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A Passive Membrane Photobioreactor for the Isolated Cultivation of Algal Resource Utilizing Selectivity (ICARUS), with Wastewater as a Feedstock

by

Ivy L.C. Drexler

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Engineering Science
Department of Civil and Environmental Engineering
College of Engineering
University of South Florida

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Dedication

To Mike, my husband,

The smartest person I know.

And our little babe.

And to my parents,

Who taught me that with humor

I'll always get by.

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Abstract

Renewed momentum in the microalgae industry due to commercial interest in biofuels and bioproducts is driving the need to increase the economic competitiveness of large-scale microalgal production. Current knowledge of membrane systems common in other disciplines, such as environmental engineering, marine science, and biomedicine, are relevant to algae production. With pore sizes ranging from microns to angstroms, membranes provide tailored functions for solid/liquid separation (cell retention, biomass concentration and dewatering), gas/liquid separation (gas delivery and removal), and solute/liquid separation (bioproduct recovery, feedstock preparation and effluent recycling) that are problematic or not possible with other technologies. Though membranes have great potential to facilitate cultivation and harvesting, challenges in energy reduction and fouling mitigation need to be overcome for long-term, cost-effective applications. This body of research includes a thorough literature review of membrane applications in the algal industry and three experimental studies investigating ways to improve the cultivation and harvesting of microalgal species in wastewater.

The first study investigated the growth of native and augmented algal communities in various growth media. Algal monocultures (*Chlorella sorokiniana* and *Botryococcus braunii*) and algal communities native to clarifiers of a wastewater treatment plant were batch cultivated in 1) clarified effluent following a BOD removal reactor (PBCE), 2) clarified effluent following a nitrification reactor (PNCE), and 3) a reference medium (RM). After 12 days, all algal species achieved nitrogen removal between 68-82% in PBCE and 37-99% in PNCE, and phosphorus

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removal between 91-100% in PBCE and 60-100% in PNCE. The pH of the wastewater samples increased above 9.8 after cultivation of each species, which likely aided ammonia volatilization and phosphorous adsorption. Both monocultures grew readily with wastewater as a feedstock, but *B. braunii* experienced significant crowding from endemic fauna. In most cases, native algal species' nutrient removal efficiency was competitive with augmented algal monocultures, and in some cases achieved a higher biomass yield, demonstrating the potential to utilize native species for nutrient polishing and algal biomass production.

In the second study, the isolated cultivation of algal resource utilizing selectivity (ICARUS) process was conceived and developed. ICARUS integrates a passive membrane photobioreactor configuration with wastewater as a growth medium. Eleven membranes of varying porosity and materials were examined based on characteristics and resulting algae productivity. Four ICARUS series (40kDa-PVDF, 0.53 g L^{-1}, 14.1 mg; 0.1µm-PVDF, 0.43 g L^{-1}, 16.6 mg; 12kDa-RC, 0.35 g L^{-1}, 14.5 mg; 0.2 µm-CA, 0.41 g L^{-1}, 14.5 mg) had a final cell density and mass yield that was significantly higher than that of suspended culture (0.25 g L^{-1}, 9.1 mg). Optimal pore size range was identified to be 50-1000 kDa. Six additional series (0.2µm-CA, 0.1µm-PVDF, 40kDa-PVDF, 12kDa-RC, 3.5kDa-PVDF, and 3kDa-RC) also sustained significantly longer exponential growth phases than the suspended cultures. The ICARUS series maintained an average pH of 9.55, which was significantly lower than the average pH of 10.21in the suspended culture. Membrane characteristics affecting the variability in microalgae productivity were evaluated in 2D and generalized linear models.

In the third study, select membranes from the laboratory experiments in Chapter 5 (12kDa-RC, 40kDa-PVDF, 7µm-NY) were tested in extended field conditions at a wastewater treatment plant, where the movement of dissolved constituents and biomass productivity were

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compared to that of closed suspended series. All ICARUS series had higher biomass productivity (RC, 2.87 g L^{-1}; PVDF, 10.6 g L^{-1}; NY, 8.45 g L^{-1}) than the suspended series (0.38 g L^{-1}), which was due to both a longer exponential growth phase and passive dewatering in the ICARUS series. Dissolved ions passed readily across each membrane, and no nutrient limitation was apparent in any series. Gas exchange was slower than expected, which may have been due to external and internal attached growth utilizing gases at the membrane surface. However, dissolved oxygen concentration did not limit algal growth, and adequate carbon dioxide was available to regulate ICARUS pH. In fact, the ICARUS series maintained an average pH of 7.6, whereas the pH of the control series reached 9.8-10.5. The invasion of endemic wastewater species was dependent on pore size; the RC and PVDF series maintained a monoculture, but the NY series had severe contamination.

The resulting research has demonstrated a proof-of-concept of a new microalgal cultivation method which may reduce the cost of large-scale cultivation efforts integrated at wastewater treatment plants or within existing algal production facilities. Investigating various wastewater effluents, membranes, and algal strains has allowed for recommendations for the operation of scaled-up systems. Future research should focus on mechanisms and characteristics of biofouling as well as the operation of a field scale prototype. By improving large scale algal cultivation, algal biofuels may become more economically competitive with fossil fuels or other renewables, enhancing their participation in the country's diverse energy portfolio.

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Chapter 1: Introduction

1.1 Introduction

Microalgae are a diverse group of aquatic organisms capable of surviving in virtually any type of environmental condition. Due to their ability to utilize a heterotrophic, mixotrophic, or autotrophic metabolism, microalgae take advantage of favorable conditions in even the harshest of environments. Many algal species, such as *Chlorella, Haematococcus,* and *Spirulina*, are cultivated for numerous bioproducts which contribute significantly to the global economy (Raja et al., 2008). Microalgae have been investigated extensively in the U.S. through the Aquatic Species Program (Sheehan et al., 1998), a research initiative focused on generating transportation fuels from microalgae by improving their lipid yield. Although the two decades-long investigation yielded many advances, many of the obstacles identified by the program are still dogging the algal industry today, such as triggering lipid production and engineering effective and economic production systems.

As the global population continues to climb above seven billion, demand for energy will increase, as will the production of waste (i.e., wastewater, industrial emissions). However, the supply of fossil fuels will continue to decline, requiring a sustainable fuel source to fill this gap for future generations. In theory, microalgae require less land area than other land-based biofuels, such as corn, soybeans, or canola (Chisti, 2007), giving a practical advantage over their competitors. Because of their adaptability, microalgae are able to utilize resources (such as

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wastewater and industrial flue gases) that other land-based biofuels cannot. In fact, large-scale cultivation may require that these resources are used as feedstock in order to maintain the economic and environmental competitiveness of microalgal cultivation with other biofuels and fossil fuels (Clarens et al., 2010; Clarens et al., 2011; Beal et al., 2012).

Depending on the species and cultivation conditions, microalgae can theoretically produce 20 to 100x the lipids of other land-based biofuels (Chisti, 2007) and can be a substantial contributor in replacing fossil fuel demand (Brennan and Owende, 2010). Reducing the economic burden of harvesting and dewatering would contribute significantly to reducing the overall cost of processing microalgae to transportation fuels. Addressing cultivation issues, such as reducing culture collapse due to invasion of grazers, meeting specific biological requirements, and taking advantage of marginal land, would further reduce the economic and environmental footprint of a large-scale cultivation system.

Membranes have been used extensively in the algal industry for cultivation and harvesting (Chapter 3), but many require energy intense active filtration. On the other hand, passive membrane systems (e.g. dialysis or osmosis) move dissolved constituents or waters diffusionally based on a concentration gradient. In particular, dialysis membranes have been used to grow algae in wastewater (Schultz & Gerhardt, 1969; Dor, 1975; Hoover et al., 2011; Wiley et al., 2013) and have been successfully used to increase culture cell density (Jensen et al., 1972; Powers et al., 1976; Vincent & Silvester, 1979). Higher cell densities reduce the energy and chemical input requirements of harvesting, dewatering, and processing.

A number of patents exist on dialysis growth of algae, capitalizing on the high cell density, passive design, and flexible configuration opportunities (Gerhardt, 1965; Calabrese, 1995; Dor, 1977; Trent, 2010). The most well-known process combining wastewater treatment,

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membrane application (dialysis and forward osmosis), and microalgal cultivation is NASA's Offshore Membrane Bag Enclosure for Growing Algae (OMEGA) and Algae Systems' membrane bag process. However, these systems suspend algal culture directly in wastewater, whereas the process presented in this dissertation separates the algae from wastewater for enhanced crop protection.

Although raceway ponds and various geometries of closed photobioreactors (PBRs) have been extensively studied, many challenges remain with both cultivation methods (Ugwu, 2008; Posten, 2009). Closed systems are more productive than open systems, (Chist, 2007; Mata et al., 2010) but typically exert a higher energy demand. Raceways risk bacterial contamination, compromising the quality of media and harvested biomass. Despite challenges, there is a shift toward open pond cultivation, as it appears to be the most energetically and economically sustainable option for large scale (Clarens et al., 2011). This shift may be unnecessary, however, if a low-energy closed system, such as the process presented in this dissertation, can safely increase crop yields while taking advantage of cheaper resources for cultivation.

The research objective of this dissertation was to develop a new microalgal cultivation process, *Isolated Cultivation of Algal Resource Utilizing Selectivity (ICARUS)*, based on a passive membrane design. The proposed cultivation process is a hybrid of an open raceway pond and closed photobioreactor, creating a unique batch culture configuration exposed to a continuous and selective influx of fresh nutrients. The algae are cultivated in a semi-closed reactor in batch configuration, but the membrane allows for open and continuous exchange between the wastewater growth media.

The ICARUS process was conceived to create denser algal cultures (thereby decreasing harvesting and dewatering efforts), reduce invasion of grazers when using wastewater as a

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feedstock, and minimize the external inputs and land footprint required in conventional algal cultivation. It was hypothesized that utilizing a passive membrane configuration that protects the algae from biological interference (grazers and competitors), yet takes advantage of the diffusional movement of desirable constituents based on concentration gradients would promote denser, more homogenous cultures than growing algae directly suspended in wastewater. The microalgae cultures would reach higher densities because of longer exponential growth periods (due to a higher available nutrient pool) and lower species competition. The ICARUS configuration would float within current wastewater treatment reactors or algal cultivation lagoons, taking advantage of existing infrastructure and space. The objective of the dissertation was to demonstrate a proof-of-concept of this new microalgal cultivation process in laboratory and field conditions.

The work in this dissertation contributes to the growing body of knowledge regarding cultivation of microalgae species in wastewater. The overall work is divided into three sub-experiments with independent objectives. The objective of the first study was to explore potential sites at a wastewater treatment plant to integrate an algal photobioreactor. Two clarified wastewater effluents with different dominant nitrogen sources were investigated for their potential to grow four algal communities. Wastewater was chosen as a growth medium due to its abundance, reliability, and relatively consistent nutrient concentration. Municipal wastewater has been used successfully as a growth medium in many studies (Pittman et al., 2011), with reasons for success and failure well documented. Biomass productivity and nutrient removal capability of augmented algal monocultures were compared to that of native wastewater algal communities to select the optimal species and growth medium for subsequent studies. Batch experiments

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sought to identify potential operational challenges with integrating algal cultivation into a wastewater treatment process train.

Based on the findings of the first study, a new cultivation process, the ICARUS process, was proposed and tested in laboratory and field conditions. ICARUS utilizes a membrane to maintain a physical barrier between the wastewater growth medium and the algal culture. Nutrients and gases pass across the membrane diffusionally via a concentration gradient, passively delivering constituents abundant in wastewater (i.e., carbon dioxide, nutrients) and removing waste products (i.e., dissolved oxygen) from the algal culture. The physical separation has many potential advantages: decoupling the culture from the medium increases the available nutrient pool; the physical barrier protects the algal culture from grazers (e.g., rotifers); the physical barrier protects the integrity of an algal monoculture that may otherwise be overrun by competing endemic species; the passive passage of nutrients and gases does not require an added energy input; and the configuration can take advantage of the existing footprint and infrastructure of a wastewater treatment plant.

The purpose of the second study was to establish the proof-of-concept of the ICARUS cultivation method under laboratory conditions. The objective was to explore membranes of varying materials, porosity, and thickness in order to identify characteristics that promote high cell density and allow adequate passage of nutrients and gases into the growth chamber. Biomass productivity and pH was compared to that of suspended cultures. The laboratory studies also sought to identify operational challenges that may be encountered in field experiments.

The third study investigated the ICARUS method when submerged in a continuous flow of wastewater at a wastewater treatment plant. The experimental design compared biomass growth in ICARUS to control cultures and investigated changes in dissolved constituent and gas

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concentrations based on series conditions. The field study sought to investigate invasion of endemic wastewater species as well as identify operational challenges to consider for scale-up designs.

The algal biofuel industry is resurging with the recognition that alternative sustainable fuels are essential to maintain (and increase) the quality of life for the world's inhabitants. However, many challenges remain within the algal industry regarding resource allocation, invasion of grazers, and the economics of cultivation, harvesting, and processing. The objective of this research was to develop a new method for cultivating algae directly in wastewater, utilizing resources once considered 'wastes.' The main challenges addressed with the new method are to improve culture density and reduce species competition, thereby improving the overall economics of cultivation.

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Chapter 2: Materials and Methods

2.1 Introduction

Various analytical methods were needed to complete the research experiments described in subsequent chapters of this dissertation, including colorimetric methods, solids analyses, and use of probes. Although methods and materials specific to each experiment are described in short in subsequent chapters, this chapter will describe each analytical method in detail. Calibration curves and descriptions of chemical reactions occurring in assays are included as appropriate. Algae (*Chlorella sorokiniana* and *Botryococcus braunii*) stock cultivation and care is also described.

2.2 Colorimetric Methods

2.2.1 Optical Density

Optical density was measured using the single wavelength setting on a spectrophotometer (Hach DR/4000, Loveland, CO, USA). Algae cultures were agitated to homogenize the culture prior to sample removal. Approximately 3.5 mL of sample was placed into a clean 1 cm square plastic cuvette. The cuvette was wiped with a tissue to remove oils, dirt, or other foreign matter from the outside of the cuvette. Another cuvette was filled with approximately 3.5 mL of deionized water, which served as a blank. The optical density (as relative absorbance units) of the algae culture was measured at 680 nm to coincide with the absorbance range of chlorophyll pigments. However, because the pigment content can change with environmental conditions or

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algal growth phase (Griffiths et al., 2011) and generally decreases with growth limitations, the absorbance of the algal culture at 750 nm was also measured in the experiments in Chapter 6. The absorbance at 750 nm captures the content of algae and bacteria independent of pigment content in the cells and has been shown to remain steadier at varying growth phases and environmental conditions (Griffiths et al., 2011). A ratio of the absorbance at 680 nm and 750 nm was used to monitor chlorophyll content, and therefore, potential growth limitations during cultivation.

2.2.2 Ammonia-Nitrogen

Ammonia-nitrogen was measured using the salicylate method and conducted with the Hach High Range Test'N'Tube™ Method 10031 (Hach, Loveland, CO, USA). Vials were prefilled with 5 mL of distilled water to which 1 mL of sample was added. A blank was prepared identically to the sample vials using distilled water in place of the sample. Two reagent packets, the Ammonia Salicylate Reagent Powder Pillow (sodium salicylate and sodium nitroferricyanide) and the Ammonia Cyanurate Reagent Powder Pillow (sodium dichloroisocyanurate, lithium hydroxide, sodium citrate and sodium tartrate) were added to the sample vials. Monochloramine was formed when ammonia present in the samples reacted with chlorine. Upon the addition of salicylate, 5-aminosalicylate was formed, which became oxidized with the addition of a sodium nitroprusside catalyst. The result was a blue-colored compound which mixed with excess reagent to show a green-colored solution proportional to the amount of ammonia present in the sample. All reactions took place over 20 minutes; at the end of the reaction period, the optical density (as relative absorbance units, RAU) of each sample vial was measured in a spectrophotometer (Hach DR/4000, Loveland, CO, USA) and internally compared

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to the optical density of the blank at 655 nm. The RAU was converted to mg NH_4-N L^{-1} by an internal calibration curve.

2.2.3 Nitrate-Nitrogen

Nitrate-nitrogen was measured using the chromotropic acid method and conducted with Hach Nitraver® X High Range Test'N'Tube™ Vials Method 10020 with a range of 0 to 30.0 mg L^{-1} (Hach, Loveland, CO, USA). A 1 mL aliquot of sample was added to the mixture of distilled water and sulfuric acid present in the vial. The vial was inverted ten times to mix, and the "blank" reading of the vial's optical density (as relative absorbance units, RAU) was taken on a spectrophotometer (Hach DR/4000, Loveland, CO, USA) at 410 nm. A NitraVer X Reagent B Powder Pillow (chromotropic acid (disodium salt), white quartz sand, sodium metabisulfite) was added to the vial. The vial was inverted ten times to mix, then given a five minute reaction period. During the reaction period, the chromotropic acid reacted with nitrate in the highly acidic conditions of the vial, causing a yellow color to appear proportional to the nitrate present. The optical density of the vial (as RAU) was measured again at 410 nm and internally compared to the "blank" reading taken five minutes prior. The RAU was converted to mg NO_3-N L^{-1} by an internal calibration curve.

2.2.4 Total Phosphorous

Total phosphorous was conducted with the Hach Total Phosphorous High Range (1.0 to 100.0 mg L^{-1}) Test'N'Tube™ Vials Method 10127, which is adapted from Standard Methods 4500 B-C (Eaton et al., 2005). The blank and sample vials were prepared by adding 5 mL of deionized water or sample, respectively, to a Hach vial, which contained a mixture of distilled water and sulfuric acid. Both the blank and sample vials were treated identically in the following steps. A Potassium Persulfate Powder Pillow (potassium persulfate) was added to each vial. The

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vials were then allowed to digest for 30 minutes at 150°C, where the hot, acidic conditions allowed the persulfate to convert organic phosphates to orthophosphates. At the end of the digestion period, the vials were removed from the incubator and allowed to cool to room temperature. Next, 2 mL of 1.54N sodium hydroxide solution was added, and the vials were mixed; a 0.5 mL aliquot of molybdovanadate reagent (ammonium molybdate, ammonium metavanadate, sulfuric acid and demineralized water) was then added and the vial was mixed again. A seven minute reaction period allowed the orthophosphates time to react with the molybdate in the acid medium, resulting in a phosphate/molybdate complex. In the presence of vanadium, the complex created yellow molybdovanadophosphoric acid. The optical density (RAU) of each sample vial was measured in a spectrophotometer (Hach DR/4000, Loveland, CO, USA) and internally compared to the optical density of the blank at 420 nm. The RAU was converted to mg PO_4 L^{-1} by an internal calibration curve in the spectrophotometer.

2.2.5 Total Nitrogen

Total nitrogen was measured using the Persulfate Digestion Method and conducted with the Hach Total Nitrogen High Range (10.0 to 150.0 mg L^{-1}) Test'N'Tube™ Vials Method 10072. A Total Nitrogen Persulfate Reagent Powder Pillow (potassium persulfate) was added to a HR Total Nitrogen Hydroxide Digestion Vial (sodium hydroxide, demineralized water). A 0.5 mL aliquot of deionized water and sample was added to the blank and sample vial, respectively. Vials were shaken for 30 seconds and placed in a preheated incubator at 105°C for 30 minutes. Under hot, alkaline conditions, nitrogen in all forms was converted to nitrate. When vials cooled to room temperature, one Total Nitrogen Reagent A Powder Pillow (sodium metabisulfite) was added to each vial, vials were shaken for 15 seconds, and a three minute reaction took place. At this stage, sodium metabisulfite removed interferences with halogen oxides. Next, a Total

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Nitrogen Reagent B Powder Pillow (sodium metabisulfite, urea, chromotropic acid, disodium salt, white quartz sand) was added to the vial, vials were shaken for 15 seconds, and a two minute reaction took place. Chromotropic acid reacted with nitrate in the acidic conditions to form a yellow complex. A 2 mL aliquot from the Hydroxide Digestion Vial was transferred to a Total Nitrogen Reagent C Vial, and the new vial was inverted ten times. After a five minute reaction period, the optical density (RAU) of the sample vial was measured on a spectrophotometer (Hach DR/4000, Loveland, CO, USA) at 420 nm and internally compared to the blank vial. The RAU was converted to mg N L^{-1} by an internal calibration curve.

2.2.6 Chemical Oxygen Demand

Chemical oxygen demand (COD) was measured using a digestion method approved by the U.S. EPA for wastewater analyses (Standard Method 5220 D) (Eaton et al., 2005) and conducted with Hach Chemical Oxygen Demand High Range (20 to 1500 mg L^{-1}) Test'N'Tube^{TM} Vials Method 8000. A 2 mL aliquot of distilled water or sample was added to the blank and sample vials (mercuric sulfate, silver sulfate, chromic acid, sulfuric acid, demineralized water), respectively. Test'N'Tube vials contained. The vials were inverted several times to mix and placed in a preheated incubator at 150°C for two hours. During incubation under acidic and high temperature conditions, the potassium dichromate oxidized the organic compounds in the sample, which reduced the dichromate ion to green chromic acid. Silver present in the vial acted as a catalyst, whereas mercury shielded interferences with chlorides. Once vials were cooled to room temperature, the optical density (RAU) was measured at 620 nm in a spectrophotometer (Hach DR/4000, Loveland, CO, USA) by internally comparing it to the optical density of the blank. The RAU was converted to mg COD L^{-1} by an internal calibration

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curve. The concentration of COD reported by this test is defined by the amount of oxygen consumed per liter of sample subjected to the conditions of the test.

2.2.7 Soluble Protein Assay

Proteins were determined using the Lowry Protein Assay as described in Ferlita (2011). Prior to testing, samples were vacuum filtered (Whatman GF 934-A), and the filtrate was used for analyses. Five reagents were needed for the Lowry Protein Assay: Reagent A (4% sodium carbonate in 0.2N sodium hydroxide solution) was prepared by dissolving 0.8 g of sodium hydroxide (NaOH) in 100 mL of distilled water, then adding 4.0 g of sodium carbonate to 96 mL of the 0.2N NaOH solution; Reagent B (2% copper sulfate solution) was prepared by dissolving 0.5 g copper sulfate in 24.5 mL of distilled water; Reagent C (4% sodium tartrate solution) was prepared by dissolving 2.0 g of sodium tartrate in 48 mL of distilled water; Reagent D (Reagents A, B, C in a 100:1:1 ratio); Reagent E (1:1 mixture of Folin's reagent and deionized water). Details on reagent preparation can be found in Ferlita (2011). Each sample set was conducted in triplicate when adequate sample volume was available; otherwise samples were conducted in duplicate.

A 2 mL aliquot of deionized water or sample was added to 20 mL glass vials for the blank or sample tests, respectively. A 2 mL aliquot of Reagent D was then added, and samples were covered tightly with aluminum foil and left to react for 10 minutes. Next, a 0.4 mL aliquot of Reagent E was added, and each vial was vortex mixed. Vials were covered tightly with aluminum foil and allowed to react for 30 minutes. A 3.5 mL aliquot of the sample was transferred to clean 1 cm plastic cuvettes. The optical density (RAU) of the sample cuvettes was compared to that of the blank at 550 nm on a spectrophotometer (Hach DR/4000, Loveland, CO, USA).

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To relate absorbance to the protein concentration, a calibration curve using Bovine Serum Albumin was generated. The calibration curve for experiments conducted in this body of work was prepared based on Table 2.1 and is shown in Figure 2.1. Each sample was prepared in duplicate at minimum. Furthermore, two independent dilution series were combined in one calibration curve.

Table 2.1. Solution preparation to generate the protein calibration curve for the Lowry Protein Assay. BSA=Bovine Serum Albumin.

Protein (BSA) Concentration (mg L^{-1})	**Vol. BSA Stock Solution (mL)**	**Vol. of DI Water (mL)**
100	10.0	0.0
75	7.5	2.5
50	5.0	5.0
25	2.5	7.5
10	1.0	9.0
0	0.0	10.0

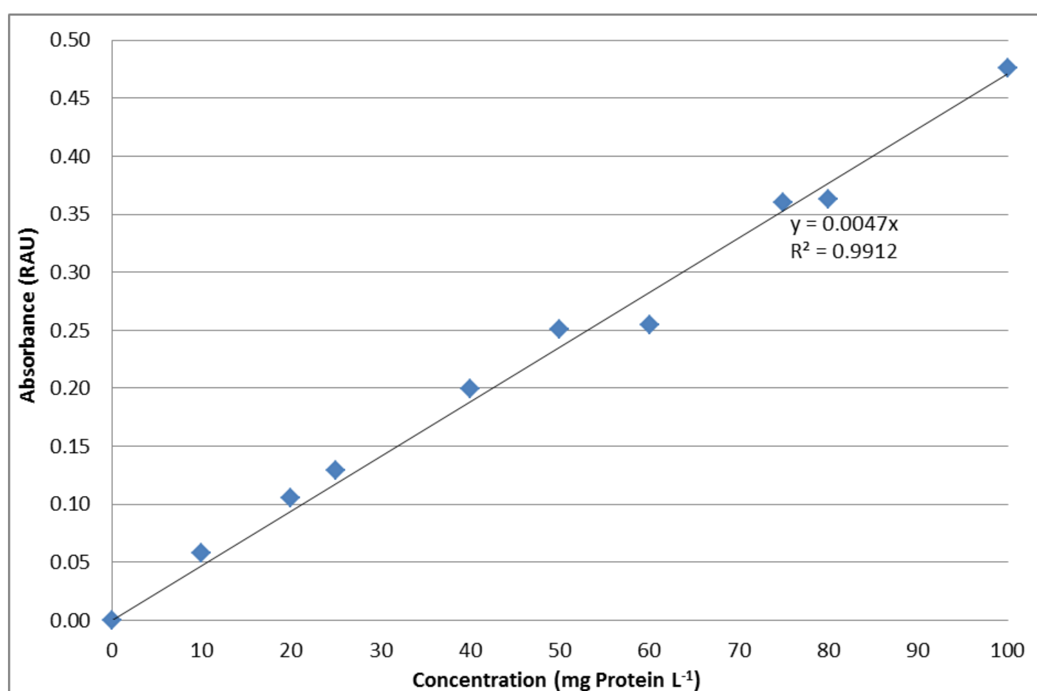

Figure 2.1. Calibration curve for the Lowry Protein Assay using Bovine Serum Albumin as a standard. RAU: relative absorbance units.

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2.2.8 Soluble Carbohydrate Assay

Carbohydrate concentration was determined using an assay described previously in Ferlita (2011). The assay required the use of a phenol reagent (5% wt/V phenol/deionized water) and a sulfuric acid reagent (0.5% wt/V hydrazine sulfate/sulfuric acid). The phenol reagent was prepared by dissolving a 25 g aliquot of ultra-pure phenol in 500 mL of distilled water. The sulfuric acid reagent was prepared by dissolving 2.5 g of hydrazine sulfate in 500 mL of concentrated sulfuric acid.

Prior to analysis, samples were vacuum filtered (Whatman GF 934-A), and the filtrate was used for the analysis. A 0.5 mL aliquot of sample was added to glass 20 mL test tubes in triplicate. A blank was prepared with 0.5 mL of carbohydrate-free water and treated identically to sample vials. Under the fume hood, a 0.5 mL aliquot of the phenol reagent was added to each sample. Next, a 2.5 mL aliquot of the sulfuric acid reagent was added to the test tube while vortex mixing, being careful not to allow the solution to splash out of the top of the tube while mixing. The resulting reaction generated heat, so care was taken when handling sample vessels. Similarly, reagents used were hazardous, so appropriate personal protective equipment (i.e., coat, goggles, gloves) was always worn. The sample vessels were covered tightly with aluminum foil and allowed to cool in a dark place for at least one hour (color remained stable for 24 hours). Samples were vortex mixed prior to transferring 3.5 mL aliquots to clean 1 cm plastic cuvettes. The optical density (RAU) was read on a spectrophotometer (Hach DR/4000, Loveland, CO, USA) at 490 nm, which internally compared the RAU of the sample to that of the prepared blank.

To relate absorbance to the carbohydrate concentration, a calibration curve was generated using dextrose (Figure 2.2). A dextrose stock solution was made by dissolving 0.100 g of

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dextrose in 1 L of distilled water. Subsequent points of the calibration curve were generated at minimum in duplicate based on the concentrations in Table 2.2.

Table 2.2. Solution preparation to generate the carbohydrate calibration curve.

Dextrose Concentration (mg mL^{-1})	**Vol. of Stock Solution (µL)**	**Vol. of DI Water (µL)**
100	500	0,0
70	350	150
50	250	250
30	150	350
10	50	450
0	0,0	500

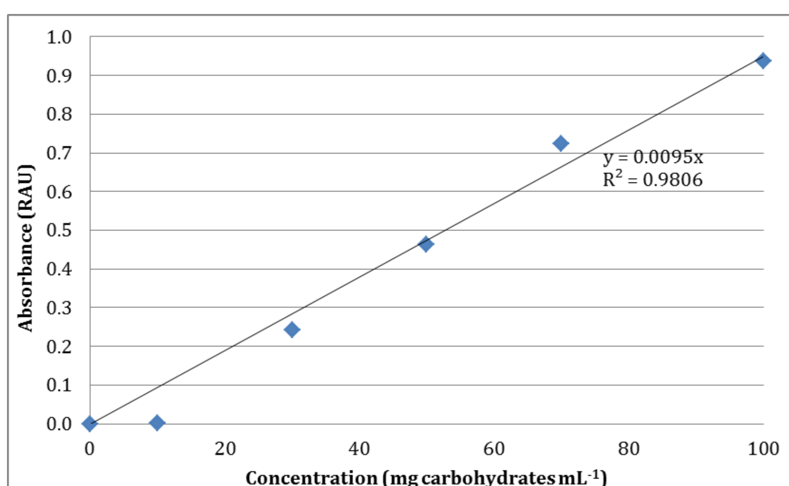

Figure 2.2. Calibration curve for carbohydrate assay using dextrose as a standard. RAU: relative absorbance units.

2.3 Solids

2.3.1 Total Suspended Solids

Total suspended solids (TSS) (also referred to as dry weight in subsequent chapters) analyses were conducted in accordance with the Standard Methods 2540D (Eaton et al., 2005). Glass fiber filters (Whatman 934-A) were rinsed with deionized water, placed in aluminum weigh dishes (Fisherbrand, fluted, 64mmx19mm), and heated in an oven (Fisher ISOTEMP® 100 Series Model116G) at 105°C for a minimum of one hour. Filter assemblies were removed

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from the oven, cooled in a desiccator, and pre-weighed. Samples were shaken to ensure homogeneity and a known volume of sample was filtered through the prepared filters. When specified in the methods sections of accompanying chapters, mixed culture algal samples were homogenized (LabGen 125, Cole Parmer) prior to filtering. Samples prepared for analyses in Chapter 6 were also rinsed with 20 mL of 0.5M ammonium bicarbonate as described by Zhu and Lee (1997) and subsequently rinsed with 20 mL of deionized water. After filtering, samples were placed back in the oven for a minimum of one hour before removal; samples were cooled to room temperature in a desiccator and reweighed. Total suspended solids/dry weight was calculated as described in Standard Methods 2540D (Eaton et al., 2005) and shown in Equation 2.1, where Wt_2 is the weight of the filter assembly and sample (g), Wt_1 is the weight of just the filter assembly (g), and V is the volume of sample filtered (L).

$$(Wt_2 - Wt_1) \;/\; V = TSS \qquad \text{Eq. 2.1}$$

2.3.2 Volatile Suspended Solids

Volatile suspended solids (VSS) analyses were conducted using the filtered samples prepared for total suspended solids. Filter assemblies were placed in a muffle furnace (Thermolyne 48000) at 550°C for 15-20 minutes according to Standard Methods 2540E (Eaton et al., 2005). Filter assemblies were cooled to room temperature in a desiccator and reweighed. Volatile suspended solids were calculated as described in Standard Methods 2540E and shown below in Equation 2.2, where Wt_2 is the weight of the filter assembly and sample (g), Wt_3 is the weight of the filter assembly and sample after burning in the muffle furnace (g), and V is the volume of sample filtered (L).

$$(Wt_2 - Wt_3) \;/\; V = VSS \qquad \text{Eq. 2.2}$$

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2.3.3 Mass Yield

The total volume of an algal culture was measured using an appropriately sized graduated cylinder. The total mass yield (MY) of the culture was calculated by multiplying the total suspended solids or volatile suspended solids concentration (g L^{-1}) by the total volume (L) of the culture, as shown in Equation 2.3.

$$SS\ (g\ L^{-1})\ *\ V\ (L) = MY\ (g) \qquad \text{Eq. 2.3}$$

2.4 Probeware and Other Laboratory Equipment

2.4.1 pH

The pH of all samples was measured using either glass pH electrodes (Oakton Instruments, Vernon Hills, IL, USA) and a digital pH meter (Corning pH/ion analyzer 350) or a gel-filled pH probe (Vernier Instruments, Beaverton, OR, USA) and a LabQuest2 portable digital interface or LabView software (Vernier Instruments, Beaverton, OR, USA). Prior to sample reading, the calibration of the probes was checked using calibration solutions of pH 4.0, 7.0, and/or 10.0 (Fisher Scientific, Pittsburg, PA), and probes were recalibrated if the reading was more than +/- 0.05 pH units from the expected value of the calibration solution. Probes were rinsed with deionized water and dried with tissue between each sample reading. The pH electrodes were stored in long-term storage solution between uses, prepared by adding 10 g of solid potassium chloride to 100 mL of pH 4 buffer.

2.4.2 Conductivity

Conductivity was measured using an alternating current conductivity probe in conjunction with a LabQuest2 portable digital interface or LabView software (Vernier Instruments, Beaverton, OR, USA).The probe had a range of 0 – 20,000 µS cm^{-1}. The calibration of the conductivity probe was checked with a standard 500 mg L^{-1} sodium chloride solution

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prepared in the laboratory, and the probe was recalibrated with the standard solution as needed. The conductivity probe was rinsed with deionized water and dried with tissue between each sample reading. The probe was allowed to stabilize for at least three minutes prior to sample reading.

2.4.3 Dissolved Oxygen Concentration

Dissolved oxygen concentration was measured using a Clark-type polarographic electrode membrane probe with a LabQuest2 portable digital interface or LabView software (Vernier Instruments, Beaverton, OR, USA). The probe had a range of 0 – 15 mg L^{-1} and automatic temperature compensation from 5-35°C. The probe was calibrated by submerging the probe in Sodium Sulfite Calibration Solution (Vernier Instruments, Beaverton, OR, USA) and recording the zero point. The probe was rinsed, dried, and placed in the calibration bottle just above (i.e., not whetted by) 0.25 inches of deionized water. The second calibration point (8.51 mg L^{-1}) was based on 760 mm of barometric pressure at sea level and a temperature of 24°C. Upon sampling, dissolved oxygen concentration was logged for 30 seconds, and the average value was recorded. The probe was rinsed with deionized water and gently dried with a tissue between each sample measurement.

2.4.4 Temperature

Temperature was measured using a manual thermometer. The thermometer was rinsed and gently dried with a tissue between each sample measurement. The thermometer was placed in the sample medium and allowed to stabilize prior to taking a measurement.

2.4.5 Carbon Dioxide Concentration

Free carbon dioxide concentration was measured with a portable dissolved carbon dioxide analyzer (OxyGuard CO2, OxyGuard International AS, Denmark). The analyzer was

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calibrated by the following steps and as described in the OxyGuard CO2 manual. First, the distilled water used for calibration was checked to be sure the carbon dioxide content was below 10 mg L^{-1}. Approximately 100 mL of distilled water was poured into the calibration beaker, and the value was read after a five minute waiting period. The value (4 mg L^{-1}) was noted as the Raw Water value. A 1 mL aliquot of calibration solution (OxyGuard CO2) was injected into the calibration beaker with the distilled water; the calibration fluid absorbed all free carbon dioxide. When the reading stabilized, the "zero" adjustment screw was turned until the display read "0." The calibration beaker was emptied, rinsed thoroughly, and refilled with approximately 100 mL of distilled water. Approximately 3 mL of pH conditioner (OxyGuard CO2) was added to the beaker and stirred until dissolved. The probe was replaced in the beaker and the stirrer turned back on. After five minutes, the value (10 mg L^{-1}) was recorded as the "A" value (if value was higher than 20 mg L^{-1}, the water would have been discarded and a new source of water would have been found). A 1 mL aliquot of calibration solution was added with a syringe and allowed to stir for ten seconds without the probe. The probe was replaced and a reading was taken after five minutes (value "B", 58 mg L^{-1}). The calibration value was calculated as described by OxyGuard and noted in the following equations:

$$U = 50 \times A/(B-A) \qquad \text{Eq. 2.4}$$

$$K = 50 + U \qquad \text{Eq. 2.5}$$

The "Slope" screw was adjusted to display the new "K" value as calculated. In the field study described in Chapter 6, "U" = 10.41 and "K" = 60.41.

Field samples were taken by lowering the probe into the sample stream and allowing the probe to stabilize for at least 15 minutes. Laboratory samples were measured using the

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calibration beaker with stirrer after allowing each sample to stabilize for five minutes. The probe and beaker were washed at least three times with deionized water between each sample reading.

2.4.6 Flow

Water flow at the field site was measured using a flow rate sensor that measures the velocity of water using a propeller and a LabQuest2 portable digital interface (Vernier Instruments, Beaverton, OR, USA). The sensor had a range of 0 – 4.0 m s^{-1} and a resolution of 0.0012 m s^{-1}. For measurements in Chapter 6, the flow rate sensor was placed in the clarifier trough directly next to the upwell weir with the propeller facing the water flow. The average flow over 30 seconds was recorded.

2.4.7 Total Organic Carbon and Total Nitrogen

Liquid total organic carbon (TOC) and total nitrogen were measured using a Total Organic Carbon analyzer (Shimadzu TOC-V CSH), which included a Total Nitrogen detector (Shimadzu TNM-1) (Shumadzu Scientific Instruments, Inc., Columbia, MD). TOC was measured by combusting the sample at 680°C and measuring the gas using a Non-Dispersive Infrared (NDIR) sensor. The nitrogen monoxide detected upon combustion, measured by chemiluminescence, was used to measure total nitrogen. Samples were vacuum filtered (Whatman GF 934-A) prior to analysis. Additionally, samples were acidified externally with 1N HCl and sparged with Ultra Zero Grade Air for two minutes (to remove inorganic carbon) prior to measuring. The area output of analyses were transformed to concentration using calibration curves produced using standard solutions of potassium hydrogen phthalate (KHP) and potassium nitrate.

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2.5 Algae Stock Cultivation

The *Chlorella sorokiniana* (UTEX#246) and *Botryococcus braunii* (UTEX #572) stock cultures were ordered in agar slant test tubes from the University of Texas Culture Collection of Algae. The tubes were placed in the incubator upon receipt, where they were subjected to the same conditions as stock cultures. The incubator was fitted with rails and holders to accommodate different sized Erlenmeyer flasks (ranging from 100 mL to 500 mL). Algae stocks were shaken at 100-150 rpm (Lab-Line Incubator-Shaker, Melrose Park, IL) under a 12h/12h photoperiod of approximately 21 W m^{-2} during light hours. Light was provided by four fluorescent bulbs that ran the width of the incubator. Stock cultures were not artificially aerated.

