hydrogen yields, especially when more complex feedstocks are used. Third, sensitivity of the hydrogenase enzyme to hydrogen partial pressures hampers process efficiency and lowers yields. Hydrogen removal strategies such as aggressive stirring regimes or gas sparging add to the process energy investment. The remaining engineering challenges to be addressed include appropriate reactor design, difficulty sustaining long-term continuous hydrogen production, and scale-up from the laboratory to commercial and industrial scales.

The goal of this research was to overcome the most serious of these challenges by (1) developing a membrane technology, embedded with hydrogen-producing mixed consortia and hollow fibers, to produce and capture hydrogen from dissolved phase organics in wastewater, (2) testing these composite hollow fiber membranes for hydrogen

production and capture in continuous stirred tank reactors fed synthetic wastewater, and (3) conducting batch tests of real wastewaters to determine feedstock potential for future continuous biological hydrogen production experiments using the composite hollow fiber membranes.

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Chapter 2: A modular technology for fermentative hydrogen production and capture from wastewater

2.1 Introduction

Despite the inherent chemical energy potential of wastewater, current wastewater treatment practices expend a considerable amount of energy removing dissolved energy-dense compounds. The water and wastewater treatment sector accounts for approximately 3% of energy use nationally (USEPA, 2006), which is similar to that in other developed countries (Curtis, 2010). A typical municipal wastewater treatment plant allots more than 50% of its total energy use to aeration (Curtis, 2010), converting the reduced chemical energy within wastewater into CO_2 and biomass growth. If treated differently, however, the chemical energy potential of wastewater could be captured into usable forms of energy while simultaneously reducing subsequent treatment demands.

Anaerobic treatment is used to generate methane, or with the application of new technologies, other forms of energy such as hydrogen (Guest et al., 2009; McCarty et al., 2011). Indeed, anaerobic digestion is used primarily used to harvest energy in the form of methane biogas from high strength industrial wastewaters or wastewater solids after secondary aerobic treatment has already occurred. By inhibiting the naturally occurring methanogens that consume hydrogen and acetate within the anaerobic digestion process, however, the degradation of the dissolved organic compounds in wastewater can be redirected to produce hydrogen. Hydrogen, when produced biologically, is regarded as a renewable and attractive clean energy source as a result of its energy density and clean-burning properties. Laboratory studies have demonstrated that a range of industrial waste

streams are able to be used for fermentative biohydrogen production, including noodle manufacturing waste (Noike, 2002), rice winery wastewater (Yu et al., 2002), filtered leachate of waste biosolids (Wang et al., 2003), sugarbeet wastewater (Hussy et al., 2005), palm oil mill effluent (O-Thong et al., 2008), and pig waste slurry (Kotsopoulos, 2009). The highest yield was demonstrated by Ueno et al. (1996), achieving a yield of 343 mL hydrogen/g hexose using sugar factory wastewater. Likewise, biohydrogen production has been achieved with various reactor configurations, including batch (Lay et al., 2012; Maintinguer et al., 2011), semi-batch (Buitron and Carvajal, 2010), continuous operation (Hussy et al., 2005; Tapia-Vanegas et al., 2013; Zhu et al., 2013), packed bed, fluidized bed (Lin et al., 2009), and membrane bioreactors (Oh et al., 2004; Lee et al., 2007). A review of fermentative hydrogen production by Li and Fang (2007) found that most studies were conducted using carbohydrate-based synthetic wastewaters, with hydrogen yields of continuous reactors ranging from 64 mL hydrogen/g hexose to 308 mL hydrogen/g hexose.

Despite the potential, technical challenges remain that prevent full-scale wastewater biohydrogen production from being economically feasible. Previously studied hydrogen production has often required pre-treatment of the influent wastewater to deactivate hydrogen-consuming methanogens and prevent interspecies hydrogen transfer. Pretreatment techniques have included heating, acidifying, or autoclaving (Lay et al., 2010; Lee et al., 2007; Sharma and Li, 2010; respectively), which reduce the net energy gained from the process and are not typically feasible at a realistic scale. Because

hydrogen production is less favorable when the hydrogen partial pressure is high, removing excess hydrogen from the system is also critical, which can be difficult at a large scale. In fact, in many studies, biogas was captured passively, which offers little control and can require significant infrastructure upgrades to existing facilities, such as the retrofitting of large tanks with air-tight covers and mixers or the construction of new closed tanks. Alternatively, if hydrogen was removed via nitrogen sparging, as has been performed in some studies (Hussy et al., 2003; Show et al., 2011), it would result in a significant operational cost that would negate energy savings/gains.

Two recent engineering advances, microbial encapsulation and the development of very efficient gas transfer methodologies, present us with the opportunity to develop a biohydrogen generation system that has the potential to overcome these challenges. Encapsulation of bacteria into polymers or membranes allows localization and control of microbial communities, thereby eliminating the need to pretreat the wastewater. Indeed, microorganisms have been immobilized within sol-gel polymers (Karube et al., 1976; Gill and Ballesteros, 2000), embedded within silica gel nanoparticles (Reategui et al., 2012), trapped within latex-coatings (Gosse et al., 2012), and encapsulated within electrospun fibers (Liu et al., 2009; Klein et al., 2009; Tong et al., 2013), all while demonstrating cell viability. In addition, microporous hollow fibers provide high surface areas for gas transfer control in aqueous environments (Gabelman and Hwang, 1999; Y. Fang et al., 2002; Clapp et al., 2004; Lauterbock et al., 2012), offering a modular, energy efficient method for adding or removing gas from a system.

The objective of this research was to make use of these advances to develop a scalable and modular technology to produce and capture hydrogen from dissolved phase organics in wastewater. This technology was based on the membrane-encapsulation of hydrogen-producing mixed consortia on hollow fiber membranes for efficient hydrogen collection and removal (Figure 2-1). These composite hollow fiber membranes were submerged into continuous stirred tank reactors (CSTRs) fed synthetic wastewater and tested for hydrogen production and capture. Additionally, batch tests of real wastewaters were conducted to determine feedstock potential for future continuous biological hydrogen production experiments using the composite membrane modules. Louis Sigtermans performed the batch experiments, developed the methodology for module construction and application, developed the methodology for the CSTR experiments, and performed the CSTR experiments with membranes constructed of cast polyvinyl alcohol; lab work on the CSTR experiments using electrospun membranes was contributed by Dr. Ana Lucia Prieto.

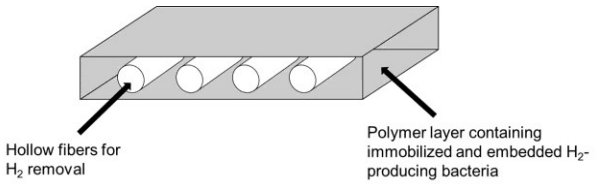

Figure 2-1. Schematic of composite membrane for biohydrogen production and capture from wastewater.

