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Algae to Propellants: Biosynthesis of 1,2-Propanediol from Algal Derived Sugars

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Abstract

Propylene glycol dinitrate (PGDN) is an important component used in the formulation of energetic materials. A renewable, environmentally-friendly and effective production technique for the production of 1,2-propanediol, an important precursor to PGDN, is desired. While fermentation of 1,2-propanediol has been achieved, it suffers from major limitations such as expensive feed sugars and relatively low yields. It is hypothesized that algae sugars could serve as a feedstock for the microbial synthesis of 1,2-propanediol, thereby reducing production costs and enhancing sustainability.

Algal biomass produced from an Algal Turf Scrubber® has proven to have significant potential as a source of fermentable materials. However, to commercialize the production of 1,2-propanediol and other bulk chemicals from algae, growth rates need to be optimized. It has been suggested that the addition of carbon dioxide gas can enhance the growth rates of algae. This dissertation demonstrates a novel method of gas delivery to thin-film aqueous systems utilizing a unique hollow fiber membrane manifold that is ideal for use in ATS® systems. It was determined that hollow fiber membranes are more effective at delivering gas to liquid compared to traditional bubbling and a porous diffuser, particularly at shallow depths. A mass transfer model was developed to describe the transport of carbon dioxide gas into water in such systems.

In addition to the need for increased growth rates, further challenges lie in the recovery of fermentable materials from algal biomass. While methods of extraction have been developed for other algae components, such as algal oils and proteins, most of the techniques are not suitable for the recovery of fermentable carbohydrates. Very few techniques have focused on the extraction of carbohydrates, and those that do lack economic efficiency due to long processing times and high costs associated with enzymes and/or energy requirements. By combining

mechanical means of disruption, such as abrasive material, with sonication, it could be possible to reduce processing times, thereby lowering energy costs. Based on this hypothesis, a novel technique for the extraction of algal sugars was developed. This technique, deemed "sonic abrasion," was found to extract nearly twice as much sugar than sonication alone, and adequate carbohydrate concentrations for use in fermentation processes could be achieved.

Finally, the feasibility of utilizing algal derived sugars to ferment 1,2-propanediol from *Thermoanaerobacterium thermosaccharolyticum* was investigated using a 10 g/l synthetic algal sugar mixture. When compared to a 10 g/l glucose feed, it was found that the synthetic algal sugar mixture was capable of producing nearly twice as much 1,2-propanediol whilst producing fewer by-products.

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Finally, I would like to thank the SMART scholarship and the University of Arkansas Graduate School Doctoral Academy Fellowship for the financial support I received.

Dedication

I dedicate this dissertation to my amazing, loving husband, Walter. You have provided constant support and encouragement every step of the way. You believe in me always, even in the moments I find it hard to believe in myself. You truly make my life more fulfilling, and words cannot express how much I appreciate your patience and your gentle spirit. You are always my calm when things get crazy. Without you, I honestly don't think I could have made it through this process, not to mention this journey we call life. I am so blessed to have you by my side, and thank God every day for giving me more than I could have ever thought to ask for.

Also, what kind of crazy dog lady would I be if I didn't dedicate this to my sweet Pit Bulls? They have been with me since the beginning of graduate school, many moves and many roommates later, and I can genuinely say they have made my graduate studies more enjoyable. I could have the worst day ever in lab, leave feeling completely disheartened, and come home to those silly pups and their wagging tails, and I always knew it was going to be okay. Thank you, Aspen and Koda, for being a daily reminder to enjoy the little things in life.

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Abbreviations

ATS®: Algal Turf Scrubber®

BTTN: Butanetriol Trinitrate

DHAK: Dihydroxyacetone Kinase

DHAP: Dihydroxyacetonephosphate

dI: Deionized

DNS: 3,5-Dinitrosalicylic Acid

DO: Dissolved Oxygen

HFM: Hollow Fiber Membrane

HMP: Hexose Monophosphate

HRAP: High Rate Algal Ponds

IPA: Isopropyl Alcohol

MFA: Metabolic Flux Analysis

OD: Optical Density

PBR: Photobioreactor

1,2-PD: 1,2-Propanediol

PEEK: Polyether Ether Ketone

PGDN: Propylene Glycol Dinitrate

PYG: Peptone-Yeast-Glucose

TAL: Triacetic Acid Lactone

TATB: Triamino Trinitrobenzene

List of Papers

L. Merriman, A. Moix, R. Beitle, J. Hestekin. Carbon dioxide gas delivery to thin-film aqueous systems via hollow fiber membranes. Submitted to Journal of Membrane Science, May 2013.

L. Woods, M. Riccobono, N. Mehan, J. Hestekin, R. Beitle. Synergystic effect of abrasive and sonication for release of carbohydrate and protein from algae. Separation Science and Technology, 2011, 46:4, 601-604.

CHAPTER 1

Introduction and Objectives

1.1 Introduction

The increasing demand for a more economical and environmentally benign means of producing commodity chemicals (such as alcohols and diols) has recently renewed interest in the production of these types of products via fermentation processes. In particular, 1,2-propanediol has gained attention as a candidate for microbial synthesis due to its application as an energetic precursor, as well as a variety of other industries. The pathways to ferment this chemical from sugars are recognized. However, some major limiting factors associated with the successful commercialization of this process involve high cost of raw materials and relatively low yields.

The need for a sustainable alternative fuel source has reintroduced the idea of developing biofuels from algal matter. A lot of research has been invested in the production of biodiesel from algae oils (Chisti 2007; Pokoo-Aikins *et al*. 2009; Williams and Laurens 2010; Chen *et al.* 2012; Demirbas 2009; Griffiths and Harrison 2009; Scott *et al.* 2010). Recent studies have indicated that algae sugars can also be utilized in fermentation processes for the production of biofuels, such as ethanol and butanol (Ueda *et al.* 1996; Hirona *et al.* 1997; Bush *et al*. 2006; Potts *et al.* 2011; Ellis *et al.* 2012). However, to our knowledge, the use of algae sugars as a feedstock for the production of other bulk chemicals has been scarcely explored.

While algae have demonstrated significant potential as a source of raw materials for fermentation processes, more research is necessary to address the development of efficient

methods to optimize growth rates of algae biomass. Several recent studies have suggested that the addition of carbon dioxide gas to the algal growth medium can increase biomass production rates in algae growth applications such as photobioreactors and raceway ponds (Chinnasamy *et al.* 2009; Westerhoff *et al.* 2009; Douskova *et al.* 2009). Bubbling and enclosed hollow fiber membrane manifolds have been investigated as a means of gas delivery in these types of systems (Ferreira *et al.* 1997; Kumar *et al.* 2010; Carlvalho and Malcata 2001). Little to no research has been done in this area for immobilized algae growth applications such as the Algal Turf Scrubber® (ATS®) technology.

ATS® technology, as will be described shortly, uses indigenous algal species to remediate water of nitrogen and phosphorous, to capture carbon dioxide, and finally, to produce the building blocks for many bulk chemicals. Improvements in algae growth can be achieved by combining existing technologies such as algal turf scrubbing® and hollow fiber membranes. Algal sugars can be extracted from the organism and then used as raw materials for the biosynthesis of valuable chemicals, such as 1,2-propanediol. Fermentation of such chemicals is extremely beneficial from an environmental standpoint, as it does not deplete non-renewable resources, nor does it require hazardous chemical use as do the traditional methods of chemical synthesis. Algae, which are often considered a waste product from the perspective of wastewater treatment, as a source of raw material could also make the microbial production of commodity chemicals, such as 1,2-propanediol, more economically appealing. A schematic of the overall process is outlined in **Figure 1.1** below.

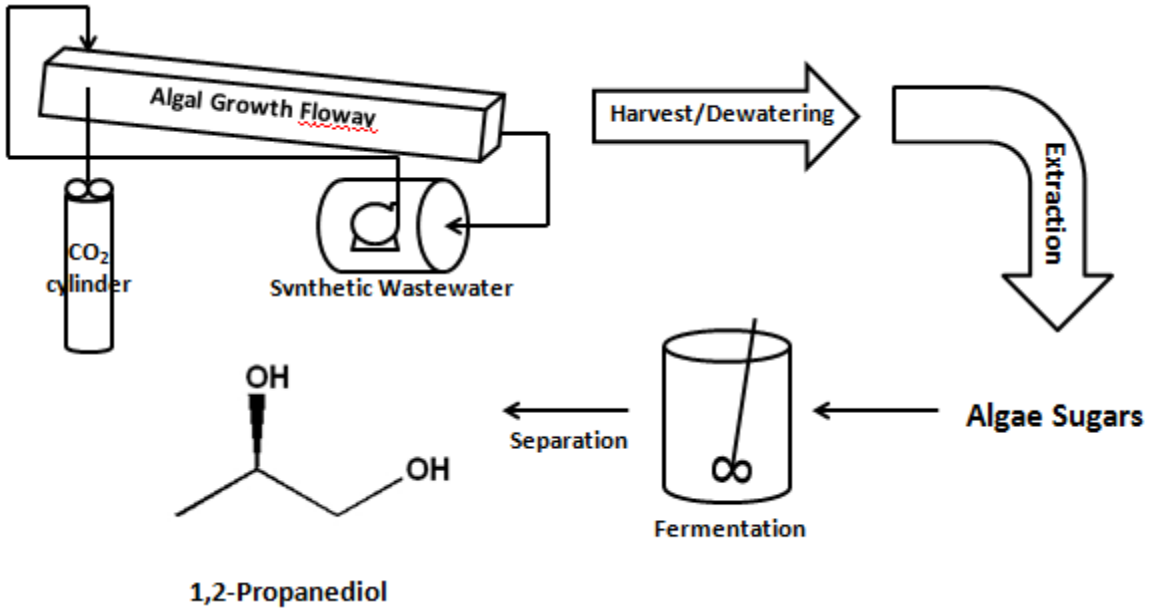

Figure 1.1: Schematic of the overall process to produce 1,2-propanediol from algal sugars harvested from an ATS® system.

1.2 Project Objectives

The three primary objectives of this project were:

1. Investigate the utility of hollow fiber membranes as a means of carbon dioxide gas delivery in a large scale algal raceway system and develop a mass transfer model for CO_2 delivery into thin-film aqueous systems.
2. Develop of novel method for the extraction of algal sugars by combining sonication and abrasives.
3. Examine the bacterial production of 1,2-propanediol using 5- and 6-carbon sugars representative of algal derived sugars.

1.3 References

Bush, R. and K. Hall (2006). Process for the production of ethanol from algae. US Patent No. 7,135,308 B1. November 14, 2006.

Carvalho, A. and F. Malcata (2001). "Transfer of carbon dioxide within cultures of microalgae: plain bubbling versus hollow-fiber modules." *Biotechnology Progress.* 17: 265-272.

Chen, L., T. Liu, W. Zhang, X. Chen and J. Wang (2012). Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. *Bioresource Technology.* 111: 208-214.

Chinnasamy, S., B. Ramakrishnan, A. Bhatnagar and K. Das (2009). "Biomass production potential of wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO_2 and temperature." *Int. J. Mol. Sci*. 10: 518-532.

Chisti, Y (2007). Biodisel from microalgae. *Biotechnology Advances.* 1-13.

Demirbas, A. (2009). Production of biodiesel from algae oils. *Energy Sources, Part A.* 31: 163-168.

Douskova, I., J. Doucha, K. Livansky, J. Machat, P. Novak, D. Umysova, V. Zachleder, V. and M. Vitova (2009). "Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs." *Appplied Microbiology and Biotechnology.* 82: 179-185.

Ellis, J., N. Hengge, R. Sims and C. Miller (2012). Acetone, butanol, and ethanol production from wastewater algae. *Bioresource Technology.* 111: 491-495.

Ferreira, B., H. Fernandes, A. Reis and M. Mateus (1998). "Microporous hollow fibres for carbon dioxide absorption: mass transfer model fitting and the supplying of carbon dioxide to microalgal cultures." *J. Chem. Technol. Biotechnol.* 71: 61-70.

Griffiths. M. and S. Harrison (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol.* 21: 493-507.

Hirano, A., R. Ueda, S. Hirayama and Y. Ogushi (1997). CO_2 fixation and ethanol production with microalgae photosynthesis and intracellular anaerobic fermentation. *Energy.* 22:2-3, 137-142.

Kumar, A., X. Yuan, A. Sahu, J. Dewulf, S. Ergasand H. Langenhove (2010). "A hollow fiber membrane photo-bioreactor for CO_2 sequestration from combustion gas coupled with wastewater treatment: a process engineering approach." *J. Chem. Technol. Biotechnol.* 85: 387-394.

Pokoo-Aikins, G., A. Nadim, M. El-Halwagi and V. Mahalec (2010). "Design and analysis of biodiesel production from algae grown through carbon sequestration." *Clean Technology Environmental Policy.* 12: 239-254.

Potts, T., J. Du, M. Paul, P. May, R. Beitle, J. Hestekin (2011). The production of butanol from Jamaica bay macro algae. *Environmental Progress & Sustainable Energy.* 31(1), 29-36.

Scott, S., M. Davey, J. Dennis, I. Horst, C. Howe, D. Lea-Smith and A. Smith (2010). Biodiesel from algae: challenges and prospects. *Current Opinion in Biotechology.* 21: 277-286.

Ueda, R., S. Hirayama, K. Sugata and H. Nakayama (1996). Process for the production of ethanol from microalgae. US Patent No. 5,578,472. November 26, 1996.

Westerhoff, P., Q. Hu, M. Esparza-Soto, W. Vermaas (2010). "Growth parameters of microalgae tolerant to high levels of carbon dioxide in batch and continuous-flow photobioreactors." *Environmental Technogy.* 31(5): 523-532.

Williams, P. and L. Laurens (2010). Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetic & economics. *Energy Environ. Sci.* 3: 554-590.

CHAPTER 2

Literature Review and Background Studies

2.1 ATS® technology as a source of biomass

Algal Turf Scrubbing® (ATS®) is a patented technology that was originally developed by Walter Adey in the early 1980s (Adey 1982) and is gaining more and more interest as a form of wastewater treatment and source of algal biomass (Adey *et al.* 2013; Mulbry *et al.* 2008; Mulbry and Wilkie 2001; Mulbry *et al.* 2005; Kebede-Westhead *et al.* 2006; Pizarro *et al*. 2002; Pizzaro *et al.* 2006). It is an attached algae growth system that consists of a slightly inclined (typically 1-2 degrees) floway or trough in which water flows down, and the walls of the trough should be elevated so that water flows evenly across the width of the floway. The flat surface of the trough contains a high surface area polymeric or carpet-like matrix upon which the microalgae spores can attach and the algae grows to form an algal "turf." A light source, either natural or artificial, is provided. Water is introduced to the floway in a periodic surging fashion to enable the algae to attach and to increase the turbulence within the system. This enhances the mixing of the nutrients in the water, which in turn promotes algae growth. At the end of the floway, clean water "scrubbed" of pollutants is returned to its source. Algal biomass is periodically harvested to ensure maximum growth (Adey 1982; Adey 1998). While ATS® systems are not the only method available for algae production, there are some specific parameters that set it apart from other technologies, such as photobioreactors and open ponds.

Photobioreactors (PBRs) are closed systems used to grow algae. These systems typically consist of large tubular reactors constructed of either glass or plastic, where carbon dioxide is introduced to water and nutrients in the presence of light. These reactors can be vertical,

horizontal or conical, and cultures are recirculated by a pump or airlift system (Ugwu *et al.* 2008; Chisti 2007; Lehr and Posten 2009). The major benefit of PBRs is the ability to control the cultivation conditions of the system (Ugwu *et al.* 2008; Wu and Merchuk 2002). Mixing can be optimized and contamination can be easily prevented, making it possible to grow specific strains of algae (Ugwu *et al.* 2008). However, the major drawback to this type of technology is the high cost associated with the production of the algae. Photobioreactors are often placed indoors, requiring artificial light to be supplied. There are dark regions within photobioreactors that do not directly receive light, which makes mixing necessary to ensure adequate light is supplied (Chisti 2007; Wu and Merchuk 2002). However, some species of algae are very sensitive to shear stress, and this mixing can damage the algal cells (Chisti 2007). Furthermore, photobioreactors require a continuous supply of soluble inorganic carbon whereas open systems can achieve some CO_2 directly from the atmosphere. It is also necessary to remove the oxygen produced by the algae from the growth medium, as high levels of dissolved oxygen decrease photosynthetic activity, thereby inhibiting the production of biomass (Becker 1994; Chisti 2007). The transparent materials are also an added expense and make scale-up difficult (Chisti 2007; Ugwu *et al.* 2007). While these closed systems can be very beneficial for growing algae, they are not necessarily the most economically feasible. Because algal turf scrubbers use solar energy as a light source and also serve as a wastewater treatment method, the cost associated with algae growth for the production of fermentable materials is quite low in comparison to photobiorectors (Adey *et al.* 2011).

Open ponds are another form of commonly used suspended algae growth technology. Some open ponds, such as high-rate algal ponds (HRAPs), are somewhat similar to ATS®

systems in that they are typically set up in a raceway formation and mixing is enhanced by wave pumps, baffles or paddle wheels (Chisti 2007; Moheimani and Borowitzka 2006; Hoffman 1998), and they often use wastewater as a nutrient source (De Godos *et al.* 2010; Hoffman 1998). Other open ponds are just circular ponds or aerated lagoons that do not necessarily integrate mixing capabilities. These systems are well studied and a lot of research has been done to optimize their design (Hoffman 1998; Oswald 1988). While raceways typically cost less to build and maintain, they suffer from evaporative loss as well as low productivities when compared with photobioreactors (Chisti 2007). However, one of the primary concerns that separates these suspended algae growth systems from the ATS® is the ease of harvesting. In an attached algal growth apparatus, harvest can be accomplished by a simple mechanical means such as vacuuming, whereas in an open pond harvesting is much more difficult and more energy intensive (Hoffman 1998).

Algae produced via an Algal Turf Scrubber® system removes carbon dioxide from the atmosphere and remediates polluted water of nitrogen and phosphorous while producing the building blocks for the production of commodity chemicals (e.g., biodiesel from oils, or diols and alcohols from the cellulosic component of the algae) (Adey *et al.* 2011; Craggs *et al.* 1996). Previous research has indicated that ATS® systems are capable of producing up to 45 grams algal dry weight per square meter per day, and carbohydrates account for approximately 25% of the dry weight of algae harvested from an ATS® (Adey *et al.* 2011). Based on these comments, algae produced via an ATS® displays excellent potential as a candidate for large-scale fermentation processes. ATS® technology could therefore result in a significant decrease in the consumption of petrochemicals. As the demand for commodity chemical products increases, the

development of renewable resources is an important component of sustainable consumption, which will be beneficial to future generations. Furthermore, the use of algae as a feedstock also benefits society because it is not competing with food sources such as soybean and corn; in addition, algae can be cultivated on marginal land that would otherwise not be suitable for crop production (Dismukes *et al.* 2008; Moheimani and Borowitzka 2006).

2.2 Delivery of carbon dioxide to optimize algae productivity

The use of algae as a form of carbon capture is well recognized. Algae take in carbon dioxide from the atmosphere and use it during photosynthesis to produce algal biomass, which can then be used as a raw material for fermentation processes. Approximately 400 ppm carbon dioxide is currently present in the atmosphere (Dlugokencky and Tans 2013). Previous research has indicated that some species of algae can tolerate as much as 20% CO_2 (v/v) (Westerhoff *et al.* 2010). It has been demonstrated that the addition of CO_2 to the algal growth medium can enhance biomass growth rates (Westerhoff *et al.* 2010; Chinnasamy *et al.* 2009). A particular study by Chinnasamy et *al.* (2009) revealed that the growth rates of a specific strain of *Chlorella vulgaris* was enhanced by the addition of up to 12% (v/v) CO_2, and declined sharply from 12-20% with the peak productivity at 6% CO_2. It is important to note, however, that high concentrations of carbon dioxide can be toxic to algae because it drastically reduces the pH of the growth medium over time. Since most microalgae grow best around a pH of 7-9, it is necessary to maintain the pH of the system for optimum results. This can be done by the simple addition of a low molarity sodium hydroxide or potassium hydroxide solution to the growth medium.

One of the major issues limiting the use of carbon dioxide for this application is the high cost of pure CO_2. For this reason, flue gas from power plants, which typically contains between 10-20% CO_2, has sparked interest as a source of carbon dioxide gas. The effects of flue gas on algal growth have been under investigation, and productivity of algal growth has been realized (Westerhoff *et al.* 2010; Matsumoto *et al*. 1995; Brune *et al*. 2009; Doucha *et al*. 2005; Pokoo-Aikins *et al*. 2010; Douskova *et al*. 2009; Matsumoto *et al*. 1996). Douskova et *al*. (2009) stated that algal growth rates for *Chlorella vulgaris* were actually higher when flue gas containing 10-13% CO_2 (v/v) was used, compared to a control mixture of air and 11% CO_2 (v/v). This was attributed to the fact that there was less dissolved oxygen (DO) present in the flue gas mixture. Another study by Matsumoto et *al*. (1996) suggested that other components, such as NO gas, present in flue gas mixtures can actually serve as valuable nutrients to the microaglae. Flue gas has great potential as a carbon dioxide source for the growth of microalgae because it would be the most economically and environmentally viable option, as it would help to reduce greenhouse gas emissions from power plants.

Another important consideration for the addition of carbon dioxide gas to improve growth rates of algae is the method by which gas is delivered. The most common method of CO_2 delivery used in algae growth systems is the direct injection of gas into the growth medium (Douskova *et al*. 2009; De Godos *et al*. 2010), which is usually achieved by bubbling gas through an open tube. Another technique commonly used is gas bubbling via a porous diffuser (Carvalho and Malcata 2001; Kumar *et al*. 2010). While these methods offer a simplistic way to supply CO_2 to growth systems, they suffer in terms of mass transfer. As a result, there is risk that a lot of CO_2 may be lost to the atmosphere. In an effort to develop a delivery method that is more

effective in terms of mass transfer, hollow fiber membranes have also been investigated for use in algae growth applications (Carvalho and Malcata 2001; Ferreira *et al*. 1998; Kumar *et al*. 2010). Hollow fiber membranes can supply tiny microbubbles over an increased surface area, which would allow for superior mass transfer efficiencies to be achieved, with minimal amounts of CO_2 released back into the atmosphere. Additionally, the same productivity can be achieved whilst utilizing lower gas pressures, in comparison to conventional systems, thereby reducing production costs.

Ferreira *et al.* (1998) derived a mathematical model for the mass transfer of carbon dioxide gas into algal growth medium via a hollow fiber membrane system using simple equations. Carbon dioxide was supplied via a traditional HFM module where the fibers were encased in a cylindrical tube and potted at both ends. Gas entered one end of the tube, and liquid was circulated over the shell side of the fibers from a 500 mL reservoir, which was closed to the atmosphere. As carbon dioxide was added, pH measurements were taken and the carbonic acid dissociation equilibrium relationships were used to determine the concentration of carbon in the system. To determine the mass transfer coefficient, a mass balance of the inorganic carbon was utilized. Initially, they used a 0.1 M sodium hydroxide solution to calculate the mass transfer to the system, before performing experiments with algal culture. To determine the effects of CO_2 to algal growth, bubbling and hydrophobic hollow fiber membranes were used to deliver a 1% (v/v) carbon dioxide mixture to 1 dm^3 *Chlorella vulgaris* culture at 30°C. Dissolved oxygen and pH were measured. Although algae growth was comparable for both methods of gas delivery, the concentrations of dissolved oxygen in the system were lower when the hollow fiber membranes were used (Ferreira *et al*. 1998).

Another study was conducted by Carvalho and Malcata (2001), who investigated plain bubbling, hydrophobic HFMs and hydrophilic HFMs as a means of carbon dioxide gas delivery to microalgae cultures. Two 1 L closed glass vessels were used. The authors implemented the methods of Ferreira et *al*. in their mass transfer measurements, and had similar findings. They determined that the highest carbon concentrations in the media were achieved when the hydrophobic membranes were used, followed by bubbling, then hydrophilic membranes. Furthermore, slight improvements in biomass productivity were observed when the fiber modules were utilized.

Kumar et *al*. (2010) investigated the effects of gas delivery via hollow fibers for various inlet, outlet and liquid concentrations of carbon dioxide. They, too, derived a set of mass transfer equations, and measured the effects of the gas delivery through the HFMs in a photobioreactor, using synthetic wastewater as a growth medium. Different liquid velocities were also considered. Gas chromatography was used to measure liquid CO_2 concentrations. They observed increases in both CO_2 fixation rates and algal biomass productivity when the hollow fiber membranes were used compared to conventional PBRs.

In each of these studies, higher liquid phase velocities resulted in increases in mass transfer efficiencies (Carvalho and Malcata 2001; Ferreira *et al*. 1998; Kumar *et al*. 2010). This is to be expected because the overall mass transfer coefficient is highly dependent upon the resistance of the liquid phase mass transfer. When liquid velocities are increased, mixing is enhanced, thereby reducing the thickness of the boundary layer on the membrane surface and

diminishing the mass transfer resistance in the liquid phase (Ferreira *et al*. 1998; Kumar *et al*. 2010).

The mass transfer coefficient, k_L, can be calculated either experimentally or from the Sherwood number, *Sh*. When both convective and diffusive mass transfer are taking place, the Sherwood number is commonly used in mass transfer calculations and can be calculated from a correlation in the following form:

$$Sh = \frac{k_L d}{D} = \alpha Re^{\beta} Sc^{1/3}$$

where *d* is the characteristic length and *D* is the diffusion coefficient for carbon dioxide gas in the liquid media. The Sherwood number is a function of both the Reynolds number, *Re*, and the Schmidt number, *Sc*, which can be calculated from the velocity and kinematic viscosity of the liquid. Previously developed correlations for calculating *Sh* are available in literature for different flow conditions and various geometries, and the coefficient, α, and exponent, β, can either be used from published correlations or determined experimentally. Ferreira et *al*. (1998) used these equations as well, and found that their assumption of the overall mass transfer coefficient being dominated by the mass transfer coefficient in the liquid phase to be valid. The authors provide a table of mass transfer correlations using *Sh*, *Re* and *Sc* for hollow fiber modules from literature. With such variation in the correlations, it was suggested that k_L is dependent upon the diameter of the fibers, number of fibers used and the separation of the fibers within the hollow fiber module (Ferreira *et al*. 1998).

Some of the major drawbacks associated with the use of hollow fibers in algal growth applications include the potential damage to the fibers during harvest, pressure drop through the membrane (Kumar *et al*. 2010), and membrane fouling (Ferreira *et al.* 1998; Kumar *et al*. 2010). After culture experiments were conducted, Ferreira et *al*. (1998) performed further analysis of the mass transfer for the module, again using the NaOH solution, and a 15% decrease in the overall mass transfer coefficient was realized. They attributed this to fouling, as well as the fact that hydrophobic character of the membranes can diminish with use. These considerations are important when choosing a membrane material and determining an operating time for the algal growth system before replacing the fibers.

All of the aforementioned studies that utilize hollow fiber membranes as a delivery method for carbon dioxide gas to suspended algal culture using either closed flasks or photobioreactors. Although closed systems allow for more environmental control, the large-scale production of bulk chemicals from algal feedstocks is not feasible from an economic standpoint. Coupling algae growth with wastewater treatment could have a profound impact for the commercialization of this process. Very few studies have investigated the delivery of CO_2 to open and/or attached-growth systems. To our knowledge, there are no studies that utilize hollow fiber membranes for gas delivery in thin-film, attached algal growth systems, such as ATS® technology.