To transfer the culture into liquid medium (typically Bold 1NV), a metal spatula was sterilized by dipping the spatula in ethanol and heating with a flame. The cooled spatula was inserted into the tube (being careful to maintain tube at a horizontal so dust would not fall into it) and a chunk of algae was scraped from the test tube lawn. The agar and algae complex was placed in a small petri dish with about 50 mL of Bold 1NV medium. Bold 1NV medium was made with the following components: $NaNO_3$ (2.94 mM); $CaCl_2$ (0.17 mM); $MgSO_4$ (0.3 mM); K_2HPO_4 (0.43 mM); KH_2PO_4 (1.29 mM); NaCl (0.43 mM); P-IV Metals Solution, Vitamin B_{12}, Biotin, Thiamine per UTEX Bold 1NV recipe (The detailed recipe can be found at the UTEX website, http://web.biosci.utexas.edu/utex/mediaDetail.aspx?mediaID=15). The petri dish was placed 12-18 inches under fluorescent lights at a 12/12 photoperiod. Typically, in about one week, the culture was dense enough to transfer to a flask.

Once the algae culture in the petri dish reached a density of at least approximately 0.500 RAU at 680 nm (see section 2.2.1 for details on measuring optical density), it was transferred to a larger flask. As the dilution rate should not exceed 1:10, the new volume should not exceed

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500 mL. However, to ensure adequate inoculant, stock cultures were often transferred to 100 mL or 250 mL flasks filled 2/3 with sterilized Bold medium such that the RAU of the new culture was approximately 0.100 at 680 nm. The flask should not be filled more than 3/4 to leave ample room for the fluid to swirl while shaking. The petri dish culture was agitated with a pipet (drawing and dispensing culture back into the dish) while holding the cover over the dish, then withdrawn and transferred to the flask. Once the culture was transferred, a Styrofoam stopper was placed over the top of the flask.

New stock cultures were seeded every 3-4 weeks to maintain a fresh stock with a similar procedure to that described above. Duplicate flasks were filled to 2/3 capacity with sterilized Bold medium and seeded so that the optical density was at least 0.100 RAU at 680 nm. The new cultures were labeled with the date of transfer. Three generations of cultures (the new stock and two previous generations) were kept in case of a culture collapse, but older generations were discarded down the sink after bleaching or autoclaving.

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Chapter 3: Membrane Applications for Microalgae Cultivation and Harvesting: A Review[1]

3.1 Introduction

The algae industry is an important part of the global economy. In addition to bioproducts, algae are increasingly recognized for their use in the bioenergy and waste management sectors. As commercial demands for algal biofuel and bioproducts increase, new methods to cultivate, harvest, and separate biomass and bioproducts from growth medium become increasingly important, especially as harvesting can be energy intensive and costly. This review focuses on the science and technology of membranes for the cultivation and harvesting of microalgae biomass and bioproducts, drawing on knowledge in water treatment and applications relevant to commercial algae production.

3.1.1 Algae Background

Algae are a biologically diverse group of organisms that are important in global commerce, with annual revenues of about 1.25 billion USD (Raja et al., 2008) and 6 billion USD (Pulz & Gross, 2004) in the micro- and macro-algae industries, respectively. Depending on the species, algae can contain a high content of vitamins, minerals, protein, pigments, carbohydrates, or polyunsaturated fatty acids (Morineau-Thomas et al., 2002); algae are useful for biofuels and a variety of bioproducts such as nutritional supplements, animal and fish feed, fertilizers and

[1] The contents of this chapter are published in: Drexler ILC & Yeh DH (2014) Membrane applications for microalgae cultivation and harvesting: a review. Reviews in Environmental Science and Bio/Technology 13(4):487-504. The final publication is available at Springer via http://link.springer.com/article/10.1007/s11157-014-9350-6

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cosmetics (Morineau-Thomas et al., 2002; Molina Grima et al., 2003; Chisti, 2007; Harun et al., 2010), as shown in Table 3.1.

Most algae are phototrophs and require sunlight, water, carbon dioxide, and nutrients (namely nitrogen and phosphorus) to grow. Algae can be auto-, hetero-, or mixotrophic (Liang et al, 2009), making cultivation with a variety of feedstocks possible. For example, many growth requirements can be met using alternative resources, such as water and nutrients in wastewater or carbon dioxide in industrial flue gas (Mata et al., 2010). Algae can effectively treat a variety of wastewater streams, such as municipal, agricultural, or industrial waste, and the cultivated biomass can be anaerobically digested for biogas or converted to liquid fuels (Chisti, 2007).

Microalgae production can be challenging. First, the efficient delivery of carbon dioxide during algae cultivation can be problematic. Second, due to the small size of single-cellular microalgae (3-30 μm diameter) and the dilute concentration of cultures (generally less than 0.5 g dry biomass L^{-1}) (Molina Grima et al., 2003), separating, harvesting, and dewatering algal cells and bioproducts can also be difficult and costly. A variety of conventional technologies (e.g., centrifugation, sedimentation, flocculation, screening and belt press) have been applied at different stages of algal growth, each with their own advantages and disadvantages. Finally, the appropriate recycling of growth medium or recycling of wastewater is paramount to the long-term sustainability and economics of microalgae production. In recent years, membranes have successfully been applied to address all of the above challenges.

3.1.2 Membrane Background

Membranes are used for separation, providing a thin physical barrier that selectively restricts the passage of solvents (liquids and gases) and solutes (ions, biomolecules, colloids, cells, suspended solids), depending on solvent/solute properties (e.g., size, charge, solubility, and

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other chemical compositions) and membrane characteristics (e.g., pore size and distribution, charge, surface roughness, and material). Membrane pore size (and distribution) generally govern which constituents can pass through a membrane, but selection can also be affected by membrane fouling. Figure 3.1 depicts the rejection of solutes by macrofiltration (MaF), microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO, also known as hyperfiltration).

Active filtration is typically characterized by a pressure gradient (transmembrane pressure), where the solute is selectively rejected and the filtrate (permeate) is either drawn across the membrane with positive feed-side pressure, negative permeate-side pressure, or a combination thereof. Active filtration includes dead end filtration, which is prone to fouling, and tangential flow filtration (TFF), which can create shear due to the parallel movement of the feed flow. Passive filtration (e.g., dialysis and forward osmosis) relies on the movement of a solute or solvent across the membrane as a result of concentration gradients. Passive filtration, used to concentrate biomass or bioproducts, generally requires less energy than active filtration. Common membrane modules and typical flow configurations are shown in Figures 3.2 and 3.3, respectively.

For algal cultivation and harvesting, membranes provide unique functions for solid/liquid separation (cell retention, biomass concentration and dewatering), gas/liquid separation (gas delivery and removal), and solute/liquid separation (bioproduct recovery, feedstock preparation, and effluent recycling) that are problematic or not possible with other technologies.

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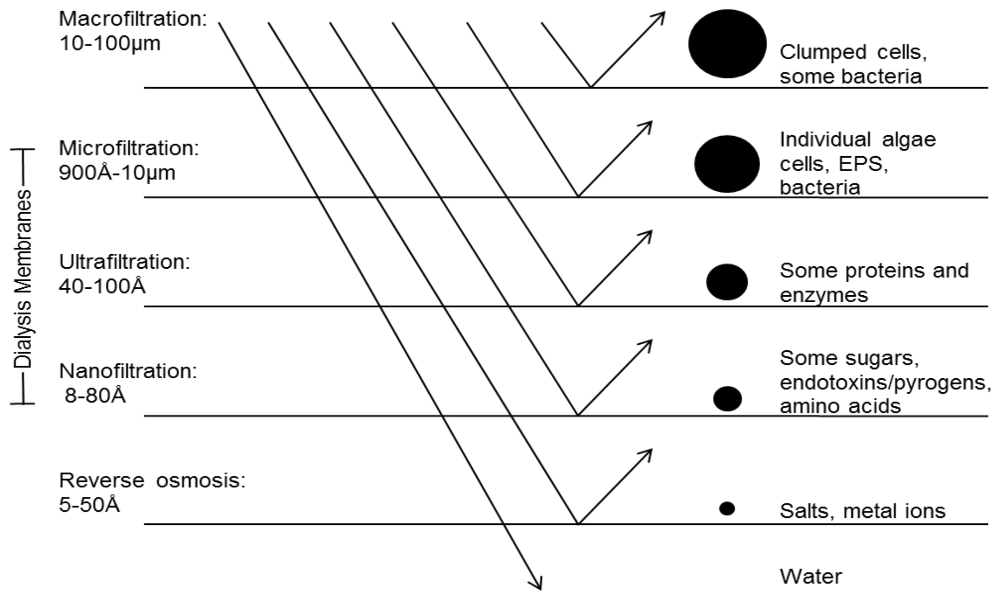

Figure 3.1. Filtration spectrum demonstrating algal products rejected based on membrane pore size.

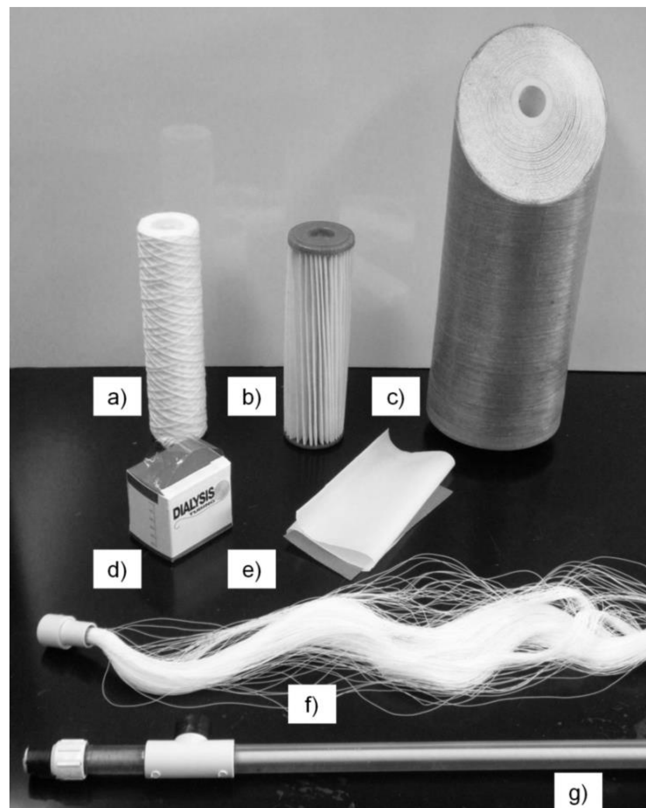

Figure 3.2. Various types of membranes and configurations. a) string-wound cartridge; b) pleated sheet cartridge; c) spiral wound element; d) dialysis tubing; e) flat sheet; f) hollow fiber; g) tubular.

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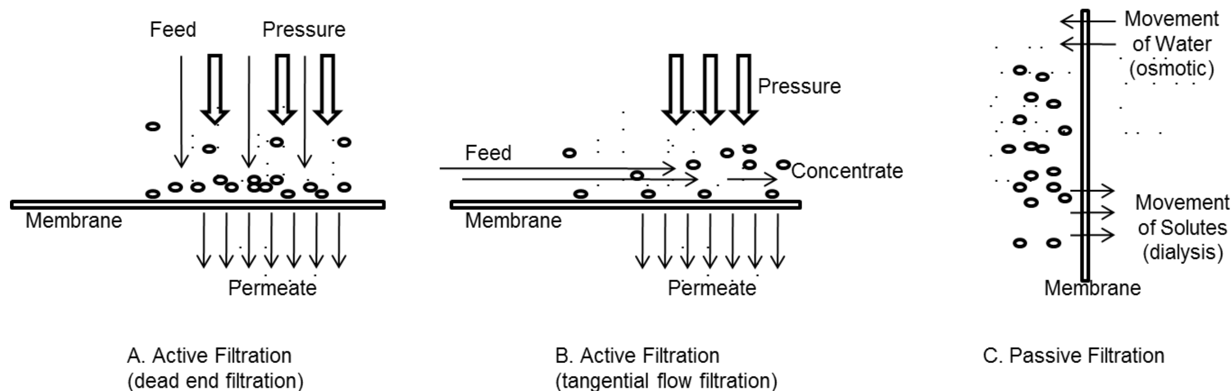

Figure 3.3. Examples of fluid flow and/or particle flow across a membrane in various configurations.

3.1.3 Membrane Materials Used in the Algal Industry

Membrane material selection in the algal industry may depend on culture concentration, species characteristics, and flow parameters. A list of studies that investigated various algal species, membrane configuration, material, and pore size is shown in Table 3.2. Typical membrane materials include cloth and nonwoven fabric for MaF; polyvinylidene fluoride (PVDF), polyacrylonitrile (PAN), polyether sulfone (PES) and polysulfone (PS) for MF/UF; cellulose acetate (CA) and polyamide for NF/RO; cellulose ester (CE) and regenerated cellulose (RC) for dialysis; polypropylene and PVDF for gas delivery; and silicon-containing polymeric membranes (such as polyvinytrimethylsilane, PVTMS) for gas separation.

After studying eleven (Rossi et al., 2004) and eight (Rossignol et al., 1999) membranes of different pore size and material, those with a pore size range of 40-100 kDa (about the range of UF membranes) were recommended for long term use. De Baerdemaeker et al. (2013) found similar results, with UF membranes demonstrating better flux and fouling resistance than MF membranes. PVDF membranes maintained the highest permeability compared to polyvinyl chloride (PVC) and polyethersulfone/polyvinylpyrollidone (PES/PVP) membranes after critical flux measurement tests including filtration, relaxation, and backwash cycles. Acrylic membranes

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were found to be the easiest to clean (compared to PAN, PVDF, and PES membranes) (Rossi et al., 2004), and PAN-type UF membranes were determined the best material for cell recovery (Rossignol et al., 1999).

Surface charge and hydrophobicity are also a consideration when selecting membrane material, as these characteristics can affect membrane fouling. Decreased electrostatic attractions on neutral membranes can reduce fouling potential. Alternatively, negatively charged membranes may repel similarly charged algal cells or organic material. The combination of charge and hydrophobicity may also affect performance. For example, negatively charged hydrophilic membranes (Rossignol et al., 1999; Sun et al., 2013) have been recommended for longer term operation and have been shown to foul less easily by extracellular and natural organic matter, oils, and grease than hydrophobic membranes (Hung & Liu, 2006; Babel & Takizawa, 2010). Furthermore, fouling on hydrophobic membranes tends to be less reversible (Babel & Takizawa, 2010). Choice of membrane material can affect fouling potential, thereby impacting both membrane performance and longevity.

3.2 Cultivation

Algae can be cultivated on a large scale in open systems (e.g., high rate algal ponds, raceways and lagoons) and closed systems (e.g., photobioreactors or membrane bags) (Mata et al., 2010); more often membranes are used to cultivate algae in closed systems. Due to their flexibility, membranes can be utilized for cultivation in many ways, such as concentrating algae biomass to reduce footprint or increase treatment capacity, treating growth medium for recirculation, or delivering carbon dioxide for improved mass transfer.

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3.2.1 Cell Retention or Isolation with Passive Filtration

Dialysis membranes have been used extensively for bacterial cultivation and bioproduct production and harvesting, and these methods hold promise for algae cultivation as well (Schultz and Gerhardt, 1969). Dialysis cultivation decouples the algae culture from its growth medium by separating the two liquids with a semi-permeable membrane barrier. The physical separation from grazing organisms may be extremely important especially in wastewater, as rotifers can destroy an algal culture in just a few days (Huang et al., 2013). Nutrient transport occurs via concentration gradients, therefore requiring minimal energy. Decoupling the algae culture from the growth medium has allowed researchers to investigate growth kinetics (Skipnes et al., 1980), species interactions (Vincent & Silvester, 1979), and toxicity tolerances (Powers et al., 1976), which may be important for facilities interested in specific traits of a target algae species.

Early studies have shown that algal cell density achieved via dialysis culture exceeded that of suspended cultures in natural environments (Jensen et al., 1972) and wastewater (Dor, 1975; Blais et al., 1984). Dialysis cultivation keeps grazing and predatory organisms away from growing algae, removes toxic byproducts, and increases nutrient availability. If algae cultivation is integrated with wastewater treatment processes, denser cultures may achieve better nutrient removal efficiency (Xu et al., 2014) and require a smaller reactor footprint to achieve similar yields. Denser cultures require less energy and fewer inputs to dewater, which also improves process economics.

The *Offshore Membrane Enclosures for Growing Algae* (OMEGA), patented and licensed by NASA, is a membrane bag system used to cultivate freshwater algae with wastewater. The OMEGA system is floated in seawater, where forward osmosis is used to passively dewater the algae culture. If the bags are broken, freshwater algae die when exposed to

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salty conditions, preventing the threat of invasive species release. The bags can subsequently be cleaned and reused after the algae have been harvested (Hoover et al., 2011; Wiley et al., 2013).

The use of floating membrane bags has two additional advantages. In arid environments (e.g., southwestern United States), evaporation in algae ponds (up to 80 inches of water annually) is problematic (Hanson, 1991). Maintaining the algae culture and growth medium in a closed system reduces water loss and can save the cost of algal pond augmentation. Furthermore, the water in which the membrane bag floats provides a heat sink, which can help shield the algal culture from wide temperature swings throughout the day.

3.2.2 Algae Membrane Photobioreactors

Algae membrane photobioreactors incorporate MF or UF membranes into a closed cultivation process; closed systems are typically more productive than open systems, such as raceways or ponds (Chisti, 2007; Mata et al., 2010), with an average production of 0.14 g L^{-1} in open systems compared to 4 g L^{-1} in closed systems (Chisti, 2007). During cultivation, membrane systems can accommodate high cell densities, which may decrease the land footprint required and increase nutrient removal efficiency in treatment applications (Singh & Thomas, 2012; Xu et al., 2014). Although used more often for controlled cultivation of algae in industry, algae MBRs have recently been used in wastewater treatment, such as treating digester effluent (Prieto, 2011; Ruiz-Martinez et al., 2012; Singh & Thomas, 2012). The algae membrane photobioreactors in these studies were able to remove 50-100% of ammonia, 35% of nitrate, and 60-100% of phosphate in continuous operation, despite fluctuating feed quality. Although the average algal cell density was just 0.5 g L^{-1} in one study (Prieto, 2011), the transmembrane pressure of 0.06 Bar was well below values of 0.34 Bar typically reported elsewhere in similar

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studies (Danquah et al., 2009a; Danquah et al., 2009b), which suggests that the density of the algal culture could further increase without affecting permeate flux.

In addition to treating domestic wastewater, a membrane photobioreactor was used to increase cell retention time to treat industrial wastewater from an electronics factory supplemented with nutrients (Zhen-Feng et al., 2011). Although the algal culture did not grow well in continuous flow due to metals toxicity, phosphorous and nitrate removal were significant. Similarly in a recent study, a continuous flow photobioreactor treating synthetic wastewater achieved an algal cell density of 4.8 g/L and phosphorous removal of 66% (Xu et al., 2014). Integrating a photobioreactor cultivation with wastewater treatment provides a cheap source of nutrients and water, while still taking advantage of the high cell densities of closed systems.

3.2.3 Growth Medium Recirculation or Wastewater Treatment

External inputs, such as freshwater, nutrients, or carbon dioxide are among the most significant economic investments in large-scale algal cultivation (Clarens et al., 2010). In fact, the limitation of these resources may tip the scale to where algal cultivation is no longer economically competitive (Pittman et al., 2011). Therefore, it may be desirable or necessary to recirculate growth medium or obtain nutrients from alternative sources, such as wastewater. Compared to most water treatment technologies, membranes have a competitive advantage in treating alternative nutrient streams due to ease of automation, relatively small footprint required, and low sensitivity to many influent water quality parameters (e.g., pH, temperature, dissolved nutrients). Similarly, effluent water from large-scale cultivation efforts such as aquaculture (Castaing et al., 2010) or supernatant from algal harvesting efforts (Chen et al., 2013) can be treated with a membrane system prior to environmental discharge.

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Organic substances, often present in surface water and wastewater, may likewise need to be removed from recirculated growth medium to prevent accumulation. Membranes have been used successfully to clean ballast water and filter toxic algae, total suspended solids, and turbidity out of seawater used for algal cultivation (Castaing et al., 2010; Castaing et al., 2011). A combination of RO and UF membranes also effectively removed extracellular polymeric substances produced by algae and/or bacteria (Van Nevel et al., 2012). RO membranes also removed algal toxins (e.g., such as domoic acid, saxitoxin, brevetoxin, and okadaic acid) from intake water at desalination plants in lab-scale and field monitoring efforts (Seubert et al., 2012). Not only may such algal excretions be toxic to people and the environment, but the excretions have been shown to increase the pH of water and the potential to form disinfection byproducts, such as chloroform (Her et al., 2004).

Because membranes can effectively remove toxins and other constituents from source water, they can be used to treat growth medium for recirculation or discharge to the environment. Membranes can remove turbidity to ensure adequate light penetration when waste management is integrated with algal cultivation. One approach is to utilize membranes to remove suspended solids, leaving dissolved nutrients in solution for algal assimilation in recirculated medium.

3.2.4 Gas Delivery and Separation

Airlift bioreactors are commonly used for mixing and gas delivery due to low operating costs (Futselaar et al., 2009), but airlift photobioreactors may suffer from limitations in mass transfer and unevenly distributed algal biomass, which affects light and nutrient availability (Fan et al., 2007). In contrast, membranes have been used in algal cultivation to improve mass transfer and enhance gas delivery for carbon dioxide uptake. Improved carbon dioxide availability

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increases algal growth rates, thereby enhancing algal biomass or bioproduct production (Pires et al., 2012).

When coupled with a higher interfacial area, membranes can increase gas exchange efficiency by discharging smaller, more uniform bubbles and increase the retention time of those bubbles in solution (Cheng et al., 2006; Fan et al., 2008). In fact, an external hollow fiber membrane module increased the retention time of gas bubbles tenfold compared to conventionally pumped gas delivery in an ordinary photobioreactor (Cheng et al., 2006). Similarly, a membrane sparged helical tubular photobioreactor used to cultivate *Chlorella vulgaris* improved carbon fixation 0.95-5.40 times over conventional photobioreactors (Fan et al., 2007). The concentration of delivered carbon dioxide could be decreased from 1.0 to 0.2% when the fixation rate was increased from 80 to 260 mg L^{-1} $hour^{-1}$ (Cheng et al., 2006). If the amount of carbon dioxide fed to an algal reactor can be decreased, the external operating costs and the overall economics of cultivation will be improved.

Although hollow fiber membranes can be used to decrease bubble size and increase sparger area (Fan et al., 2007), the length and number of membranes must balance bubble production surface area and contact time in the reactor (Fan et al., 2007). Intense bubble diffusion to mix the culture can cause cloudiness, which could impede light penetration and algal growth (Fan et al., 2008). Reactor design is therefore critical to optimize production. To combat undesirably intense mixing, a membrane sparged photobioreactor was designed to accommodate a high surface area to volume ratio for light exposure, reduced self-shading and convective gas and liquid flows, and could be scaled up for higher volumes (Fan et al., 2008).

Membranes can also be used to clean waste industrial gases (‘free sources of carbon dioxide) for algal cultivation. Gas separation membranes remove NO_x and SO_x (which could

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reduce the pH of algal cultures) from flue gas prior to injection in an algal photobioreactor (Pires et al., 2012). An innovative solar powered bioreactor integrated algal and purple bacteria cultivation with anaerobic digestion. The reactor used a 0.2 µm PVTMS gas separation membrane to separate the biogas from anaerobic digestion into methane (for biogas) and carbon dioxide (used to feed the algae photobioreactor) (Teplyakov et al., 2002). This system demonstrates how membranes can be integrated into a multilevel production system, where waste effluents are recycled for other processes.

Membranes may also be used to harvest gases (e.g., oxygen and hydrogen) from algal cultures. A 0.2 µm polytetrafluoroethylene (PTFE) porous membrane was used to separate oxygen from algal growth medium (Cogne et al., 2005). Because the oxygen recovery was successfully used to estimate algae growth at considerable precision and accuracy, the membrane unit was recommended for artificial life support systems in space. Furthermore, as high dissolved oxygen can inhibit algal photosynthesis (Fan et al., 2008), removing oxygen is important to optimize growth in large scale cultivation. Hydrogen, generated by blue-green algae and slated for use as biofuel, has also been collected using membrane photobioreactors (Markov, 1999).

3.3 Harvesting

Due to the energy and capital costs associated with harvesting algae in large scale production facilities, it is critical to select and design an appropriate harvest method (Molina Grima et al., 2003). Common harvesting techniques include filtration, gravity sedimentation, flocculation, flocculation + flotation, or centrifugation. Forward osmosis, though less common, can also be used (Hoover et al., 2011; Buckwalter et al., 2013). Although high solids content in feed water may accelerate fouling potential, membrane filtration is generally less sensitive to chemical speciation, feed water quality parameters (e.g., pH) and algal biomass properties (e.g.,

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settleability) than chemical harvesting processes, such as coagulation or flocculation (Babel et al., 2002; (Morineau-Thomas et al., 2002); Her et al., 2004; Ghernaout et al., 2010).

Another appeal of membrane filtration is that it is minimally disruptive to harvested biomass. Membranes cause minimal stress to the algal cells and avoid chemical additives (e.g., flocculants or pH adjustments required for flocculation) that may otherwise degrade the quality of harvested biomass or bioproducts (Wicaksana et al., 2012). Membrane systems, however, may require more energy and are susceptible to fouling (Wicaksana et al., 2012). Knowledge from the water treatment field may provide insight to potential challenges and solutions for the use of membranes in large scale algal harvesting efforts.

3.3.1 Biomass Concentration (Dewatering)

Although membranes are not typically used in large scale processes due to the high flux required (Molina Grima et al., 2003), there is the potential to use membrane filtration to concentrate and dewater in smaller scale processes. Despite lower initial flux, UF membranes have demonstrated better flux over a longer period than MF membranes, probably because the larger pores of the MF membranes are more prone to fouling (Rossignol et al., 1999; Rossi et al., 2004). MF is recommended if daily flow volumes are less than 2 m^3 day^{-1}, but it is most likely more economical to centrifuge volumes larger than 20 m^3 day^{-1} (Rossignol et al., 1999). When flows fall between this range, other system attributes, such as membrane material and system flow rate, may be considered. Microstrainers, vibrating screen filters (Uduman et al., 2010), and cloth filtration (Shelef et al., 1984) may be alternative filtration methods if small pore sizes are not necessary.

Algal species characteristics, such as algal cell size, cell specific gravity, or the species' ability to autoflocculate may also influence choice of harvesting method (Uduman et al., 2010).

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Cell size is especially important, as filtration tends to work better with larger microalgae (such as *Spirulina platensis*) rather than smaller diameter microalgae (such as *Dunaliella* and *Chlorella*) (Harun et al., 2010). If cells are fragile, MF membranes may be suitable (Molina Grima et al., 2003).

Membrane fouling can be turned to an advantage for dewatering. Algae cake formation (i.e., dynamic foulant layer) may reduce large pore sizes in materials such as cloth textiles, thereby providing better straining as the cake layer develops. Similar to a belt filter press used to dewater sludge, algae can be collected on a membrane surface and actively (by pressure) or passively (by gravity) drained. Dewatered algae are scraped off the membrane surface into a collection basin, much like current drum, disc, or belt filters collect sludge. Various filtration and screening devices that may be appropriate to harvest algae with minimal energy are reviewed in Shelef (1984).

3.3.1.1 Biomass Separation with Dead End Filtration

Dead end filtration (shown in Figure 3.3a) is typically more successful when filtering low concentration solutions, as fouling (via cake formation) can occur quickly. Therefore, because algal cultures tend to be higher in density at harvest, it would generally not be economical to use dead end filtration to harvest. Batch investigations of filter materials and foulant characteristics have demonstrated that rapid fouling occurs with this configuration (see Table 3.2 for specific studies using dead end filtration).

Although dead end filtration is not typically used because of fouling concerns, two studies (Bilad et al., 2012; Bilad et al., 2013) extrapolated the energy requirement, and therefore the operational costs, of a lab-scale submerged flat sheet membrane to harvest microalgal cultures. The studies showed that dead end filtration is energetically competitive with other

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harvesting methods, such as flocculation or vacuum filtration. The systems investigated experienced a moderate to low level of membrane fouling yet achieved high flux. Biomass was concentrated up to 10 times the initial concentration (to a density of 3.5 g L^{-1}) (Bilad et al., 2012) or 6 times the initial concentration (to a density of 1.52 g L^{-1}) with the addition of a magnetically induced vibrating membrane (Bilad et al., 2013). However, this concentration factor and cell density is much lower than that achieved using other configurations, as described below. Because of the relatively low density, Bilad et al. (2013) suggest coupling an initial membrane filtration step with centrifugation.

3.3.1.2 Biomass Separation with Tangential Flow Filtration

During TFF (also known as cross-flow filtration) feed water passes parallel to the membrane while permeate passes through perpendicularly (see Figure 3.3b). Because of its ability to highly concentrate algae feedstock, TFF can be an economical first step for dewatering. TFF concentrated *T. suecica* 150 times its initial concentration (up to 8.8% w/v solids). The process required 2.06 kWh m^{-3}, which was less energy than that required for flocculation, centrifugation, or vacuum filtration and comparable to the energy requirement of pressure filtration. The improved energy efficiency allows for a faster return on investment for the harvesting equipment (Danquah et al., 2009b). Similar results were achieved with *N. oculata* (Bhave et al., 2012) where hollow fiber and tubular membranes concentrated the algal biomass 75x to a density of 150 g L^{-1} with an estimated energy requirement of 0.3-0.7 kWh m^{-3}.

Results from algal experiments conducted in the laboratory are often meant to represent algal populations in their natural environment. However, when generations of algae are cultivated in the laboratory, the biology of the organism, such as motility and morphology, may be impacted, which may affect the applicability of results outside the laboratory. To address this

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phenomenon, TFF was used to harvest and concentrate natural species from freshwater sources for laboratory experiments, such that experimental results could be more appropriately extrapolated to natural populations. Algae have been successfully concentrated 5-40 times, without damaging cell structures, including motility features and reproductive capabilities (Petrusevski et al., 1995). TFF may be a strategy to preserve the integrity of the algal cells during harvest if it is desirable for certain morphological characteristics to remain intact.

TFF configurations may increase shear stress of fragile algae cells. Distressed algae may release algogenic organic material which could exacerbate fouling (Rossi et al., 2004). High shear may also cause premature release of bioproducts due to algae cell breakage (Rossi et al., 2004). If bioproduct recovery (such as lipids) is important post-harvest, premature breakage could lead to substantial economic losses. Cell breakage and lysis could also lead to further fouling. Large scale TFF may be limited by the replacement costs of membranes and pumping requirements (Chen et al., 2011). As such, harvesting objectives must be carefully considered in system design, and TFF may be most successful used in conjunction with other strategies to harvest and dewater cultures.

3.3.1.3 Biomass Separation with Passive Membranes

Passive membrane systems, such as dialysis or forward osmosis, rely on concentration gradients for the movement of solutes or water (see Figure 3.3c). These methods are particularly attractive due to their lower energy costs. Dialysis algal cultivation in various media has been shown to achieve higher cell densities than algal cultures suspended directly in growth medium (Jensen et al., 1972; Dor, 1975; Vincent & Silvester, 1979; Blais et al., 1984); the higher cell density achieved in dialysis may reduce harvesting costs in subsequent dewatering steps.

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Forward osmosis also has potential as an initial dewatering step, where algae growth medium is passively removed using a draw solution (Buckwalter et al., 2013). The availability of an affordable and recoverable draw solution is strongly tied to economic feasibility (Zhao et al., 2012), and the choice of draw solution may also affect fouling potential. For example, draw and feed solutions that contain divalent magnesium ions can cause severe and irreversible fouling due to reverse diffusion (Zhou et al., 2013). A membrane spacer can help alleviate fouling and improve flux in these cases.

Forward osmosis is also used to concentrate algal biomass in the OMEGA system (described previously in section 3.2.1). Although operational challenges remain, forward osmosis may be used in conjunction with other dewatering methods, where upfront passive design can reduce energy requirements further down the processing train.

3.3.2 Bioproduct Separation

Many algal species produce commercially-attractive bioproducts, such as neutraceuticals, antioxidants, toxins, or pigments (shown in Table 3.1). Harvesting these bioproducts can be challenging due to their solubility, small molecular weight, or dilute concentrations. Due to its flexibility, membrane filtration can be used to separate bioproducts from growth medium, aid purposeful cell disruption to free intracellular products, or harvest gases (such as oxygen).

Dialysis membranes are effective to isolate non-diffusible products or concentrate diffusible ones (Jensen et al., 1972; Cooper et al., 1983). Due to the passive nature of these membranes, a low energy demand is required and bioproducts can be harvested with minimal disturbance to the larger culture. Although dialysis has been used extensively to harvest and study bacterial bioproducts (Schultz & Gerhardt, 1969), the methods are transferable to algal cultures as well.

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Marennine, a bioproduct of the marine diatom *Haslea ostrearia,* used in anticancer research and cosmetics, was recovered with submerged ultrafiltration membranes (Rossignol et al., 2000a). Because hydrostatic pressure was used to backwash, researchers did not have to pump the algal culture across the membrane, which decreased the energy demand and shear stress. The configuration isolated approximately three times the marennine concentration achieved in conventional systems that did not use membranes.

A subsequent study (Rossignol et al., 2000b) compared the submerged membrane reactor to that of an agar gel block inoculated with *H. ostrearia* . Researchers found a lower marennine concentration in the bulk solution and lower volumetric and specific productivities from immobilized algal cells. Lower marennine production may have been due to slower diffusion of the bioproduct from the agar block; the higher concentration with the block impeded further production. The authors suggested to surround the gel block with a membrane to improve bioproduct recovery and prevent the release of immobilized algal cells.

Shear stress caused by hydrodynamic forces may limit membrane applications in pressure driven algal cultivation (Rossignol et al., 2000b), as excessive shear stress may cause cell breakage and premature release of bioproducts (Rossi et al., 2004). Shear stress could be minimized by careful selection of membrane type or configuration; Castaing et al. (2011) found that when filtering aquaculture feed water with hollow fiber microfiltration membranes, the low hydrodynamic shear did not rupture toxic algal cells. Because submerged membranes eliminate the need to recirculate medium, there is less potential for cell damage and toxin and EPS release with these systems (Castaing et al., 2010). However, if cell breakage and subsequent release of desirable components could be controlled, deliberate shear caused by tangential flow or dead end filtration could be advantageous to harvest certain intracellular products.

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3.4 Membrane Fouling

Membrane fouling may be the principal challenge in large scale filtration applications. It can be costly, both in operation (increased energy inputs) and capital (equipment replacements). However, fouling propensity can be somewhat mitigated with material selection, as noted in section 3.3.1.3, or with operation, as discussed in section 3.3.4.3. This section draws from literature in the drinking water industry to discuss fouling mechanisms, characteristics, consequences, and mitigation as the topics relate to the algal industry.

3.4.1 Fouling Mechanisms

Although fouling plagues membrane systems, a recent study found that algae membrane bioreactors exhibited less fouling than membrane systems operated in activated sludge (Xu et al., 2014). Membrane fouling due to algae may be caused by a multitude of factors, including membrane characteristics (discussed briefly in section 3.1.3), media constituents, and algal growth conditions. Algal excretions (commonly referred to as extracellular polymeric substances (EPS), extracellular polysaccharide substances, algogenic organic matter, and extracellular organic matter) produced during growth or intracellular products released upon cell lysis can contribute to fouling.

Fouling potential may be affected by the amount of EPS per cell volume of algae, the composition of the EPS (i.e., hydrophobic portions of EPS can cause irreversible fouling) (Qu et al., 2014)), and the colloidal properties of organics (Chiou et al., 2010). Polymers of alginate monomers are responsible for different types of fouling, where one alginate monomer combination (MG block) causes severe pore blockage and another (MM block) contributes to cake fouling (Meng & Liu, 2013).

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Although fouling during drinking water and wastewater treatment may be caused by other mechanisms (i.e., adsorption, pore blockage, concentration polarization/scaling, or biofilm and cake formation), fouling associated with algae has been found to be due mostly to cake formation (Morineau-Thomas et al., 2002; Castaing et al., 2010), and, to a lesser degree, pore blockage or hydrophobic adhesion (Qu et al., 2014). Cake formation generally rises with increasing algal feed concentration (Babel & Takizawa, 2010), and the EPS found in algal cakes makes it more difficult for permeate to travel between cells (Morineau-Thomas et al., 2002; Babel & Takizawa, 2010). The cake layer may advance membrane fouling more rapidly, as the denser fouled layer does not have the same pore space availability that would have otherwise allowed the passage of smaller particulates (Zhang et al., 2013b). On the other hand, cake formation creates additional filtration that may prevent further pore blockage and fouling within the membrane (Meng & Liu, 2013). Furthermore, hydrophilic portions of the cake layer may impede water flow due to interactions with hydrogen bonds (Qu et al., 2014).

The algal growth phase may also influence the fouling potential of the culture during harvest. Cultures in early exponential phase contained significantly fewer biopolymers and humic-like substances and also fouled less (Zhang et al., 2013b) than those in stationary growth phase, which had higher amounts of humic-like substances. In another study, TFF achieved a higher concentration of algal culture (48x concentrated) with less energy (0.39 kWh m^{-3}) when algae were harvested during a low growth rate (stationary) phase as opposed to a high growth rate (exponential) phase (23x concentration, 0.51 kWh m^{-3}) (Danquah et al., 2009a).

Algal growth conditions similarly influence fouling potential. In optimal conditions, the negative charge on fast growing algae causes cells to repel each other, which reduces their fouling potential (Babel et al., 2002). Under suboptimal conditions such as very high temperature

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and light intensity algae produce more EPS which leads to higher fouling potential. For example, hot and dry summer weather dramatically increased the cake resistance of algae cultivated in outdoor ponds compared to the cake resistance during cooler months (Babel et al., 2002). Similarly, in a study comparing micro- and ultrafiltration, when the algal feed temperature was increased from 20-24°C, flux increased. However, increasing the temperature from 24-28°C did not effect a similar increase in flux, most likely due to increased EPS production (Sun et al., 2013). *A. platensis* increased EPS production and bioflocculated under light stress (Rossi et al., 2008). It may be possible to mitigate fouling potential by carefully controlling environmental conditions, such as light intensity or temperature, of an algae culture prior to harvest.

3.4.2 Foulant Characteristics

Although membrane foulants are composed of many constituents (intact algal cells, extracellular excretions, and dissolved organic matter), the major component is EPS. The specific composition and release of EPS may change with species, growth phase, and growth conditions, but EPS commonly contains "glycolic acids, carbohydrates, polysaccharides, amino acids, peptides, organic phosphorous, enzymes, vitamins, hormonal substances, inhibitors, and toxins" (Her et al., 2004). Its composition is most often dominated by polysaccharides and proteins (Her et al., 2004; Chiou et al., 2010; Zhang et al., 2010; Wicaksana et al., 2012), with high amounts of mycosporine-like amino acids and phycobilin in the proteinaceous portion (Her et al., 2004). In fact, 7-50% of the carbon assimilated by algae is routed to EPS (Babel et al., 2002). Large amounts of carboxylic functions may cause EPS to be negatively charged when pH exceeds 9.5 (Rossi et al., 2004), which may help mitigate fouling potential on negatively charged membranes. In general, algal EPS has very different characteristics than other natural organic matter (Henderson et al., 2008), which may complicate fouling potential during harvesting.