2.2 Methods

2.2.1 Preparation of microorganisms for encapsulation

A pure strain of *Clostridium butyricum* (American Type Culture Collection, Manassas, VA) was used for initial hydrogen production experiments. Cultures were maintained on petri plates containing Reinforced Clostridial Medium agar (see recipe below) and

incubated at 36 °C. A hydrogen-producing mixed culture was produced by taking sludge from a mesophilic laboratory anaerobic digester and heat-treating at 95 °C for 40 minutes to deactivate hydrogen-consuming methanogens. Cultures were maintained by inoculating serum bottles containing synthetic wastewater with heat-treated sludge seed and incubating at 36 °C for 24 hours.

Illumina sequencing of bacterial 16S genes was used to analyze the microbial community. Briefly, the 16S rRNA genes were amplified through PCR with primers designed to target the V3 region of the gene. In addition to the gene binding sequences, these primers also have sequences to bind to the Illumina sequencing primers, tags to identify different samples, and sequences to attach to the Illumina platform. Sequencing was performed by the University of Minnesota Genomics Center with an Illumina MiSeq (2 x 150). Data was analyzed using Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al., 2010) run through the Minnesota Supercomputing Institute. The analysis identified approximately 97% of microbial community as belonging to families *Bacillaceae* and *Clostridiaceae* (Appendix Figure A).

2.2.2 Media and synthetic wastewater

Reinforced Clostridial Medium (RCM), developed by Hirsch and Grinstead (1954), was used for pure culture experiments. The medium contained the following per liter of deionized water: 3 g yeast extract, 10 g meat extract, 10 g peptone, 5 g glucose, 1 g soluble starch, 5 g sodium chloride, 3 g sodium acetate, and 0.5 g cysteine hydrochloride.

The synthetic wastewater was used for mixed culture experiments and was designed to represent high-strength municipal wastewater. This medium was modified from that used by Klatt and LaPara (2003) and contained the following per liter of deionized water: 3750 mg gelatin, 1750 mg starch, 960 mg polyoxyethylene-sorbitan monooleate, 80 mg yeast extract, 80 mg casamino acids, 150 mg ammonium chloride, 500 mg sodium bicarbonate, 25 mg sodium phosphate monobasic, 30 mg potassium phosphate dibasic, 40 mg magnesium chloride, 60 mg calcium chloride, and 0.1 mL SL7 trace mineral solution (Biebl and Pfennig, 1981). This synthetic wastewater had a chemical oxygen demand (COD) of 8500 mg/L and a pH of 6.5.

2.2.3 Membrane construction

2.2.3.1 Cast polyvinyl alcohol (PVA)

An 8.3% (w/v) aqueous solution of Elvanol 71-30 (DuPont, Wilmington, DE) was heated to near boiling under constant stirring until dissolved. The solution was cooled to room temperature and degassed under vacuum for 5 minutes. A washed and weighed microbial culture, either the pure culture or the mixed heat-treated consortia, was gently stirred into the solution. The control membrane was prepared without the addition of organisms.

A 14 cm × 7 cm section of hydrophobic polyethylene hollow fiber membrane fabric (340 μm ID, 390 μm OD, model EHF390; Mitsubishi Rayon, New York, NY) was placed on a polytetrafluoroethylene casting block. Approximately 7 g of polymer solution was poured over the hollow fiber fabric onto the casting block and spread evenly over an area of 10 cm × 7 cm, leaving the ends of hollow fiber fabric exposed on each side. The total

biomass encapsulated within the experimental membrane ranged from 1.65 to 4.66 mg dry weight.

After drying, the ends of the hollow fiber membranes were plumbed into manifolds (Masterflex silicone L/S 17 tubing; Cole-Parmer, Vernon Hills, IL) and bonded using Silicone II Household Glue (GE, Huntersville, NC) (Figure 2-2). PVA membrane modules were submerged in 1% (w/v) aqueous boric acid for 4 minutes to cross-link the polymer.

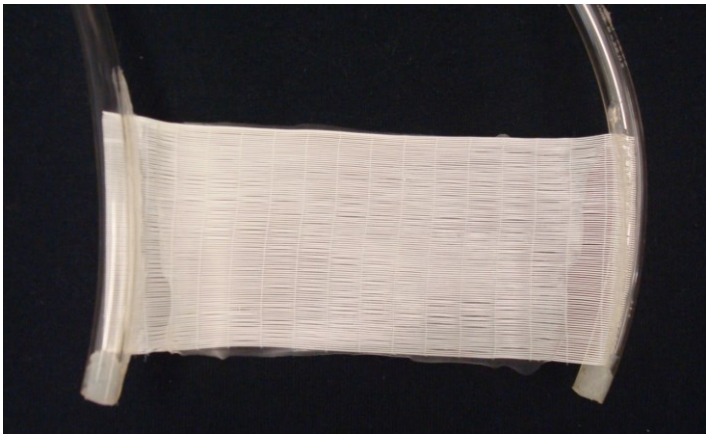

Figure 2-2. Photo of PVA composite membrane module.

2.2.3.2 Electrospun microtubules

The heat-treated anaerobic consortia were also encapsulated in electrospun microtubules as described in Klein et al. (2009). Briefly, a core polymeric solution containing the consortia and a shell polymer solution were co-spun using a spinneret with two coaxial capillaries. No cells were mixed into the core solution for the control membranes. The core solution consisted of 5 wt % polyethylene oxide (PEO) 600 K in water. The shell solution was 9 wt % polycaprolactone (PCL) 80 K and 1 wt % polyethylene glycol (PEG) 6 K dissolved in a mixture of chloroform and dimethylformamide, 9:1 (w/w). All

polymers and solvents were purchased from Sigma-Aldrich (St. Louis, MO). The electrospun tubules were collected on a stationary plate on top of which sat a 14 cm × 7 cm section of hollow fiber fabric. Spinning was performed for approximately 1.5 hours on each side of the hollow fiber fabric, with even coverage over a 10 cm × 7 cm area. The ends of the hollow fibers were then plumbed into manifolds as described above. The total biomass encapsulated was approximately 0.3 mg dry weight for the electrospun bioactive membrane.

2.2.4 Bioreactor design

Membrane modules were tested for hydrogen production in 2.75 L headspace-free continuous stirred tank reactors (CSTRs) constructed of poly(methyl methacrylate) (Figure 2-3). A peristaltic pump (Masterflex L/S variable speed drive pump; Cole-Parmer) controlled the flow rate of feedstock influent. Gastight reactor covers sloped up at a 9% grade towards the liquid effluent port, ensuring that any excess evolved biogases would not form a permanent headspace in the reactor. The membrane module manifolds were mounted to the cover and supplied with ultrahigh purity N_2 sweep gas at a starting nominal flow rate of 8 mL/min. All reactors were operated at room temperature (nominally 22 °C), operated at a nominal hydraulic residence time (HRT) of 18 hours, and fed either RCM or synthetic wastewater for the monoculture and mixed consortia experiments, respectively.