2.3 Recovery of algal sugars as a source of raw material for fermentation

One of the necessary steps in the overall process of algae to 1,2-propanediol involves the extraction of fermentable sugars from the algal biomass. Traditional methods used in the disruption of algal cells focus primarily on the extraction of fatty acids and methyl esters, which

can then be converted to biodiesel. These techniques are not necessarily suitable for recovering fermentable material from algae because they involve the use of harsh, organic solvents, such as hexane or methanol (Wang and Wang 2012; Aresta *et al*. 2005; Hossain *et al*. 2008; Nagle *et al*. 1990; Nys *et al.* 1998). In addition to algal oils, the recovery of many other high-value components of algae has received notable attention (Zachleder 1984; Sastra and Rao 1984; Herrero *et al.* 2006). Several studies have focused on the recovery of materials for their antioxidant, antiviral and antimicrobial capabilities (Sastra and Rao 1984; Herrero *et al.* 2006; Caccamese *et al.* 1981; Witvrouw and Clercq 1997). Very little research has been performed to tap in the potential of carbohydrate fractions present in algae. Those that have typically involve multi-step processes that are both time and energy intensive (Zhang *et al.* 2010; Yaich *et al.* 2013; El-Rafie *et al.* 2013; Rodriguez-Jasso *et al.* 2011; Shekharam *et al.* 1987). Enzymatic digestion has also been considered (Dumay *et al.* 2013), but is rather impractical for bulk chemical production from algae due to the costs associated with the enzymes. In order for the process of using algae as a feedstock for the biosynthesis of bulk chemicals to be economical, efficient methods of recovering fermentable material from algae must be developed (Woods *et al.* 2010).

One such method is acid hydrolysis. Potts *et al*. (2011) from the University of Arkansas Chemical Engineering department conducted a study to determine optimum conditions for acid hydrolysis. Based on observations from early hydrolysis studies, they found that drying the algae first was helpful in extracting the maximum amount of fermentable material. Samples of the macroalgae *Ulva lactuca* were air dried and ground before being subjected to acid hydrolysis. Acid hydrolysis was done by using 100 g of ground algae in 1 liter of dilute sulfuric acid and

autoclaved at 125^{o}C. They performed a set of experiments varying the acid concentration as well as the autoclave time, and found that using 2% sulfuric acid for 30 minutes was adequate to extract sugars for fermentation. Following hydrolysis, sodium hydroxide was added to the hydrolysate to adjust the pH and solids were removed by centrifugation and filtration. The resultant media was then sterilized for 30 minutes at 125 ^{o}C to prepare the samples for fermentation studies.

Another study by by Zhang *et al*. (2010) utilized a multi-step process for the extraction of polysaccharides from algae. The algae were dried and cut before they were autoclaved in water for 3-4 hours over a temperature range of 115°-125°C. Specific conditions were chosen based on each of the five species of algae used. Following steam sterilization, the samples were filtered and the resultant solution was dialyzed over a 48 hour period before it was concentrated under pressure. A 75% ethanol solution was used to wash the samples before they were lyophilized to produce the polysaccharides. Other studies have been performed using similar techniques involving hydrolysis followed by solvent wash (Yaich *et al.* 2013; El-Rafie *et al.* 2013).

An alternative method is sonication, a cell disruption technique that employs high-frequency sound waves (Agrawal *et al.* 2003; Kapuchu *et al.* 2000; Lorinez *et al.* 2004). However, due to high energy costs associated with power requirements, this technique is not typically considered economically feasible for large scale production of bulk chemicals from algae. Combining sonication with other techniques, such as solvent extraction and enzyme degradation, as a means to improve algal lipid extraction yield have been investigated (Wang and Wang 2012; Neto *et al*. 2013). However, as previously mentioned, these studies have focused on

the recovery of lipids from algae, rather than carbohydrates, and involve harsh solvents and/or expensive enzymes. By combining mechanical means of disruption, such as abrasive material, with sonication, it could be possible to reduce processing times, thereby lowering energy costs. Based on this hypothesis, the development of a novel extraction technique for the recovery of algal sugars was sought (Woods *et al.* 2010).

2.4 Fermentation of 1,2-propanediol

2.4.1 Biosynthesis of energetic precursors

New methods of microbial synthesis in the production of feedstocks for energetic materials are of particular interest to the Department of Defense. The precursors of many high-value compounds, including binders, oxidizers, plasticizers, and primary explosives, are typically made by a single manufacturer that is based outside of the United States. Because of this, the availability of these chemicals is never more than a safety incident or business decision away from putting our national security at risk. Therefore, the Navy is seeking alternative means of independently producing these materials safely and economically. Implementing fermentation processes for the production of these precursors could lead to increased availability of energetic components, improved sustainability by reducing the need for organics from non-petroleum-derived feedstocks, and the significant reduction of toxic waste.

Microbial processes have been employed to synthesize a variety of products, and these processes are generally considered to be environmentally benign. By mimicking or adapting specific chemical reactions that take place within biological organisms, biochemical engineers can discover new avenues to high-value compounds that have previously been impractical (Grogan 2005). Bacterial production optimized by strain improvement via metabolic engineering

offers significant advantages over traditional synthetic methods. The pathway specificity within microorganisms presents biochemical engineers with the opportunity to generate products that would normally require several steps for reaction and purification. This creates the potential for improved yield while reducing labor requirements. In addition to the potential for simplified synthesis and purification, bioengineered microorganisms are far more environmentally-friendly means of production compared to traditional chemical synthesis. Biological organisms typically function best at ambient temperatures and pressures, and are generated from renewable resources. Fermentation processes require aqueous environments which create the potential for reductions in hazardous waste. Each of these factors enhances the safety and sustainability of feedstock production.

Although the ability of living organisms to produce useful feedstocks cleanly and efficiently has been known for quite some time, the tools and knowledge necessary to customize these metabolic pathways are still being expanded upon. By utilizing and manipulating previously known yet unexploited biosynthetic pathways, more efficient means of producing feedstock materials, such as those leading to nitramines, high nitrogen content explosives, or other undiscovered energetics could be realized.

The Frost research group at Michigan State University has explored the microbial synthesis of a few energetic material precursors. One such investigation was the synthesis of butanetriol by Niu *et al*. (2003). Butanetriol is produced commercially from the reduction of esterified malic acid by $NaBH_4$, which is then is nitrated to butanetriol trinitrate (BTTN) for use in modern propellants as an energetic plasticizer (Schofield *et al.* 2002). In an effort to produce

butanetriol without generating large quantities of borate waste products, they employed enzymes from three different microbes *E. coli* constructs were assembled with dehyrdrogenases and dehydratases from *Pseudomonas fragi* and benzoylformate decarboxylase from *Pseudomonas putida*. Other dehydrogenase and dehydrtase enzymes necessary for the biosynthesis of butantriol were native to *E. coli*. They achieved a 25% and 35% yield of D-1,2,4-butanetriol and L-1,2,4-butanetriol, respectively. While further strain improvements are required to increase product yields, they established an alternative to large-scale production of butanetriol through complete microbial synthesis (Niu *et al.* 2003).

In another study, the Frost group proposed a chemoenzymatic process to synthesize phloroglucinol from D-glucose (Zha *et al.* 2003). They engineered biosynthesis pathway for the production of triacetic acid lactone (TAL) utilizing a fatty acid synthase from *Brevibacterium ammoniagenes*. TAL can then be converted, via a chemical catalyst, to phloroglucinol, which is a key component of the energetic compound 1,3,5-triamino-2,4,6-trinitrobenzene (TATB). Frost's group was further successful in producing practical quantities of phloroglucinol from a microbial process that involved the insertion of plasmids containing PhlD, a polyketide synthase, into *Pseudomonas fluorescens* and *E. coli* (Achkar *et al.* 2004). These studies have triggered even more interest in microbial synthesis of other energetic precursors.

2.4.2 Chemical synthesis of 1,2-propanediol

While there are multiple target products that are of interest to the Navy, the most easily-synthesized proposed target is 1,2-propanediol, a precursor to propylene glycol dinitrate (PGDN). PGDN is produced from the continuous nitration of 1,2-propanediol, and is the

primary component of Otto fuel, a propellant used in torpedoes. 1,2-Propanediol (1,2-PD or propylene glycol) is a major commodity chemical in the United States, with over 1 billion pounds produced each year. It is used in a wide variety of other applications including, but not limited to, unsaturated polyester resins, detergents, pharmaceuticals, cosmetics, nutrition products, antifreeze and deicing agents (Cameron *et al*. 1998; Bennett *et al.* 2001; Berrios-Rivera *et al.* 2003; Saxena *et al*. 2010). Traditional chemical synthesis of this diol involves the hydration of propylene oxide, which is derived from propylene, a nonrenewable petrochemical (Altaras and Cameron 1999; Altaras *et al.* 2001). This can be accomplished by two different methods: a chlorohydrin process or hydroperoxide process. In the chlorohydrin process, propylene and chlorine gas react to form propylene chlorohydrin, which is then combined with caustic soda to generate propylene oxide. The major drawback to this route is that caustic soda and chlorine are required in stoichiometric ratios and the chemicals are not reused, resulting in high costs associated with hazardous waste (Cameron *et al*. 1998; Bennett *et al.* 2001; Saxena *et al.* 2010). Two hydroperoxide processes are available. In the first one, isobutene is oxidized to produce *t*-butyl hydroperoxide. This is followed by a catalytic reaction between *t*-butyl hydroperoxide and propylene to yield and 1:1 mole ratio of propylene oxide to *t*-butyl alcohol. Isobutene is replaced by ethylbenzene in the second hydroperoxide process, and phenylethanol is produced as a coproduct. The major limitation of the hydroperoxide process is that the market for the coproducts directly influences the economic analysis of propylene oxide production. While phenylethanol can be used to produce styrene, *t*-butyl alcohol is of very little value (Cameron *et al*. 1998; Bennett *et al.* 2001). Each of these methods yields a racemic mixture of the R and S forms of 1,2-propandiol (Cameron *et al*. 1998; Altaras and Cameron 1999; Altaras *et al.* 2001), which are shown in **Figure 2.1**. Because these chemical methods require a

nonrenewable resource and generate toxic waste (Saxena *et al*. 2010), alternative, environmentally benign routes are desired.

Another recently described route to 1,2-propanediol relied on treating glycerol (a readily available metabolic product) with copper chromite catalyst at moderate temperature and pressure (Dasari *et al.* 2005). While encouraging, this technique requires the use of a catalyst whose supply is listed as potentially-threatened according to The Technical Cooperation Program Critical Materials Technical Panel.

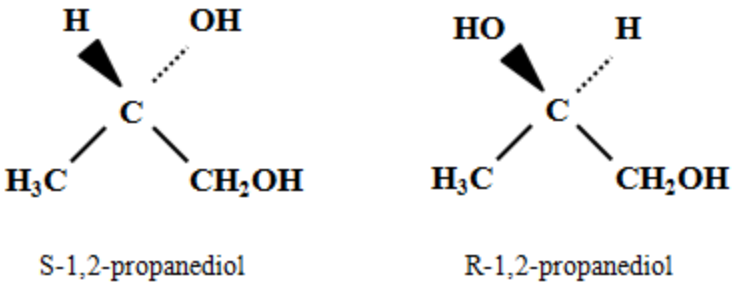

Figure 2.1: Chemical structure of 1,2-propanediol.

2.4.3 Microbial pathways to 1,2-propanediol

The laborious process currently involved in generating propanediol and its dependence on non-renewable fossil fuels suggests that the development of biosynthetic pathways would be a wise investment toward mitigating possible supply issues. Cameron *et al*. (1998) discuss three approaches for producing 1,2-PD from renewable feedstocks: 1) The catalytic hydrogenation of sugars, 2) the fermentation of sugars to lactic acid and subsequent catalytic hydrogenation of lactic acid esters, and 3) the direct fermentation of sugars. They report that, while each of these have various advantages and disadvantages associated with them, direct fermentation shows the most potential due to advances in metabolic engineering, as well as its overall simplicity. Two general pathways are recognized for the direct fermentation of sugars to form 1,2-propanediol, and are shown in **Figures 2.2** and **2.3**.

Direct fermentation of 6-deoxyhexose sugars (i.e., rhamnose and fucose) can be accomplished by several native organisms, such as *E. coli*, *Bacteroides ruminicola*, *Bacillus macerans*, *Salmonella typhimurium* and various yeasts (Saxena *et al.* 2010). L-rhamnose, the less expensive of the two sugars, sells for over $300 per kilogram, making this route infeasible commercially (Cameron *et al*. 1998; Saxena *et al*. 2010; Altaras and Cameron 1999). The pathway of these two sugars to produce 1,2-PD is shown in **Figure 2.2.**

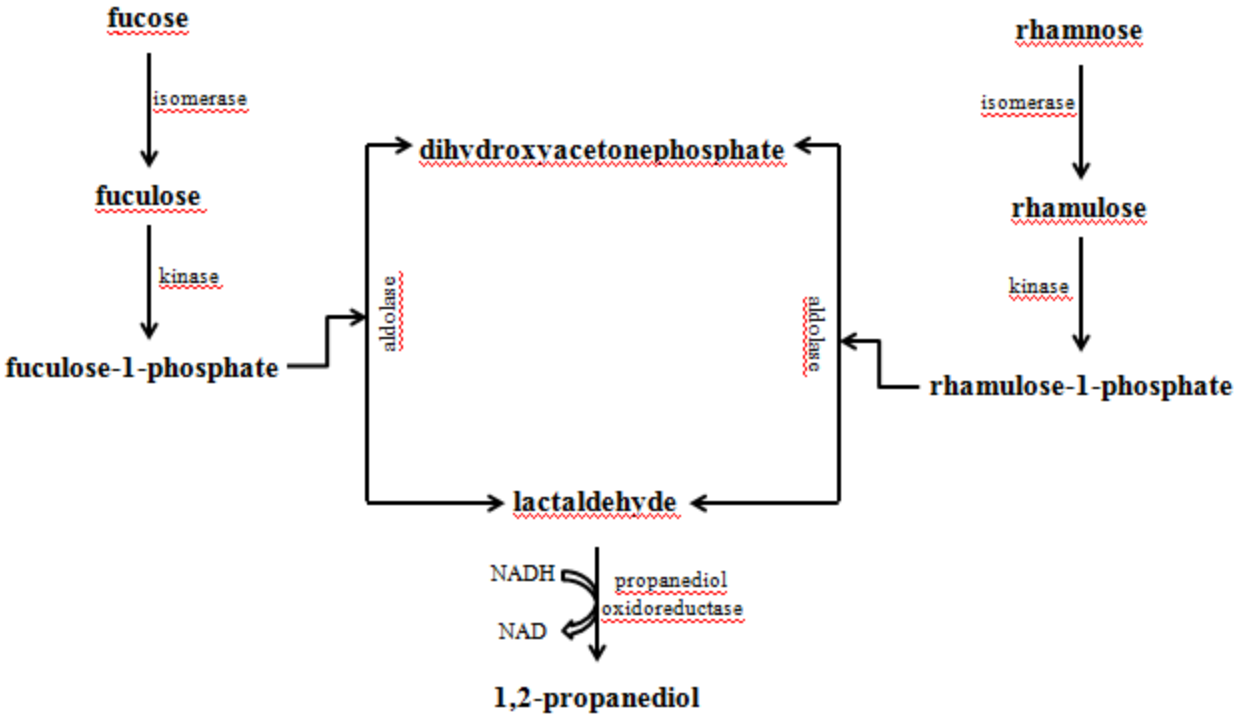

Figure 2.2: Schematic representation of metabolic pathways for the production of 1,2-propanediol from deoxy sugars.

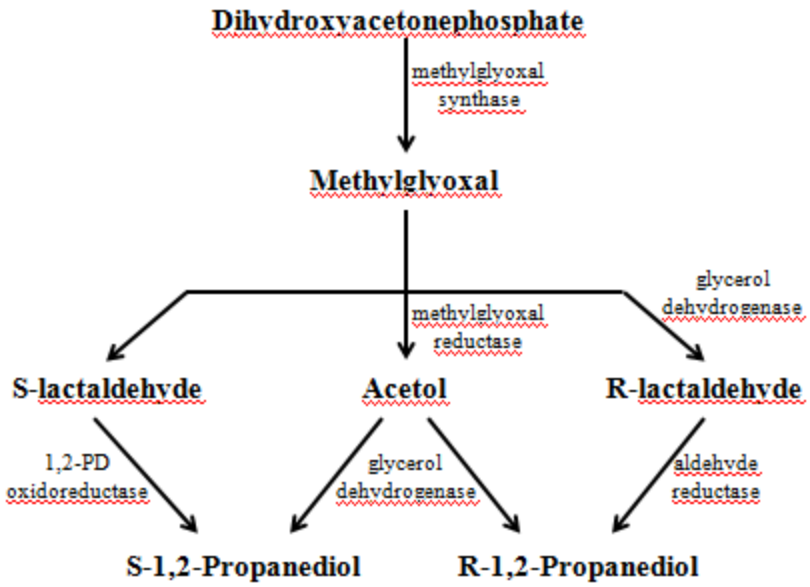

Figure 2.3: Schematic representation of metabolic pathway for the production of 1,2-propanediol from DHAP.

Thermoanaerobacterium thermosaccharolyticum is known to produce R-1,2-propanediol from common sugars, such as glucose and xylose. The glycolytic intermediate dihydroxyacetonephosphate (DHAP) is dephosphorylated by methylglyoxal synthase to form methylglyoxal, which is then reduced to either lactaldehyde or acetol. Glycerol dehydrogenase and methylglyoxal reductase are the other enzymes involved in this reduction. Further reduction to 1,2-PD is accomplished by native activity (Cameron *et al.* 1998). This pathway is demonstrated in **Figure 2.3**.

While 1,2-PD production can be accomplished natively by this anaerobe, the titers are rather low, and improvement by metabolic engineering is limited in that the organism is not very well characterized at present (Altaras and Cameron 1999). In a specific study by Altaras *et al* (2001), it was discovered that biomass such as corn, wood byproducts and cheese whey could serve as a feedstock, and *T. thermosaccharolyticum* could ferment a broader list of sugars than previously realized. They even reported that, when compared to individual sugar fermentation, mixed sugars doubled the yield of 1,2-PD from similar values of consumed sugar. This shows promise in regards to using algae as a feedstock for 1,2-PD production, as extraction processes from algal biomass typically yield a mixture of sugars.

Wild-type *E. coli* is not known to produce 1,2-PD from common sugars. However, metabolic engineering has proven to be a successful means of enhancing the production of 1,2-PD from certain *E. coli* strains using glucose as a substrate. Altaras and Cameron (1999) reported that *E. coli* AG1 that has been genetically modified to overexpress glycerol dehydrogenase and/or methylglyoxal synthase enzymes have proven to produce 1,2-PD from

glucose fermentation under anaerobic conditions. Furthermore, because *E. coli* is well-characterized, further optimization and strain improvement can be accomplished by deleting competing pathways, as demonstrated by Berrios-Rivera *et al.* (2003). Clomburg and Gonzalez (2011), who used glycerol as a feedstock for 1,2-PD production, further demonstrated strain improvement by overexpressing glycerol dehydrogenase and methylglyoxal synthase, as well as expressing ATP-dependent dihydroxyacetone kinase (DHAK) from *C. freundii* in place of PEP-dependent DHAK native to wild-type K12 *E. coli* strain MG1655. In addition to the overexpression of certain enzymes key to the synthesis of 1,2-PD, they performed gene deletions which blocked the pathways for the production of organic acids such as lactate and acetate. They also found the co-production of ethanol to be very valuable as it helped maintain redox balance and ensured ATP generation. The relevant pathways involved in the metabolism of glycerol to 1,2-PD and other fermentation products are depicted in **Figure 2.4**.

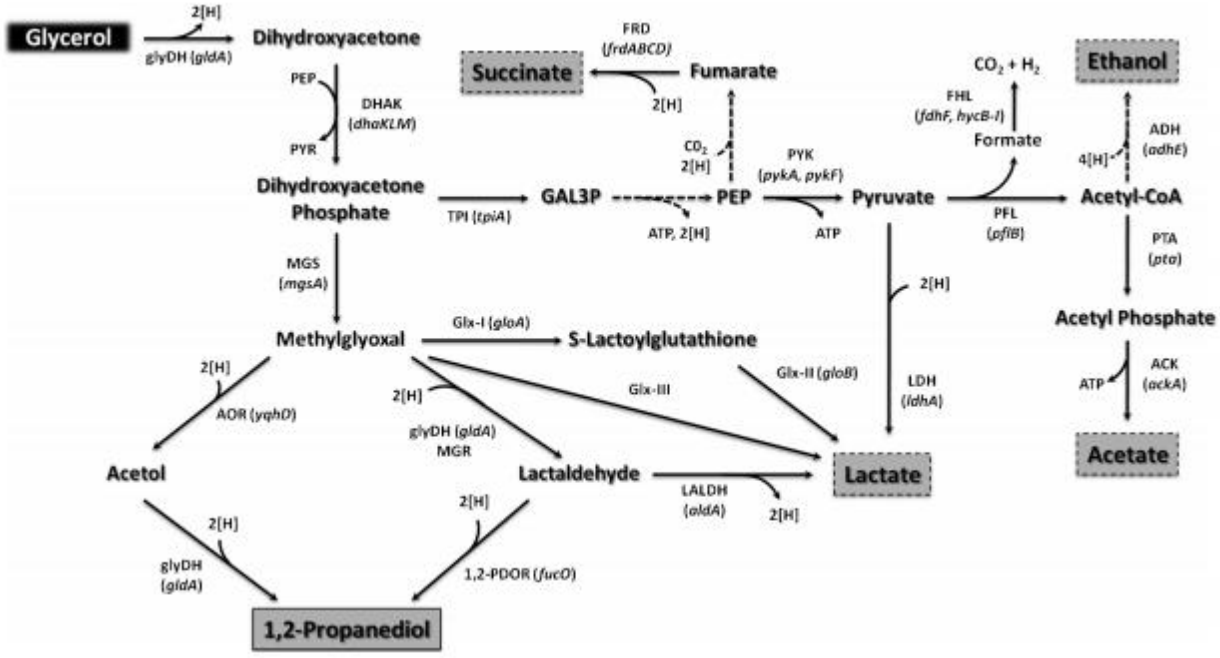

Figure 2.4: *E. coli* pathways involved in the synthesis of 1,2-PD and other fermentation products during the fermentative metabolism of glycerol (Clomburg and Gonzalez 2011).

2.5 References

Achkar, J., M. Xian, H. Zhao and J. Frost (2005). Biosynthesis of Phloroglucinol. *J. Am. Chem. Soc.* 127: 5332-5333.

Adey, W. (1982). Algal turf scrubber. US Patent No. 4,333,263. June 8, 1982.

Adey, W. (1998). Algal turf water purification method. US Patent No. 5,851,398. December 22, 1998.

Adey, W., P. Kangas and W. Mulbry (2011). Algal turf scrubbing: Cleaning surface waters with solar energy while producing a biofuel. *Bioscience.* 61(6): 434-441.

Adey, W., H. Laughinghouse IV, J. Miller, L. Hayek, J. Thompson, S. Bertman, K. Hampel and S. Puvanendran (2013). Algal turf scrubber (ATS) floways on the Great Wicomico River, Chesapeake Bay: productivity, algal community structure, substrate and chemistry. *Journal of Phycology.* 49(3): 489-501.

Agrawal, P. and A. Pandit (2003). Isolation of a-glucosidase from Saccharomyces cerevisiae: cell disruption and adsorption. *Biochemical Engineering Journal*. 15: 37-45.

Altaras, N. and D. Cameron. (1999) Metabolic engineering of a 1,2-propanediol pathway in *Escherichia coli*. *Applied and Environmental Microbiology.* 1180-1186.

Altaras, N., M. Etzel and D. Cameron (2001). Conversion of sugars to 1,2-propanediol by *Thermoanaerobacterium thermosaccharolyticum* HG-8. *Biotechnology Progress.* 17: 52-56.

Aresta, M., A. Dibenedetto, M. Carone, T. Colonna and C. Fragale (2005). Production of biodiesel from macroalgae by supercritical CO_2 extraction and thermochemical liquefaction. *Environmental Chemistry Letters*. 3: 136-139.

Becker, E. (1994). Microalgae: Biotechnology and Microbiology, Vol. 10, J. Baddiley (Ed.), Cambridge Univ. Press, Cambridge, New York.

Bennett, G. and K. San (2001). Microbial formation, biotechnological production and applications of 1,2-prop
anediol. *Appl. Microbiol. Biotechnol.* 55: 1-9.

Berrios-Rivera, S., K. San and G. Bennett (2003). The effect of carbon sources and lactate dehydrogenase deletion on 1,2-propandiol production in *Escherichia coli*. *J. Ind. Micobiol. Biotechnol.* 30: 34-40.

Brune, D., T. Lundquist and J. Benemann (2009). Microalgal biomass for greenhouse gas reductions: potential for replacement of fossil fuels and animal feeds. *Journal of Environmental Engineering.* 135(11): 1136-1144.

Caccamese, S., R. Azzolina, G. Furnari, M. Cormaci and S. Grasso (1981). Antimicrobial and antiviral activies of some marine algae from eastern Siciliy. *Botanica Marina.* 24: 362-367.

Cameron, D., N. Altaras, M. Hoffman and A. Shaw (1998). Metabolic engineering of propanediol pathways. *Biotechnology Progress.* 14: 116-125.

Carvalho, A. and F. Malcata (2001). Transfer of carbon dioxide within cultures of microalgae: plain bubbling versus hollow-fiber modules. *Biotechnology Progress.* 17: 265-272.

Chinnasamy, S., B. Ramakrishnan, A. Bhatnagar and K. Das (2009). Biomass production potential of wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO_2 and temperature. *Int. J. Mol. Sci*. 10: 518-532.

Chisti, Y (2007). Biodisel from microalgae. *Biotechnology Advances.* 1-13.

Clomburg, J. and R. Gonzalez (2011). Metabolic engineering of *Escherichia coli* for the production of 1,2-propanediol from glycerol. *Biotechnology and Bioengineering.* 108(4): 867-879.

Craggs, R., W. Adey, K. Jenson, M. St. John, B. Green and W. Oswald (1996). Phosphorous removal from wastewater using an algal turf scrubber. *Water Science and Technology*. 33(7): 191-198.

De Godos, I., S. Blanco, P. Gracia-Encina, E. Becares and R. Munoz (2010). Influence of flue gas sparging on the performance of high rate algae ponds treating agro-industrial wastewaters. *Journal of Hazardous Materials.* 179: 1049-1054.

Dasari, M., P. Kiatsimkul, W. Sutterlin and G. Suppes (2005). Low-pressure hydrogenolysis of glycerol to propylene glycol. *Applied Catalysts A: General*. 281: 225-231.

Dismukes, G., D. Carrieri, N. Bennette, G. Ananyev and M. Posewitz (2008). Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. *Current Opinion in Biotechnology.* 19(3): 235-240.

Dlugokencky, E., and P. Tans (2013). Trends in Atmospheric Carbon Dioxide. Earth System Research Laboratory, National Oceanic & Atmospheric Association. [Online]. Available: http://www.esrl.noaa.gov/gmd/ccgg/trends/global.html. [2013, May 19].

Doucha, J., F. Straka and K. Livansky (2005). Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. *Journal of Applied Phycology.* 17: 403-412.

Douskova, I., J. Doucha, K. Livansky, J. Machat, P. Novak, D. Umysova, V. Zachleder, V. and M. Vitova (2009). Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Applied Microbiology and Biotechnology.* 82: 179-185.

Dumay, J., N. Clément, M. Morançais and J. Fleurence (2013). Optimization of hydrolysis condition of *Palmaria palmate* to enhance R-phycoerythrin extraction. *Bioresource Technology.* 131: 21-27.

El-Rafie, H., M. El-Rafie and M. Zahran (2013). Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. *Carbohydrate Polymers*. 96: 403-410.

Ferreira, B., H. Fernandes, A. Reis and M. Mateus (1998). Microporous hollow fibres for carbon dioxide absorption: mass transfer model fitting and the supplying of carbon dioxide to microalgal cultures. *J. Chem. Technol. Biotechnol.* 71: 61-70.

Grogan, G. (2005). Biotransformations. *Annual Reports on the Progress of Chemistry, Section B.* 101: 192-209.

Herrero, M., A. Cifuentes and E. Ibañez (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-productions, algae and microalgae: A review. *Food Chemistry.* 98(1): 136-148.

Hoffmann, J. (1998). Wastewater treatment with suspended and nonsuspended algae. *J. Phycol.* 34: 757-763.