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The molecular weight distribution of EPS can change with species and growth condition, which in turn affects fouling characteristics. When stressed algae were grown in nutrient free water, the molecular weight distribution of EPS was found to be less than 1000 kDa, but when grown in NSII nutrient rich medium, the distribution was greater than 2000 kDa (Babel et al., 2002). Others (Percival & Foyle, 1979; Eteshola et al., 1996) have reported molecular weight distributions of EPS in the range of 4000 - 6000 kDa in synthetic medium. Qu et al. (2014) found that EPS in the range of 0100 kDa. to 45 μm contributed most significantly to fouling. However, the hydrophilic non-acidic smaller molecular weight (7-11 kDa) portion of EPS was the highest contributor to fouling on a PVC UF membrane, and carbohydrates contributed more to fouling than proteins (Zhang et al., 2013a).

It should be noted, however, that the studies described above examined different species, and EPS release and fouling potential can vary depending on the species. For example, membrane filtration of *Chlorella* sp. achieved higher flux permeation than filtration of *P. purpureum*, mainly due to smaller cell size of *Chlorella* sp. (which allowed more flow between cells) and lower EPS production under similar growth conditions (Morineau-Thomas et al., 2002). Therefore, it is important to understand the growth responses, and in particular the EPS characteristics, of an algal culture to be harvested before selecting membrane type, harvest schedule, or environmental conditions.

Transparent exopolymeric particles (TEP), a subgroup of EPS, have recently been recognized as a previously overlooked but major factor in biofouling of drinking water processes (Berman et al., 2011). TEP, like other EPS, may be secreted by bacteria, algae, or diatoms, but TEP can also form abiotically from dissolved organic matter (Berman and Holenberg, 2005). TEP are negatively charged and may assemble in various configurations, such as sheets, bundles,

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clumps, or aggregates. Because of their stickiness, TEP may help establish a scaffolding for biofilm formation (which may be formed by cross-linked alginate blocks) (Meng & Liu, 2013) that simultaneously provides nutrition to microorganisms (Berman et al., 2011). In fact, many argue that when TEP sticks to a membrane surface, it is the first step to create a biofouling layer (Berman and Holenberg, 2005; Berman et al., 2011). Appropriate pretreatment steps (coagulation/sand filtration or ultrafiltration/reverse osmosis) can remove fractions of TEP in source water to mitigate biofouling later in the harvesting process (Van Nevel et al., 2012).

3.4.3 Fouling Control

Although fouling is a real and consistent concern with membrane processes, proper design and operation can help minimize it. For example, TFF reduces fouling potential by shearing the membrane. As too much shear stress could damage algae, fluid velocity and configuration must take into account algal hardiness (Rossi et al., 2008). Maintaining a high fluid velocity or mixing or intermittent/continuous bubbling across the membrane surface may also reduce fouling potential (Rossi et al., 2004; Wicaksana et al., 2012; Sun et al., 2013). Bubbling and mixing introduce additional energy inputs and still may not be able to prevent all irreversible fouling. Similar methods are recommended for dialysis culture, as gentle stirring kept fouling off a dialysis membrane turbidostat for two weeks (Skipnes et al., 1980). Membranes can also be coated with a functional coating material such as hydrophilic polyvinyl alcohol (PVA) (Hwang et al., 2013) which increased flux by 36% compared to non-treated membranes. Tailored designs can improve the economic performance by reducing fouling potential.

Backwashing or chemical cleaning can occasionally restore membrane flux, but algae related fouling (particularly on hydrophobic membranes (Babel & Takizawa, 2010) or by hydrophobic EPS (Qu et al., 2014)) is often irreversible. Algal cells of some species (such as

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Chlorella sp.) dissociate with decreased transmembrane pressure which can help reverse fouling; EPS, however, may cause cells to remain attached to the membrane even if transmembrane pressure is reduced (Morineau-Thomas et al., 2002). Membrane surface roughness can also increase when fouled which may not be completely reversible (Castaing et al., 2010) and could lead to further fouling. Chemical coagulation and pretreatment can alleviate fouling potential, as EPS can form complexes with metal ions and settle out (Zhang et al., 2013b). However, chemical additives create external costs and may threaten the integrity of harvested biomass. Likewise, ozone pretreatment reduces fouling potential on hydrophilic membranes (Hung & Lui, 2006), but coupling membrane filtration with other pretreatment methods introduce additional costs and complexity. Models can be used to better predict cake formation and flux decline which may aid in operation, maintenance, and design of the system (Zhang et al., 2010).

3.5 Additional Considerations (Energy and Costs)

As harvesting can account for a significant amount of algae production costs (Brennan and Owende, 2010), appropriate selection of harvesting method is critical for economic viability. Even within similar technologies, such as centrifugation or filtration, significant differences in energy usage and concentration factors exist (Molina Grima et al., 2003), which makes it challenging to compare harvest methods. Energy requirements may vary depending on when the method is used (i.e., in bulk harvesting or thickening) because of the varying density of the algal culture. Filtration is typically used in thickening, which is inherently a more energy intensive step to concentrate algae slurry (Brennan & Owende, 2010). Therefore, to properly compare the energy footprint of different harvesting methods, the timing of these methods within the harvesting process should be considered.

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Nonetheless, some energy comparisons have been conducted in the literature. Membrane filtration, though potentially energy intensive, can be used to concentrate algae and maintaining their biological structure (Petrusevski et al., 1995). In fact, the concentration factor achieved relative to energy input can be competitive with centrifugation (Molina Grima et al., 2003). An early economic assessment of submerged MF flat sheet membranes showed that the energy requirement for this configuration can be less than that of polymeric flocculation, tangential flow or vacuum filtration, and stand-alone centrifugation (Bilad et al., 2012). Similarly, using hollow fiber or tubular membranes can reduce the energy demand to dewater by at least 80% over centrifugation or froth filtration while the membranes also renovated growth medium to acceptable levels for recirculation (Bhave et al., 2012). A magnetically induced membrane vibration system can keep energy requirements low (Bilad et al., 2013). Furthermore, coupling membrane filtration with centrifugation can decrease energy consumption from 0.5 kWh/kg to 0.169 kWh/kg (DeBaerdemaeker et al., 2013), a significant decrease in scaled-up applications.

Most research studies have been conducted as lab or bench scale, making it difficult to extrapolate to full-scale systems. For instance, a pilot study of a submerged wastewater treatment MBR consumed 5-6 kWh m^{-3} for aeration, which is quite high compared to a full-scale plant (0.23 kWh m^{-3}) (Bilad et al., 2012). Another case study of a full-scale submerged MBR treating wastewater reported an energy demand of 0.64 kWh m^{-3}; this energy requirement was assumed to drop to 0.40 kWh m^{-3} if the membranes were used for algae harvesting and not wastewater treatment (Bilad et al., 2012). Actual energy consumption estimates based on lab tests varied from 0.25-0.48 kWh m^{-3} depending on algal species and concentration of feed (Bilad et al., 2012). Energy demand for airlift MBRs treating sewage are in the range of 0.25-0.7 kWh m^{-3},

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which overlaps the range of energy demand for conventional activated sludge systems of 0.3-0.4 kWh m^{-3} (Prieto, 2011).

In comparison, typical energy consumption of other common dewatering methods are: centrifugation, 8 kWh m^{-3}; sedimentation, 0.1 kWh m^{-3}; vibrating screen filter, 0.4 kWh m^{-3}; chamber filter press, 0.88 kWh m^{-3}; TFF, 2.06 kWh m^{-3}; flocculation and dissolved air flotation, 10-20 kWh m^{-3}; electrocoagulation, 0.8-1.5 kWh m^{-3} (Uduman et al., 2010). For a thorough review of more advantages and disadvantages of various dewatering methods, see Uduman et al. 2010.

Although membrane filtration, and in particular active pressure filtration, can have a high energy demand relative to other methods, the demand is much lower than other frequently used methods of centrifugation and dissolved air flotation. Perhaps the best solution is not to use one technology in isolation, but to adapt various methods at appropriate times throughout the harvesting and dewatering process. For instance, membrane filtration or gravity sedimentation can be utilized in an initial dewatering step, followed by a centrifuge or dissolved air flotation unit (which would have a higher energy requirement but can achieve a higher solids content).

3.6 Conclusion

Algae biomass production is slated to rise in the coming decades with increased global interest in biofuels and bioproducts. Membrane systems are useful in multiple areas of the cultivation and harvesting process, and continuous developments in system configuration and design may make membrane systems more energetically competitive with other methods. Although fouling remains a challenge to large-scale membrane applications, continued research in polymer science or interfacial phenomena may help develop materials that are better able to resist fouling. Finally, technologies and techniques used in other industries may be applicable to

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large-scale algal cultivation and harvesting and should therefore be considered when developing new system designs.

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Table 3.1. Summary of algal bioproducts, applications, and species. This table demonstrates the ubiquity of algal products across different industries in the global economy, as well as potential membrane applications in their cultivation and/or harvesting.

Product	Product Application	Species or Group	References	Membrane Application
Alginate	Food additive, pharmaceuticals; used in membrane fouling studies	*Macrocystis* sp., *Laminaria* sp., *A. nodosum*, *Durvillaea* sp., *Lessonia* sp.	Jensen, 1993	Separation of bioproducts; alginate used to immobilize cells in treatment applications, can be used in tandem with a membrane; harvest brown seaweed
Agar	Food additive, microbiology	*Gelidium* sp., *Gracilaria* sp., *Gellidiella* sp., *Pterocladia* sp.	Jensen, 1993	Harvesting large algal cells
Biomass	Aquaculture feed	*I. galbana, T. suecica, Scenedesmus* sp., *Chlorella* sp., *H. cervicornis, C. crenulata, P. valderianum, S. platensis, P. tricornutum, Pavlova* sp., *Chaetoceros* sp., *Nannochloropsis* sp., *Skeletonema* sp., *Thalassiosira* sp.	Mata, 2010; Raja, 2008; Spolaore, 2006	Treating growth media for recirculation; treating wastewater for discharge; delivery of gases to aquaculture ponds
	Livestock feed	Spirulina, *Porphyridium* sp., *L. digitata, C. reinhardtii, Arthrospira* sp.	Harun, 2010; Raja, 2008; Spolaore, 2006	Harvesting/dewatering biomass
	Health food, food supplement, feed surrogates	*A. platensis, C. vulgaris, C. ellipsoidea, Porphyra* sp., *Laminaria* sp., *Undaria* sp., *H. fusiforme, D. salina, Arthrospira* sp., *A. flos-aquae*	Pulz, 2004; Mata, 2010; Harun, 2010; Raja, 2008; Spolaore, 2006	Separation of bioproducts; harvesting biomass
Carotenoids, astaxanthin, β-carotene	Health food, food supplement, feed additives, pharmaceuticals	*H. pluvialis, D. salina, Muriellopsis* sp.	Pulz, 2004; Mata, 2010; Raja, 2008	Separation of bioproducts; harvesting biomass
Carrageenan	Food additive, biomedical	*Eucheuma* sp., *C. crispus, Gigartina* sp., *F. lumbricalis, Hypnea* sp.	Jensen, 1993	Separation of bioproducts
Chlorophyll	Pharmaceuticals, cosmetics, food pigmentation	Cyanobacteria sp. (chlorophyll-*a*); *Chlorella* sp., brown and red algae (chlorophyll-*b*)	Harun, 2010	Separation of bioproducts
Fatty acids	Pharmaceuticals, nutrition, cosmetics, baby food, animal nutrition	*O. aurita, I. galbana, P. triconutum, N. oculata, T. pseudonana, P. viridis, Nannochloropsis* sp., *S. mangrove, C. cohnii, A. caryerea, T. aureum, Porphyridium* sp., *Arthrospira* sp., *Nitzschia* sp.	Pulz, 2004; Mata, 2010; Harun, 2010; Raja, 2008; Spolaore, 2006	Separation of bioproducts
Immune modulators	Pharmaceuticals, nutrition	*L. majuscule*	Pulz, 2004	Separation of bioproducts

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Table 3.1. (Continued).

Lipids	Fuel production	*P. triconutum, B. braunii, Nanochloropsis* sp., *I. galbana*	Pulz, 2004; Metzger, 2005; Harun, 2010; Raja, 2008	Harvesting/dewatering biomass; gas delivery to cultures
Nori	Food	*Porphyra* sp.	Jensen, 1993	Harvesting biomass
Phycocyanin	Cosmetics	*A. platensis*, Cyanobacteria	Pulz, 2004	Separation of bioproducts
Polysaccharides	Pharmaceuticals, cosmetics, nutrition	*P. cruentum, C. pyrenoidosa, C. ellipsoidea*	Pulz, 2004; Mata, 2010	Separation of bioproducts

Table 3.2. Illustrative examples of membrane applications with respect to algal research.

Focus	Species	Flow	Configuration	Material	Pore Size	Reference
Role of EPS[a] in fouling	*Chlorella* sp. and *P. purpureum*	Classical TFF[b] cell; annular swirling decaying flow module	Flat sheet	PVDF[c], Iris 3065	40 kDa	Morineau-Thomas et al., 2002
	C. vulgaris, Chodatella sp., *Microcystis* sp.	Dead end filtration cells (stirred)	Flat sheet	RC[d], UF[e]	10 kDa	Chiou et al., 2010
Seasonal variation of EPS[a] production	*Chlorella* sp.	Dead end	Disc filter	PVDF[c]	0.45 µm	Babel et al., 2002
Investigate membrane fouling and critical flux	Blue-green algae	TFF[b]	Flat sheet	NF[f] 200	360 Da	Her et al., 2004
	A. platensis	TFF[b]	Flat membrane (Rayflow module)	ATZ[g]	50 kDa	Rossi et al., 2008
	C. sorokiniana	TFF[b]	Flat sheet	Anopore inorganic disc membrane PVDF[c]	0.2 µm 0.2 µm	Wicaksana et al., 2012
Algal cake deposition	*Chlorella* sp.	Dead end	Disc filter	PVDF[c] CE[h]	0.45 µm 0.45 µm	Babel &Takizawa, 2010
Monitoring algal growth rate	*T. pseudonana, S. costatum, P. tricornutum*	Pump assisted dialysis	Flat sheet	Acropor, nylon reinforced Nucleopore	3 µm 2 µm	Skipnes et al., 1980
Species interaction	*M. aeruginosa, C. pyrenoidosa*	Dialysis	Dialysis tubing	Unspecified material	12-14 kDa	Zhang et al., 2007
Biomass production	*S. obliquus*	Dialysis	Dialysis tubing	RC[d]	24 Å	Dor, 1975

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Table 3.2. (Continued)

Harvesting *A. platensis*	*A. platensis*	TFF[b]	Flat membrane (Rayflow module)	PES[i], PAN[j], PVDF[c]	3k-1.5 µm	Rossi et al., 2004
Harvesting marennine	*H. ostrearia*	TFF[b]	Flat sheet (Rayflow module)	PAN[j] (IRIS 3038)	40 kDa	Rossignol et al., 2000a
Harvesting biomass	*Chlorella*	Dead end	Flat sheet	6 types of Fluoropolymer	10 kDa - 0.1 µm	Sun et al., 2013
Concentrating algae biomass	Native species: *S. hantzschii, S. astraea, Cyclotella* sp., *R. minuta,* small green algae	TFF[b]	Hollow fiber Flat sheet	Pellicon Cassette System, Millipore	0.45 µm 0.45 µm	Petrusevski et al., 1995
Filtering toxic algae	*H. triqueta*	Submerged unit	Hollow fiber	PSU[k]	10 kDa, 300 kDa, 0.2 µm	Castaing et al., 2010
Water quality monitoring	11 species including: *T. pseudonana, P. tricornutum, S. costatum, T. nitzschoides*	Dialysis	Dialysis tubing Disc filters	RC[d] Sartorius-Membrane filter GmbH	Unspecified porosity	Jensen et al., 1972
CO_2 Delivery	*C. vulgaris*	Photobioreactor	Hollow fiber	Microporous PP[l]	328 µm (ID[n])	Cheng at el., 2006
	C. vulgaris	Photobioreactor	Hollow fiber	3 types of PVDF[c], (with PEG[m] additive)	0.273 µm, 1.492 µm, 1.517 µm	Fan et al., 2007
	C. vulgaris	Photobioreactor	Hollow fiber	PVDF[c] PP[l]	1.49 µm 0.27 µm	Fan et al., 2008

[a]EPS=extracellular polymeric substances, [b]TFF=tangential flow filtration, [c]PVDF=polyvinylidene fluoride, [d]RC=regenerated cellulose, [e]UF=ultrafiltration, [f]NF=nanofiltration, [g]ATZ=Zr-O2+tiO2 on alumina support, [h]CE=cellulose ester, [i]PES=polyether sulfone, [j]PAN=polyacrylonitrile, [k]PSU=polysulfone, [l]PP=polypropylene, [m]PEG=polyethylene glycol, [n]ID=inner diameter

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Chapter 4: Nutrient Removal and Biomass Production by Native and Augmented Algal Populations at a Municipal Wastewater Treatment Plant[2]

4.1 Introduction

Although algae have most commonly been used in wastewater treatment lagoons for nutrient polishing prior to discharge, microalgae cultivation could be sustainably integrated into advanced wastewater treatment plants (WWTP), expanding infrastructure that serves dual purposes and effectively uses wastes as resources. Not only may integration decrease the environmental footprint and increase the energy return on investment of the two systems (Clarens et al., 2011; Beal et al., 2012), but without it, the economic feasibility of current algal cultivation technology is somewhat dubious (Pittman et al., 2011). Advanced wastewater treatment plants not only contain adequate nutrients to sustain algae growth, but integrating the two processes can contribute economic benefits to plant operation (Drexler et al., 2014).

Many algal species can grow in municipal, industrial, and agricultural wastewater and have been used in numerous wastewater treatment applications (Hoffman, 1998). Aside from reducing loading in wastewater, algal biomass can be harvested for many bioproducts, including biofuels, fertilizers, or fish feed (Mata et al., 2010).

This study involved the cultivation of two native algal communities derived from wastewater clarifiers (attached biofilm) and two monocultures (*Chlorella sorokiniana* and

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[2] This chapter has been published previously. ©IWA Publishing (2014). The definitive peer-reviewed and edited version of this article is published in Water Science and Technology (DOI: 10.2166/wst.2014.340) and is available at www.iwapublishing.com. Copyright release form can be found in Appendix F.

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Botryococcus braunii) augmented with endemic cultures in unsterilized wastewater effluents. *Chlorella* sp. were chosen because of their relatively fast growth rate, resiliency, and nutritional value (de-Bashan et al., 2008). *B. braunii* grows much slower but produces large quantities of lipids, making it a desirable species for biofuel production (Metzger, 2005). If lipids were released into the wastewater stream, the resulting COD could be treated by activated sludge processes. Both species can be digested for biogas (Frigon et al., 2013). Because native species already thrive in the desired environment and pose a smaller threat of becoming an invasive species, phycoprospecting may reduce risks in algae-wastewater integration and identify appropriate native species for biofuel (Wilkie et al., 2011). Phycoprospecting may be especially convenient if the end use of algal biomass is less sensitive to algal composition. Therefore, native algal communities were also examined.

The aim of this study was to compare the biomass yield and nutrient removal efficiencies of four algal populations at two stages of an advanced WWTP and a reference medium.

4.2 Materials and Methods

4.2.1 Algae Stock Cultivation

Algal monocultures were obtained from the University of Texas (UTEX) Culture Collection of Algae. *C. sorokiniana* (UTEX #246) and *B. braunii* (UTEX #572) stock cultures were acclimated in reference medium and Bold 1NV medium (UTEX, web.biosci.utexas.edu/utex/media.aspx) prior to batch tests. During the acclimation stage, both cultures were shaken at 150 rpm (Lab-Line Incubator-Shaker, Melrose Park, IL) at a 12/12 photoperiod (3000 lux) at room temperature (25°C). Reference medium contained the following components: $NaNO_3$ (1.47 mM); NH_4Cl (2.95 mM); K_2HPO_4 (0.43 mM); KH_2PO_4 (1.29 mM); $CaCl_2$ (0.25 mM); $MgSO_4$ (0.30 mM); NaCl (0.43 mM); $NaHCO_3$ (0.17 mM); Na_2SiO_3 (0.15

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mM); citric acid (0.16 mM); ferric citrate (0.14 mM); $CuSO_4$ (0.08 µm); $ZnSO_4$ (0.15 µm); $CoCl_2$ (0.84 µm); $MnCl_2$ (0.061 µm); Na_2MoO_4 (0.052 µm); $FeCl_3$ (0.36 mM); $ZnCl_2$ (0.037 mM); Vitamin B_{12}, thiamine, biotin per UTEX Bold 1NV recipe. Native algal communities (A and B) were harvested from the respective clarifier weirs, transported in cold storage, and inoculated in batch experiments the same day.

4.2.2 Experimental Design

Algae were cultivated using clarified process streams from two sequential stages (BOD removal and nitrification) of the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL, USA, described previously in Drexler et al. (2014) and illustrated in Figure 4.1. Wastewater used as growth media was 1) clarified effluent following a high purity oxygen carbonaceous BOD removal reactor (PBCE) and 2) clarified effluent following a nitrification reactor (PNCE). These sampling locations were chosen because of differing dominant nitrogen sources, (ammonia in the PBCE and nitrate in the PNCE), and better light penetration in clarified effluent than activated sludge. For comparison, algae were cultivated in 3) reference medium (RM) containing both nitrogen sources.

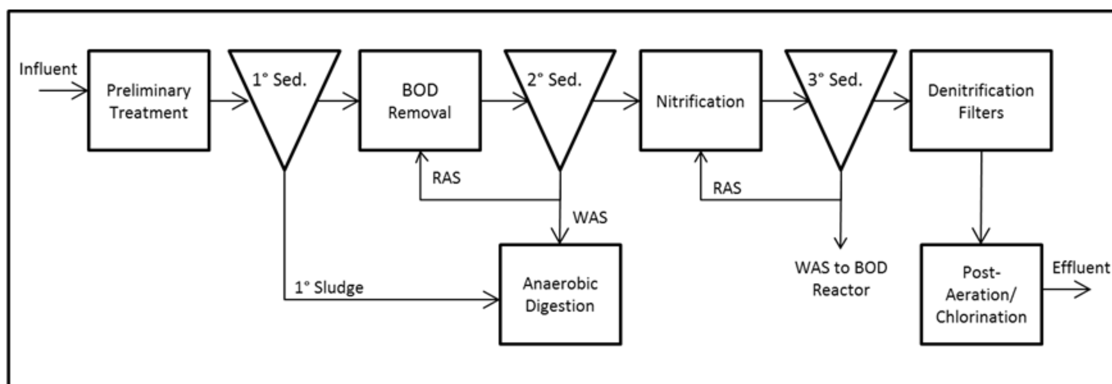

Figure 4.1. Process flow schematic at the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL. Note: WAS=waste activated sludge; RAS=return activated sludge.

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Cultivation bioassays were conducted in 12-day batch tests in the laboratory, utilizing four algal populations: *Chlorella sorokiniana, Botryococcus braunii,* an algal biofilm community native to the post-BOD removal clarifier (community A), and an algal biofilm community native to the post-nitrification clarifier (community B). Each population was cultivated in three types of growth media (PBCE, PNCE, and RM), resulting in a 4x3 experimental matrix. Initial water quality conditions of the media are shown in Table 4.1, where the difference in nitrogen speciation between PBCE and PNCE is evident. Although increasing the pH of the RM was considered, it was not adjusted since the pH remained within the range preferred by *C. sorokiniana* and typical of activated sludge.

Table 4.1. Average initial conditions for post-BOD removal clarified effluent (PBCE), post-nitrification clarified effluent (PNCE), and reference medium (RM) between four batch studies.

Parameter	PBCE	PNCE	RM
Soluble nitrogen	34.3(4.0)	34.5(1.9)	46.3(4.3)
Ammonia-N	29.1(1.9)	0.0(0.0)	20.8(0.3)
Nitrate-N	1.1(0.5)	30.9(1.7)	22.2(0.5)
Phosphate-P	7.3(3.1)	7.8(4.5)	151.2(12.4)
Soluble COD	58.8(11.8)	35.8(9.5)	92.0(3.1)
pH	7.4(0.1)	7.6(0.2)	6.5(0.3)

Standard deviation is shown in parentheses. All units are in mg/L, except for pH.

Wastewater was collected at the beginning of each species' batch test, which, due to variations at the wastewater plant, resulted in slightly different characteristics among the series. However, ammonia and nitrate concentrations among the series were fairly stable (RSD of 6.5% and 5.4%, respectively), and, after inoculation, phosphorous concentrations were not limiting in any case. Because the intention of the study was to test the feasibility of growing the algal species in unaltered wastewater to mimic field conditions, wastewater was not autoclaved or augmented with nutrients prior to experimental use.

Statistical comparisons of the initial and final conditions (pH, total and soluble nitrogen, ammonia, nitrate, phosphate, COD, total suspended solids (TSS), and optical density (OD)) were

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made using the Student's t-test. Comparisons with p-values less than 0.05 ($p<0.05$) were considered 'significant' and are referred to accordingly.

Triplicate Erlenmeyer flasks (500 mL for *C. sorokiniana*, communities A&B; 125 mL for *B. braunii* due to limited available inoculant) were filled with growth medium (400 mL for *C. sorokiniana*, communities A&B; 100 mL for *B. braunii*) and inoculated with *C. sorokiniana* or *B. braunii* until the biomass density was approximately 0.09 g/L. Native species were dewatered (but not dried) to remove remnant free liquid (Whatman glass fiber filters 934-AH), and a known amount of biomass (approximately 0.1 g) were added to each flask. All flasks were cultivated in the conditions described above.

4.2.3 Analytical Methods

Water quality tests were conducted using the following HACH methods: chemical oxygen demand (COD) (method #8000), total nitrogen (TN) (method #10072), nitrate-nitrogen (method #10020), ammonia-nitrogen (method #10031), and phosphate (method #10127). Phenol-sulfuric acid carbohydrate assays and Lowry protein assays were conducted to measure soluble carbohydrate and protein (Ferlita, 2011). Optical density (OD) was measured at 680 nm (HACH DR/4000 spectrophotometer), and pH was measured with Oakton pH probes. Total suspended solids (TSS) were measured according to Standard Methods 2540D. (Method development for analytical methods can be found in Appendix B).

4.3 Results and Discussion

4.3.1 pH Changes

In wastewater treatment, pH regulates biological health, and physical-chemical processes. All algal communities significantly increased the pH in both wastewater samples from a range of 7.4-7.6 to a range of 9.8-11.0, most likely due to the consumption of alkaline compounds

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(Uusitalo, 1996) and the photosynthetic removal of carbon dioxide, as the cultures were not artificially aerated. The largest pH increase of the RM occurred when cultivating *Chlorella* sp. (from 6.0 to 6.8) and algal community A (from 6.5 to 7.8), but increased just slightly when cultivating *B. braunii* (from 6.6 to 6.8) and decreased when cultivating algal community B (from 6.8 to 6.3). The pH change in the RM was not significant due to the addition of the buffering agent $NaHCO_3$.

Although activated sludge processes generally operate within a pH range of 6-8, other physical chemical processes may require an alkaline pH. Strategically placing an algal reactor ahead of an alkaline treatment step could be advantageous for reducing chemical inputs. On the other hand, drastic pH increases may be harmful to downstream pH-sensitive processes. The extent of pH elevation may be lower in continuous growth at a WWTP than the batch bioassays due to the constant supply of carbon dioxide rich wastewater following aerobic respiration. However, microalgae grown in a photobioreactor integrated ahead of nitrification may consume alkalinity needed by nitrifiers; the consumption of carbon dioxide should be incomplete, as to leave adequate carbon dioxide in solution for nitrifying autotrophs. The net impact of algae-induced pH changes on biological processes in a full-scale integration deserves further study.

4.3.2 Total and Soluble Nitrogen

The total nitrogen removal efficiency of communities A&B was not significantly different than that of *C. sorokiniana* in PBCE, and outperformed *C. sorokiniana* in PNCE (Figure 4.2). The removal efficiency of *B. braunii* was higher than the native species communities in all but one batch, but all nitrogen removal may not be solely attributable to *B. braunii* due to the presence of endemic fauna in unsterilized wastewater.

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The final soluble nitrogen concentrations were significantly lower than the initial values in all samples (except community A in RM). Results agree with previous batch *Chlorella* sp. studies that achieved 61-86% (Li et al., 2011; Zhu et al., 2013) removal. With the exception of *B. braunii*, all species achieved greater removal of soluble nitrogen in the PBCE than the PNCE. Soluble nitrogen removal in the RM was considerably less than that in wastewater effluents. As native species' removal was comparable to augmented cultures, phycoprospecting may be as effective and less operationally intense than maintaining augmented cultures.

4.3.3 Ammonia-Nitrogen

All species achieved significantly higher ammonia removal in PBCE than the RM (Figure 4.2). The final ammonia concentrations were significantly lower than the initial concentrations in all bioassays, and ammonia removal in the PBCE agreed with previous studies of *Chlorella* sp. that achieved 60-100% (Ruiz-Marin et al., 2010; Li et al., 2011) removal. Ammonia removal by the monocultures was not significantly different than that of the native species in PBCE, except *B. braunii* batch cultures achieved significantly higher removal than community A.

The location-specific nitrogen balance profiles for each algal population are presented in Figure 4.3. Ammonia removal in batch cultures of *C. sorokiniana, B. braunii,* and community B cultivated in PBCE all appear to be aided by volatilization due to elevated pH. Volatilization has previously been found to be highly correlated with free ammonia concentration in algae ponds (Zimmo et al., 2003). In previous studies (Gustin & Marinsek-Logar, 2011), when pH rose above 10, aeration aided ammonia volatilization reached above 80%; although flasks were not aerated in this study, unaccounted for ammonia in PBCE batch tests were between 32-45%, which is within a reasonable range of unaided volatilization for the pH range of this study. Ammonia removal could also have been aided by struvite precipitation. Furthermore, pH remained below

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8.0 in the RM (due to the buffering agent added) for all batch tests, and overall ammonia removal was significantly less in the RM than both wastewater effluents.

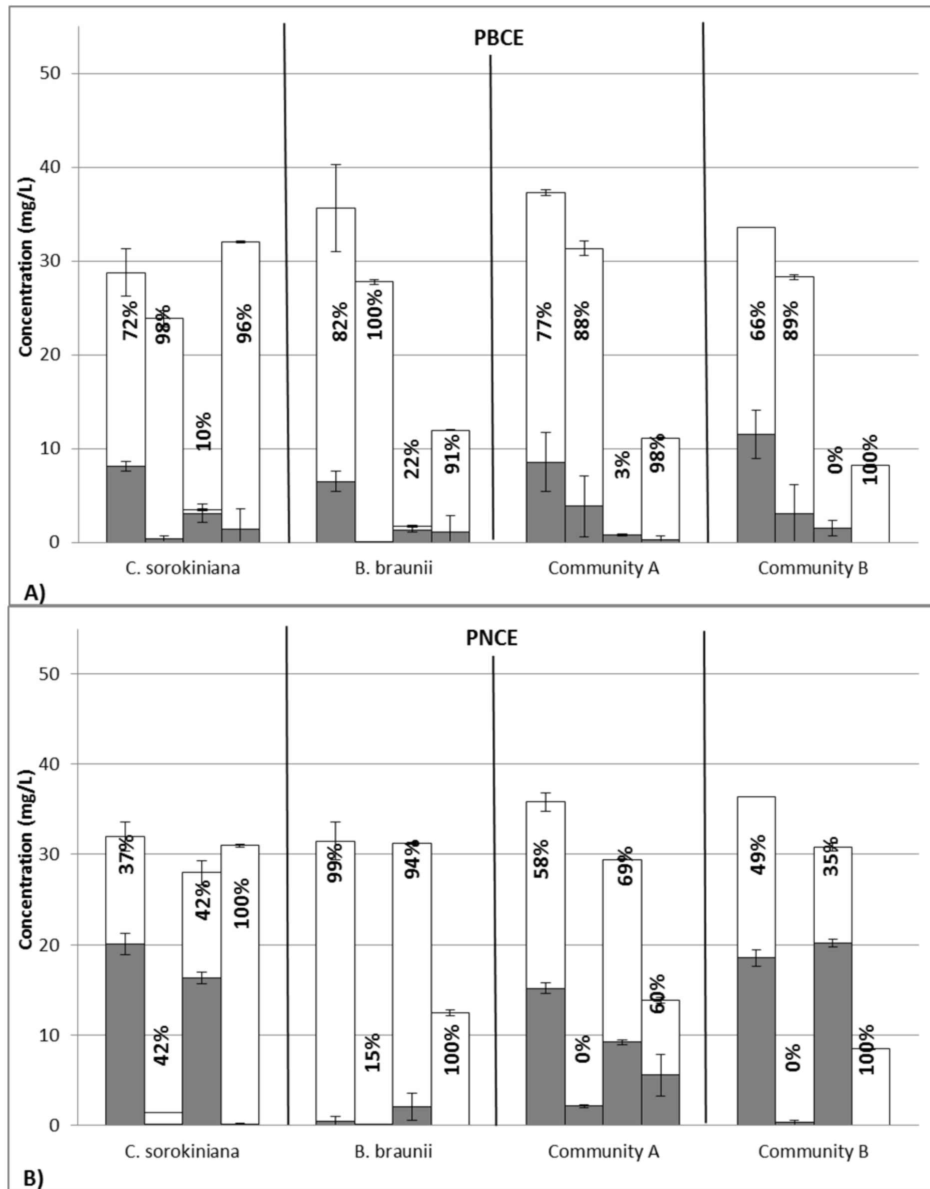

Figure 4.2. Change in soluble nitrogen, ammonia-N, nitrate-N, and phosphate-P after 12 day cultivation of four algal populations in three growth media. Bars of each group represent the following from left to right: soluble nitrogen, ammonia-nitrogen, nitrate-nitrogen, and phosphate-phosphorous. Initial concentration is shown as white bars; final concentration is shown as black bars. Error bars depict one standard deviation. PBCE, post-BOD removal clarified effluent; PNCE, post-nitrification clarified effluent; RM, reference medium.

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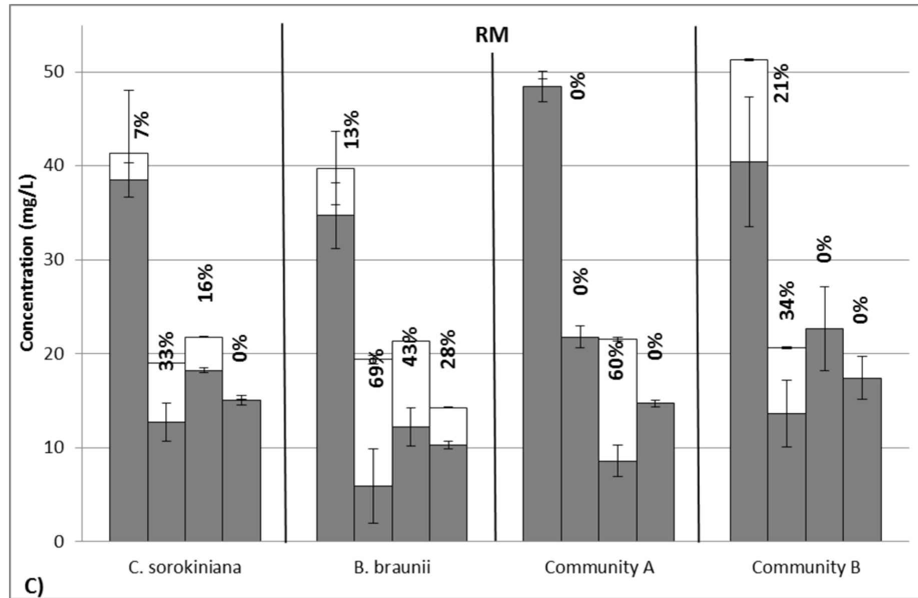

Figure 4.2. (Continued)

4.3.4 Nitrate-Nitrogen

Nitrate removal was significant for all algal populations in the PNCE, as shown in Figure 4.2. *B. braunii* achieved a considerably higher nitrate removal efficiency (94%) than all other species (35-69%), which agrees with previous work (Sawayama et al., 1992), where *B. braunii* achieved near complete removal of nitrate in batch tests. However, due to background fauna, not all of the nitrate removal may be attributable to *B. braunii*.

Gaps in the nitrogen balance profiles (Figure 4.3) observed in the PNCE and RM may be explained (aside from experimental error) by denitrification. Previous work (Zimmo et al., 2004) has shown that denitrification contributes significantly (approximately 25% of total nitrogen loss) to nitrogen removal in algae-based ponds, and bacteria genetically similar to denitrifying bacteria have previously been found in wastewater process streams immediately following nitrification (Ghosh & Love, 2011). Denitrification by coexisting bacteria (in background wastewater or introduced with inoculant) was considered a possible cause of unaccounted for nitrogen removal even though conditions in this study may be different than field conditions.

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Unfortunately, direct measure of denitrification was not possible, and genetic testing of the algal bacteria communities was not conducted to verify the presence of denitrifying organisms. Denitrification is, therefore, merely a potential explanation.

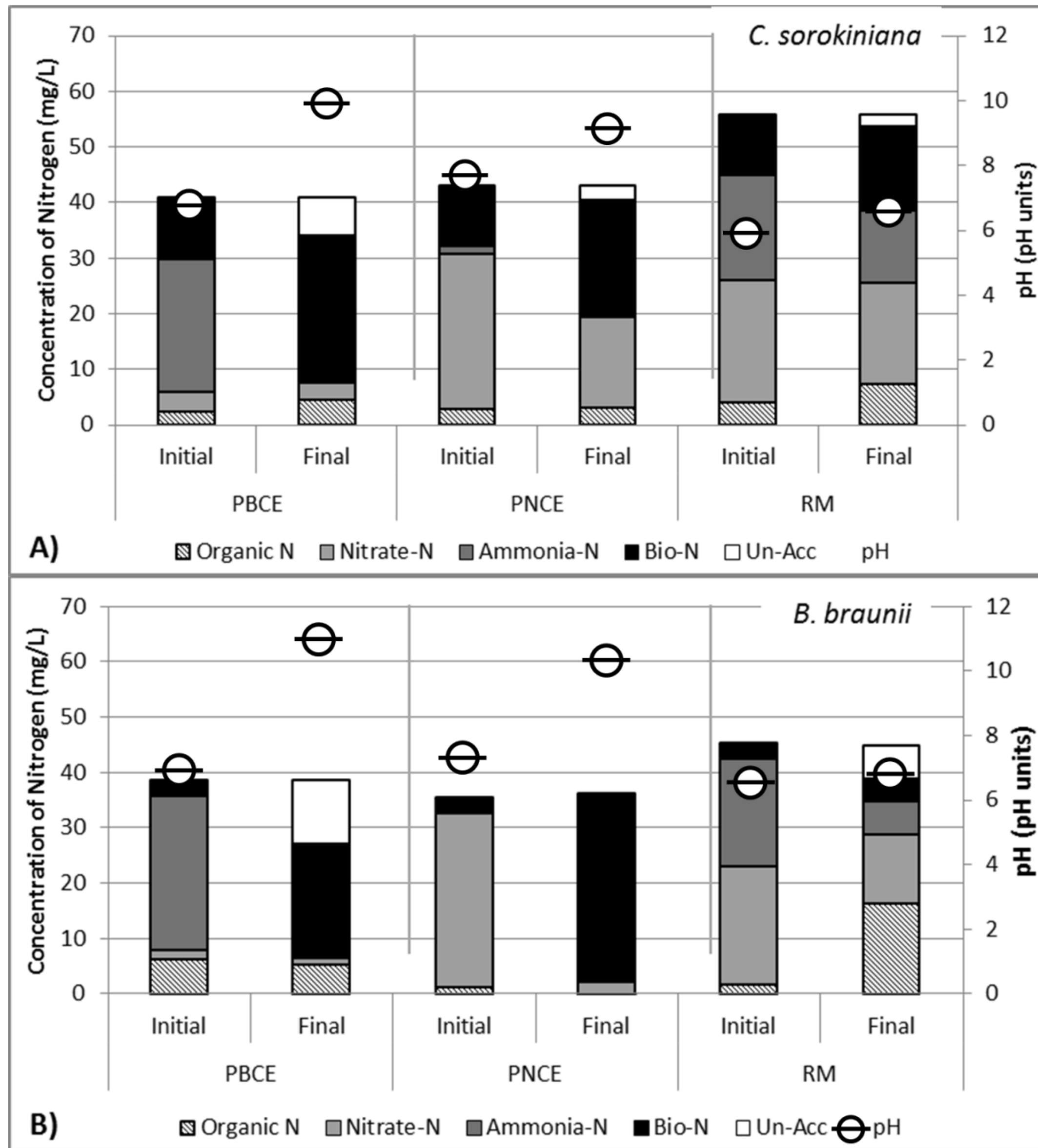

Figure 4.3. Nitrogen profiles of four algal populations cultivated in three growth media. Bio-N is the mass of nitrogen bound in biomass. Un-Acc is nitrogen that is not accounted for in measured tests or calculations; it could represent volatized or denitrified nitrogen. PBCE, post-BOD removal clarified effluent; PNCE, post-nitrification clarified effluent; RM, reference medium.