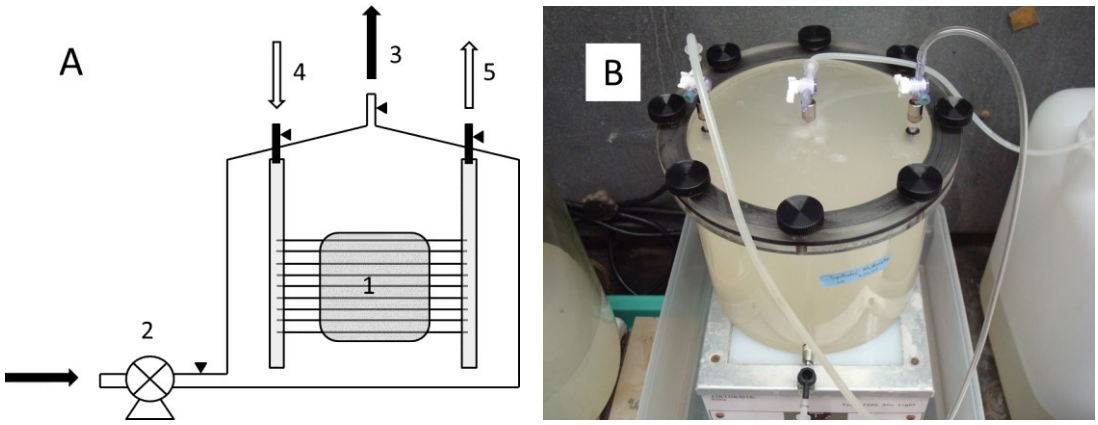

Figure 2-3. (A) Schematic of headspace-free CSTR setup. (1) Hollow fiber membrane module; (2) Feedstock influent pump; (3) Feedstock effluent and off-gas port; (4) Sweep gas influent port; (5) Sweep gas and biogas effluent port; (▲) Sampling locations. (B) Photo of the reactor setup.

Liquid and gas samples were taken from their respective influent and effluent ports at 8-24 hour intervals. The following parameters were measured throughout the duration of the experiments: reactor pH and temperature, flow rate and composition of sweep gas effluent, volume and composition of off-gas, flow rate of reactor influent, and reactor influent and effluent COD concentrations.

2.2.5 Experimental setup

2.2.5.1 Effect of membrane chemistry

Two membrane chemistries were tested: cast PVA and PEG-PCL/PEO electrospun microtubules. The chemistries were compared for their hydrogen production rates, yield, and capture efficiency, in addition to their ability to retain encapsulated biomass.

2.2.5.2 Effect of bacterial encapsulation

The PVA membrane chemistry was tested both with and without (negative control) the encapsulated heat-treated sludge consortia to distinguish between hydrogen production originating from membrane-bound biomass and suspended biomass. The contributions of

the encapsulated versus planktonic microorganisms to the total hydrogen production rate and yield were determined.

2.2.5.3 Effect of feed pH and sweep gas flow rate

The bioactive PVA membrane system was tested under two sweep gas flow rates, 8 mL/min and 2 mL/min, to determine the effect on hydrogen capture by the hollow fiber membrane. It was hypothesized that a higher sweep gas flow rate would improve hydrogen capture by providing a stronger driving force for the hydrogen into the membrane. The effect of feed pH on hydrogen production by the bioactive PVA membrane system was also investigated, so the reactor was supplied synthetic wastewater of pH 6.5, 8.5, and 9.3. The pH of the feedstock was adjusted by increasing the concentration of sodium bicarbonate. It was hypothesized that a higher influent pH would improve hydrogen production by raising the pH in the reactor as well. The system was allowed to reach steady state and operate for at least 8 days after each parameter change.

2.2.5.4 Effect of feedstock, continuous feed

Bioactive PVA membranes were tested in systems fed with wastewater obtained from the Metropolitan Wastewater Treatment Plant in Saint Paul, MN. Two experiments were performed with centrate feed and one experiment was performed with secondary influent. All experiments were run for approximately 10 days.

2.2.5.5 Effect of feedstock, batch feed

Batch hydrogen production experiments were performed in serum bottles (160 mL). Each bottle contained 50 mL of feedstock and 1 mL of heat-treated sludge culture (planktonic) as seed. Additionally, 1.5 mL of 1 M NaOH was added to bottles containing

dairy manufacturing wastewater to adjust the pH from 4.6 to 6.3. Some of the feedstocks were sterilized by autoclaving (designated as such in the results). Each feedstock was tested in triplicate serum bottles. The bottle headspace was sampled and the gas composition determined after incubation at 36 °C for 24 hours.

The feedstocks tested were: municipal wastewater primary influent, secondary influent, and centrate from the Metropolitan Wastewater Treatment Plant in Saint Paul, MN, and dairy manufacturing wastewater from a facility in Farmington, MN (Kemps LLC). Feedstocks had the following characteristics: 165 mg/L COD, primary influent; 105 mg/L COD, secondary influent; 1326 mg/L COD, centrate; 55,000 mg/L COD, dairy waste. After collection, wastewater samples were stored at 4 °C until used. Synthetic wastewater (as described above) was also tested for comparison.

2.2.6 Analytical methods

The composition of biogas (hydrogen and methane) was measured using a gastight syringe to inject samples onto a gas chromatograph (model 6890; Agilent Technologies, Santa Clara, CA) equipped with a thermal conductivity detector and a packed column (Supelco molecular sieve 13X 45/60, 10 ft × 1/8 in × 2.1 mm; Sigma-Aldrich, St. Louis, MO) with nitrogen as the carrier gas. Injection volumes ranged from 10 μL to 500 μL as needed to maintain the signal within calibration values. The detection limit was 0.02% (v/v) for hydrogen and 0.08% (v/v) for methane. Standards (1% hydrogen in nitrogen; 99% methane) were obtained from Sigma-Aldrich. Ultra high purity nitrogen was used for blanks.

Evolved (off-gas) and dissolved gasses from the continuous reactors were sampled from the liquid effluent port by drawing off the combined liquid and gas effluent with a gastight syringe, shaking for one minute to allow liquid-headspace equilibration, and injecting a portion of the syringe headspace onto a gas chromatograph. The liquid/gas volume ratio was determined by measuring feedstock flow rate and off-gas volume.

COD and pH were measured using COD digestion vials and colorimeter and a bench pH meter, as described in Tan et al. (2013). Briefly, Hach (Loveland, CO) high range COD digestion vials containing 2 mL of filtered sample (diluted as necessary) were placed in a heating block set to 150 °C for 2 hours. COD measurements were made with a Hach DR/890 colorimeter. Gas and liquid flow rates were measured volumetrically.