Hossain, A., A. Salleh, A. Boyce, P. Chowdhury and M. Naqiuddin (2008). Biodiesel Fuel Production from Algae as Renewable Energy. *American Journal of Biochemistry and Biotechnology*. 4(3): 250-254.

Kapucu, H., N. Gulsoy and U. Mehmetoglu (2000). Disruption and protein release kinetics by ultrasonication of Acetobacter peroxydans cells. *Biochemical Engineering Journal*. 5: 57-62.

Kebede-Westhead, E., C. Pizarro and W. Mulbry (2006). Treatment of swine manure effluent using freshwater algae: Production, nutrient recovery, and elemental composition of algal biomass at four effluent loading rates. *Journal of Applied Phycology.* 18: 41-46.

Kumar, A., X. Yuan, A. Sahu, J. Dewulf, S. Ergasand H. Langenhove (2010). A hollow fiber membrane photo-bioreactor for CO_2 sequestration from combustion gas coupled with wastewater treatment: a process engineering approach. *J. Chem. Technol. Biotechnol.* 85: 387-394.

Lehr, F. and C. Posten (2009). Closed photo-bioreactors as tools for biofuel production. *Current Opinion in Biotechnology.* 20: 280-285.

Lorinez, A. (2004). Ultrasonic Cellular Disruption of Yeast in Water-based Suspensions. *Biosystems Engineering*. 89(3): 297-308.

Matsumoto, H., N. Shioji, A. Hamasaki, Y. Ikuta, Y. Fukuda, M. Sat, N. Endo and T. Tsukamoto (1995). Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. *Applied Biochemistry and Biotechnology.* 51/52: 681-692.

Matsumoto, H., A. Hamasaki, N. Sioji and Y. Ikuta (1996). Influence of CO_2, SO_2 and NO in flue gas on microalgae productivity. *Journal of Chemical Engineering of Japan.* 620-624.

Moheimani, N. and M. Borowitzka (2005). The long-term culture of the coccolithophore *Pleurochrysis carterae* (Haptophyta) in outdoor raceway ponds. *Jounral of Applied Phycology.* 18: 703-712.

Mulbry, W., and A. Wilkie (2001). Growth of benthic freshwater algae on dairy manures. *Journal of Applied Phycology.* 13: 301-306.

Mulbry, W., E. Kebede-Westhead, C. Pizarro and L. Sikora (2005). Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. *Bioresource Technology*. 96: 451-458.

Mulbry, W., S. Kondrad, C. Pizarro, E. Kebede-Westhead (2008). Treatment of dairy manure effluent using freshwater algae: Algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. *Bioresource Technology*. 99: 8137-8142.

Nagle, N. and P. Lemke (1990). Production of Methyl Ester Fuel from Micoralgae. *Applied Biochemistry and Biotechnology*. 24/25: 355-361.

Neto, A., R. de Souza, A. Leon-Nino, J. da Costa, R. Tiburcio, R. Nunes, T. Sellare de Mello, F. Kanemoto, F. Saldanha-Correa and S. Gianesella (2013). Improvement in microalgae lipid extraction using a sonication-assisted method. *Renewable Energy.* 55: 525-531.

Niu, W., M. Molefe and J. Frost (2003). Microbial synthesis of the energetic material precursor 1,2,4-butanetriol. *J. Am. Chem. Soc.* 125: 12998-12999.

Nys, R., S. Dworjanyn and P. Steinberg (1998). A new method for determining surface concentrations of marine natural products on seaweeds. *Marine Ecology Progress Series.* 162: 79-87.

Oswald, W. (1988). Large-scale algal culture systems (engineering aspects), in M. Borowitzka and L. Borowitzka (Eds.), Micro-algal Biotechnology, Cambridge University Press, Cambridge. 357-394.

Pizarro, C., W. Mulbry, D. Blersch and P. Kangas (2006). An economic assessment of algal turf scrubber technology for treatment of dairy manure effluent. *Ecological Engineering.* 26: 321-327.

Pizarro, C., E. Kebede-Westhead and W. Mulbry (2002). Nitrogen and phosphorous removal rates using small algal turfs grown with dairy manure. *Journal of Applied Phycology.* 14: 469-473.

Pokoo-Aikins, G., A. Nadim, M. El-Halwagi and V. Mahalec (2010). Design and analysis of biodiesel production from algae grown through carbon sequestration. *Clean Technology Environmental Policy.* 12: 239-254.

Potts, T., J. Du, M. Paul, P. May, R. Beitle, J. Hestekin (2011). The production of butanol from Jamaica bay macro algae. *Environmental Progress & Sustainable Energy.* 31(1): 29-36.

Rodriguez-Jasso, R., S. Mussatto, L. Pastrana, C. Aguilar and J. Teixeira (2011). Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. *Carbohydrate Polymers*. 86: 1137-1144.

Sastra, V. and G. Rao (1994). Antibacterial substances from marine algae: Successive extraction using benzene, chloroform and methanol. *Botanica Marina.* 37: 357-360.

Saxena, R., P. Anand, S. Saran, J. Isar and L. Agarwal (2010). Microbial production and applications of 1,2-propanediol. *Indian J. Microbiol.* 50(1): 2-11.

Schofield, D., M. Bailey and J. Monteith (2002). Process for the preparation of butane triols. US Patent No. 6,479,714 B1. November 12, 2002.

Shekharam, K., L. Venkataraman and P. Salimath (1987). Carbohydrate composition and characterization of two unusual sugars from the blue green alga, *Spirulina platensis*. *Pytochemistry.* 26(8): 2267-2269.

Ugwu, C., H. Aoyagi and H. Uchiyama (2008). Photobioreactors for mass cultivation of algae. *Bioresource Technology.* 99(10): 4021-4028.

Wang, G. and T. Wang (2012). Lipid and biomass distribution and recovery from two microalgae by aqueous and alcohol processing. *J. Am. Oil Chem. Soc.* 89: 335-345.

Westerhoff, P., Q. Hu, M. Esparza-Soto, W. Vermaas (2010). "Growth parameters of microalgae tolerant to high levels of carbon dioxide in batch and continuous-flow photobioreactors." *Environmental Technogy.* 31(5): 523-532.

Witvrouw, M. and E. Clercq (1997). Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *General Pharmacology: The Vascular System*. 29(4): 497-511.

Woods, L., M. Riccobono, N. Mehan, J. Hestekin and R. Beitle (2011). "Synergistic effect of abrasive and sonication for release of carbohydrate and protein from algae." *Separation Science and Technology.* 46(4): 601-604.

Wu, X. and J. Merchuk (2002). Simulation of algae growth in a bench-scale bubble column reactor. *Biotechnology and Bioengeering.* 80(2): 156-167.

Yaich, H., H. Garna, S. Besbes, M. Paquot, C. Blecker and H. Attia (2013). Effect of extraction conditions on the yield and purity of ulvan extracted from *Ulva lactuca. Food Hydrocolloids.* 31: 375-382.

Zachleder, V. (1984). Optimization of nucleic acids assay in green and blue-green algae: Extraction procedures and the light-activated diphenylamine reaction for DNA. *Arch. Hydrobiol. Suppl.* 67(3): 313-328.

Zha, W., Z. Shao, J. Frost and H. Zhao (2003). Rational pathway engineering of type I fatty acid synthase allows the biosynthesis of triacetic acid lactone from D-glucose in vivo. *J. Am. Chem. Soc.* 126: 4534-4535.

Zhang, Z., F. Wang, X. Wang, X. Liu, Y. Hou and Q. Zhang (2010). Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. *Carbohydrate Polymers.* 82(1): 118-121.

CHAPTER 3

Preparatory Work and Preliminary Studies

3.1 Development of hollow fiber membrane manifold

Hollow fiber membranes are typically encased in cylindrical cartridges of various lengths and diameters. While this module may be sufficient for use in closed systems, it is not the most practical for use in an open, attached-growth system such as the ATS®. Because of this, a unique hollow fiber membrane (HFM) manifold (**Figure 3.1)** has been constructed in order to perform gas delivery experiments. Polyether ether ketone (PEEK) hollow fiber membranes (Porogen) have been looped in sections of varying length and the open ends of the fibers were placed through one-half-inch CPVC elbows. The fibers were secured in place by filling the elbows with Conathane (Cytec Industries), a polyurethane epoxy resin, leaving approximately ¼-½ inch of the ends of the fibers sticking out of the male end of the CPVC elbow for gas to enter the fibers. After the polymer was given adequate time to cure, the manifold was completed by installing the hollow fiber elbows into ½ inch CPVC T connections in ½ inch CPVC pipe. End caps were put on both ends of the pipe and one end has a hose barb, where carbon dioxide enters through flexible plastic tubing from a compressed gas cylinder.

This design is truly ideal in that it allows gas to be delivered directly to the location it is utilized by the algae, by placing it on the surface of the floway beneath the mesh/carpet upon which the algae attaches. Furthermore, this manifold offers flexibility in design and may be modified for different geometries (length of fibers, number of elbows, etc.). It also allows for modifications to be made for use in other applications. Finally, this simple design allows for easy

replacement of the hollow fiber membranes in the event that the fibers get damaged or become fouled.

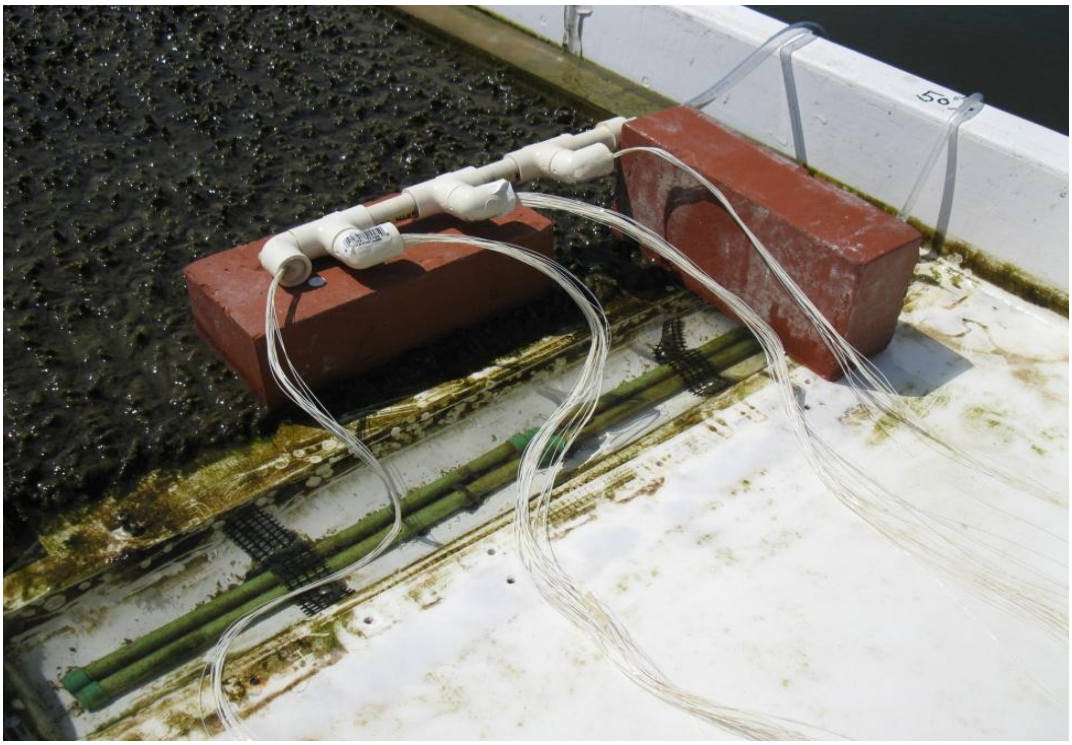

Figure 3.1: Picture of hollow fiber membrane manifold constructed for use in ATS® system.

3.2 Wicomico River preliminary experiments

Preliminary CO_2 delivery test experiments have been performed at a land based ATS® on the Great Wicomico River in Virginia. A HFM manifold consisting of four elbows, with eight fibers per elbow and 20 ft. sections of fiber was installed at the 40-60 ft. position on an 80-ft. floway. This position was chosen because it is known that, in these types of systems, CO_2 availability depletes over the length of the ATS®, thus it would not be efficient to pre-inject the gas into the water at the feed position. Biomass samples were harvested from various positions along the length of the floway to determine the effects of carbon dioxide gas delivery over a two month period. Samples from the same points along the floway were taken both without CO_2 and with CO_2 being added to the system. The picture below **Figure 3.2** demonstrates the location of the manifold as well as the biomass sample points.

Figure 3.3 shows the algae productivity in $g/m^2/day$ vs. the various positions on the floway from which algae samples were harvested. There was a similar or modest gain in the algae productivity over the two month period when the carbon dioxide was added compared to when there was no CO_2 addition. However, the addition of CO_2 resulted in a drastic pH drop over the length of the floway following the HFM manifold. As mentioned before, too low of a pH can have a negative impact on algae growth. Since the pH was not controlled in these experiments, yet similar to increased algae growth was realized, it is expected that significantly higher productivities can be achieved with simple pH control. Further, a change in speciation from diatoms and blue green algae to only blue green algae was also observed. This provided evidence that carbon dioxide delivery via hollow fibers could be extremely beneficial, as there

are some potential processing advantages associated with this taxonomy alteration, such as increased oil/carbohydrate content and simplified extraction.

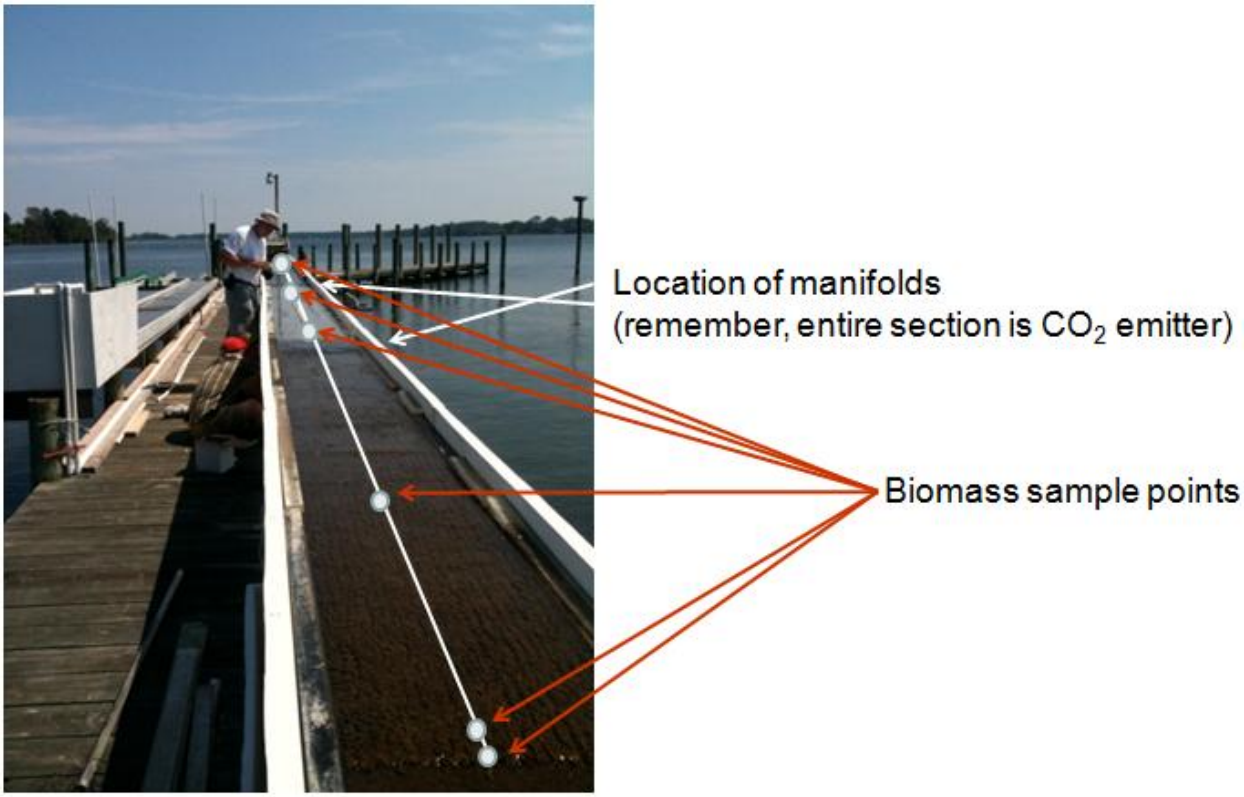

Figure 3.2: Location of hollow fibers and biomass sample points in Wicomico River ATS system.

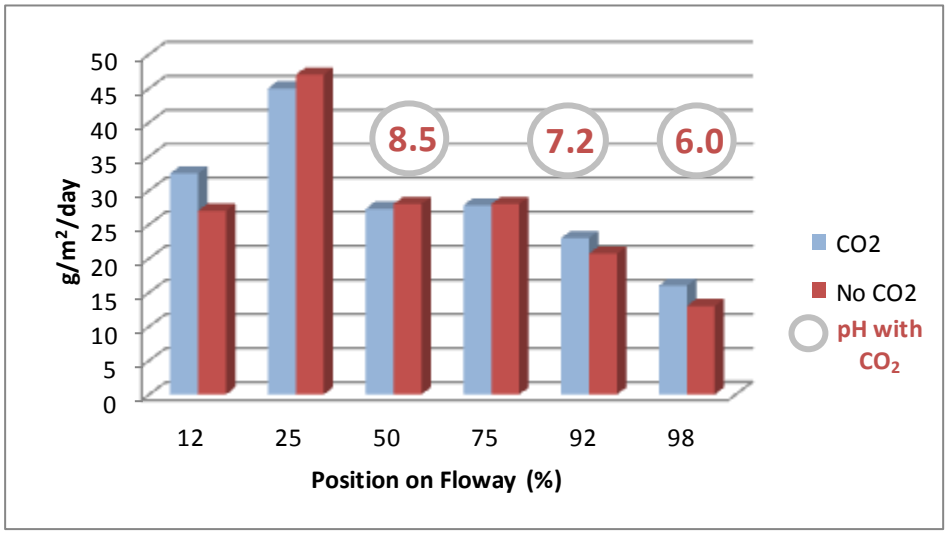

Figure 3.3: Effects of CO_2 on algae productivity and pH.

3.3 Building of lab scale ATS®

A laboratory scale ATS® has been constructed in-house to model carbon dioxide gas delivery, and ultimately to determine its effects on algae growth, in open, thin-film aqueous systems. The apparatus consists of a 14 feet long by two feet wide floway with elevated edges. The width of the floway has been leveled, and there is a slight slope (one inch drop in four feet) down the length of the floway to facilitate the flow of water evenly throughout the system. A piece of carpet has been placed on the flat surface of the floway to eventually serve as the surface for algae attachment and growth. Water is introduced to the system by pumping it, via a sump pump, through one-inch reinforced tubing into two-inch PVC pipe from a 255 gallon tank into a dump bucket at the front/top of the system, which operates in a surging fashion, dispensing the water at regular, periodic intervals. A trough was built at the back end of the floway to catch the water, which then exits the floway through one-inch reinforced tubing and is returned to the bottom of the tank for recycle. Two ball valves have been installed in the PVC pipe to regulate the flow rate of the water entering the system. One of the ball valves installed in the PVC piping serves as a bypass from the pump to provide adequate mixing of nutrients in the tank. To ensure as much control as possible in an open system, the apparatus has been built inside a greenhouse on campus at the University of Arkansas to avoid contamination. Natural sunlight serves as the primary light source. A picture of the apparatus is shown in **Figure 3.4**.

There are a few additions to the laboratory scale floway, which are currently in progress, that are necessary for carrying out all of the experiments related to gas delivery and algae growth. Temperature and pH probes will be utilized at both the top and bottom of the flat surface on the floway, as well as in the reservoir, in order to monitor the system conditions.

Artificial seawater, with added nutrients necessary for algal growth, will be used as growth medium. Peristaltic pumps will be installed to deliver nutrients to the system as needed. It is expected that the water in the system will evaporate over time, thus a volume controller will be installed in the reservoir to ensure that the volume of the water remains constant, as well as the nutrient and salt concentration in the solution. This will be extremely important to control during the modeling phase because the diffusivity, and ultimately the mass transfer coefficient, is dependent on the content of the water solution.

The laboratory scale ATS® system and HFM manifold have been designed with the intent of assigning an undergraduate student to continue research in this area. Initially, experiments will be conducted to model the gas delivery of carbon dioxide to the system using the two methods, hollow fibers and direct injection. For the direct injection method, CO_2 gas will be bubbled directly into the water tank via a piece of plastic tubing from the compressed gas cylinder. A hollow fiber manifold has been placed on the flat surface of the floway underneath the carpet that was discussed in Section 3.3. This will aid in protecting the fibers during algae harvest in later experiments. The goal of this system is to model the mass transfer of carbon dioxide gas delivery in a thin-film, aqueous system, and ultimately to determine the effects carbon dioxide addition via hollow fibers on algae growth.

Figure 3.4: Picture of laboratory scale floway at the University of Arkansas.

3-A Appendix – Chapter 3

3-A.1 Benchtop algal growth system

Prior to building the greenhouse ATS® system, a smaller, bench scale algal growth system was built. The floway consisted of a 5 ft. by 2 ft. stainless steel trough with a flat bottom and elevated sides, much like an ATS®. A light source was supplied by installing fluorescent lamps above the trough. The front end of the trough was elevated by placing wood shims beneath the flat portion of the stainless steel, and a 2x4 piece of wood was cut to fit the width of the floway and was installed perpendicular to the floway surface to ensure that water did not escape from the top of the apparatus. Approximately six inches from the 2x4, another 2x4 was cut to fit the width of the floway, and was placed parallel to the floway surface. Both 2x4s were sealed around the edges with silicone to prevent leaking and the ends of the pieces of wood. Aquarium gravel was placed between the two pieces of wood to provide a pallet in which water could fill up and then evenly distribute across the width of the floway. At the end of the trough, there was a large plastic Rubbermaid container where the water collected, and there was a small submersible pond pump in the tank that pumped water up to a pipette washer that sat on a shelf above the trough via 1 in. reinforced tubing. Once the pipette washer filled up, it would drain through a dishwasher hose into a 1 in. diameter PVC pipe. The PVC pipe had a rubber stopper on the opposite end of the dishwasher hose, and several small holes were drilled in the PVC pipe on one side with about ¼ inch spacing. The PVC pipe rested on top of the pallet of gravel, and water was released over the width of the trough periodically. Plastic mesh was installed on top of the flat surface of the stainless steel trough, which would serve as a place for algae to attach. Magnets were placed on top of the mesh to keep it from floating. The system was seeded with algae from the pilot scale ATS® operated by the University of Arkansas in Springdale by clipping sections of the polymeric mesh from that system and placing them on the surface of the

benchtop floway. After approximately one month, the algae began to fill up the entire trough. Algae was harvested every 2-4 weeks, as needed, to maintain decent growth rates. A picture of the bench scale floway is shown in **Figures 3-A.1** and **3-A.2**.

This bench scale floway was built to determine optimum nutrient conditions for algae growth, as well as to perform the initial testing of the hollow fibers and verify the effects on algae growth. In order to determine the optimum nutrient conditions, experiments were performed using various plant fertilizers with different levels of nitrogen and phosphorous, and algae growth and nutrient levels were analyzed over time. For each plant fertilizer tested, experiments were carried out for two weeks. Algae growth was determined at the end of the two-week period by harvesting the algae with a Shop-Vac, and centrifuging it to remove as much water as possible. The algae was then weighed to determine a value in $g/m^2/day$. Water samples were taken from the Rubbermaid tub daily and water quality was analyzed using nitrogen, phosphorous and total organic carbon test kits from Hach. In addition, algae growth experiments were performed with the addition of CO_2 via the HFMs and without CO_2 to determine if there was a noticeable effect on growth rates.

While the experiments were planned out well and were carried out carefully to avoid as much error as possible, unfortunately no significant differences were observed in the algae growth for the various parameters tested. It was determined that the size of the floway simply was not adequate to gain meaningful data. It is significant to note that, around this same time, the Springdale ATS® was being shut down due to lack of funding. Because of this, we decided to work with our collaborators at the Smithsonian Institute to test our hollow fiber membranes in

their pilot scale ATS® at the Wicomico River in Virginia. As discussed in section 3.2, preliminary experiments went well and the data looked promising. Unfortunately, however, we were reliant on our collaborators to perform most of the analytical work, and they had a different vision for the project than we did. It was at that point that we decided to build the laboratory scale ATS® to conduct experiments with our hollow fiber membrane bundles.

Figure 3-A.1: Photograph of bench scale algal growth floway, seeded with sections of algae from Springdale ATS®.

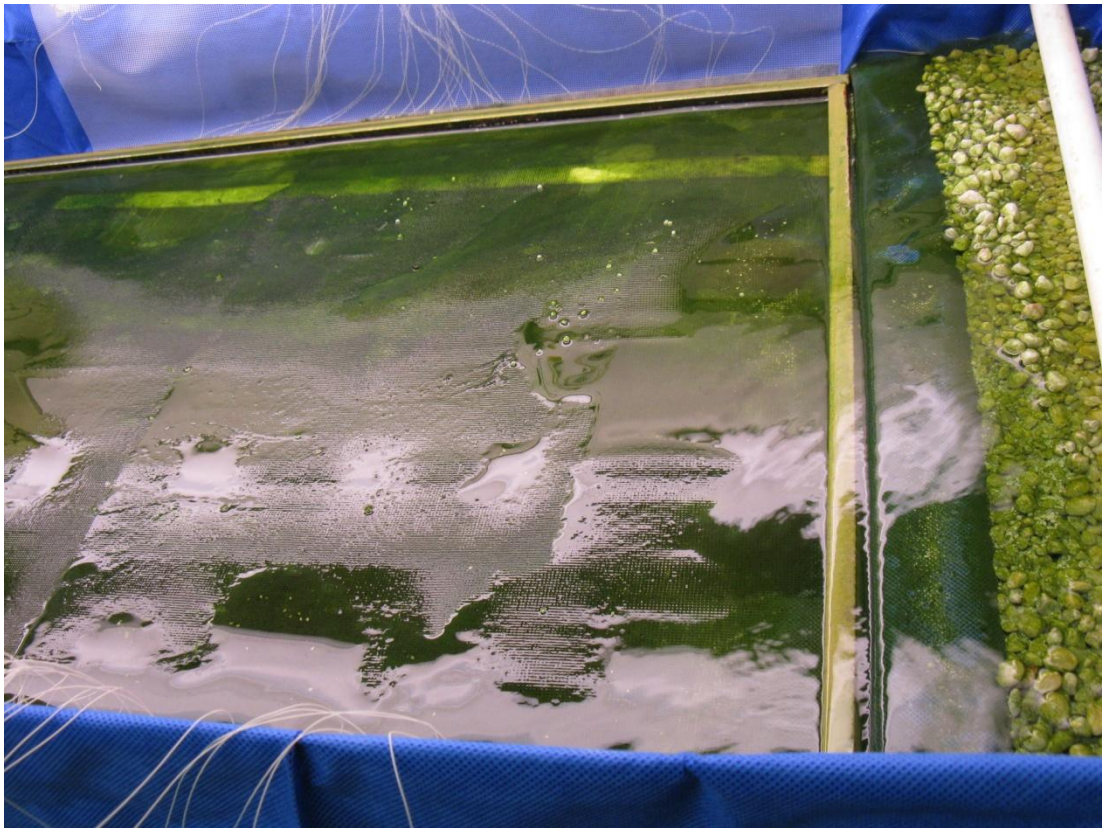

Figure 3-A.2: Photograph of algae growth with hollow fibers installed.

3-A.2 Development of hollow fiber manifold

Section 3.1 depicts the final product for the development of the hollow fiber manifold used in the gas delivery experiments involving the Wicomico River pilot scale ATS® as well as the laboratory experiments to compare the effects of CO_2 delivery via HFMs to other methods. This section is to demonstrate the process that led to coming up with that design.

