Assessing Outdoor Algal Cultivation in Panel and Raceway Photobioreactors for Biomass and Lipid Productivity

by

Everett Eustance

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved July 2015 by the
Graduate Supervisory Committee:

Milton Sommerfeld, Co-Chair
Peter Fox, Co-chair
Paul Westerhoff

ARIZONA STATE UNIVERSITY

August 2015

ABSTRACT

Over the past decade, there has been a revival in applied algal research and attempts at commercialization. However, the main limitation in algal commercialization is the process of cultivation, which is one of the main cost and energy burdens in producing biomass that is economically feasible for different products. There are several parameters that must be considered when growing algae, including the type of growth system and operating mode, preferred organism(s), and many other criteria that affect the process of algal cultivation. The purpose of this dissertation was to assess key variables that affect algal productivity and to improve outdoor algal cultivation procedures. The effect of reducing or eliminating aeration of algal cultures at night, in flat panel photobioreactors (panels), was investigated to assess the reduction of energy consumption at night. The lack of aeration at night resulted in anoxic conditions, which significantly reduced lipid accumulation and productivity, but did not affect log phase biomass productivity. In addition, the reduction in aeration resulted in lower pH values, which prevented ammonia volatility and toxicity. Raceways are operated at deeper cultivation depths, which limit culture density and light exposure. Experimentation was accomplished to determine the effects of decreasing cultivation depth, which resulted in increased lipid accumulation and lipid productivity, but did not significantly affect biomass productivity. A comparison of semi-continuous cultivation of algae in raceways and panels in side-by-side experiments showed that panels provided better temperature control and higher levels of mixing, which resulted in higher biomass productivity. In addition, sub-optimal morning temperatures in raceways compared to panels were a significant factor in reducing algae biomass productivity. The results from this research

indicate that increasing lipid productivity and biomass productivity cannot be completed simultaneously. Therefore, the desired product will determine if lipid or biomass productivity is more crucial, which also dictates whether the system should be operated in batch mode to either allow lipid accumulation or in semi-continuous mode to allow high biomass productivity. This work is a critical step in improving algal cultivation by understanding key variables that limit biomass and lipid productivity.

DEDICATION

This dissertation is dedicated to my family who I love dearly. To my parents Camie and Randy Eustance and my sister Chanee Eustance who all showed me how to work hard and made sacrifices so that I could pursue my dream. To my wife Jessica Eustance, who has always supported me and has been amazing, as I have worked towards getting my Ph.D. To my baby girl Brianne Eustance, who has been my inspiration during these last few months.

ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Milton Sommerfeld. Without his guidance and support, I would not have had the freedom to pursue the research that will help shape the future of algal cultivation.

I would also like to thank my committee members, Dr. Peter Fox and Dr. Paul Westerhoff, for their support and questioning during this process.

Additionally, I would like to thank Dr. Robert Gardner who initially mentored me during my Master's degree, as he taught me a strong foundation for conducting excellent research.

I would also like to thank Joshua T. Wray, Shahrzad Badvipour, and Emil Puruhito, who are excellent co-authors and colleagues, which provided a great community for collaboration during this process.

Lastly, I would like to thank the technicians of the Arizona Center for Algae Technology and Innovation for their help and support during this process. Specifically, I would like to thank Sarah Arrowsmith for her help with the biochemical analysis that was completed during this process.

TABLE OF CONTENTS

Page

CHAPTER Page

CHAPTER Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

1. INTRODUCTION

1.1. Commercialization of Algae

Over the past decade, there has been a revival in applied algal research and attempts at commercialization. This has been spurred by several different pathways including the use of algae for 1) bioremediation of polluted waters and wastewaters, 2) the use of algae biomass to replace fossil fuels, 3) the potential for unique high-value products, and 4) the use of algal biomass as an animal and fish feed.

1.1.1. Algae for Bioremediation Purposes

Since the 1950s, algae have been studied for their use for bioremediation purposes. The first articles written in the 1950s characterize the role of algae in sewage oxidation ponds and their ability for enhanced nutrient removal. Research in this area has been one of the few areas within the algal field to maintain continued interest since its inception in the 1950s. Algal bioremediation began with municipal wastewater due to the organisms' ability to remove large quantities of nitrogen and phosphorus. Many studies have shown that many green algae are capable of removing essentially all nitrogen and phosphorus from traditional municipal wastewater. As the Environmental Protection Agency (EPA) continues to decrease the discharge limits on existing facilities, better methods will need to be employed to efficiently remove nitrogen and phosphorus, which have been linked as the primary nutrients for downstream eutrophication.

In addition to municipal wastewater treatment, algae have been evaluated for bioremediation of animal wastewaters, including dairies and pig farms and the liquid effluent from anaerobic digesters.

1.1.2. Algae as an Energy Source

A major area of focus has been in finding clean, renewable energy sources to replace fossil fuels. Currently, research has diversified into two main sectors: mechanical/chemical methods of capturing energy such as wind turbines and solar panels. The other research area focuses on the biological production of energy from crops such as corn and sugar cane for ethanol and camolina and soybean for biodiesel. The reason that these alternative energy sources can be enticing compared to traditional wind and solar is the ability to store energy in the biomass. Wind turbines and solar panels convert energy from one form into electricity, which in large quantities cannot be stored, whereas biological sources create chemical compounds such as starches or lipids that can be converted into fuel allowing for on-demand use. An area that has seen a significant increase in research and funding over the past decade is the cultivation of microalgae for the production of biodiesel. Compared to terrestrial crops, algae are capable of producing greater yields of biomass and lipids (Chisti 2007). Furthermore, current research is focused on assessing the best method to extract the most energy from the algae biomass. The two methods being investigated are utilizing lipids to produce fatty acid methyl esters and hydrothermal liquefaction, which utilizes the entire cell to produce a bio-crude product that can be inserted into existing crude oil processing plants. However, as researchers have continued to study and improve the process of producing fuel from

algae, both life cycle and techno economic assessments have indicated that algae are currently and in the near future unlikely to be economically viable without utilizing their valuable co-products.

1.1.3. Algae for High-Value Products

Depending on the organism being cultivated, additional processing of the biomass or lipids can produce high-value products (HVP). These can be a wide variety of chemicals, but the main HVPs currently being pursued are carotenoids and omega-3 fatty acids. Carotenoids are utilized by algae for collection of light and for protection of the cell by preventing the formation of reactive oxygen species (Blankenship 2002). Carotenoids have developed a significant market based on their anti-oxidant properties, which the most commonly known are β-carotene and astaxanthin (Markou and Nerantzis 2013; Pérez-López et al. 2014). β-carotene is a precursor to vitamin A and is often associated with carrots due to the distinct orange color (Hejazi et al. 2004). Additionally, β-carotene is used for its natural pigmentation as a food-coloring agent. Astaxanthin is considered one of the strongest antioxidants in nature and is used in aquaculture to improve the pink color in shrimp and salmon (Guerin et al. 2003; Spolaore et al. 2006).

1.1.4. Algae as Animal Feed or Fertilizer

Recent legislation in the State of Arizona has designated algae as a crop, meaning algaculture is agriculture (Trentacoste et al. 2015). Algae utilize light and carbon dioxide more efficiently than terrestrial crops, which allows for significantly higher growth rates

(Williams and Laurens 2010). Estimates indicate that algae can produce between 60 to 180 tonnes/ha/yr of biomass depending on growth conditions, while estimates for alfalfa, which algae has the potential to replace, is approximately 18 tonnes/ha/yr in Arizona (Hu et al. 1998a; Moheimani and Borowitzka 2007; Williams and Laurens 2010).

Biofuels are typically produced from lipids in the algal cell, which leaves a significant amount of biomass left unused. This spent biomass consists of high levels of protein and carbohydrates. Two options for this residual biomass that have widely been discussed are its use as either a protein additive for livestock and fish feed or as an organic fertilizer for farms (Spolaore et al. 2006). Another option for algae lipids is to utilize certain species that are known to have higher levels of omega-3 fatty acids, which can be used to change the fatty acid profile of dairy cows' milk (Franklin et al. 1999; Vahmani et al. 2013; Shingfield et al. 2013).

1.2. Limitations to Commercialization of Algae

However, over the past decade, few companies have successfully turned algae or algal products into a commercial business. This is due to limitations, both energetically and economically, in all areas of producing, harvesting, and refining the algal biomass. The area with the largest uncertainty in both energetics and economics is the initial algal cultivation process. This is because there are several approaches to growing algae. These design considerations include the use of photobioreactors or raceways, batch or semi-continuous cultivation, preferred organism(s), and many other criteria that can change the outcomes of both the life cycle analysis and the techno-economic analysis. These issues

have led many researchers and analysts to focus on downstream processes without consideration of the source of the input, which has drastic effects on all downstream processes. This includes the type of algae and the culture density, which affects the efficiency of harvesting and the amount of water needed to be removed from the biomass, respectively. The type of algae used and when it is harvested significantly impacts the biochemical composition of the algae biomass, which changes what the algae can be used for and how much energy and cost is required for either cultivation or refining of the algae biomass for the desired products.

1.3. Project goal: Improving the Algal Cultivation Process

The objectives of this dissertation were to improve the feasibility of algae as a commercial endeavor by assessing and improving current algal cultivation processes associated with either lipid productivity or biomass productivity as described below:

1) Reducing or eliminating aeration of algal cultures at night, when high levels of aeration/mixing are not needed, will decrease unnecessary energy consumption of growing algae with the benefit of reducing ammonia volatility and toxicity. However, eliminating aeration at night will cause anoxia to occur, which will increase starch utilization to maintain the same metabolic rate. What affect will reducing aeration in cultures at night have on culture productivity and its biochemical composition? Will reducing aeration prevent ammonia volatility from occurring?
2) Traditionally raceway ponds are operated at depths between 15 and 30 cm; however, flat-panel photobioreactors are traditionally operated at 1 to 10 cm in

thickness/depth for optimal productivity and increased culture density. In shallower raceways, algae will respond differently to the increased light concentration reaching the cell compared to being absorbed by the water and other particulates, which can affect productivity and biochemical composition. Will reducing the culture depth increase biomass productivity and/or lipid productivity?

3) Semi-continuous and continuous growth can improve culture productivity by maintaining a log phase culture. Furthermore, what is the achievable biomass productivity in cultivations systems such as raceways and panels? By comparing raceways and panels in a side-by-side experiment, is it possible to eliminate external environmental factors in assessing productivity? Which system has higher biomass productivity? What may be limiting growth in the different cultivation systems?

These three main goals are major issues associated with algal cultivation that need to be addressed to improve the feasibility of algae as a future commodity.

1.4. Overview of Dissertation

The remainder of the dissertation highlights the work and knowledge of improving algal cultivation in the photobioreactors and raceway ponds located on the Arizona Center for Algae Technology and Innovation (AzCATI) field site at Arizona State University Polytechnic campus in Mesa, Az. The project is outlined in 6 chapters: Literature review, 3 manuscript chapters, synthesis chapter and an overall summary chapter.

The literature review is a detailed assessment required to interpret the results in the following manuscripts. The literature review is split into 4 sections. What do algae need to grow? This is focused on the nutrients CO_2, phosphorus and nitrogen. The major focus on this section is the necessity to utilize ammonium for industrial scale cultivation of algae and provides extensive information on why this is important. It also provides information to show that growth on ammonium as a N-source requires increased levels of CO_2 for increased growth rates, which currently is problematic in raceways. How are algae grown? This highlights panel photobioreactors and raceways, along with methods of improving growth rate, decreasing cost and providing information on the possible pathways for large-scale facilities. What effects algal growth? This discusses the effects of pH, nutrient limitation and CO_2 availability on system productivity. What are the benefits of using algae over terrestrial crops? The differences in maximum achieved productivities of both algae and energy/food crops are described and compared to the theoretical limit of photosynthesis.

The research is focused on the use of *Scenedesmus acutus* strain LB-0414 (strain 0414) and Strain LB-0424 (strain 0424). The latter was isolated in non-cooled panels in July 2014 after a haboob dust storm. Cultures were grown in 55 L flat-panel photobioreactors with a light-path length of 4.5 – 5 cm and in raceway ponds with an area of 30.37 m^2.

Research for the dissertation was split into three manuscript chapters with distinct goals. The purpose of the first manuscript was to reduce energy requirements and to prevent ammonia volatilization and toxicity from occurring at night when the culture pH

shifted due to CO_2 being shutoff. Results showed that eliminating aeration at night prevented the pH of the culture from increasing and allowing for volatilization of ammonia from occurring. However, by shutting of aeration, the cultures experienced extended periods in anoxic conditions, which delayed lipid accumulation but did not affect biomass productivity during the log phase. Intermittent sparging at night was utilized to provide additional oxygen to increase lipid accumulation, while maintaining lower pH values and reducing energy consumption at night by greater than 95%. The second manuscript focuses on growth in algal raceways at different depths. The goal of this manuscript is to highlight the importance of selecting the optimal culture depth and areal culture density to minimize light limitation and improve lipid accumulation for batch growth. Results show that providing cultures with excess levels of nitrogen can maintain higher levels of cellular protein, but prevents cultures from accumulating lipids. In addition, decreasing culture depth improved the areal lipid yield. The third manuscript focuses on semi-continuous growth in panels and raceways. The purpose of this manuscript was to determine the difference in biomass productivity being achieved in panels and larger (30.37 m^2) raceways to determine what factors limited culture productivity. This includes the impact of seasonal changes on daily average solar irradiance and culture temperature, which play a significant role in the productivity of the algal cultures.

The synthesis chapter covers how these three manuscripts provide a detailed view on how to improve algal cultivation feasibility by looking at the key variables for lipid accumulation and productivity, and biomass productivity. The chapter also provides a discussion of how the three manuscripts impact the field of algal cultivation. Finally, the

conclusion chapter highlights the key findings from each manuscript, along with general conclusions developed in the synthesis chapter and suggestions on future research that should be completed to further improve the feasibility of algal cultivation.

2. BACKGROUND

2.1. What is Required for Algal Growth

Algae require light and three macronutrients that are not abundant enough in natural waters. These are carbon, phosphorus and nitrogen.

2.1.1. Carbon source

Carbon is typically provided through culture aeration in the form CO_2, which can be utilized directly or converted into dissolved inorganic carbon (DIC) as shown in Equation 2.1.

$$\boldsymbol{CO_{2\,(g)} \leftrightarrow CO_{2\,(aq)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+} \qquad \textit{Eq 2. 1}$$

Previous studies have shown that levels of CO2 found in ambient air are not sufficient to maintain high levels of productivity in dense cultures and that the optimal range is somewhere between 1 and 5% or beyond if dealing with ultra-high cell density cultures (Gardner et al. 2012; Hu et al. 1998b; Eustance et al. 2013). Additionally many cultures have shown inhibition at levels above 10%, making flu gas a possible carbon source without the need of dilution (Doucha et al. 2005; Matsumoto et al. 1997; Negoro et al. 1991; Hanagata et al. 1992). In highly productive cultures, the available DIC is important to prevent carbon limitation. Figure 2.1 shows the significant impact that both pH and the percent CO_2 in the aeration gas have on DIC. Most algal cultures are grown at a pH between 6 and 9, which indicates that bicarbonate is the main form for DIC and can be directly utilized by some strains (Shiraiwa et al. 1993). To increase the ability to utilize bicarbonate algae have also developed a series of enzymes known as carbonic

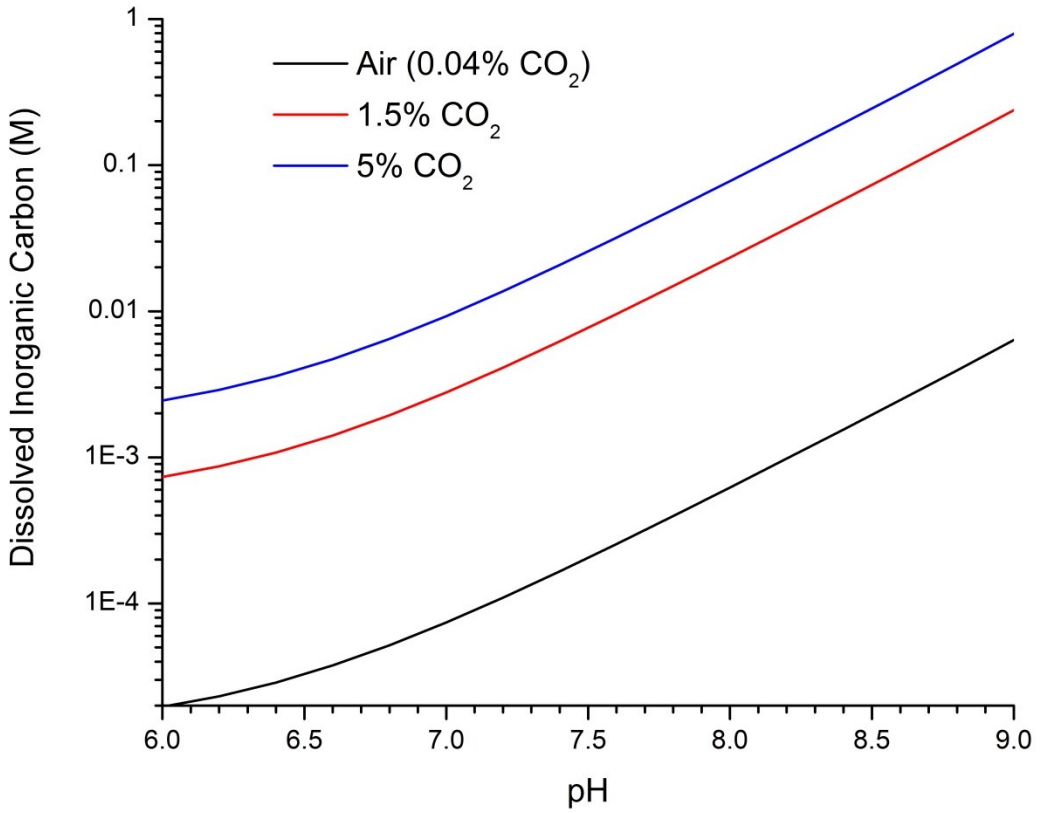

Figure 2.1: Graph showing the increase in dissolved inorganic carbon (DIC) with an increase in pH along with how the percent CO_2 in the gas phase affects total DIC.

anhydrases, which depending on its location converts bicarbonate to CO_2 for transport across membranes or CO_2 back to bicarbonate to prevent the CO_2 from leaving the cell (Beardall et al. 1998; Giordano et al. 2005). This is known as the CO_2 or Carbon Concentrating Mechanism and allows for significantly higher concentrations of carbon within the cell compared to the concentration in the surrounding environment. However, it has been shown that the carbon concentrating mechanism is active in the presence of low levels of CO_2, which has led to the suggestion of using bicarbonate as the primary carbon source and to induce lipid accumulation (Gardner et al. 2012; Gardner et al. 2013; Wensel et al. 2014).

2.1.2. Phosphorus

Phosphorus is a key element required for biological function and is used in ATP, nucleotides and phospholipids. With an expanding global requirement for food and biofuel crops, the cost and potential limitation of phosphate has already shown a drastic increase in the price of fertilizers and it is estimated that global reserves may be deplete in as few as 50 years (McGill 2012; Scholz and Wellmer 2013). However, algae are capable of growing in a variety of environments including municipal and livestock wastewaters, which tend to have an abundance of phosphorus. Previous research has shown that algae are very efficient at removing excess phosphorus from wastewater. Originally, it was deemed as a luxury uptake of phosphorus and stored internally (Powell et al. 2008; Powell et al. 2009). However, some researchers believe that phosphate uptake is a two-stage process beginning with surface adsorption followed by transport through the cell membrane, which accounts for the rapid removal of phosphate from the media (Sanudo-Wilhelmy et al. 2004; Yao et al. 2011). The ability of algae to complex greater amounts of phosphorus than what is needed for assimilation is one of the main reason algae have been suggested for tertiary treatment of municipal wastewater (Zhang et al. 2008).

2.1.3. Nitrogen

The nitrogen utilization of algae compared to terrestrial based crops is between 55 and 111 times greater than for rapeseed (ha^{-1} $year^{-1}$) (Sialve et al. 2009). This increase is due to the significant increase in biomass productivity. Using wastewater as a nitrogen

and phosphorus source at an average concentration of 40 mg-N/L and 3 mg-P/L will require 2.5 m^3 of wastewater to produce 1 kg of dried algal biomass, where nitrogen is the limiting factor (Lundquist et al. 2010; Sedlak 1991). However, the value 40 mg- N/L is a general average for wastewater entering a municipal treatment facility. The concentration that exits facilities is significantly lower due to the incorporation of the nitrification/denitrification cycle that converts incoming nitrogen into N_2 gas (Rittmann and McCarty 2001). Therefore, other wastewater sources will be needed to provide nitrogen. Within municipal treatment facilities, one of the main burdens is the production of sludge that is typically sent to landfills. Sludge can be processed further through anaerobic digestion (Rittmann and McCarty 2001). Upon digestion the solids are separated leaving a centrate that is high in ammonia and phosphorus that can be utilized for algal growth (Cabanelas et al. 2013). Another potential source for nitrogen is wastewater from dairies and other animal feedlot operations, which tend to have highly concentrated wastewater lagoons (Chen et al. 2012; Dodd 1979; Godos et al. 2009; Kebede-Westhead et al. 2006; Mulbry et al. 2008; Buchanan et al. 2013).