2.2.7 Calculations and data analysis

2.2.7.1 Yield, rate, and temperature-adjusted yield calculations

Hydrogen capture yields were calculated using the following equation:

$$Y_c = \frac{\varphi_{H,sg}\, Q_{sg}}{C_{hex}\, Q_f} \quad (1)$$

where $\varphi_{H,sg}$ is the volume fraction of hydrogen in the sweep gas sample, Q_{sg} is the sweep gas effluent flow rate, C_{hex} is the hexose concentration of the feedstock, Q_f is the feed flow rate, and Y_c is the hydrogen capture yield in mL/g hexose.

The rate of hydrogen escape via off-gas and dissolution in reactor effluent was determined by the following equation:

$$R_e = \frac{\varphi_{H,ss}\, Q_{og} + \frac{\varphi_H}{k_H} Q_f}{2.75} \quad (2)$$

where $\varphi_{H,ss}$ is the volume fraction of hydrogen in the liquid effluent sample syringe headspace, Q_{og} is the off-gas flow rate, k_H is the specific dimensionless Henry's Law constant, Q_f is the feed flow rate, 2.75 is the reactor volume in L, and R_e is the rate of hydrogen escape from the reactor in mL/day/L.

To compare hydrogen capture yields of the composite membranes to hydrogen yields obtained in similar studies from published literature, differences in operating temperature were accounted for. The effect of temperature on biological activity can be approximated by a doubling of metabolic rates for every 10 °C increase. A linear relationship between the ratio of the observed yield over the predicted yield at 22 °C and the operating temperature was used to derive the following equation:

$$Y_{22} = Y_T \left(\frac{T}{10} - \frac{6}{5}\right)^{-1} \quad (3)$$

where Y_T is the observed yield, T is the operating temperature of the study in question, and Y_{22} is the estimated yield at 22 °C.

2.2.7.2 Statistical tests

A t-test assuming equal variance was used to compare hydrogen capture yields under continuous operation at different feed pH conditions and sweep gas flow rates. Only data points beyond 2 HRT (1.5 days) after a parameter change were grouped together to prevent non-steady state data from being included in the statistical test.

2.3 Results

2.3.1 Cast PVA membrane

The membrane prepared for this experiment contained 4.66 mg encapsulated biomass by dry weight. Figure 2-4 shows that hydrogen was produced from synthetic wastewater and was captured by the hollow fibers with an approximate yield of 45-50 mL/g hexose during the period of most stable operation (Day 5 – Day 27). For comparison, the theoretical yield is 2 moles of hydrogen per mole of glucose when the end product is butyrate, but 4 moles of hydrogen when the end product is acetate. A complete stoichiometric conversion of one gram hexose to butyrate would yield 272 mL hydrogen at 25 °C. Conversion to acetate would yield 544 mL hydrogen. This experiment was between 8-18% efficient based on these benchmarks. During the same period, the observed hydrogen capture rate ranged between 78 mL/day/L and 122 mL/day/L. A COD reduction of 5.3% was measured between the influent and effluent at 45 days, demonstrating transformation of the dissolved organic matter. Colonization of the reactor liquor either by bacteria leaking from the membranes or otherwise present in the laboratory was observed, resulting in suspended, hydrogen-producing biomass. Reactor upset occurred around Day 28, most likely as a result of an increase in effluent tubing diameter, changing the internal pressure of the reactor. This, compounded by the pH rising outside of the optimal levels (pH 6.5; Wongthanate et al., 2014) due to a temporary decrease in microbial activity, likely started a cycle of microbial dormancy and further decline in hydrogen production beginning around Day 37. Throughout the experiment, the buffering capacity of the feedstock was such that the pH in the reactor was typically in the 4-5 range.

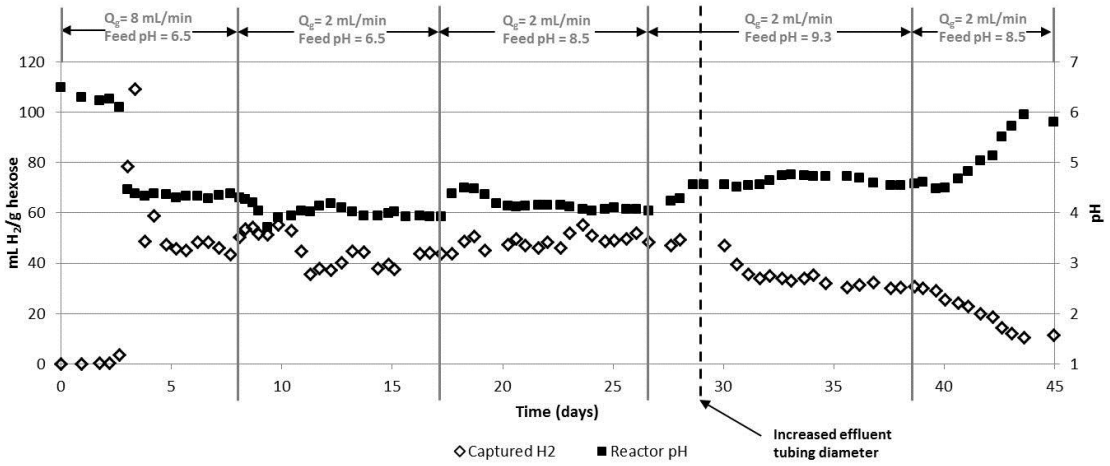

Figure 2-4. Time series of hydrogen capture yield and reactor pH of a trial of the biologically active PVA-hollow fiber composite membrane. Changes in sweep gas flow (Q_g) and influent feed pH are indicated with arrows at top.

Analysis of hydrogen capture data showed differences in yield when feed pH and sweep gas flow rates varied. With influent pH held constant, a higher sweep gas flow rate (8 mL/min compared to 2 mL/min) resulted in a higher hydrogen yield ($p<0.05$). Likewise, at a constant gas flow rate, a feed pH of 8.5 resulted in a higher hydrogen yield than a feed pH of 6.5 ($p<0.05$) (Appendix, Figure B). Both of these results were consistent with expectations.

Figure 2-5A indicates that between 30-80% of all hydrogen generated in the reactor was captured by the hollow fibers, with the remainder exiting the reactor with the liquid effluent, either dissolved or as bubbles in the flow. Capture efficiency decreased linearly throughout the experiment until reactor upset around Day 37 (Fig 2-5A). The hydrogen capture rate remained stable around 100 mL hydrogen/day/L between Days 5 – 27, while the escape of hydrogen via the liquid effluent increased over the same period (Fig 2-5B).

Hydrogen production yield (escaped and captured hydrogen combined) peaked at 120 mL/g hexose on Day 30. This represented a maximum conversion efficiency of 44% assuming butyrate as the end product. Contributing factors lowering the observed hydrogen production efficiency (compared to stoichiometric ideal) include the formation of other reduced end products (i.e. lactate, ethanol), substrate conversion into biomass growth, and inhibition of hydrogenase by high hydrogen partial pressures.