As mentioned previously, hollow fibers membranes are typically used in cylindrical cartridges. Because of the application we desired to use it for, we thought it would be best if the manifold could allow the fibers to lay flat beneath the algae growth surface in our attached growth floway systems. **Figure 3-A.3** shows the initial design attempt. The manifold was constructed by placing a piece of silicone tubing on the end of a plastic fitting. The hollow fibers were fed through the plastic fitting and tubing, and the open end of the tubing was clamped with heavy duty paper clips in an effort to prevent the epoxy from leaking out of the bottom of the manifold. A two-part heavy duty epoxy was purchased from Lowe's to pot the fibers into the fitting. This design was unsuccessful for a variety of reasons, mainly due to epoxy leaking from the open end of the tubing before it had a change to cure. Also, the epoxy resin that was chosen was too thick to get between each of the fibers, thus there were many air gaps formed in the epoxy as it cured, which would mean the manifold would be insufficient for gas delivery.

Figure 3-A.3: Initial design attempt for HFM manifold.

The second attempt, shown in **Figures 3-A.4** and **3-A.5** provided even more insight that led to our final design. We chose the polyurethane epoxy, Conathane, because it had a lower viscosity than many of the other resins we looked into. This meant that it would fill the spaces between the fibers more effectively, living little to no air gaps as it cured. We also did away with the silicone tubing from the previous attempt, and tried using cotton balls as a way to prevent the epoxy from leaking out of the end of the plastic fitting. Two plastic fittings were used, with the idea that one end would be where gas would enter the manifold and the other would have a pressure gauge attached to see if there was any pressure drop over the length of the fibers. HFMs were fed through the fitting with the open ends of the fibers protruding approximately ½ inch from each of the male ends. These alterations resulted in a far more promising HFM manifold which was the one we first tested in our bench scale algal floway. However, the major issue with this design was that the fibers had to be bent where the cotton ball was placed at the base of the fitting. Unfortunately, this crushed the fibers, making them less effective for gas delivery, and created increased risk for fiber breakage near the location that the fibers were bent. Furthermore, the ends of the manifold where the fibers were potted could not be placed underneath the growth surface, which meant there was a significant portion of the fiber area that were not protected by the growth mat and were very susceptible to breakage.

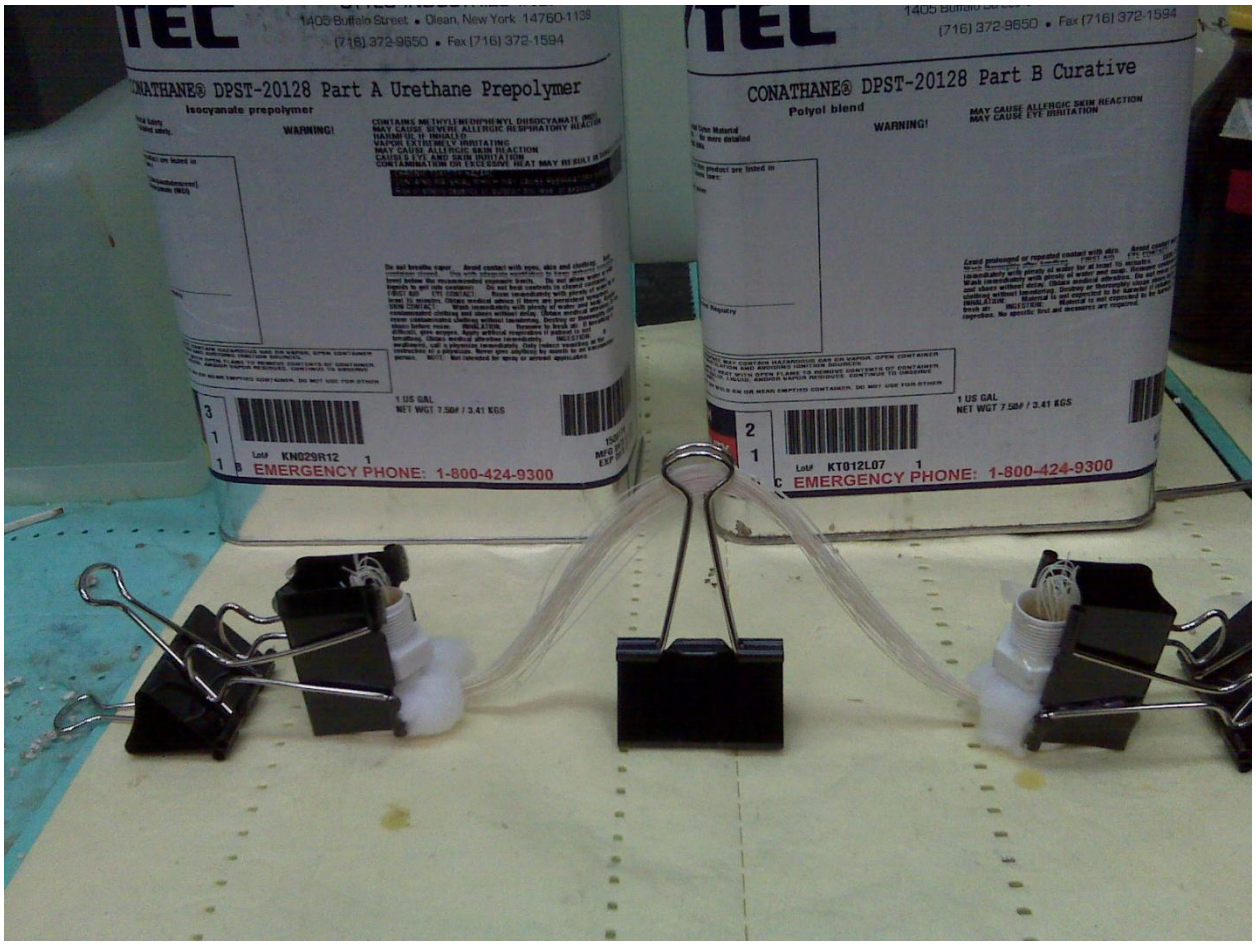

Figure 3-A.4: Second design attempt for HFM manifold.

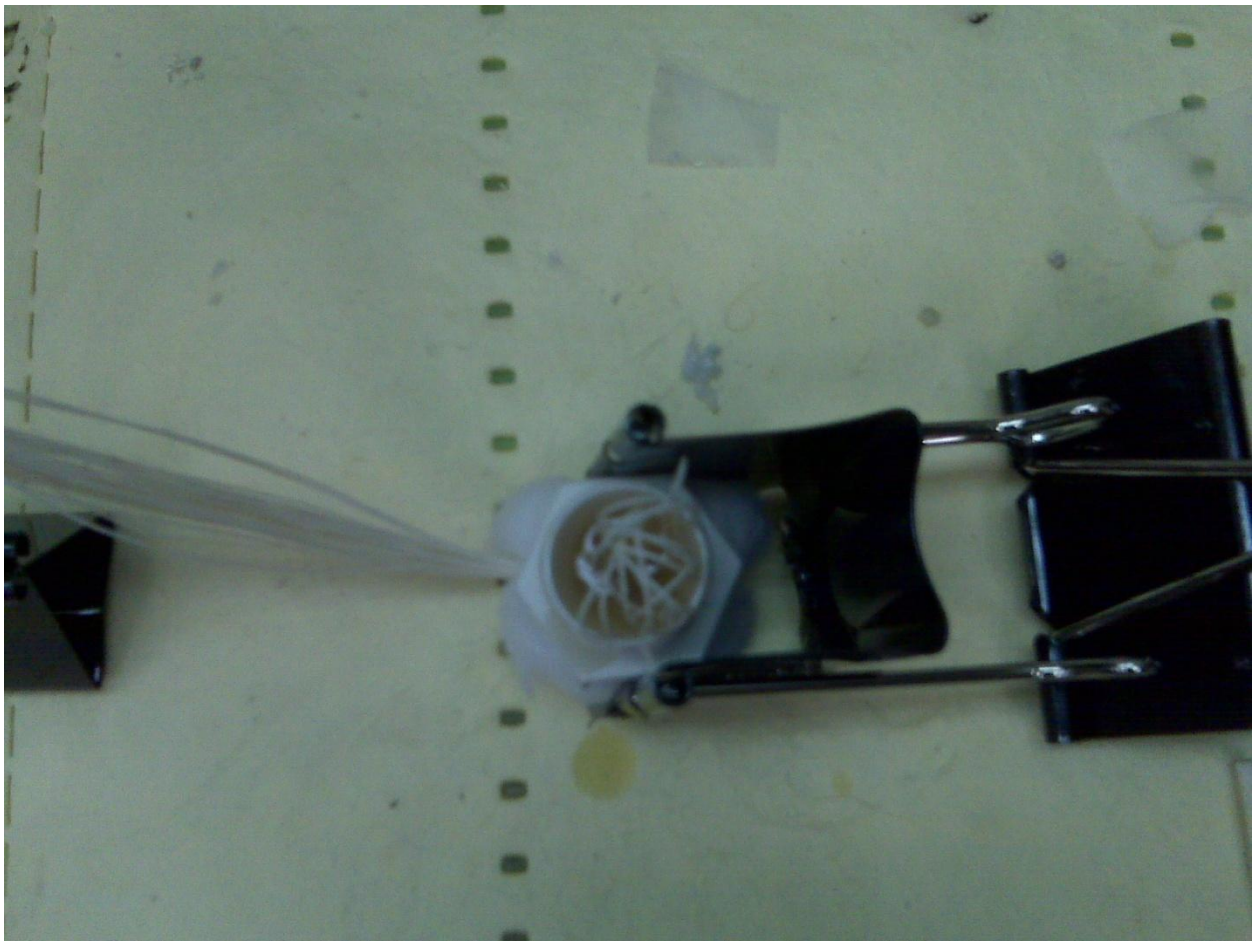

Figure 3-A.5: Second design attempt for HFM manifold – top view to show open ends of the fibers protruding from fitting.

A very different approach was taken in the next attempts by cutting a narrow, lengthwise gap in two 1-inch diameter PVC pipes. The idea was to place each of the open ends of the fibers through the channel towards the center of the PVC pipe, which would then be sealed with epoxy to hold the fibers in place. End caps would be placed on each end of the PVC pipes. A hose barb was to be installed on one of the end caps, which would serve as the entry point for the gas delivery. The PVC pipes could then be placed on either side of the floway, and the fibers could lie safely below the growth mat, which we hoped would minimize the risk of breakage.

While the Conathane was perfect for ensuring that the outer surfaces of the fibers were completely immersed in epoxy, the viscosity was too low to stay in play long enough to adequately fill the channel that was cut in the PVC pipe, leaving several gaps once the polymer cured. A couple of different options were used in an effort to keep the Conathane in place long enough to cure and fill the channel along the PVC pipes, including aluminum foil, gauze and thick, disposable lab towels. A picture of these attempts is shown in **Figure 3-A.6.**

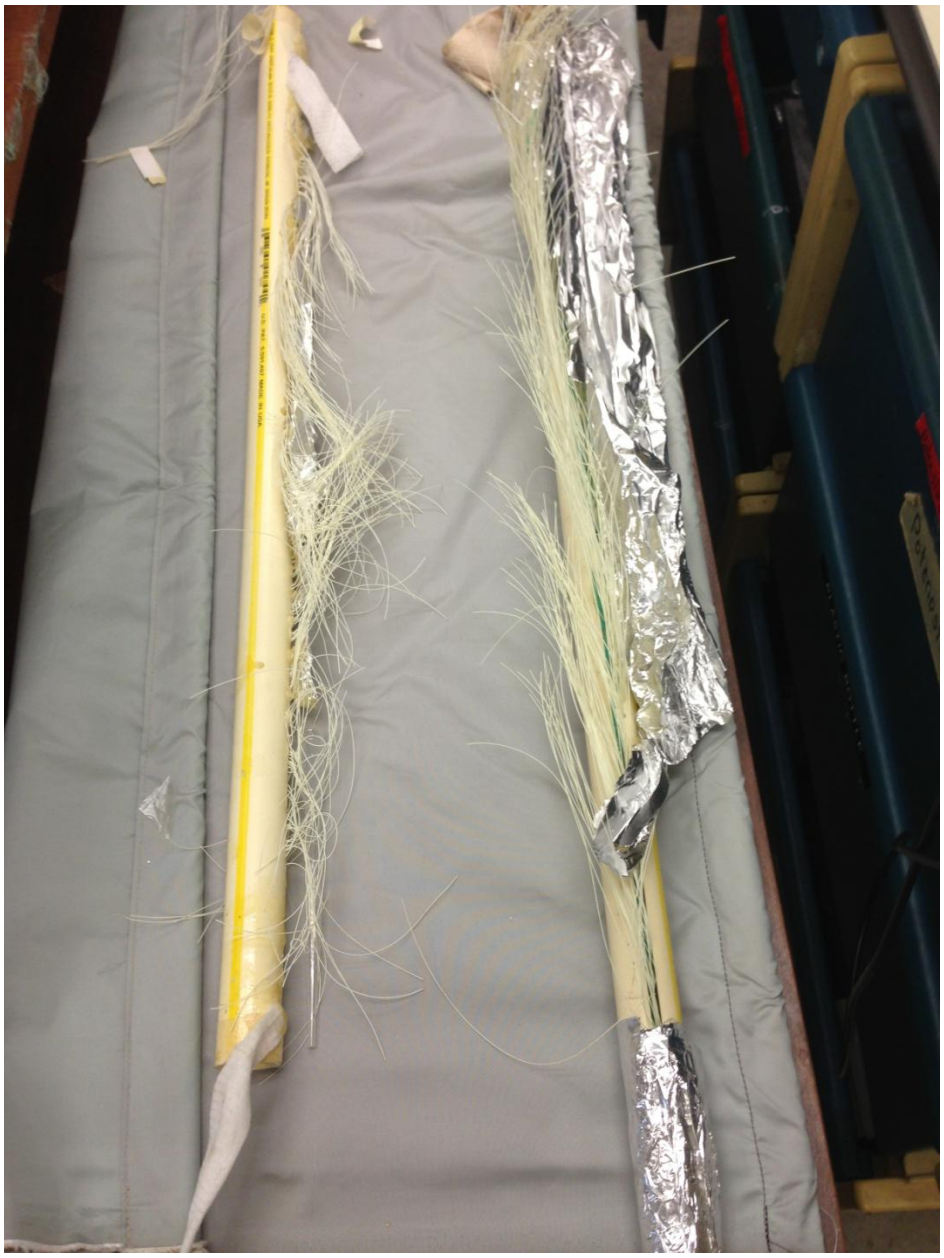

Figure 3-A.6: HFM manifold design attempts involving the use of PVC pipe and other materials.

The gauze worked fairly well to keep the Conathane in place until it cured completely, so a similar attempt was made utilizing the PVC pipe with the channel cut along one side of the pipe. But in an effort to mitigate some of the risk of air gaps where fibers were placed inside the channel, we decided to use only one PVC pipe and loop the fibers. This design attempt is shown in **Figure 3-A.7**. While it worked better than any of the previous attempts, it was still not ideal due to the fact that there were still areas in the epoxy where there were small gaps that gas could escape, causing a loss of pressure in the fibers when the manifold was tested.

Through trial and error and by combining this attempt with the second attempt, we arrived at the idea for the final manifold design discussed in Section 3.1. It was decided that by using elbow fittings rather than straight fittings, there would be no need for any kind of cotton or gauze to prevent the epoxy from leaking. The elbows could simply be placed in a vice grip and held in place until the polymer was given adequate time to cure completely. By looping sections of fiber in the elbows, and using shorter sections of PVC pipe and T connections to attach the elbows, it is possible to create a manifold that can be customized for a variety of different geometries. The finals design also makes it very easy to replace a section of fibers in the event that they become damaged or fouled by simply removing the old fiber elbow and installing a new one into the T connection.

Figure 3-A.7: HFM manifold design attempt involving the use of PVC pipe with gauze and looping the fibers.

CHAPTER 4

Carbon dioxide gas delivery to thin-film aqueous systems via hollow fiber membranes

A paper submitted to the *Journal of Membrane Science* (May 2013)

Lauren Merriman, Alex Moix, Robert Beitle, Jamie Hestekin

4.1 Abstract

Three methods of gas delivery, namely bubbling via an open tube, a porous diffuser and a unique hollow fiber membrane (HFM) manifold, were tested to compare the effects of mass transfer for CO_2 gas delivery into water. Carbon dioxide gas was delivered to a deionized water bath at liquid depths of 1.5, 3 and 5 inches. A gas flow rate of 0.1 liter per minute (LPM) was used and pH was measured over time for each trial, and the carbonic acid dissociation relationships were used to calculate the total amount of carbon in the system for each time point. The hollow fiber membranes proved to be a far superior method to introduce the CO_2 gas into the system than both bubbling and a commercial diffuser, and demonstrated even greater superiority at more shallow depths; which makes them ideal for use in thin-film algae growth systems such as the Algal Turf Scrubber® (ATS®) technology. Further experiments were performed using the HFM manifold at the 1.5" depth and temperatures were varied at 20º, 30º and 40º Celsius to determine the effects of temperature on the mass transfer. An evapoporometry technique and a scanning electron microscope (SEM) were used to estimate the average pore size for the HFMs. Bubble sizes for each method were estimated by photography as well as a bubble size prediction model developed by Ramakrishnan for the open tube and HFMs. The effective mass transfer coefficient, k_La, was determined from the carbon values averaged from three trials

for each set of the parameters tested. Finally, a parallel mass transfer model was developed to calculate the carbon concentration in the system over time for the hollow fiber membranes. This model shows that the hollow fibers on the membranes, before they release, are quite important to the mass transfer of carbon dioxide into the system.

4.2 Introduction

Hollow fiber membranes (HFMs) have traditionally been used in separations because of their high surface area to volume ratio. For the same reason, they have also been used for bubbling applications and gas-liquid contacting. Gabelman and Hwang [1] offered an early review of hollow fiber membranes for many different contacting operations in the carbonation of beverages, protein extraction, VOC removal, etc. Their review noted that hollow fibers are an effective method of adding gases to liquid due to the large surface area and small bubbles produced. Recently, gas-liquid contacting has become important in hollow fiber membrane applications, as gas can be used to clean the fibers and reduce fouling [2-4]. In these applications, air sparging has been found to be a more effective cleaning method than traditional backwashing. Many studies involving membrane bioreactors, or using membranes to enhance growth rates of fermentation processes by collecting cells while allowing product removal, use hollow fiber membranes as well [5-7]. The use of hollow fiber membranes to enhance algae growth via the addition of carbon dioxide gas has also been explored [8-14].

Several recent studies have indicated that the addition of carbon dioxide or flue gas to algal growth medium can enhance biomass production rates in algae growth applications such as photobioreactors and raceway ponds [8-20]. It is estimated that the atmosphere contains

approximately 400 ppm carbon dioxide [21], but it has been demonstrated that up to 20% CO_2 (v/v) can be tolerated by some species of microalgae [8]. An important developmental consideration for the enhancement of algae growth by addition of carbon dioxide gas is the method of gas delivery. Typically, CO_2 delivery to the growth medium is performed by directly bubbling the gas into the growth system [10-11]. This is usually performed by either injecting the gas through a tube, or by bubbling gas into the media through a porous diffuser [12,14]. However, these methods are not particularly effective in terms of mass transfer and much of the CO_2 is lost to the atmosphere, thus resulting in higher production costs. Because of this, gas delivery via hollow fiber membranes has also been investigated for algal growth applications [12-14]. Gas delivery via porous hollow fibers would allow for higher mass transfer efficiencies to be accomplished due to the increased surface area over which mass transfer can occur. Lower gas pressures can also be implemented with the same productivity, in comparison to conventional systems. In addition, hollow fiber modules can reduce feedstock costs because the unused gas can be recirculated [12]. As a result, minimal amounts of CO_2 would be released back into the atmosphere.

Carvalho and Malcata [12] conducted a study to compare the effects of carbon dioxide transfer to microalgae cultures via plain bubbling versus hollow fiber membrane modules. Both hydrophobic and hydrophilic membranes were investigated. They noticed increases in biomass productivity when the fiber modules were used, and it was determined that carbon concentration in the media was highest with hydrophobic membranes, followed by bubbling and then hydrophilic membranes. Ferreira et *al*. [13] compared the effects of CO_2 addition to algal growth using hydrophobic hollow fiber membranes and bubbling. They determined that, while algae

growth was comparable for both methods of gas delivery, when CO_2 was supplied via hollow fibers, the dissolved oxygen concentration in the system was lower. Another study by Kumar et *al*. [14] investigated the effects of hollow fibers in a photobioreactor that used synthetic wastewater as a growth medium. They used various inlet, outlet and liquid concentrations of carbon dioxide, as well as varying liquid velocities. The authors stated that, in hollow fiber membrane photobioreactors, both algal biomass growth and CO_2 fixation rates were higher than in those without the fiber modules. However, in all of these cases, traditional hollow fiber membranes have been used. To date, there has yet to be a study of HFMs in an open system where the bubbles can come in direct contact with the growth media without running it through a module.

A common theme among each of these studies is that higher mass transfer efficiencies were achieved by increasing liquid phase velocities [12-14], which makes sense because as the turbulence of the system is increased, the thickness of the laminar boundary layer next to the surface of the membrane would decrease, thereby reducing the mass transfer resistance of the liquid phase and increasing the mass transfer coefficient [13-14]. It is important to note that CO_2 should be supplied in adequate amounts for the system to absorb [13], as excess gas would only be released back into the atmosphere and provide no added benefit to the growth system.

It should be noted that all of the previously discussed investigations involving hollow fibers for carbon dioxide delivery to algal cultures have used either closed flasks or photobioreactors. While these closed systems offer more control over the growth environment, they are not cost effective for the large scale production of biofuels from algae feedstocks. Very

little research has been performed on CO_2 delivery to open systems. In addition, all of the aforementioned studies also used an enclosed hollow fiber module, where the ends of the hollow fibers are potted, and the fiber bundle is placed inside an enclosed manifold. To our knowledge, there are no studies that involve the use of hollow fibers as a method of gas delivery in thin-film, attached algal growth systems, such as the ATS® system [23-26], in which the growth system is only a few inches deep. The results of this study can also be used to show how mass transfer will occur in hollow fibers where the bubbling cannot be as easily seen as our manifold systems.

4.3 Theory

4.3.1 Mass Transfer Model

Ferreira et *al*. [13] used simple equations to derive a model for the mass transfer of carbon dioxide into growth medium using a hollow fiber membrane system. The overall mass transfer coefficient, K_L, of carbon dioxide into the media for the hollow fiber membranes can be determined from the individual mass transfer coefficients in the liquid phase, membrane and gas phase. K_L is calculated by taking the reciprocal of the sum of three mass transfer resistances in series, namely the resistance of the liquid phase, $1/k_L$, membrane material, $1/k_M$, and gas phase, $1/k_G$:

$$K_L = \frac{1}{\frac{1}{Hk_G}+\frac{1}{Hk_M}+\frac{1}{k_L}} \quad (1)$$

where H is Henry's Law constant. Due to the relatively low solubility of carbon dioxide gas in water, Henry's constant is large. Furthermore, gas-phase diffusivity is four orders of magnitude larger than liquid-phase diffusivity [22], and diffusivity is directly proportional to the mass

transfer coefficient. Thus it can be estimated that the overall mass transfer is dominated by mass transfer in the liquid phase. Therefore the resistances to mass transfer for the gas phase and the membrane material are negligible, and the expression can be reduced to:

$$\frac{1}{K_L} \approx \frac{1}{k_L} \tag{2}$$

The authors used a sodium hydroxide solution to determine the mass transfer coefficient by measuring the pH of the solution and using the dissociation equilibrium relationships for carbonic acid before performing experiments with algal culture. A mass balance of the inorganic carbon was used to determine the mass transfer coefficient:

$$V\frac{dC}{dt} = K_L A(C^* - C) \tag{3}$$

Where V is volume of the liquid phase, A is the gas-liquid interfacial area, C* is the dissolved CO_2 concentration at the boundary layer, and C is the dissolved CO_2 concentration in the bulk liquid phase. This equation can also be expressed in terms of an effective mass transfer coefficient, k_La, when the geometries are not known or not easily separated from the mass transfer coefficient [22]:

$$\frac{dC}{dt} = k_L a(C^* - C) \tag{4}$$

By integrating the above equation at the limits of C=0 at t=0 and C=C at t=t, the equation becomes:

$$C = C^*(1 - e^{\frac{-K_L A t}{V}}) \quad (5)$$

The Sherwood number, *Sh*, is typically used in mass transfer calculations when both convective and diffusive mass transfer must be taken into consideration. The Sherwood number is a function of both the Reynolds number, *Re*, and the Schmidt number, *Sc*, and is represented by the following correlation:

$$Sh = \alpha Re^{\beta} Sc^{1/3} \quad (6)$$

The coefficient, α, and the exponent, β, can be found either experimentally or from previously developed correlations for the Sherwood number, which are available in literature for various geometries under laminar and turbulent flow conditions. The relationships for the Sherwood, Reynolds and Schmidt numbers are further defined by the following equations, respectively:

$$Sh = \frac{k_L d}{D}, \qquad Re = \frac{du}{\nu} \qquad \text{and} \qquad Sc = \frac{\nu}{D}$$

where *d* is the characteristic length, *D* is the diffusion coefficient for carbon dioxide in the media, *u* is the liquid velocity and *ν* is the kinematic viscosity of the liquid. The mass transfer coefficient, k_L, can easily be calculated from these relationships.

4.3.2 Evapoporometry Model

Evapoporometry was developed by Krantz [27] and operates upon the principal that a volatile wetting liquid will evaporate from a membrane progressing from largest to smallest pores due to the relationship between the pore radius, *r*, and the instantaneous vapor pressure, *P'* (6). This is described by the Kelvin equation,

$$ln\frac{P'}{P} = -\frac{2\sigma V}{RTrcos\theta} \tag{6}$$

where *P* is the vapor pressure of the liquid under normal conditions, σ is the surface tension, *V* is the liquid molar volume, *R* is the gas constant, *T* the absolute temperature, and Θ is the contact angle. In this system, vapor pressure is proportional to evaporation rate according to Irving Langmuir's evaporation equation,

$$W = (P_v - P_P)\sqrt{\frac{m}{2\pi RT}} \tag{7}$$

where *W* is the evaporation rate, P_v is the vapor pressure of the liquid, P_P is the partial pressure of the vapor in the gas, and *m* is the mass of each vapor molecule assuming P_P is negligible because the system is open to the environment and *T* remains constant. An equation describing pore radius as a function of instantaneous evaporation rate can then be derived from Eq. (6),

$$r = -\frac{2\sigma V}{RTcos\theta \ln\left(\frac{W'}{W^\circ}\right)} \tag{8}$$

where *W'* is the instantaneous evaporation rate and *W°* is the normal evaporation rate of the free standing liquid layer found before the volatile liquid begins evaporating from the membrane [27].

4.3.3 Bubble Size Prediction Model

The average bubble size produced by the hollow fiber membranes was determined mathematically based on a two-step mechanism of bubble formation proposed by Ramakrishnan [28]: step 1 being the expansion stage and step 2 being the detachment stage (2). By making a force-balance at the end of the first step Ramakrishnan developed the following equation,

$$V_E^{5/3} = \frac{11}{192\pi(\frac{3}{4\pi})^{2/3}g} Q^2 + \frac{3}{2(\frac{3}{4\pi})^{1/3}g} \times \frac{\mu}{\rho} Q V_E^{1/3} + \frac{\pi D\gamma}{g\rho} V_E^{2/3} \quad (9)$$

Where

$$A = 1 + \frac{96\pi r_E \mu}{11\rho Q}$$

$$B = \frac{16g}{11Q}$$

$$C = \frac{16\pi D\gamma cos\theta}{11\rho Q}$$

$$G = \frac{3\mu}{2\left(\frac{3}{4\pi}\right)^{\frac{1}{3}}\left(\frac{11}{16}\rho\right)}$$

and V_E is the volume of the force-balance bubble (cm^3), g is the acceleration due to gravity (cm/sec^2), Q is the volumetric flow rate of gas (cm^3/sec), μ is the viscosity of the continuous phase (g/cm sec), ρ is the density of the liquid (g/cm^3), D is the orifice diameter (cm), γ is the surface tension (dyn/cm), r_E is the radius of the force-balance bubble (cm), and θ is the contact angle (deg).