2.1.3.1. Nitrate

The most common nitrogen source used in algal research is nitrate, which is in stark contrast to the most abundant source of nitrogen in the form ammonium. This is because nitrate is considered to be a more stable form of nitrogen (in terms of pH and temperature) for research, which has shown that lipid concentrations increase with respect to an increase in the pH of the medium (Gardner et al. 2011; Guckert and Cooksey 1990). Within the cell, nitrate is converted to ammonium in the chloroplast and

has a large impact on the carbon-nitrogen metabolism (Turpin 1991). The reducing power for nitrate assimilation comes from the photosynthetically generated electron flow, and utilizes approximately 20% of the total electron flow, while the rest is directed towards photosynthetic CO_2 fixation (Turpin 1991). However, using nitrate from traditional sources in a large-scale system would make the entire process cost prohibitive (Lundquist et al. 2010)

2.1.3.1.1. Nitrate Uptake in the Presence of Low CO2 and its pH Effect

As with other nitrogen sources in the presence of low CO_2, nitrate uptake is reduced due to a co-transport requirement (Turpin 1991). However, the process of nitrate uptake releases hydroxyl ions to maintain cell neutrality, which increases the pH of the medium (Fuggi et al. 1981). The pH increase allows for an increase in DIC equilibrium of the medium, which would potentially allow for a higher cell concentration being achieved at a faster rate (Eustance et al. 2013). In addition, increased pH has shown to increase the TAG content of *Scenedesmus* sp. WC-1 and *Coelastrella* sp. PC-3 by preventing cell-cycling (Gardner et al. 2011). However, this concept may decrease the overall lipid productivity of the system by stopping growth and therefore reducing biomass productivity.

2.1.3.2. Urea

Urea is one of the main nitrogen sources found in raw municipal wastewater. The urea can be utilized by algae; however, in large-scale facilities, the presence of bacteria will degrade a significant portion of the urea into ammonium prior to uptake. When algae uptake urea it is hydrolyzed to ammonium using the enzyme complex ATP:urea

amidolyase or hydrolytically cleaved using urease (Bekheet and Syrett 1977; Leftley and Syrett 1973; Hodson et al. 1975). The enzymatic degradation of urea has the additional benefit of producing intracellular bicarbonate, which is readily utilizable. In addition, the uptake of urea does not require the translocation of either a proton or hydroxyl ion to maintain cell neutrality, which provides a more stable baseline pH for control purposes.

2.1.3.2.1. Urea Production

Urea is used extensively as a fertilizer and is produced commercially from ammonia and CO_2 (Ramírez and Worrell 2006). Current urea production plants are placed near industrial ammonia production facilities. The main energetic cost in producing urea is making ammonia, which accounts for 80% of the energy (Rollinson et al. 2011). This is because facilities produce ammonia from atmospheric nitrogen and hydrogen from natural gas (Schlögl 2003). Urea is then produced using anhydrous ammonia and CO_2 at high pressures in the presence of a catalyst (Gowariker et al. 2009). Current total production rates for urea from all industrial facilities are estimated to be approximately 222 Mt/year (Rollinson et al. 2011). Based on current crude oil production rates and the amount of nitrogen needed for algal biomass production, if all industrially produced urea were utilized for algae to biodiesel, there would only be enough biodiesel or bio-crude to replace approximately 10 to 20 percent of the current crude oil production rate (BP 2014; Chisti 2008). However, Rollinson et al. highlights that the annual human production of urea is more than 400 times the current industrial production rate (2011). This highlights the continued interest in using wastewater to grow algae; however, urea is quickly hydrolyzed in the presence of bacteria producing

ammonium (Udert et al. 2003). The concerns of quantity of industrially produced urea and the rapid degradation of urea in non-sterilized conditions indicate that urea will most likely not be a viable nitrogen source for algae growth.

2.1.3.3. Ammonium

The main concern with growing algae on ammonium is the potential for decreased or inhibited growth (Bongers 1956; Dvořáková-Hladká 1971; Elrifi et al. 1988; Eustance et al. 2013; Guy et al. 1989; Ludwig 1938; Thacker and Syrett 1972). There are two main causes for decreased productivity in algal systems: incorrect pH and carbon limitations.

2.1.3.3.1. Decreasing pH

A major issue associated with algal growth on ammonium is the decrease in pH associated with the translocation of protons out of the cell to maintain cell neutrality during ammonium utilization (Fuggi et al. 1981). Acidification reduces the amount of DIC, exacerbating any existing carbon limitations and prevents algae from maintaining faster growth rates (Eustance et al. 2013). Therefore, algal growth on ammonium has a lower pH limit for optimum growth rate based on the carbonate system set at a pH of 6.35, the point at which bicarbonate becomes the dominant form of carbon in the medium. The actual pH of the medium should be closer to a pH value of 7.0 to 7.5 (depending on ammonium concentration) to allow for increased DIC (Abeliovich and Azov 1976; Norici and Giordano 2002). The release of protons during ammonium uptake is counteracted by reducing a portion of the alkalinity of the water, which is

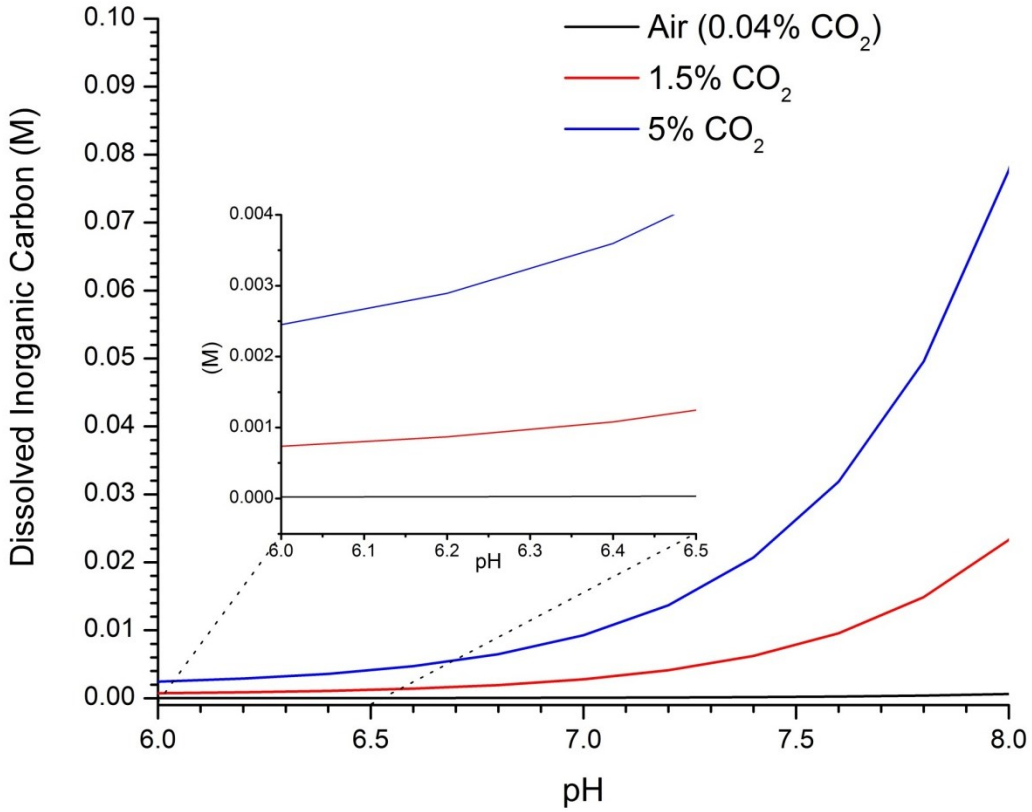

Figure 2.2: Effects of pH and percent CO_2 in gas phase on total DIC. Insert highlights the limited DIC available between pH 6 and 6.5.

accounted for by the carbonate system and natural organic matter (NOM) (Baird and Cann 2005). However, it is important to recognize that not all of the alkalinity can be used to counteract a given concentration of ammonium. The reason for this is alkalinity is traditionally measured by titrating to a pH at which all carbonate/bicarbonate is removed from the medium. This occurs at a pH of approximately 4.5, which previous research has shown that a pH below 5, for non-acidophilic algae, can cause cell-cycle inhibition (Eustance et al. 2013; Xin et al. 2010a). Based on DIC, the pH of a culture should be maintained above 6.35, the point at which bicarbonate is present at an equal concentration to dissolved CO_2. pH levels above this will drastically increase in DIC as shown in Figure 2.2. Furthermore, as with most systems, a constant pH is desired for system stability, therefore, some systems will require the addition alkalinity. In many

cases, for algae, it is suggested that sodium bicarbonate can be utilized to neutralize the pH acidification and provide immediate DIC (Gardner et al. 2012; Gardner et al. 2013; White et al. 2013).

2.1.3.3.2. Effects of Low CO_2 Concentrations

When algae are grown on ammonium, increased levels of CO_2 are required to maintain high levels of growth independent of the medium's pH. This is due to the interactions within the carbon-nitrogen metabolism. When algae are grown on ammonium, the cells increase anaplerotic reactions to replace TCA cycle intermediates that are used for the increase in amino acid synthesis (Elrifi et al. 1988; Norici et al. 2002; Turpin et al. 1991; Vanlerberghe et al. 1990b). This is accomplished by increasing the production of phosphoenolpyruvate carboxylase (PEPC), the main enzyme utilized by chlorophytes in anaplerotic reactions (Norici et al. 2002). In many cases, the carbon requirements associated with anaplerotic reactions compete with carbon utilized for photosynthetic reactions. Increased levels of PEPC increase the amount of carbon diverted to anaplerotic reactions instead of carbon used for photosynthesis and growth (Turpin et al. 1991). This is due to the greater affinity for the carbon exhibited by PEPC compared with RUBISCO (Guy et al. 1989). Therefore, in a low carbon environment, the algal cell will become growth inhibited due to carbon limitation at a lower cell concentration in the presence of ammonium than in other nitrogen sources due to the increased anaplerotic reaction (Eustance et al. 2013). Low carbon can also inhibit ammonium uptake into the cell, as is the case with nitrate, CO_2 must be present for uptake to occur (Amory et al. 1991). Thus to ensure high productivity and effective

bioremediation of nutrients, algae grown on ammonium will require increased CO_2 concentrations.

2.1.3.3.3. Free Ammonia Inhibition

The pKa of ammonium to ammonia is approximately 9.2 depending on temperature (Emerson et al. 1975). High concentrations of ammonium have been linked to algal growth inhibition due to increased concentrations of free ammonia within the medium (Abeliovich and Azov 1976; Azov and Goldman 1982; Peccia et al. 2013). This inhibition can be avoided by keeping the pH below 7.5, which equates to approximately 1.5% of the ammonium being in the free ammonia form or by maintaining lower levels of nitrogen in the system. If the algae are grown on very high concentrations of ammonium that can be found in animal wastewaters, the pH of the medium should be decreased to below 7 to decrease the concentration of free ammonia to approximately 0.5% of the total ammonium concentration. The inhibitory effect of ammonia is strain specific, and most strains show a 50% reduction in growth rate at above 0.5 mM free ammonia (Abeliovich and Azov 1976; Azov and Goldman 1982). Maintaining a pH of seven during photosynthetic activity is obtainable through acidification of the medium with CO_2, which provides additional carbon required for growth on ammonium and reduces ammonia inhibition. In addition to growth inhibition, free ammonia is volatile, which at increasing pH values will transfer to the atmosphere due to the high aeration rates in photobioreactors or high surface area in raceways. This decreases the amount of nitrogen available reducing biomass concentrations and increases the emissions.

2.1.3.3.4. Interaction of Ammonium with Other Nitrogen Sources

It has been shown that ammonium has an inhibitory effect on the utilization of both nitrate and urea (Dortch 1990; Fernández et al. 2004; Molloy and Syrett 1988). Ammonium can inhibit nitrate and urea utilization by limiting the uptake of the other nitrogen sources (Dortch 1990; Ingemarsson et al. 1987; Larsson et al. 1985; Molloy and Syrett 1988). Therefore, it does not seem likely that nitrate can be utilized to balance the proton production of ammonium with the production of hydroxyl ions.

2.1.3.4. Best nitrogen source to study

At this time, many researchers have identified ammonium and urea as two of the few viable nitrogen source for large-scale production of algae (Lundquist et al. 2010; Sedlak 1991). This is due to several key factors. First, incoming municipal wastewater is composed of urea and ammonium, which account for approximately 60% and 40%, respectively, of the total nitrogen available at approximately 40 mg-N*L^{-1} (Sedlak 1991). Urea is an acceptable nitrogen source for algal growth; however, ammonium partially inhibits urea utilization, and urea is rapidly degraded by bacteria into ammonium and bicarbonate (Solomon et al. 2010). Furthermore, large-scale algal facilities will need to use anaerobic digestion or other methods to process the unused algal biomass. This will generate onsite CO_2, energy and a method to recycle nutrients. In methods that recycle nitrogen, ammonium is produced from the degraded proteins (de Boer et al. 2012; Sialve et al. 2009). Therefore, industrial-scale algal facilities will most likely utilize ammonium for growth to help create a closed loop to reduce the cost associated with nutrient input. However, nitrate can be produced from ammonium through the nitrification process that

is completed in most current wastewater treatment facilities. However, nitrification requires high levels of aeration, which will significantly impact the (E)ROI of an algal facility.

2.1.4. Sources for Low Cost Nutrients

Carbon, nitrogen, and phosphorus are readily available nutrients within different waste streams. Measures to curb pollution are becoming increasingly strict, which will require many facilities to evaluate new methods of remediation. It is often suggested that algae can be grown utilizing CO_2 from flue gas and obtain the required nitrogen and phosphorus from wastewaters (Cabanelas et al. 2013; Chen et al. 2012; Garcia et al. 2000; Oswald et al. 1957; Park et al. 2010; Woertz et al. 2009a; Woertz et al. 2009b).

2.1.4.1. What waste streams can be utilized?

One of the major concerns with growing algae for biofuel production is the acquisition of nutrients. Wastewater and flue gas provide significant levels of carbon, nitrogen and phosphorus and are currently being treated without providing any benefit, but algae can utilize both to produce biofuels and other by-products. Wastewater is a general term that can apply to any number of sources including municipalities, agriculture, industrial etc. Since the 1950s researchers have assessed algae's capability to remove nitrogen and phosphorus from municipal wastewater (Ludwig et al. 1951; Oswald et al. 1957; Hoffmann 1998; Cabije et al. 2009; Picot et al. 2009; Woertz et al. 2009a). However, many facilities currently use a one sludge approach to remove nitrogen through nitrification/denitrification, which converts the ammonia to nitrite then nitrate and finally to nitrogen gas (Rittmann and McCarty 2001). This removes a

significant amount of the dissolved nutrients. Flue gas has high levels of CO_2, which is critical for maintaining high growth rates in algae (Brune et al. 2009; Doucha et al. 2005; Douskova et al. 2009). Algae have the capability of reducing the CO_2 levels in flue gas from fossil fuels, which will reduce the amount of new CO_2 entering the atmosphere.

2.1.4.1.1. Carbon Dioxide from Flue Gas

Several studies have assessed the ability of algae to grow on flue gas. This is due to the high concentrations of CO_2 from the combustion of fossil fuels. In addition to being able to utilize the CO_2, algae are also capable of reducing the levels of SO_X and NO_X (Matsumoto et al. 1997; Nagase et al. 1998; Negoro et al. 1991; Yoshihara et al. 1996).

2.1.4.1.2. Reclaimed Water from Municipal

In current wastewater facilities utilizing a nitrification/denitrification process to remove organic nitrogen, the final effluent is lacking sufficient nutrients for algal growth. Analysis of local wastewater systems indicated that the waters had high levels of phosphorus compared to the available nitrogen and iron.

2.1.4.1.3. Anaerobic digester centrate from Municipal sludge

In wastewater treatment plants the two main pathways for incoming usable nutrients is either off gassing as N_2 or incorporation into sludge. In many facilities, the sludge is dewatered and sent to landfill. However, some facilities have started to utilize anaerobic digesters to further process the sludge. The digested sludge is dewatered prior to application as a fertilizer. This water, known as centrate, has a high concentration of

ammonium and contains humic and fulvic acids causing turbidity and a decrease in light transmittance. Therefore, it is proposed to combine the centrate with the reclaimed municipal water for better productivity

2.1.4.1.4. Dairy/Animal Waste

Commercial animal feedlots provide a significant source of poorly managed wastewaters. Currently many facilities use large lagoons, which release ammonia and greenhouse gases (Leytem et al. 2011). However, some facilities are beginning to utilize controlled anaerobic digestion to help capture the methane being released (Park et al. 2010). This also provides a source of CO_2, and the effluent has high levels of ammonium and phosphorus for algae growth and is similar to the centrate found in anaerobic digestion of municipal sludge.

2.2. How are Algae Grown?

Traditionally there are two distinct options for algae growth: raceways and photobioreactors.

2.2.1. Raceways

Algal raceway ponds (Fig. 2.3) were developed in the 50s by Oswald for use in wastewater treatment (Oswald et al. 1957). The simplicity of the raceway has been maintained with only minor enhancements, which allows for easy scalability. Furthermore, raceways look favorable compared to bioreactors in LCA and TEA studies due to the lower capital input and lower energy demand during cultivation. In addition to raceway ponds, facilities have utilized shallow open ponds for the production of β-

carotene (Borowitzka and Moheimani 2013a). However, this is limited to high-value products due to the extremely low productivity and the requirements for high salinity to minimize biological contamination.

Figure 2.3: Photo of large raceway at AzCATI facility in Mesa, Az.

The main tradeoffs in using raceways over photobioreactors are lower productivities and lower biomass densities, which will require more land to grow and process more water to harvest the same amount of biomass. The productivity can be improved if carbon limitation is avoided, the raceway is provided with sufficient mixing, and the culture is operated semi-continuously. Outdoor productivities in raceways have been reported above 40 g/m^2/day, significantly higher than 15 g/m^2/day, which is currently considered a standard productivity for algal LCA/TEAs (Moheimani and Borowitzka 2007). The low biomass density is due to the operating depth of raceways, which is commonly set between 15 and 30 cm (Heussler 1985; Moheimani and Borowitzka 2007; Sompech et al. 2012). This is in stark contrast to current photobioreactors, which tend to have an optimal productivity between 1 and 10 cm in

path length or depth depending on the cultivation parameters (Hu et al. 1998b; Richmond and Cheng-Wu 2001). The limitation in minimum raceway depth is attributed to the increase in energy required to overcome greater frictional losses (Oswald 1988). Oswald calculated the maximum distance between paddlewheels using the Manning equation with different raceway depths and found that by increasing the raceway depth from 10 cm to 30 cm the maximum distance between paddlewheels increased almost 12-fold. In addition, decreasing the raceway operating depth will theoretically increase the raceway temperature due to having a smaller heat capacity (Béchet et al. 2011; Oswald 1988). One way to overcome both of these concerns is the use of an inclined system that feeds into a reservoir as shown in Figure 2.4 (Doucha and Lívanský 1995; Heussler 1985; Waller et al. 2012; Masojídek et al. 2011). This allows gravity to overcome the frictional losses due to the bottom and sides of the channel and can use a sump pump or an Archimedes screw pump in place of the traditional paddlewheel to lift the culture to the beginning of the channel.

Figure 2.4: Left: photo of system in Trebon, Cz (Borowitzka and Moheimani 2013a) Right: photo of ARID system in Tucson, Az.

However, raceways have several concerns that are highlighted when completing a risk assessment. Because the raceways are more open to the environment and have fewer controls compared to bioreactors, raceways tend to have lower productivities, higher

contamination of both predators and competing species, and have higher chances of raceway crashes (Borowitzka and Moheimani 2013a; Borowitzka 1999; Chisti 2007). One of the most common suggestions to eliminate the concerns of predation and contamination is to operate under extremophilic conditions. These conditions include high and low pH, high temperature and high salinity (Moll et al. 2014; Rodolfi et al. 2009; Schenk et al. 2008; Wensel et al. 2014; Selvaratnam et al. 2014). However, extremophiles are not feasible if the environment will require extensive modification to become appropriate for the selected organism. High temperature increases the volatility of ammonia and reduces the solubility of gases. Similarly utilizing an acidophile will limit the transfer of CO_2 by limiting the equilibrium concentration of DIC, which is the driving force for CO_2 transfer. Higher pH levels will allow for an increased DIC in the medium; however, ammonium will need to be dosed to prevent volatilization and toxicity (Wensel et al. 2014). Using extremophiles is limited to existing extreme environments because it would not be feasible to drastically modify natural conditions to cultivate a selected organism.

2.2.2. Bioreactors

Because of the limitations in control and contamination in open raceway systems, there has been significant effort to assess and reduce the energy associated with photobioreactors. Photobioreactors are commonly split into two distinct groups: flat-panel or tubular design.

Flat-panel reactors have been used extensively for research on algae for biofuel production, high-density cultures, high-value products and many other elements due to

their simplicity in design and easy scalability. Over the last few decades flat-panel reactors have increased in complexity as modification have been implemented to improve productivity and efficiency while reducing capital cost (Kumar et al. 2011; Marxen et al. 2006; Slegers et al. 2011; Zhang et al. 2001; Rodolfi et al. 2009; Hu et al. 1998a; Hu et al. 1998c). The main principle behind flat-panel reactors is the use of aeration to provide mixing for the culture. One of the biggest discrepancies in the algal field is determining the optimal light-path length for productivity. Previous research has shown that the optimal productivity can occur between 1 and 10 cm (Hu et al. 1998b; Richmond and Cheng-Wu 2001). Hartig et al. (1988) attempted to correlate optimal biomass productivity to areal density of biomass. The determination was that the optimal density was 45 g-dw/m^2 of illuminated surface. These values indicate that a reactor with a 10 cm light-path length will have the best productivity at 0.45 g/L and a thin flat-panel reactor with a light-path length of 1 cm will have an optimal density of approximately 4.5 g/L. This is a 90 percent reduction of water needed to be removed during harvesting to produce the same quantity of biomass. One of the main reasons for decreasing the light-path length is to reduce the amount of light being attenuated based on the Beer-Lambert law (Eq 2.2) (Blankenship 2002).

$$\boldsymbol{A = \varepsilon c l = log \frac{I_0}{I}} \qquad \textit{Eq 2. 2}$$

Where A is absorption, ε is the extinction coefficient, c is concentration of particles and l is the light path length. This equation shows that to increase the biomass concentration, the light path length needs to decrease for the same amount of light to be attenuated. This has been continuously demonstrated by the differences in the optimal

biomass concentration being used for raceways and photobioreactors (Hu et al. 1998b; Hu et al. 1998c; Gordon and Polle 2007; Singh and Sharma 2012).