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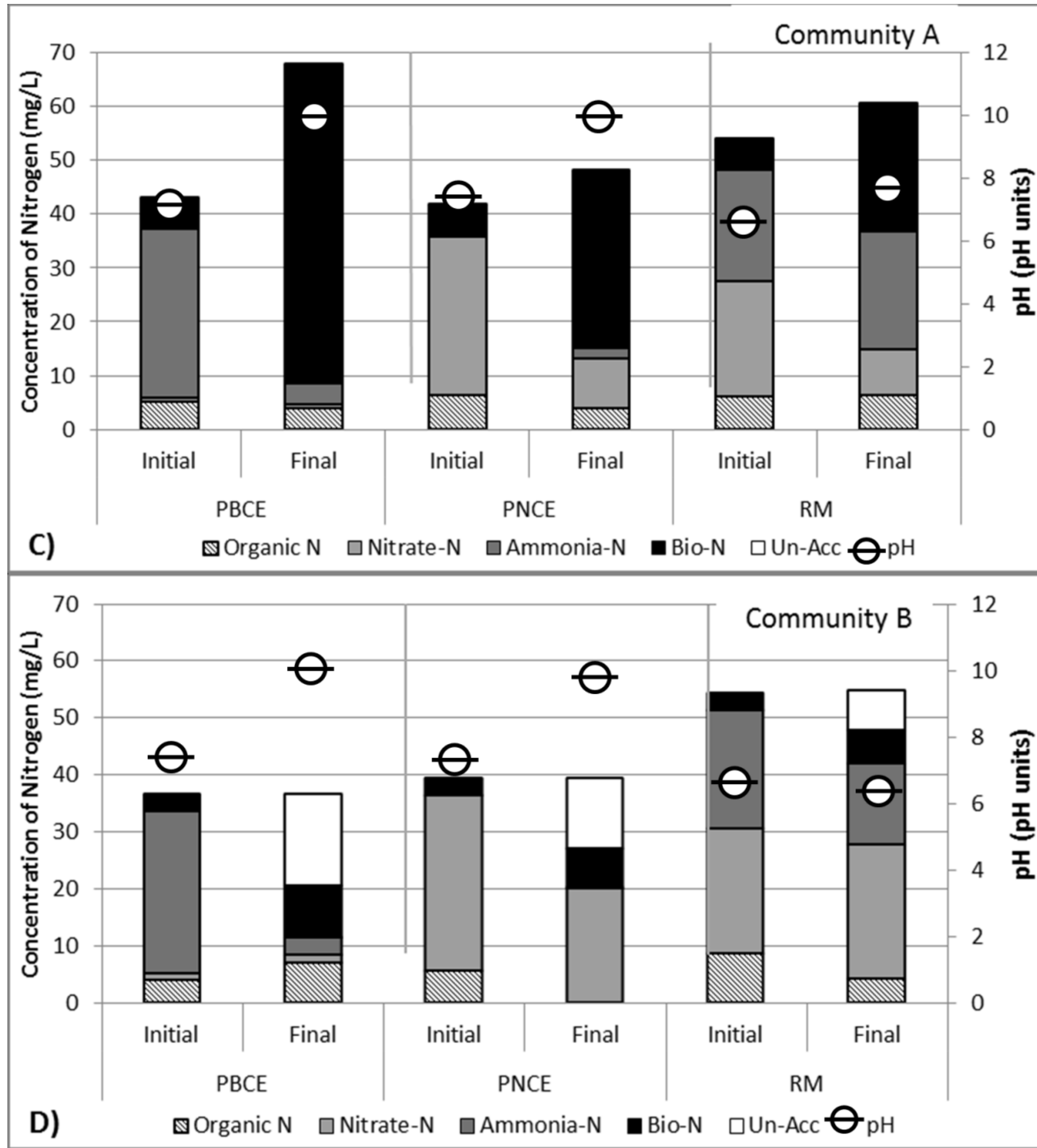

Figure 4.3. (Continued)

4.3.5 Nitrogen Preference of Algae Species

In the presence of both ammonia and nitrate nitrogen, many algal species, including *Chlorella sp.*, will preferentially utilize ammonia (Ruiz et al., 2011). *B. braunii* has previously been grown successfully in nitrate dominated secondary wastewater (Sawayama et al., 1992; An et al., 2003). Accordingly, it was hypothesized that the native species would prefer the nitrogen species dominant in their respective clarifier.

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To investigate nitrogen preferences, the RM included both nitrogen species. Although *C. sorokiniana* and *B. braunii* achieved significant removal of both ammonia and nitrate in the RM, the removal efficiency of ammonia was significantly higher than that of nitrate (Figure 4.2C). The native species appeared to prefer the opposite nitrogen species as was expected, as community A had significant removal of nitrate and community B had significant removal of ammonia. Differences in the environmental conditions in batch tests compared to the native environment (i.e., attached versus suspended growth, light intensity) may have selected for different dominant species (with potentially different nitrogen preferences) than would be dominant in the native environment.

The amount of nitrogen measured in the final biomass of community A greatly exceeded the initial soluble nitrogen available (Figure 4.3C), suggesting either analytical error and/or nitrogen fixation. Previous studies identifying algal species in secondary treated wastewater (Ghosh & Love, 2011) noted the presence of cyanobacteria, which have been shown to fix nitrogen. Nitrogen fixed by community A cultivated in the PBCE, PNCE, and RM amounted to approximately 25 mg/L, 6.5 mg/L, and 6.5 mg/L, (corresponding to approximately 57%, 15%, and 12% of the initial available nitrogen). Although it is possible that laboratory conditions favored nitrogen fixing bacteria, it is unlikely that nitrogen fixation occurred because ammonia and nitrate remained at the end of the experiments. It is more likely that the difference is explained by experimental error or a yet unidentified factor.

4.3.6 Phosphate-Phosphorous

Due to algal assimilation and/or adsorption, phosphate-phosphorous removal was significant for all algal communities in both wastewater effluents (Figure 4.2). An abiotic trial, where the pH of the RM was artificially raised, confirmed that phosphate did not precipitate

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significantly until pH values were higher than 8. Increased pH, such as in the wastewater effluents, has been shown to encourage phosphate precipitation and adsorption to algal cells (Zhu et al., 2013) and may have aided phosphorous removal. Phosphate removal was somewhat higher than previously reported for *Chlorella* sp. in domestic sewage treatment in batch cultivation (12-92% (Ruiz-Marin et al., 2010; Li et al., 2011)), but similar to that found previously by *B. braunii* in secondary wastewater (Sawayama et al., 1992). In the PBCE, the native communities provided equal removal efficiency as the augmented cultures. Although community A had significantly less phosphorous removal in the PNCE than other species, the community still achieved an average of 60% removal. The phosphorous removal in RM was not significant (except in the *B. braunii* batch tests), where pH did not exceed 8 in any case due to the presence of a buffering agent. Although the RM had a much higher phosphorous concentration than the clarified effluent, phosphorous is not considered toxic to algae (Carpenter *et al.* 1998) and therefore would not have a detrimental effect on growth. Similarly, the elevated phosphorous concentration would not offer an advantage, since all cultures began with adequate N:P ratio (Redfield 1958) and excess phosphorous concentrations.

Communities A and B achieved comparable phosphate removal as the augmented cultures. If an algal-integrated WWTP did not require a monoculture with specific traits, cultivating phycoprospected populations that could achieve the same nutrient removal may be more economical and less difficult than maintaining an augmented culture.

4.3.7 Organic Carbon

The final soluble COD concentration increased significantly from an initial (33-82 mg/L) to final (76-141 mg/L) concentration range when cultivating *C. sorokiniana* in all media. Soluble COD also increased when cultivating *B. braunii* (from 84 mg/L to 227 mg/L) and community A

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(from 60 mg/L to 107 mg/L) in the PBCE. Similarly, soluble COD increased in the PNCE (from 25 to 49 mg/L) after cultivating community B.

The findings are contrary to two similar batch test studies (Li et al., 2011; Zhu et al., 2013) which achieved a minimum of 67% soluble COD removal depending on environmental factors. Both studies, however, were run on high strength wastewater with COD concentrations at least four times higher than those in this study. Hence, heterotrophic processes were likely dominant. Though COD removal was rapid in the first 2-3 days of the experiments, it eventually reached steady state above 100 mg/L. A batch study cultivating *B. braunii* in secondarily treated domestic sewage with lower nutrient loading also observed an increase in soluble organic carbon (Sawayama et al., 1992).

An increase in soluble COD could be due to an increased production of extracellular polymeric substances (EPS), which can be induced via environmental stresses (Babel et al., 2002) and should be accompanied by increased soluble carbohydrate, protein, and/or organic nitrogen (Her et al., 2004). Slight increases of these components did occur, though not significantly, in the batch cultures (Table 4.2). Organic nitrogen concentration increased when *C. sorokiniana* was cultivated in PBCE and PNCE (from 2.5 to 4.4 mg/L and 2.8 to 3.0 mg/L, respectively). Soluble carbohydrate and protein increased when cultivating *B. braunii* (from 20.1 to 26.8 mg/L and 16.3 to 16.7 mg/L, respectively) and community A (from 8.9 to 41.0 mg/L and 10.2 to 17.6 mg/L, respectively) in PBCE. Similar increases in carbohydrate were observed when cultivating community B in the PNCE and PBCE (from 6.9 to 12.9 mg/L and 7.7 to 11.8 mg/L, respectively).

Although EPS may contribute to internal organic loading, it could help reduce the external chemical demand required for denitrification if readily biodegradable and integrated

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ahead of denitrification. The effect of algal cultivation on soluble COD concentration in continuous culture and subsequent processes is deserving of further study.

Table 4.2. Data summary of the initial and final soluble organic nitrogen, COD, carbohydrate, and protein concentration of each experimental series.

Species	Med.	Organic-Nit		COD		Carbohydrate		Protein	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
B. braunii	PBCE	6.2	5.1	84	227	20.1	26.8	16.3	16.7
	PNCE	1.3	0.1	69	66	21.9	10.6	18.8	7.7
	RM	1.6	16.5	120	86	26.8	11.5	10.5	8.8
C. sorokiniana	PBCE	2.5	4.4	43	115	8.6	5.8	13.1	10.2
	PNCE	2.8	3.0	33	76	8.3	3.9	11.1	9.1
	RM	11.9	7.6	82	141	8.6	7.7	10.3	13.7
Comm. A	PBCE	5.2	3.9	60	107	8.9	41.0	10.2	17.6
	PNCE	6.4	3.9	45	74	6.8	54.0	9.9	11.6
	RM	6.1	18.0	90	90	8.0	26.8	10.3	3.0
Comm. B	PBCE	4.1	7.0	73	73	7.7	11.8	16.9	10.6
	PNCE	5.6	0.0	25	49	6.9	12.9	13.2	10.0
	RM	8.6	4.2	92	81	19.1	5.9	7.1	8.2

4.3.8 Final Yield

Both monocultures grew readily in the wastewater effluents, confirming the feasibility of municipal wastewater as a feedstock for algae cultivation. The TSS yield of *C. sorokiniana* and *B. braunii* was significantly higher in both wastewater effluents than the RM (Figure 4.4). The TSS yield of communities A and B were significantly higher in the PBCE than the PNCE. Because batch cultivation occurred in unsterilized wastewater, some biomass yield could be attributed to growth of endemic species.

The final concentration of *C. sorokiniana* and *B. braunii* cultivated in wastewater effluents was lower than that reported in other studies (An et al., 2003; Ruiz et al., 2011). Because the N:P ratio in all batch tests was lower than 16:1 (Redfield, 1958), phosphorous was not considered to be limiting in any case. Instead, the lower yield is most likely a result of not aerating the bioassays, or a suboptimal light regime, as carbon dioxide and light are important factors for algal growth.

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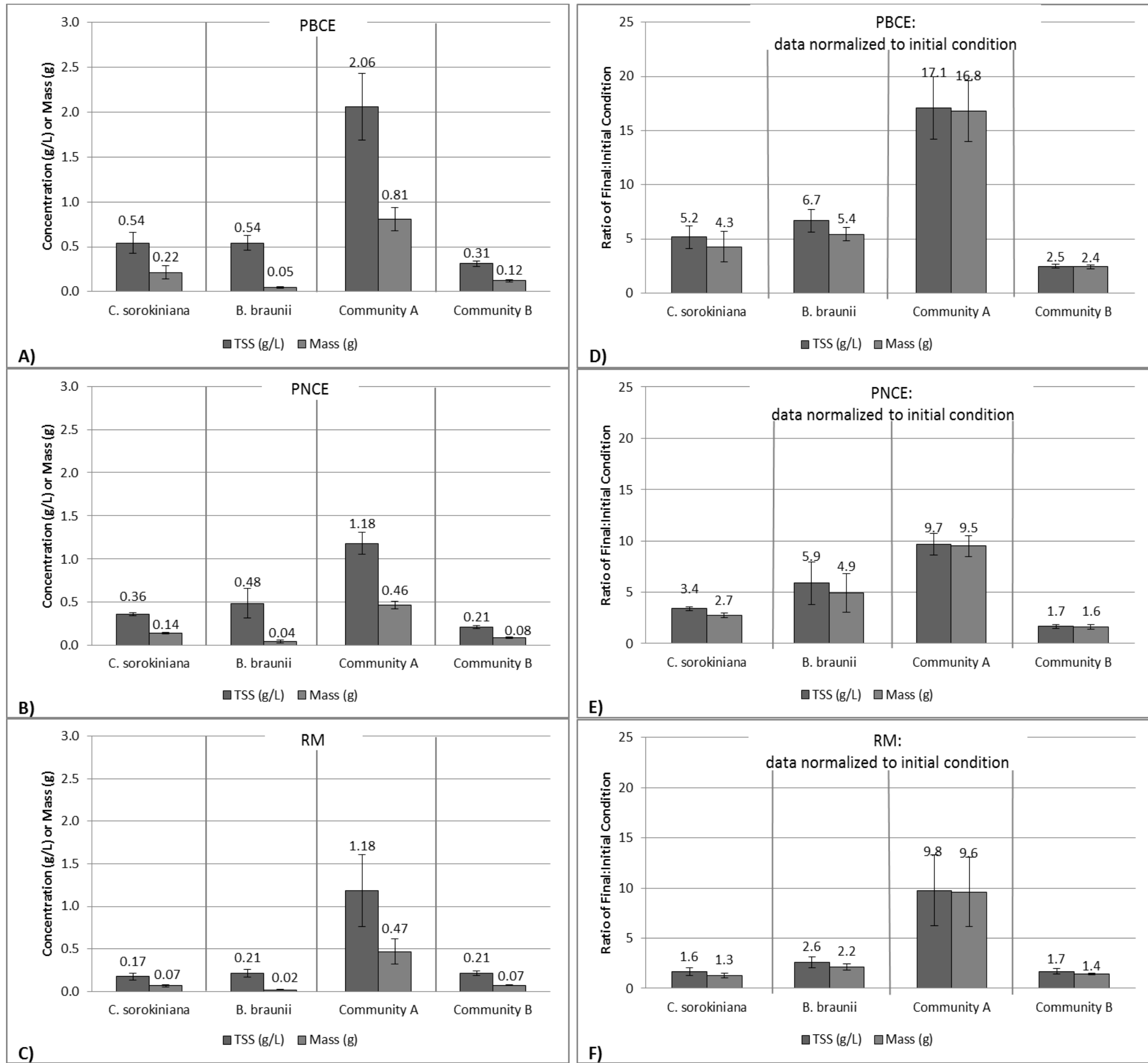

Figure 4.4. Final yield of four algal populations cultivated in three growth media. Yield is presented as total suspended solids (TSS) and mass as dry weight (A-C) and normalized to initial TSS and mass as dry weight (D-F). PBCE, post-BOD removal clarified effluent; PNCE, post-nitrification clarified effluent; RM, reference medium.

When final yield was normalized to initial concentration, *B. braunii* had a significantly higher final TSS than *C. sorokiniana* and community B when grown in both wastewater effluents. Other faster growing endemic species in the unsterilized wastewater overtook *B. braunii* in the batch tests. *C. sorokiniana* had a significantly higher final TSS than community B in both wastewater effluents. Community A, however, had the highest final TSS of all species in

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both wastewater effluents. Similar trends were seen in mass yield as dry weight for each species and growth media. Since native species produced as much or more biomass than augmented cultures in many cases, if biomass volume is the goal of integration (i.e., for biogas production or fertilizers), native species may be an acceptable alternative to augmented monocultures.

Growth rate was calculated by drawing the slope of the line through the growth curve during the exponential growth phase (considered days 0-3 in this study) after the data had been normalized to the first order kinetics equation:

$$X_t = X_o \exp^\wedge kt \qquad \text{Eq. 4.1}$$

where X_t is the concentration at time t, X_o is the initial concentration, k is the slope of the line (i.e., the growth rate, d^{-1}), and t is the time when the measurement was taken.

Specific growth rate was only determined for the *C. sorokiniana* series, due to other cultures' heterogeneity inhibiting the ability to take a representative sample without significant culture disruption. The specific growth rate of *C. sorokiniana* was significantly higher in both wastewater samples than the RM (0.11 d^{-1}), and the growth rate in the PBCE (0.52 d^{-1}) was significantly higher than the PNCE (0.40 d^{-1}). The growth rates were within the range of those previously reported for *Chlorella* sp. in wastewater (Ruiz et al., 2011; Zhu et al., 2013). The lower growth rate in the RM may have been due to the lack of algal-bacterial interactions in the sterilized medium.

The growth rate in the wastewater media was much higher than the reference medium (Figure 4.5). *C. sorokiniana* had the highest growth rate in the PBCE, which was expected based on the preliminary data in Appendix A. According to the growth curve there was not a lag phase, as algae grew in all cases from Day 1. The exponential phase lasted about three days in each case as well; afterwards a growth limitation presumably appeared, such as low carbon dioxide, high

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pH, or limited nutrients. All series then entered a stationary phase which was maintained for the duration of the study. None of the series demonstrated a death phase within the time period of the study. The culture in PNCE showed a slight decline after Day 8, which may have entered a death phase if the experimental period was longer.

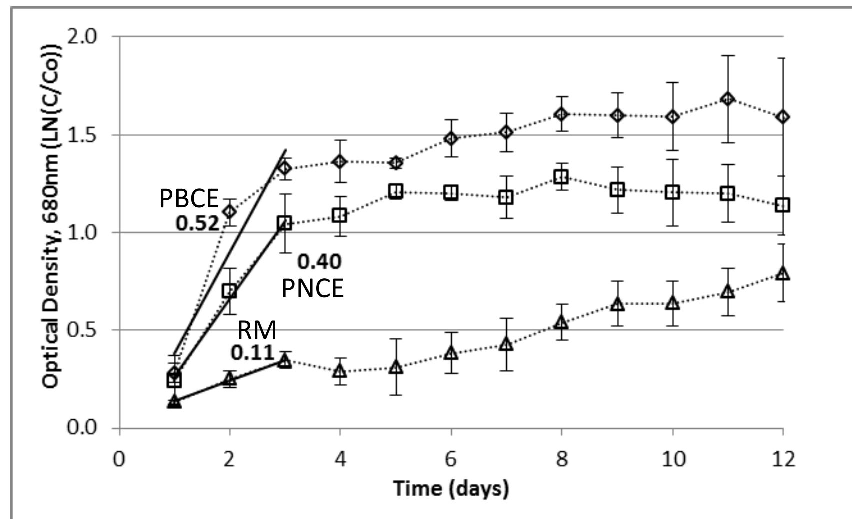

Figure 4.5. Growth curve of *C. sorokiniana* grown in post-BOD removal clarified effluent (PBCE), post-nitrification clarified effluent (PNCE), and reference medium (RM). Optical density was normalized to first order kinetics. Straight lines show fitted growth curves, with calculated growth rates adjacent to them. Error bars show one standard deviation of triplicate batch tests.

4.3.9 Community Interactions

Because wastewater effluents were not sterilized to better simulate scale-up conditions, diverse endemic algae and bacteria (Ghosh & Love, 2011), were cultivated along with target species. Although community profiling tests were not conducted on the final communities, qualitative inspection suggested that *B. braunii* was overcrowded by endemic species in the wastewater effluents (to a lesser extent in the PNCE than the PBCE), while remaining dominant in the RM (Figure 4.6). Although grazers were not quantified at the end of the experiment, it could be assumed that low *B. braunii* cell counts could also be due to grazing in addition to competition. Microscopic inspection of communities A and B also indicated that the dominant

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groups shifted when grown in different growth media. The fast growing *C. sorokiniana* remained dominant in all series. Due to species competitiveness, it may be difficult to maintain the dominance of a slow-growing monoculture with wastewater as a feedstock, unless a means of segregation or isolation is implemented, such as a membrane bioreactor (Sawayama et al., 1992; Prieto, 2011), that may reduce competition while allowing passage of nutrients.

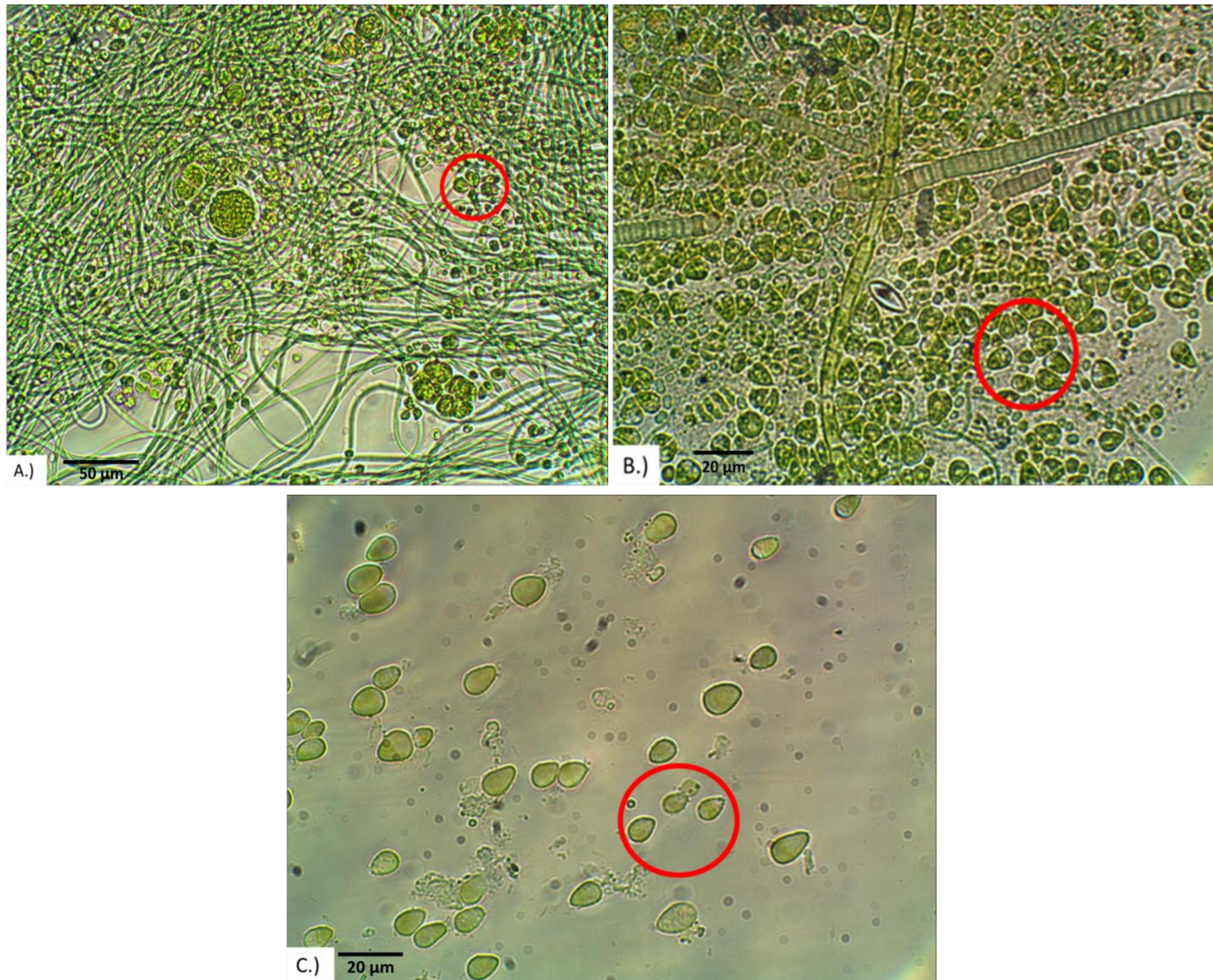

Figure 4.6. Final algal populations after batch cultivation of *B. braunii* in three growth media. A.) unsterilized clarified effluent following a post-BOD removal reactor, PBCE (5x), B.) unsterilized post-nitrification clarified effluent, PNCE (20x), and C.) sterilized reference medium, RM (20x). Circles highlight examples of *B. braunii* cells. Scales are approximate.

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4.4 Conclusion

Algal biofilm communities native to the clarifiers at two stages in an advanced wastewater treatment plant had similar nutrient removal and biomass production capability as other extensively studied monocultures (*C. sorokiniana* and *B. braunii*.). After 12 days, all algal species achieved nitrogen removal between 68-99%, and phosphorus removal between 60-100% in clarified effluents. All species investigated achieved higher yield in the ammonia-dominated PBCE, which may be where future algal photobioreactors should be incorporated. If a treatment plant integrates algae cultivation and is less concerned with specific biomass traits, cultivating native species can provide similar nutrient removal and biomass production benefits compared to augmented algal monocultures. Elevated pH in the batch series was significant and could be advantageous in continuous operation if an algal reactor was placed ahead of a treatment process that required an elevated pH. Increased soluble COD concentrations could be used to decrease external carbon inputs for denitrification. Both topics and the degradability of the soluble COD produced deserve further study. Not surprisingly, significant endemic species growth was apparent in unsterilized wastewater; if clean monocultures are required or predation is a concern, an isolation mechanism in the algal reactor may be recommended.

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Chapter 5: Isolated Cultivation of Algal Resource Utilizing Selectivity (ICARUS) – Laboratory Investigation

5.1 Introduction

Microalgae sales as nutritional supplements, animal and fish feed, fertilizers, cosmetics and other bioproducts, and biofuels (Mulbry et al., 2006; Chisti, 2007; Harun et al., 2010; Milledge, 2011) contribute significantly to the global economy (Raja et al., 2008). Microalgae have also gained renewed momentum as a potential biofuel, due to their rapid growth rate, natural production of oils, and ability to grow on non-arable lands in diverse climates.

However, in order to make algae cultivation economically competitive, cheap and reliable sources of water, nutrients, and carbon dioxide must be available. Wastewater is a consistent source of these necessities, is conveniently collected in centralized wastewater treatment plants, contains adequate nutrients for large-scale growth (Drexler et al., 2014), and has already been used to grow algae in various contexts (Hoffman, 1998). In fact, integrating wastewater treatment into large-scale algae cultivation may be necessary for favorable economics and investment returns (Clarens et al., 2011; Pittman et al., 2011; Beal et al., 2012).

Due to the relatively small size of microalgae and the dilute conditions in which they are cultivated (Molina Grima et al., 2003), harvesting can be extremely difficult, energy intense (Murphy & Allen, 2011), and account for up to 20-30% of total cultivation costs (Brennan & Owende, 2010). Therefore, a passive system requiring fewer inputs and energy is needed to grow

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a more concentrated algal culture for subsequent dewatering steps. Furthermore, species competition and predation can cause algal culture collapse (Huang et al., 2013) and threaten the integrity of harvested algae (Chapter 4), especially when using wastewater as a growth medium.

Membranes have been used in various capacities for cultivating and harvesting algae in multiple arenas in the algal industry (Chapter 3). Though many applications require active filtration, dialysis membranes provide a physical barrier, but allow diffusional passage of constituents (i.e., dissolved molecules or gases) based on a concentration gradient. Dialysis membranes have been used in limited studies to grow algae in wastewater (Schultz & Gerhardt, 1969; Dor, 1975; Hoover et al., 2011; Wiley et al., 2013) with promising results. Other studies (Jensen et al., 1972; Powers et al., 1976; Vincent & Silvester, 1979) have shown that dialysis culture increases the cell density of a target species when compared to a culture grown directly in medium. Not only does dialysis culture promote higher cell density, but it also provides a physical barrier against grazers and undesirable species in growth medium.

The proposed process, isolated cultivation of algal resource utilizing selectivity (ICARUS), uses a dialysis membrane to decouple an algal culture from a wastewater growth medium, thereby increasing the available nutrient pool. A conceptual diagram of ICARUS is illustrated in Figure 5.1. The goal of this study was to evaluate eleven membranes to determine which membrane characteristics promoted the longest exponential growth phase and highest cell density. The experimental design tested the hypothesis that ICARUS would extend the exponential growth phase and increase cell yields compared to algal cultures grown directly suspended in wastewater. Furthermore, ICARUS would regulate growth medium pH by facilitating gas exchange with surrounding wastewater. This study is a first step in screening materials and providing a proof of concept for subsequent scale-up systems.

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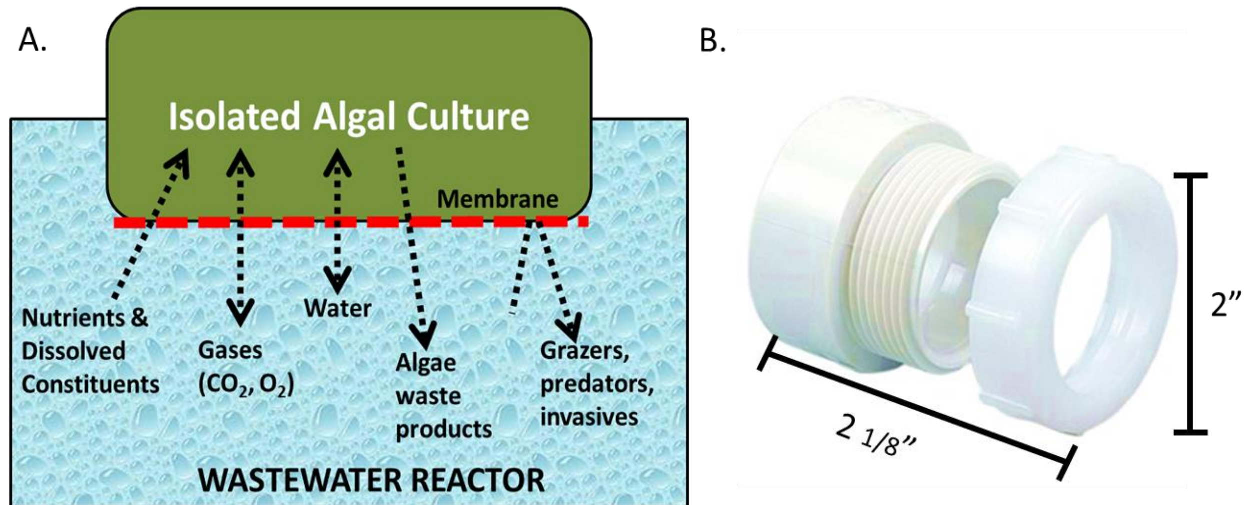

Figure 5.1. (A.) Conceptual principles of the isolated cultivation of algal resource utilizing selectivity (ICARUS) process, and (B.) expanded view of PVC trap adapters used as ICARUS pods. Algal culture is separated from wastewater growth medium using a membrane. Via concentration gradients, nutrients and gases pass between ICARUS culture and wastewater.

5.2 Methods

5.2.1 Stock Culture

Chlorella sorokiniana stock culture (University of Texas Culture Collection of Algae, culture #246) was cultivated in batch configuration in Bold 1NV medium. *C. sorokiniana* was chosen because it has a relatively fast growth rate, a robust nature, and commercial value in various markets. Bold 1NV contained the following components: $NaNO_3$ (2.94 mM); $CaCl_2$ (0.17 mM); $MgSO_4$ (0.3 mM); K_2HPO_4 (0.43 mM); KH_2PO_4 (1.29 mM); NaCl (0.43 mM); P-IV Metals Solution, Vitamin B_{12}, Biotin, Thiamine per UTEX Bold 1NV recipe. Algae stocks were shaken at 150 rpm (Lab-Line Incubator-Shaker, Melrose Park, IL) under a 12h/12h photoperiod of approximately 21 W m^{-2} during light hours. Stock cultures were not artificially aerated.

5.2.2 Experimental Design

ICARUS pods were constructed from polyvinyl chloride (PVC) plumbing valve adapters (Figure 5.1B) with an inner diameter of 2” (5.08 cm), which provided a membrane surface area

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of approximately 3.14 in^2 (20.3 cm^2) and an approximate inner volume of 6.7 in^3 (108 mL). Membranes were sandwiched between two pieces of the adapter, which provided a watertight seal when threaded. The active side of the anisotropic PVDF membranes faced the inner chamber of the ICARUS pod. The top of the pod is open to the atmosphere. ICARUS pods were placed in a bench-top recirculating raceway reactor with four parallel tracks (as shown in Figure 5.2) (earlier iterations of ICARUS pods are described in Appendix A). Each track was filled with four liters of clarified effluent (PBCE) originating from a pure oxygen carbonaceous removal reactor at the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL (described previously in Drexler et al. (2014) and illustrated in Figure 4.1). Clarified effluent was independently circulated in each track at a flow rate of 400 mL min^{-1}. Although medium was added to replace that lost via evaporation, the raceway was essentially operated as a batch reactor. The raceway reactor was kept at room temperature (approximately 25°C) and exposed to a 12h/12h photoperiod of 20 W m^{-2}.

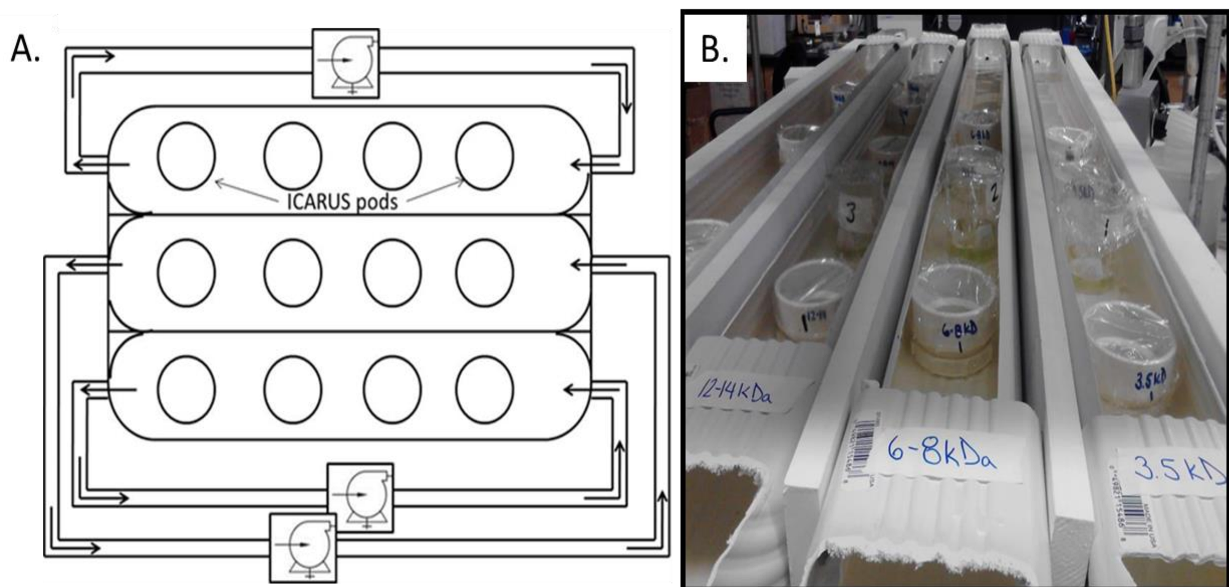

Figure 5.2. (A.) Schematic of laboratory ICARUS raceway, and (B.) photograph of laboratory raceway set up featuring ICARUS pods within each raceway.

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ICARUS pods, filled with 40 mL of deionized water, were allowed to equilibrate in the clarified effluent for two hours to prevent algal cell lysis upon inoculation. Each series was tested in at least two independent trials, each time in triplicate pods, using clarified effluent collected on separate occasions. The average water quality of the wastewater used in all trials is shown in Table 5.1. Similarly, triplicate glass beakers (100 mL) were filled with 40 mL of clarified effluent and placed in the raceway under the same temperature and light intensity. Algal cultures cultivated in beakers will be referred to throughout the paper as suspended culture.

Table 5.1. Average concentration of water quality parameters of clarified effluent during all ICARUS trials.

Parameter	Units	Concentration
Total suspended solids	$g\ L^{-1}$	0.03(0.5)
COD (total)	$mg\ L^{-1}$	60(17)
COD (soluble)	$mg\ L^{-1}$	52(12)
Total nitrogen (total)	$mg\text{-}N\ L^{-1}$	31(7)
Total nitrogen (soluble)	$mg\text{-}N\ L^{-1}$	30(7)
Ammonia	$mg\text{-}NH4\ L^{-1}$	27(4)
Nitrate	$mg\text{-}NO3\ L^{-1}$	3.3(3.6)
Phosphate	$mg\text{-}PO4\ L^{-1}$	4.5(2.3)
pH	pH units	7.4(0.1)

Standard deviations are shown in parentheses.