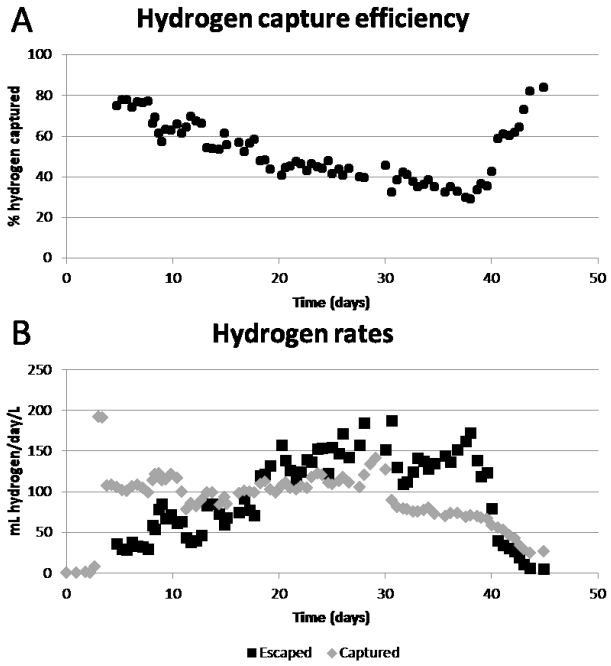

Figure 2-5. (A) Time series of hydrogen capture efficiency of the biologically active PVA-hollow fiber composite membrane; (B) Time series of hydrogen capture and escape rates of the same membrane.

2.3.2 Negative control cast PVA membrane

Hydrogen production and capture by the control PVA membrane system was observed at yields similar to that in the bioactive PVA membrane trial (Fig 2-6). Although hydrogen was produced and captured in the control system, this is an experimental artifact. Hydrogen-producing bacteria were able to colonize the control reactor suspension despite

not being intentionally seeded. Because of their spore-forming ability, these microorganisms were likely a persistent contaminant present on lab utensils and equipment, despite efforts to sterilize and segregate control trial supplies. Hydrogen production and capture in the negative control indicates suspended biomass provides a significant contribution to total hydrogen production within these systems, and that the hollow fibers are extracting dissolved-phase hydrogen from the suspension. The use of sterilized feed meant there was no competition for substrate from native microflora, which allowed hydrogen producers to colonize the suspended phase, and resulted in no competition for hydrogen from methanogens within the wastewater. This allowed for capture of hydrogen produced in the suspended phase, which would not be expected otherwise. If implemented at full-scale, non-sterilized feed would be used; a membrane with no embedded microorganisms, therefore, would be expected to result in no hydrogen production or capture.

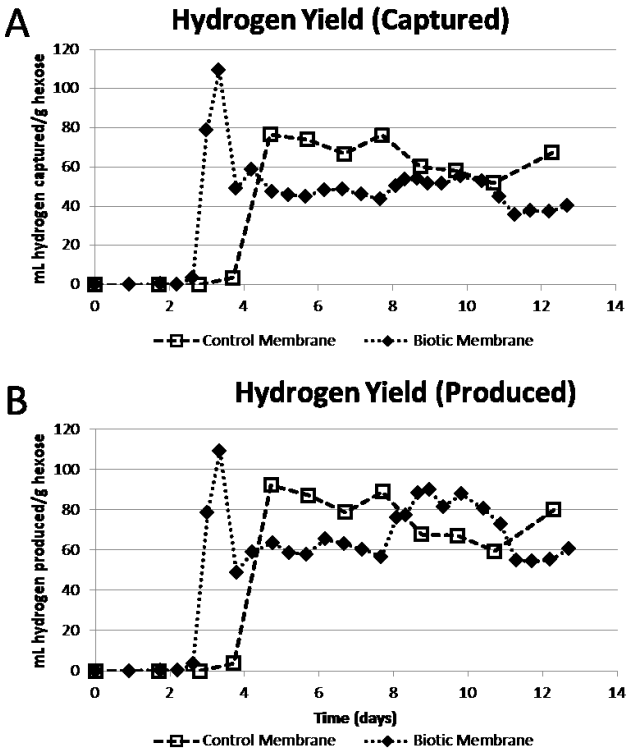

Figure 2-6. (A) Comparison of hydrogen capture yields between the biologically active and control PVA-hollow fiber composite membranes; (B) Comparison of hydrogen production yields between the same membranes.

2.3.3 Electrospun membrane

Figure 2-7 shows that the electrospun membrane was able to produce and capture hydrogen as well. The PVA membrane module outperformed the electrospun membrane, yielding approximately twice as much hydrogen (20-30 vs. 45-50 mL captured/g hexose). Differences between the two membranes include chemistry, degree of polymer attachment to the hollow fibers, and quantity of encapsulated biomass (4.7 mg for the PVA vs. 0.3 mg for the electrospun microtubules). It is thought that the lower quantity of encapsulated biomass was the primary reason for the lower hydrogen production in this membrane. The lag time of the electrospun membrane trial was significantly longer (190 hours vs. 65 hours), likely also due to the smaller amount of encapsulated biomass. COD

reduction between the influent and effluent varied between 1 and 16% removal throughout the run. As in the PVA trials, colonization of the reactor liquor by bacteria leaking from the membranes or otherwise present in the laboratory was observed.

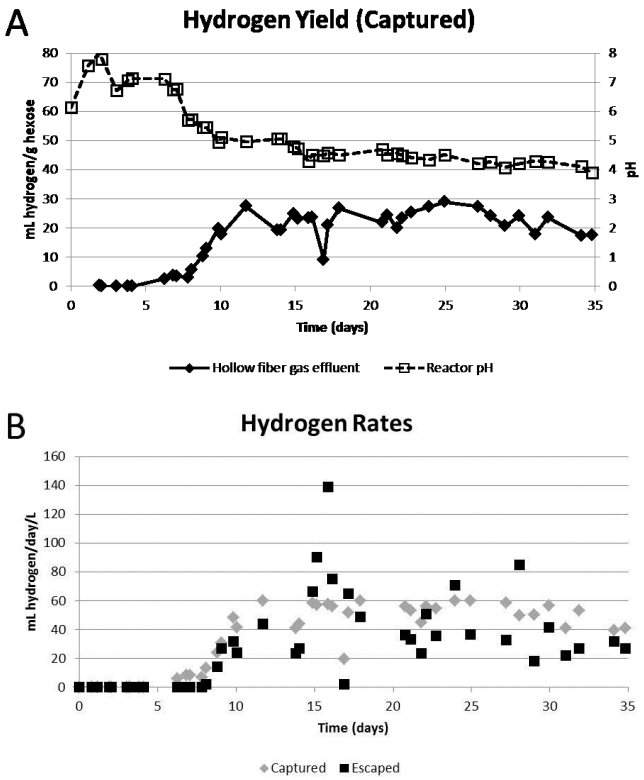

Figure 2-7. (A) Time series of hydrogen capture yield and reactor pH of a trial of the biologically active electrospun fiber-hollow fiber composite membrane; (B) Time series of hydrogen capture and escape rates of the same membrane.