A major concern associated with the use of flat-panel reactors is the high capital cost compared to most raceway systems. This has led to research focused on replacing the need for glass or acrylic to maintain shape, and to be replaced with a soft plastic bag held in place with a steel enclosure as shown in Figure 2.5. These systems require significant infrastructure to maintain a constant light path as shown on the left in Figure 2.5 the culture path length fluctuates several centimeters between the rigid posts. This can be minimized by creating a lattice structure as shown in the photo on the right in Figure 2.5 which prevents the flexible plastic from bowing and increasing the light-path length.

Figure 2.5: Left: photo of Pseudocolumn Reactor at AzCATI Facility. Center: photo of research scale flat-panel reactors at AzCATI. Right: Photo modified from Acién Fernández et al. (2013) of design patented by Tredici and Rodolfi to minimize variation in light-path length.

The second design type commonly found in photobioreactors is tubular design as shown in Figure 2.6. These typically consist of an airlift or pump to raise the culture to the top of a long helical tube design before returning to the initial airlift or pump station. One of the main limitations associated with the tubular design is scalability. It is typically estimated that algal culture can flow through approximately 100 m of tubing before the dissolved oxygen levels become inhibiting (Weissman et al. 1988). This indicates that tubular photobioreactors will be designed with a set footprint and scale up would require replication of the module instead of expanding the size of one unit. This can be

problematic, as it will prevent the system from utilizing larger pieces of equipment that would be more efficient. However, because of their closed design, the tubular photobioreactors would be more appropriate for products requiring low contamination such as astaxanthin and omega-3 fatty acids (Molina Grima et al. 2003; Markou and Nerantzis 2013).

Figure 2.6: Tubular photobioreactor photo (Acién Fernández et al. 2013).

For both tubular and flat-panel photobioreactors, one of the largest drawbacks is the requirement for some type of heat exchanger to remove the excess solar energy that is not converted into biomass. This increases the capital cost and the operating costs of the system compared to raceways, which typically only utilize evaporation to maintain lower temperatures.

2.2.3. Trade-off between two pathways

Both raceways and photobioreactors have significantly different advantages and disadvantages. Raceways have lower capital cost, operating cost, and energy requirements that provide a more favorable (E)ROI, but due to the risks associated with lower productivities and higher contamination and raceway crash concerns many people are still in favor of utilizing photobioreactors.

2.3. What Affects Algal Growth?

Previous research has shown that many factors play a significant role in the overall biomass productivities as well as the composition of the biomass. Three of the more significant factors are pH, nutrient source/limitation and CO_2 concentration.

2.3.1. pH

Biomass productivity can be highly variable depending on the pH of the environment. Many strains perform optimally within the range of 6 to 9. However, as previously mentioned growth on ammonium requires a pH below 7.5. Previous research has shown that having a pH that is extremely high (>10) or low (<5) has a significant impact on biomass productivity by causing cell-cycle inhibition (Eustance et al. 2013; Gardner et al. 2012). In many organisms an increase in pH leads to an increase in TAG accumulation (Gardner et al. 2011). Other research has looked at certain species of *Chlorella* in acidic environments caused cessation of growth and the release of maltose into the media (Dorling et al. 1997; McAuley et al. 1996). The critical factor in manipulating the pH for an increase in a desired product requires the cessation of biomass growth, which will most often reduce the overall production rate of the culture.

2.3.2. Nutrient Sources and Limitation

The nitrogen source used for growing algae can have a significant impact on the algal cell and the environment. Growth on ammonium can acidify the medium limiting growth or cause ammonia toxicity at higher pH values. Growth on nitrate will increase the pH of the medium, which is beneficial by increasing the amount of DIC available.

Additionally, growth on ammonium compared to nitrate can change the composition of the biomass and the required level of CO_2 to prevent growth limitations (Eustance et al. 2013; Lourenco et al. 2002).

Removal of nutrients from the medium causes the organism to undergo stress. This forces the algal cell to stop growth and switch to creating higher levels of storage compounds including TAGs or starch. Previous research has shown that limitations in nitrogen, phosphorus, iron and silica can all cause an increase in TAGs (Moll et al. 2014; Liu et al. 2008; Liang et al. 2013; Xin et al. 2010b).

2.3.3. CO_2 concentration and Availability

As previously discussed, the availability of DIC can play a significant role on biomass productivity by being a limited resource. The concern for CO_2 availability is dependent on many factors in the system. One of the biggest concerns is the mass transfer of CO_2 from the gas into the liquid phase, and is the biggest issue with delivering carbon to the culture. The transfer of CO_2 depends on culture temperature, bubble size, column height, total flow rate, culture pH and density, the system being open or closed, and the concentration of CO_2 in the gas phase. Typically, DIC is monitored in algal cultures using pH primarily to determine if DIC is becoming limiting.

2.4. What are the Benefits of using Algae over Terrestrial Plants?

One of the biggest reasons algae have been the focus of research for animal feed and biofuels is the high biomass productivity that can be achieved compared to terrestrial based crops as shown in Table 2.1. The high theoretical limit stems from the efficient use

of 12% of solar energy being converted into biomass. However, taking into account respiration, photorespiration and other metabolic activities drop the efficiency by nearly 30% as shown in Table 2.1 as the lower theoretical limit, which shows a realistic maximum biomass productivity (Melis 2009). In algal growth, yields are traditionally discussed in areal productivity with units of $g/m^2/day$. The high and low theoretical limits correlate to approximately 110 and 77 $g/m^2/day$, respectively. However, Table 2.1 shows that current projects have not been able to reach the theoretical limits with either raceways or reactors. This is mostly due to sub-optimal growth conditions.

Table 2.1: Modified from Williams and Laurens (2010) highlighting the high annual biomass production compared to current terrestrial crops.

Crop	Biomass Productivity (mton/ha/year)	Source
Theoretical Limit High	410	(Williams and Laurens 2010)
Theoretical Limit Low	280	(Melis 2009)
Sugar Cane	74-95	(Williams and Laurens 2010)
Switch Grass	8-20	(Williams and Laurens 2010)
Corn	8-34	(Williams and Laurens 2010)
Alfalfa	6-18	(U.S. Department of Agriculture 2013)
Photobioreactor achieved	182	(Williams and Laurens 2010)
Raceway achieved	60	(Williams and Laurens 2010)

Annual alfalfa productivity within Arizona for 2012 averaged 8.3 tons/acre/year or 18.6 mton/ha/yr (U.S. Department of Agriculture 2013). While the annual productivity currently being achieved by algae in raceway ponds is closer to 60 mton/ha/yr as shown in Table 2.1 modified from Williams and Laurens (2010). 60 mton/ha/yr represents 16 $g/m^2/d$; however, outdoor yields in raceways and bioreactors for short term research projects have reached above 40 and 50 $g/m^2/d$, respectively (Hu et al.

1998a; Moheimani and Borowitzka 2007). Both of these yields were achieved in semi-continuous or continuous harvesting modes, which is ideal for high protein and lower lipids. This also highlights the concern for using batch systems. Log phase growth is significantly higher than the growth rate found during cultivation for lipid accumulation. This has been one of the main driving points for the use of hydrothermal liquefaction (HTL) on whole biomass in place of transesterification of lipids (Frank et al. 2013). This is currently a highly debated topic among professionals in the algal industry.

Algal species are extremely diverse in biochemical composition, cell size and structure, and optimal growth environment. This allows algae to be utilized for a variety of purposes. Algae are currently utilized for production of high value products including astaxanthin, β-carotene, and omega-3s. In addition algae are used for production of agar-agar, carrageenan , and nutritional supplementation (Borowitzka 1997). Current utilization is limited to higher valued products due to the high cost of producing large quantities of algal biomass.

The purpose of this dissertation was to improve the feasibility of growing algae for commercial products. This was done by assessing different technologies including photobioreactors and raceway ponds. Research focused on methods to decrease the energy expenditure in photobioreactors by decreasing aeration requirements at night. Research in raceways focused on the capability of growing algae for lipids or increasing the biomass productivity though semi-continuous growth.

3. MANUSCRIPT 1: THE EFFECTS OF LIMITING NIGHT TIME AERATION ON PRODUCTIVITY AND LIPID ACCUMULATION OF *SCENEDESMUS DIMORPHOUS* TO MINIMIZE AMMONIA VOLATILIZATION

Contributions of Author and Co-Authors

Author: Everett Eustance

Contributions: Conceived the study, collected and analyzed data, and wrote the manuscript

Co-author: Joshua T. Wray

Contributions: Collected data, assisted with study design, discussed results, and edited manuscript.

Co-author: Shahrzad Badvipour

Contributions: Collected data, discussed results, and edited manuscript.

Co-author: Milton R. Sommerfeld

Contributions: Discussed the results and implications, commented and edited the manuscript.

Manuscript Information Page

Everett Eustance*, Joshua T. Wray, Shahrzad Badvipour, Milton R. Sommerfeld
Journal Name: Algal Research
Status of Manuscript:
____Prepared for submission to a peer-reviewed journal
____Officially submitted to a peer-reviewed journal
____Accepted by a peer-reviewed journal
__x_Published in a peer-reviewed journal
Published online April 12th, 2015

*School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ, 85287; Everett.eustance@asu.edu

3.1. Introduction:

It is frequently emphasized that algal cultivation processes must reduce energy consumption and utilize wastewaters as a nutrient source to improve the feasibility of growing algae for biofuels (Peccia et al. 2013; Gendy and El-Temtamy 2013; Borowitzka and Moheimani 2013b). One way to significantly reduce the energy consumption of cultivation is to minimize the mixing and/or aeration of cultures at night. However, reducing aeration/mixing at night and using wastewaters dictate the pH and dissolved oxygen (DO) levels at night, which may affect culture productivity.

3.1.1. Reducing Culture Mixing at Night

In algal cultivation, one of the main energy consumptions is the aeration for photobioreactors and paddlewheels for ponds to maintain high productivity. The amount of aeration/mixing required by algal cultures is significantly lower at night when the cultures are respiring and do not have access to light. Previous research has indicated that the nightly biomass loss associated with respiration is approximately 10% of the previous day's growth rate (Burris 1977; Geider 1992). Lower metabolic activity and minimal mixing required without sunlight suggests that nighttime aeration can be significantly reduced. One of the main issues related to algal production systems is heat loss at night and the resultant decrease in temperature due to the high surface area to volume ratio (SA/V). Multiple researchers have assessed the use of central collecting tanks to reduce radiative heat loss by decreasing the SA/V, which improves daytime culture temperatures and productivities (Doucha and Lívanský 1995; Waller et al. 2012). However, central holding tanks increase the chance of anoxic/anaerobic conditions due to the low SA/V,

which may affect the cultures' productivities. Algal cultures utilize respiration at night for cell maintenance, which leads to anoxic/anaerobic conditions unless there is sufficient oxygen available. Lacking oxygen forces the culture to shift from aerobic respiration to mixed acid fermentation (Atteia et al. 2013; Hirano et al. 1997; Vanlerberghe et al. 1990a). This requires a significant increase in starch consumption to account for the decreased ATP produced from anaerobic respiration associated with the Pasteur Effect (Peavey et al. 1983). Aerobic respiration provides approximately 30 ATP/glucose, while mixed-acid fermentation produces only 5 ATP/glucose (Atteia et al. 2013). Because lipids are too reduced to be utilized as an energy source in anoxic conditions, the algal culture will only be able to consume starch for energy (Müller et al. 2012).

Previous research has utilized dark anaerobic conditions for ethanol or H_2 gas production (Miura et al. 1982; Ohta et al. 1987; Vanlerberghe et al. 1990a; Ueno et al. 1998). Ueno et al. (1998) assessed dark fermentation and found that under anaerobic conditions *Chlorococcum littorale* consumed 23% of its internal storage of starch in the first 12 hours, while Ohta et al. (1987) reported *Chlamydomonas* MGA 161 consumed 53% of its starch in the first 3 hours. However, it is important to note that both sets of experiments were conducted on algae acclimated to continuous light and were not focused on the accumulation of lipids, but on either ethanol or H_2 production.

Miura et al. (1982) is one of the few papers to assess H_2 production by *Chlamydomonas reinhardtii* that was exposed to a light/dark cycle with a shifting aerobic/microaerobic environment, and was found to utilize up to 90% of its stored starch each night. Maeda et al. (1996) showed that in the same organism over the course of 12

hours the starch content under dark anaerobic conditions decreased from 36% to 9% (w/w), while under dark aerobic conditions the organism dropped to 21.6% starch (w/w). However, these papers did not focus on the production of lipid or the change in growth rate associated with cycling between aerobic/anoxic conditions during a diurnal cycle. Further investigation is therefore needed.

3.1.2. Wastewaters

Several wastewater sources have been suggested for algal production, including centrate from municipal anaerobic digesters and dairy lagoon wastewater (DLW). Almost all wastewater sources being considered contain ammonium as the nitrogen source. Previous research has shown that growing algae on ammonium as the nitrogen source in place of nitrate can have an impact on the cell's physiology and lipid content. Furthermore, it changes the environmental conditions necessary for optimal algal growth.

In algal cultures grown on nitrate, high pH levels are desired for increased TAG accumulation and for limiting contamination from predators and competing species (Gardner et al. 2011; Moll et al. 2014). However, high pH is undesirable for growth on ammonium. The equilibrium point between ammonium (NH_4^+) and free ammonia (NH_3) is approximately 9.3 (Emerson et al. 1975). At a pH of 7.3, there is at least 1% of the total ammonia found as free ammonia. This illustrates a significant issue, as free ammonia is both toxic and volatile. From the biological perspective, algae may exhibit lower levels of productivity at higher pH values. This is challenging as many facilities utilize alkalophilic algae such as *Spirulina* and operate at pH values above 9. If these facilities transition from nitrate to ammonium, they will have significant toxicity and

volatilization. Previous research has shown growth inhibition in cultures with a pH greater than 8 and a total ammonia concentration of 2 mM (Abeliovich and Azov 1976). Furthermore, Azov and Goldman (1982) showed that a concentration of 1.2 mM of free ammonia caused a 50 % decrease in carbon assimilation. This suggests that maintaining lower pH values is critical when cultures are grown using ammonium.

In ammonium-based cultures, sufficient buffering capacity or alkalinity is required to prevent acidification of the medium (Eustance et al. 2013; Xin et al. 2010a; Wett and Rauch 2003). In concentrated wastewaters like centrate and DLW with high levels of ammonia, the medium has enough buffering capacity from the alkalinity and natural organic matter to prevent the pH from acidifying due to ammonium uptake by the biomass (Li et al. 2011; Woertz et al. 2009a; Bonmati and Flotats 2003; Eustance et al. 2013). However, centrate and DLW are typically alkaline with pH levels above 8, which increases the concentration of free ammonia causing toxicity and volatilization (Azov and Goldman 1982). During the day, the pH is lowered due to acidification from increased levels of CO_2, which shift the pH closer to that of the CO2/bicarbonate pk_a of 6.35. However, it is not feasible to utilize CO_2 to maintain culture pH at night. Therefore, cultures will have a higher chance of ammonia volatility and toxicity as the medium returns to higher pH values. One method to reduce the impacts of free ammonia at night is to minimize aeration/mixing, which significantly reduces the cultures ability to achieve equilibrium with the lower CO_2 content of the atmosphere by limiting the surface area available for gas transfer. This preserves higher levels of CO_2 in the medium, therefore maintaining a lower medium pH. However, as previously stated, this can cause anoxia and affect culture productivity.

This paper is focused on reducing and removing nightly aeration from cultures grown in flat-panel photobioreactors to assess its impact on culture productivity and biochemical composition of the biomass. The use of flat-panel photobioreactors allows for the monitoring of cultures in a homogenous, controlled environment and provides information on how the algae biomass composition is influenced by the different environmental conditions.

3.2. Materials and Methods

3.2.1. Organisms and Cultivation

All experiments were carried out using *Scenedesmus dimorphous* (Strain LB 0414) cultured in outdoor research grade flat-panel acrylic photobioreactors at the Arizona Center for Algae Technology and Innovation (AzCATI) field-site in Mesa, AZ. The photobioreactors were placed in an array of 24 acrylic flat-panel reactors with North-South facing exposure measuring 46” (1.17 m) in width by 46” (1.17 m) in height and approximately 1.5” (3.8 cm) in depth (thickness) or path length. The panels have an internal volume of 60 L; however, reactors were filled with approximately 55 L of medium to account for the volume required for aeration. Aeration was provided by small drilled holes (~1/32” (0.8 mm)) in 1/2” (1.3 cm) PVC located at the bottom of the reactor at a rate of approximately 0.5 vvm. During daylight hours CO_2 was added to the aeration line to provide a concentration of 1.5% CO_2 (v/v). For two experiments, two tanks were set up with secondary low-flow aeration lines to provide between 0.02 and 0.04 vvm aeration at night. This was completed using 3/8” (9.5 mm) piping with 1/32” (0.8 mm) holes. The reactors contained an internal 1/2" (1.3 cm) stainless steel cooling line

connected to an evaporative cooling system. Cultures were grown in modified BG-11 medium with either ammonium chloride or ammonium bicarbonate as the nitrogen source. Because these experiments utilized tap water, magnesium sulfate, calcium chloride and sodium carbonate were not added to the modified BG-11 medium. For cultures growing on ammonium as the nitrogen source, sodium bicarbonate was dosed to prevent acidification if the source was ammonium chloride; however, sodium bicarbonate was not dosed if ammonium bicarbonate was the nitrogen source. The nitrogen concentration of BG-11medium for experiments was reduced to 25% of its original strength to provide for growth to an optimal culture density capable of depleting the media of nitrogen and reaching maximum lipid potential of the alga in a timely manner. The composition of the modified BG-11medium was 236 mg /L of NH_4Cl (BDH) or 348.8 mg /L of NH_4HCO_3 (J.T. Baker), 9.58 mg/L K_2HPO_4 (BDH), 1.58 mg/L Citric Acid Monohydrate (Sigma-Aldrich), 1.31 mg/L of Ammonium Ferric Citrate (Alfa Aesar), 0.71 mg/L H_3BO_3 (Sigma-Aldrich), 0.445 mg/L $MnCl_2 \cdot 4H_2O$ (Sigma-Aldrich), 0.06 mg/L $ZnSO_4 \cdot 7H_2O$ (Sigma-Aldrich), 0.10 mg/L $Na_2MoO_4 \cdot 2H_2O$ (Sigma-Aldrich), 0.02 mg/L $CuSO_4 \cdot 5H_2O$ (Sigma-Aldrich), 0.1 mg/L $Co(NO_3)_2 \cdot 6H_2O$ (Sigma-Aldrich). Tap water used averaged 180 mg/L Na, 75 mg/L Ca, 70 mg/L S, 24 mg/L Mg, and 8 mg/L K with a hardness of 280 mg /L as $CaCO_3$.

3.2.2. Monitoring

Temperature, pH, and dissolved oxygen (DO) were continuously monitored using a Neptune Apex controller (Neptune Systems, LLC.), which also provided pH control using sodium bicarbonate for cultures grown on ammonium chloride. The Apex also

controlled solenoid valves to turn aeration on and off at programmed intervals and to turn on the secondary aeration line for the continuous low-flow aeration experiment. Due to the lack of multiple DO probes, the decrease in oxygen at night was only monitored in one tank and assumed to have similar levels in all replicate tanks. For experiments with different nightly aeration, the probe was switched between tanks every couple of days.

3.2.3. Analytical

3.2.3.1. Biomass Density

Ash-free dry weight (AFDW) was measured to assess growth performance. Glass filters (VWR 696 glass microfiber 1.2µm) were ashed for 4 hours at 500°C prior to initial weighing. Duplicate samples were collected by filtering 10 to 20 mL per filter (depending on culture density). Samples were placed into an oven at 60°C overnight. The filters with biomass were weighed to determine dry cell weight and then ashed at 500°C for 4 hours to determine the AFDW and ash content of the biomass.

3.2.3.2. Phosphorus and Nitrogen

Nitrogen and phosphorus levels were monitored using flow-injection analysis with a Lachat QuikChem 8500 capable of simultaneous measurement of nitrate, ammonium, and phosphate levels of each sample. Duplicate samples were analyzed.

3.2.3.3. Lipids

Biodiesel potential (BP) was measured using direct transesterification following the procedure "Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by *in situ* Transesterification" outlined by NREL (Van Wychen and Laurens 2013b). It is

important to note that FAME's only account for between 50 and 80 percent of the total lipids that are measured gravimetrically.

3.2.3.4. Carbohydrates

Carbohydrates were measured using the colorimetric method "Determination of Total Carbohydrates in Algal Biomass" developed by NREL (Van Wychen and Laurens 2013a).

3.2.3.5. Proteins

Protein content was determined using Bio-Rad DC Protein Assay (Bio Rad) with γ-globulin as the standard. 1-2 mg of freeze-dried algal biomass was placed in a micro-centrifuge tube with 1 mL of reagent A and heated at 60°C for 60 min, with intermittent vortexing. 100 µL of the resulting solution was transferred into a clear test tube with an additional 500 µL of reagent A and 4.0 mL of reagent B. The samples were vortexed and incubated at room temperature for 15 minutes prior to measuring absorption at 750 nm using a Hach DR 5000 Spectrophotometer.