Beakers and pods were inoculated with a known volume of *C. sorokiniana* stock culture until the optical density at 680 nm was approximately 0.100, which corresponded to an approximate cell density of 0.05 $g\ L^{-1}$. Pods and beakers were lightly covered with plastic wrap to minimize water loss through evaporation. Each trial period lasted seven days.

5.2.3 Membrane Characteristics

Membrane materials selected were regenerated cellulose (RC) (SpectrumLabs, Rancho Dominguez, CA), cellulose acetate (CA) (Sterlitech, Kent, WA), polyvinylidene fluoride (PVDF) (Orelis, Salindres, France), and nylon (NY) (Component Supply Company, Fort Meade, FL). RC is a common material in dialysis filtration and has been used previously in algal studies

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(Jensen et al., 1972). PVDF, more commonly used in pressure filtration for water/wastewater, fouling studies and algal harvesting, was chosen because of its durability and larger pore range (Babel et al., 2002; Morineau-Thomas et al., 2002; Rossi et al., 2004; Babel & Takizawa, 2010). CA is less commonly used in algal studies, but was selected because of its higher pore range, durability, and surface characteristics. NY was selected due to its higher pore range and relatively inexpensive cost compared to other materials. Both RC and CA membranes were hydrophilic (per manufacturers' specifications), which have been shown to foul less than hydrophobic membranes in long-term operation (Rossignol et al., 1999; Hung & Liu, 2006; Babel & Takizawa, 2010; Sun et al., 2013). PVDF is generally a hydrophobic material with a hydrophilic surface modification for enhanced water wetting. The hydrophobicity of the NY membranes was unavailable. Membranes spanned a wide range of porosities, centered on a 40 kDa pore size recommended from previous studies (Rossignol et al., 1999; Rossi et al., 2004; DeBaerdemaeker et al., 2013). Characteristics of the eleven membranes tested are shown in Table 5.2 and cross-sections of selected membranes are shown in Figure 5.3.

Table 5.2. Characteristics of membranes screened in ICARUS trials.

Material	Porosity	Relative Permeability	Relative Thickness	Thickness (nm)
RC	3.5 kDa	6.8	1.0	29
RC	6-8 kDa	7.9	2.1	61
RC	12-14 kDa	8.2	1.2	36
CA	0.2 µm	9.4	2.7	76
PVDF	3 kDa	1.0	6.6	190
PVDF	10 kDa	1.4	4.8	138
PVDF	40 kDa	6.9	4.8	138
PVDF	0.1 µm	9.4	5.0	144
NY	7 µm	8.4	2.3	66
NY	15 µm	9.3	1.9	55
NY	31 µm	10.4	2.0	56

Relative permeability and thickness were calculated as described in Section 2.4. RC=regenerated cellulose; CA=cellulose acetate; PVDF=polyvinylidene fluoride; NY=nylon.

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5.2.4 Analytical Methods

Daily cell growth of each pod and beaker was measured via optical density (OD), after agitating the culture, at 680 nm on a spectrophotometer (HACH DR/4000, Loveland, CO, USA). OD was used because samples could be returned to pods and beakers after reading as to not disrupt the volume and concentration gradients of dialysis pods. A calibration curve (r^2=0.96) was developed by measuring dry weight (Standard Methods, 2540D, with the addition of an ammonium bicarbonate rinse (Zhu & Lee, 1997)) on algae stock culture and test samples when available. The resulting curve was used to convert optical density to dry weight in each trial. Glass pH electrodes were used to measure daily pH (Oakton Instruments, Vernon Hills, IL, USA).

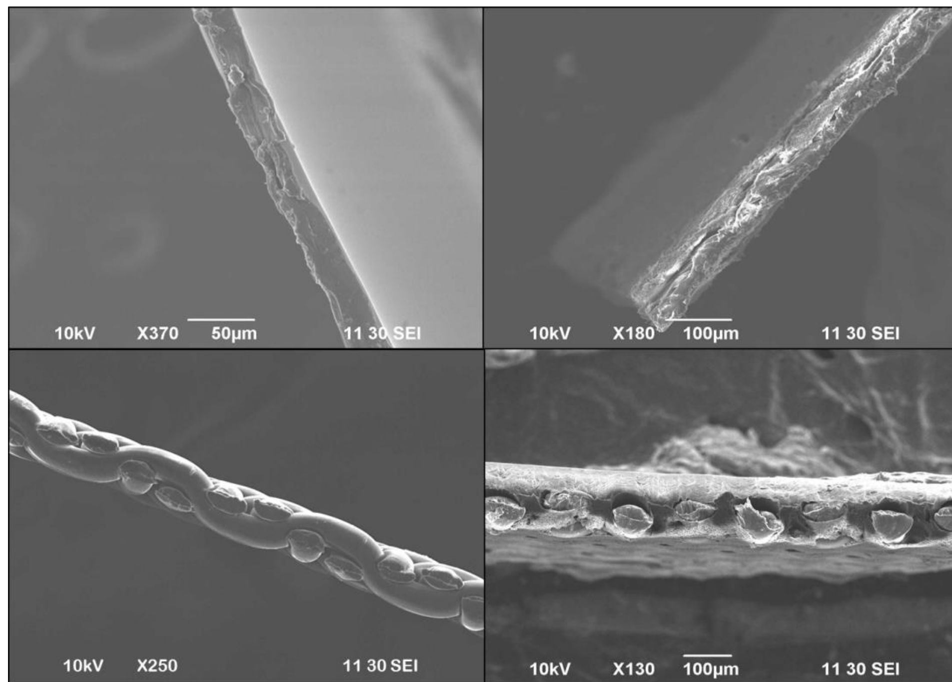

Figure 5.3. Scanning electron microscope photographs of the cross-sectional area of examples of each membrane material screened in the study. Clockwise from top left: 3.5kDa-RC (regenerated cellulose), 0.2µm-CA (cellulose acetate), 40kDa-PVDF (polyvinylidene fluoride), and 7µm-NY (nylon).

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It was hypothesized that membrane thickness and permeability would be the two main characteristics affecting ICARUS performance. Hence, these properties were characterized for each membrane. It was assumed that other characteristics, such as surface roughness or hydrophobicity, would not have a significant effect on algae growth at this stage of the screening process as fouling was not the focus of this study. Membrane thickness was measured using scanning electron microscopy (JEOL, Peabody, MA, USA). Membrane permeability was measured by filling raceways with a sodium chloride solution (500 mg L^{-1}). Sodium chloride was chosen as a model compound because its diffusivity (as calculated by the Hayduk-Laudie correlation) of $1.60x10^{-5}$ cm^2 sec^{-1} falls in the middle of the diffusivity range of target constituents (ammonium, phosphate, and nitrate) of 1.36 $x10^{-5}$ to 2.03 $x10^{-5}$ cm^2 sec^{-1}. Also, the molecular weight and charge of ammonium is similar to that of sodium. Triplicate ICARUS pods were fitted with membranes as described in section 5.2.2 and filled with 50 mL of distilled water. Conductivity inside the ICARUS pods was measured (Vernier conductivity probe, Beaverton, OR, USA) at ten minute intervals for two hours. Permeability was defined as the slope of the conductivity curve (beginning after a 10 minute lag period), and relative permeability was defined as the permeability normalized to the smallest permeability value (in this case, the 3.5kDa-PVDF membrane). Relative permeability and thickness of each membrane are shown in Table 5.2.

Specific growth rate was determined by calculating the slope of the growth curve (transformed to first order kinetics) during the first three days of cultivation. The length of the exponential growth phase was determined by calculating the slope between each data point on the growth curve. Exponential growth was considered to have occurred if the slope between data points was greater than 0.25 day^{-1}, which is in the lower range of previously reported growth

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rates of *C. sorokiniana* (0.2-1.32 d^{-1} depending on COD and nutrient loading) (Ruiz et al., 2011; He et al., 2013; Zhu et al., 2013). All statistical tests were conducted using SAS 9.3 (Cary, NC, USA), except the generalized linear model, which was developed using R Commander (GNU General Public License, John Fox, McMaster University). Relationships with p-values less than 0.05 ($p<0.05$) were considered *significant* and are refer to as such (unless otherwise specified).

5.3 Results

5.3.1 Growth Characteristics and Biomass Yield

5.3.1.1 Final Cell Density

High cell density is important in algae cultivation because subsequent processing steps require cultures to be a thick slurry (15-20% solids) that is often 200-2000x the initial culture concentration (Uduman et al., 2010). Starting the dewatering process at a higher density will require less energy and fewer external inputs to reach a target concentration for downstream processing (i.e., for biofuel production), improving the total process economics.

On the lab scale, the cell density of ICARUS series did not reach the density of 4 g L^{-1} typical of photobioreactors (Chisti, 2007), which was expected as the geometry of ICARUS pods and environmental conditions were not optimized at this stage. In most cases, however, the cell density was well above the 0.14 g L^{-1} density typical of raceway cultures (Chisti, 2007), even in suspended cultures. Four of the ICARUS series (40kDa-PVDF, 0.53 g L^{-1}; 0.1μm-PVDF, 0.43 g L^{-1}; 12kDa-RC, 0.35 g L^{-1}; 0.2 μm-CA, 0.41 g L^{-1}) had a final cell density that was significantly higher than that of the suspended culture (0.25 g L^{-1}). The suspended culture had a significantly higher cell density than the 15 μm (0.06 g L^{-1}) and 31 μm (0.03 g L^{-1}) NY membranes, with no significant difference among the remaining five series.

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In general, membranes with porosities in the range of 12kDa to 0.2 μm achieved a higher cell density than those less than or greater than this range (Figure 5.3A-C). The 40kDa-PVDF membrane had a significantly higher density than nine of the other series, which agrees with previous studies that recommended 40kDa pore size for microalgae harvesting (Rossignol et al., 1999; Rossi et al., 2004; DeBaerdemaeker et al., 2013). Similarly, the 0.1μm-PVDF and 0.2μm-CA had significantly higher cell density than seven series. It is hypothesized that the pore sizes in this range allow efficient passage of nutrients and gases, while maintaining the algae cells within the ICARUS culture.

Because the larger pore sizes allowed algae cells to leak through the membrane at the beginning of the trial, the 15 μm and 31 μm NY membranes had a significantly lower cell density than nearly all other series (Figure 5.4F). Although NY is cheaper compared to other materials tested, the large pore size may exclude its use in ICARUS cultivation, unless an algal biofilm layer (essentially a self-forming dynamic membrane layer) could be established rapidly to prevent cell loss. The larger pore size may also allow endemic organisms and grazers to invade the algal culture, organisms that may otherwise be held back by smaller pore sizes. However, if a larger algal species (such as *Spirulina*) was cultivated instead of *Chlorella*, the large pore size may be less of a concern in regard to biomass loss.

5.3.1.2 Final Mass Yield

Although cell density is important for subsequent dewatering steps, it may not be the best measurement of final yield, as water loss during cultivation may merely be concentrating the culture. High evaporation rates in large-scale systems can cause operational challenges, i.e., replacing growth medium or correcting salinity (Borowitzka & Moheimani, 2013). Passive

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water loss, however, if predictable and timed advantageously, can strategically be used to concentrate cultures just prior to harvest.

Although the cell density in the 40kDa-PVDF series was significantly higher than that of most other series, the mass yield was only significantly higher than five, including the suspended culture. A similar trend was seen with the 0.2µm-CA membrane, which had a significantly higher density than seven series, but only significantly higher mass yield of five. The 0.1µm-PVDF series had a significantly higher cell density and mass than seven series. In the cases where cell density did not translate to significantly higher mass yield, water loss could account for the discrepancy. Water loss among ICARUS pods may fortuitously be a potential mechanism for passive dewatering and should be investigated further in field studies.

Four membrane series achieved significantly higher mass yield than the suspended cultures (40kDa-PVDF, 0.1µm-PVDF, 12kDa-RC, and 0.2µm-CA). In fact, the 40kDa-PVDF, 0.1µm-PVDF, and 0.2µm-CA series had the highest mass yield of all series considered. series outperforming suspended cultures were the larger pore sizes of each membrane material (except nylon), which suggests that the larger pore sizes facilitated stronger growth by allowing faster movement of constituents across the membrane. The difference is especially apparent within the PVDF membrane group, where the 40kDa and 0.1µm both reach significantly higher mass yields than the two smaller pore sized PVDF membranes tested. Differences among RC membrane series were not significant. When membrane series were broken into groups based on porosity (small, 3kDa-12kDa; medium, 40kDa-0.2µm; large, 7µm-31µm), the medium range had a significantly higher mass yield than the small, large, or suspended cultures, which agrees with earlier studies that recommended a 40kDa pore size for algae harvesting (Rossi et al., 2004; Rossignol et al., 1999; DeBaerdemaeker et al., 2013).

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There appears to be an upward limit of porosity, as the two series (15µm-NY and 31µm-NY) that had significantly lower mass yield than suspended cultures (and the majority of other series as well) had much larger pore sizes (Figure 5.5). Although the 7µm-NY membrane series performed significantly better than the larger porosity NY membranes, it did not have significantly higher growth than any other series. As shown in the normalized growth curve (Figure 5.4F), series with relatively large pore sizes lost some biomass in the initial stage of the trials from which they never completely recovered.

5.3.1.3 Specific Growth Rate and Exponential Growth Phase

Trends in specific growth rate generally followed that of final yield, which would be expected (Table 5.3), but there were fewer significant differences among series' specific growth rates. Specific growth rate is generally affected by environmental conditions (i.e., sunlight, nutrient, and carbon dioxide availability). Because there is a biological limit to an organism's specific growth rate based on given environmental conditions, it is not unexpected that variability in specific growth rate among series is not as significantly different as variability among yield outcomes, as few environmental conditions were changing throughout the study. Differences among growth rates are most likely due to differences in nutrient and carbon dioxide availability (a function of membrane characteristics), as light intensity and temperature remained constant in all trials. All series (except the 31µm-NY) achieved average specific growth rates in the range previously reported for *Chlorella* sp. (Ruiz et al., 2011; He et al., 2013; Zhu et al., 2013).

Only two series (40kDa-PVDF and 0.1 µm-PVDF) had significantly higher growth rates than the suspended culture, compared to four series yielding a significantly higher cell density and mass yield. Although the growth of the cultures began at a similar pace, the exponential

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phase of the ICARUS series was sustained longer, thereby promoting higher overall growth by the end of the trial (Table 5.4).

Series whose exponential growth phase lasted five days or longer had a significantly higher final cell density than those series whose exponential phase only lasted four or fewer days. Similarly, series with exponential phases longer than five days had significantly higher mass yields than those with exponential phases lasting three days or less. Six individual series (0.2μm-CA, 0.1μm-PVDF, 40kDa-PVDF, 12kDa-RC, 3.5kDa-PVDF, and 3kDa-RC) had significantly longer exponential growth phases than the suspended cultures, four of which achieved significantly higher productivity.

Table 5.3. Significant differences ($p<0.05$) among final cell density and mass yield of all series in ICARUS and suspended trials.

	3kDa	10kDa	40kDa	0.1μm	3.5kDa	6kDa	12kDa	0.2μm	7μm	15μm	31μm	Susp
3kDa			(+)	(+)*				(+)		(-)*	(-)*	
10kDa			(+)	(+)*			(+)	(+)			(-)*	
40kDa	(-)	(-)			(-)	(-)*	(-)		(-)*	(-)*	(-)*	(-)*
0.1um	(-)	(-)			*-	(-)*			(-)*	(-)*	(-)*	(-)*
3.5kDa									(-)	(-)*	(-)*	
6kDa				(+)				(+)*		(-)	(-)*	
12kDa	(-)	(-)							(-)	(-)*	(-)*	(-)
0.2um	(-)	(-)							(-)	(-)*	(-)*	(-)
7um				(+)							(-)*	
15um			(+)	(+)	(+)	(+)	(+)	(+)	(+)		*+	(+)*
31um	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)			(+)*
Susp			(+)	(+)			(+)	(+)		(-)	(-)	

Mass Yield (lower left) — Cell Density (upper right)

Final cell density relationships are shown in the upper right (grey) of the table, and final mass yield relationships are shown in the bottom left (white). Symbols in parentheses depict whether the series yield in the top row is greater than (+) or less than (-) the series yield in the left hand column. The * symbol is shown if the specific growth rate between the two series was significant. In general, trends in specific growth rate follow that of cell density; when cell density was not significant but specific growth rate was, a symbol next to the * depicts the relationship between the top row and left hand column series.

The PVDF, CA, and RC membrane groups all achieved significantly longer average exponential phases than the NY membrane group. When membrane groups were broken into size categories, the exponential phase of the medium porosity group (40kDa-0.2μm) was significantly longer than all other categories, including the suspended culture. The small porosity category

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(3kDa-12kDa) also had significantly longer exponential phases than the suspended and large porosity groups.

In many cases, ICARUS demonstrated higher yield, faster growth rate, and longer exponential phase than microalgae grown in suspended cultures. The use of the dialysis membrane decouples the algae culture from the growth medium, increasing nutrient availability, as ICARUS pods drew from four liters of wastewater compared to suspended cultures which drew from 40 mL. Increased nutrient availability, along with pH regulation, is suspected to be a main mechanism for prolonging the exponential growth phase in ICARUS series, leading to higher algal productivity.

Table 5.4. Growth characteristics of ICARUS cultivated *C. sorokiniana* using various membranes compared to suspended culture.

Membrane Type	Avg. Specific Growth Rate (day^{-1})	Exponential Growth Phase (days)	Final Yield Dry Weight ($g\ L^{-1}$)	Final Yield Mass (mg)	Areal Density ($g\ m^{-2}$)
3.5kDa-RC	0.42(0.08)	3.6(0.5)	0.31(0.04)	12.1(3.9)	6.0(2.0)
6kDa-RC	0.35(0.22)	3.1(0.8)	0.28(0.09)	10.1(3.3)	5.0(1.6)
12kDa-RC	0.47(0.07)	3.8(0.7)	0.35(0.07)	14.5(2.4)	7.1(1.2)
3kDa-PVDF	0.44(0.03)	3.5(0.8)	0.22(0.04)	8.6(2.1)	4.2(1.0)
10kDa-PVDF	0.40(0.03)	3.3(0.5)	0.20(0.03)	7.7(1.1)	3.8(0.6)
40kDa-PVDF	0.57(0.14)	4.4(1.0)	0.53(0.14)	14.1(2.6)	7.0(1.3)
0.1µm-PVDF	0.61(0.04)	5.7(1.0)	0.43(0.03)	16.6(1.8)	8.2(0.9)
0.2µm-CA	0.55(0.13)	5.8(1.0)	0.41(0.16)	14.5(5.4)	7.1(2.7)
7µm-NY	0.40(0.08)	3.2(0.7)	0.17(0.06)	10.4(3.4)	4.9(2.0)
15µm-NY	0.26(0.05)	2.6(1.0)	0.06(0.01)	3.9(0.8)	1.9(0.4)
31µm-NY	0.09(0.04)	1.4(0.5)	0.03(0.01)	1.9(0.6)	0.9(0.3)
Suspended	0.43(0.08)	2.2(0.8)	0.25(0.12)	9.4(4.9)	4.1(1.1)

Values are averages with standard deviation in parentheses. RC=regenerated cellulose; CA=cellulose acetate; PVDF=polyvinylidene fluoride; NY=nylon. Replicates were as follows: suspended culture, n=18; 3kDa-PVDF, 10kDa-PVDF, n=6; all other series, n=9.

5.3.1.4 Algal Health

Microscopic investigation of the algae cultivated in ICARUS and suspended growth was conducted in select trials. Based on visual inspection, it appeared that ICARUS cultivation

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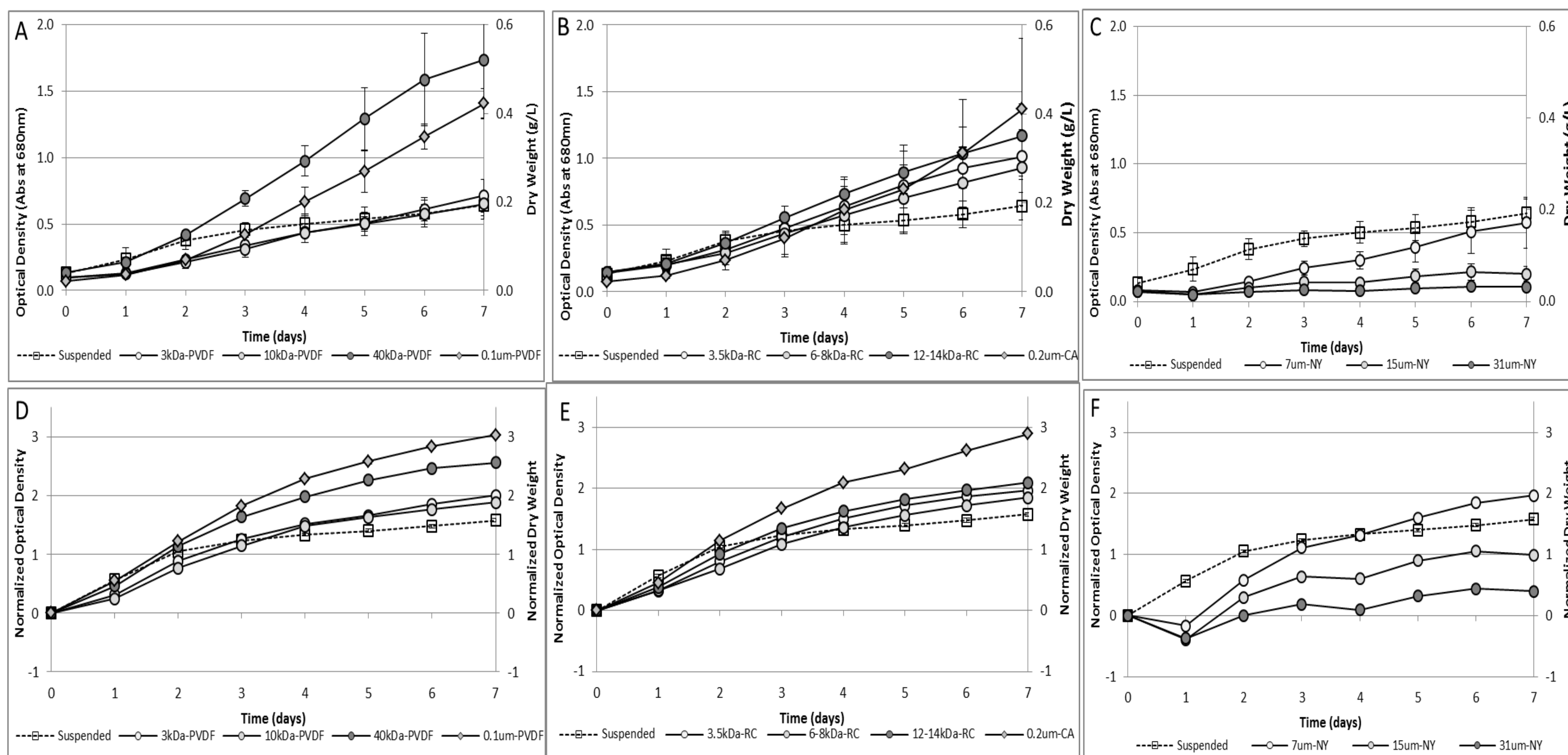

Figure 5.4. Average growth curves of ICARUS and suspended cultures. Growth is illustrated as optical density and dry weight (A-C), optical density and dry weight normalized to first order kinetics (LN(C/Co)) (D-F), and final mass yield (G-I). RC=regenerated cellulose; CA=cellulose acetate; PVDF=polyvinylidene fluoride; NY=nylon. Sample sizes were as follows: suspended culture, n=18; 3kDa-PVDF, 10kDa-PVDF, n=6; all other series, n=9. Errors bars depict one standard deviation.

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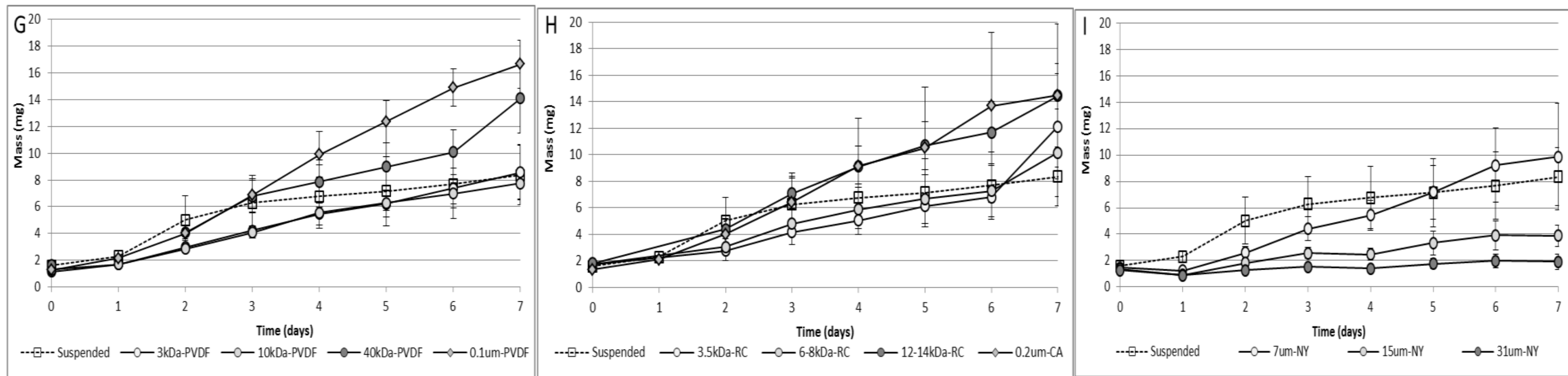

Figure 5.4. (Continued)

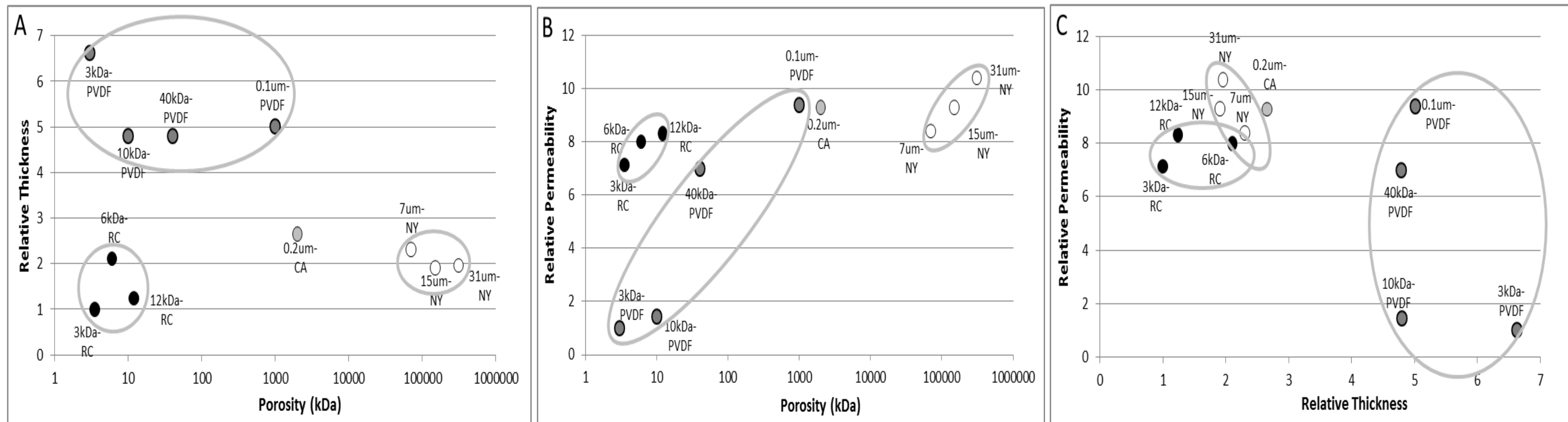

Figure 5.5. Relationships between membrane characteristics (porosity, relative thickness, relative permeability, and material). Circles and series shading highlight clustering within membrane materials.

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maintains a monoculture much better than a culture suspended directly in wastewater. Furthermore, the algae in ICARUS culture appeared healthier (greener, rounder) than the suspended cultures (Figure 5.6).

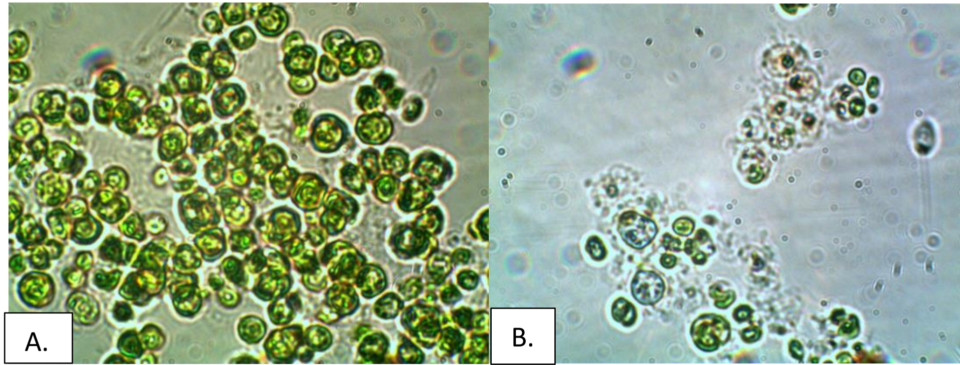

Figure 5.6. Microscopic images (100x) of *C. sorokiniana* grown in (A.) 0.1 µm PVDF membrane pod and (B.) directly in wastewater.

5.3.2 Changes in pH

It was hypothesized that ICARUS would not only increase nutrient availability to cultures, but would also regulate gas exchange. As algae photosynthesize, carbon dioxide decreases, which in turn can elevate pH or change alkalinity (Uusitalo, 1996). Stabilizing pH in large-scale cultivation can be challenging, and elevated pH can inhibit algal growth (Goldman et al., 1982). Similarly, elevated dissolved oxygen concentration, produced during photosynthesis, can also inhibit algal growth (Shelp & Canvin, 1980). Gas exchange within cultures is therefore very important.

Despite differing membrane characteristics, the variability in pH between membrane series was small, which was expected since gases should move freely across the MF and UF membranes tested. The ICARUS series maintained an average pH of 9.55, which was significantly lower than the average of 10.21 in suspended culture (Figure 5.7). It is expected that this difference would increase in continuous flow conditions when carbon dioxide was constantly

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replenished. Both the ICARUS and suspended cultures had a significantly higher pH than the raceway medium; ideally ICARUS pH would more closely match its surrounding medium. Optimizing these relationships should be investigated in further studies.

Culture pH increased in the first three days of each trial, which coincided with the exponential growth phase (Figures 5.4 and 5.5). The initial pH increase is starker in the suspended cultures, followed by a slight decrease, whereas the trend is more gradual in ICARUS series. In fact, on day three, the pH in suspended cultures averaged 10.71, compared to just 9.18 in the ICARUS series. Elevated pH appears to restrict exponential phase and has been shown to inhibit *Chlorella* sp.(Goldman et al., 1982) growth; by regulating pH, as in the ICARUS series, the exponential growth phase is allowed to last longer.

As pH regulation suggests that carbon dioxide is freely moving across the membrane, it is suspected that the ICARUS process will also aid in passively removing dissolved oxygen from photosynthesizing cultures. As elevated oxygen levels can inhibit exponential growth (Shelp & Canvin, 1980), faster removal may enhance productivity. As such, pH and dissolved oxygen regulation are areas deserving of further study.

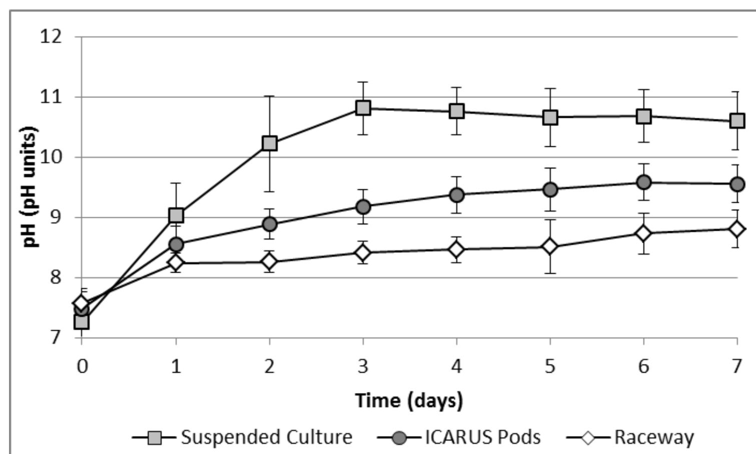

Figure 5.7. Average pH of algae in ICARUS series compared to suspended cultures and raceway medium. Error bars depict one standard deviation.

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5.3.3 Membrane Characteristics and Final Yield

5.3.3.1 Analysis of Individual Membrane Characteristics

Membrane characteristics, such as material, porosity, permeability, and thickness, may all effect the movement of constituents across a membrane and therefore affect algae growth. Two dimensional plots (Figure 5.5) show the relationship between membrane characteristics. As expected, porosity and permeability have a positive correlation, whereas correlations among other characteristics are not as clear. Thickness is the sum of the active membrane layer plus a backing; the backing may contribute to thickness but not necessarily decrease permeability. Membranes tend to clump based on material within each figure, which is due to similar construction (thickness) or pore range. Although other characteristics, such as surface roughness or hydrophobicity, may affect movement of constituents, the four selected characteristics were assumed to have the largest effect in this study, as the trials were short enough that fouling was not expected to be an issue. However, in scale-up studies, other characteristics should be considered.

The effect of individual membrane characteristics on biomass production (dry weight and mass yield) is shown in Figure 5.8. Biomass yield increased with increasing porosity until about 50-1000kDa, when increasing porosity had a detrimental effect on biomass productivity (Figure 5.8D). This trend confirms the findings of this study regarding productivity, growth rate, and exponential growth phase, where medium pore size series performed significantly better than other series in all categories. Therefore, membranes within the range of 50-1000kDa would be recommended for scale-up to maximize productivity. Membrane series tend to clump based on material when relating productivity to thickness, which would be expected since material is closely related to membrane construction (Figure 5.8B&E).

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Permeability may take into account porosity and thickness, but could also be a function of surface roughness, hydrophobicity, and the distribution of pores across the membrane surface. When comparing biomass productivity to relative permeability, layering occurs within the results. A decreasing trend occurs with the nylon membranes; as permeability increases, biomass productivity decreases. This supports the prior hypothesis that microalgae may escape across the membrane with increasing porosity and permeability. RC and PVDF membranes demonstrated the opposite trend (Figure 5.8C&F); as permeability increases, productivity increases. This is most likely because the porosity still falls on the upward curve of the dome relationship in porosity (Figures 5.8A&D). If permeability were primarily influenced by porosity, the same dome relationship apparent in porosity would occur with permeability. Because this is not the case, permeability must be influenced by other variables. Because a 2D analysis cannot account for all characteristics simultaneously, nor can it account for the interactions among variables, a generalized linear model was used to further investigate membrane characteristics

5.3.3.2 Analysis of Multiple Variables and Variable Interactions

A generalized linear model (GLM) was developed (R Commander, GNU General Public License, John Fox, McMaster University) to determine which membrane characteristic of permeability, porosity, thickness, or material or the interactions thereof contributed most to the variability in biomass production (as dry weight and mass yield). The objective of the model was to determine which characteristics should be focused on for scale-up studies and whether other characteristics not considered may be influencing productivity.

Although including membrane material as a variable may limit the model's application outside this study (i.e., if scale-up studies use different materials), it was important to include in this model to determine the effect of embodied characteristics within a material beyond those

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specifically measured, such as roughness, hydrophobicity, or pore distribution. Any future predictive models would probably exclude material as a variable, but would include other membrane characteristics as needed. Although it can be affected by other characteristics, such as porosity and thickness, permeability is considered a separate variable. This was justified because the trends relating permeability to productivity do not match either porosity or thickness. Including all variables and interactions within the model yielded the lowest AIC of all model iterations tested (Table 5.5).

The results of the GLM with the optimal fit (Equation 1, Table 5.5) are shown in Table 5.6. More parameters contributed to the variability of dry weight than to mass, which would be expected; water loss (which affects dry weight more than mass) may be a function of many variables, whereas total mass may only be affected by nutrient availability and/or environmental conditions. Individually, thickness did not significantly affect dry weight, but its interaction with permeability was significant. In fact, permeability is indeed a function of numerous variables regarding its effect on dry weight variability, as permeability significantly contributed to the variability in dry weight individually and through interactions with three other variables. Based on trial data and the performance of the NY membrane group, it is not surprising that the NY material significantly affected dry weight variability individually as well as its interaction with permeability and porosity, which contributed to algal biomass loss. Exclusive of material, the characteristics most significantly affecting dry weight variability in scale-up trials are the remaining three characteristics investigated (permeability, porosity, and thickness), with most emphasis on permeability and porosity. Although other membrane characteristics may be investigated in future studies, the three selected in this study found were to be a foundational and significant.

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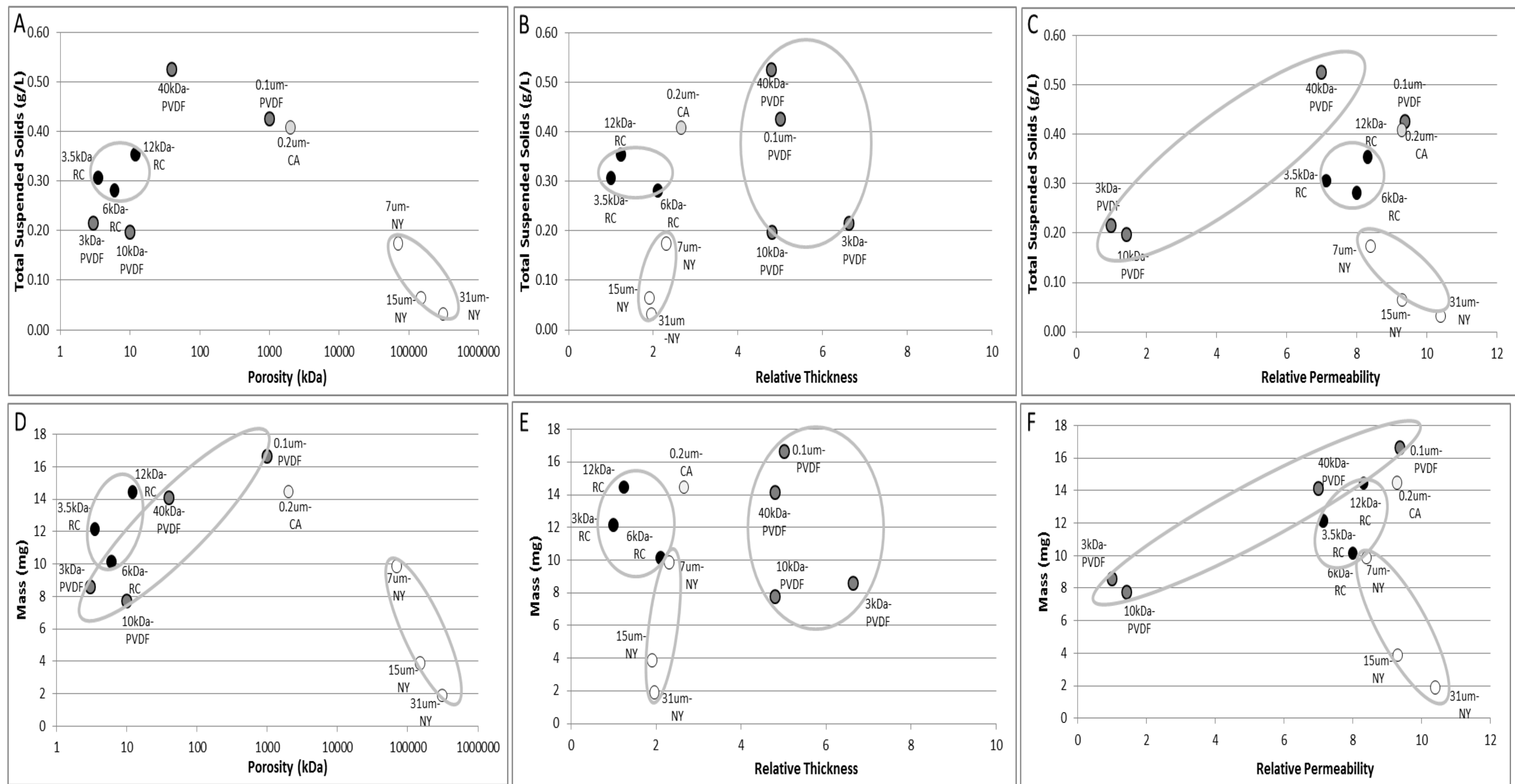

Figure 5.8. Relationship between membrane characteristics and algae final dry weight and mass. Characteristics include material, porosity, thickness, and permeability. Dry weight is shown in A.-C., and mass is shown in D.-F.. Circles and series shading highlight clumping of data points based on membrane material.