2.3.6 Real feedstock

Batch experiments (Table 2-1) showed that municipal wastewater feedstocks were compatible with the mixed microbial consortia, with sterilized secondary influent giving the highest yield in batch tests, equivalent to the yield observed using synthetic septage. pH adjusted dairy wastewater yielded the highest gross production of hydrogen but because of its high COD content, and hydrogenase inhibition at high hydrogen partial pressures, the sealed bottle batch tests did not allow the dairy wastewater to reach its full

yield potential. Non-sterilized feedstocks generally resulted in lower yields, probably because of substrate competition and hydrogen consumption from native microorganisms. It should be noted, however, that because of the higher incubation temperatures and batch mode of operation, these results are not indicative of yields under ambient temperatures and continuous operation but only serve to compare feedstocks.

Table 2-1. Batch study biohydrogen yields of raw and autoclaved wastewater samples.

Yield (mL hydrogen/g COD)	**Raw**	**Autoclaved**
Primary Influent	5.02	8.06 (± 0.30)
Secondary Influent	10.56	16.42 (± 6.65)
Centrate	0.62	0.79 (± 0.02)
Dairy Manufacturing Waste (pH adjusted)	2.43	2.07 (± 0.86)
Synthetic Wastewater	N/A	18.7

CSTR studies performed using a variety of real wastewaters and biologically active PVA membranes did not show hydrogen production. In addition, a study was performed in which a reactor being fed synthetic wastewater with stabilized hydrogen production was switched to a feed of secondary influent, and hydrogen production ceased (Appendix Figure C). These results indicated that the lack of hydrogen production was not attributable to a failure of encapsulated bacterial spores to germinate. More likely, the chemistry of the feedstocks did not favor hydrogen production. The low COD concentration of the secondary influent suggests that even if hydrogen production had occurred at yields similar to those in the batch study (16.4 mL/g COD), hydrogen concentrations would be expected to be too low to be detected (5×10^{-4} mL hydrogen/mL sweep gas sampled). The pH of the reactors was much higher (6-7.5 for centrate, 7-8 for secondary influent) than the synthetic wastewater trials. Because of the low COD concentrations, volatile fatty acids would not have been produced at sufficient

concentrations to lower the pH to levels observed with synthetic wastewater. In addition, centrate wastewater by its nature contains few simple carbohydrates but is abundant in complex and recalcitrant organics, resulting in lower yields as observed in the batch study. A lesser reason for the lack of hydrogen production may have been the inability of the PVA polymer to prevent leakage of microorganisms into and out of the membrane, compromising the effectiveness of the system. The membranes prepared for the centrate trials contained 1.65 mg and 2.18 mg encapsulated biomass by dry weight. The membrane prepared for the secondary influent trial contained 2.19 mg biomass by dry weight.

2.4 Discussion

The results of the continuous reactor experiments are summed up by three main conclusions. First, the composite membrane technology was demonstrated to be successful in producing and capturing hydrogen from high-strength synthetic wastewater, with maximum captured yields of 25-50 mL/g hexose during the periods of most stable operation (Figures 2-4, 2-7). Second, membranes constructed from two different polymer chemistries were able to produce and capture hydrogen, demonstrating flexibility and potential for optimization of materials to accommodate a diversity of needs. Finally, hydrogen production and capture in the PVA negative control was comparable to that in the biologically active PVA membrane trial, but this was likely due to an experimental artifact and the advantages of encapsulation may only be apparent when non-sterile feedstocks are used and competition for substrate and hydrogen becomes a factor.

Batch trials of real wastewater showed potential for harnessing energy from several municipal feedstocks and demonstrated the ability of the hydrogen-producing bacterial culture to metabolize substrates within the wastewater. Issues preventing hydrogen production and capture in real feedstock CSTR trials may include inefficient substrate utilization of complex organic matter, competition for substrate and/or hydrogen by native microorganisms within the wastewater, inability of membrane chemistry to prevent leakage of microorganisms, or unfavorable feedstock chemistry for hydrogen-producing bacteria such as high pH, low COD/carbohydrate concentrations, and recalcitrant organic matter. In fact, system pH has been recognized as one of the most important environmental factors affecting biological hydrogen yield, with several CSTR studies reporting an optimum pH range between 5.2 and 6.0 (Oh et al., 2004a; Zhang et al., 2008; Fang and Liu 2002).

Although studies of the membrane system using real wastewater did not yield hydrogen, we have learned what factors are important for optimizing hydrogen production and capture. First, membrane chemistry is very important, playing a dual role in protecting the encapsulated hydrogen-producers as well as ensuring that the hydrogen produced is captured by adjacent hollow fibers. There are more membrane chemistries that promise better cell retention such as silica gels (Mutlu et al., 2013) that will need to be investigated. Second, the choice of feedstock is important. Hydrogen producers prefer simple carbohydrates (i.e. dairy wastewater) over complex organics (i.e. centrate) as demonstrated by the batch experiments. Additionally, low pH inhibits methanogens that

may consume hydrogen (Chen et al., 2002). pH levels in the successful trials (Figures 2-3 and 2-6) using synthetic wastewater were low (between 4 and 5) and self-regulating, whereas the real wastewater trials maintained buffered pH levels closer to neutral. Although different membrane chemistries and coatings should be able to address other issues, there may be some wastewater feeds that are too dilute to generate adequate organic acids to lower the pH to a suitable range for sustained hydrogen production. Industrial wastewaters such as dairy manufacturing waste or sugar beet wastewater have the correct characteristics, however, and will need to be investigated in the future.

Estimated yields at 22 °C in similar studies from published literature (using similar feedstocks, seed cultures, and reactor types) range from 64 mL to 116 mL hydrogen/g hexose (Table 2-2). Although our hydrogen yields were slightly lower, the technology developed in the research described here offers some critical advantages. Previous studies, while successfully demonstrating hydrogen production, have been hampered by technological limitations such as hydrogenase inhibition by high hydrogen partial pressures, necessitating removal strategies such as aggressive stirring regimes (Lay, 2000) or gas sparging (Hussy et al., 2003) to improve hydrogen production. Additionally, the need to prevent interspecies hydrogen transfer in non-sterile conditions results in process strategies such as feedstock heat treatment which lowers the net process energy balance, and operating at low pH or short retention times which inhibit methanogen growth but result in lower hydrogen yields, especially when utilizing more complex feedstocks (Das et al., 2008). Lastly, many reactor designs for hydrogen

production are difficult to scale-up from the laboratory to commercial and industrial scales.