3.2.4. Experimental Procedures

The experiments were completed at different times throughout the year in duplicate (Table 3.1), except for the control cultures grown in August and September that were grown without replicates as previous experiments had shown insignificant differences among control duplicates. The control cultures were continuously aerated at night with 0.5 vvm (same as daytime aeration), which provided a baseline to account for differences associated with changes in the environment and inoculum. The results cover

Table 3.1: Timeline and description of experiments with different nighttime aeration conditions and ammonium sources. Cultures were grown with continuous aeration (0.5 vvm) during the day.

Experimental Condition	Apr. 8^{th}-17^{th}	Apr. 23^{rd}-May 7^{th}	Aug. 22^{nd} – Sept. 1^{st}	Sept. 14^{th}-24^{th}
Continuous Aeration	NH_4Cl; NH_4HCO_3	NH_4HCO_3	NH_4HCO_3	NH_4Cl; NH_4HCO_3
Intermittent Aeration w/ NH_4HCO_3	-	1 min/ 60 min; 1 min/ 30 min	0.5 min/5 min; 0.5 min/10 min	0.5 min/ 20 min
Low Flow Aeration w/ NH_4HCO_3	-	-	~0.04 vvm	~0.02 vvm
No Aeration	NH_4Cl; NH_4HCO_3	NH_4HCO_3	-	NH_4HCO_3

four types of scenarios for the aeration conditions at night: Continuous aeration, no aeration, intermittent sparging (using 0.5 vmm aeration), and very low continuous aeration (0.02 to 0.04 vvm). The first two sets of experiments were started on April 8, 2014 and April 23, 2014 and operated for 9 and 14 days, respectively. The experiment started on April 8, 2014 consisted of continuous aeration and no aeration grown on either ammonium chloride with pH control or ammonium bicarbonate. The experiment started on April 23, 2014 consisted of no aeration, sparging at either 1 min per 60 min or 1 min per 30 min, and continuous aeration. The second set of experiments started August 22, 2014 and September 14, 2014 and both were run for 10 days. The experiment started on August 22, 2014 consisted of intermittent sparging at either 0.5 min per 5 min or 0.5 min per 10 min, low continuous aeration attempting to maintain 80% DO saturation, and a single continuous aeration as a control. The experiment started on September 14, 2014 consisted of no aeration, intermittent sparging at 0.5 min per 20 min, low continuous aeration attempting to maintain 60% DO, and two, single continuous aeration controls

grown on either ammonium chloride with pH control or ammonium bicarbonate. Cultures were sampled daily for AFDW in the afternoon during log phase and less often during the stressed phases due to slower growth. The biochemical composition of the biomass for the April 23rd experiment was analyzed on days 10 and 14, while the August 22nd experiment had biomass analyzed for days 4, 7 and 10. The September 14th experiment had biomass analyzed for days 3, 7 and 10, where days 3 and 7 were measured at sunset (6:30 pm) and the following sunrise (6:00 am) to determine the impact of the dark cycle on biochemical composition under different aeration conditions. Because these experiments were being completed in an outdoor environment with many variables, the data represent specific time points and utilize control cultures for comparisons to minimize the impact of uncontrolled variables on the data analysis.

3.3. Results:

3.3.1. Comparison of Full and No Aeration at Night

To determine the impact of eliminating aeration at night, cultures were grown under four different conditions: the cultures were grown in duplicate with either ammonium bicarbonate (NH_4HCO_3) or ammonium chloride (NH_4Cl) with sodium bicarbonate ($NaHCO_3$) pH control and with or without aeration at night. Figure 1 shows the pH for the different conditions during one representative night of each experiment. In the presence of aeration, cultures grown on NH_4HCO_3 had a higher pH, averaging between 8.2 and 8.5 during the night associated with the increase in alkalinity provided from HCO_3^-, while growth on NH_4Cl tended to have a slightly lower pH (ca. 8.0).

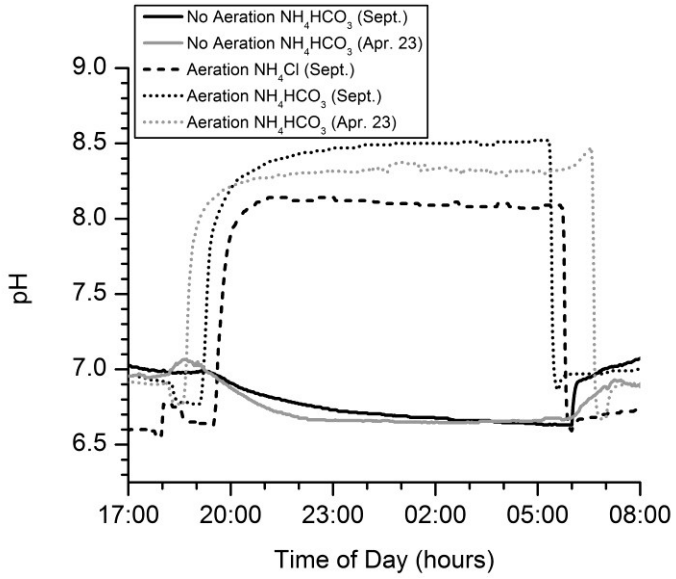

Figure 3.1: Mean nighttime pH for duplicate cultures grown in the log phase in April and September under different aeration conditions: no aeration at night, continuous aeration grown on NH_4Cl, and continuous aeration grown on NH_4HCO_3.

Figure 1 also shows the cultures without aeration at night in an attempt to eliminate ammonia volatilization and minimize ammonia toxicity. This was accomplished by turning off aeration prior to CO_2 being shut off at night. This minimized the gas transfer with the atmosphere and slowed the off-gassing of excess CO_2, which helped maintain a lower pH (Figure 3.1). The pH in cultures grown on ammonium without aeration did not increase. Instead, the cultures exhibited an initial pH acidification corresponding to decrease in dissolved oxygen (DO), which was attributed to respiration (Figure 3.2). However, the acidification continued after DO was eliminated in the cultures, indicating the alga was undergoing anaerobic respiration. Figure 3.3 shows the AFDW for cultures aerated and non-aerated at night and suggests that there is minimal impact to the biomass productivity during the log phase, but that cultures without aeration tend to have lower biomass densities during the stress phase.

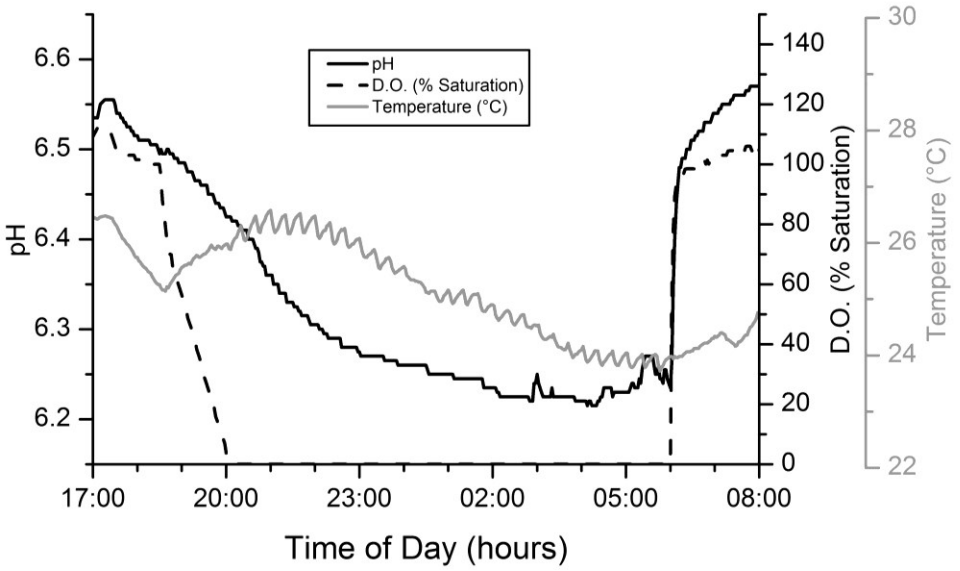

Figure 3.2: pH, dissolved oxygen, and temperature in a non-aerated at night culture during the log growth phase.

Figure 3.3 also shows ammonium concentrations in the media, and highlights that cultures with aeration removed the ammonia from the media at a faster rate, most likely due to ammonia off-gassing since the cultures had comparable growth rates. Table 3.2 shows the biochemical composition of the biomass at different time points for cultures continuously aerated and not aerated at night. The cultures were sampled during log phase, late log/early stress phase, late stress phase and at the time of harvest. It is important to note that the late stress phase for the April 23rd experiment is the same number of days for the harvest samples in the other experiments. Table 3.2 shows that the aerated cultures at the time of harvest had a higher lipid composition.

To verify that cultures being aerated at night exhibited ammonia volatility, a negative control was completed where panels were either aerated or not aerated with tap water and 45 mg-N/L, as NH_4HCO_3, to monitor the change in concentration during two consecutive nights without biomass assimilation. This time period was selected as experiments tended to have significant levels of ammonium in the medium during the

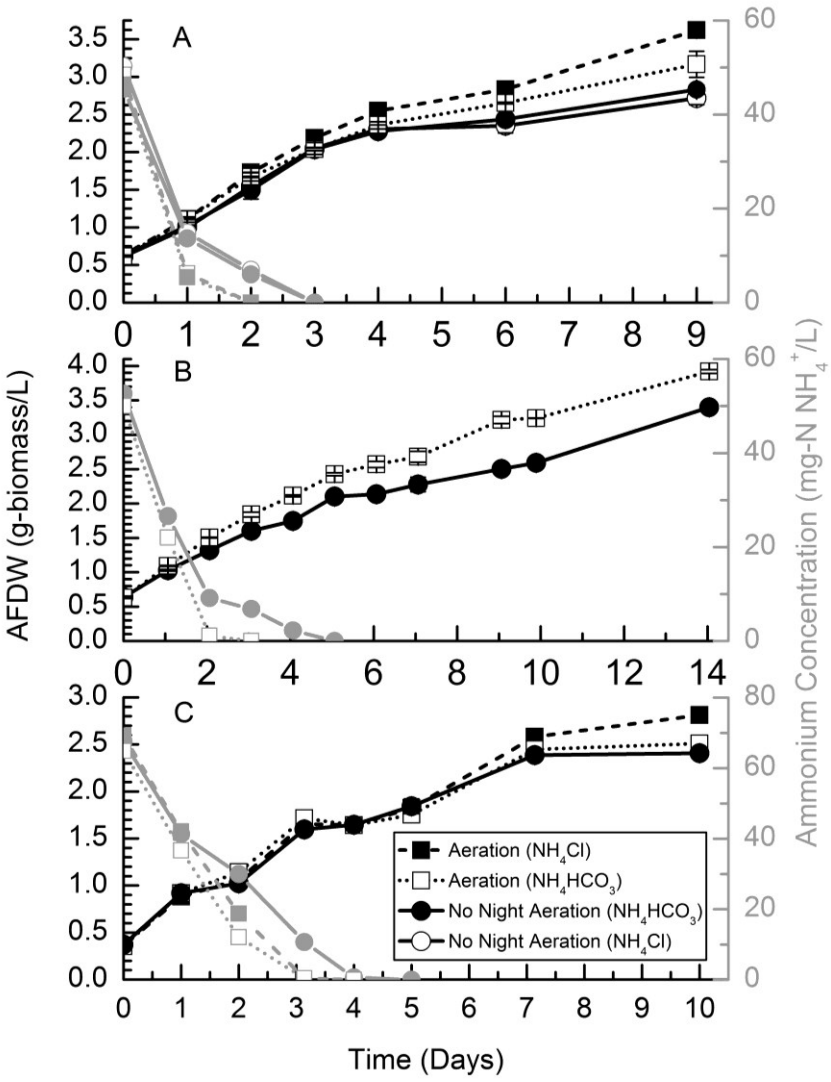

Figure 3.3: Mean and min-max values for AFDW (left y-axis with black lines) and ammonium concentration (right y-axis with gray lines) for experiments conducted in duplicate starting A) April 8 B) April 23 and C) September 14, 2014. Cultures were grown with either continuous aeration or no aeration at night, and grown on either NH4HCO3 or NH4Cl.

first two nights. The results showed that cultures with aeration at night had lost 6.8 ± 3.6% of the total ammonia, while cultures without aeration did not show any change in concentration. The large variability may be attributable to differences in aeration rate and bubble size possibly due to limits in accurate aeration rate settings and calcification from hard water, which can decrease the size of the aeration orifices, resulting in one culture losing 4.3% of the total ammonium, while the other lost 9.3%.

Table 3.2: Biochemical composition of strain LB 0414 with and without aeration at night during different growth phases. All experiments were completed in duplicate with the Avg ± S.D. being reported except for continuous aeration completed in August, which was a single culture. The two individual controls in September were combined to be reported as Avg ± S.D.

Experiment	Phase	Time of Day	AFDW g-biomass/L	Protein % DW	Carbs % DW	BP % DW
No Aeration[1,3]	Log	Sunset[3]	1.60 ± 0.06	20.5 ± 1.9	46.4 ± 1.8	9.9 ± 0.6
		Sunrise[3]	1.47 ± 0.04	20.6 ± 0.3	37.7 ± 1.4	10.3 ± 0.2
	Early Stress	Sunset[3]	2.39 ± 0.01	16.1 ± 0.2	46.5 ± 1.2	18.5 ± 0.8
		Sunrise[3]	2.24 ± 0.03	16.8 ± 0.7	46.5 ± 1.7	19.2 ± 0.3
	Late Stress	Afternoon[1]	2.59 ± 0.03	15.5 ± 0.2	42.9 ± 1.1	21.5 ± 1.1
	Harvest	Afternoon[1]	3.40 ± 0.06	15.4 ± 0.4	37.9 ± 1.4	26.8 ± 1.0
		Afternoon[3]	2.41 ± 0.00	14.4 ± 0.3	39.0 ± 1.1	23.0 ± 0.9
Continuous Aeration [1,2,3]	Log	Afternoon[2]	1.76	27	42	11
		Sunset[3]	1.68 ± 0.04	21.7 ± 0.5	41.4 ± 2.2	10.2 ± 0.1
		Sunrise[3]	1.57 ± 0.03	23.2 ± 1.9	39.3 ± 3.8	12.9 ± 0.7
	Early Stress	Afternoon[2]	2.07	16	37	27
		Sunset[3]	2.51 ± 0.09	17.0 ± 0.5	37.6 ± 2.1	26.4 ± 1.3
		Sunrise[3]	2.31 ± 0.13	18.6 ± 0.4	30.6 ± 1.1	29.3 ± 0.7
	Late stress	Afternoon[1]	3.24 ± 0.00	17.6 ± 1.5	26.5 ± 3.6	35.7 ± 2.1
	Harvest	Afternoon[1]	3.92 ± 0.04	14.6 ± 0.5	24.7 ± 0.6	35.5 ± 1.1
		Afternoon[2]	2.25	16	32	33
		Afternoon[3]	2.66 ± 0.21	16.6 ± 0.3	26.4 ± 2.7	34.4 ± 1.0

[1] Experiment started on April 23, Late stress = Day 10; Harvest = Day 14
[2] Experiment started on August 22, Log = Day 4; Early Stress = Day 7; Harvest = Day 10
[3] Experiment started on September 14, Log = Day 3; Early Stress = Day 7; Harvest = Day 10

3.3.2. Effects of Intermittent Sparging

To determine the effects of intermittent sparging, culture tanks were set up with different aeration/sparging conditions. Tanks were run in duplicate with intermittent sparging of 1 min per 60 min, 1 min per 30 min, 0.5 min per 20 min, 0.5 min per 10 min, or 0.5 min per 5 min. Figure 3.4 shows that the pH for the intermittently sparged cultures fluctuated due to the introduction and removal of O_2 and CO_2, respectively. Furthermore, the pH of the cultures increased throughout the night as the level of CO_2 in the medium approached equilibrium with air as shown by the continuously aerated cultures.

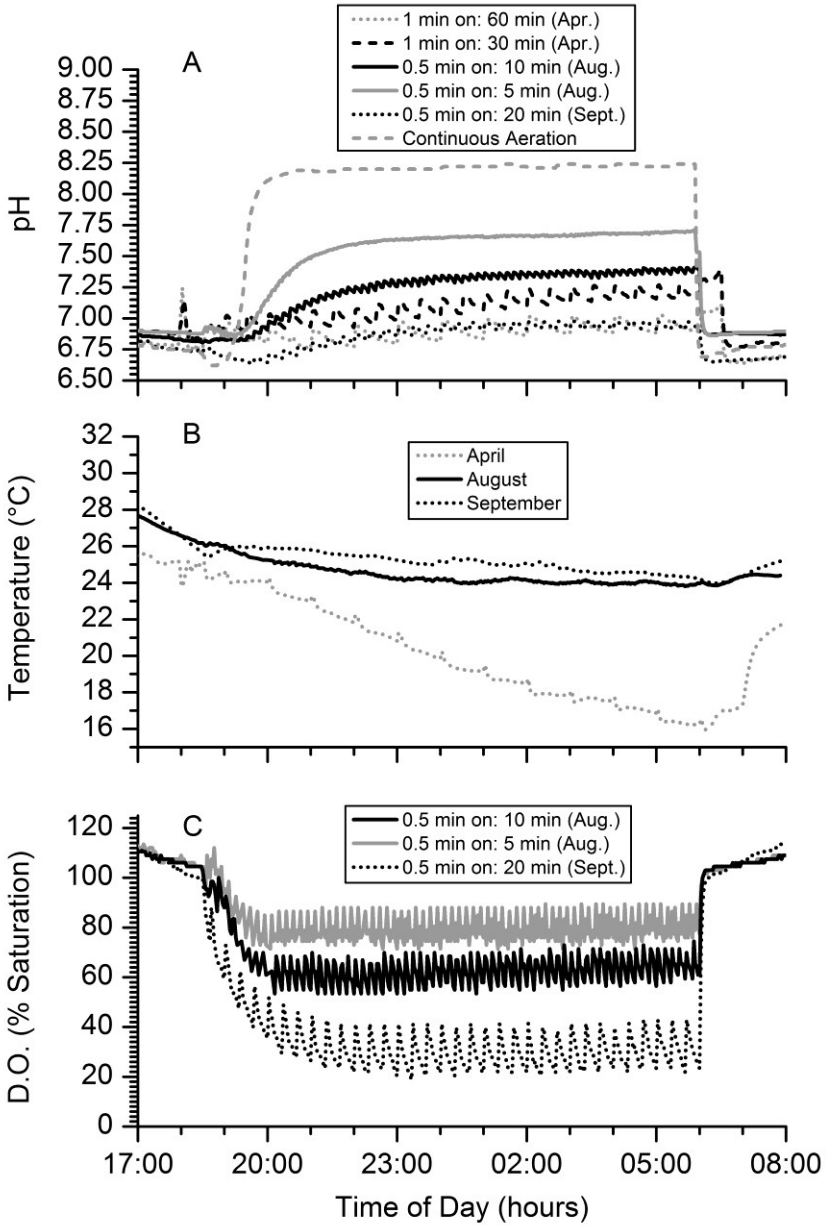

Figure 3.4: A) Nighttime pH for log phase cultures grown with different sparging frequencies at night: 1 min/60 min, 1 min/30 min, 0.5 min/20 min, 0.5 min /10 min, 0.5 min /5 min, and continuous aeration. B) Nighttime temperature for cultures grown in April, August, and September. C) Dissolved oxygen for cultures sparged 0.5 min /5 min and 0.5 min /10 min grown in August, and 0.5 min/20 min grown in September.

Figure 3.4 shows DO levels for three intermittent sparging conditions: 0.5 min: 20 min, 0.5 min: 10 min and 0.5 min: 5 min, which showed that the DO % saturation fluctuated by 15 to 20 %, with averages of 35, 60, and 80 % saturation at night, respectively. The

graph also indicates that the rate of respiration was higher during the first few hours as the DO decreased at a faster rate than the intermittent sparging can replace initially, but by the middle of the night, the DO remained within a similar DO % saturation range. Figure 3.5 shows that the different sparging frequencies had little impact on the AFDW compared to the continuous aeration cultures. However, Table 3.3 shows that the cultures lagged behind in lipid accumulation. Figure 3.5 also shows a significant difference in growth for the August 22nd experiment. This is attributed to unfavorable environmental conditions, including high culture temperatures (i.e., Strain LB 0414 does not perform well above 30°C).

Table 3.3: Biochemical composition of strain LB 0414 with different sparging frequencies at night during different growth phases. All experiments were completed in duplicate with the Avg ±S.D. being reported.