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The variables significantly affecting mass yield variability were much less diverse than those affecting dry weight. Neither material nor porosity significantly affected mass yield individually or through interactions with other characteristics. Permeability and the interaction between permeability and thickness were the only two variables to contribute significantly to the variability in dry weight, which may be due to the contribution that permeability has on nutrient and gas availability. Therefore, in scale-up studies, these two characteristics may be emphasized in membrane selection.

Table 5.5. Equations and associated Akaike Information Criterion (AIC) for GLMs determining variability among dry weight (DW) and mass productivity.

Equation	DW AIC	Mass AIC
(1) = permeability + porosity + thickness + material + (permeability* porosity) + (permeability*thickness) + (permeability*material) + (porosity*thickness) + (porosity*material) + (thickness*material)	-181.02	477.21
(2) = permeability + porosity + thickness + material	-163.49	489.11
(3) = permeability + porosity + material+ (permeability*porosity) + (permeability*material) + (porosity*material)	-160.9	480.25
(4) = permeability + porosity + thickness + (permeability*porosity) + (permeability*thickness) + (porosity*thickness)	-157.71	491.48
(5) = permeability + porosity	-129.74	492.9

Expanded results of Equation 1 are shown in Table 5.6. Symbol * shows interaction terms.

The significance of different characteristics' contributions to productivity variability may be important in scaling up, as dry weight and mass have different characteristics contributing to their variability. Depending on the goal of the project, maximizing cell density or overall mass yield, different characteristics may be desired when selecting the membrane material.

5.4 Conclusion

ICARUS, a new process of cultivating and harvesting microalgae in wastewater, was proposed and tested. In many cases, ICARUS series yielded a higher cell density and higher mass yield than the suspended cultures. The pore range of 50-1000kDa produced the highest

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Table 5.6. Variables and interactions contributing significantly to the variability among biomass productivity using GLM Equation 1 in Table 5.5.

Variable/Interaction	Effect on Dry Weight	Effect on Mass
Permeability	*	*
Porosity	***	
Material(NY)	***	
Material(RC)	***	
Permeability:Porosity	***	
Permeability:Material(NY)	***	
Permeability:Thickness	*	**
Porosity:Material(NY)	***	

Significance codes: ***=0.001; **=0.01; *=0.05

productivity, suggesting this range should be investigated for scale-up. ICARUS series regulated pH better than the suspended cultures, which helped to extend the exponential growth phase. Different membrane characteristics contributed to the variability among biomass production and should be considered for scale-up design. Although the current study provides a proof-of-concept foundation for the proposed method, future field studies should investigate gas and nutrient exchange, fouling, and grazer dynamics.

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Chapter 6: Isolated Cultivation of Algal Resource Utilizing Selectivity (ICARUS) – Field Evaluation

6.1 Introduction

As the world's population continues to rise, the demand for energy and resources and the production of waste will also increase. Innovative pathways to recover resources from wastes (Mehta et al., 2014) may be necessary to satiate global food and energy demands into the future. Microalgae are regaining popularity as a potential pathway due to their ability to grow in diverse environments with recycled inputs (i.e., wastewater, sea water, flue gases) and the wide variety of products derived from them (Mata et al., 2010). Despite its significant contribution to the global economy in other sectors (Raja et al., 2008), large-scale algal production as a feedstock for biofuels remains challenging.

Microalgae are typically small in size (3-30 μm) and cultivated in relatively dilute concentrations (generally less than 0.5 g dry biomass L^{-1}) (Molina Grima et al., 2003), which makes harvesting and dewatering microalgal cultures particularly problematic and expensive, accounting for 20-30% of algal biofuel production costs (Brennan & Owende, 2010). External inputs, such as nutrients, freshwater, and carbon dioxide, also add a substantial environmental burden (Clarens et al., 2010). Using wastewater as a growth medium, which embodies a free source of nutrients, water, and carbon dioxide, has been shown to potentially decrease the environmental impact (Clarens et al., 2011), operating costs (Drexler et al., 2014), and increase

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the return on investment (Beal et al., 2012) of integrating algae cultivation with wastewater treatment. However, utilizing wastewater may increase the chances of introducing grazers and invasive species to a microalgal culture (Chapter 4), which can lead to algal culture collapse (Huang et al., 2013).

A recently introduced cultivation method, the isolated cultivation of algal resource utilizing selectivity (ICARUS) (Chapter 5), addresses each of these major cultivation and harvesting challenges. The passive membrane configuration takes advantage of concentration gradients between an algal culture and a wastewater growth medium. Nutrients and gases flow freely across the membrane, but biological interferences (grazers and competitive species) within wastewater are kept separate by the physical membrane barrier. Previous studies have shown that dialysis cultivation encourages a higher cell density than suspended cultures (Chapter 5; Dor, 1975); a more concentrated culture will require less dewatering downstream, saving energy and increasing the economic feasibility of the entire process.

Building on previous laboratory scale experiments of ICARUS (Chapter 5), this study tested the configuration *in-situ* in an activated sludge clarifier. The experimental design compared biomass growth in ICARUS to control cultures and investigated changes in dissolved constituent and gas concentrations based on series conditions. Grazer invasion and external biofouling is also assessed.

6.2 Methods

6.2.1 Algae Stock Culture

Microalgae (*C. sorokiniana,* UTEX #246, University of Texas (UTEX) Culture Collection of Algae) stock culture was grown in Bold 1NV medium. Bold 1NV contained the following components: $NaNO_3$ (2.94 mM); $CaCl_2$ (0.17 mM); $MgSO_4$ (0.3 mM); K_2HPO_4 (0.43

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mM); KH_2PO_4 (1.29 mM); NaCl (0.43 mM); P-IV Metals Solution, Vitamin B_{12}, Biotin, Thiamine per UTEX Bold 1NV recipe. Stock cultures were shaken at 150 rpm (Lab-Line Incubator-Shaker, Melrose Park, IL, USA) at a 12/12 photoperiod (21 W m^{-2}) at room temperature (25°C). Stock cultures were not artificially aerated. *C. sorokiniana* was chosen because *Chlorella* sp. grow relatively quickly (0.2-1.32 d^{-1} depending on COD and nutrient loading) (Ruiz et al., 2011; He et al., 2013; Zhu et al., 2013), have been successfully grown in wastewater (Chapter 4), and have commercial value and biofuel potential (Mata et al., 2010).

6.2.2 Experimental Design

Glass canning jars (approximately 4 oz, 100 mL) with two-piece lids (Figure 6.1) were filled with 80 mL of growth medium (i.e., tap water or wastewater). Each jar had an approximate membrane surface area of 4 in^2 (25.8 cm^2). Experimental series were prepared as shown in Table 6.1 and will subsequently be referred to by their Reference Tag throughout the manuscript. Series including algae were inoculated with 5 mL of stock culture such that the biomass density was approximately 0.04 g L^{-1}. Eight jars were prepared for each series, and two jars were sacrificially sampled every five days during the twenty day study period. The inner lid of the ICARUS jars were replaced with membranes and overlaid with a rubber gasket which created a water tight seal when threaded with the band. Membrane materials chosen were nylon (NY), regenerated cellulose (RC), and polyvinylidene fluoride (PVDF); characteristics and justification for selection were as described in Chapter 5. The active side of the anisotropic PVDF membranes faced the inner chamber of the ICARUS pod. Non-ICARUS jars (blanks and controls) were fitted with the manufacturer's air and water tight lids and bands, thus were sealed from any nutrient or fluid exchange with the clarified effluent.

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Figure 6.1. Glass jars used as ICARUS pods (A.) without algae and (B.) with algae inoculated.

All jars were confined in a wire cage lid-side down and lowered into the upwelling weirs of the clarifier following the post-BOD carbonaceous removal reactor (Figure 6.2) at the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL (described previously in Drexler et al. (2014) and illustrated in Figure 4.1) for 20 days in early spring. The location was chosen because previous experiments showed that *C. sorokiniana* preferred the ammonia-dominated clarified effluent (Chapter 4). Due to cellular respiration and oxidation of organic material in the carbonaceous removal reactor, the clarified effluent is also rich in carbon dioxide.

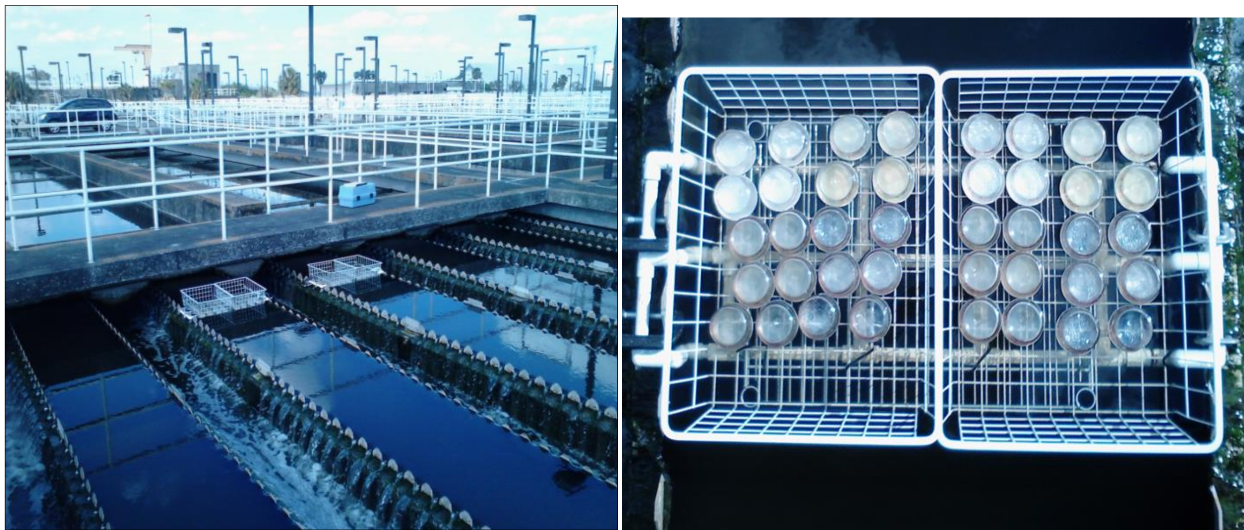

Figure 6.2. Site location of experimental set up in the activated sludge clarifier at the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL.

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Table 6.1. Experimental design of series in the field study.

Reference Tag	Porosity	Membrane Material	Growth Medium
NY*	7 µm	NY	tap water
PVDF*	40 kDa	PVDF	tap water
RC*	12-14 kDa	RC	tap water
NY-B	7 µm	NY	tap water
PVDF-B	40 kDa	PVDF	tap water
RC-B	12-14 kDa	RC	tap water
B-WW	n/a	n/a (lids)	wastewater
B-TW	n/a	n/a(lids)	tap water
C-WW*	n/a	n/a(lids)	wastewater
C-TW*	n/a	n/a (lids)	tap water

The '*' symbol indicates which of the series were inoculated with algae. NY = nylon; PVDF = polyvinylidene fluoride; RC = regenerated cellulose; B=blank; C=control.

6.2.3 Analytical Methods

The initial carbon dioxide concentration (OxyGuard CO2, OxyGuard International AS, Denmark), pH (Oakton Instruments, Vernon Hills, IL, USA), dissolved oxygen concentration and conductivity (Vernier Instruments, Beaverton, OR, USA), and optical density at 680 and 750 nm (HACH spectrophotometer DR/4000, Loveland, CO, USA) of each jar were measured prior to deployment. The dry weight (Standard Methods 2540D with an ammonia bicarbonate rinse recommended by Zhu & Lee (1997)) was measured in representative jars prepared identically to those deployed, as to not alter the starting volume of study jars. Every five days, jars were collected from the clarifier, and membrane lids were replaced with airtight lids immediately after extraction. Jars and membrane lids were transported in cold, dark storage to the laboratory, where all parameters were measured the same day. Jars remained in cold dark storage in the laboratory until microscope investigations using a hemacytometer were conducted within one day of sampling.

The air temperature, photosynthetically action radiation (PAR), and light intensity at the experimental site were monitored with HOBO data logging equipment (Onset Computer

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Corporation, Bourne, MA, USA) every fifteen minutes during the study period. Dissolved carbon dioxide concentration (OxyGuard CO2, OxyGuard International AS, Denmark), pH, dissolved oxygen concentration, water flow, and conductivity (Vernier Instruments, Beaverton, OR, USA) and temperature at the experimental site were periodically measured throughout the study. Site conditions are summarized in Table 6.2.

The specific growth rate and length of exponential growth phase were calculated as described in Chapter 5. The optical density at 680 nm was measured to estimate the chlorophyll content of the algal cultures throughout the experiment. Chlorophyll content can decrease with growth limitations, such as nitrogen starvation, or at certain growth phases, such as stationary or late-stationary growth phases (Griffiths et al., 2011). Because chlorophyll content has been shown to vary with environmental conditions and stages of the growth cycle (Griffiths et al., 2011), the optical density of the culture was also taken at 750 nm, a wavelength outside the range of any algal pigments but that measures the presence of cells. The ratio between the two wavelengths was used to estimate the chlorophyll content of the algal culture at different stages of the experiment, calculated as shown in Equation 1.

$$(OD_{680}-OD_{750})/OD_{750} = CR \qquad \text{Eq. 6.1}$$

where OD_{680} and OD_{750} are the optical density (as relative absorbance units) of the cultures at 680 nm and 750 nm, respectively, and CR is the chlorophyll ratio.

6.3 Results and Discussion

6.3.1 Final Yield

Culture cell density is important for downstream dewatering processes, where the target solids content at the harvest, slurry, and cake phase is 0.02-0.06%, 2-7%, and 15-20%, respectively (Uduman et al., 2010). The higher the cell density at the start of the dewatering

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Table 6.2. Site conditions during the ICARUS field study.

Parameter	Units	Average	Maximum	Minimum
Carbon dioxide	mg L^{-1}	87(14)	111	63
Dissolved oxygen	mg L^{-1}	2.9(2.1)	7.4	1
pH	pH units	6.7(0.1)	7.1	6.6
Conductivity	µS cm^{-1}	2029(351)	2782	1395
Water temperature	°C	13.0(3.7)	19.4	8.3
Flow	m sec^{-1}	0.1(0.04)	0.166	0.013
Air temperature	°C	21.6(4.3)	31.9	9.0
PAR	µE	395	2554	1.2
Light intensity	W m^{-2}	202	1277	0.6

Standard deviations are shown in parentheses. Water quality parameters are the result of grab samples, whereas atmospheric conditions are the result of continuously logged data. PAR=photosynthetically active radiation.

process (i.e., closer to harvest), the less energy or other inputs (i.e., coagulants) may be needed to reach the target solids content. The C-WW series was the first to surpass the 0.02% solids target on Day 5, but growth stagnated and the solids content only increased to 0.4% by the end of the trial. The RC, PVDF, and NY series were near the target on Day 5, and well surpassed the target range by Day 10 with an average solids content of 0.10%, 0.09%, and 0.08%, respectively. In fact, by Day 20, the RC series had an average solids content more than10x that of the target (0.29%). The NY (0.85%) and PVDF (1.06%) series had an average solids content more than 40x higher than the target harvest range (Figure 6.3).

Although the algal culture is not quite dense enough to be considered a slurry (2-7% solids), the geometry of the photobioreactor and the environmental conditions have not yet been optimized. Field study results already well surpass those of laboratory conditions (Chapter 5), suggesting that better design should further improve system performance.

Due to the configuration of the jars and quiescent internal culture, most of the algae biomass settled on the membrane, which was then scraped back into the liquid volume of the jar prior to measuring final yield. The algae cake that formed was a thick slurry, and if scraped and measured directly would have a much higher solids content than the resuspended yields. Scale up

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configurations of ICARUS should take advantage of gravity settling and passive dewatering, and both mechanisms should be investigated in further studies.

Similar trends are noted in the cell density of ICARUS series. By Day 10, all ICARUS series had surpassed the cell density and mass yield of the control and blank series (Table 6.3). The final cell density of the inoculated ICARUS series was well above the final cell density of any other series, including the NY-B series, which had a comparable density due to invasion of endemic species. The PVDF (10.6 g L^{-1}) and NY (8.45 g L^{-1}) series achieved a cell density that was more than double the 4 g L^{-1} typically seen in photobioreactors (Chisti, 2007). Higher cell densities were the result of sustained exponential growth, but also of passive dewatering that concentrated the cultures. Although reaching the target cell density occurred on Day 20, it is expected that optimizing reactor geometry, inoculation density, and harvest regime will boost productivity and decrease the time to achieve target densities.

Though reaching 7x the density of the C-WW series, the RC series did not nearly achieve the density of the other ICARUS series. Based on previous laboratory-scale tests (Chapter 5), it was expected that the PVDF series would achieve the highest productivity, with the NY and RC series following. This outcome was indeed observed in the in field trials. The C-WW series, without access to atmospheric carbon dioxide, achieved a similar cell density (0.25 g L^{-1}) after five days to open system suspended series conducted in the laboratory (Chapter 5). The final mass yield of the ten experimental series followed a similar pattern as cell density.

The sharp increase in the cell density of the ICARUS series in the last five days of the experiment is partly due to water loss during this period (Figure 6.3A,D). Mass yield, which accounts for water loss, shows a steadier growth throughout the study (Figure 6.3B,E). When daily mass yield is normalized to the initial mass, the exponential growth phase becomes more

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apparent (Figures 6.2C,F). The specific growth rate of each series based on normalized mass yield during each trial quartile is shown in Table 6.3. The specific growth rates of the ICARUS series, B-WW, C-WW, and NY-B are in the range previously reported for *Chlorella* sp (Chapter 5; Zhu et al., 2013; He et al., 2013; Ruiz et al., 2011), though on the lower end. This outcome could be due to suboptimal reactor geometry, poor mixing within ICARUS, or limited nutrient or gas availability. The high growth rate and yield in the NY-B series is due to the invasion of endemic species and is not reflective of a *C. sorokiniana* culture.

The objective of large-scale cultivation is to sustain exponential growth to optimize productivity, and in some cases, nutrient removal; scale-up configurations should therefore account for the growth phase in the harvesting regime. The exponential growth phase was sustained up to ten days in the RC, PVDF, NY, NY-B, and B-WW series. The duration was more than double that achieved in previous laboratory experiments testing the same series (Chapter 5). The exponential phase lasted five days in the C-WW series, which although twice as long as laboratory trials (Chapter 5), was half that of ICARUS series in field studies. The longer exponential phase in the field is likely attributed to the higher availability of carbon dioxide and nutrients compared to laboratory conditions. Similarly, the longer exponential growth phase of ICARUS series compared to the C-WW series was likely due to the increased availability of carbon dioxide and nutrients, and the ability to expel wastes (such as dissolved oxygen).

The chlorophyll content of microalgae can change with environmental conditions and can be used as an indicator of growth limitations (Rosko & Rachlin, 1977; Boussiba et al., 1999; He et al., 2013). The chlorophyll ratio (Eq. 1) is used as a measure of chlorophyll content, and changes in the ratio may indicate a growth limitation, such as low nitrogen or high light intensity. The chlorophyll ratio of both the C-WW and C-TW series were the same on Day 0; however, as

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the C-WW series grew, the ratio increased through the exponential growth phase, then declined as growth was limited. The ratio of the C-TW series rapidly declined after Day 0, due to nutrient limitations in the tap water. Similarly, the ratio of the B-WW series increased during exponential growth phase, then decreased and steadied during the stationary phase.

Table 6.3. Average final yield after 20 days of growth in each series and specific growth rate (normalized to first order kinetics) at sampling intervals throughout the study period.

Series	Final Yield				Specific Growth Rate			
	Dry Weight ($g\ L^{-1}$)	Mass Yield (g)	Areal Density ($g\ m^{-2}$)	Percent Solids (%)	Day 0-5	Day 5-10	Day 10-15	Day 15-20
RC	2.87	0.082	13.23	0.287	0.37	0.26	0.03	-0.05
RC-B	0.21	0.010	1.61	0.021	0.00	0.13	-0.01	0.38
PVDF	10.6	0.176	28.31	1.058	0.37	0.29	0.10	0.11
PVDF-B	0.03	0.002	0.29	0.003	0.00	0.14	-0.02	0.04
NY	8.45	0.114	18.33	0.845	0.30	0.31	0.13	0.05
NY-B	5.55	0.073	11.79	0.555	-0.06	0.53	0.22	0.21
B-Tap	0.01	0.001	0.15	0.001	0.00	-0.09	0.15	-0.03
B-WW	0.35	0.027	4.38	0.035	0.49	0.30	-0.01	0.07
C-Tap	0.05	0.004	0.69	0.005	-0.09	0.08	0.12	0.02
C-WW	0.38	0.031	4.92	0.038	0.52	0.00	0.06	0.02

The chlorophyll ratio of the inoculated ICARUS series increased in the first five days, then remained steady during the exponential growth phase (Figure 6.4). The chlorophyll ratios continued to be steady, with a slight decline, between Days 15 and 20 (with more dramatic decreases in the RC and NY on Day 15, which may be attributed to sampling error). Although the ratios were not increasing, there was not a significant drop by the end of the study period, as seen with the control series, which may indicate that there was not a severe growth limitation during the study period. The slight increase in the chlorophyll ratio of the non-inoculated ICARUS series is most likely due to endemic invasion, which is verified in Figures 6.3 and 6.11.

6.3.2 Movement of Constituents

Conductivity was measured as a composite surrogate of all target species (i.e., nutrients) to ensure that dissolved constituents were able to pass through the membrane. Historical

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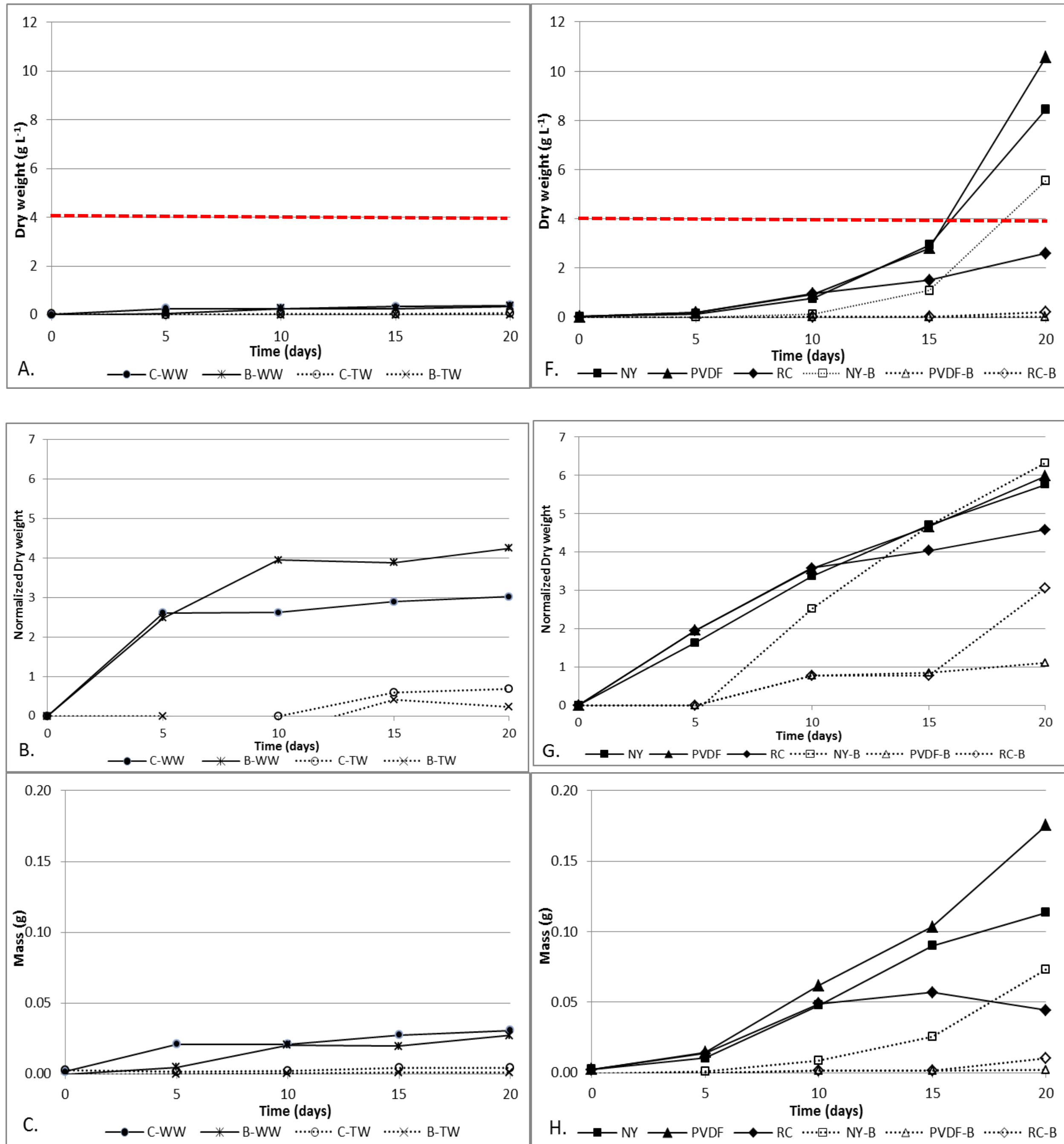

Figure 6.3. Final yield as cell density, mass, and percent solids in the control (A-E) and ICARUS (F-J) series as raw data and normalized to the initial condition. The red dotted lines in (A) and (D) depict a target concentration of 4 g L^{-1}, a typical concentration of closed photobioreactors.

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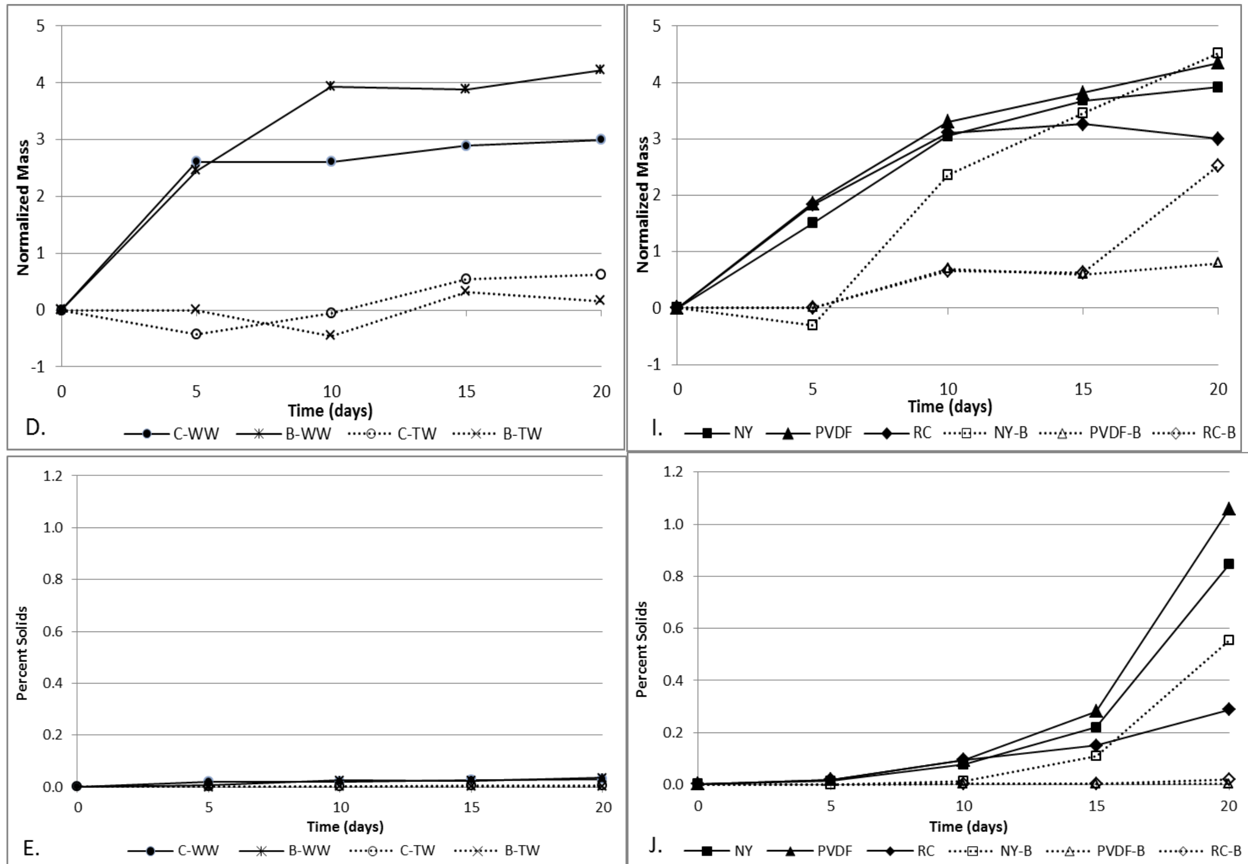

Figure 6.3 (Continued)

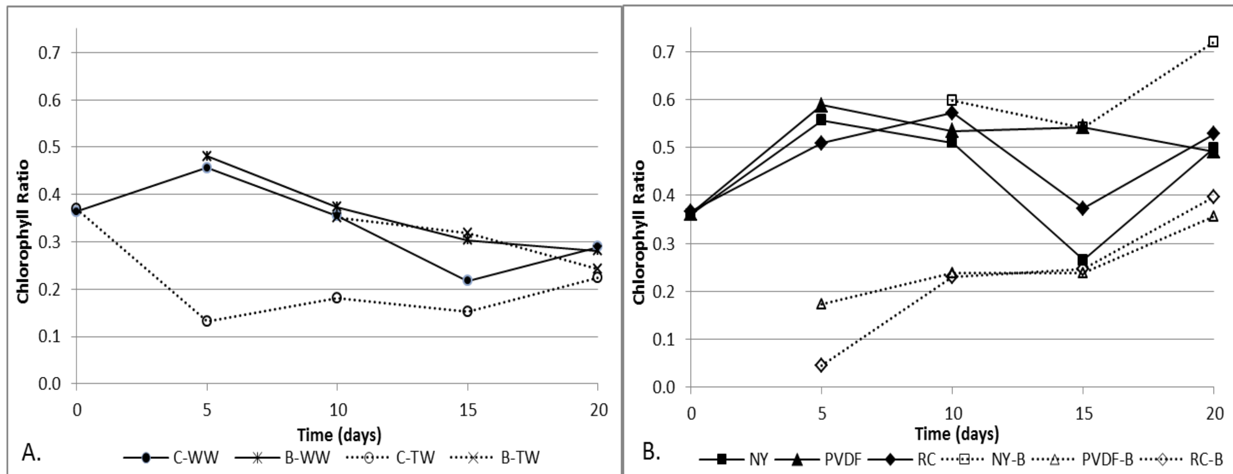

Figure 6.4. Chlorophyll ratio of each experimental series throughout the duration of the study.

ammonia and phosphate (Chapter 3) concentrations at the upwell clarifiers was previously determined to be 29 mg L^{-1} and 7 mg L^{-1}, respectively, with an N:P ratio of 4.1:1, which is well

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below that recommended by the Redfield ratio (16:1) (Redfield, 1958). These conditions indicate adequate nutrients and carbon dioxide are available at this stage of the treatment process to cultivate algae (Drexler et al., 2014; Chapter 4; Chapter 5).

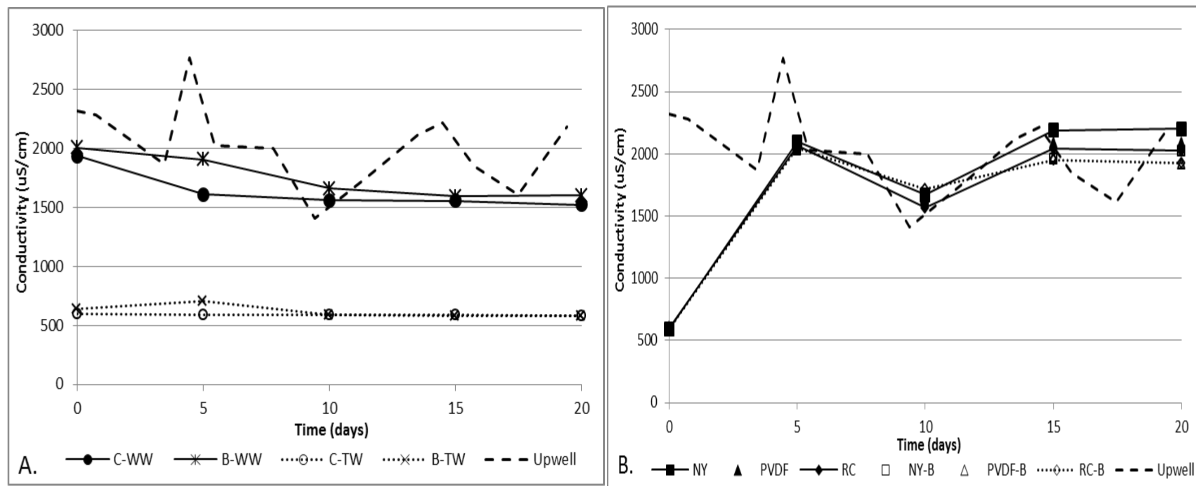

Figure 6.5. Conductivity in each series compared to the conductivity in the upwell clarifier weir.

The conductivity of ICARUS series followed the trends seen in the upwell weir of the clarifier, suggesting that dissolved constituents easily passed across the membrane and became available to the algal culture (Figure 6.5). Although it is possible that conductivity could remain constant while nutrients were depleted, it is unlikely. In fact, conductivity decreased in the C-WW and B-WW closed system series with increasing algal growth, coinciding with depleting nutrients. If productivity outpaced diffusion in the ICARUS series, a decrease in conductivity would have been expected in these series as well. However, conductivity remained constant. Furthermore, the ICARUS series did not crash at the end of the exponential growth phase, as would be expected if nutrients were severely depleted. As expected, the conductivity in C-TW and B-TW series remained steady throughout the study, as minimal to no growth occurred in these series. In summary, conductivity appeared to be a suitable measure of nutrient passage,

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demonstrating that nutrient availability was sufficient throughout the study period for ICARUS series.

Dissolved oxygen (DO) concentration can indicate photosynthetic activity in microalgal cultures, and elevated DO levels can inhibit exponential growth (Shelp & Canvin, 1980)effluent was 13°C, yielding an oxygen saturation concentration of approximately 10.5 mg L^{-1} (U.S. EPA, accessed 2014). The average DO of the inoculated ICARUS series (7.9 m L^{-1}) was well below saturation throughout the duration of the study, but maintained a concentration well above that of the surrounding wastewater (3.3 mg L^{-1}) (Figure 6.6). Because the DO concentration remained relatively constant in ICARUS series throughout the study and increases in DO concentration did not coincide with the end of the exponential growth phase, DO concentration was not considered a major limiting factor. However, a previous study using *C. pyrendoisa* detected an irreversible 24% photosynthetic inhibition when oxygen levels were 100% saturated and a reversible 12% inhibition at 50% saturation (Shelp & Canvin, 1980). Although the DO concentration did not reach 100% saturation, it did reach above 50%. Scale-up configurations should optimize oxygen removal to prevent photosynthetic inhibition.

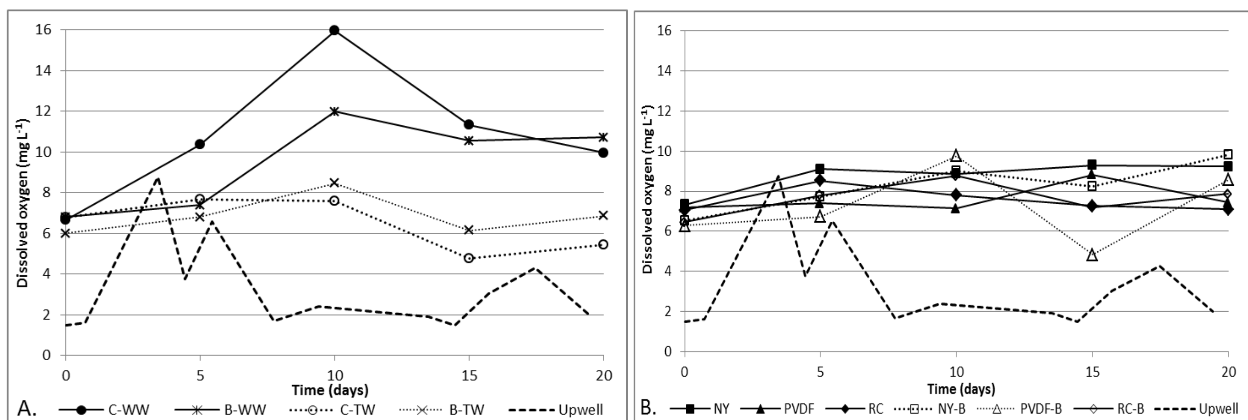

Figure 6.6. Dissolved oxygen concentration of each experimental series throughout the duration of the study. The thick dotted line shows the background dissolved oxygen concentration in the upwell weir of the clarifier.

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It was expected that DO would quickly equilibrate with the clarified effluent, effectively diffusing outwards across ICARUS membranes. However, oxygen produced via photosynthesis outside the ICARUS jars (i.e., attached algal growth on the metal frames supporting the experiment and the external side of the membranes) potentially decreased the concentration gradient directly below and at the membrane surface. Because field DO readings were taken outside the metal frames, this potential increase directly below the jars was not captured. The reduced concentration gradient may have slowed the DO exiting the jar, which may also have contributed to water loss in the ICARUS series (discussed further in Section 6.3.3). In scale up configurations, maintaining a steep DO gradient with surrounding wastewater may be important to prevent inhibition, but could affect passive dewatering efforts prior to harvesting.