Ramakrishnan determined that the second step occurs when the bubble's base has covered a distance that is equal to the radius of the force balance bubble and developed the following equation:

$$r_E = \frac{B}{2Q(A+1)}\left(V_F^2 - V_E^2\right) - \left(\frac{C}{AQ}\right)\left(V_F - V_E\right) - \frac{3G}{2Q\left(A-\frac{1}{3}\right)}\left(V_F^{\frac{2}{3}} - V_E^{\frac{2}{3}}\right) \quad (10)$$

where V_F is the final volume of the bubble (cm^3) [28]. Thus, Equation 10 should give the radius of the bubble as it detaches from the hollow fiber.

4.4 Experimental

4.4.1 Carbon Dioxide Delivery

Three methods of gas delivery were investigated, namely an open tube, a commercial porous diffuser, and a hollow fiber membrane (HFM) manifold. In all experiments, pure CO_2 gas was delivered from a compressed gas cylinder through flexible plastic tubing and a 0.04-0.5

LPM acrylic flowmeter (VWR). The regulator pressure was set at approximately 30 psi and a flowrate of 0.1 LPM was used. This flowrate was chosen based on the maximum allowable flowrate due to the backpressure created from the hollow fibers, and was used for all delivery methods as a control.

For the open tube experiments, a short section of ½ inch CPVC pipe was attached to the flexible tubing coming from the flowmeter with a hose barb fitting. A 12-inch industrial porous diffuser was used as the porous diffuser and was attached to the flexible tubing. HFM manifolds were constructed using hydrophobic polyether ether ketone (PEEK) hollow fiber membranes (Porogen). Eight fibers were looped in sections of 19 and 26.5 inches, for a total fiber length of 304 and 424 inches, respectively. The open ends of the fibers were placed through ½ in. CPVC elbows. The fibers were secured in place by filling the elbows with Conathane (Cytec Industries), a polyurethane epoxy resin, until all fibers were thoroughly coated with no gaps left between the epoxy and the inner wall of the CPVC elbow. Approximately ¼-½ inch of the open ends of the fibers were left protruding from the male end of the CPVC elbow for gas to enter into the fibers. After the polymer was given adequate time to cure, the manifold was completed by installing the elbow into a ½ inch CPVC connection that was used in the open tube experiments. A picture of the HFM bundled is shown in **Figure 4.1**. This manifold offers flexibility in design and may be modified for different geometries (length of fibers, number of elbows, etc.) if necessary, and is ideal for the ATS® application because it can be placed beneath the growth surface mat in order to deliver the CO_2 to the media directly where the algae grows.

All experiments were performed in an Isotemp waterbath (FisherScientific) with inside dimensions of approximately 18x11.5 inches. Deionized water was added to the bath at depths of 1.5, 3 and 5 inches, for a total volume of approximately 5, 10 and 16.7 liters, respectively. A solution of 0.2 N NaOH was used to bring the pH value to ~8 at the beginning of each experiment. A pH meter was used to record the initial pH, and carbon dioxide gas was delivered to the system at 0.1 LPM for 25-45 minutes. Sample volumes of 3 mL were taken and the pH values were recorded every minute for the first five minutes, every 2-3 minutes from 5-15 minutes, and every 5 minutes thereafter for up to 45 minutes or until the pH value remained constant. The water was stirred prior to each sampling to ensure the solution was well mixed. All experiments were done in triplicate and fresh deionized (dI) water was used each time. Due to the buoyancy of the HFMs in the water, a plastic canvas was cut to fit the dimensions of the water bath and was placed over the fibers. Small stainless steel weights were used to ensure that the HFM manifold was submerged and the fibers did not float to the surface of the water. Using the plastic canvas in the experimental setup was also ideal because it is similar to the mesh or carpet that would be used in an ATS® system. As a control, the plastic canvas was also used in the experiments with the open tube and the porous diffuser.

Water depth was the primary parameter considered to compare the performance of the HFM manifold to the other two methods of gas delivery. First, experiments were performed at water depths of 1.5, 3 and 5 inches at room temperature (20°C), and each of the three gas delivery methods was tested. To determine how water temperature may affect the mass transfer in thin-film aqueous systems, further experiments were performed using the HFM manifold. These experiments were carried out as previously described at a water depth of 1.5 inches, but

the water bath was set to temperatures of 20, 30 and 40°C. Again, experiments were done in triplicate for each temperature. An average pH value for each time point was calculated from the three values recorded for each experiment.

4.4.2 Evapoporometry

A diffusion chamber was fabricated using a rectangular Plexiglas container with inside dimensions of 1 7/8" by 7/8". The lid was removed and a sample of hollow fiber membranes with unknown pore size was glued to the bottom of the container with the same Conathane epoxy used in the HFM manifold. Enough epoxy was added to prevent lateral leakage without completely submerging the fibers. A micropipette was used to add 2 ml of 100% isopropyl alcohol (IPA) to fully submerge the fibers. A Mettler Toledo AB104-S/FACT microbalance and Windmill 7 Data Acquisition Software were used to log the mass of the evapoporometry apparatus over time as the IPA evaporated. A data point was collected every 30 seconds until all the IPA had evaporated and was analyzed using Excel by forward differencing the mass-time data to solve for the instantaneous evaporation rate. The same experiment was used on a 100 nm Nuclepore flat sheet membrane in a cylindrical diffusion chamber with a 4 cm base to serve as a control.

4.4.3 Scanning Electron Microscope

The average pore size of a small sample of the hollow fiber membranes used in the experiments was also determined using a Phenom FEI tabletop SEM. A gold sputter coater was used to coat the sample 10 nm thick to allow for the surface morphology to be reflected. The

pore size of the membranes was hand calculated from the pictures taken by the SEM, which are depicted in **Figure 4.2**.

4.4.4 Prediction of Bubble Size

Bubble sizes were determined using two different techniques. For the first, photographs were taken with a Nikon D3100 through the wall of a glass rectangular aquarium, using a ruler to estimate the size of the bubbles for each method of gas delivery. Several pictures were taken using all three gas delivery methods, and 15-25 bubbles were measured visually to the nearest 0.5 mm to approximate the average bubble diameter for each method. Examples of the photographs taken are shown in **Figure 4.3**. The model developed by Ramakrishnan [28] was used as a second method to predict the average bubble size produced by the HFMs as well as the open tube used in the CO_2 bubbling experiments.

4.5 Results and Discussion

4.5.1 Gas Delivery

The total concentration of carbon in the system over time was calculated from the averaged pH values using the carbonic acid dissociation relationships [29-30] mentioned above and TK Solver to solve the equations simultaneously. The results for total carbon concentration versus time were plotted for each method of gas delivery at each depth tested, shown in **Figure 4.4**. From these data it is evident that the HFM manifold is far superior to both the diffuser and the open tube bubbling methods in terms of gas delivery to the system, particularly at shallow depths. At the 1.5” depth, the HFM manifold was able to deliver over 60 times the amount of carbon to the system as the open tube bubbling, and more than 6 times the amount of carbon as

the porous diffuser, in as little time as 25 minutes. As the water depth increases, the superiority of the HFMs to the other methods decreases slightly, but the manifold still serves as a more effective means of delivery for all depths tested. The experimental data for the variable temperatures at 1.5" depth did not show a significant correlation between temperature and the mass transfer coefficient, as depicted in **Figure 4.5.** This was expected over the range of temperatures tested due to the fact that the mass transfer coefficient, kL, is proportional to the absolute temperature. It suggests that HFM will behave similarly over most ranges it is used in an algae growth system.

4.5.2 Membrane Pore Size Determination

A 100 nm Nuclepore flat sheet membrane was used to test the validity of the evapoporometry system. It was found to have an average pore diameter of 103.59 nm using evapopopormetry. This is a 3.59% error from the average pore size reported by the manufacturer (100 nm). Thus, we assumed that system worked well with predicting pore diameters of membranes in this range. Data from three trials using the HFMs showed that the average pore diameter is 26.79 nm with a standard deviation of 13.23 nm. **Figure 4.6** shows the pore size distribution as a function of percent of total pores. As this shows we have a significant amount of pores in the range of 5 nm to 50 nm with little pore area above 50 nm (expect possible defects above 100 nm). This is consistent with the SEM pictures (**Figure 4.2**) and we thus later used this data to predict bubble size.

4.5.3 Bubble Size Prediction

The model developed by Ramakrishnan [28] was used to predict the average bubble size produced by the HFMs as well as the tube used in the CO_2 bubbling experiments. The orifice diameter used in the model for the hollow fiber membranes was 26.79 nm, determined by evapoporometry, and the orifice diameter used for the tube was 1.6 cm, the measured inner diameter of the CPVC elbow. Calculations were done at a flow rate of 0.01 LPM and assuming a contact angle of 0° between the water and the hollow fiber membranes and the tube. The average volume of the bubbles produced by the HFMs and the tube were predicted to be 3.77E-2 cm^3 and 3.67E-1 cm^3 respectively. From the photographs taken with the camera, bubble diameters were estimated to be approximately 2.5±0.4 mm for the HFMs, 1.5±0.6 mm for the diffuser, and 7.5±2.4 mm for the open tube. Thus, assuming spherical bubbles, the bubble volumes were calculated to be 8.18E-3 cm^3, 1.77E-3 cm^3 and 2.23E-1 cm^3, respectively. However, if you compare the range of possible bubble volumes suggested by the standard deviation in visual inspection you get 4.85E-3 to 1.28 E-2 cm^3 for HFMs and 6.95 E-2 to 5.08 E-1 cm^3 for the open tube. This range includes the sizes seen for the open tubes but is still slightly outside what we have seen with the HFMs but is on the same order of magnitude.

4.5.4 Mass Transfer Modeling

The previously described mass transfer correlation for the effective mass transfer coefficient was used to determine the k_La for each delivery method at each depth and temperature by integrating equation 4 and calculating the slope of the line for $-\ln(C^*-C/C^*-C_o)$ vs. time. The value used for C* was 0.26 M, which was the maximum total carbon concentration observed in all of the experiments. The linear portion of the graph, thus the values for the total

carbon concentration from 3 to 15 minutes, was used in the calculations. The k_La values for each set of parameters tested are reported in **Table 4.1**. From experimental observations it was noted that, as gas was delivered to the system via the hollow fiber membrane manifold, tiny microbubbles would form on the surface of the HFMs and stay attached for a short time (approximately 4-15 seconds). These bubbles would coalesce with adjacent bubbles until a large enough bubble was formed and became buoyant enough to release from the HFMs before rising through the water. This can be visualized by the two pictures on the right in **Figure 4.3**. This phenomenon is attributed to the hydrophobicity of the PEEK membrane material. This observation also provides an explanation for the results achieved by Carvalho and Malcata [12], where they concluded that hydrophilic membranes were less effective than bubbling, whilst the highest carbon concentration was achieved with the hydrophobic membranes. It is likely that, due to the HFMs being trapped inside a module, it was not evident that the bubbles were adhering to the membrane surface.

Based on these observations, it was determined that in order to provide an accurate model, the effective mass transfer coefficient should be further broken down into two terms: one for the contribution for the bubbles that remain on the HFM surface and another for the contribution of the bubbles rising through the solution. Equation (3) was modified to depict the parallel mass transfer from the two contributions and is represented by following equation, which was used to develop the model:

$$\frac{dC}{dt} = K_{L-HFM}A_{HFM}/V(C^* - C) + K_{L-liq}A_{liq}/V(C^* - C) \quad (11)$$

where $K_{L\text{-}HFM}$ and $K_{L\text{-}liq}$ are the individual mass transfer coefficients for the bubbles on the membrane surface and those rising through the liquid, respectively, A_{HFM} is the total area of the bubbles on the HFM surface at any point in time, A_{liq} is the total area of the bubbles rising through solution at any point in time, V is the total volume of the liquid, and C* and C are total concentrations of carbon in the system as described above.

By combining equations (3) and (4) and integrating equation (11), we get:

$$-\ln\left(\frac{C^*-C}{C^*-C_o}\right) = k_L a * t = (K_{L-HFM}\frac{A_{HFM}}{V} + K_{L-liq}\frac{A_{liq}}{V}) * t \quad (12)$$

or

$$k_L a = K_{L-HFM}\frac{A_{HFM}}{V} + K_{L-liq}\frac{A_{liq}}{V} \quad (13)$$

A_{HFM} was determined visually from the photographs taken to estimate bubble size. A short segment of fiber was measured from the photographs and it was found that there were approximately 7 bubbles on the HFM surface per inch of fiber length. This number was multiplied by the total fiber length (304 inches) to obtain the total number of bubbles, which was then multiplied by the surface area of a bubble. The bubble diameter used for the HFM mass transfer modeling was 0.25 cm, as determined from the photographs. A_{liq} was calculated by approximating the number of bubbles in the liquid at a given time from the gas flow rate of 0.1 LPM, the liquid height for the given trial, and a bubble rise velocity of 1 ft/s [31], then multiplying that number by the surface area of a bubble with diameter 0.25 cm. Due to the fact

that, when the fibers had gas flowing through them, the fibers would rise approximately ½ inch from the base of the water bath, a liquid height of 1, 2.5 and 4.5 inches was used for the 1.5", 3" and 5" depths, respectively. The liquid volume (V) was calculated based on the same approximation. $K_{L\text{-}HFM}$ and $K_{L\text{-}liq}$ were calculated using Excel Solver by performing a least squares analysis with the constraint that $K_{L\text{-}HFM}$ was constant for all three depths and minimizing the standard deviation between the values for $K_{L\text{-}liq}$. The assumption that the individual mass transfer coefficients are constant regardless of the depth of the liquid is valid because the characteristic length in the Sherwood equation is the bubble diameter, which is independent of the water depth. Based on these assumptions, $K_{L\text{-}HFM}$ was calculated to be 0.84 cm/s and $K_{L\text{-}liq}$ was calculated to be 51.4 cm/s. By evaluating equation (12) with the parameters as described and the individual mass transfer coefficients, we end up with:

$$-\ln\left(\frac{C^*-C}{C^*-C_o}\right) = k_L a * t = (0.84 * \frac{A_{HFM}}{V} + 51.4 * \frac{A_{liq}}{V}) * t \qquad (14)$$

Since the initial (C_o = 0) and saturation (C* = 0.26) concentrations of total carbon are known, equations (5) and (14) can now be used to evaluate what the total concentration of carbon in the system will be at any time, t. **Figure 4.7** shows how the model compares to the actual experimental values. As depicted in **Figures 4.4** and **4.5**, there is a short lag phase of approximately three minutes at the beginning of each experiment. This is likely due to the time necessary for the HFMs to fill up with gas before they begin to deliver gas to the system. To account for this lag phase, the time scale for the experimental data was adjusted in **Figure 4.7** by three minutes, such that t = 3 became t = 0 in order to more accurately represent when CO_2 delivery actually began. As the graph demonstrates, the validity of the model is strong, and the

total concentration of carbon in the system at any time point, t, can be predicted from the model quite well. The hydrophobicity of the HFMs is very significant, particularly at shallow depths. The relative contribution of the mass transfer term for the bubbles adhering to the membrane surface at 1.5 inches is 67.2%, and 32.8% of the mass transfer is attributed to the bubbles rising through the solution. These numbers are nearly reversed in the case of the 5" depth, where 68.7% of the mass transfer is due to the rising bubbles and only 31.3% is from the bubbles on the surface of the hollow fibers. Thus in a shallow depth gaseous delivery systems, such as an ATS® or inside a hollow fiber module, hydrophobic membranes will deliver more carbon dioxide than their hydrophilic counterparts.

4.6 Conclusions

Three methods of gas delivery were investigated at different depths of water in an open system. Of the three methods, the hollow fiber membranes proved to be the most efficient means of delivering carbon dioxide gas into water for each depth tested. The superiority of the HFMs at the shallowest depth is particularly significant due to its potential application in an ATS® system. Further experiments were performed with the HFMs at a depth of 1.5" and three different temperatures to determine what effect, if any, temperature had on the mass transfer. The experimental data confirmed that temperature does not have a significant effect on the carbon concentration in the system. From this it can be concluded that the geometries of the system (i.e., water depth/volume, bubble diameter, fiber length and number of fibers) are the primary factors that affect the mass transfer to the system. Evapoporometry was used to approximate the pore sizes of the HFMs, as well as a membrane of known pore sizes as a control. The evapoporometry model suggested a pore size of approximately 27 nm for the HFMs, which

was a reasonable value based on SEM pictures. Bubble sizes were predicted from the orifice diameter of the HFMs and open tube used in the bubbling experiments using the Ramakrishnan model. Photographs were also taken through a glass aquarium wall and a ruler was used to estimate the bubble diameter visually. These bubble sizes were used to determine some of the parameters necessary to develop a mass transfer model for the HFMs. It was observed that, due to the hydrophobic nature of the membrane material, bubbles would form on the surface of the HFMs before releasing and rising up through the solution. Because of this, it was determined that in order to develop an accurate model, the mass transfer equation should be broken down into two terms: one for the contribution of the bubbles on the fiber surface and one for the contribution of the bubbles rising through the liquid. Finally, a mass transfer model was developed, which can accurately predict the carbon concentration in the system over time.

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4.8 References

[1] A. Gabelman, S. Hwang. Hollow fiber membrane contactors. J. Memb. Sci., 159 (1999) 61-106.

[2] A. Robles, M. Ruano, F. Garcia-Usach, J. Ferrer. Sub-critical filtration conditions of commercial hollow-fibre membranes in a submerged anaerobic MBR (HF-SAnMBR) system: The effect of gas sparging intensity. Bioresour. Technol., 114 (2012) 247-254.

[3] L. Liu, Z. Ding, Y. Lu, R. Ma. Modeling the bubbling enhanced microfiltration for submerged hollow fiber membrane module. Desalination, 256 (2010) 77-83.

[4] J. Tian, Y. Xu, Z. Chen, J. Nan, G. Li. Air bubbling for alleviating membrane fouling of immersed hollow-fiber membrane for ultrafiltration of river water. Desalination, 260 (2010) 225-230.

[5] W. Yang, N. Cicek, J. Ilg. State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America. J. Memb. Sci., 270 (2006) 201-211.

[6] H. Yu, Y. Xie, M. Hu, J. Wang, S. Wang, Z. Xu. Surface modification of polypropylene microporous membrane to improve its antifouling property in MBR: CO_2 plasma treatment. J. Memb. Sci., 254 (2005) 219-227.

[7] H. Yu, M. Hu, Z. Xu, J. Wang, S. Wang. Surface modification of polypropylene microporous membrane to improve their antifouling property in MBR: NH_3 plasma treatment. Sep. Purif. Tech., 45 (2005) 8-15.

[8] P. Westerhoff, Q. Hu, M. Esparza-Soto, W. Vermaas. Growth parameters of microalgae tolerant to high levels of carbon dioxide in batch and continuous-flow photobioreactors. Environ. Technol., 31 (2010) 523-532.

[9] S. Chinnasamy, B. Ramakrishnan, A. Bhatnagar, K. Das. Biomass production potential of wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO_2 and temperature. Int. J. Mol. Sci., 10 (2009) 518-532.

[10] J. Doucha, K. Livansky, J. Machat, P. Novak, D. Umysova, V. Zachleder, M. Vitova. Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. Appl. Microbiol. Biotechnol., 82 (2009) 179-185.

[11] I. De Godos, S. Blanco, P. Gracia-Encina, E. Becares, R. Munoz. Influence of flue gas sparging on the performance of high rate algae ponds treating agro-industrial wastewaters. J. Hazard. Mater., 179 (2010) 1049-1054.

[12] A. Carvalho, F. Malcata. Transfer of carbon dioxide within culture of microalgae: plain bubbling versus hollow-fiber modules. Biotechnol. Prog., 17 (2001) 265-272.

[13] B. Ferreira, H. Fernandes, A. Reis, M. Mateus. Microporous hollow fibres for carbon dioxide absorption: mass transfer model fitting and the supplying of carbon dioxide to microalgal cultures. J. Chem. Technol. Biotechnol, 71 (1998) 61-70.

[14] Kumar, A., Yuan, X., Sahu, A., Dewulf, J., Ergas, S., Langenhove, H. "A hollow fiber membrane photo-bioreactor for CO_2 sequestration from combustion gas coupled with wastewater treatment: A process engineering approach." J. Chem. Technol. Biotechnol, 85 (2010) 387-394.

[15] H. Matsumoto, N. Shioji, A. Hamasaki, Y. Ikuta, Y. Fukuda, M. Sat, N. Endo, T. Tsukamoto. Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. App. Biochem. Biotechnol., 51/52 (1995) 681-692.

[16] D. Brune, T. Lundquist, J. Benemann. Microalgal biomass for greenhouse gas reductions: potential for replacement of fossil fuels and animal feeds. J. Environ. Eng., 135 (2009) 1136-1144.

[17] J. Doucha, F. Straka, K. Livansky. Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. J. Appl. Phycol., 17 (2005) 403-412.

[18] G. Pokoo-Aikins, A. Nadim, M. El-Halwagi, V. Mahalec. Design and analysis of biodiesel production from algae grown through carbon sequestration. J. Clean Technol. Env., 12 (2010) 239-254.

[19] I. Douskova, J. Doucha, K. Livansky, J. Machat, P. Novak, D. Umysova, V. Zachleder, M. Vitova. Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. Appl. Microbiol. Biotechnol., 82 (2009) 179-185.

[20] H. Matsumoto, A. Hamasaki, N. Sioji, Y. Ikuta. Influence of CO_2, SO_2 and NO in flue gas on microalgae productivity. J. Chem. Eng. Jpn., 1996, pp. 620-624.

[21] E. Dlugokencky, P. Tans. (2013). Trends in Atmospheric Carbon Dioxide. Earth System Research Laboratory, National Oceanic & Atmospheric Association. [Online]. Available: http://www.esrl.noaa.gov/gmd/ccgg/trends/global.html. [2013, May 19].

[22] J. Welty, C.E. Wicks, R.E. Wilson, G.L. Rorrer. Gas-liquid mass transfer operations in well-mixed tanks, in: J. Welter, L. Sapira and P. McFadden (Eds.), Fundamentals of Momentum, Heat, and Mass Transfer, Fifth ed., Wiley, Danver, Massachusetts, 2008, pp. 603-610.

[23] W. Adey. Algal turf scrubber. US Patent No. 4,333,263. June 8, 1982.

[24] W. Adey. Algal turf water purification method. US Patent No. 5,851,398. December 22, 1998.

[25] E. Becker, Microalgae: Biotechnology and Microbiology, Vol. 10, J. Baddiley (Ed.), Cambridge Univ. Press, Cambridge, New York, 1994.

[26] J. Hoffmann. Wastewater treatment with suspended and nonsuspended algae. J. Phycol., 34 (1998) 757-763.

[27] W. Krantz, A. Greenberg, E. Kujundzic, A. Yeo, S. Hosseini. Evapoporometry: A novel technique for determining the pore-size distribution of membranes. J. Memb. Sci., 438 (2013) 153-166.

[28] S. Ramakrishnan. Studies in bubble formation-I bubble formation under constant flow conditions. Chem. Eng. Sci., 24 (1969) 731-747.

[29] International Atomic Energy Agency. Environmental Isotopes in the Hydrological Cycle: Principles and Applications, Vol. 1. Chemistry of carbonic acid in water. Vienna, 2000, pp. 87-98.

[30] H. Ruud. (2012). Carbon dioxide in water equilibrium. [Online]. Available: http://www.pwtag. org/researchdocs/Used%20Ref%20docs/52%20Carbondioxide%20in%20water%20equilibrium.pdf. [2013, January 30].

[31] D. Steinmeyer, W. Penney, B. Crocker. Liquid-Gas Systems, in: R. Perry, D. Green, J. Maloney (Eds.), Perry's Chemical Engineers' Handbook, Sixth ed., McGraw Hill, New York, 1984, pp. 18-18–18-61.

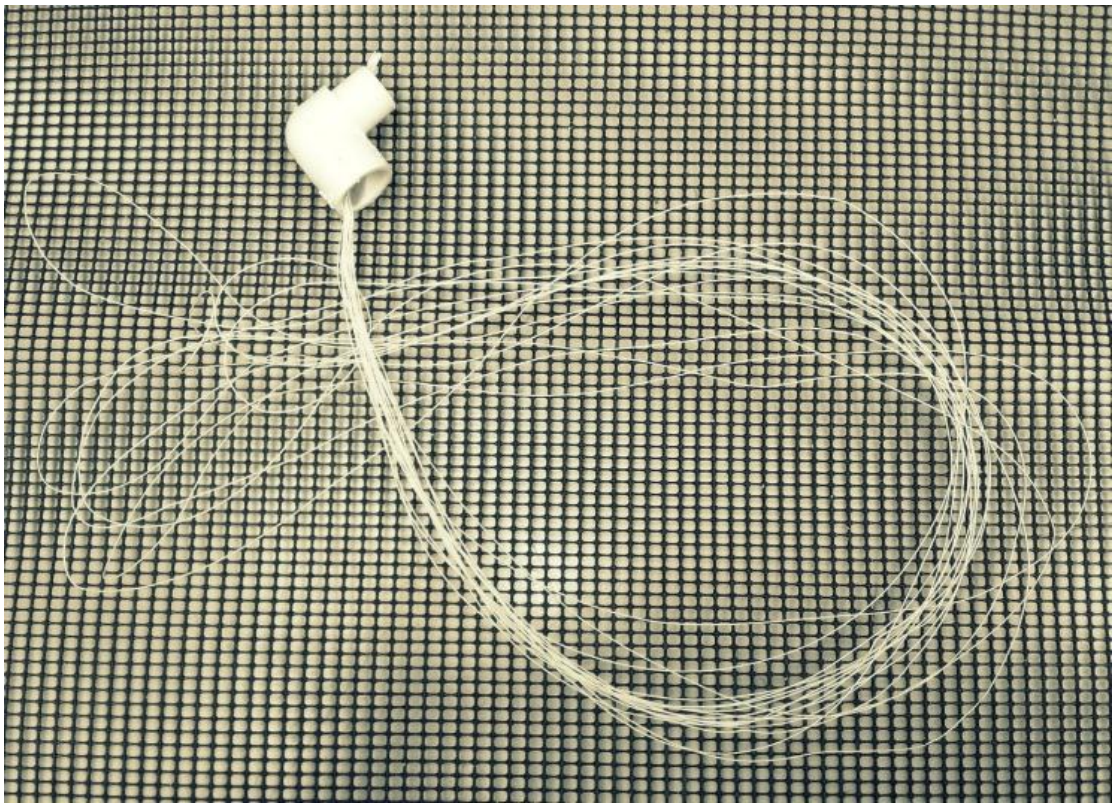

Figure 4.1: Hollow fiber membrane bundle.