Experiment (min:min)	Phase	Time of Day	AFDW g-biomass/L	Protein % DW	Carbs % DW	BP % DW
1.0:60[1]	Late stress	Afternoon	3.23 ±0.09	16.9 ± 0.7	35.9 ± 0.9	26.7 ± 1.5
	Harvest	Afternoon	3.91 ±0.10	15.2 ± 0.6	30.4 ± 1.1	33.5 ± 2.6
1.0:30[1]	Late stress	Afternoon	3.37 ±0.27	14.9 ± 0.5	32.0 ± 0.6	32.0 ± 1.1
	Harvest	Afternoon	4.11 ±0.19	13.2 ± 0.6	28.0 ± 1.9	36.4 ± 1.6
0.5:20[3]	Log	Sunset	1.61 ±0.04	21.7 ± 0.7	43.5 ± 1.4	10.2 ± 0.4
		Sunrise	1.47 ±0.03	25.3 ± 2.4	37.0 ± 3.1	11.8 ± 0.6
	Early Stress	Sunset	2.45 ±0.03	16.2 ± 0.8	42.0 ± 1.6	22.4 ± 0.5
		Sunrise	2.42 ±0.13	17.0 ± 0.3	39.5 ± 2.2	24.2 ± 1.4
	Harvest	Afternoon	2.69 ±0.09	14.8 ± 0.6	34.6 ± 1.8	28.6 ± 1.6
0.5:10[2]	Log	Afternoon	1.56 ±0.10	27.7 ± 1.6	43.4 ± 3.7	10.6 ± 0.8
	Early Stress	Afternoon	2.06 ±0.11	17.9 ± 1.0	38.1 ± 4.5	23.0 ± 0.6
	Harvest	Afternoon	2.12 ±0.11	15.4 ± 0.3	35.4 ± 2.9	26.1 ± 0.9
0.5:5[2]	Log	Afternoon	1.59 ±0.13	26.8 ± 1.5	40.7 ± 3.1	10.5 ±1.0
	Early Stress	Afternoon	1.99 ±0.22	16.4 ± 0.3	38.5 ± 3.6	23.0 ± 1.0
	Harvest	Afternoon	2.02 ±0.11	15.3 ± 0.8	37.2 ± 0.8	27.6 ± 1.5

[1] Experiment started on April 23, Late stress = Day 10; Harvest = Day 14
[2] Experiment started on August 22, Log = Day 4; Early Stress = Day 7; Harvest = Day 10
[3] Experiment started on September 14, Log = Day 3; Early Stress = Day 7; Harvest = Day 10

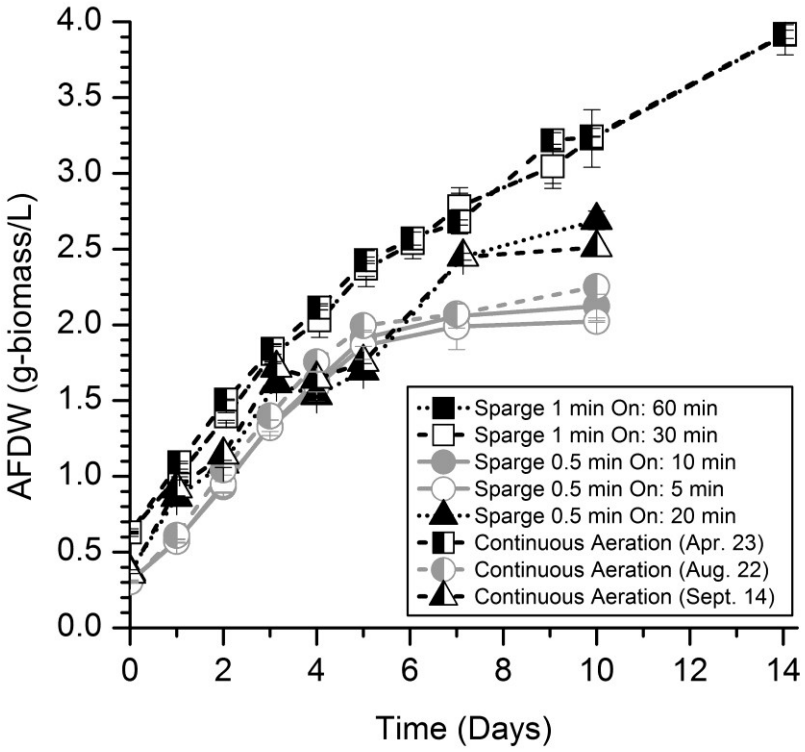

Figure 3.5: Mean and min-max values for AFDW of cultures grown in April, August or September being sparged at different intervals. Cultures grown in April (squares) were the continuous aeration control, 1 min/hour and 1 min/30 min. Cultures grown in August (circles) were continuous aeration control, 0.5 min/10 min and 0.5 min/5 min. Cultures grown in September (triangles) were the continuous aeration control and 0.5 min/20 min. Controls in August and September did not have replicates.

3.3.3. Effects of Continuous Low-Flow Aeration at Night

To determine the effects of continuous low-flow aeration at night, which consisted of a secondary aeration line providing between 0.02 and 0.04 vvm, duplicate tanks were operated with DO saturations maintained near 60% or 80%. Values lower than these were hard to achieve due to equipment limitations. Figure 3.6 shows the pH, temperature, and DO for a respective night during the log phase of growth, which compared to the intermittently sparged cultures, provided a more consistent DO saturation. Figure 3.7 shows that the cultures have a similar AFDW compared to cultures

with high levels of aeration at night, but Table 3.4 shows that the cultures did not reach the same level of lipid accumulation.

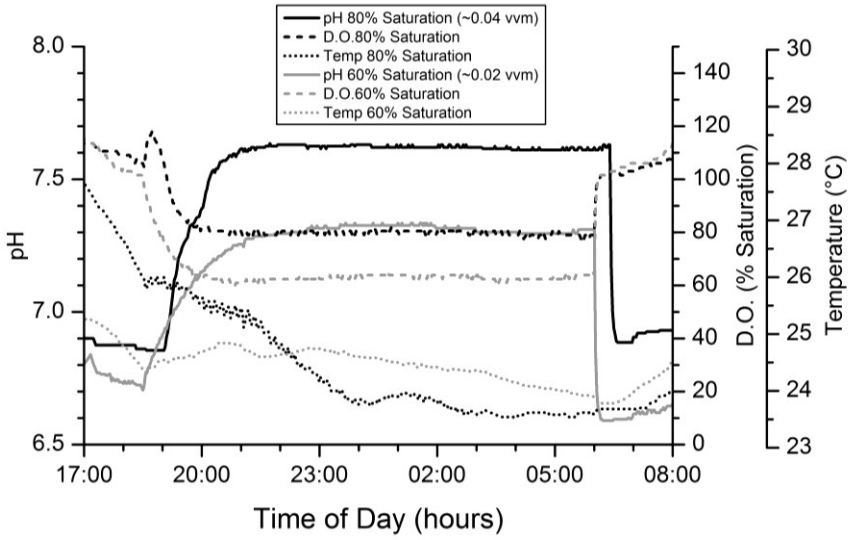

Figure 3.6: pH, dissolved oxygen, and temperature for log phase cultures grown with low-flow aeration at night to provide either 80% DO saturation (~0.04 vvm) or 60% DO saturation (~0.02 vvm).

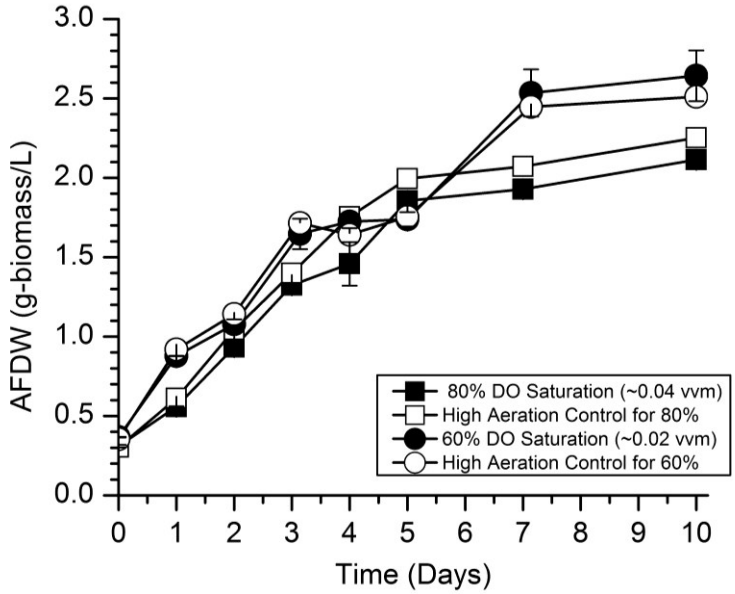

Figure 3.7: Mean and min-max values for AFDW for low-flow aeration at night cultures completed in duplicate with either 80 % DO saturation or 60 % DO saturation. For comparison, continuous high-flow aeration cultures are provided as controls for 80% and 60%.

Table 3.4: Biochemical composition of strain LB 0414 with low aeration (approximately 60% or 80% DO saturation) at night during different growth phases. All experiments were completed in duplicate with the Avg ±S.D. being reported.

Experiment	Phase	Time of Day	AFDW g-biomass/L	Protein % DW	Carbs % DW	BP % DW
60% (~0.02vvm)[3]	Log	Sunset	1.65 ±0.14	20.4 ± 2.5	41.4 ± 1.8	9.6 ± 0.0
		Sunrise	1.53 ±0.05	23.7 ± 1.0	36.9 ± 1.8	11.5 ± 0.9
	Early Stress	Sunset	2.53 ±0.21	15.9 ± 0.3	40.3 ± 1.3	23.3 ± 0.1
		Sunrise	2.30 ±0.16	16.8 ± 1.1	37.5 ± 1.0	24.4 ± 0.3
	Harvest	Afternoon	2.64 ±0.23	14.9 ± 0.3	33.5 ± 1.6	30.3 ± 0.4
80% (~0.04vvm)[2]	Log	Afternoon	1.45 ±0.19	25.7 ± 3.3	41.9 ± 3.2	10.3 ± 0.2
	Early Stress	Afternoon	1.93 ±0.04	16.0 ± 0.6	39.5 ± 4.1	20.9 ± 0.3
	Harvest	Afternoon	2.11 ±0.04	14.8 ± 1.1	37.4 ± 3.7	27.3 ± 0.5

[2] Experiment started on August 22, Log = Day 4; Early Stress = Day 7; Harvest = Day 10
[3] Experiment started on September 14, Log = Day 3; Early Stress = Day 7; Harvest = Day 10

3.4. Discussion:

3.4.1. Anoxic/Anaerobic Respiration

Aeration/mixing is critical for algal cultivation because it provides a well-mixed, homogeneous environment and increases CO_2 transfer for rapid growth. However, high levels of aeration and mixing at night increases ammonia volatility and toxicity. This can be minimized by reducing or eliminating nightly aeration, but also increases the chance of anoxic culture conditions. Previous literature has assessed the use of dark anaerobic conditions for a fermentative process from algae to produce ethanol and H_2; however, these studies did not assess the impact on final lipid accumulation (Ueno et al. 1998; Miura et al. 1982; Peavey et al. 1983; Ohta et al. 1987; Maeda et al. 1996). In the current study, total carbohydrates (but not starch) was measured and showed significantly smaller drops in carbohydrates from 46.4% to 37.7% under dark anaerobic conditions and from 41.4 to 39.3% under dark aerobic conditions compared to the studies completed by Miura

et al. (1982), Ueno et al. (1998), and Ohta et al. (1987). Furthermore, the levels of total carbohydrates remained significantly higher in non-aerated at night cultures during nutrient depletion (38.2 ± 1.3% compared to aerated cultures at 25.6 ± 2.0%), which may also explain the lower biodiesel potential (BP) shown in Table 3.2. Starch is preferentially accumulated over lipids due to the increased efficiency of producing starch from the C_3 pathway (Johnson and Alric 2013). In order for lipid production to occur, algae produce a two-carbon molecule from a three-carbon pathway resulting in an initial energy loss of 33%. This is a significant loss in exponentially growing organisms where carbon and energy from light are already limiting resources for growth. However, lipids are preferentially stored over starch in Strain LB 0414 as shown in previous research and in Tables 2, 3 and 4, where the cultures increased in BP and decreased in carbohydrate content during extended periods of nutrient depletion (Gardner et al. 2013; Fernandes et al. 2013). When starch is consumed for energy it produces 5.3 ATP per carbon fixed during glucose oxidation, while fatty acids produce 6.7 ATP per carbon during β-oxidation (Johnson and Alric 2013). The higher ratio of energy to mass of fatty acids may explain why algae entering nutrient stress will increase lipid accumulation in preparation for an extended period of nutrient deprivation. As nutrient depletion occurs, the algal cell-cycle becomes arrested since there is not enough total protein or RNA/DNA precursors to commit to the next step in cell division, possibly allowing for the organism to waste energy shunting carbon to lipid production (Zachleder et al. 2002; Fernandes et al. 2013). This is seen macroscopically as the algal culture shifts from a bright green color to brown as chlorophyll content decreases, biomass productivity decreases, and lipid content increases (Gardner et al. 2013). However, the cultures that underwent

extended periods of anoxia at night did not exhibit the traditional color transition and maintained a dark green color closer to that associated with the late log growth phase. This lack of transition was also observed in the analytical data (Table 3.2), where anoxic cultures had higher total carbohydrate and protein content at the end of the experiment than cultures with DO at night.

The current study shows that cultures grown seasonally, in April and September, with intermittent sparging had a delay in lipid accumulation, but were still able to achieve similar biodiesel potential as cultures with continuous aeration by the end of the experiments. In addition, cultures without aeration during the night did show an increase in lipid content from nutrient replete to nutrient deplete conditions (Table 3.2), suggesting that if the experiments had been allowed to proceed for an extended period of time the cultures could eventually reach the same level of biodiesel potential.

In addition to higher starch requirements, the delay in lipid accumulation in panels without aeration at night can also be attributed to the lower rates of ammonium uptake (Figure 3.2). This lower uptake rate occurs due to the lack of respiration needed for traditional ammonium assimilation (Vanlerberghe and Turpin 1990; Boussiba et al. 1984). However, cultures are able to assimilate small amounts of nitrogen by utilizing starch and creating alanine as a fermentative product (Vanlerberghe et al. 1991). Both the delay in nitrogen depletion and the additional consumption of starch at night resulted in a significant delay in lipid accumulation.

3.4.2. Aeration

Figures 4 and 6 showed that there are alternative methods for maintaining DO, while preventing the pH from increasing enough to allow for significant ammonia volatility and toxicity. The level of continuous aeration necessary to maintain between 60 and 80% DO saturation was below 0.04 vvm (daytime aeration was 0.5 vvm) and the intermittent sparging resulted in an overall aeration reduction between 90 and 98%. This drastically reduces aeration and resultant energy consumption, suggesting that the cultures need less than 10% of the daytime aeration to maintain high levels of productivity. Results from Table 3.2 indicates that by eliminating aeration at night the culture showed a decrease in final lipid accumulation of more than 36.4 ± 3.7% compared to cultures with continuous aeration. However, if cultures were intermittently sparged the impact on the final lipid accumulation ranged from no difference to a decrease of 21.6 ± 0.8%. It is important to note that the frequency of the intermittent sparging showed that cultures grown in April with a frequency of 1 min/hour and 1 min/30 min had a lipid content closer to that of the respective control cultures than in the more frequent interval sparging completed in August and September (Tables 2 and 3). This may be due to the cultures' temperature at night, which in April averaged below 20°C and in August and September averaged closer to 24°C (Figure 3.2). When the culture temperature was higher at night, higher levels of respiration may have occurred.

In industrial systems, CO_2 will need to be added in a different method than coarse bubble aeration to significantly improve utilization efficiency. Some authors have suggested the use of secondary membrane spargers for CO_2, while having coarse aeration

bubbling to provide mixing. This utilizes the principle that coalescence does not occur as fast in water due to the presence of algae (Eriksen et al. 1998; Poulsen and Iversen 1999). These membrane spargers can be designed to also add oxygen to the cultures at night to prevent anoxia. Another option is to control the flow rate of coarse aeration as was done in this study. This methodology showed a very consistent DO level as shown in Figure 3.6. However, it may be more economically feasible to utilize solenoids to periodically sparge the cultures as was shown in Figure 3.4. This method provides less control over the DO level, but Table 3.3 showed that the lipid productivity was similar to that with the continuous low flow as long as the culture never reached anoxia. The need to maintain DO in the algal cultures at night is critical and is important information for creating innovative designs that minimize temperature loss at night (Doucha and Lívanský 1995; Waller et al. 2012). The use of central collecting tanks will increase the chance of anoxic conditions and will require similar monitoring and aeration as shown in this study.

3.4.3. pH and Ammonium Volatilization

Utilizing NH_4Cl with $NaHCO_3$ can assist in maintaining a lower pH than NH_4HCO_3 during the night in the aerated cultures. This was accomplished by maintaining a lower alkalinity in the culture because the two different ammonium sources utilize bicarbonate (alkalinity) to counteract the acidification from ammonium uptake. Utilizing NH_4HCO_3 instead of dosing bicarbonate with NH_4Cl increased the alkalinity during the beginning of the experiment and increased the pH at night, thereby increasing the ammonia volatility rate. This is emphasized in the AFDW (Figure 3.2), which shows a difference ($p = 0.07$ in t-test with unequal variance) in the final biomass concentration

for growth on NH_4Cl and NH_4HCO_3, and an increase in final AFDW of 12.5%. This may suggest that the culture lost 12.6 ± 5.4% of its available ammonium to volatilization. However, in a negative control experiment, culture media without biomass showed that over two days 6.8 ± 3.6% of the available ammonia was volatilized, indicating other factors not being accounted might have impacted the cultures. For example, the difference could be due to changes in aeration and bubble size associated with system operation and changing characteristics of the aeration system over time.

By limiting the pH of the cultures at night, the overall biomass productivity was greater. This is expected as ammonia losses were estimated to be more than 5% in this study and other studies have suggested that in open ponds without CO_2 addition ammonia losses can be anywhere from 10 to 40% depending on pH (Park et al. 2011; Páez-Osuna et al. 1997; Nunez et al. 2001). This indicates that in an actively aerated/mixed culture, significant ammonia loss can occur at night when the pH exceeds 8.3.

Ammonia air stripping has been extensively studied, and results show that when the pH is between 8.5 and 9 and the system is operated at higher temperatures the removal efficiency ranges from 10 to 30% (Bonmati and Flotats 2003; Guštin and Marinšek-Logar 2011; Heggemann et al. 2001; Liao et al. 1995; Quan et al. 2009; Yoon et al. 2008). However, one of the main differences between the previous experiments and ammonia in aerated algal cultures is time. Most of the previous experiments focused on the removal of ammonia over the course of 1-3 hours, whereas algal cultures (with higher levels of ammonia) will experience multiple nights at a pH where 10 to 15% of the total

ammonia is unionized (Emerson et al. 1975). This will allow inefficient removal and for significant volatilization to occur, especially in highly concentrated wastewater sources.

3.5. Conclusions:

- Dissolved oxygen is required for algal biomass to accumulate lipids
- Lack of aeration of algae cultures at night resulted in anoxic conditions and anaerobic respiration, which reduced the rate of lipid accumulation and nitrogen uptake
- Cultures that were intermittently sparged at night showed a delay in lipid accumulation
- Intermittent sparging decreased aeration requirements by greater than 95% at night
- Cultures without aeration had a decrease in final BP content greater than 36.4 ± 3.7% compared to continuously aerated cultures
- Cultures with intermittent sparging had a decrease in final lipid content between 0 and 21.6 ± 0.8%, depending on how often the cultures were sparged
- Nighttime medium temperatures below 20°C in the intermittently sparged (1 min: 60 min and 1 min: 30 min) cultures had similar lipid accumulations to cultures with higher nighttime temperatures (~ 24°C) and smaller sparging timespans (0.5 min: 20 min to 0.5 min: 5 min)

3.6. Acknowledgements

The authors would like to acknowledge the Laboratory for Algae Research and Biotechnology and the Arizona Center for Algae Technology and Innovation for intellectual and equipment support and to specifically thank Sarah Arrowsmith for assistance in performing analytical assessments. Funding was provided by the Arizona Board of Regents (Regents' Innovation Fund).