Table 2-2. Estimated biohydrogen yields at 22 °C based on observed yields of similar studies. gs – gas sparging; SS – sewage sludge; ADS – anaerobic digested sludge

Reference	Carbohydrate substrate	Seed Type	Reactor Type	Temp (°C)	Yield (mL hydrogen/g hexose)	Estimated Yield at 22 °C
Fang et al. 2004	Glucose	SS	CSTR	36	260	108
Shin et al. 2004	Sucrose	ADS	CSTR	35	148	64
Hussy et al. 2003	Wheat starch	ADS	CSTR-gs	35	254	110
Noike et al. 2002	Noodle mfg. waste	ADS	CSTR	35	200	87
Hussy et al. 2005	Sugar beet wastewater	ADS	CSTR-gs	32	231	116

The system described here attempts to overcome these limitations, and has demonstrated the ability to produce and capture hydrogen while avoiding or reducing some of the energy investments required in other studies. By using hollow fibers, the energy required to supply sweep gas to the membrane is significantly less than sparging (Pankhania et al., 1994). By embedding hydrogen-producing bacteria within a semi-permeable membrane, a local environment favorable for hydrogen production can be created and sustained, intensifying biological reactivity and stabilizing the community composition. The encapsulated organisms are prevented from escaping, wanted substrates are allowed to diffuse through, and unwanted organisms cannot penetrate the membrane. This precludes the need for feedstock pretreatment. Finally, the modular nature of this technology simplifies the process of scaling up and implementation using existing infrastructure.

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Chapter 3: Conclusions and Recommendations

This study demonstrated that a composite hollow fiber membrane coated with a polymer containing encapsulated hydrogen-producing consortia is a feasible technology for producing and capturing hydrogen from an organic-rich feedstock. The demonstrated advantage of this technology is its modular design and operation. Other advantages not definitively established by the results of these experiments may include the protection and stabilization of hydrogen-producing consortia and efficient capture of generated hydrogen to limit interspecies hydrogen transfer in real wastewater systems.

Based on the results of these experiments, the energy contribution of this technology can be roughly estimated if implemented on an industrial scale under the non-optimized configuration described in this text. The calculations are purely for demonstration purposes, are simplified, and make the following assumptions: (1) hydrogen yields are equivalent to the maximum observed in experiments (50 mL/g hexose); (2) the COD of a high-carbohydrate industrial wastewater such as dairy manufacturing waste (55 g/L COD) is equivalent to the "hexose" concentration; (3) a dairy manufacturing facility produces 100,000 gal/day of wastewater. With these assumptions, a system implemented at an industrial scale could produce approximately 3.4 MWh/day. To put this number into perspective, according to the U.S. Department of Energy, the average American household consumes 29 kWh/day. This means the hydrogen produced from a manufacturing facility of this size could power 116 households. With these numbers as a

starting point, the objective of future research is to increase the efficiency of this technology to make full-scale implementation feasible.

The specific tasks of future research include: (1) demonstrating the technology's ability to produce and capture hydrogen from a real wastewater feedstock high in simple carbohydrates such as dairy manufacturing waste; (2) optimizing membrane polymer chemistries to improve encapsulated biomass retention and substrate diffusion; and (3) developing a deeper understanding of substrate and hydrogen diffusion rates between the liquid, membrane, and gas phases of the composite membrane system to determine process bottlenecks.

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Appendix

Hydrogen-producing anaerobic consortia

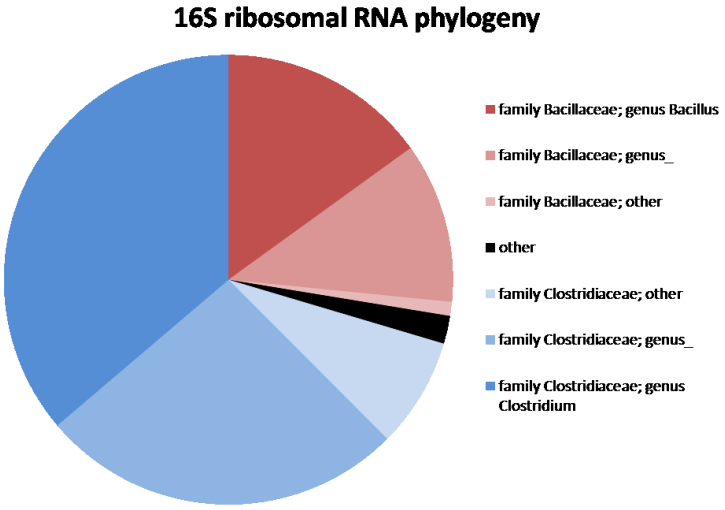

Figure A. 16S ribosomal RNA community analysis of mixed consortia obtained from heat-treating anaerobic sludge. QIIME allows analysis of high-throughput community sequencing data. "Family xxx; genus_" denotes sequences matching multiple genera within the family. "Family xxx; other" denotes sequences that are identified to family but are not matched to a specific genus.

Effect of feed pH and sweep gas flow rate

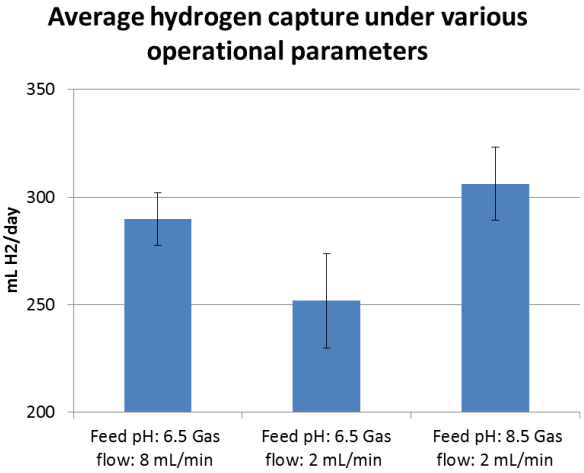

Figure B. Average hydrogen capture rates for three sets of operational conditions using data from the biotic PVA membrane experiment. Error bars represent standard deviations.