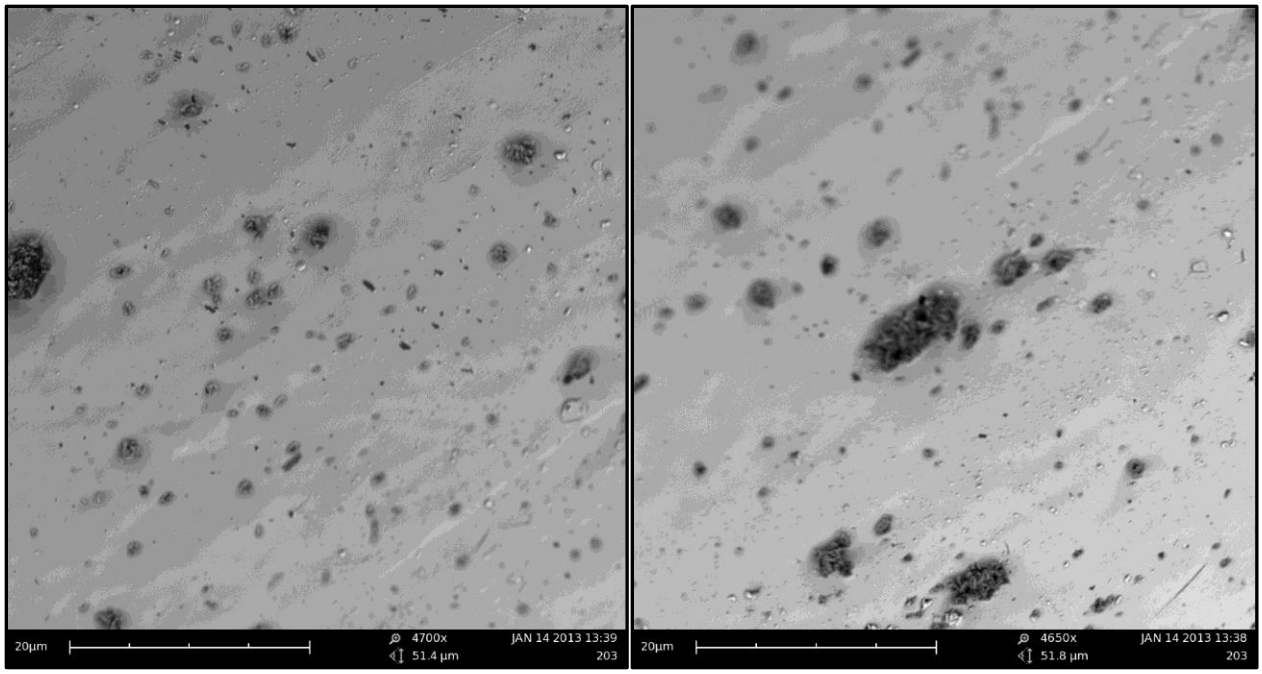

Figure 4.2: SEM pictures of the hollow fiber membranes used in the approximation of the average pore diameter.

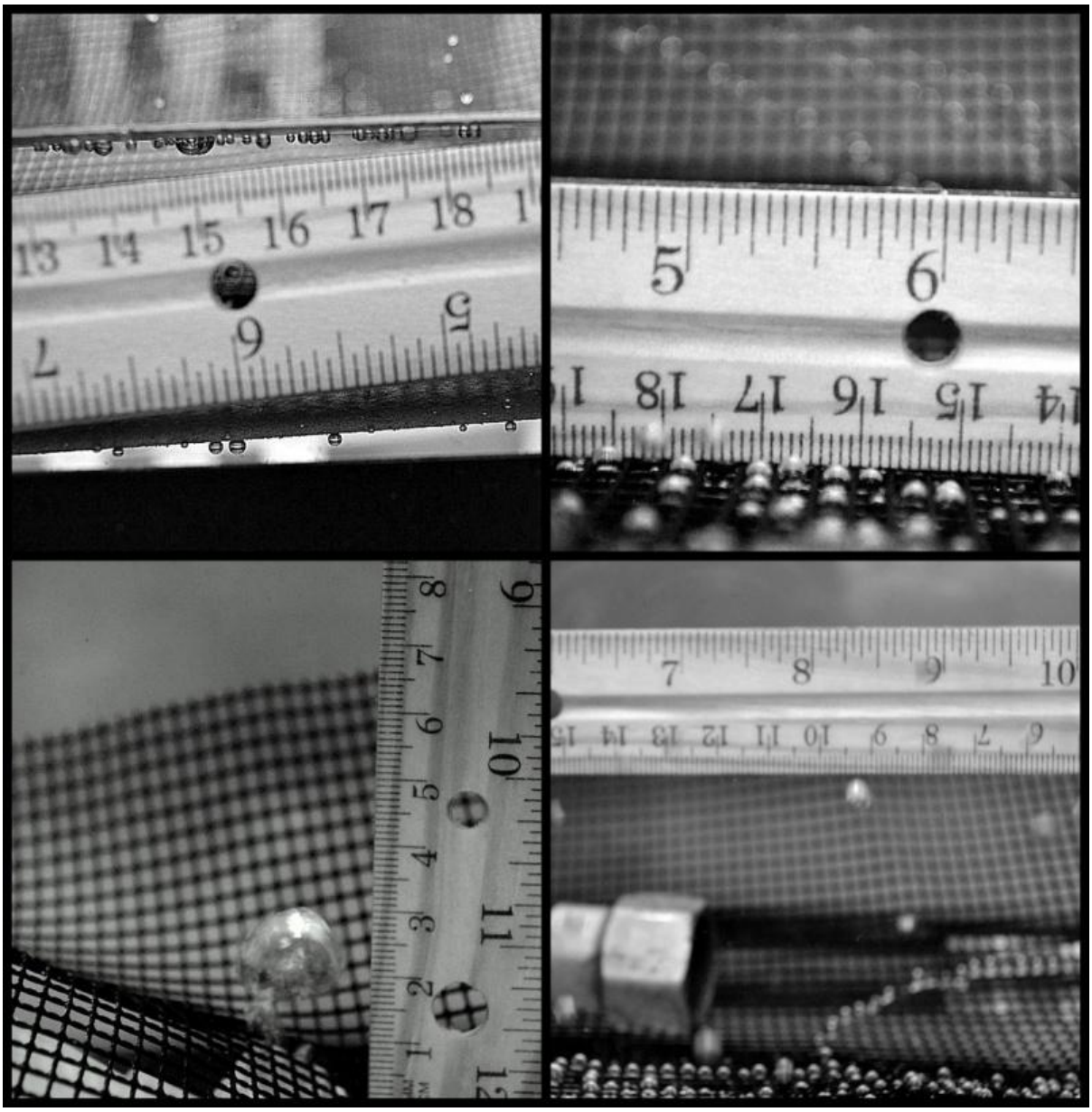

Figure 4.3: Photographs taken to estimate bubble size: diffuser (top left), open tube (bottom right), HFM surface (top right), bubbles from HFMs in solution (bottom right).

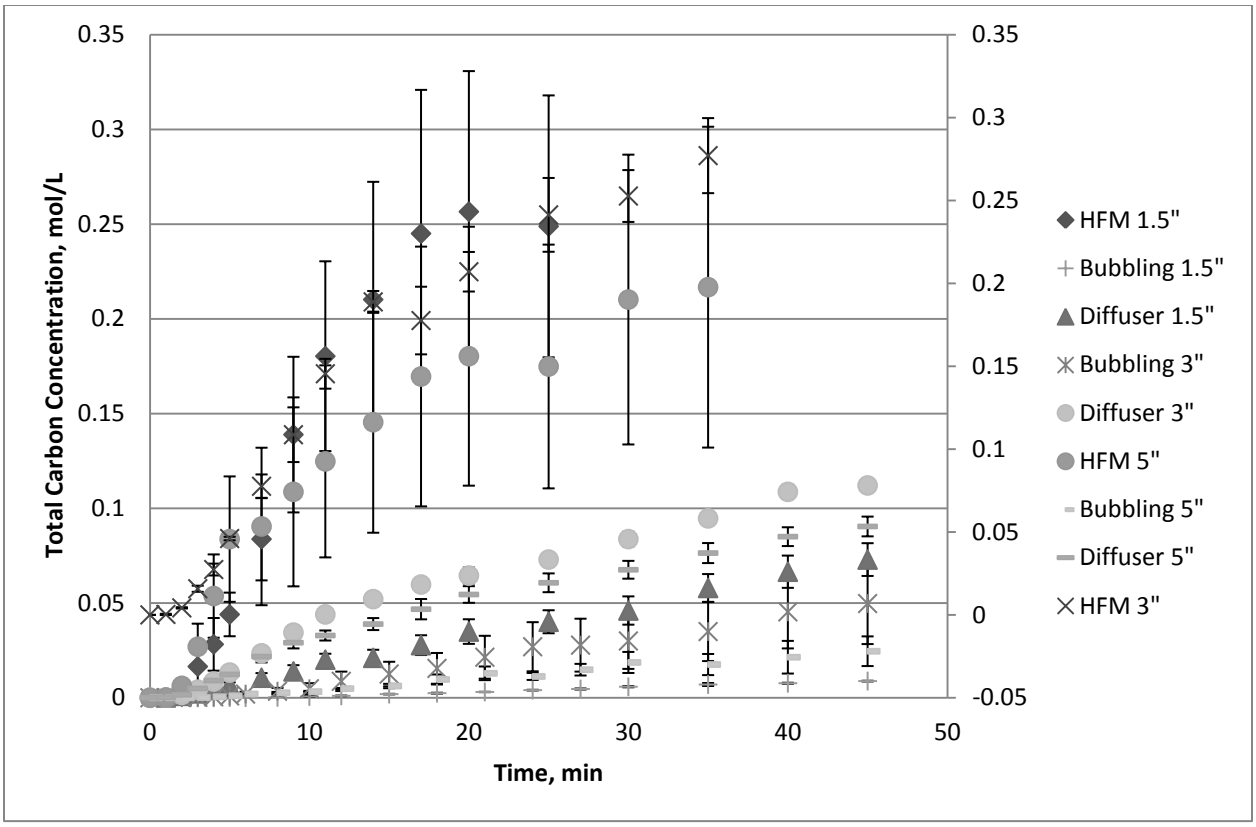

Figure 4.4: Total carbon concentration for all delivery methods at 20°C and three different depths tested.

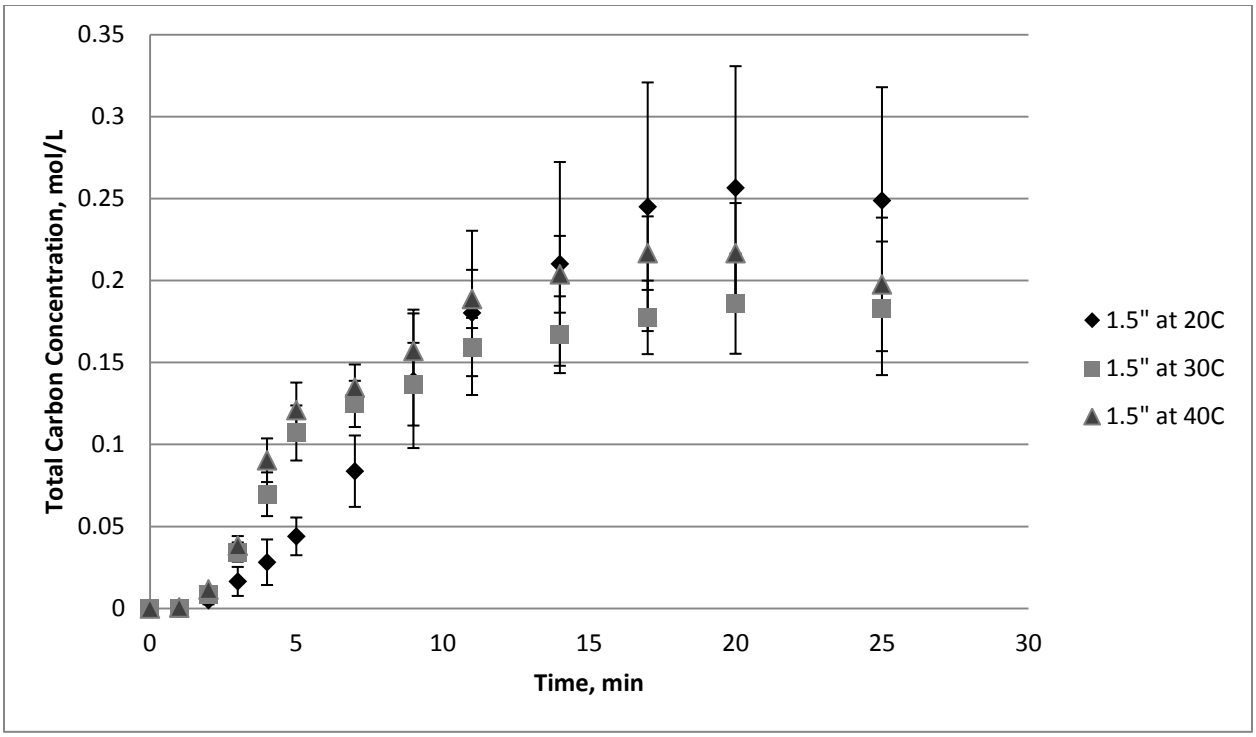

Figure 4.5: Experimental data for HFMs at 1.5” depth and variable temperatures tested.

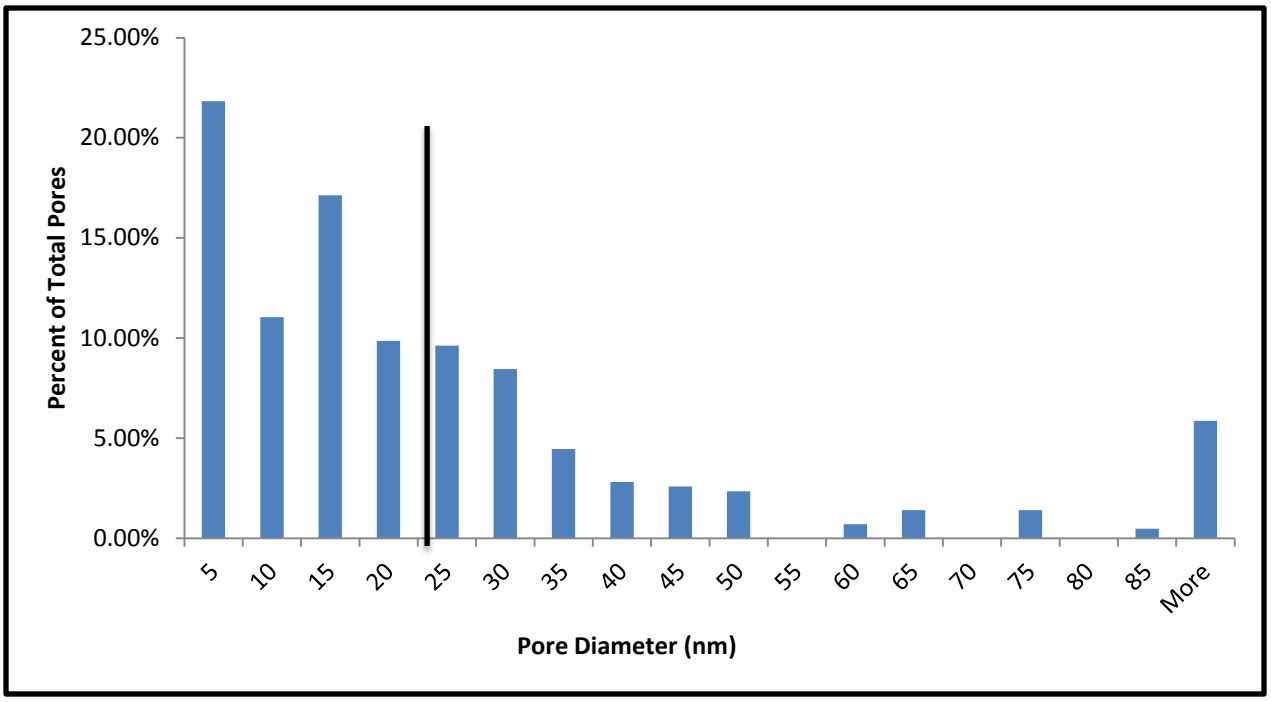

Figure 4.6: Evapoporometry determined pore size distribution as a function of percent of total pores for the hollow fiber membranes. The solid black line depicts the average pore diameter.

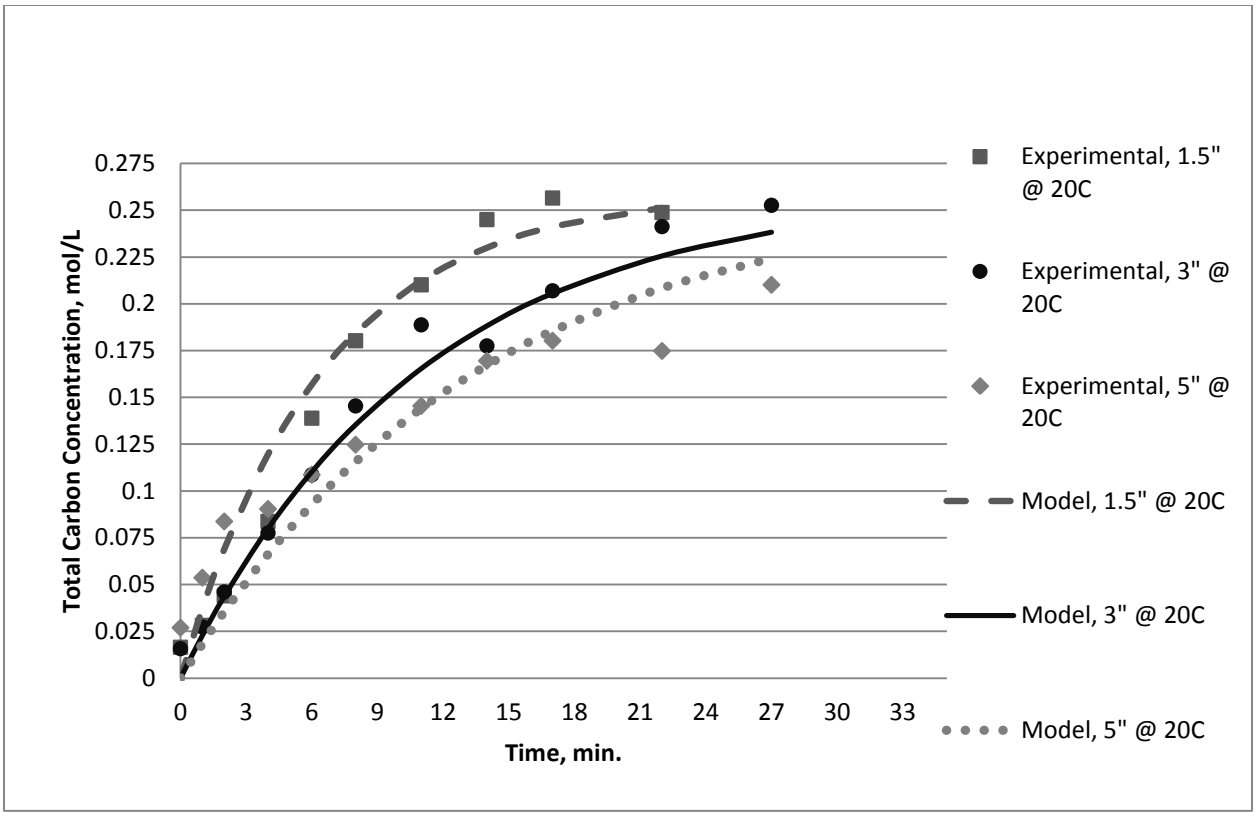

Figure 4.7: Comparison of mass transfer model and experimental data for HFMs at 20°C and different depths.

Delivery Method	Water Depth	Temperature	$k_L a$ (s^{-1})
HFM	1.5"	20°C	1.50E-01
Diffuser	1.5"	20°C	7.40E-03
Open Tube	1.5"	20°C	5.90E-04
HFM	3"	20°C	1.10E-01
Diffuser	3"	20°C	1.97E-02
Open Tube	3"	20°C	4.03E-03
HFM	5"	20°C	5.97E-02
Diffuser	5"	20°C	1.34E-02
Open Tube	5"	20°C	1.96E-03
HFM	1.5"	30°C	7.76E-02
HFM	1.5"	40°C	1.18E-01

Table 4.1: Effective mass transfer coefficients, $k_L a$, for each set of parameters tested.

4-A Appendix – Chapter 4

4-A.1 Documentation from major professor

3202 Bell Engineering Center • Fayetteville, Arkansas 72701-1201 • (479) 575-4951 • (479) 575-7926 (FAX)

College of Engineering

Department of Chemical Engineering

MEMORANDUM

To: Dean of Graduate School, University of Arkansas

From: Robert R. Beitle, PhD PE
Professor, Chemical Engineering

Re: Lauren Merriman statement of work for dissertation

This memorandum confirms that Lauren Merriman, a PhD candidate in the Ralph E. Martin Department of Chemical Engineering, completed over 51% of the work submitted as a manuscript for publication. The manuscript, reflected in her dissertation, was titled:

Carbon dioxide gas delivery to thin-film aqueous systems via hollow fiber membranes

4-A.2 Raw data and calculations

Status	Rule
Satisfied	K1 = H*HCO3/H2CO3
Satisfied	K2 = H*CO3/HCO3
Satisfied	Kw = H*OH
Satisfied	Ctot = CO3+HCO3+H2CO3
Satisfied	2*CO3+HCO3+OH = H
Satisfied	pH = -log(H)

Table 4-A.1: Example of rule sheet from TK solver (HFM at 1.5" and 20°C).

Status	Input	Name	Output	Unit	Comment
	.0000004188	K1			
LF		H	.000323593656929628		
LF		HCO3	.00032359353222671		
LF		H2CO3	.250030598022962		
	4.3E-11	K2			
LF		CO3	4.29999834291391E-11		
	6.81E-15	Kw			
		OH	2.10449118954176E-11		
LF		Ctot	.250354191598189		
L	3.49	pH			
	293.15	T			

Table 4-A.2: Example of input/output sheet from TK solver (HFM at 1.5" and 20°C).

pH	[H+]	[HCO3-]	[H2CO3]	[CO3-2]	**[Ctotal]**
7.49	3.24E-08	-2E-07	-1.37E-08	-2.36E-10	-2E-07
4.99667	1E-05	1E-05	0.000242	4.30E-11	0.00025
4.34333	4.5E-05	4.5E-05	0.004913	4.30E-11	0.00496
4.08333	8.3E-05	8.3E-05	0.016268	4.30E-11	0.01635
3.96667	0.00011	0.00011	0.027839	4.30E-11	0.02795
3.87	0.00013	0.00013	0.04345	4.30E-11	0.04359
3.73	0.00019	0.00019	0.082793	4.30E-11	0.08298
3.62	0.00024	0.00024	0.137402	4.30E-11	0.13764
3.56333	0.00027	0.00027	0.178375	4.30E-11	0.17865
3.53	0.0003	0.0003	0.207966	4.30E-11	0.20826
3.49667	0.00032	0.00032	0.242467	4.30E-11	0.24279
3.48667	0.00033	0.00033	0.253894	4.30E-11	0.25422
3.49333	0.00032	0.00032	0.246226	4.30E-11	0.24655

Table 4-A.3: Example of TK solver list variables (average of three trials - HFM at 1.5" and 20°C): pH as input, concentrations as outputs.

Depth (in)	k_La =	$K_{L-HFM}*(A_{HFM}/V_{liq})$	$(A/V)_{HFM}$	$*K_{L-HFM}$ (cm/s)	$(A/V)_{liq}$	K_{L-liq} (cm/s)
1.5	0.149989		0.123177112	0.840762493	0.000983	47.24561019
3	0.109911		0.049270845	0.840762493	0.000983	69.69390071
5	0.059693		0.027372692	0.840762493	0.000983	37.32565491
				*assume constant		51.42172194
						16.5832978

Table 4-A.4: Excel calculations used to calculate mass transfer coefficients for the model.

Time		1.5"		3"		5"	
Actual	Shifted	Model C	Experimental C	Model C	Experimental C	Model C	Experimental C
0		0	0	0	0	0	0
1		0.037130053	0.000254737	0.022842134	0.000283055	0.018435343	0.000146219
2		0.068957641	0.005003158	0.043677487	0.004229125	0.035563525	0.006292304
3	0	0	0.016499427	0	0.015758423	0	0.026935342
4	1	0.037130053	0.02820182	0.022842134	0.027351186	0.018435343	0.053681621
5	2	0.068957641	0.043983055	0.043677487	0.046052699	0.035563525	0.083737191
7	4	0.11962622	0.083737191	0.080017578	0.077557324	0.066262573	0.090409773
9	6	0.156856392	0.138900031	0.110252885	0.108675995	0.092762518	0.108675995
11	8	0.184212315	0.180281273	0.135408953	0.145440472	0.115637728	0.124760485
14	11	0.212265418	0.210165775	0.165446111	0.18876826	0.14421992	0.145440472
17	14	0.229934557	0.245006133	0.188241731	0.177534335	0.167143143	0.169550005
20	17	0.241063396	0.256545324	0.205541646	0.206966485	0.185527812	0.180278508
25	22	0.251236191	0.248801291	0.2256137	0.241279988	0.208442204	0.17483195
30	27	0.255944133		0.238287673	0.252643628	0.224306054	0.210165775
35	32	0.258122956		0.24629032	0.277002267	0.235288746	0.216713537

Table 4-A.5: Excel calculations comparing experimental calculated values to model predicted values for total carbon concentration vs. time.

CHAPTER 5

Synergistic effect of abrasive and sonication for release of carbohydrate and protein from algae

A paper published in *Separation Science and Technology* (Woods et al. 2010)

Lauren Woods, Michael Riccobono, Nathan Mehan, Jamie Hestekin, Robert Beitle

5.1 Abstract

While algae have demonstrated significant potential as a raw material due to the high intracellular concentration of carbohydrates and proteins, a primary limitation as a biofuel feedstock is due to the fact that an economically feasible method of extraction has yet to be offered. Algae samples, acquired from a local waste treatment facility, were combined with abrasive materials and subjected to ultrasonication for specific time intervals. It was found that the synergistic effect of sonication in the presence of abrasive material, such as silicon beads or sand, could extract adequate protein and carbohydrate to be utilized in fermentation processes.

5.2 Introduction

Algae are unicellular, photosynthetic organisms that grow in many diverse habitats by harvesting energy from the sun to convert water and carbon dioxide into biomass. This biomass, consisting of carbohydrates, proteins and lipids, can be converted to fuel using either catalytic (e.g. biodiesel) or fermentation routes (e.g. alcohols). One of the impediments of using algae lies in extracting material from the biomass, because algae can have extremely durable cell walls. Traditional techniques of chemical lysis have proven to be ineffective or prohibitively difficult,

with most methods of cell disruption designed to recover the fatty acids and methyl esters fraction for subsequent catalysis [1-4]. While this technology is appropriate for biodiesel production, it is not necessarily appropriate for recovery of the fermentable material, specifically carbohydrate and protein, from the algal feedstock. Few studies have examined recovery of non-oil fractions and, when reported, have focused on recovering specific small molecules such as lutein, β-phycoerythrin, phytol, β-carotene, membrane proteins, etc., rather than that deemed raw material for ethanol or butanol production [5-10]. In order to commercialize algal biofuel production, an effective and economical method for the extraction of fermentable materials must be developed.

Ultrasonic cell disruption, the focus of this investigation, employs high-frequency sound waves to destroy cell walls [11-13]. It is typically encountered on laboratory scales, but has found applications in larger systems [14, 15]. As a possible improvement to reduce the time involved and hence energy costs, it was hypothesized that the combination of sonication with the addition of abrasive materials would enhance carbohydrate / protein release. The term sonic abrasion will be used to describe sonication of algae in the presence of a material that aids with mechanical disruption. We report on the use of sonic abrasion for the release of carbohydrate / protein fractions from algal biomass recovered from a wastewater treatment facility, and demonstrate that is shows promise due to the short processing time and to the properties of the material generated by the technique.

5.3 Materials and Methods

Algae were bulk-harvested from an Algal Turf Scrubber located in Springdale, AR. By visual analysis, the majority of the collected sample was determined to be the green filamentous algae, *Cladophora*. The algae were cleaned of foreign debris and towel-dried in a thin sheet by applying pressure until water was no longer visibly absorbed by unsoiled towels. It was then divided into five gram samples to be individually treated. Algae samples were sonicated with inert debris to measure the effect of abrasive particles on cell lysis. Two forms of abrasives were selected based on particle size, accessibility, and scalability: 0.1 mm silicon milling beads (Midwest Scientific) and river (pool) filter sand (Burton). Samples of towel-dried algae were combined with their equal weight in abrasive material and placed in 50 ml stainless-steel cups containing 25 ml of deionized water. The samples were sonicated for 2, 5, and 15 minutes with a Model 2000 Misonix Ultrasonic Cell Disruptor (Newtown, CT). As controls, algae samples were sonicated without abrasives, or were left untreated to be used as a baseline for spectrophotometer readings. A second set of controls were prepared using glycerol as a lysis agent. These samples contained 50% (v/v) glycerol. Each sample was then centrifuged for 20 minutes at 14,000 rpm to remove cell debris and abrasive particles.