3.7. References

Abeliovich A, Azov Y (1976) Toxicity of ammonia to algae in sewage oxidation ponds. Appl Environ Microbiol 31 (6):801-806

Atteia A, van Lis R, Tielens AGM, Martin WF (2013) Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochim Biophys Acta 1827 (2):210-223

Azov Y, Goldman JC (1982) Free ammonia inhibition of algal photosynthesis in intensive cultures. Appl Environ Microbiol 43 (4):735-739

Bonmati A, Flotats X (2003) Air stripping of ammonia from pig slurry: characterisation and feasibility as a pre- or post-treatment to mesophilic anaerobic digestion. Waste Manage 23 (3):261-272

Borowitzka MA, Moheimani NR (2013) Sustainable biofuels from algae. Mitig Adapt Strateg Glob Change 18 (1):13-25

Boussiba S, Resch CM, Gibson J (1984) Ammonia uptake and retention in some cyanobacteria. Arch Microbiol 138 (4):287-292

Burris JE (1977) Photosynthesis, photorespiration, and dark respiration in eight species of algae. Mar Biol 39 (4):371-379

Doucha Jí, Lívanský K (1995) Novel outdoor thin-layer high density microalgal culture system: Productivity and operational parameters. Arch Hydrobiol Suppl 76:129-147

Emerson K, Russo RC, Lund RE, Thurston RV (1975) Aqueous ammonia equilibrium calculations: Effect of pH and temperature. J Fish Res Board Can, 32 (12):2379-2383

Eriksen N, Poulsen B, Lønsmann Iversen JJ (1998) Dual sparging laboratory-scale photobioreactor for continuous production of microalgae. J Appl Phycol 10 (4):377-382

Eustance E, Gardner RD, Moll KM, Menicucci J, Gerlach R, Peyton BM (2013) Growth, nitrogen utilization and biodiesel potential for two chlorophytes grown on ammonium, nitrate or urea. J Appl Phycol 25 (6):1663-1677

Fernandes B, Teixeira J, Dragone G, Vicente AA, Kawano S, Bišová K, Přibyl P, Zachleder V, Vítová M (2013) Relationship between starch and lipid accumulation induced by nutrient depletion and replenishment in the microalga Parachlorella kessleri. Bioresour Technol 144 (0):268-274

Gardner R, Peters P, Peyton B, Cooksey K (2011) Medium pH and nitrate concentration effects on accumulation of triacylglycerol in two members of the Chlorophyta. J Appl Phycol 23 (6):1005-1016

Gardner RD, Lohman E, Gerlach R, Cooksey KE, Peyton BM (2013) Comparison of CO_2 and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. Biotechnol Bioeng 110 (1):87-96

Geider R (1992) Respiration: Taxation without representation? In: Falkowski P, Woodhead A, Vivirito K (eds) Primary Productivity and Biogeochemical Cycles in the Sea, vol 43. Environmental Science Research. Springer US, pp 333-360

Gendy TS, El-Temtamy SA (2013) Commercialization potential aspects of microalgae for biofuel production: An overview. Egypt J Pet 22 (1):43-51

Guštin S, Marinšek-Logar R (2011) Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. Process Saf Environ Prot 89 (1):61-66

Heggemann MH, Warnecke HJ, Viljoen HJ (2001) Removal of ammonia from aqueous systems in a semibatch reactor. Ind Eng Chem Res 40 (15):3361-3368

Hirano A, Ueda R, Hirayama S, Ogushi Y (1997) CO2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. Energy 22 (2–3):137-142

Johnson X, Alric J (2013) Central carbon metabolism and electron transport in *Chlamydomonas reinhardtii*: Metabolic constraints for carbon partitioning between oil and starch. Eukaryot Cell 12 (6):776-793

Li Y, Chen Y-F, Chen P, Min M, Zhou W, Martinez B, Zhu J, Ruan R (2011) Characterization of a microalga *Chlorella sp*. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour Technol 102 (8):5138-5144

Liao PH, Chen A, Lo KV (1995) Removal of nitrogen from swine manure wastewaters by ammonia stripping. Bioresour Technol 54 (1):17-20

Maeda I, Miyashiro M, Hikawa H, Yagi K, Miura Y, Mizoguchi T (1996) Acceleration of starch degradation by suppression of H_2 evolution in *Chlamydomonas sp*. MGA161. Biosci, Biotechnol, Biochem 60 (6):975-978

Miura Y, Yagi K, Shoga M, Miyamoto K (1982) Hydrogen production by a green alga, *Chlamydomonas reinhardtii*, in an alternating light/dark cycle. Biotechnol Bioeng 24 (7):1555-1563

Moll KM, Gardner RD, Eustance EO, Gerlach R, Peyton BM (2014) Combining multiple nutrient stresses and bicarbonate addition to promote lipid accumulation in the diatom RGd-1. Algal Research 5 (0):7-15

Müller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu R-Y, van der Giezen M, Tielens AGM, Martin WF (2012) Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol Mol Biol Rev 76 (2):444-495

Nunez VJ, Voltolina D, Nieves M, Pina P, Medina A, Guerrero M (2001) Nitrogen budget in *Scenedesmus obliquus* cultures with artificial wastewater. Bioresour Technol 78 (2):161-164

Ohta S, Miyamoto K, Miura Y (1987) Hydrogen evolution as a consumption mode of reducing equivalents in green algal fermentation. Plant Physiol 83 (4):1022-1026

Páez-Osuna F, Guerrero-Galván SR, Ruiz-Fernández AC, Espinoza-Angulo R (1997) Fluxes and mass balances of nutrients in a semi-intensive shrimp farm in north-western Mexico. Mar Pollut Bull 34 (5):290-297

Park JBK, Craggs RJ, Shilton AN (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 102 (1):35-42

Peavey DG, Gfeller RP, Gibbs M (1983) Presence of a Pasteur Effect in algae. Physiologie Vegetale 21 (3):337-342

Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman JB (2013) Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends Biotechnol 31 (3):134-138

Poulsen BR, Iversen JJL (1999) Membrane sparger in bubble column, airlift, and combined membrane–ring sparger bioreactors. Biotechnol Bioeng 64 (4):452-458

Quan X, Wang F, Zhao Q, Zhao T, Xiang J (2009) Air stripping of ammonia in a water-sparged aerocyclone reactor. J Hazard Mater 170 (2–3):983-988

Ueno Y, Kurano N, Miyachi S (1998) Ethanol production by dark fermentation in the marine green alga, *Chlorococcum littorale*. J Ferment Bioeng 86 (1):38-43

Van Wychen S, Laurens LML. NREL (2013a) Determination of total carbohydrates in algal biomass.

Van Wychen S, Laurens LML. NREL (2013b) Determination of total lipids as fatty acid methyl esters (FAME) by *in situ* transesterification.

Vanlerberghe GC, Feil R, Turpin DH (1990) Anaerobic metabolism in the N-limited green alga *Selenastrum minutum*: I. Regulation of carbon metabolism and succinate as a fermentation product. Plant Physiol 94 (3):1116-1123

Vanlerberghe GC, Joy KW, Turpin DH (1991) Anaerobic metabolism in the N-limited green alga *Selenastrum minutum*: III. Alanine Is the product of anaerobic ammonium assimilation. Plant Physiol 95 (2):655-658

Vanlerberghe GC, Turpin DH (1990) Anaerobic metabolism in the N-limited green alga *Selenastrum minutum*: II. Assimilation of ammonium by anaerobic cells. Plant Physiol 94 (3):1124-1130

Waller P, Ryan R, Kacira M, Li P (2012) The algae raceway integrated design for optimal temperature management. Biomass Bioenergy 46 (0):702-709

Wett B, Rauch W (2003) The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. Water Res 37 (5):1100-1110

Woertz I, Feffer A, Lundquist T, Nelson Y (2009) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng-ASCE 135 (11):1115-1122

Xin L, Hong-ying H, Ke G, Jia Y (2010) Growth and nutrient removal properties of a freshwater microalga *Scenedesmus sp*. LX1 under different kinds of nitrogen sources. Ecol Eng 36 (4):379-381

Yoon H, Lim JH, Chung HK (2008) Ammonia removal model based on the equilibrium and mass transfer principles. B Kor Chem Soc 29 (3):555-561

Zachleder V, Bišová K, Vítová M, Kubín Š, Hendrychová J (2002) Variety of cell cycle patterns in the alga Scenedesmus quadricauda (Chlorophyta) as revealed by application of illumination regimes and inhibitors. Eur J Phycol 37 (3):361-371

4. MANUSCRIPT 2: THE EFFECTS OF CULTIVATION DEPTH, AREAL DENSITY AND NUTRIENT LEVEL ON LIPID ACCUMULATION OF *SCENEDESMUS ACUTUS* IN OUTDOOR RACEWAY PONDS

Contributions of Author and Co-Authors

Author: Everett Eustance

Contributions: Conceived the study, collected and analyzed data, and wrote the manuscript

Co-author: Joshua T. Wray

Contributions: Collected data, assisted with study design, discussed results, and edited manuscript.

Co-author: Shahrzad Badvipour

Contributions: Collected data, discussed results, and edited manuscript.

Co-author: Milton R. Sommerfeld

Contributions: Discussed the results and implications, commented and edited the manuscript.

Manuscript Information Page

Everett Eustance*, Joshua T. Wray, Shahrzad Badvipour, Milton R. Sommerfeld
Journal Name: Journal of Applied Phycology
Status of Manuscript:
____Prepared for submission to a peer-reviewed journal
__x_Officially submitted to a peer-reviewed journal
____Accepted by a peer-reviewed journal
____Published in a peer-reviewed journal
Submitted: May 15th, 2015

*School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ, 85287; Everett.eustance@asu.edu

4.1. Introduction:

4.1.1. Productivity

Microalgae show a significant decrease in overall biomass productivity during the process of nitrogen depletion when most of the lipid accumulation occurs (Eustance et al. 2013; Eustance et al. 2015; Gardner et al. 2011; Moll et al. 2014; Breuer et al. 2012; Stephenson et al. 2010). Outdoor productivities in raceways have been reported above 40 $g/m^2/day$; however, these productivities are not maintained throughout the year with average productivities closer to 10 to 15 $g/m^2/day$ (Moheimani and Borowitzka 2007, 2006). Furthermore, high productivity is often associated with semi-continuous growth, as most research has shown a significant decrease in biomass productivity during conditions that stress biomass for lipid accumulation (Eustance et al. 2015; Eustance et al. 2013; Gardner et al. 2012; Gardner et al. 2011). For example, nitrogen deprivation in flat-panel photobioreactors decreased from an average of 10 - 15 $g/m^2/day$ during log phase (peak of 22 $g/m^2/day$) to an average of 4 - 7 $g/m^2/day$ during stress phase (Eustance et al. 2015). This may be attributed to a decrease in chlorophyll as cultures repurpose its internal nitrogen sources and the increase in energy to convert incoming carbon into lipids instead of carbohydrates (Gardner et al. 2013; Johnson and Alric 2013).

One of the biggest difficulties in the algal field is determining the optimal light-path length for productivity in outdoor systems as solar radiation differs by location and season. Previous research has shown that the optimal productivity can occur between 1 and 10 cm for flat-panel photobioreactors, while raceways are operated between 10 and 30 cm in depth with 15 cm being the most common (Moheimani and Borowitzka 2006;

Grobbelaar 2013; Hu et al. 1998b; Richmond and Cheng-Wu 2001). For comparison, these cultivation systems can be assessed using areal density and productivity. This also provides the best method for assessing photosynthetic efficiency of the PAR flux. Previous research has highlighted that optimal growth occurs in raceways when the areal density of the culture is between 40 and 150 g/m^2 depending on culture depth and the species being cultivated (Hartig et al. 1988; Grobbelaar et al. 1995). Hartig et al. (1988) concluded that the optimal areal density was between 40 and 45 g/m^2; based on a depth of 10 to 30 cm, in which the optimal volumetric density would vary from 0.45 g/l down to 0.13 g/l. The greater depth significantly increases the amount of water to be removed during the harvesting process. However, if the depth were reduced to 6 or 7 mm as is observed in the cascade reactor, the volumetric density would be greater than a ten-fold increase (Doucha and Lívanský 2009). In addition, Grobbelaar et al. (1995) determined that by reducing the depth and with the high mixing rates, the cascade reactor increased the optimal areal density close to 150 g/m^2. This reduces the amount of cultivation water by a factor of more than 30 compared to traditional raceways, which has a significant impact on downstream processing.

4.1.2. Limitations in Decreasing Raceway Cultivation Depth

Decreasing cultivation depth has historically been cited as being limited by two factors: friction and temperature instability (Béchet et al. 2011; Oswald 1988; Grobbelaar 2013). Shallow raceways require more paddlewheels per square meter of surface area to overcome frictional losses of the raceway surface (Oswald 1988). Oswald calculated the maximum distance between paddlewheels using the Manning equation with different

raceway depths and found that by increasing the raceway depth from 10 cm to 30 cm the maximum distance between paddlewheels increased almost 12-fold. This is because the frictional loss attributed to the raceway surface remains the same, while the amount of energy required to move the additional water increases allowing for a larger distance between paddlewheels. Additionally, it has been documented that decreasing the raceway operating depth will theoretically increase the temperature instability due to having a smaller heat capacity (Béchet et al. 2011; Oswald 1988). This means that the culture's productivity may decrease as the temperature deviates farther from the optimal cultivation temperature (Moheimani and Borowitzka 2007; Ras et al. 2013; Vonshak et al. 2001; Waller et al. 2012). However, deep cultivation depths significantly reduce the operating temperature as cultures loose large amounts of heat at night and take longer to warm up in the morning (Crowe et al. 2012; Waller et al. 2012).

4.1.3. Diurnal Temperature Fluctuations

One of the biggest impacts on culture productivity is the diurnal temperature fluctuation. Research has shown that cultures tend to have 4 to 5 °C range between the optimal temperature and the point where cultures begin to die from high temperatures (Ras et al. 2013). This has drastic effects on culture productivity if the temperature spikes unexpectedly, but can be minimized by rotating algal crops to accommodate the different temperature ranges. However, suboptimal morning temperatures cause photoinhibition and reduced enzyme activity, which reduces the productivity of cultures for a majority of the available growing season (Ras et al. 2013; Vonshak et al. 2001). Previous research has attempted to address the concerns of suboptimal morning temperatures associated

with nightly heat loss by creating a basin in the ground to minimize the surface area to volume (Waller et al. 2012). This research showed a significant improvement in maintaining the cultivation temperature and productivity. However, the design depends on gravity and pumping for circulation, the feasibility of which is unknown at this time. To improve morning cultivation temperatures in traditional raceway ponds, it is possible to decrease the raceway depth, which utilizes the temperature instability of a smaller heat sink to increase the rate at which the cultures reach a desired temperature range.

4.1.4. Lipid accumulation

Determination of algal strains' capability for lipid accumulation is often accomplished in flasks and indoor or outdoor photobioreactors (Rodolfi et al. 2009; Griffiths and Harrison 2009; Přibyl et al. 2012; Lourenco et al. 2002). However, these systems typically operate under highly controlled conditions, which significantly increase the productivity and lipid accumulation. Raceway ponds, as mentioned above, have several environmental and design differences from photobioreactors and indoor cultivation methods that significantly affect the cultures ability to accumulate lipids within a similar time frame to controlled experimentation. The purpose of this study was to investigate the effects of different nutrient concentrations and cultivation depths on the areal density, areal productivity, and lipid accumulation of Scenedesmus *acutus* strain LB 0414 during the winter months in Mesa, AZ in larger, outdoor, research-scale raceways.

4.2. Materials and Methods

4.2.1. Organisms and Cultivation

All experiments were carried out using *Scenedesmus acutus* (Strain LB 0414) cultured in outdoor raceway ponds at the Arizona Center for Algae Technology and Innovation (AzCATI) field-site in Mesa, AZ. This strain was recently reclassified from *Scenedesmus dimorphous* based on 18s identification. Experiments were completed in three identical raceway ponds each consisting of two channels 6.1 m long, 1.7 m wide with two ends each with a radius of 1.78 m providing a total area of 30.37 m^2. Velocity of raceways was set to an average linear flow of 25 cm/s. During the day CO_2 was added through a Sweetwater diffusion stone measuring 30 cm long and 5 cm wide at a rate of 5 L/min. Inoculating cultures were grown in 60 L flat panel photobioreactors with Modified BG-11 Medium. The composition of the Modified BG-11 Medium was 1.5 g/L $NaNO_3$ (Alfa Aesar), 38.3 mg/L K_2HPO_4 (BDH), 6.32 mg/L Citric Acid Monohydrate (Sigma-Aldrich), 5.24 mg/L of Ammonium Ferric Citrate (Alfa Aesar), 2.84 mg/L H_3BO_3 (Sigma-Aldrich), 1.78 mg/L $MnCl_2 \cdot 4H_2O$ (Sigma-Aldrich), 0.24 mg/L $ZnSO_4 \cdot 7H_2O$ (Sigma-Aldrich), 0.40 mg/L $Na_2MoO_4 \cdot 2H_2O$ (Sigma-Aldrich), 0.08 mg/L $CuSO_4 \cdot 5H_2O$ (Sigma-Aldrich), 0.4 mg/L $Co(NO_3)_2 \cdot 6H_2O$ (Sigma-Aldrich). Because these experiments utilized tap water, magnesium sulfate, calcium chloride and sodium carbonate were not added to the Modified BG-11 Medium. Tap water used averaged 180 mg/L Na, 75 mg/L Ca, 70 mg/L S, 24 mg/L Mg, and 8 mg/L K with a hardness of 280 mg /L as $CaCO_3$. Approximately 75 to 100 L of culture was transferred to the raceways, where cultures were grown in Modified BG-11 Medium with industrial fertilizers

replacing the nitrate and phosphate sources: ammonium iron citrate was used for an iron source and no additional micronutrients were added. The cultures utilized calcium nitrate 15.5-0-0 (YaraLiva Tropicote) for a nitrogen source, and Blooming and Rooting 9-58-8 (Ferti-lome) for a phosphorus source. The concentration of nutrients was manipulated to obtain optimal growth and lipid accumulation conditions.

4.2.2. Monitoring

Temperature and pH were continuously monitored using a Neptune Apex controller (Neptune Systems, LLC.). Ambient conditions were measured with an Argus weather station capable of recording ambient temperature, relative humidity, and light intensity (Argus Control Systems, LLC.).

4.2.3. Analytical

4.2.3.1. Biomass Density

Ash-free dry weight (AFDW) was measured in duplicate samples to assess growth performance. Glass filters (VWR 696 glass microfiber 1.2µm) were ashed for 4 hours at 500°C prior to initial weighing. Duplicate samples were collected by filtering 10 to 20 mL per filter (depending on culture density). Samples were placed into an oven at 60°C overnight. The filters with biomass were weighed to determine dry cell weight and then ashed at 500°C for 4 hours to determine the AFDW and ash content of the biomass.

4.2.3.2. Nitrate

Nitrate-nitrogen levels were monitored using flow-injection analysis with a Lachat QuikChem 8500 with samples being analyzed in duplicate.

4.2.3.3. Lipids

Lipids were expressed as biodiesel potential (BP) and measured using direct transesterification following the procedure "Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by *in situ* Transesterification" outlined by NREL (Van Wychen and Laurens 2013b). It is important to note that FAME's only account for between 50 and 80 percent of the total lipids that are measured gravimetrically.

4.2.3.4. Carbohydrates

Carbohydrates were measured using the colorimetric method "Determination of Total Carbohydrates in Algal Biomass" developed by NREL (Van Wychen and Laurens 2013a).

4.2.3.5. Proteins

Protein content was determined using Bio-Rad DC Protein Assay (Bio Rad) with γ-globulin as the standard. 1-2 mg of freeze-dried algal biomass was placed in a micro-centrifuge tube with 1 mL of reagent A and heated at 60°C for 60 min, with intermittent vortexing. 100 µL of the resulting solution was transferred into a clear test tube with an additional 500 µL of reagent A and 4.0 mL of reagent B. The samples were vortexed and incubated at room temperature for 15 minutes prior to measuring absorption at 750 nm using a Hach DR 5000 Spectrophotometer.

4.3. Results

4.3.1. Varying Nitrogen Concentration

Two experiments were conducted to determine the approximate nitrogen concentration required for cultures to accumulate lipids within a relatively short time period (approximately 14 days). The first experiment conducted from October 27, 2014 to November 8, 2014 (October experiment) had the raceways operating at 20, 30, and 60 mg-N as NO_3^-/L, with a cultivation depth of 9 cm. The second experiment conducted from January 3, 2015 to January 26, 2015 (January experiment) had the raceways inoculated with a concentration of 20, 60, and 110 mg-N as NO_3^-/L, with a cultivation depth of 7.5 cm. Figure 4.1 shows the ash free dry weight (AFDW), areal density, areal productivity, nitrogen concentration, culture and ambient temperature, and sunlight intensity for A) October experiment and B) January experiment. The results show that cultivation for the October experiment had peak productivity between 9.0 and 12.7 g/m^2/day for the cultures with 20 and 60 mg-N/L, respectively, that occurred at areal densities of 29.9 and 39.2 g/m^2. The results also show that all three cultures had a drop in productivity the following day irrespective of the nitrate concentration. This drop is attributed to a change in the weather as shown by a significant decrease in ambient and culture temperature over the next few days of the experiment. Figure 4.1 also shows that cultivation did not completely remove the 60 mg-N/L over the course of the experiment. This is highlighted in Table 4.1, which shows the biochemical composition of the October experiment at peak productivity (day 4), nutrient depletion for 20 and 30 mg-N/L (day 7), and the point of harvest (day 12). The results show that cultivation at 20 mg-N/L

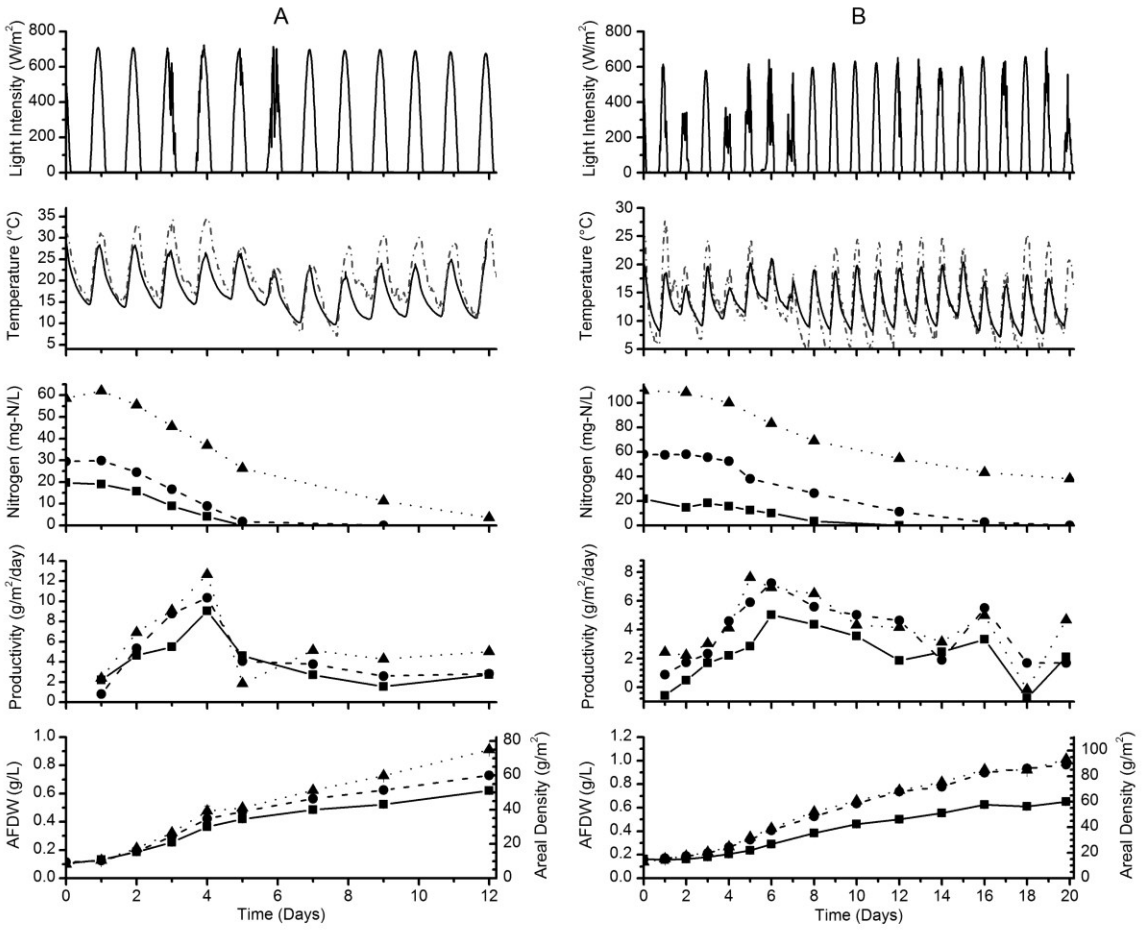

Figure 4.1: AFDW, areal density, productivity, nitrogen concentration, sunlight intensity, average culture temperature (solid line) and ambient temperature (dash dot line) for cultures grown from A) October 27, 2014 to November 8, 2014 and B) January 6, 2015 to January 26, 2015. Cultures grown in October were provided with nitrogen levels of 20 mg-N/L (filled square/solid line), 30 mg-N/L (filled circle/dashed line), and 60 mg-N/L (filled triangle/dotted line) and operated at a depth of 9 cm. Cultures grown in January with nitrogen levels of 20 mg-N/L (filled square), 60 mg-N/L (filled circle), and 110 mg-N/L (filled triangle) and operated at a depth of 7.5 cm.

compared to 60 mg-N/L at 9 cm improved biodiesel potential to 20.0 ± 0.1% compared to 12.5 ± 0.5%, respectively. However, due to the slower growth rate attributed to nitrogen stress, the total biodiesel produced for each was 0.124 g-FAME/L (10.2 g-FAME/m^2) compared to 0.114 g-FAME/L (9.36 g-FAME/m^2) an increase of only 8.7% for nitrogen deficient cultures compared to nitrogen sufficient cultures.