As expected, the DO concentration increased at the beginning of the study in the C-WW and B-WW series, due to photosynthetic activity, but fell as algal growth declined. The DO concentration of the C-WW series reached 16.0 mg L^{-1}, nearly 160% saturation, on Day 10. The increase coincided with the end of the exponential growth phase in this series, suggesting high DO concentration may have inhibited growth. The B-TW and C-TW series showed a slight decrease in DO concentration after Day 10, possibly due to respiration.

Carbon dioxide is a necessary input for photosynthesis, and its dissolved concentration can also influence the pH of growth media. The average carbon dioxide concentration in the upwell clarifier was 87 mg L^{-1}, which was well above the estimated saturation point of carbon dioxide (approximately 0.84 mg L^{-1}; calculations regarding carbon dioxide saturation are shown in Appendix C). The high concentration is due to microbial respiration in the pure oxygen carbonaceous treatment step occurring just prior to the clarifier. Although other activated sludge effluents would also have carbon dioxide concentration available due to respiration, the unique

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layout of the treatment plant used in this study grants access to water highly enriched with carbon dioxide unavailable in conventional activated sludge biological process schemes. Similar opportunities should be mined in other plant layouts for scale-up configurations.

The carbon dioxide concentration in the C-WW series declined sharply in the first five days (Figure 6.7), which coincided with the end of the exponential growth phase and sharp increase in culture pH. The carbon dioxide concentration in the B-WW series decreased at a slower rate, reaching zero at Day 10, which coincided with the longer exponential growth phase of this series. Although the C-WW and B-WW series ended with the same mass yield and cell density, the algal inoculant in the C-WW series speeded up the productivity, but also depleted resources faster. The carbon dioxide limitation and subsequent elevation of pH was likely a limiting growth factor in the closed system series. The carbon dioxide concentration increased in the C-TW and B-TW series in the latter half of the study, most likely due to respiration, which coordinated with a decline in the dissolved oxygen concentration during the same time frame.

The carbon dioxide concentration in the ICARUS series increased in the first five days as the jars equilibrated with the surrounding wastewater, but decreased through the exponential growth phase and beyond (Figure 6.7). In no case did the ICARUS series completely equilibrate with the clarified effluent, which was unexpected. The ICARUS series not inoculated with algae, and therefore not removing carbon dioxide internally, equilibrated at approximately 40 mg L^{-1} with surrounding effluent, signaling that the low internal concentration may not only be due to photosynthetic uptake by ICARUS cultures. The slow equilibration is probably due to the attached growth (i.e., on the metal frames and external membrane surface) outside the ICARUS jars utilizing carbon dioxide during photosynthesis, thereby decreasing the concentration gradient in the effluent immediately below the jars and at the membrane interface.

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The carbon dioxide concentration on Days 15 and 20 decreased with increasing pore size of the inoculated series. The RC series had the lowest productivity and the highest dissolved carbon dioxide concentration. The PVDF series had a higher cell density and mass yield than the NY series, which should have led to lower carbon dioxide concentration in the PVDF series. However, the NY series had higher external biofilm mass, which could have been utilizing carbon dioxide at the membrane interface and slowing the rate of exchange. In fact, the carbon dioxide concentration slightly increased in the ICARUS treatments from Day 15 to Day 20, when the external biofilm was decreasing (see Section 6.3.4). Lower biofilm on the outside of the jar may have improved the concentration gradient, allowing more carbon dioxide to enter the jars.

The carbon dioxide concentration did not approach zero in any ICARUS series, suggesting that carbon dioxide was still able to diffuse even if at a slower rate than expected. It is also possible that carbon dioxide was actually diffusing more rapidly than could be assessed by a single grab sample, but was being taken up by the algae cake on the internal side of the membrane before it could dissolve into the ICARUS medium. In this case, carbon dioxide would not be limiting, as the carbon dioxide concentration that penetrated the algae cake would be excess carbon dioxide stored as a reserve. Scale-up studies should devise means to minimize the external attached growth to ensure a steeper concentration gradient and more rapid delivery of carbon dioxide to the algal culture.

Due to changes in alkalinity (Uusitalo, 1996) as a result of carbon dioxide and nutrient uptake, algae cultivation typically elevates pH. Microalgae can be sensitive to large pH swings, and *Chlorella* sp. in particular have been shown to prefer a pH range between 6-8 (Rachlin & Grosso, 1991). Although the pH in the ICARUS series remained slightly higher than the clarified

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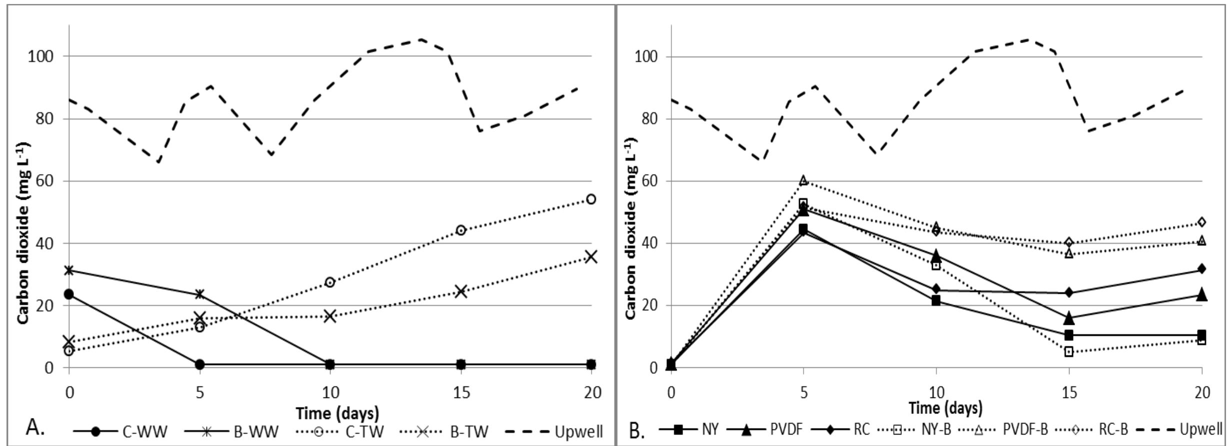

Figure 6.7. Carbon dioxide concentration in experimental series throughout the duration of the study compared to the carbon dioxide concentration in the clarified effluent.

effluent, all series remained in the ideal range of 6-8 (average of 7.63) (Figure 6.8), despite decreasing carbon dioxide concentration. There was little difference between inoculated and non-inoculated ICARUS series, implying algae growth had limited influence on pH regulation in the open systems.

The pH of the C-WW series increased from 7.6 to 9.8 during the exponential growth phase, before settling at 9.8. The B-WW series increased sharply from Day 5 (7.5) to Day 10 (10.5) before flattening around 10.5, which also coincided with the exponential growth phase. Similar results were seen in laboratory experiments that were open to the atmosphere (Chapter 5), signaling that increased growth in ICARUS is due primarily to carbon dioxide exchange with wastewater and not the atmosphere. As expected, the pH of the C-TW and B-TW series did not change much throughout the study. In typical full-scale algae cultivation, suspended cultures would likely require external inputs (i.e., carbon dioxide or buffering agents) to maintain pH in an ideal range, since carbon dioxide depletion in suspended cultures both open and closed to the atmosphere quickly exhaust the dissolved supply. In contrast, ICARUS cultures would not require additional inputs, as long as an appropriate medium was chosen (such as biologically

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treated wastewater), improving the economic and environmental feasibility of ICARUS over other cultivation configurations.

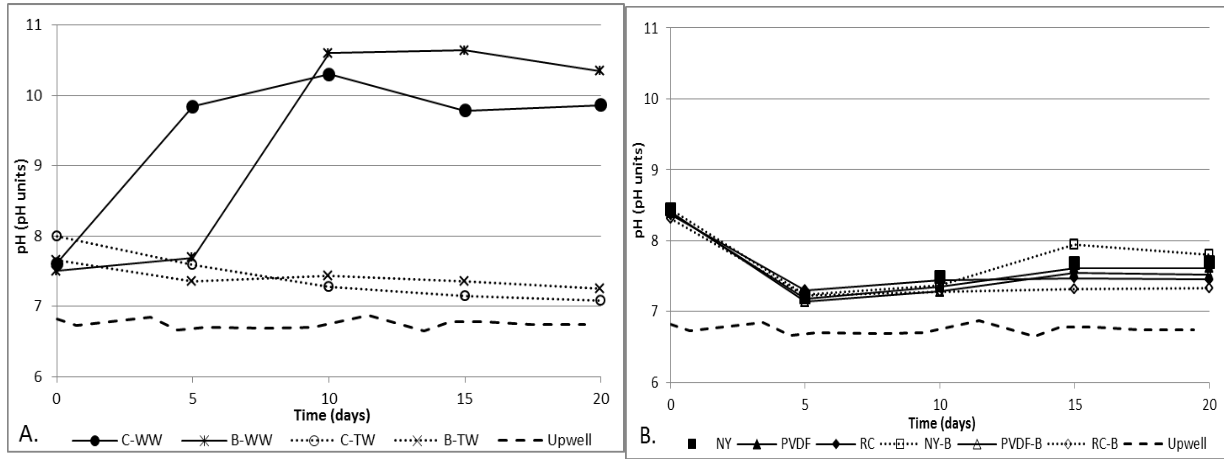

Figure 6.8. The pH of experimental series throughout the duration of the study compared to the pH of the upwell clarifier weir.

It should be noted that light limitation in the ICARUS culture could have limited the exponential growth phase, even though adequate PAR was available during the study. The internal medium was not mixed in any series, leaving algae cultures to settle on the bottom of the jars. As the cell density increased, especially in the ICARUS series, it would be more difficult for light to penetrate the algae cake. Unfortunately, light limitation at this resolution was not measured, but should be considered in future scale-up configurations. If the algae cultures were mixed and kept in suspension, the potential light limitation may be reduced. However, gravity settling of the cultures may prove to be an important harvesting mechanism for scale-up configurations, so trade-offs between mixing the culture to enhance light delivery and allowing the culture to settle for harvesting should be considered.

6.3.3 Volume Loss

Volume loss could be caused by many factors, including membrane porosity, thickness, permeability, material, oxygen produced via photosynthetic activity, and external biofilm

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thickness. Oxygen production was estimated using the empirical formula and stoichiometric relationships in Drexler et al. (2014). These factors and their interactions were analyzed with a generalized linear model (GLM) (R Commander, Gaussian distribution) to determine which factors or interactions had the most significant effect on the variability of water loss in the ICARUS series. Of the factors measured, oxygen produced and the interaction of oxygen production and permeability had the most significant ($p<0.01$) effect on water loss.

As passive water loss may prove to be an important advantage of a closed ICARUS configuration, where cultures could be concentrated prior to harvest, a predictive model should be developed that accounts for algal biomass production, photosynthetic oxygen production, membrane permeability, and reactor configuration. Predicting the timing of water loss will optimize harvesting regimes by taking advantage of the passive dewatering mechanism, harvesting algae when the cell density is highest.

The estimated amount of oxygen produced by algal productivity in this study was compared to the actual water loss measured. Results are shown in Figure 6.9 as a linear regression ($r^2=0.43$) and overlaid with measured results. In most cases, the model overpredicted volume loss, i.e., more oxygen is produced than water is leaving the system. Therefore, in future iterations of a water loss model, other mechanisms (i.e., oxygen entering solution, oxygen passing across the membrane, oxygen leaving with displaced water, and amount of oxygen stored in the headspace) should be considered. (Further discussion of water loss and recommendations for building a model to predict water loss due to photosynthetic oxygen production can be found in Appendix C).

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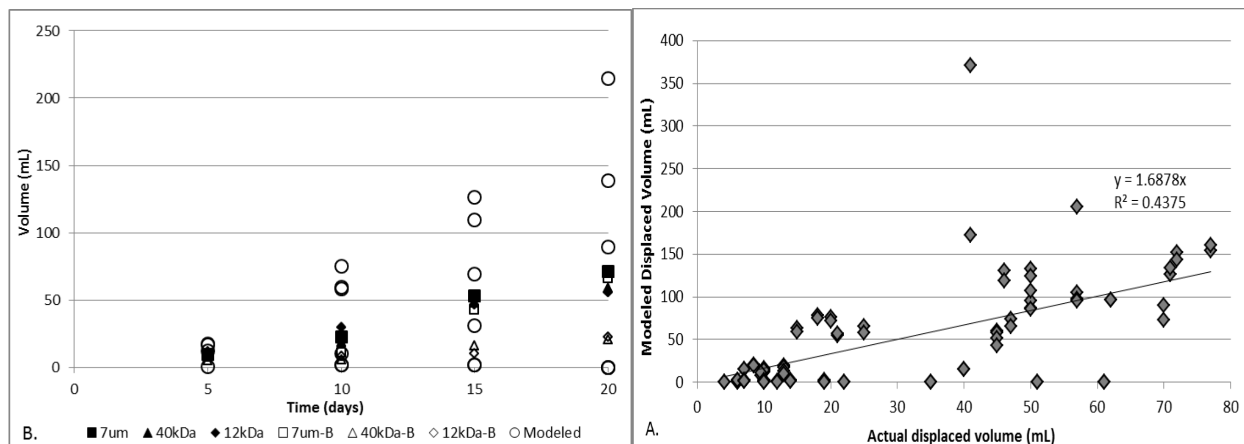

Figure 6.9 Results of preliminary water loss model. (A.) Linear regression model comparing modeled water loss to measured water loss and (B.) model prediction overlaid on measured results of water loss.

6.3.4 Biofilm Formation External to Membrane

An attached biofilm of predominately endemic filamentous algae grew on the external side of all the ICARUS membranes. The biofilm mass of the RC-B, PVDF, and NY series peaked on Day 10, whereas the biofilm mass of the RC and NY-B series peaked on Day 15 (Figure 6.10). All series were declining by Day 20, except the PVDF-B series, which continued to gain biomass. The decline in biofilm mass could be due to biological or physical mechanisms. As the biofilm grew larger, it may have reached a concentration of organisms that attracted a critical mass of grazers. As the increasing population of grazers fed on the biofilm, the biofilm mass decreased. Alternatively, as the biofilm became thicker it also became heavier, making it easier for it to slough off the membrane if agitated.

Biofilm formation on the external side of ICARUS is an important subject of investigation for scale up activities. Attached growth can limit the exchange of nutrients or gases passing across the membrane, decreasing the availability of these constituents to the microalgal culture and lowering the concentration gradient at the membrane surface. Attached biofilm can

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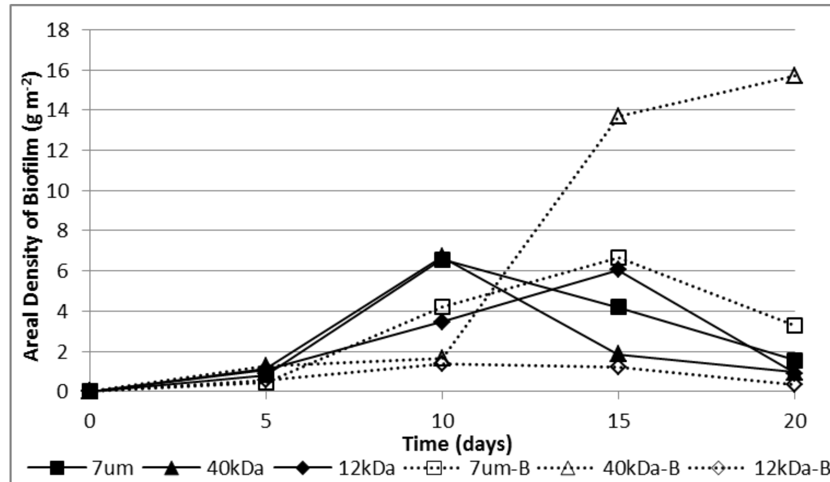

Figure 6.10. Progression of biofilm growth attached to the external side of ICARUS membranes.

also hinder harvesting efforts if the external biofilm contaminates the target culture. Also, as in the case with the NY series, once a filamentous algae establishes itself on the outside of the membrane, it can more easily penetrate the membrane pores and invade the ICARUS culture. The dynamics of the duplex biofilm system (with bacteria on the outside and algae on the inside) should be studied and modeled, as biofilms and fouling could greatly affect algal performance in scale-up configurations.

6.3.5 Invasive and Grazing Organisms

The ICARUS series had various success in maintaining a pure *Chlorella* sp. culture through the duration of the study (Figure 6.11). The PVDF and RC series maintained predominately a monoculture in the inoculated jars, with few grazers or invasive species present when examined under the microscope. Few invasive organisms were present in the PVDF-B and RC-B series as well, which is corroborated with the minimal growth of these series. The NY series allowed significant endemic species, likely including grazing rotifers, to pass across the membrane. Both the NY and NY-B series achieved high yields, but the resulting culture was not

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predominantly *Chlorella* sp. Community diversity increased throughout the duration of the experiment, with filamentous algae dominating along with *C. sorokiniana* by Day 20.

If the end goal of algal cultivation is to increase biomass production for nutrient removal or to use the biomass for less specific end-uses (i.e., digestion or fertilizer) then maintaining a monoculture may not be a priority (Chapter 4). When using the appropriate porosity, ICARUS can protect an algae culture from grazers, which is important for preventing culture collapse, maintaining a monoculture when using wastwater as a feedstock, and keeping the target culture from leaking through the membrane. Typical suspended cultures would not have the added advantage of the physical membrane barrier afforded in ICARUS, leaving suspended cultures vulnerable to grazers and contamination.

The PVDF and NY membranes were easier to clean and were more durable and easy to handle than the RC membrane. The RC membrane ripped easily, buckled when whetted, and occasionally slid out from the gasket, compromising culture integrity. The PVDF and NY membranes held their shape and did not slide from the gasket. However, the large pore size of the NY membrane allowed significant invasive growth in the jars, which also compromised the integrity of the *Chlorella* sp. monoculture. Based on material properties and ease of handling, the PVDF membrane in the pore size tested would be recommended for scale-up.

6.4 Conclusion

ICARUS series, and in particular the PVDF series, had a higher biomass productivity (solids content, mass yield, and cell density) than non-ICARUS suspended series. The higher productivity is due to a longer exponential growth phase and increased nutrient and carbon dioxide availability compared to closed series. As nutrients and carbon dioxide did not appear

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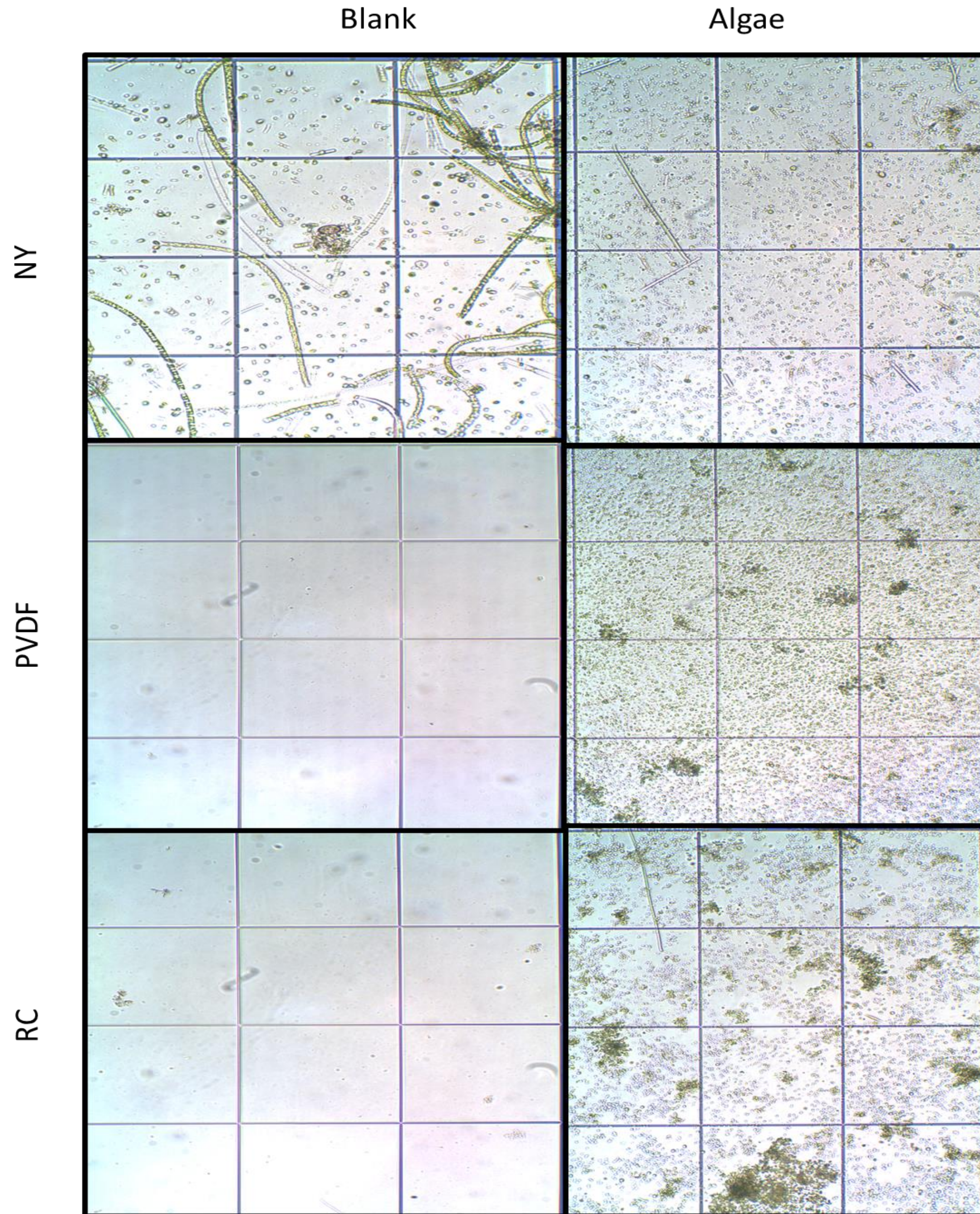

Figure 6.11. Community diversity in the ICARUS series on Day 20 of the experimental period. All microscope photographs were taken at 5x.

limiting, growth limitations may be due to elevated dissolved oxygen concentrations or light availability in the algae cake. External attached growth is a challenge for optimizing concentration gradients, and external biofilm growth should be modeled for future scale-up configurations. Membrane porosity had a significant effect on community dynamics within the ICARUS series, with larger pore sizes leading to contamination by endemic species.

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Chapter 7: Conclusions and Recommendations

7.1 Conclusion

Global populations are projected to increase, even as supply of fossil fuels and other resources decline. Meanwhile, demand for energy and resources will not subside, which will require sustainable solutions for renewable energy. Microalgae are one potential part of a renewable energy portfolio, a solution that can simultaneously recycle waste (i.e., wastewater and industrial flue gases) while addressing global energy and resource shortages. However, cultivation, harvesting, and processing methods must be improved to decrease the environmental footprint and increase the economic competitiveness of large-scale microalgal cultivation. Membrane applications, in particular passive configurations, may be used to address many current bottlenecks, such as cell density, nutrient delivery, and protection from grazers.

This body of work provides a foundational proof-of-concept of a passive membrane cultivation system – the Isolated Cultivation of Algal Resource Utilizing Selectivity (ICARUS). Preliminary batch studies lent insight into species selection, effective types of wastewater for cultivation, and potential problems that may be encountered in scale-up (i.e., culture contamination, competition, and predation). Laboratory and field studies demonstrated that with certain membrane types ICARUS cultivation yields a higher biomass density and productivity than an algal culture suspended directly in growth medium. The solids content of ICARUS cultures was also higher than that of non-ICARUS suspended cultures, which is promising for

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scale-up harvesting and dewatering efforts. It is expected that by optimizing photobioreactor geometry and harvesting schedules, the solids content can be increased, which may eliminate subsequent dewatering interventions now currently necessary.

Numerous membrane types were tested, and the effects of membrane characteristics on productivity were investigated. Many variables (i.e., permeability, porosity, and material) and their interactions with other variables affected culture density, whereas permeability was the main contributor to variability in mass productivity. Productivity peaked with a pore range of 50-1000kDa, which was also an ideal range for maintaining the integrity of an algal monoculture. Membranes above and below this range also achieved high productivity, but often at the expense of culture homogeneity. Based on the results of this work, it would be recommended to use a PVDF material in the pore range of 40-1000kDa for scale-up applications.

The current studies were limited to four membrane materials, but other membrane types could be considered. Further investigation of the more economical cloth textile membranes may improve capital investment in scale-up studies, if pore size can be reduced (or fouling can be controlled to reduce pore size and distribution). Regenerated cellulose would not be recommended for scale-up applications, as the material was quite fragile, slippery, and difficult to maintain in place. Cellulose acetate and PVDF membranes were both durable and easy to handle and clean. Cellulose acetate was not tested in field conditions but could be in future studies. Other materials typically used for MF/UF filtration, such as polyacrylonitrile (PAN), polyether sulfone (PES) and polysulfone (PS), may be more suitable than conventional dialysis materials such as regenerated cellulose, due to durability. These materials could be investigated in future field studies.

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Regardless of porosity, adequate passage of nutrients and gases occurred across all membranes tested. ICARUS cultures maintained a lower pH range than suspended cultures, which was due to carbon dioxide exchange with a larger volume of wastewater. Growth limitations in the batch tests may have been due to low carbon dioxide availability (and therefore elevated pH), whereas limitations in the field may be attributed to low light penetrating into the algae cake layer. Gas exchange was slower in the field than expected, which may have been due to attached growth on the external side of the membrane and experimental apparatus. Field studies suggest that oxygen produced in the closed ICARUS system displaced the growth medium, passively dewatering the algae culture.

7.2 Research Limitations and Recommendations for Future Research

The literature review identified several research thrusts that should be investigated to fill current research gaps in the algal industry. The most prominent bottlenecks are improving the economics of harvesting and dewatering; exploiting renewable sources of nutrients, water, and carbon dioxide; and minimizing culture collapse by predation. The experiments detailed in this body of work addressed each of these challenges, but further research must be conducted to fully understand the exchange mechanisms of the proposed method and adequately prepare for system scale-up.

The first study (described in Chapter 4) investigated the use of wastewater as a renewable source of nutrients, water, and carbon dioxide while also assessing the productivity of native communities alone and native communities augmented with algal monocultures. This study was limited in that the initial algal communities were not genetically characterized nor was the community change genetically tracked throughout the study. Further studies on algal community dynamics, especially in the context of wastewater treatment, may be beneficial if native species

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are to be cultivated in scale-up. Shifts in community populations were identified, but could not be quantified with the methods employed. Future studies could use metagenomics to better quantify community shifts during cultivation. Similarly, community shifts may have been due to the batch conditions of the experiment, and different results may have been realized in a continuous system. ICARUS may better track community shifts, as the initial algal seed will remain separate from the wastewater. Furthermore, ICARUS would allow for a continuous flow of nutrients outside the membrane, while maintaining the original culture in quasi-batch conditions. As native populations have been shown to be just as productive and effective at nutrient removal than augmented monocultures, further studies of native populations using ICARUS may be useful for scale-up operations when monocultures are not necessary. Further studies could investigate species competition or screen native species for potential desirable characteristics (tolerance to high nutrient loads, large pH swings, low temperature ranges, etc.).

The second study (described in Chapter 5) involved the laboratory screening of eleven types of membranes to be used in ICARUS scale-up studies and compared biomass productivity to that of suspended cultures. The study was limited in the number and type of membranes that could logistically be screened. Other materials or pore sizes (such as other cloth textiles with a tighter pore size) may be considered in future studies. Although membrane characteristics investigated in this study showed significant contribution to variability in productivity, other membrane characteristics (such as surface roughness, charge, or hydrophobicity) should be considered in future GLM analyses. A predictive model of biomass productivity based on membrane characteristics (assuming environmental conditions are equal) could be developed if further membrane types or characteristics were included. This model could be useful in scale-up

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designs. Results of lab experiments suggest that the mechanisms of gas and nutrient exchange, membrane fouling, and grazer dynamics should be investigated.

Select membranes from Chapter 5 were tested in ICARUS configuration in a wastewater clarifier in Chapter 6. Productivity and nutrient availability in ICARUS were compared to a control series. This study was limited in the number of replicates that could logistically be deployed for the extended study. If repeated, it would be recommended to have triplicate jars for each series and sampling day. At the time of experimental design, it was unknown when productivity would peak; extending the study beyond the 20 day period may reveal more insight into external biofilm dynamics and passive dewatering via oxygen production, and establish a theoretical limit to growth. Future studies should quantify the ability of ICARUS cultivation to maintain a monoculture in extended field conditions, including beyond the 20 day study. It may be useful for harvesting recommendations to determine if there is a threshold of time in which endemic species can be safely kept out. Passive dewatering may prove to be a powerful advantage of ICARUS and should be investigated in more detail in future field studies. Although many potential growth limitations were ruled out in the field study, the mechanisms limiting growth could not be definitively demonstrated. Light penetration through the algae cake should be investigated, as well as optimal submersion depth in the growth medium. The movement of individual nutrient species (i.e., nitrogen, phosphorous, vitamins) and/or toxins (i.e., algal wastes, EPS) across the membrane should also be investigated, rather than collectively measuring these constituents as conductivity. Ion selective electrodes (ISEs) or fine-tipped micro ISEs may be used for this purpose.

Biofouling is a major complication of membrane technology and continues to hinder membrane-based harvesting and dewatering efforts in the algal industry. Therefore, future

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studies should investigate the mechanisms of biofouling both on the internal side (presumably made up of algal foulants) and the external side (presumably made up of both algal and bacterial foulants). Specifically, studies could focus on the mechanisms (i.e., physical vs. biological) causing the external biofilm to decrease after Day 15 in the third study. Foulant composition could be measured using ATR-FTIR, SEM, or confocal microscope. Shotgun metagenomics could also be used to determine which microbes inhabit the biofilm and what type or metabolic processes they are undergoing. Characterizing the foulants on various membrane types may help with membrane selection for scale-up or determine an appropriate membrane cleaning regime and schedule.

The dynamic fouling layer may be a large contributor to the length of the exponential growth phase, as the biofilm layer appears to be limiting mass transfer of gases and can change the membrane properties. Ideally, scale-up configurations would not have to be concerned with an external fouling layer that limited the exchange of constituents, but in reality, it would be virtually impossible to eliminate all biofouling. Future studies should focus on the development of this biofilm layer and how to encourage growth that may be beneficial for cultivation. For example, if a larger pore size membrane was selected because it is more economical, developing a purposeful biofilm layer (in essence, a self-forming dynamic membrane layer) may aid in decreasing the pore size and distribution, thereby limiting invasion of endemic wastewater species. Undesirable biofouling could be minimized through reactor design (discussed further in Section 7.3).

The three studies identified and addressed the operational challenges of pH regulation and species invasion. ICARUS cultivation appeared to regulate culture pH better than a non-ICARUS suspended culture, due to gas exchange with surrounding wastewater. Maintaining a

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regulated pH may be important for integrating an algal photobioreactor into a wastewater treatment process as to not disturb the biological community in downstream treatment steps. However, the elevated pH may be useful if it occurs just before coagulation or flocculation. Elevated pH may also be used to provide favorable environmental conditions for algal species that can tolerate basic conditions in order deter growth of other invasive organisms that cannot.

The studies also showed that ICARUS could maintain a monoculture even in extended field conditions. Algae grown in wastewater are often slated for anaerobic digestion due to its heterogeneity upon harvest. However, biogas production is not particularly lucrative when compared to other value-added algal bioproducts, such as biodiesel. ICARUS allows for the homogenous cultivation of higher value algae crops while still taking advantage of the 'free' sources of nutrients, freshwater, and carbon dioxide.

Although the current body of research has laid the foundation for ICARUS, further research should be done to ensure that effective, efficient, and appropriate steps to scale-up are taken. The method promises high cell densities and improved productivities over conventional suspended growth, but the mechanisms determining growth have not yet been fully characterized. Current data shows promise for scaling-up, and recommendations for further studies have been clearly defined.

7.3 Scale Up Considerations

A major direction of future research should focus on the scale up of the system. Numerous potential ICARUS configurations have been filed in a provisional patent submitted to the US Patent and Trademark Office. Scale-up systems should first consider the goals of the project (i.e., biomass production, nutrient polishing, need for monoculture) to aid in membrane selection, species selection, and ICARUS configuration. For example, based on the results of this

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research, membrane pore sizes between 40 kDa - 0.1 µm would be recommended to maintain a monoculture. However, if the algal biomass was slated for anaerobic digestion (and therefore did not require homogeneity) a membrane material with a larger pore size and distribution could be used. Also, pore size and distribution may depend on the algal species cultivated; *Chlorella* sp. might leak through a membrane that would retain *Spirulina*.

Future designs should optimize reactor geometry based on site infrastructure conditions, inoculant density, and harvest regimes. The current studies were conducted in a rectangular upwell clarifier, where the configuration of the ICARUS reactor may look quite different than that deployed in a circular clarifier. Reactor depth would most likely not exceed 1 m in either case in scale-up configurations to maintain adequate light penetration through the culture. However, an ICARUS reactor could still be deployed in turbid water, such as an activated sludge basin, since the reactor floats on top and would not be affected by growth medium turbidity.

As the current studies took advantage of the unique layout of the HFCAWTP, which offered an ammonia and carbon dioxide rich clarified effluent for algal cultivation, scale up studies should also consider innovative placement in existing treatment streams. An ICARUS reactor (with a tighter NF membrane) may work in high-ammonia sidestream treatment, where mass transfer limitations across the membrane may dampen and artificially lower the ammonia concentration to a tolerable level for algal growth.

Lastly, scale-up designs should minimize external attached algal growth that may retard exchange at the membrane. In the field study, the ICARUS jars were laid directly on top of a basket which was easily colonized by filamentous algae. Large-scale ICARUS pods should not have scaffolding directly below that would encourage excessive filamentous growth that could migrate to the ICARUS pods. Also, as algae are harvested from the inner growth chamber, the

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outer surface of the membrane could be cleaned. Perhaps a coating could be used on the external side of the membrane to minimize the ability of unwanted organisms from becoming established.

The cost of photobioreactors (PBRs) tends to be higher than that of open raceway ponds, due to energy inputs for pumping, aeration, and temperature regulation. However, it is expected that a scaled-up configuration of ICARUS will be less expensive to operate than conventional PBRs because no additional energy is required for aeration or temperature regulation. Minimal energy may be required for pumping and/or mixing the growth medium or potentially moving the ICARUS reactor within the wastewater reactor. As ICARUS is a hybrid open/closed system (open to the wastewater growth medium, but closed to the atmosphere and undesirable wastewater biology), the cost will likely fall between that of typical open and closed systems.

Although ICARUS may be more costly to operate than an open raceway system, co-locating the reactor in a wastewater treatment plant decreases the costs of external inputs (fertilizers, fresh water, carbon dioxide) which should improve overall economics. Similarly, the potential for producing a significantly higher cell density than an open system also improves the process' economic feasibility. According to Chisti (2007), a typical raceway pond of 0.3 m deep and a volumetric productivity of 0.117 kg m^{-3} d^{-1}, would require 7828 m^2 to produce 100,000 kg of algal biomass annually. The current study achieved a volumetric productivity of 0.5 kg m^{-3} d^{-1}, which would require 1826 m^2 to produce 100,000 kg of algal biomass annually. The area equates to approximately 70% of the clarifier area following the post-BOD removal reactor at the Howard Curren Advanced Wastewater Treatment Plant, the 96 MGD plant where this study took place. If the clarifier area following post-BOD removal and post-nitrification was included, the available area could potentially produce more than 280,000 kg of algal biomass per year. The biomass produced could be a higher value crop than that typically grown in wastewater, as the

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membrane preserves culture homogeneity. At least theoretically, algal production could be adequate to justify installing ICARUS reactors in existing treatment plant infrastructure given the productivity demonstrated in this body of work. Pilot studies should be explored, at minimum, to obtain better field data to extrapolate productivity potential.

The proposed method promotes a higher cell density than both open and closed reactors while safely utilizing ‘free’ nutrients, freshwater, and carbon dioxide in wastewater. Grazers and other undesirable members of the complex biological community of wastewater are effectively separated from the algal biomass, allowing the growth of a higher value homogenous culture. The high cell density produced in ICARUS culture may eliminate dewatering steps farther down the processing stream, saving energy and external inputs for algal biofuel production. Although much research is needed in regard to mechanisms of growth and biofilm formation, the initial results of laboratory and field studies warrant scale-up trials of the proposed method.

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Appendices

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Appendix A. ICARUS Technology Development - Preliminary Results

A.1 Preliminary Methods

This Appendix includes Supplementary Material originating from preliminary experiments conducted by Breeyn Greer under the supervision of the Author through the Research Experience for Undergraduates – Tampa Interdisciplinary Environmental Research Program (NSF Award #1200682) during the summer of 2011. The experimental objectives were two-fold: 1) demonstrate the feasibility of growing microalgae in dialysis bags suspended in various types of growth media and compare cell density of dialysis bag cultures to that of suspended culture; 2) demonstrate the difference in permeability among membranes of varying pore sizes.

The first objective was tested by filling dialysis bags (12-14 kDa regenerated cellulose, SpectrumLabs, Rancho Dominguez, CA) with 40 mL of deionized water and submerging them in 400 mL of growth medium (clarified effluent following a post-carbonaceous BOD removal reactor, post-nitrification clarified effluent, belt filter press supernatant, and Bold 1NV synthetic medium) (Figure A1). Dialysis bags were clamped (SpectrumLabs, Rancho Dominguez, CA) at the bottom and fitted with sampling tubes at the top (such that the seal around the sampling tubes was water tight). Erlenmeyer flasks (500 mL) were filled with 400 mL of growth medium for suspended cultures. The experimental set ups were inoculated with *C. sorokiniana* (UTEX #246) such that the initial cell density in the bagged and suspended culture was the same. Experimental treatments in Trial 1 were shaken at 150 rpm (Lab-Line Incubator-Shaker, Melrose Park, IL) under a 12h/12h photoperiod of approximately 21 W m^{-2} during light hours. Because the dialysis bags ripped within a few days of inoculation in the first trial, subsequent trials were shaken at 50 rpm under the same photoperiod. Experimental cultures were not artificially aerated.

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Figure A1. Experimental set ups for preliminary ICARUS tests using dialysis bags of regenerated cellulose and cellulose acetate. Photographs courtesy of Breeyn Greer.

Culture growth was measured via optical density at 680 nm on a spectrophotometer (HACH DR/4000, Loveland, CO, USA) after agitating the culture. Because samples could be returned to experimental treatments after reading, optical density was minimally disruptive to the volume and concentration gradients of dialysis bag treatments. The specific growth rate of each culture was calculated using first order kinetics as outlined in previous chapters (Chapters 4, 5, and 6) of this work.