The concentration of carbohydrates was determined using a Beckman-Coulter DU 800 Spectrophometer (Fullerton, CA). To quantify carbohydrate release, the phenol-sulfuric acid reaction was used. This simple method of analysis was chosen because the reagents are inexpensive, and it provides rapid, reproducible results (Dubois et al., 1955). Directly following centrifugation, 0.5 ml of each supernatant was separated into glass test tubes. The samples were then mixed with 0.5 ml of 5% phenol solution and 2.5 ml concentrated sulfuric acid. These

experiments were performed in a cold water bath to prevent overheating of the glass tubes upon reaction of the reagents. After an incubation time of 15 minutes, the samples were analyzed at a fixed wavelength of 488 nm. Further characterization of the material was performed using an Aminex HPLC column with refractive index monitor. For HPLC runs, the mobile phase was 0.5% sulfuric acid.

To quantify protein release, a BioRad DC Protein Assay kit was used (Hercules, CA). 100µl of each sample was mixed with 500µl of reagent A (an alkaline copper tartrate solution) in a test tube and vortexed. 4.0ml of reagent B (a dilute Folin Reagent) was then added to each test tube and vortexed. Following a 15 minute incubation time, absorbances were read at 750nm, and the values were compared to a BSA standard curve to determine concentration.

5.4 Results

Data were taken from the spectrophotometer readings, and the concentrations of protein and carbohydrate present in the extract were determined in g/l using a standard BSA curve for the BioRad assay and a standard glucose curve for the phenol-sulfuric acid assay. For each time/abrasive combination, the procedure was performed three times to determine an average value. Time course trajectories of carbohydrate / protein release are presented in **Figures 5.1 and 5.2.** When algae was sonicated without any treatment or in the presence of glycerol, samples presented approximately 0.7 mg/ml glucose equivalent. Compared to these controls, the addition of either abrasive material increased the carbohydrate release 1.6 fold, yielding a mixture that contained approximately 1.1 mg/ml. Time course trajectories showed that when abrasives were present the carbohydrate release occurred faster, with about seventy percent of

the release occurring within the first five minute period. Both silica and river sand yielded similar final results. As indicated by the figures, gains in were made when abrasives were combined with ultrasonic cell disruption. Note that the concentration of glucose equivalent and protein are on the order of a gram per liter (g/l) because of the experimental conditions chosen for the work (volume of fluid used to process the algae).

Figure 5.2 shows that protein release was compromised by the addition of the abrasive material. All treatments, within the first five minutes of sonication, released 60 – 70 percent of the total protein released. However, when either sand or silica was added, the amount of protein recovered at the end of the treatment decreased by 15%. This decrease was attributed to nonspecific adsorption of protein, typical of silica via ion exchange or similar mechanism. Note however, despite this unfortunate effect, the material prepared by sonication with abrasive produces acceptable level of protein as a raw material for fermentation.

Various sugars were identified using HPLC analysis. **Table 5.1** lists several C5 and C6 sugars, identified by retention times, present in the samples after sonication. C5 sugars included xylose and arabinose, and C6 sugars included glucose, galactose, and mannose. Xylose, which had an average value of about 1 g/l, comprised the majority of the carbohydrate recovered, representing approximately 60% of the recovered fermentable material that was identified. Glucose and arabinose were the second and third most abundant sugar, with values of 0.37 and 0.18 g/l respectively. Other carbohydrates were present based on the presence of additional peaks within the chromatogram (data not shown) with retention times significantly shorter than glucose. Note that the phenol sulfuric acid values used to track release of carbohydrates will not

map one to one with the HPLC values, because carbohydrate values reported via acid treatment will under report values. It was, however, very encouraging that the two assay methods for carbohydrate was on the same order of magnitude and the two major constituents were captured with the phenol sulfuric assay, as this method would be favored for quick analysis compared to HPLC.

5.5 Discussion

It has been demonstrated that sonic abrasion may be useful for the recovery of the carbohydrate / protein fraction from algae. Currently, 0.1 mm silica and 1 mm river sand have been tested as abrasive materials. The former was chosen because it was hypothesized that smaller abrasives would perform favorably by utilizing less power during sonication, and the latter was chosen because of availability and inexpensive material. These two test abrasives will continue to serve as materials of interest, and will be examined alongside materials with a range of particle diameters and hardness. It will also be necessary to scale the process from the small batches to larger ones in order to demonstrate feasibility.

The ratio of abrasive:biomass:water will be examined to seek optimal combinations based on our initial findings. The ratio chosen as a starting point was 1:1:5 (weight basis), which has routinely yielded 0.10 - 0.30 g carbohydrate equivalent per gram dry algae. It is likely that this ratio of abrasive:biomass:water can be varied widely, with the amount of water and abrasive substantially cut at the expense of longer sonication time. Smaller overall volumes will favor fermentation, because as the concentration of available carbohydrate and protein increase, higher volumetric productivities of biofuels may be realized. Note that our current formulation results

in a medium that contains at minimum 1 g/l glucose equivalent, determined by the phenol sulfuric acid method of analysis. This value does not account for branched sugars like starch and xylohexaose which are likely present. Thus, it is likely that as we proceed with fermentation experiments there will be more available carbon to produce either ethanol or butanol via fermentation with Clostridium or similar species capable of catabolism with five carbon sugars.

5.6 Acknowledgements

The authors would like to thank the following sources of support: the NSF Research Experience for Undergraduates program (MR and ND), and the University of Arkansas. LW is a Department of Defense SMART Research Fellow.

5.7 References

1. Aresta, M., Dibenedetto, A., Carone, M., Colonna, T., Fragale, C., Production of biodiesel from macroalgae by supercritical CO_2 extraction and thermochemical liquefaction. Environmental Chemistry Letters, 2005. 3: p. 136-139.

2. Hossain, A., Salleh, A., Boyce, A., Chowdhury, P., Naqiuddin, M., Biodiesel Fuel Production from Algae as Renewable Energy. American Journal of Biochemistry and Biotechnology, 2008. 4(3): p. 250-254.

3. Nagle, N., Lemke, P, Production of Methyl Ester Fuel from Micoralgae. Applied Biochemistry and Biotechnology, 1990. 24/25: p. 355-361.

4. Nys, R., Dworjanyn, S., Steinberg, P., A new method for determining surface concentrations of marine natural products on seaweeds. Marine Ecology Progress Series, 1998. 162: p. 79-87.

5. Benavides, J., Rito-Palomares, M., Simplified two-stage method to B-phycoerythrin recovery from Porphyridium cruentum. Journal of Chromatography B, 2006. 844: p. 39-44.

6. Ceron, M., Campos, I., Sanchez, J., Acien, F., Molina, E., Fernandez-Sevilla, J., Recovery of Lutein from Microalgae Biomass: Development of a Process for Scenedesmus almeriensis Biomass. Journal of Agricultural and Food Chemistry, 2008. 56: p. 11761-11766.

7. Disch, A., Schwender, J., Muller, C., Lichtenthaler, H., Rohmer, M., Distribution of the mevalonate and glyceraldehyde phosphate/pyruvate pathways for isoprenoid biosynthesis in unicellular algae and the cyanobacterium Synechocystis PCC 6714. Biochemical Journal, 1998. 333: p. 381-388.

8. Hedenskog, G., Enebo, L., Vendlova, J., Prokes, B., Investigation of Some Methods for Increasing the Digestibility in Vitro of Microalgae. Biotechnology and Bioengineering, 1969. XI: p. 37-51.

9. Nagao, R., Ishii, A., Tada, O., Suzuki, T., Dohmae, N., Okumura, A., Iwai, M., Takahashi, T., Kashino, Y., Enami, I., Isolation and characterization of oxygen-evolving thylakoid membranes and Photosystem II particles from a marine diatom Chaetoceros gracilis. Biochemica et Biophysica Acta, 2007. 1767: p. 1353-1362.

10. Popov, N., The efficacy of methods for the destruction of algae cells and possibilities for keeping the soluble protein complex. Comptes rendus de I' Academie bulgare des Sciences, 1974. 27(2): p. 243-246.

11. Agrawal, P., Pandit, A., Isolation of a-glucosidase from Saccharomyces cerevisiae: cell disruption and adsorption. Biochemical Engineering Journal, 2003. 15: p. 37-45.

12. Kapucu, H., Gulsoy, N., Mehmetoglu, U., Disruption and protein release kinetics by ultrasonication of Acetobacter peroxydans cells. Biochemical Engineering Journal, 2000. 5: p. 57-62.

13. Lorinez, A., Ultrasonic Cellular Disruption of Yeast in Water-based Suspensions. Biosystems Engineering, 2004. 89(3): p. 297-308.

14. Destaillats, H., Lesko, T., Knowlton, M., Wallace, H., Hoffmann, M., Scale-up of sonochemical reactors for water treatment. Industrial and Engineering Chemistry Research, 2001. 40: p. 3855-3860.

15. Gogate, P., Mujumdar, S., Thampi, J., Wilhelm, A., Pandit, A., Destruction of phenol using sonochemical reactors: scale up aspects and comparison of novel configuration with conventional reactors. Separation and Purification Technology, 2004. 34(1-3): p. 25-34.

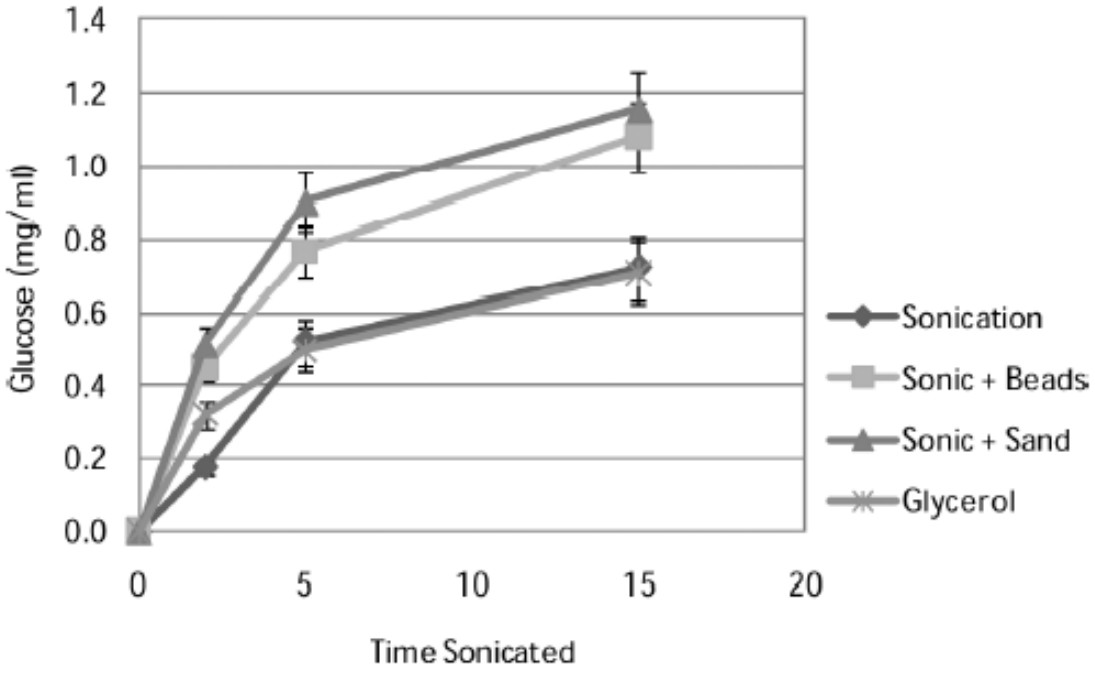

Figure 5.1: Time course trajectories for carbohydrate release under various treatments.

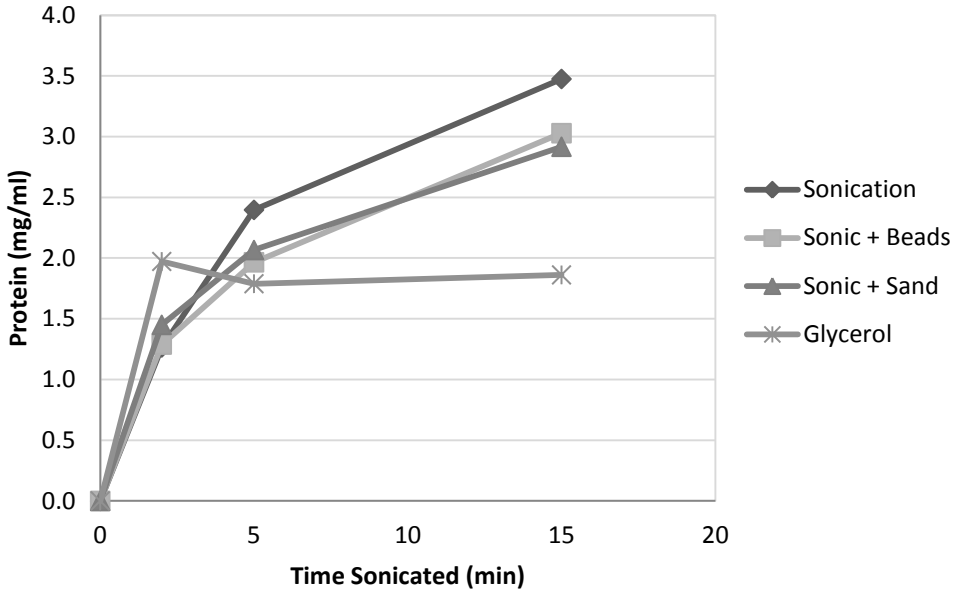

Figure 5.2: Time course trajectories for protein release under various treatments.

Carbohydrate	Concentration (g/l)
xylose	0.97
glucose	0.37
arabinose	0.18
galactose	0.09
mannose	0.06

Table 5.1: Summary of HPLC analysis of carbohydrates released via sonic abrasion.

5.8 Appendix – Chapter 5

5.8-A.1 Permissions

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Title: Synergistic Effect of Abrasive and Sonication for Release of Carbohydrate and Protein from Algae

Author: L. Woods, M. Riccobono, N. Mehan et al.

Publication: Separation Science and Technology

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5.8-A.2 Documentation from major professor

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College of Engineering
Department of Chemical Engineering

MEMORANDUM

To: Dean of Graduate School, University of Arkansas

From: Robert R. Beitle, PhD PE
Professor, Chemical Engineering

Re: Lauren Merriman statement of work for dissertation

This memorandum confirms that Lauren Merriman, a PhD candidate in the Ralph E. Martin Department of Chemical Engineering, completed over 51% of the work submitted as a manuscript for publication. The manuscript, reflected in her dissertation, was titled:

Synergistic effect of abrasive and sonication for release of carbohydrate and protein from algae.

CHAPTER 6

Microbial production of 1,2-propanediol from algal derived sugars

6.1 Abstract

A native strain of *Thermoanaerobacterium thermosaccharolyticum* was utilized in a preliminary study to examine the conversion of common sugars to 1,2-propanediol. A defined medium consisting of salts, vitamins and a 10 g/L sugar solution that is representative of what could be derived from algae was used to investigate the hypothesis that sugars extracted from algae could be converted to 1,2-propanediol via an anaerobic fermentation process. Medium consisting of 10 g/L glucose was also used as a substrate to serve as a benchmark. Products were measured by GC analysis and HPLC analysis was used to monitor sugar consumption. It was determined that 1,2-propanediol yields were nearly doubled when the synthetic algal sugar media was used versus the glucose media.

6.2 Introduction

Successful studies involving the production of energetic precursors such as butanetriol [1], triacetic acid lactone [2] and phloroglucinol [3] from genetically engineered microorganisms have sparked interest in further developing pathways to produce microbial synthesis of other components used in formulations of energetic material. 1,2-propanediol, a precursor to propylene glycol dinitrate (PGDN), is of particular interest to the Navy. PGDN is the primary component of Otto fuel, a propellant used in torpedoes and produced by continuous nitration of 1,2-propanediol.

Thermoanaerobacterium thermosaccharolyticum is a thermophilic anaerobe that is capable of producing R-1,2-propanediol from common 5- and 6-carbon sugars, such as glucose and xylose. Dihydroxyacetonephosphate (DHAP), an intermediate in glycolysis, is converted to methylglyoxal by the enzyme methylglyoxal synthase. Methyglyoxal is then reduced to either lactaldehyde, by glycerol dehydrogenase, or acetol, methyglyoxal reductase. Finally, reduction to 1,2-PD is achieved by native activity [4]. It is hypothesized that sugars recovered from algae could serve as a feedstock for the fermentation of 1,2-propanediol from *T. thermosaccharolyticum*, thereby reducing costs whilst enhancing sustainability.

While the production of 1,2-PD can be accomplished natively by *T. thermosaccharolyticum*, the titers are somewhat low. Furthermore, improvements by metabolic engineering are limited because the organism is not very well characterized [5]. Optimum culture conditions for 1,2-propanediol production via *T. thermosaccharolyticum* (formerly *Clostrdium thermosaccharolyticum*) have been established at 60°C, a pH of 6.0 and fermentation under a nitrogen atmosphere [6]. In a specific study by Altaras *et al*, it was discovered that biomass such as corn, wood byproducts and cheese whey could serve as a feedstock, and *T. thermosaccharolyticum* could ferment a broader list of sugars than previously realized [7]. This shows promise in regards to using algae as a feedstock for 1,2-PD production, as extraction processes from algal biomass typically yield a mixture of sugars. However, they used initial sugar concentrations of 30 g/l in their fermentation processes, which is very high in comparison from what would likely be seen in algae feedstocks.

An investigation by Potts *et al.* (2010) revealed that acid hydrolysis could be used to release sugars from algae and convert them into fermentable sugars. They reported that *Ulva lactuca*, a macroalgae rich in carbohydrates, could produce 6-18 g/L of glucose equivalent, with an average yield of 15.2 g/L, as revealed by 3,5-dinitrosalicylic acid (DNS) assay. They noted, however, that the assay only measures reducing sugars, meaning that some of the sugars measured may not be fermentable [8]. Therefore, lower concentrations of sugars should be considered to better assess the viability of using algae as a feedstock for *T. thermosaccharolyticum* fermentation to produce 1,2-propanediol.

6.3 Materials and methods

6.3.1 Fermentation

Initial fermentation experiments were carried out by culturing *Thermoanaerobacterium thermosaccharolyticum* HG-8 (ATCC 31960) under anaerobic conditions at 60°C in 125 ml serum bottles. Lyophilized *T. thermosaccharolyticum* was obtained from ATCC. Stock cultures were prepared by removing 10 ml of peptone-yeast-glucose (PYG) media from a pre-sterilzed Hungate tube (ATCC) with a syringe and needle, adding the media to the bacteria, and quickly drawing the bacteria back into the syringe and transferring it back into the Hungate tube. The stock culture was then placed in a water bath at 60° for a period of 2-3 days. Once the stock culture was healthy, a 5 ml inoculum was transferred under anaerobic conditions to a serum bottle containing approximately 80 mL of ATCC 1465 media prepared with 10 g/l glucose. Cultures were split every 24-48 hours to maintain healthy growth by transferring 10 ml of fresh medium from a sterilized serum bottle to a growing culture bottle, then transferring 10 ml of cell

culture back to the fresh media. Splits were performed under nitrogen gas to maintain anaerobic conditions, and cultures were kept in a water bath set at 60°.

Batch preliminary fermentation experiments were performed using serum bottles with approximately 80 ml of ATCC 1465 media with initial sugar concentrations of 10 g/l and inoculums of 10 ml. This concentration was chosen because 10 g/l is a feasible amount of sugar one could expect to obtain from an algae hydrolysis process. A sugar mixture representative of what could be extracted from algae was prepared to assess the ability of *T. thermosacchrolyticum* to produce 1,2-propanediol from algal derived sugars. The synthetic algal sugar mixture consisted of 5 g/l glucose, 1.5 g/l xylose, 1.5 g/l arabinose, 1 g/l galactose and 1 g/l mannose, for a total sugar concentration of 10 g/l. Glucose media (10 g/l) was also used as a bench mark for comparison. Because the stock culture was started on PYG media, at least three serial transfers were performed on both glucose media and the sugar mixture media prior to the start of the experiments. Both glucose and sugar mixture experiments were done in two sets, duplicates of each, with staggered inoculation times in an effort to develop a curve of 1,2-propanediol production over time. Samples of approximately 3 ml were drawn from the serum bottles using a sterile needle and syringe every 3-9 hours. Samples were filtered using 0.45 μm polyethersulfone syringe filters (VWR) before placing approximately 1 ml each into a GC and HPLC vial to prepare them for analysis.

Scaled-up preliminary fermentation experiments were performed using a 1.5-L Applikon bioreactor at 60°C and a pH of 6.0. A nitrogen purge was used and dissolved oxygen was monitored to ensure anaerobic conditions were maintained. Approximately 120 ml of healthy

culture was used to inoculate 1 L of ATCC 1465 media. Initial sugar concentrations of 10 g/l were used as described previously for the serum bottle experiments. Experiments were carried out for 60-70 hours to ensure enough time for maximum sugar consumption, and samples were taken every 3-9 hours and approximately 5 ml were removed from the bioreactor at each sample time. Optical density measurements were taken at 600 nm using a Beckman-Coulter spectrophotometer to monitor cell growth over time for the bioreactor experiments. Samples were then filtered using 0.45 μm polyethersulfone syringe filters (VWR) to prepare them for HPLC and GC analysis.

6.3.2 Analytical methods

High performance liquid chromatography with a Waters HPLC and refractive index detector was utilized to monitor the consumption of carbohydrates in the fermentation broth over the course of the experiments. An Aminex HPX-87N column (Bio-Rad) was used at 80°C and a flow rate of 0.6 ml/min with Milli-Q water as the mobile phase. Standard samples were prepared from glucose, xylose, arabinose, mannose and galactose (Sigma-Aldrich).

Time-course samples were also analyzed by gas chromatography to measure the production of 1,2-propanediol and by-products throughout the course of the experiments. Analysis was performed using a Shimadzu Model 2014 gas chromatograph operating with a flame ionization detector at 300°C, and automated sample injector at 300°C, and a Phenomonex FFAP 30 m x 0.32 mm x 0.25 μm capillary column. The temperature profile was 3 minutes at 35°C, 20 ml/min to 180°C, and 3 minutes at 180°C. Standard samples were prepared from ACS grade 1,2-propanediol, ethanol and acetic acid.

6.4 Results and Discussion

T. thermosaccharolyticum fermentations yielded 1,2-propanediol, acetic acid and ethanol, as determined by gas chromatography analysis. Examples of the GC chromatograms are shown in **Figures 6.1** and **6.2**. Retention times for the 1,2-propanediol, acetic acid and ethanol were 8.8, 7.8 and 2.9 minutes, respectively. **Figures 6.3** and **6.4** show the production of 1,2-propanediol, as well as acetic acid and ethanol by-products for the bioreactor experiments using glucose and the sugar mixture, respectively. The production of 1,2-propanediol, acetic acid and ethanol are individually compared in **Figures 6.5, 6.6 and 6.7**, respectively, for the two media options. 1,2-propanediol yield was greater when synthetic algal sugar mix was used compared to the glucose media, as shown in these figures. Optical density measurements were taken to monitor cell growth. For the glucose medium, a maximum optical density measurement of 1.4 was observed at 15 hours after inoculation. A maximum OD reading of 1.08 was reached at 24 hours after inoculation for the sugar mixture. While higher OD readings were achieved with the glucose-only media, as shown in **Figure 6.8**, the production of 1,2-propanediol was up to 65% greater when the sugar mixture feed was used **(Figure 6.5)**. A maximum 1,2-propanediol concentration of 1.4 g/L (0.14 g/g sugar) was achieved from the synthetic algal sugar mixture, compared to only 0.83 g/L (0.083 g/g sugar) from the glucose feed. Note that these values are given as grams of product per gram of sugar fed, not sugar consumed. Final concentrations of 1,2-PD were 1.2 g/L and 0.73 g/l for the sugar mixture and glucose, respectively. Based on **Figure 6.5**, it appears that most of the 1,2-propanediol production was accomplished within 21 hours of inoculation for both media options tested. While 1,2-PD production was greater when the sugar mixture was used, it is important to note that the concentrations of acetic acid and ethanol were both higher

when the glucose medium was used. At the 21 hour point, the concentrations of ethanol, acetic acid and 1,2-PD were approximately 3.1 g/L, 1.6 g/L and 0.78 g/L, respectively, for a total amount of 5.5 g product per gram of glucose fed. For the sugar mixture medium, these values were approximately 1.4 g/L ethanol, 0.92 g/L acetic acid and 1.2 g/L 1,2-propanediol, for a total amount of 3.5 g product per gram of sugar fed. Because glucose is typically a preferred substrate, it was expected that the total product concentration would be higher for the medium containing only glucose as a substrate. More research must be done to determine why 1,2-PD production was higher when the sugar mixture was used and which sugar(s) are preferred for the conversion to 1,2-propandiol. In the glucose reactor, acetic acid and ethanol continued to be produced for approximately 40 hours followed by a noticeable drop in concentration, while acetic acid production for the sugar mixture reactor continued to increase and ethanol concentrations fluctuated throughout the 70 hour incubation time **(Figures 6.6** and **6.7)**. This could be attributed to the fact that a 70% ethanol solution was used to clean the needle prior to taking samples each time, so it is likely that some of the ethanol showing up in the analysis is a result of the efforts to maintain sterile conditions, and not all of it is attributed to bacterial production. It is possible that, in the glucose reactor, once all of the sugar is depleted, the bacteria began to recycle acetic acid as a carbon source [9] .

Results for the serum bottle experiments were similar to the bioreactor experiments in that more 1,2-PD was produced when the sugar mixture medium was used when compared to the glucose medium, and because of the smaller volumes used, product yields were quite low. It is also important to note that, due to the smaller volumes and sample availability, pH and dissolved

oxygen were not controlled for the serum bottle experiments. For this reason, all numbers given were from the analysis of the bioreactor experiments.

Unfortunately, the data obtained from HPLC analysis for the sugar consumption was not reliable. For each sample, duplicate injections were performed, and drastically different measurements were seen for the two injections of the same sample. A sample chromatogram is shown in **Figure 6.9**. Major peak shifting and magnification of the peaks was observed. These problems were discussed with the column manufacturer, and various attempts were made to solve the issues. However, the column had become damaged from previous incorrect use, and it was determined that the column would need to be replaced before further experiments could continue. While exact sugar concentrations throughout the course of the experiments could not be determined, it was possible to at least estimate the time at which the sugars were consumed. When the glucose-only medium was used, glucose was completely consumed within 18 hours of inoculation. For the medium containing the synthetic algal sugar mixture, although the sugars were never completely consumed, maximum consumption was reached within 24 hours of inoculation.

6.5 Conclusions

Two media options containing 10 g/l of sugar were investigated to assess the possibility of using algae as a feedstock for 1,2-propanediol production from *T. thermosaccharolyticum*. In these preliminary experiments using a 1.5 L bioreactor, concentrations of 10 g/l proved to be adequate to produce 1,2-propanediol in measureable concentrations. Furthermore, a 65% increase in 1,2-propanediol production was observed when a 10 g/l synthetic algal sugar mixture

was used, compared to 10 g/l glucose media. While further experiments will need to be performed to confirm these results and to better understand the sugar utilization of this microbe, this is promising because it suggests that the fermentation of 1,2-PD from algal sugars is not only a viable option, it is possible that higher concentrations can be produced from an algae sugar mixture rather than a traditional glucose feed. This could potentially result is decreased production costs as well as contribute to a more sustainable process.

6.6 Acknowledgements

Special thanks to the SMART scholarship for providing funding, as well as the Naval Air Warfare Center – Weapons Division in China Lake, California for use of the facilities and some of the supplies to perform some of this work. I would also like to thank Tom Potts and Alex Lopez for their assistance with the analytical equipment.

6.7 References

[1] Niu, W., M. Molefe and J. Frost (2003). Microbial synthesis of the energetic material precursor 1,2,4-butanetriol. *J. Am. Chem. Soc.* 125: 12998-12999.

[2] Zha, W., Z. Shao, J. Frost and H. Zhao (2003). Rational pathway engineering of type I fatty acid synthase allows the biosynthesis of triacetic acid lactone from D-glucose in vivo. *J. Am. Chem. Soc.* 126: 4534-4535.