Figure 4.1 also shows the data for cultivation during the January experiment, which were inoculated with initial concentrations of 20, 60, and 110 mg-N/L at a depth of 7.5 cm to determine how the increase in nitrogen would affect growth and total biodiesel produced. The peak productivity levels of 5.0 and 7.6 g/m^2/day for the cultures grown with 20 and 110 mg-N/L, respectively, occurred at areal densities of 26.7 and 32.1 g/m^2. However, the raceways with 60 and 110 mg-N/L were able to maintain higher levels of productivity for a longer period. The results show that cultures inoculated with 110 mg-N/L did not increase in productivity or areal density compared to the culture inoculated with 60 mg-N/L, indicating the additional nutrients were not being utilized. Table 4.1 also shows the biochemical composition for the raceways operated during the January experiment, which highlights the difference in biodiesel potential for cultures with different nitrogen concentrations. At the point of harvest (day 20) cultures had 21.4 ± 1.0%, 13.4 ± 0.1%, and 9.3 ± 0.6% for raceways with initial nitrogen concentration of 20, 60, and 110 mg-N/L, respectively, which corresponds to a total biodiesel production of 0.139, 0.130, and 0.094 g-FAME/L and 12.8, 11.9, and 8.7 g-FAME/m^2.

The peak productivity levels in the January experiment are significantly lower than the peak productivities in the October experiment, which is attributed to the significantly lower peak sunlight intensities and culture temperatures due to the different seasons (Fig. 4.1). The experiment also went longer due to cloudy weather during the log phase, shown in Figure 4.1 by the low light intensity, which also limited the peak productivity of the culture.

Table 4.1: Biochemical composition of *Scenedesmus acutus* strain 0414 during different stages of growth under different nutrient levels and the same depth.

Initial Nitrogen	Time point	AFDW	Carbohydrates	Protein	BP[3]
mg-N/L		g-biomass/L	% (w/w)	% (w/w)	% (w/w)
20[1]	Day 4	0.36 ± 0.00	21.6 ± 2.4	34.3 ± 1.4	8.2 ± 0.2
	Day 7	0.49 ± 0.00	30.4 ± 2.9	28.4 ± 2.8	15.6 ± 0.0
	Day 12	0.62 ± 0.01	24.6 ± 0.4	24.9 ± 0.4	20.0 ± 0.1
30[1]	Day 4	0.43 ± 0.00	29.6 ± 0.2	31.5 ± 3.5	11.4 ± 0.5
	Day 7	0.56 ± 0.01	30.5 ± 1.3	32.1 ± 1.2	12.5 ± 0.6
	Day 12	0.73 ± 0.01	25.3 ± 0.4	28.2 ± 0.5	17.4 ± 0.5
60[1]	Day 4	0.48 ± 0.04	32.4 ± 0.5	27.4 ± 2.2	10.0 ± 0.2
	Day 7	0.62 ± 0.01	26.2 ± 0.1	28.6 ± 2.6	9.4 ± 0.1
	Day 12	0.91 ± 0.01	25.0 ± 0.5	32.7 ± 2.5	12.5 ± 0.5
20[2]	Day 6	0.29 ± 0.00	26.0 ± 4.7	34.8 ± 0.1	12.2 ± 0.1
	Day 14	0.55 ± 0.01	23.0 ± 3.9	36.8 ± 2.0	18.1 ± 0.4
	Day 20	0.65 ± 0.01	25.6 ± 0.3	29.8 ± 0.7	21.4 ± 1.0
60[2]	Day 6	0.41 ± 0.00	17.6 ± 1.7	40.6 ± 0.2	7.3 ± 0.3
	Day 20	0.97 ± 0.01	21.1 ± 0.6	43.0 ± 0.9	13.4 ± 0.1
110[2]	Day 6	0.42 ± 0.00	16.8 ± 0.5	46.7 ± 2.8	7.4 ± 0.0
	Day 14	0.82 ± 0.01	12.4 ± 2.6	43.4 ± 1.8	8.3 ± 0.1
	Day 20	1.01 ± 0.01	14.5 ± 1.6	43.0 ± 0.9	9.3 ± 0.6

[1] Experiment conducted from October 27, 2014 to November 8, 2014
[2] Experiment conducted from January 6, 2015 to January 26, 2015
[3] BP= Biodiesel potential

4.3.2. Varying Culture Depth

The second series of experiments tested the impact on cultures with different depths at the same areal density. The first experiment was conducted from December 3, 2014 to December 18, 2014 (December experiment) with raceway depths of 9 cm, 18 cm, and 24 cm. The second experiment was conducted from January 29, 2015 to February 12, 2015 (February experiment) with raceway depths of 7.5 cm, 15 cm, and 20 cm. Figure 4.2 shows the AFDW, areal density, areal productivity, nitrogen concentration, culture

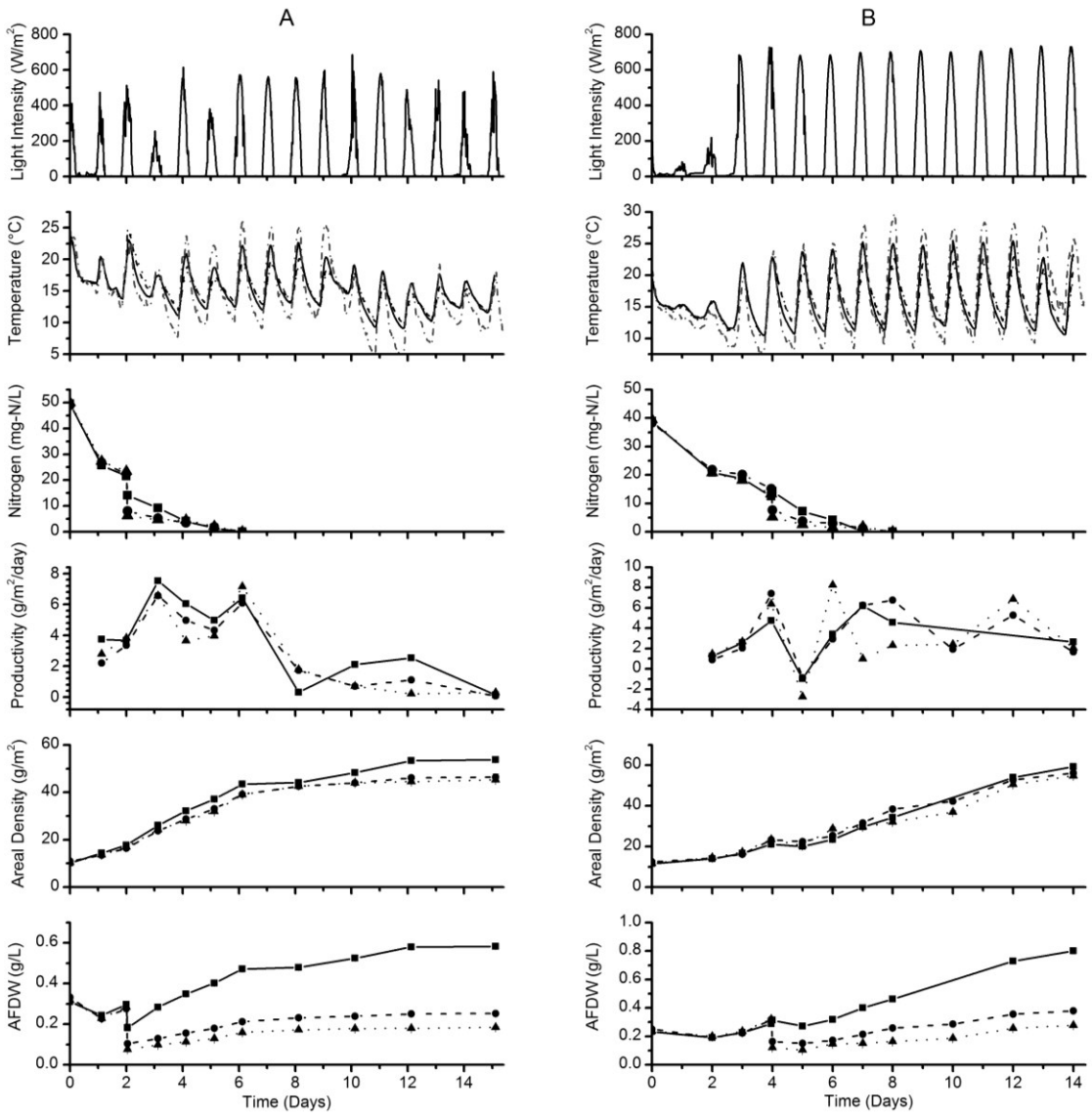

Figure 4.2: AFDW, areal density, productivity, nitrogen concentration, sunlight intensity, culture temperature and ambient temperature (dash dot line) for cultures grown from A) December 3, 2014 to December 18, 2014 and B) January 29, 2015 to February 12, 2015. Cultures in December were grown at depths of 9 cm (filled square/solid line), 18 cm (filled circle/dashed line), and 24 cm (filled triangle/dotted line) with the same areal concentration of nitrogen (mg-N/m2). Cultures in January/February were grown at different depths: 7.5 cm (filled square), 15 cm (filled circle), and 20 cm (filled triangle) with the same areal concentration of nitrogen (mg-N/m2).

and ambient temperature, and sunlight intensity for A) December experiment and B) February experiment. As shown in Figure 4.2, raceways were cultivated at a lower depth for the first few days to allow the biomass to acclimatize to the dilute conditions before increasing the volume to final operating depths to minimize and prevent photoinhibition

Table 4.2: Biochemical composition of *Scenedesmus acutus* strain 0414 during different stages of growth under different depths and the same areal nutrient concentrations (mg-N/m^2).

Culture Depth	Time point	AFDW	Carbohydrate	Protein	BP
cm		g-biomass/L	% (w/w)	% (w/w)	% (w/w)
9[1]	Day 5	0.40 ± 0.01	29.2 ± 2.1	32.1 ± 2.0	12.6 ± 1.2
	Day 10	0.52 ± 0.01	25.1 ± 1.0	26.0 ± 1.0	16.2 ± 0.0
	Day 15	0.58 ± 0.01	31.7 ± 0.7	25.7 ± 1.0	18.1 ± 0.3
18[1]	Day 5	0.18 ± 0.01	25.7 ± 3.8	32.2 ± 0.8	14.0 ± 1.1
	Day 10	0.24 ± 0.00	22.8 ± 2.5	34.1 ± 3.8	16.0 ± 0.7
	Day 15	0.25 ± 0.01	26.7 ± 1.4	29.5 ± 2.6	17.1 ± 0.2
24[1]	Day 5	0.13 ± 0.00	24.0 ± 2.7	33.1 ± 3.8	11.9 ± 0.1
	Day 10	0.18 ± 0.00	24.5 ± 0.5	31.6 ± 0.6	13.5 ± 0.6
	Day 15	0.18 ± 0.00	26.7 ± 1.4	28.9 ± 1.0	14.2 ± 0.1
7.5[2]	Day 7	0.40 ± 0.00	28.9 ± 0.5	32.0 ± 0.0	13.6 ± 0.3
	Day 14	0.80 ± 0.02	29.8 ± 3.4	22.3 ± 2.3	21.4 ± 0.3
15[2]	Day 7	0.21 ± 0.00	26.9 ± 3.3	30.8 ± 0.8	13.3 ± 0.2
	Day 14	0.38 ± 0.00	34.5 ± 2.3	22.4 ± 1.2	19.8 ± 0.5
20[2]	Day 7	0.15 ± 0.00	25.0 ± 0.5	30.7 ± 0.9	13.0 ± 0.1
	Day 14	0.28 ± 0.00	32.6 ± 2.8	20.0 ± 2.6	17.4 ± 0.2

[1] Experiment conducted from December 3, 2014 to December 18, 2014
[2] Experiment conducted from January 29, 2015 to February 12, 2015

and photobleaching. The dilution is shown in Figure 4.2 as a sudden drop in the AFDW of the culture, while the areal density remained the same. Because these experiments were evaluating the impact of culture depth on lipid accumulation, nutrient loading was based on the area of the raceways and not the volume. Therefore, the deeper raceways received the same total amount of nutrients as the shallow raceways as indicated in Figure 4.2 by the same initial nitrogen concentration in different raceways prior to the changing cultivation depth. Figure 4.2 shows that during the February experiment the cultures exhibited some form of inhibition that prevented growth from day 4 to day 5.

The effect was not attributed to the dilution of the culture, as the raceway operated at 7.5 cm was not diluted as the raceways that were operated at 15 and 20 cm.

Table 4.2 shows the biochemical composition of the raceways operated at different depths in the December and February experiments. The results show that the biochemical composition at the point of harvest (day 15) for the raceways operated at 9, 18, and 24 cm in the December experiment were 18.1 ± 0.3%, 17.1 ± 0.2%, and 14.2 ± 0.1%, respectively, indicating a small drop in lipid accumulation with depth. The total areal biodiesel potential of the raceways was 9.79, 7.93, and 6.43 g-FAME/m^2 for raceways at 9, 18, and 24 cm, respectively, which shows that as raceway depth decreases the amount of biodiesel produced increased by 52% in the December experiment. Table 4.2 also shows the biochemical analysis for the February experiment with the harvested biodiesel potential (day 14) for the raceways operated at 7.5, 15, and 20 cm at 21.4 ± 0.3%, 19.8 ± 0.5%, and 17.4 ± 0.2%, respectively. The total areal biodiesel potential of the raceways was 12.69, 11.12, and 9.52 g-FAME/m^2 for raceways at 7.5, 15, and 20 cm, respectively, which shows that as raceway depth decreased, the amount of biodiesel produced increased by 33% for the February experiment. For both December and February experiments, the overall increase in total areal biodiesel produced by using shallow raceways (7.5 and 9 cm) compared to deep raceways (20 and 24 cm) was greater than 3.17 g-FAME/m^2.

4.3.3. Log, Stress and Overall Cultivation Biomass Productivities

Table 4.3 shows the log phase, stress phase, and overall cultivation productivities for the October, December, January, and February experiments. Results show that log

Table 4.3: Average culture productivity during log phase, stress phase and overall cultivation time. Log and stress phase are assumed to end and start, respectively, from the time point after nitrogen deficiency in the medium is detected.

	Cultivation phase	Different Nitrogen Concentrations (mg-N/L)						Different Culture Depths (cm)						Flat Panel[5]
		20[1]	30[1]	60[1]	20[2]	60[2]	110[2]	9[3]	18[3]	24[3]	7.5[4]	15[4]	20[4]	
Volumetric Productivity g/L/day	Log	0.060	0.054	0.070	0.028	0.041	0.044	0.045	0.021	0.016	0.029	0.022	0.013	0.3
	Stress	0.027	-	-	0.019	-	-	0.015	0.003	0.002	0.056	0.020	0.019	0.17
	Overall	0.045	0.054	0.070	0.025	0.041	0.044	0.031	0.013	0.001	0.041	0.021	0.016	0.2
Areal Productivity g/m^2/day	Log	4.9	4.4	5.9	2.6	3.7	4.0	4.2	3.9	4.2	2.8	3.2	2.5	12.5
	Stress	2.3	-	-	1.8	-	-	1.4	0.5	0.4	4.2	3.0	3.8	6.9
	Overall	3.7	4.4	5.9	2.3	3.7	4.0	2.9	2.3	2.3	3.4	3.1	3.1	9.5

[1] Experiment conducted from October 27, 2014 to November 8, 2014
[2] Experiment conducted from January 6, 2015 to January 26, 2015
[3] Experiment conducted from December 3, 2014 to December 18, 2014
[4] Experiment conducted from January 29, 2015 to February 12, 2015
[5] Previously published flat panel experimental data (Eustance et al. 2015)

phase productivity for the cultures ranged from 2.5 to 5.9 g/m^2/day. The results also show that cultures that achieved nutrient depletion for lipid accumulation had productivities between 0.4 and 4.2 g/m^2/day. However, the values for the February experiment are higher due to cloudy weather during the first three days, which allowed the algal culture to remove additional nutrients without simultaneous growth as shown in Figure 4.2. This allowed the alga to have better growth after nutrient depletion of the medium, increase in stressed phase productivities, but with decreased log phase productivities. The overall productivities for the February experiment were within the range of productivities for the other experiments that experienced nutrient depletion.

4.3.4. Photoinhibition and Photobleaching

Experiments were set up to provide a large range of areal densities in order to determine the optimal areal density for peak productivity. However, in doing so, many cultures exhibited photoinhibition and photobleaching. Two experiments were attempted starting November 9, 2014 and November 18, 2014 with cultivation at different depths. Cultures on November 9 were inoculated at a depth of 7.5, 15, and 22.5 cm with an areal density of approximately 5 to 6 g/m^2, with volumetric densities of 0.067, 0.040, and 0.025 g/L, respectively. For the raceways at 7.5 and 15 cm depths, the cultures experienced significant photoinhibition, but did not die. However, the raceway at 22.5 cm experienced photobleaching within a 4-hour period. Raceways started on November 18 were initially inoculated at the same depth of 5 cm with an average areal density of 5.8 ± 0.5 g/m^2 and a volumetric density of 0.11 ± 0.01 g/L. These cultures did not photobleach; however, they exhibited significant photoinhibition that prevented growth. When

comparing to the October, December, January, and February experiments, the cultures had starting areal densities of 8 to 15 g/m^2 and volumetric densities of 0.10 to 0.33 g/L and exhibited only minor levels of photoinhibition. This demonstrates that both areal and volumetric densities are critically important in preventing significant levels of photoinhibition.

4.4. Discussion

4.4.1. Culture Depth

Oswald (1988) calculated that shallow raceways require more paddlewheels per raceway surface area to overcome frictional losses. By increasing the raceway depth from 10 cm to 30 cm, the maximum distance between paddlewheels increased almost 12-fold. This is critical as it reduces the amount of energy being utilized for cultivation, and is considered the base reasoning as to why most raceways are currently operated at deeper levels. However, research has not definitively assessed the impact of culture depth on lipid accumulation, but previous research has shown some potential in the reduction in raceway depth for improving biomass productivity during the winter (Moheimani and Borowitzka 2007). The research showed that during the winter decreasing cultivation depth increased biomass productivity, but showed that the same culture during the summer had significantly lower biomass productivities. However, this may be due to the culture exceeding the optimal temperature for growth since the experiments used the same organism for both seasons. As was shown in Table 4.3, the algal cultures did not exhibit a significant difference in overall culture productivity at different depths, but did show a small difference in cultures with higher levels of nitrate. The productivity levels

observed in these experiments were low and consistent with the range found by previous research for growth during the winter months (Crowe et al. 2012; Moheimani and Borowitzka 2006, 2007). This is attributed to the suboptimal morning temperatures causing photoinhibition and reducing enzyme activity and is an area of active research (Crowe et al. 2012; Ras et al. 2013; Vonshak et al. 2001).

Table 4.2 showed the biodiesel potential (BP) for cultures at different depths. When comparing across the depths, shallow cultures (7.5 and 9 cm) had a greater increase compared to deeper raceways. For the culture at 7.5 cm from day 7 to day 14 cultures changed from 13.6 ± 0.3% BP to 21.4 ± 0.3% BP, while the raceway at 20 cm went from 13.0 ± 0.1% BP to 17.4 ± 0.2% BP. This is further exemplified when assessing the total biodiesel produced on an areal basis where the raceway at 7.5 cm had an accumulation of 12.69 g-FAME/m^2, while the raceway at 20 cm accumulated 9.52 g-FAME/m^2. This was also shown for the experiment completed in December where the raceway at 9 cm accumulated 9.79 g-FAME/m^2 and the raceway at 24 cm accumulated 6.43 g-FAME/m^2. Therefore, cultivation depth should be considered as a critical factor in the accumulation of lipids for biodiesel.