The difference in permeability (second objective) was tested by measuring the movement of non-purgeable organic carbon (NPOC) and dissolved total nitrogen (TN) (TOC Analyzer, Shimadzu, Japan) and vitamin B12 (optical density at 360 nm (Du et al., 1998), HACH DR/4000, Loveland, CO, USA) across the dialysis membrane. Cellulose ester dialysis membranes with porosities of 0.5-1 kDa, 8-10 kDa, 100 kDa, and 1000 kDa (SpectrumLabs, Rancho Dominguez, CA) were tested. Samples were taken at varying intervals, and the permeability coefficient for each membrane and measured constituent was calculated based on the following equation (Schlutz and Gerhardt, 1969):

$$\ln\left(1-\frac{S_I}{S_e}\right) = -\left(\frac{1}{V_1}+\frac{1}{V_2}\right) * P_m * A_m * t \qquad \text{Eq. A1}$$

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where V_1 and V_2 are the inside and outside volumes, respectively, P_m is the permeability coefficient, A_m is the area of the membrane, and t is time. S_I is the initial concentration inside the dialysis bag, and S_e is the equilibrium concentration between the two solutions, defined as

$$S_e = \frac{(S_2 * V_2)}{(V_1 + V_2)} \qquad \text{Eq. A2}$$

where S_2 is the concentration in the bulk volume outside the bag. Nutrient concentrations were measured by removing a 1 mL aliquot of sample from the volumes inside and outside the dialysis bag, diluting in 19 mL of distilled water, and analyzing on the TOC Analyzer. A calibration curve was used to transform area to concentration of NPOC and TN. The calibration curves used are illustrated in Figure A2. Volumes were measured at the beginning of the experiment, and membrane area was measured directly or determined by the surface area to volume ratio provided by the membrane manufacturer.

A.2 Results of Preliminary Experiments

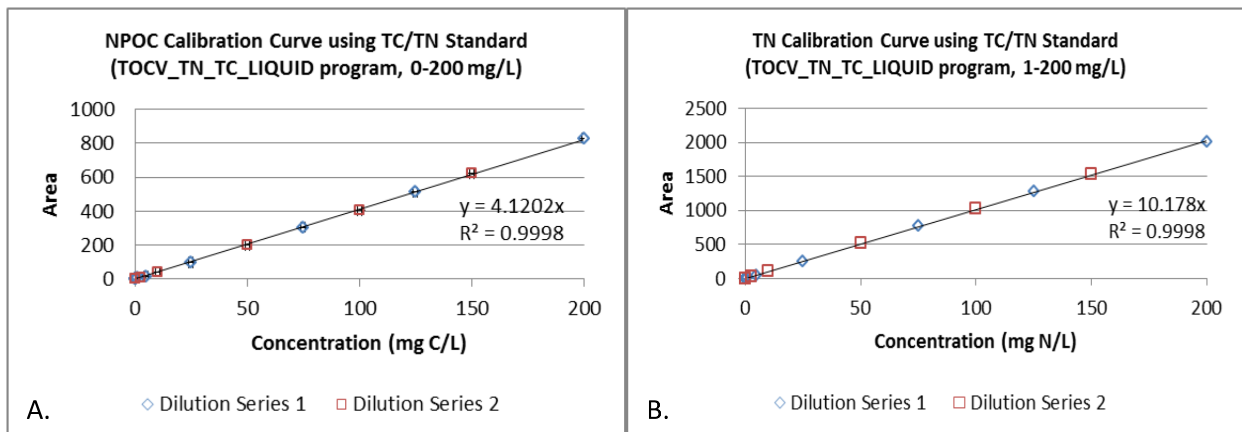

Figure A2. Calibration curves used for (A.) NPOC and (B.) TN analyses.

The growth rate of algae in the two experimental set ups were similar when grown in the same effluent, but slightly different among growth media, which was expected (Figure A3). Algae did not grow well in the belt press filtrate; therefore, specific growth rate data for this series is not shown. The growth rate standard deviation was much larger when all trials (two

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different shaking speeds) were combined (Figure A3A) than when considering just trials 2 and 3 (Figure A3B) (which had the same shaking speed). Also, the mean growth rate decreased with decreasing shaking speed, which suggest the specific growth rate is sensitive to environmental conditions. Therefore, future studies should be careful to ensure environmental conditions among experimental treatments are identical.

The dialysis cultures reached a cell density much higher than that achieved in suspended cultures (Figure A4). As also demonstrated in Chapters 5 and 6, the exponential growth phase lasted longer in dialysis culture than suspended (Figure A3). *C. sorokiniana* grew successfully in all media except filtrate, which led further studies to abandon filtrate as a medium. Although preliminary results suggested promise for dialysis culture, the durability of the regenerated cellulose proved to be a challenge, as bags ripped easily and generally did not last beyond 4-5 days.

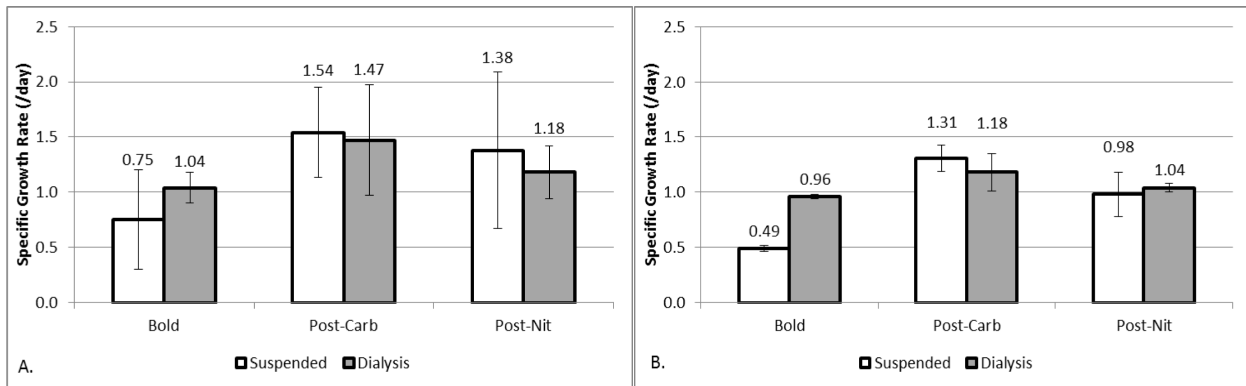

Figure A3. Specific growth rates of (A.) all trials in preliminary tests and (B.) trials 2 and 3 only.

In summary, the growth experiments demonstrated that dialysis culture had the potential to increase the length of the exponential growth phase of microalgal cultures, leading to a higher cell density than suspended cultures. However, membrane material and the configuration of the growth vessel must be strongly considered in future experiments, as preliminary tests had many challenges with leaking dialysis bags. From these early studies, we decided to use the pods and

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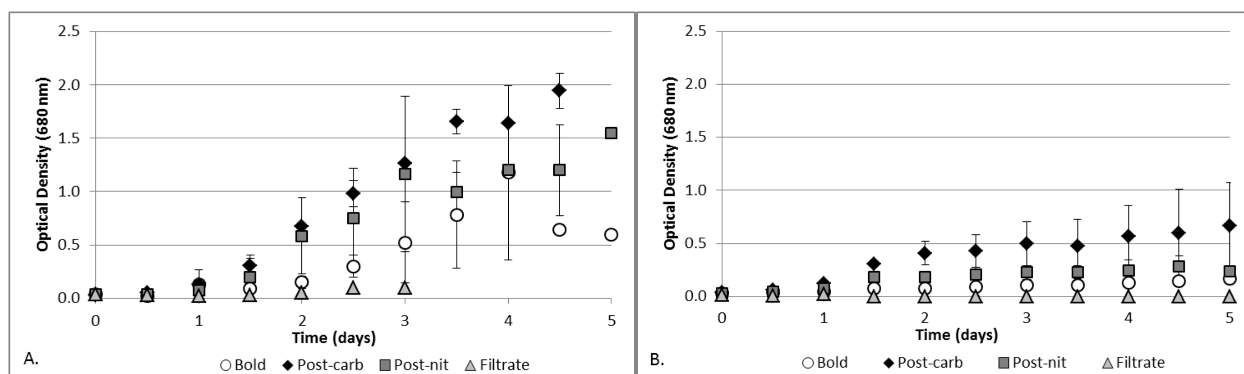

Figure A4. Optical density of algal cultures in (A.) 40 kDa regenerated cellulose dialysis bags and (B.) suspended conditions. Results depict data from four batch tests for dialysis cultures and three batch tests for suspended cultures. Data is truncated to five days due to time frame of when dialysis bags began leaking.

jars discussed in Chapters 5 and 6, with a flat membrane surface area instead of the membrane bag. It should be noted that a membrane bag may still work, if material and sealing strategy is reconsidered.

The results from the permeability tests were as expected; the movement of constituents increased with increasing porosity (Figure A5), until a threshold was reached at the higher end. Since preliminary tests were conducted on the same material, multiple membranes of varying porosity *and* material were chosen for the experiments of Chapter 5. Cellulose ester was not used in subsequent experiments because it was not readily available in flat sheets. Also, the material cracked easily and did not seem robust enough for scale-up.

The preliminary study also evaluated the effectiveness of the sampling method in the permeability tests. Total nitrogen and non-purgeable organic carbon results were not as precise as had been expected, which may be due to instrument error or the large dilution factor required with the small sample sizes available). Also, samples were removed for analysis, which changed the volume and potentially the concentration gradient of the remaining solution. Vitamin B12, in contrast, could be returned to the bulk solution after measuring, causing less disturbance to the

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volume and concentration gradient. The most reliable data was generated by measuring vitamin B12. Future experiments used conductivity as a surrogate for essential constituents, mainly because it can be measured in situ without requiring the sample to be removed from the system.

In summary, the results of the permeability tests confirmed that target constituents (i.e., carbon, nitrogen, and vitamins) could move across the dialysis membranes to become available for algae growth. Furthermore, permeability increased with increasing pore size, which would be expected, and hints at the opportunity to select for nutrient availability and/or timed delivery to induce a metabolic response (i.e., nitrogen limitation for lipid production). Lastly, the sampling protocol for permeability measurements was considered inadequate for future experiments due to the need to withdraw sample volume from the system.

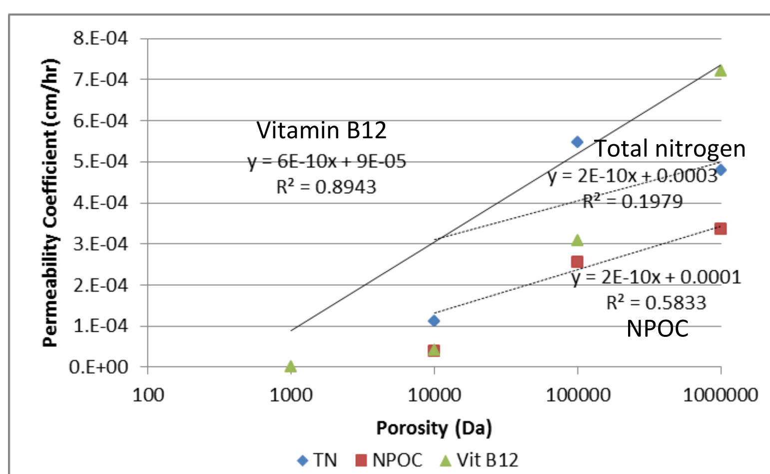

Figure A5. Permeability coefficients of cellulose ester membranes of four porosities. TN: total nitrogen; NPOC: non-purgeable organic carbon; Vit B12: vitamin B12.

A.3 References

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Appendix B. Method Development to Prepare Algae for Biomass Analyses

Preliminary experiments were conducted by Sascha Bekaan from Saxion University (Dutch Clean-Tech Community Student Exchange) in the spring and summer of 2012. Sascha worked primarily on the experiments detailed in Chapter 4, but also helped with method development to prepare algal biomass for the carbohydrate and protein assays. A summary of his results regarding various methods to test the solids portion of algal biomass can be found in Table B1. The best method was determined to be the "Measuring Raw & Filtered Sample," which admittedly was not without error. Reproducibility among samples remained a challenge. The difference between the raw and filtrate included bacteria or other constituent retained on the membrane when filtered, thereby not solely measuring carbohydrate and protein content of the algae.

Although the Filtration Method was the most reproducible, the standard deviation and coefficient of variance still remained unacceptably high. Therefore, this method (or any other listed in Table B1) was not used in the experiments detailed in this body of work. However, detailing unsuccessful methods provides a starting point for future researchers to test other methods without wasting time and resources on already failed methods.

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Table B1. Method analysis for measuring protein content, carbohydrate content, and total suspended solids (TSS) and particulate organic carbon content (POC) in microalgal biomass.

Method	Method description	Problems/suggestions	Protein	Carbohydrate	TSS/POC
Centrifuge	- Centrifuge the algae sample for 20 min at 3000 rpm - Put the pellet on an aluminum weigh dish - Dry the sample in the oven for at least 1hr at 104°C - Reweigh the sample - Suspend the algae mass with a known volume of DI water	- A little bit of algae mass left on the aluminum dish because the dishes have a rough surface - At low concentration not really possible to centrifuge the algae	-	-	-
Filtration with glass-fiber filter	- Weigh the filter on an aluminum dish - Filter a certain volume of algae sample with a vacuum pump - Dry the filter in the oven for at least 1hr at 104°C - Reweigh the filter - Measure the TSS/POC or suspend and put it in a sonicator for protein/carbohydrate assay	- Filter sticks on aluminum dish - It isn´t possible to suspend the algae mass on the filter - The filter dissolves while sonicating and after adding reagents, so you get particles	-	-	+
Filtration with membrane filter	- Weigh the filter on an aluminum dish - Filter a certain volume of algae sample with a vacuum pump - Dry the filter in the oven for at least 1hr at 104°C - Reweigh the filter - Measure the TSS/POC or suspend and put it in a sonicator for protein/carbohydrate assay	- Not usable for protein/carbohydrate analyses because you can't bring the algae mass back into solution -	-	-	+
Relationship between TSS and protein/carbohydrate	- Filter a certain volume of algae sample on a membrane filter with a vacuum pump - Put the filter in a small vial and add 10 mL of DI water - Sonicate for 10min to remove the algae mass from the filter, remove the filter and measure - Run on a second filter a TSS test with the same volume you used before	- The mass of algae in a volume changes - At low volumes the mass difference isn´t big enough to determine a good TSS - Individual for every sample	+/-	+/-	+

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Table B1. (Continued)

Relationship between optical density and protein/carbohydrates	- Make a dilution series of the sample - Measure the optical density on the spectrophotometer - Put the sample back in a vial - Run the protein/carbohydrate analysis on this sample	- Individual for every sample - High sample volume required	+/-	+/-	-
Centrifuge and drying on aluminum foil	- Centrifuge the algae sample for 20 min at 3000 rpm - Put the pellet on aluminum foil - Dry the sample in the oven for at least 1 hr at 104°C - Reweigh the sample - Put the aluminum foil in a small vial and add 10 mL water - Sonicate for 10 min - Remove the aluminum foil and measure - Reweight the aluminum foil	- Algae sticks on the aluminum foil - Foil damaged after sonicating	-	-	-
Measuring raw- and filtered sample	- Filter the sample and keep the filtrate - Run a TSS on the filter - Measure the protein/carbohydrate concentration on a raw sample and the filtrate - Calculate the concentration in the biomass		+	+	+

Analyses were conducted and the table was prepared by Sascha Bekaan. Symbols: +: method has potential for analysis indicated; -: method should not be used for analysis indicated; +/-: the method has potential but needs further refinement for the analysis indicated.

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Appendix C. Supplementary Content to Chapter 6

C.1 Calculating Carbon Dioxide Saturation

The saturation concentration of carbon dioxide was calculated using a temperature corrected Henry's constant (NIST, 2011).

$$k_H(T) = k°_{\mathbf{H}} \exp(d(\ln(k_H))/d(1/T)\ ((1/T) - 1/(298.15\ K)) \qquad \text{Eq. C1}$$

where $k_H(T)$ is the Henry's constant at temperature *T* (13°C) and $k°_{\mathbf{H}}$ is the Henry's constant for solubility at 298.15K (mol kg^{-1} bar^{-1}). Solving this equation yielded a $k_H(T)$ of 20.30 L bar mol^{-1}.

Next, the aqueous concentration of carbon dioxide was calculated based on the following equation relating atmospheric carbon dioxide to aqueous concentration at equilibrium (Benjamin, 2002).

$$[CO_2(aq)] = P_{CO2}/K_H \qquad \text{Eq. C2}$$

where $[CO_2(aq)]$ is the aqueous concentration of carbon dioxide; P_{CO2} is the partial pressure of carbon dioxide (3.9×10^{-4} bar); K_H is Henry's constant, from Eq. C1 (20.30 L bar mol^{-1}). The concentration of carbon dioxide was converted from M to mg L^{-1} by the following equation:

$$[CO2(aq)] = [CO_2(aq)](M) * MW_{CO2} * 1000\ mg\ g^{-1} = [CO_2(aq)](mg\ L^{-1}) \qquad \text{Eq. C3}$$

where $[CO_2(aq)](M)$ is 1.91×10^{-5} M; MW_{CO2} is 44 g mol^{-1}; and $[CO_2(aq)](mg\ L^{-1})$ is 0.84 mg L^{-1}. The results of these calculations were used to compare field measurements to theoretical saturation of carbon dioxide at the wastewater treatment plant.

C.2 Modeling Water Loss

Volume loss in the field study was briefly addressed in Chapter 6. This section will expand on hypotheses suggested in the Chapter, including a framework for developing a predictive model for estimating oxygen production and subsequent dewatering potential. As mentioned in the Chapter, water loss in a closed ICARUS system could be caused by many factors, including membrane porosity, thickness, permeability, material, oxygen produced via

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photosynthetic activity, external biofilm thickness, and interactions with dissolved carbon dioxide and carbonate chemistry. Oxygen production via photosynthesis was estimated using the empirical formula and stoichiometric relationships in Drexler et al. (2014).

These factors and their interactions (except dissolved carbon dioxide and carbonate chemistry) were analyzed with a generalized linear model (GLM) (R Commander, Gaussian distribution) to determine which factors or interactions had the most significant effect on the variability of water loss in the ICARUS series. Of the factors measured, photosynthetic oxygen production and the interaction of oxygen production and permeability had the most significant ($p<0.01$) effect on water loss. Although no other factor or interaction contributed significantly, the first model iteration (linear regression, described below) suggested that it may be necessary to include other factors in future modeling.

The amount of oxygen produced (estimated by the algal productivity) was compared to the actual water loss measured in the study. Results of the linear regression and overlay with measured results are shown in Figure 6.9 in Chapter 6 and reproduced below for convenience (Figure C1). Based on the overlay, it appears the model overpredicts volume loss, i.e., not all oxygen that is produced causes water to leave the system.

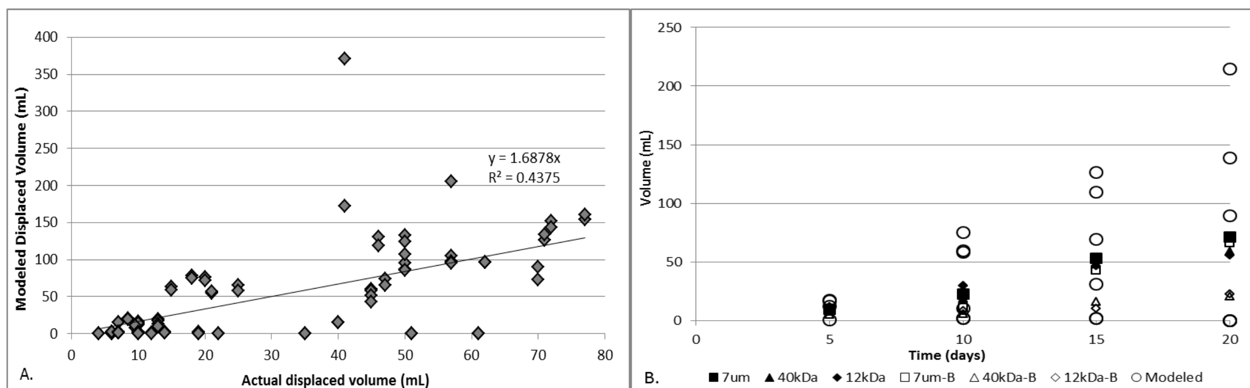

Figure C1. Figure 6.9 repeated for convenience. (A) Linear regression model comparing modeled water loss to measured water loss and (B) model prediction overlaid on measured results of water loss.

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To create a better fit, a more robust and inclusive model is suggested below (Figure C2). Unfortunately, the data the field study were not collected at the resolution needed to construct such a water loss model. The following description includes recommendations to collect pertinent data in future studies.

$$\underset{1}{\text{Oxygen produced in photosynthesis}} - \left[\underset{2}{\text{Oxygen going into solution}} + \underset{3}{\text{Oxygen diffusing through the membrane}} + \underset{4}{\text{Volume of headspace}} + \underset{5}{\text{Oxygen leaving with displaced water}} \right] = \underset{6}{\text{Volume loss of water}} - \underset{7}{\text{Loss due to sampling efforts}}$$

Figure C2. Variables suggested to be included in water loss model. It is assumed that oxygen passing into the ICARUS series across the membrane is negligible.

The terms outlined in the equation above are defined as follows:

1. Photosynthetic Oxygen Production:

$$\frac{__\text{mg algae}}{\text{(measured)}} \times \frac{\text{Mol algae}}{\text{2336 g algae}} \times \frac{\text{114.75 mol O2}}{\text{Mol algae}} \times \frac{\text{R (atm-L/mol-K)}}{\text{P (atm)}} \times \text{T (K)} = \text{mL O2 produced}$$

Eq C3

The photosynthetic oxygen production (POR) is calculated using the dry weight of algal biomass and the stoichiometric relationship in Drexler et al. (2014). However, the stoichiometry could be adjusted if a more precise relationship was known. Pressure (P) and temperature (T) can also be adjusted for field conditions. In a preliminary run of the model, both the actual mass and the change in mass were used to predict POR; the actual mass produced a more accurate prediction. It should be noted, however, that precise temperature measurements were not taken in the ICARUS jars. Dissolved oxygen is quite sensitive to temperature; obtaining an accurate temperature reading in the culture would be important for subsquent models.

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2. Volume of Oxygen Going into Solution:

In the current study, the dissolved oxygen concentration was measured on sampling days. For future modeling efforts, dissolved oxygen as well as solution temperature should be measured. Through careful data collection, this variable would be known (or could be predicted based on the solubility of oxygen at the measured temperature).

3. Oxygen Diffusing Through the Membrane:

Oxygen diffusion across the membrane is subject to Fick's Law as defined below.

$$J_O2 = -D_O2 \times \frac{dC_O2}{dz} \quad \text{(A.)} \qquad \text{And} \qquad D_O2 = 10\text{^}(A+B/T)) \times (1.0*10\text{^}-9) \quad \text{(B.)}$$

Eq C4A & B

where *J_O2* is the movement of oxygen across the membrane (mg m^{-2} sec^{-1}); *D_O2* is the diffusion coefficient of oxygen in water, calculated using Eq E4B; *dC_O2* is the measured change in carbon dioxide concentration (i.e.,the difference between the jar concentration and wastewater concentration), mg L^{-1}; *dz* is the distance in the direction of mass transfer, m.

The temperature is needed to predict partitioning between dissolved oxygen (based on solubility at given temperature) and oxygen that may enter the headspace (i.e., if solution is already saturated). Microelectrodes could be used at the membrane interface to improve the resolution of dissolved oxygen and temperature measurements inside the ICARUS culture and directly outside the membrane interface.

4. Volume of Headspace:

The amount of oxygen remaining in the headspace will be influenced by the saturation concentration of dissolved oxygen and the temperature of the solution. The volume of headspace is calculated based on jar geometry and water height. The volume filling the headspace should be

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subtracted from the total amount of oxygen produced, as oxygen will only start to push out water once the space is full.

5. Oxygen Leaving with Displaced Water:

In this study, this variable can most likely be considered negligible, as the volume of water leaving the system is relatively small. However, in scale-up configurations, it could become significant, and should therefore be included in the preliminary model. Calculating this parameter is challenging, however, because it is dependent on other variables (i.e., volume of water leaving, POR, and headspace). To prevent a circular calculation, it may have to be calculated at t_{i-1} and fed into the model to obtain the new value at t_i.

6. Volume of Water Loss:

Predicting this accumulation term is the model's ultimate goal. The term assumes that the volume of oxygen remaining after subtracting the sinks (variables 2-5) is the same volume as the displaced water. The predicted volume can be compared to field measurements to validate the model.

7. Loss Due to Sampling:

This variable is included because in the current field study, some water loss occurred throughout the sampling process due to handling (i.e., transferring jar volume from vessel to vessel for various tests, such as carbon dioxide). In the case of the field study, the average loss was 3.8 mL (STDEV 1.98 mg L^{-1}) and was determined using the loss in the blank and control series throughout the study period (5-20). In future studies that are designed specifically to capture data for the purpose of constructing the proposed model, minimal handling of samples should occur to get a more accurate reading of volume loss. For instance, only parameters pertinent to the water model (i.e., dissolved oxygen, temperature) would be measured, which

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would not require the sample to be transferred to another sample vessel. Therefore, this term may be eliminated in subsequent iterations of the model.

It should be noted that this model is useful only in the case that scale-up configurations operate in a closed system, which would allow the build-up of dissolved oxygen to force water from the cultivation vessel. The passive dewatering described would work in tandem with gravity settling and scraping to maximize solids content prior to harvest. The ability to predict water loss based on biomass production would improve harvest efficiency in scaled-up operations

C.3 References

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Appendix D. Educational Outreach

D.1 Introduction and Motivation

Although a popular topic in engineering circles, algal biofuels are largely unfamiliar to the general public, a population that normally associates 'renewable energy' with solar or wind energy. Similarly, sustainability is a theme commonly discussed at community events geared towards science and technology, but the application of technology that can contribute to sustainability is often lacking. Therefore, the concept remains fairly abstract and distant from the community member's everyday life.

To demonstrate the application of a sustainable technology, an educational booth was developed around algae biofuel and the concept of resource recovery from waste. As community events are typically geared towards a younger audience, the exhibit was meant to target a wide range of ages (from middle school to adults). A high level of interactivity was important for attracting participants to the booth and holding their attention. Having a "take-home" item was also important, as it extended the learning experience beyond the 5-10 minute interaction at the booth itself. Lastly, as many events do not provide access to water and electricity, the booth had to be largely self-sufficient.

D.2 Description of Booth

D.2.1 Algae Photobioreactors

The booth contained an algae photobioreactor with three growth chambers, as shown in Figure D1. The photobioreactors were constructed out of clear 4" PVC tubes that were about 2.5 feet long. Each tube was capped on the bottom with a PVC cap, and the top was fitted with a reducer so that the top was accessible for aeration and sampling tubes. The reactors were seated on rubber gaskets to hold them in place. The rack was constructed from a 3-tier plastic shelf.

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Circles were removed from the top shelf, allowing the reactors to slide in like a test tube rack. Each reactor was filled with a mixed algae stock (in Bold 1NV media and/or Miracle Grow©) that had previously been grown in the laboratory. As only one reactor was used to seed the Algae Bottles, it would be possible to use green dye for the other two reactors if enough algae stock was unavailable.

Figure D1. The algae photobioreactor setup used for the educational outreach booth.

Battery operated aerator pumps were used to bubble two of the reactors. The moving bubbles in the algae culture drew participants to the booth, curious as to what was happening in the green liquid. The middle reactor was bubbled using a bicycle pump attached to tubing that went to the bottom of the reactor. A sign that read "Feed Me" encouraged booth visitors to use the pump to "feed" carbon dioxide to the algae in the middle reactor.

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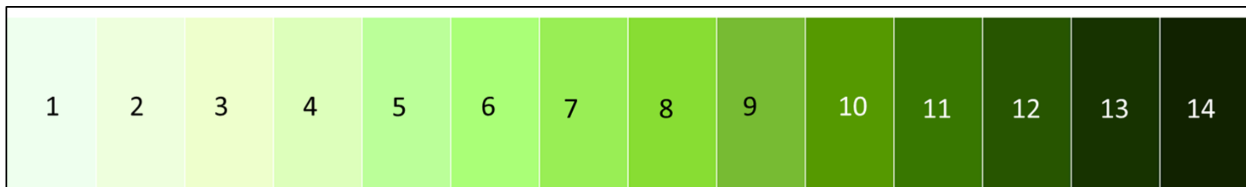
D.2.2 Algae Bottles

To ensure the learning experience continued beyond the visit to the booth, participants were given an "Algae Bottle" to take home with them. Algae Bottles were made from 8 ounce water bottles. The labels were removed, and each bottle was given a unique identification number on the cap in permanent marker. The supplementary materials shown in Figure D2 were attached to the neck of the bottle with a rubber band. The bottles were prepared (on demand) by pouring out about 2 ounces of water. Algae stock was added to each bottle such that the bottles were about a '5' on the chart in Figure D2. Lastly, a Miracle Grow© solution was added to each bottle, and the caps were replaced.



Use this **color chart** to track the growth of your algae. Go to **www.myalgaebottle.org** to **enter your data** and **compare** it to others!

Figure D2. Supplementary material attached to each Algae Bottle for the take-home activity.

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D.2.3 Other Educational Materials

Other materials were used as discussion props at the booth. A solar panel was positioned in the booth next to the photobioreactors to stimulate discussion about photosynthesis and solar energy. Microscope pictures of different algae species were blown up and laminated. The pictures were used to show visitors algal morphology and diversity. An informational poster (Figure D3) was displayed to explain the motivation behind and potential for algal biofuel in a broader context. At some events, curriculum developed by members of our lab (Bair et al., 2014) was handed out, and examples of the associated activities were displayed (Figure D4). The curriculum included a more detailed activity similar to the Algae Bottle concept, which included the opportunity for students to create their own experiment. The curriculum further emphasized applied technologies that demonstrate the concept of sustainability. The activities show students how 'wastes' (i.e., food scraps, cow manure) can become resources (i.e., compost, biogas).

D.3 Educational Narrative

The educational narrative was delivered to booth participants, and crowds generally ranged in size from 2-15 participants. The typical age depended on the event, but the narrative could be adapted depending on the age of participants.

First, the educator would ask the audience if they've heard of algae biofuels. They would then go on to discuss how algae are plants, using the microscope pictures as props. They would ask the audience what plants need to grow (i.e., sunlight, nutrients, and carbon dioxide). A participant would then "feed" the algae reactor to reinforce that algae need carbon dioxide to grow (Figure D5). The first part of the narrative emphasized photosynthesis, laying the foundation to discuss another type of "solar" energy. Last, the educator would briefly discuss

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algae biofuels, how they have the potential to be more sustainable than fossil fuels, and why it's important to start developing different kinds of renewable energy.

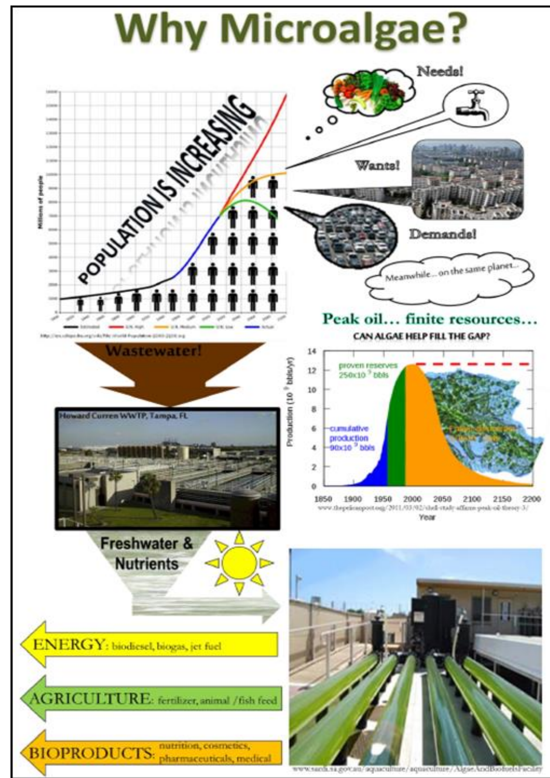

Figure D3. Informational poster used in the algal biofuel educational outreach booth.

Figure D4. Display of Biorecycling curriculum and associated activities.

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Figure D5. Demonstration items used in the educational booth. A) participant "feeding" the algae photobioreactor; B) Algae Bottles and "food" for the photobioreactors.

With a foundation of algae growth requirements, the educator would ask who would like to start their own algae photobioreactor for making algae biofuel. Each participant would be given a bottle and asked to empty about 2 ounces from the bottle. The educator would then add algae and nutrients, discussing why algae need nutrients to grow (Figure D5). The educator would then challenge the audience to think of "wastes" we may be able to use to obtain "free" sources of nutrients, water, and carbon dioxide. The educator would discuss using wastewater or industrial flue gases to grow algae in large-scale farms.

The educator would discuss how to take care of the algal photobioreactor. Participants would be asked whether they thought the reactor needed sun or air, and they were challenged to try different environments around the house. The educator then introduced the participants to the website and described how to report their algae's growth on the interactive website.

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D.4 Interactive Website

An interactive website, MyAlgaeBottle.net, was developed by the author and lab-mate Onur Ozcan to track the growth of algae in the photobioreactors disseminated throughout the community. It is hoped that this concept could be further developed to be more interactive and geographically diverse in the future. The prototype website contained five webpages: Home, How to Collect Your Data, Share Your Results, Look at Your Data, and Contact Us.

The Home Page had general information regarding algae, algal biofuel, and other products that could be made from algae biomass (Figure D6). It was intended to provide a brief refresher on the topics discussed at the booth. Future iterations of the website should include a References page where a visitor could look for more in-depth information. The Data Collection page (Figure D7) described how to use the color coded growth chart (Figure D2) to track the daily growth of algae in their photobioreactor and how to enter their data in the website. The Share Your Results Page (Figure D8) included a Google Form survey where participants could enter the daily growth information and environmental conditions of the algae in their photobioreactor. The page was linked to an excel spreadsheet using Google docs, and the data was uploaded to the Look at Your Data page using a Google API. The data was displayed in a line graph. Participants were able to compare their data to others' in the context of the environmental conditions to which their algae cultures were exposed.

Although the current website was functional, a higher level of interactivity was desired. It was hoped that the chart would initially display all data uploaded by participants, but the data could be manipulated by website visitors to view only certain data series. For example, if the visitor wanted to only view data related to bottles that had been treated similar to their own, they could turn 'off' the data that was unrelated by checking or unchecking boxes (similar to a Pivot

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Table in Microsoft Excel). Future iterations of the website should be designed to include this capability.

Other features could be added to make the experience more user-friendly. For example, highlighting the user's data on the graph would make it easier to discern their data within the sea of other data. When the user navigated to the graph to view their data compared to others, it would be obvious which data referred to their bottle. Also, in addition to each bottle having a unique bottle identification number, each bottle could be given a QR code that would link their bottle to the uploaded data. Scanning the code would bring them to the Data Collection page, making it easier to track the daily growth. As participation was fairly low, making the data entry process more user-friendly might improve the activity in the future.

Figure D6. The Home Page of MyAlgaeBottle.net, the interactive website developed in conjunction with the educational booth.

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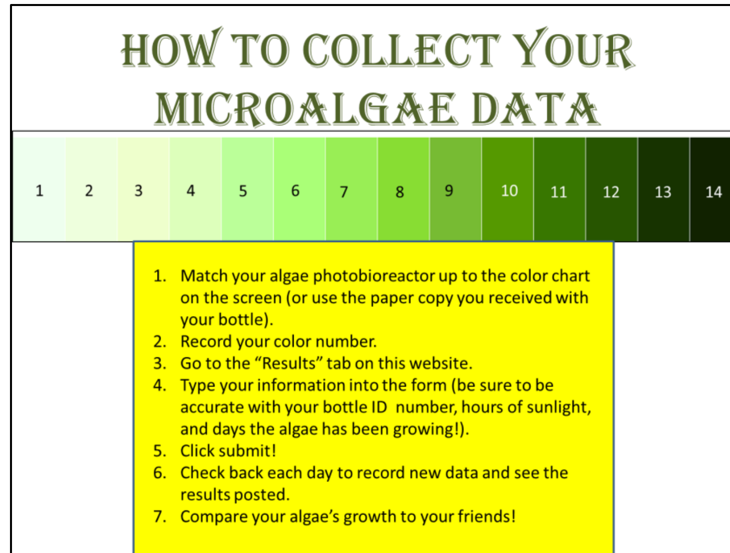

Figure D7. The How to Collect Your Data page from MyAlgaeBottle.net, the interactive website developed in conjunction with the educational booth.

Home / How to Collect Data / Share Your Results! / Look at Your Data / Contact Us

Grow Algae!

Now that you have your very own algae photobioreactor, share your data with others!

* Required

What is your photobioreactor ID number? *
This number should be located on your photobioreactor's bottle cap.

How many days has your algae been growing? *
Hint: If you got your photobioreactor on Friday, choose 1 if it's Saturday, 2 if it's Sunday, etc.

Have you kept your photobioreactor open or closed? *
Select based on the majority of the time… has the bottle cap been mostly open or mostly closed?
- Open
- Closed

Approximately how many hours of sunlight does your photobioreactor receive each day? *
- No sunlight
- 1-3 hours
- 4-6 hours
- 7-9 hours
- 10 or more hours

What is the color of your algae? *
Use the color spectrum provided with your algae bottle to estimate how dark your algae is… the darker the color, the more it's grown!

How old are you? *

Submit
Never submit passwords through Google Forms.

Powered by
This form was created outside of your domain.
Report Abuse - Terms of Service - Additional Terms

Figure D8. The Data Collection page from MyAlgaeBottle.net, the interactive website developed in conjunction with the educational booth.

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D.5 Community Events

The educational booth was displayed at three community events in the Tampa Bay Area: the St. Petersburg Science Festival (October, 2013), the University of South Florida Engineering Expo (February, 2014), and the Florida Water Environment Association Water Festival (March, 2014). All events were geared towards a K-12 audience, free, and open to the public.

The St. Petersburg Science Festival showcases STEM organizations, clubs, academic facilities, government agencies, and student researchers from the community. In close proximity to the University of South Florida's St Petersburg campus and the College of Marine Science, Florida Fish and Wildlife Conservation Commission, USGS, and NOAA, the Festival has a significant marine influence and is hosted in conjunction with an ocean-themed festival "Marine Quest." The event attracts thousands of members of the local community, predominantly from Pinellas County. At this event, over 300 Algae Bottles were given to booth visitors.

The exhibitors at the University of South Florida's Engineering Expo are predominantly student groups (such as the American Society of Civil Engineers, Engineers Without Borders, etc.), but also include local industry and government agencies. The event is geared towards student groups, typically in grades 5-12. The two-day event accommodates thousands of members of the community, with most participants coming from Hillsborough County. At this event, nearly 500 Algae Bottles were disseminated into the community.

The Florida Water Environment Association – West Coast Chapter hosted its first Water Festival in St. Petersburg in 2014. As the first year, the turnout was much lower than the other two events (less than 1000 visitors). Exhibitors included government agencies, non-profit organizations, and student groups focused on water issues. About 50 Algae Bottles were given away during this event. This event is expected to grow in coming years.

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D.6 References

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Significant content of Chapters 3 and 4 were published in *Reviews in Environmental Bio/Technology* and *Water Science and Technology,* respectively, prior to the approval of this dissertation. Copyright release forms for these published manuscripts are presented below.

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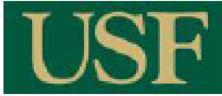

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Ivy earned her B.S. in Environmental Science from the University of San Francisco and her M.S. in Engineering Science from the University of South Florida. Her Ph.D. research focused on the cultivation of algae in wastewater using a passive membrane design. Her research interests lie in biological wastewater treatment, resource recovery (particularly in conjunction with sanitation), systems thinking, and algal biofuels. Ivy has worked in both the public and private sector of the wastewater industry and has studied in South and Central America, Australia, and Europe. Passionate about public outreach and STEM instruction, Ivy plans to dedicate her career to education.

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