Synthetic and real feedstock CSTR experiment

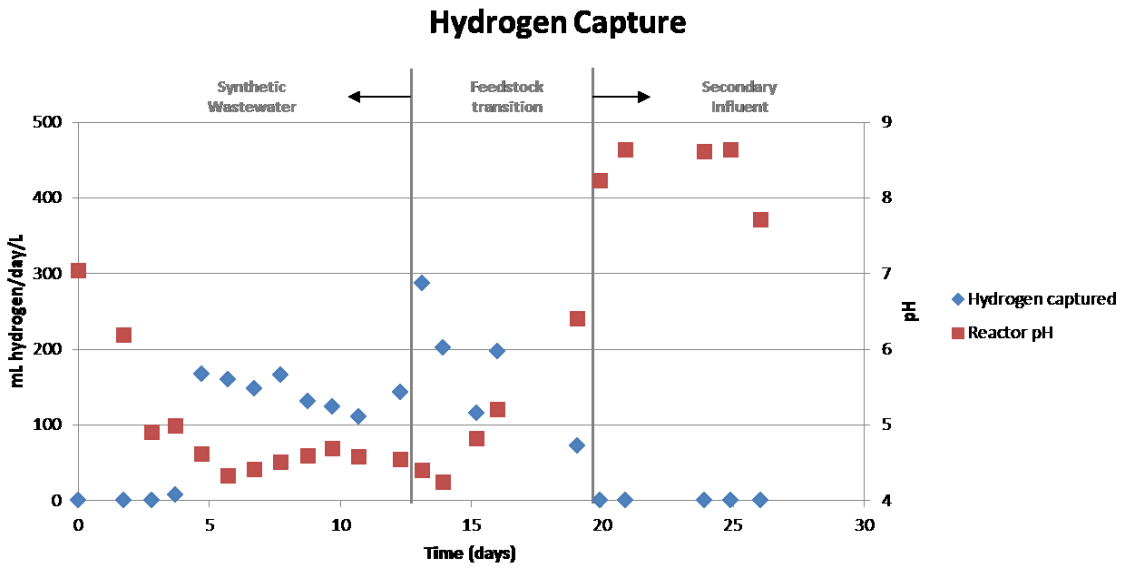

Figure C. Time series of continued negative control membrane experiment (abiotic PVA – as described in text) showing hydrogen capture rates. Experiment was begun with synthetic wastewater feedstock and then switched gradually to sterilized secondary influent (25% increments every 48 hours).

Monoculture CSTR experiments of electrospun-hollow fiber membranes

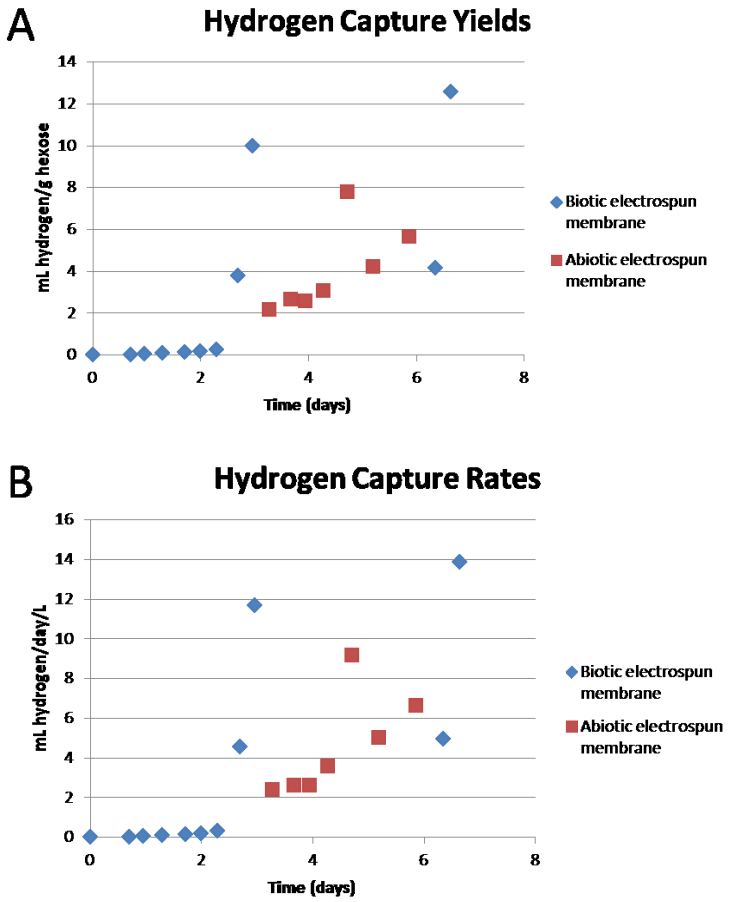

Figure D. (A) Time series of hydrogen capture yield of an experiment with a biotic electrospun fiber-hollow fiber composite membrane containing *Clostridium butyricum*, replaced with an abiotic electrospun membrane module partway through; (B) Time series of hydrogen capture rates for the same experiment.

Figure E. Photograph of reactor setup for monoculture membrane experiments.

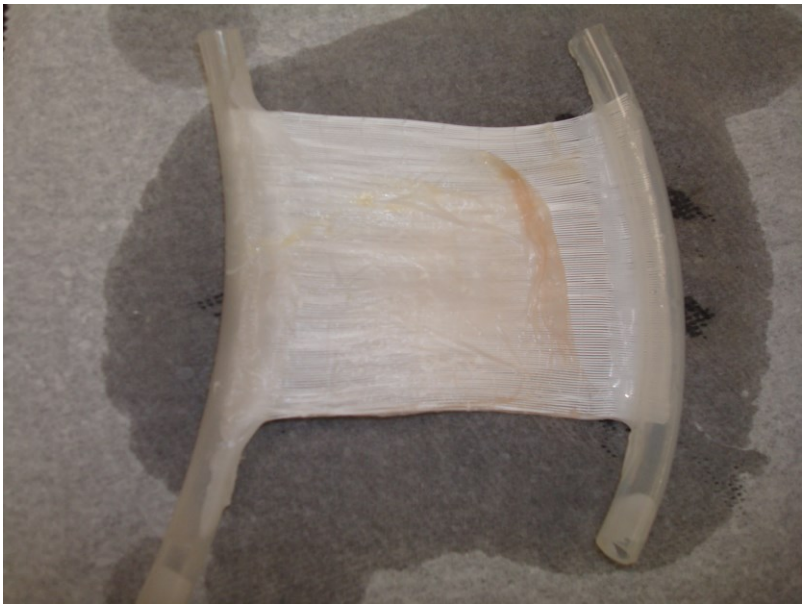

Figure F. Photograph of biotic electrospun membrane module at end of experiment. Membrane had been submerged within the reactor for five cumulative days.

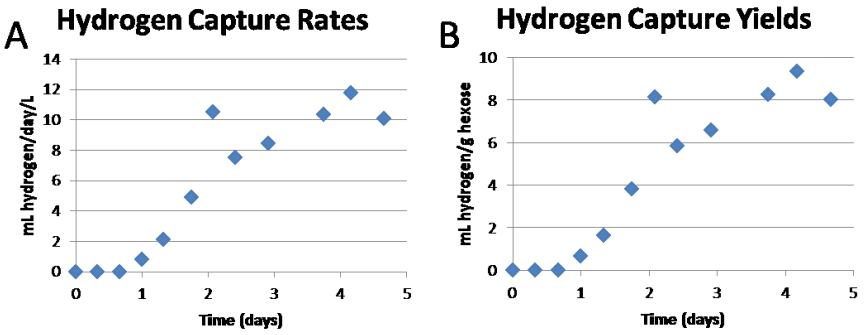

Figure G. (A) Time series of hydrogen capture rates of a trial of a bare hollow fiber membrane module; (B) Time series of hydrogen capture yields for the same experiment.