[3] Achkar, J., M. Xian, H. Zhao and J. Frost (2005). Biosynthesis of Phloroglucinol. *J. Am. Chem. Soc.* 127: 5332-5333.

[4] Cameron, D., N. Altaras, M. Hoffman and A. Shaw (1998). "Metabolic engineering of propanediol pathways." *Biotechnology Progress.* 14: 116-125.

[5] Altaras, N. and D. Cameron. (1999) "Metabolic engineering of a 1,2-propanediol pathway in *Escherichia coli*." *Applied and Environmental Microbiology.* 1180-1186.

[6] Sanchez-Riera, F., D. Cameron and C. Clooney (1987). Influence of environmental factors in the production of R(-)-1,2-propanediol by clostridium thermosaccharolyticum. *Biotechnology Letters.* 9(7): 449-454.

[7] Altaras, N., M. Etzel and D. Cameron (2001). "Conversion of sugars to 1,2-propanediol by *Thermoanaerobacterium thermosaccharolyticum* HG-8." *Biotechnology Progress.* 17: 52-56.

[8] Potts, T., J. Du, M. Paul, P. May, R. Beitle, J. Hestekin (2011). The production of butanol from Jamaica bay macro algae. *Environmental Progress & Sustainable Energy.* 31(1): 29-36.

[9] Du, J., N. Lorenz, R. Beitle and J. Hestekin (2012). Application of wafer enhanced electrodeionization in a continuous fermentation process to produce butyric acid with *Clostridium tyrobutyicum*. *Separation Science and Technology*. 47(1): 43-51.

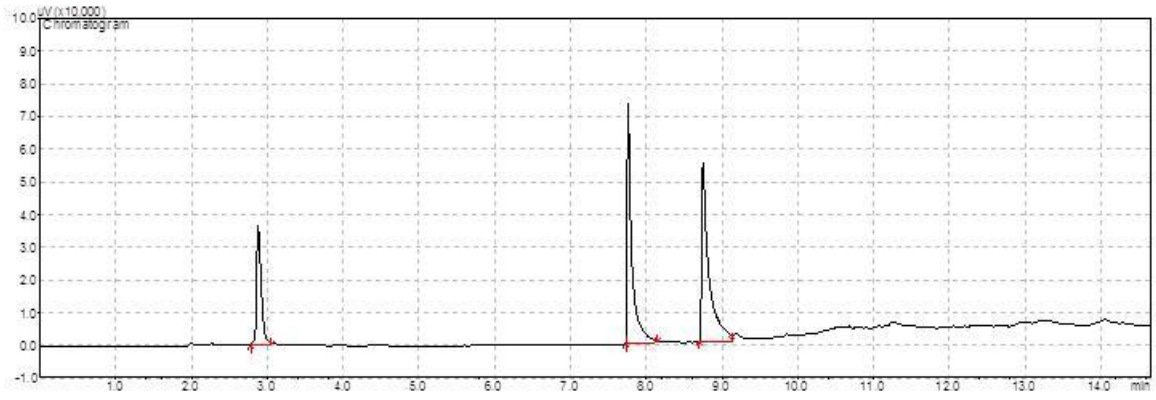

Figure 6.1: Example GC chromatogram for synthetic algal sugar mixture fermentation at 54 hours.

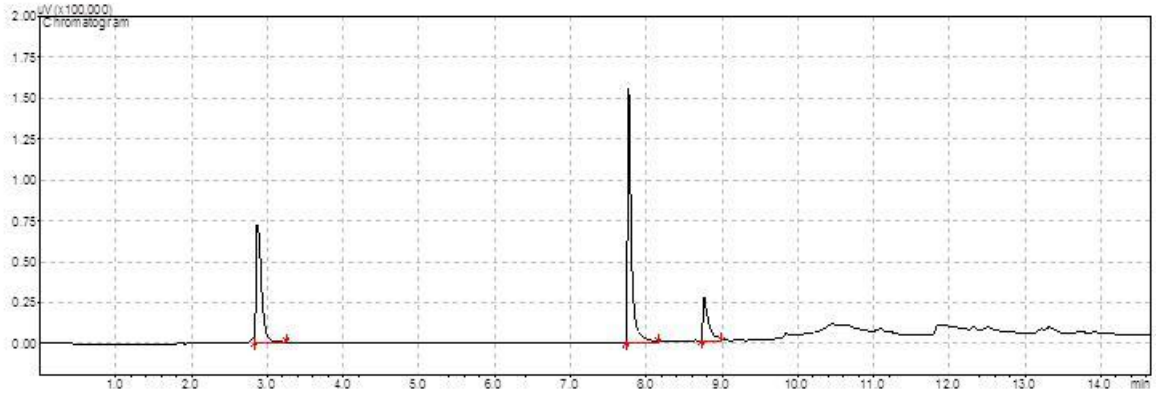

Figure 6.2: Example GC chromatogram for glucose fermentation at 39 hours.

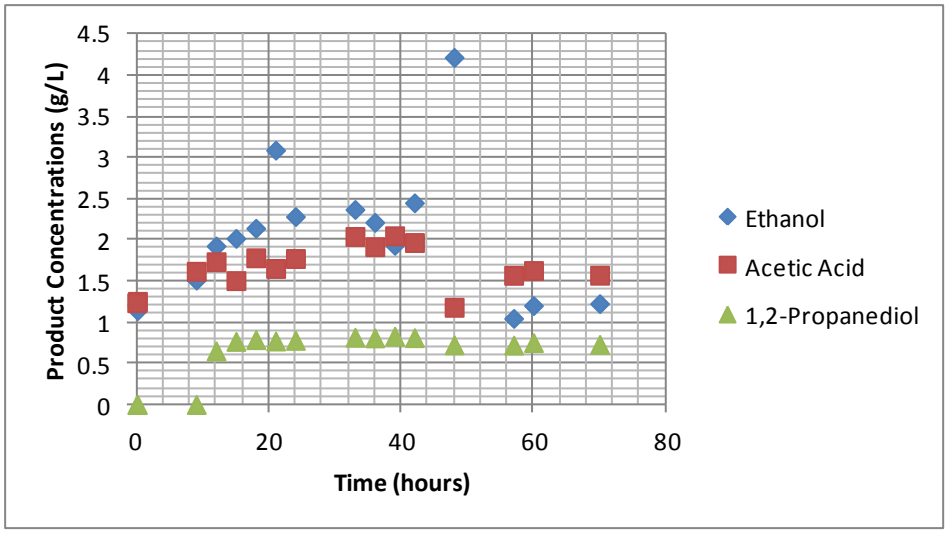

Figure 6.3: *T. thermosaccharollyticum* fermentation products determined by GC analysis for medium containing 10 g/L glucose.

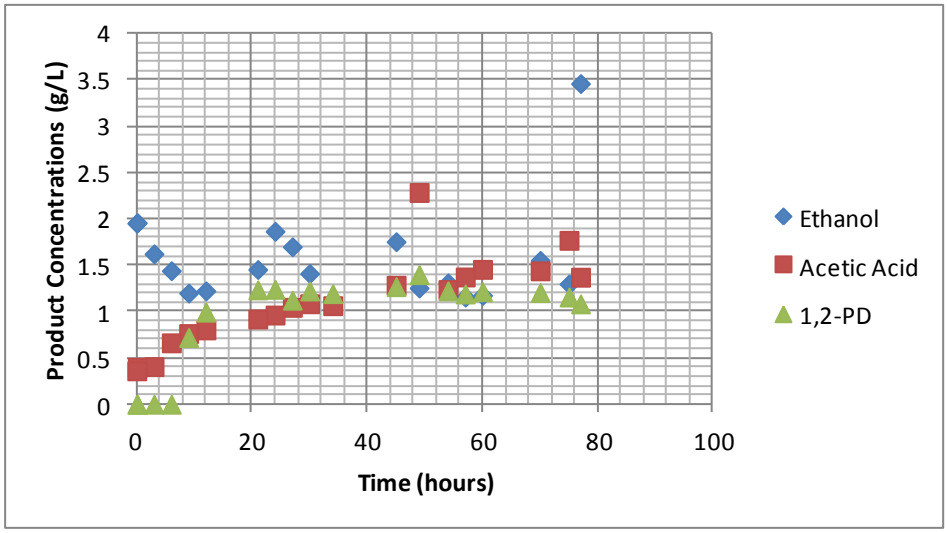

Figure 6.4: *T. thermosaccharollyticum* fermentation products determined by GC analysis for medium containing10 g/L synthetic algal sugar mixture.

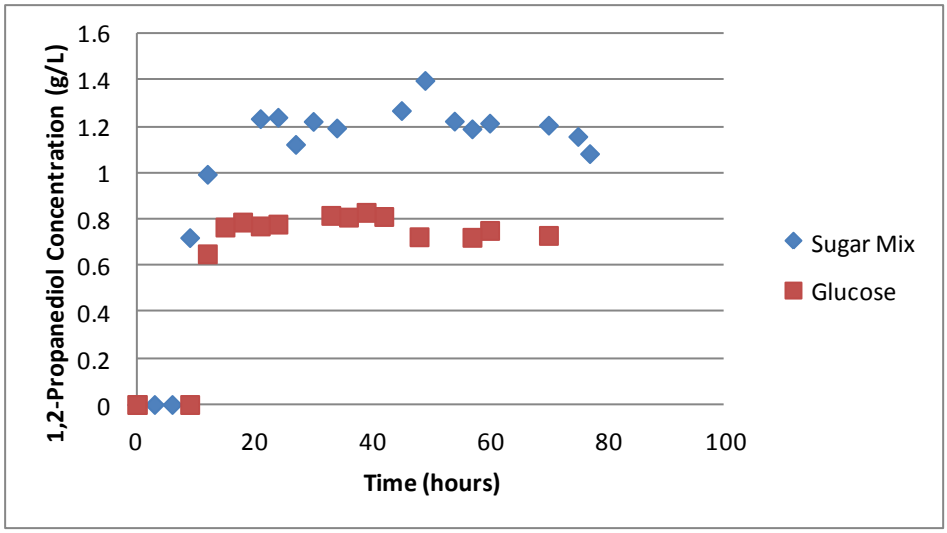

Figure 6.5: Comparison of 1,2-propanediol production for glucose and sugar mix bioreactor fermentation experiments over time.

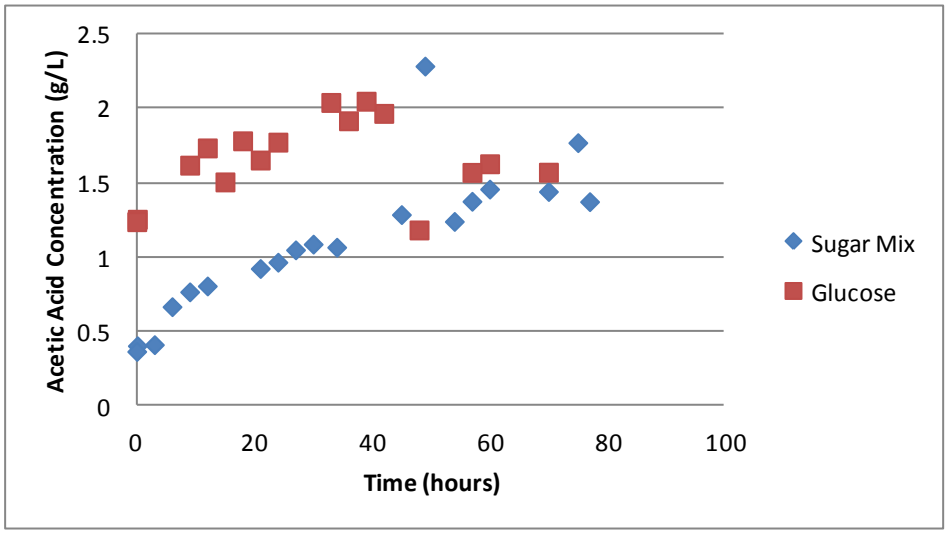

Figure 6.6: Comparison of acetic acid production for glucose and sugar mix bioreactor fermentation experiments over time.

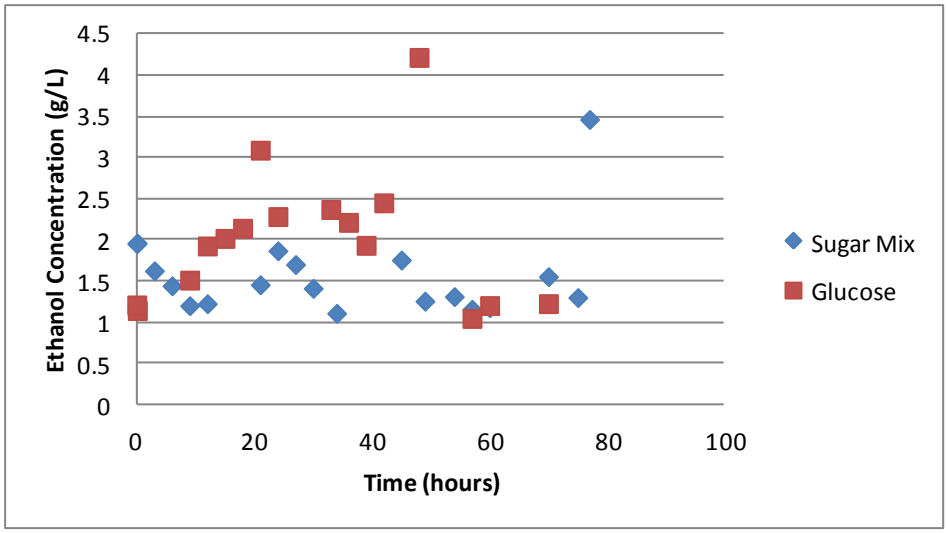

Figure 6.7: Comparison of ethanol production for glucose and sugar mix bioreactor fermentation experiments over time.

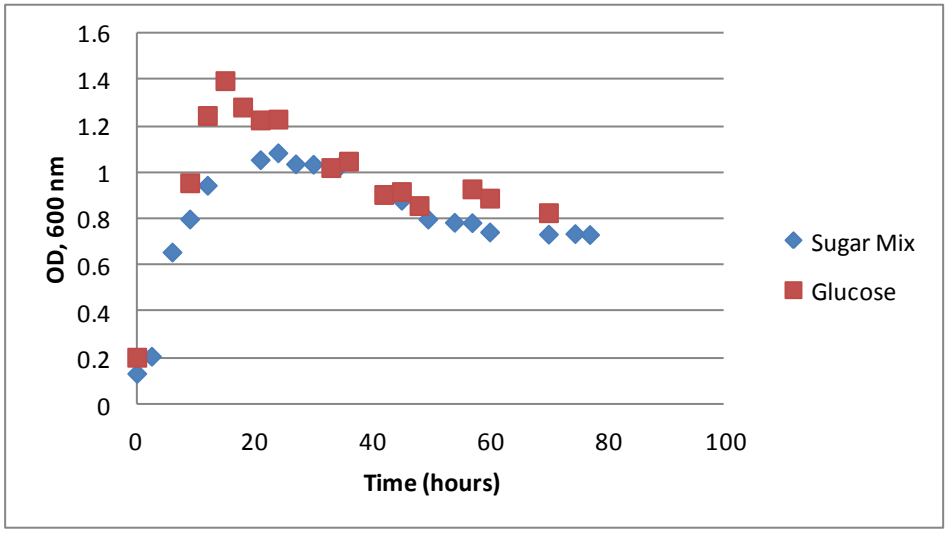

Figure 6.8: Comparison of optical density measurements for glucose and sugar mix bioreactor fermentation experiments over time.

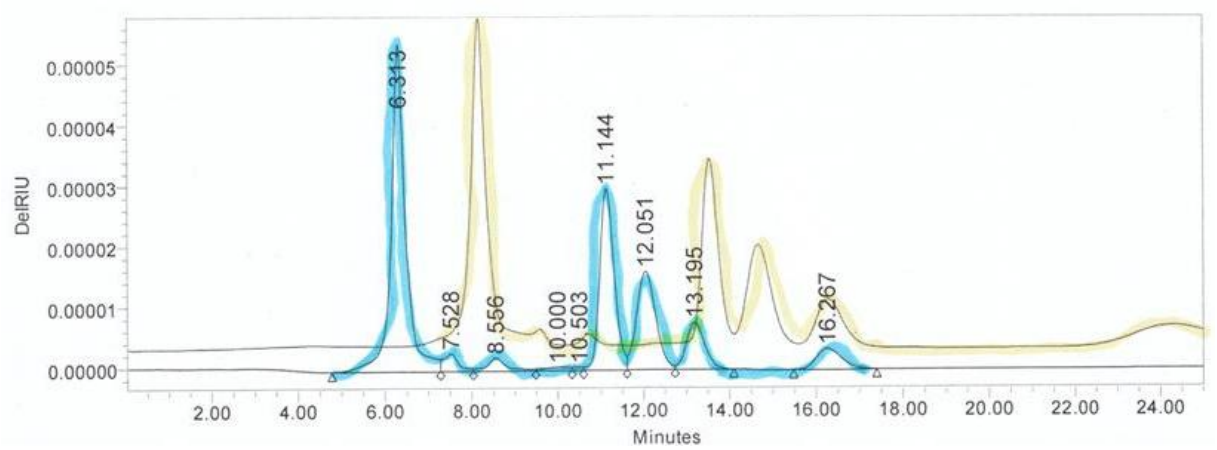

Figure 6.9: Sample HPLC chromatogram for one sample with duplicate injections to show peak shifting and magnification problems that were encountered.

6-*A Appendix – Chapter 6*

6-A.1 List of media components for ATCC 1465

ATCC medium: 1465 Basal thermophile medium

Solution 1:

NH4Cl .....0.9 g

MgCl2 . 6H2O .....0.2 g

KH2PO4 .....0.75 g

K2HPO4 .....1.5 g

Trace Element Solution (see below).....9.0 ml

10% FeSO4 . 7H2O .....0.03 ml

0.2% Resazurin.....1.0 ml

Wolfe's Vitamin Solution (see below).....5.0 ml

Trypticase Peptone (BD 211921).....10.0 g

Distilled water.....850.0 ml

Solution 2:

Yeast extract.....3.0 g

Distilled water.....100.0 ml

Solution 3:

Glucose.....5.0 g

Distilled water.....50.0 ml

Autoclave the above solutions separately at 121C for 45 minutes. Combine solutions under 100% N2 and dispense aseptically using the same gas phase. At time of inoculation add 10% sterile Na2S . 9H2O to a final concentration of 0.1%.

Trace Element Solution:

Nitrilotriacetic acid (Neutralize to pH 6.5 with KOH).....12.5 g

$FeCl_3 \cdot 4H_2O$ .....0.2 g

$MnCl_2 \cdot 4H_2O$ .....0.1 g

$CoCl_2 \cdot 6H_2O$ .....0.017 g

$CaCl_2 \cdot 2H_2O$ .....0.1 g

$ZnCl_2$ .....0.1 g

$CuCl_2$ .....0.02 g

H_3BO_3 .....0.01 g

$Na_2MoO_4 \cdot 2H_2O$.....0.01 g

NaCl.....1.0 g

Na_2SeO_3 .....0.02 g

Distilled water.....1.0 L

CHAPTER 7

Conclusions and Future Work

7.1 Carbon dioxide delivery and algae growth

Three methods of gas delivery were compared to investigate the effects of adding carbon dioxide to water at various depths and temperatures. While temperature did not seem to have an effect on the mass transfer, water depth could be considered a significant parameter. A unique hollow fiber membrane manifold was created, which proved to be far superior to both bubbling and the porous diffuser in terms of mass transfer, particularly at shallow depths. This is significant due to the potential application of hollow fiber membranes for enhancing the growth rates of algae in an Algal Turf Scrubber® system. A model was developed to accurately depict the mass transfer of carbon dioxide gas to the system.

The hollow fiber membrane manifold was tested in a pilot scale ATS®, and positive results were achieved with growth rates were comparable to those achieved prior to adding the carbon dioxide gas, despite a significant drop in pH down the length of the floway. Additionally, changes in speciation of the algae were observed, which could result in processing advantages. A laboratory scale ATS® system was built in a greenhouse at the University of Arkansas, and an undergraduate student has been given the task of performing future experiments to further investigate changes in algae growth and composition due to the effects of adding carbon dioxide to algal growth media via our unique manifold.

7.2 Sugar recovery

In Woods *et al*. (2011), it was demonstrated that sonication in the presence of abrasives (such as glass beads or sand) created a synergistic effect and more sugars were released than by sonication alone. A 60% increase in carbohydrate release was observed using this method, termed sonic abrasion, for the same power draw, when compared to the control methods we investigated. In addition, we found that it was possible to release sugars in adequate amounts for use in fermentation processes. It is important to note that this study involved algae that was bulk harvested from a local ATS® system in Springdale, Arkansas and different algal species have various sugar concentrations. It would be interesting to compare this extraction technique to that of acid hydrolysis using an algal species known for high carbohydrate content to evaluate the sugar release as well as energy requirements for each method.

7.3 Fermentation

Although lower cell densities were achieved, quantities of 1,2-propanediol production was notably higher and by-product concentrations were lower with the synthetic algal sugar mixture when compared to glucose. While bioreactor experiments need to be replicated to ensure consistent data, based on this information, it is reasonable to believe that 1,2-propanediol can be produced via microbial synthesis using sugars extracted from algae. Future work will involve replicating the experiments discussed in Chapter 6, as well as experimentation using actual algae sugars in the growth medium, as it is possible that other components present in the algae extraction could have either a positive or negative effect on the fermentation products. An algae species high in carbohydrate concentration, such as *Ulva lactuca*, should be used, and some sort of extraction technique should be employed to recover the carbohydrates. Furthermore, a new

HPLC column should be purchased to ensure accurate data. The Aminex HPX-87H column (BioRad) would be ideal because it is capable of separating sugars as well as organic acids, thus GC data can be confirmed, and any other by-products not present in the GC analysis can be recognized.

Once the experiments with *T. thermosaccharolyticum* are complete, similar experiments should be repeated with one or more of the genetically modified *E. coli* strains mentioned in Section 3.4. Environmental factors such as temperature, pH, and dissolved oxygen levels could be varied and other variables, such as agitation and incubation times, should be considered as well. The objective of these experiments would be to determine what level of anaerobicity is needed to push towards 1,2-propanediol and which conditions favor the conversion of algae sugars to DHAP, the key intermediate in the hexose monophosphate (HMP) pathway which leads to 1,2-PD production.

Because *E. coli* is well characterized and the pathways are well understood, its potential for further strain improvement is highly possible. A thorough analysis of sugars and organic acids using both HPLC and GC should be performed, and sugars concentrations should potentially be analyzed with enzymatic assays as well. Once this is done, all data necessary to perform a metabolic flux analysis (MFA) of the system would be available. The MFA model will provide specific rates of metabolite production and/or degradation of important intermediates. This will create a better understanding of how the cell is utilizing the feed source, which could aid in the future development of metabolic engineering strategies for the production of 1,2-PD from *E. coli*. Implementing these strategies could potentially lead to major improvements area of

microbial synthesis of energetic precursors, thus giving rise to the potential to transform the propellant industry and our national security.

7.4 References

Woods, L., M. Riccobono, N. Mehan, J. Hestekin and R. Beitle (2011). "Synergistic effect of abrasive and sonication for release of carbohydrate and protein from algae." *Separation Science and Technology*. 46:4, 601-604.

Vitae

Education

Chemical Engineering, University of Arkansas. Fayetteville, Arkansas.

August 2008-August 2013.	Graduate student in Chemical Engineering. Ph.D., August 2013. M.S.ChE., Conferred May 2010.
August 2007-July 2008.	Undergraduate student in Chemical Engineering. Completed deficiency courses for entrance into Chemical Engineering graduate program.

Chemistry, Northeastern State University. Tahlequah, Oklahoma.

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Experience

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Biofuel production. Gas delivery via hollow fibers for the application of algae growth: Enhancing biomass production methods and developing techniques for the extraction of carbohydrates and oils from wastewater algae for the fermentation of biobutanol.

Dr. Robert Beitle and Dr. Jamie Hestekin. Fayetteville, Arkansas.

June-Sept. 2011, May-Aug. 2012 Research Intern – SMART Program

Synthesis and Formulations Branch, Naval Air Warfare Center – Weapons Division

Fermentation. Biosynthesis of chemicals for use in propellants and energetic materials.

Dr. Joshua Carter. China Lake, California

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Have served as a T.A. for undergraduate Chemical Engineering courses including Fluid Mechanics (Dr. Shannon Servoss), Non-Equilibrium Mass Transfer (Dr. Robert Beitle), Heat Transfer (Dr. Roy Penney), Senior Chemical Engineering Lab (Dr. Robert Babcock) and Senior Design I & II (Dr. Jamie Hestekin and Dr. Robert Beitle). Fayetteville, AR.

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Coordinated mentoring programs and plan fellowship events for the women in the College of Engineering.

Dr. Julie Carrier and Dr. Heather Nachtmann. Fayetteville, Arkansas.

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Department of Chemical Engineering, University of Arkansas
Protein Purifications. Used immobilized metal affinity and hydrophobic interaction chromatographies to identify contaminating species in the purification of GFPuv protein.
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Sep. 2006-Dec. 2007 Honors Undergraduate Research.
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Polymer Electrolytes. Utilized Fourier transform infrared spectroscopy to analyze stretched induced changes in poly(ethylene oxide)-$LiCF_3SO_3$ for use in lithium ion batteries.
Dr. Chris Burba. Tahlequah, Oklahoma.

Publications

L. Merriman, A. Moix, R. Beitle, J. Hestekin. Carbon dioxide gas delivery to thin-film aqueous systems via hollow fiber membranes. Submitted to Journal of Membrane Science. 2013.

L. Woods, M. Riccobono, N. Mehan, J. Hestekin, R. Beitle. Synergistic Effect of Abrasive and Sonication for Release of Carbohydrate and Protein from Algae. Separation Science and Technology. 2010.

N. Tiwari, L. Woods, R. Haley, R. Goforth, K. Clark, M. Ataai, R. Henry, R. Beitle. Identification and Characterization of Native Proteins of Escherichia coli BL-21 that Display Affinity towards Immobilized Metal Affinity Chromatography and Hydrophobic Interaction Chromatography Matrices. Protein Expression and Purification. 2010.

C. Burba, L. Woods, S. Millar, J. Pallie. Polymer Chain Organization in Tensile-Stretched Poly(ethylene oxide)-Based Polymer Electrolytes. Electrochimica Acta. 2011.

Other Honors and Awards

- DoD Science, Mathematics and Research for Transformation Scholar (August 2010-July 2013)
- University of Arkansas Graduate School Doctoral Academy Fellow (August 2008-May

2013)

- Arkansas Chemical Engineering Graduate Students (August 2008-June 2013) – President (January 2010-May 2011), Vice President (January 2009-January 2010), Social Chair (August 2012-June 2013)
- University of Arkansas College of Engineering Dean Student Search Committee (January 2013)
- Cherokee Nation Graduate Scholar (August 2008-May 2013)
- William R. Hearst Fellow (August 2008-May 2010) - Program Coordinator for University of Arkansas Women in Engineering Program
- Society of Women Engineers (August 2008-May 2010) - Chapter Advisor
- Gates Millennium Scholar (August 2004-May 2010)
- American Chemical Society Scholar (August 2004-May 2008)
- Northeastern State University Hall of Fame (inducted in 2008)
- NSU Outstanding Senior (awarded in 2008)
- NSU University Honors Program (August 2004-December 2007)
- National Science Foundation Research Experience for Undergraduates (May 2007-July 2007)
- Who's Who Among American Colleges and Universities (2007)
- Delta Zeta Region XI Senior Scholarship Award (2007) - highest GPA in four-state region
- NSU Greek Woman of the Year (2005-2006)
- NSU Female Freshman of the Year (2004-2005)
- University of Arkansas Dean's List (Fall 2007)
- NSU President's Honor Roll (Fall 2004, Fall 2006, Spring 2007, Fall 2007)
- NSU Dean's Honor Roll (Spring 2005, Fall 2005, Spring 2006)
- Alpha Chi Honor Society
- Rho Theta Sigma Honor Society
- Kappa Mu Epsilon Mathematics Honor Society