One of the prime reasons for reducing the culture depth is to limit the amount of water being utilized during growth and being removed during the harvesting process. By reducing the cultivation depth from 15 to 30 cm to a depth of 7.5 cm, the process can eliminate 50 to 75% of the bulk water for algal cultivation. This can be further minimized by utilizing the cascade reactor, which operates at a depth of 0.6 to 1.0 cm and eliminates over 90% of the water required for cultivation (Doucha and Lívanský 2014).

4.4.2. Areal Density

Previous research has highlighted that optimal growth occurs in raceways when the areal density of the culture is between 40 and 150 g/m^2 depending on culture depth and the species being cultivated (Hartig et al. 1988; Grobbelaar et al. 1995). Hartig et al. (1988) concluded that the optimal areal density was between 40 and 45 g/m^2. This compares closely to the current study, which found peak productivity between 26.7 and 39.2 g/m^2 in batch raceways. Due to variations in environmental conditions, the areal density range is near that suggested by Hartig et al. (1988) and suggests that further refinement in the cultivation method could improve the optimal areal density. Cultivation at different depths did not show a significant difference in the optimal areal density for peak productivity as was shown in Figure 4.2.

The current study shows that a minimum areal density is required to overcome photoinhibition and prevent photobleaching during the dilution inherent with inoculating cultures. Cultures with an areal density greater than 8 g/m^2 and a volumetric density of 0.1 g/L had minimal photoinhibition, while cultures with an areal density of 5 to 6 g/m^2 exhibited photoinhibition. Furthermore, a culture at a depth of 22.5 cm and a volumetric density of 0.025 g/L was photobleached indicating that both volumetric and areal densities are important in preventing photoinhibition and photobleaching.

4.4.3. Comparison of Growth and Lipid Accumulation in Raceways to Flat-Panel Photobioreactors

One of the biggest issues in the algal field is determining the best cultivation method for lipid accumulation: flat-panel photobioreactors or traditional raceways.

Previous research (Eustance et al. 2015) using flat-panel photobioreactors (panels) provides a comparison for batch growth of the *Scenedesmus acutus* Strain LB 0414 (Table 4.3). The overall productivity for the panels compared to the most stressed raceway was 9.5 g/m^2/day to 3.4 g/m^2/day. However, when comparing the final biodiesel potential of both systems, the best-case scenario for raceway cultivation was 21.4 ± 0.3% on a volumetric density of 0.80 g/L after 14 days of cultivation, while the panels reached 35.5 ± 1.1% at a volumetric density of 3.92 g/L after 14 days. When comparing the two, based on areal properties the panel was able to achieve an areal density of 160.5 g-biomass/m^2 and areal biodiesel production of 57.0 g-FAME/m^2, while the best raceway was able to reach 59.3 g-biomass/m^2 and 12.7 g-FAME/m^2. This is a 4.5 fold increase in the lipid accumulated in the same area. However, the panel reactors have more controls that allow them to operate at a higher productivity, but require significant amounts of energy during the cultivation stage. Further improvements to raceway cultivation may improve overall lipid accumulation; however, by reducing the operating depth of the raceways, the overall lipid accumulation for biodiesel production can be increased.

4.5. Conclusions

- Decreasing cultivation depth from 20 cm to 7.5 cm and 24 cm to 9 cm affected overall biodiesel accumulation by 52% and 33%, respectively.
- A minimum operating culture density of 8.0 g/m^2 at 0.1 g/L was required to minimize initial photoinhibition of *Scenedesmus acutus*.

- Raceway pond cultures accumulating lipids had an average log-phase biomass productivity of 3.5 ± 0.9 g/m^2/day, with productivity during the stress-phase of 2.2 ± 1.4 g/m^2/day during the winter.
- Raceway cultivation showed peak productivity when cultures were at an areal density between 26.7 and 39.2 g/m^2.
- Biomass lipid accumulation was significantly lower than that achieved in previous research in outdoor flat-panel photobioreactors.

4.6. Acknowledgments

The authors would like to acknowledge the Laboratory for Algae Research and Biotechnology and the Arizona Center for Algae Technology and Innovation staff for their intellectual and equipment support.

4.7. References

Béchet Q, Shilton A, Park JBK, Craggs RJ, Guieysse B (2011) Universal temperature model for shallow algal ponds provides improved accuracy. Environ Sci Technol 45 (8):3702-3709

Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH (2012) The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. Bioresour Technol 124 (0):217-226

Crowe B, Attalah S, Agrawal S, Waller P, Ryan R, Van Wagenen J, Chavis A, Kyndt J, Kacira M, Ogden KL, Huesemann M (2012) A comparison of *Nannochloropsis salina* growth performance in two outdoor pond designs: conventional raceways versus the ARID pond with superior temperature management. International Journal of Chemical Engineering 2012:9

Doucha J, Lívanský K (2009) Outdoor open thin-layer microalgal photobioreactor: potential productivity. J Appl Phycol 21 (1):111-117

Doucha J, Lívanský K (2014) High density outdoor microalgal culture. In: Bajpai R, Prokop A, Zappi M (eds) Algal Biorefineries. Springer Netherlands, pp 147-173

Eustance E, Gardner RD, Moll KM, Menicucci J, Gerlach R, Peyton BM (2013) Growth, nitrogen utilization and biodiesel potential for two chlorophytes grown on ammonium, nitrate or urea. J Appl Phycol 25 (6):1663-1677

Eustance E, Wray JT, Badvipour S, Sommerfeld MR (2015) The effects of limiting nighttime aeration on productivity and lipid accumulation in Scenedesmus dimorphous. Algal Research 10 (0):33-40

Gardner R, Cooksey K, Mus F, Macur R, Moll K, Eustance E, Carlson R, Gerlach R, Fields M, Peyton B (2012) Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the Chlorophyte *Scenedesmus sp*. and the Diatom *Phaeodactylum tricornutum*. J Appl Phycol:1-10

Gardner R, Peters P, Peyton B, Cooksey K (2011) Medium pH and nitrate concentration effects on accumulation of triacylglycerol in two members of the Chlorophyta. J Appl Phycol 23 (6):1005-1016

Gardner RD, Lohman E, Gerlach R, Cooksey KE, Peyton BM (2013) Comparison of CO_2 and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. Biotechnol Bioeng 110 (1):87-96

Griffiths MJ, Harrison STL (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 21 (5):493-507

Grobbelaar JU (2013) Mass production of microalgae at optimal photosynthetic rates. Photosynthesis.

Grobbelaar JU, Nedbal L, Tichy L, Setlik L (1995) Variation in some photosynthetic characteristics of microalgae cultured in outdoor thin-layered sloping reactors. J Appl Phycol 7 (2):175-184

Hartig P, Grobbelaar JU, Soeder CJ, Groeneweg J (1988) On the mass-culture of microalgae - areal density as an important factor for achieving maximal productivity. Biomass 15 (4):211-221

Hu Q, Kurano N, Kawachi M, Iwasaki I, Miyachi S (1998) Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor. Appl Microbiol Biotechnol 49 (6):655-662

Johnson X, Alric J (2013) Central carbon metabolism and electron transport in *Chlamydomonas reinhardtii*: Metabolic constraints for carbon partitioning between oil and starch. Eukaryot Cell 12 (6):776-793

Lourenco SO, Barbarino E, Mancini-Filho J, Schinke KP, Aidar E (2002) Effects of different nitrogen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: an evaluation for aquaculture. Phycologia 41 (2):158-168

Moheimani NR, Borowitzka MA (2006) The long-term culture of the coccolithophore Pleurochrysis carterae (Haptophyta) in outdoor raceway ponds. J Appl Phycol 18 (6):703-712

Moheimani NR, Borowitzka MA (2007) Limits to productivity of the alga Pleurochrysis carterae (Haptophyta) grown in outdoor raceway ponds. Biotechnol Bioeng 96 (1):27-36

Moll KM, Gardner RD, Eustance EO, Gerlach R, Peyton BM (2014) Combining multiple nutrient stresses and bicarbonate addition to promote lipid accumulation in the diatom RGd-1. Algal Research 5 (0):7-15

Oswald WJ (1988) Large-scale algal culture systems (engineering aspects). In: Borowitzka MA, Borowitzka LJ (eds) Micro-algal Biotechnology. Cambridge University Press, Cambridge, pp 357-394

Přibyl P, Cepák V, Zachleder V (2012) Production of lipids in 10 strains of *Chlorella* and *Parachlorella* and enhanced lipid productivity in *Chlorella vulgaris*. Appl Microbiol Biotechnol 94 (2):549-561

Ras M, Steyer J-P, Bernard O (2013) Temperature effect on microalgae: a crucial factor for outdoor production. Rev Environ Sci Biotechnol 12 (2):153-164

Richmond A, Cheng-Wu Z (2001) Optimization of a flat plate glass reactor for mass production of Nannochloropsis sp. outdoors. J Biotechnol 85 (3):259-269

Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 102 (1):100-112

Stephenson AL, Dennis JS, Howe CJ, Scott SA, Smith AG (2010) Influence of nitrogen-limitation regime on the production by Chlorella vulgaris of lipids for biodiesel feedstocks. Biofuels 1 (1):47-58

Van Wychen S, Laurens LML. NREL (2013a) Determination of total carbohydrates in algal biomass.

Van Wychen S, Laurens LML. NREL (2013b) Determination of total lipids as fatty acid methyl esters (FAME) by *in situ* transesterification.

Vonshak A, Torzillo G, Masojidek J, Boussiba S (2001) Sub-optimal morning temperature induces photoinhibition in dense outdoor cultures of the alga Monodus subterraneus (Eustigmatophyta). Plant, Cell Environ 24 (10):1113-1118

Waller P, Ryan R, Kacira M, Li P (2012) The algae raceway integrated design for optimal temperature management. Biomass Bioenergy 46 (0):702-709

5. MANUSCRIPT 3: BIOMASS PRODUCTIVITY OF TWO *SCENEDESMUS* STRAINS OPERATED SEMI-CONTINUOUSLY IN OUTDOOR RACEWAY PONDS AND FLAT-PANEL PHOTOBIOREACTORS

Contributions of Author and Co-Authors

Author: Everett Eustance

Contributions: Conceived the study, collected and analyzed data, and wrote the manuscript

Co-author: Shahrzad Badvipour

Contributions: Collected data, discussed results, and edited manuscript.

Co-author: Joshua T. Wray

Contributions: Assisted with study design, discussed results, and edited manuscript.

Co-author: Milton R. Sommerfeld

Contributions: Discussed the results and implications, commented and edited the manuscript.

Manuscript Information Page

Everett Eustance*, Shahrzad Badvipour, Joshua T. Wray, Milton R. Sommerfeld
Journal Name: Algal Research
Status of Manuscript:
____Prepared for submission to a peer-reviewed journal
__x_Officially submitted to a peer-reviewed journal
____Accepted by a peer-reviewed journal
____Published in a peer-reviewed journal
Submitted June 26, 2015

*School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ, 85287; Everett.eustance@asu.edu

5.1. Introduction:

5.1.1. Productivity in Semi-Continuous Systems

High productivity in algal cultures is obtained through semi-continuous cultivation methods. This entails removing a percentage of the culture volume at a set frequency. Previous research in both flat-panel (panel) photobioreactors and raceway ponds (raceways) that removed a portion of the culture every 1 – 2 days were able to achieve maximum growth rates beyond 50 and 40 g/m^2/day, respectively (Hu et al. 1998a; Moheimani and Borowitzka 2006, 2007). However, these are optimal values under excellent environmental conditions. Moheimani and Borowitzka (2006) showed average productivities for different months of the year with growth during the winter significantly reduced with the productivity dropping below 3.2 g/m^2/day compared to greater than 30 g/m^2/day during the summer. The lower growth during winter can be attributed to less light and sub-optimal culture temperatures that can cause photoinhibition (Crowe et al. 2012; Ras et al. 2013; Vonshak et al. 2001). The effect of sub-optimal morning temperatures is reduced culture productivity year round. Slegers et al. (2013) predicted that controlling the temperature of open ponds would increase the yearly productivity by 5 – 10 g/m^2/day. However, considering the amount of energy being lost to the environment with a high surface area to volume, the amount of energy needed would be significantly more than that obtained from the increased biomass productivity. Another approach might be to utilize the ARID system designed at the University of Arizona, USA, which utilizes an in-ground basin to reduce the surface area to volume ratio at night (Waller et al. 2012).

Cultivation in panels requires frequent cooling to prevent high culture temperatures from lysing cells. This is attributed to the shorter path-length in panels compared to raceways, which decreases the heat capacity of the culture and consequently reduces the duration of sun exposure before the culture reaches peak temperature (Hindersin et al. 2013; Zemke et al. 2013; Grobbelaar 2013). Therefore, reducing temperatures in photobioreactors is a major energy cost and to minimize this energy consumption it would be advantageous to have seasonal algal crop rotation with organisms that can tolerate different temperatures.

5.1.2. Raceways vs Panels (Areal Density vs Volumetric)

Previous research has shown that the optimal productivity for panels occurs with a light path between 1 and 10 cm, while raceways are operated between 10 and 30 cm in depth with 15 cm being the most common (Moheimani and Borowitzka 2006; Grobbelaar 2013; Hu et al. 1998b; Richmond and Cheng-Wu 2001). For comparison, these cultivation systems can be assessed based on areal density and productivity. This also provides the best method for assessing photosynthetic efficiency of the PAR flux. Previous research has shown that optimal growth occurs in raceways when the areal density of the culture is between 40 and 150 g/m^2 depending on culture depth and the species being cultivated (Hartig et al. 1988; Grobbelaar et al. 1995). Hartig et al. (1988) concluded that the optimal areal density was between 40 and 45 g/m^2. Based on a raceway depth of 10 to 30 cm, the optimal volumetric density would be 0.45 g/l down to 0.13 g/l, while panels at the same areal density would operate at a concentration beyond 4.5 g/L for a path-length of 1 cm. In addition, Grobbelaar et al. (1995) determined that by

reducing the depth and with high mixing rates, the cascade reactor increased the optimal areal density close to 150 g/m^2, which was a volumetric density greater than 15 g/L in a system with a path-length below 1 cm. This is a significant reduction in water required for cultivation, which significantly reduces the energy expenditure during harvesting (Borowitzka 1999). However, less effort has been put into these system designs and most research has focused on raceways (Buchanan et al. 2013).

Little comparative research has been reported on cultures grown in both flat-panel photobioreactors and raceways; however, previous studies have assessed the use of tubular photobioreactors. Torzillo et al. (2012) cultivated *Phaeodactylum tricornutum* in an outdoor tubular reactor alongside a circular pond for comparison of the two systems. The results from the experiment showed that the open pond had an increase of 27% productivity compared to the photobioreactor. However, it is important to note that the tubular photobioreactor utilized a centrifugal pump for culture flow and mixing, which has a significant amount of shear and can cause a drastic decrease in productivity (Torzillo et al. 2003). This is why many cultivation systems utilize aeration for mixing or airlifting of the culture to minimize shear stress (Molina et al. 2001; Sánchez Mirón et al. 2000). Raes et al. (2014) compared *Tetraselmis sp*. in an open raceway pond and the Biocoil helical photobioreactor and showed that the organism in semi-continuous cultivation achieved 3 g/m^2/day and 0.85 g/m^2/day, respectively. The growth rate in the raceways was comparable to that obtained in previous studies; however, the study had significantly lower productivities for photobioreactors than previously reported (Eustance et al. Submitted; Hu et al. 1998a; Moheimani and Borowitzka 2007). It is important to remember that flat-panel photobioreactors are not generally considered feasible for large-

scale cultivation of algae, except for the production of high-value products; however, they provide a valuable research tool by creating a benchmark for achievable productivity.

The purpose of this study was to provide a definitive side-by-side comparison of flat-panel photobioreactors with raceway ponds for two *Scenedesmus acutus* strains in an outdoor environment during the spring growing season (end of February through the beginning of May 2015) on the Arizona Center for Algae Technology and Innovation field-site in Mesa, Arizona. Culture density, productivity, and nitrogen consumption were measured along with ambient conditions to provide information on biomass production under semi-continuous cultivation.

5.2. Materials and Methods

5.2.1. Organisms and Cultivation

All experiments were carried out using two strains of *Scenedesmus acutus*, strain LB-0414 (strain 0414) and strain LB-0424 (strain 0424), cultured in outdoor raceway ponds and flat panel photobioreactors at the Arizona Center for Algae Technology and Innovation (AzCATI) field-site in Mesa, Arizona. Strain 0414 has a maximum temperature tolerance of 30°C, while strain 0424 has a heat tolerance exceeding 45°C. Raceway experiments were completed in three identical ponds each consisting of two channels 6.1 m long, 1.7 m wide with two ends each with a radius of 1.78 m providing a total area of 30.37 m^2. Velocity of raceways was set to an average linear flow of 25 cm/s. During the day, CO_2 was added through a Sweetwater diffusion stone measuring 30 cm long and 5 cm wide at a rate of 5 L/min. Inoculating cultures were grown in 60 L flat

panel photobioreactors with Modified BG-11 Medium. The photobioreactors had a North-South facing exposure measuring 46” (1.17 m) in width by 46” (1.17 m) in height and approximately 1.5” (3.8 cm) in depth (thickness) or path length. The panels have an internal volume of 60 L; however, reactors were filled with approximately 55 L of medium to account for the volume required for aeration. Aeration was provided by small drilled holes (~1/32” (0.8 mm)) in 1/2” (1.3 cm) PVC located at the bottom of the reactor at a rate of approximately 0.5 vvm. During the day, CO_2 was added to the aeration line to provide a concentration of 1.5% CO_2 (v/v). The reactors contained an internal 1/2" (1.3 cm) stainless steel cooling line connected to an evaporative cooling system.

The composition of the Modified BG-11 Medium (1/4 bg-11) utilized in the photobioreactors was 375 mg/L $NaNO_3$ (Alfa Aesar), 9.58 mg/L K_2HPO_4 (BDH), 1.31 mg/L Citric Acid Monohydrate (Sigma-Aldrich), 5.24 mg/L of Ammonium Ferric Citrate (Alfa Aesar), 0.71 mg/L H_3BO_3 (Sigma-Aldrich), 0.445 mg/L $MnCl_2 \cdot 4H_2O$ (Sigma-Aldrich), 0.06 mg/L $ZnSO_4 \cdot 7H_2O$ (Sigma-Aldrich), 0.10 mg/L $Na_2MoO_4 \cdot 2H_2O$ (Sigma-Aldrich), 0.02 mg/L $CuSO_4 \cdot 5H_2O$ (Sigma-Aldrich), 0.1 mg/L $Co(NO_3)_2 \cdot 6H_2O$ (Sigma-Aldrich). Because these experiments utilized tap water, magnesium sulfate, calcium chloride and sodium carbonate were not added to the Modified BG-11 Medium. Tap water used averaged 180 mg/L Na, 75 mg/L Ca, 70 mg/L S, 24 mg/L Mg, and 8 mg/L K with a hardness of 280 mg /L as $CaCO_3$. During the second part of experimentation in April, the nitrogen source was changed from $NaNO_3$ to NH_4HCO_3 at 348.8 mg /L (J.T. Baker), to provide the same nitrogen concentration.

For Raceway experiments, approximately 75 to 100 L of culture was transferred to the raceways, where cultures were grown in Modified BG-11 Medium with industrial fertilizers replacing the nitrate and phosphate sources: ammonium iron citrate was used for an iron source. Nitrate, phosphate and iron were added at a concentration of 1/6 of the original concentration of Modified BG-11 Medium (1/6 BG-11 Medium), while the "micronutrients" were added at a concentration 1/50 that of Modified BG-11 (1/50 bg-11) as represented by the following concentration values. The cultures utilized calcium nitrate 15.5-0-0 (YaraLiva Tropicote) for a nitrogen source at 264 mg/L (41.2 mg-N/L), and Blooming and Rooting 9-58-8 (Ferti-lome) for a phosphorus source at 4.69 mg/L (1.13 mg-P/L). Micronutrients were added at reduced concentration compared to those for panels, at a concentration of 0.057 mg/L H_3BO_3 (Sigma-Aldrich), 0.036 mg/L $MnCl_2 \cdot 4H_2O$ (Sigma-Aldrich), 0.0048 mg/L $ZnSO_4 \cdot 7H_2O$ (Sigma-Aldrich), 0.008 mg/L $Na_2MoO_4 \cdot 2H_2O$ (Sigma-Aldrich), 0.0016 mg/L $CuSO_4 \cdot 5H_2O$ (Sigma-Aldrich), 0.008 mg/L $Co(NO_3)_2 \cdot 6H_2O$ (Sigma-Aldrich).

5.2.2. Monitoring and Control

Temperature and pH were continuously monitored using a Neptune Apex controller (Neptune Systems, LLC.). The Apex also controlled solenoid valves to turn aeration on and off at programmed intervals during the night. In addition, solenoid valves were attached to the cooling lines of the first four reactors to allow cultures to achieve higher temperature with the same cooling water. Ambient conditions were measured with an Argus weather station capable of recording ambient temperature, relative humidity, and light intensity (Argus Control Systems, LLC.). For part of the experiment, PAR was

measured on the front and backside of the photobioreactors using Li-190 sensors attached to a Li-1400 Logger (Li-COR Biosciences).

5.2.3. Analytical

5.2.3.1. Biomass Density

Ash-free dry weight (AFDW) was measured in duplicate to assess growth performance. Glass filters (VWR 696 glass microfiber 1.2µm) were ashed for 4 hours at 500°C prior to initial weighing. Duplicate samples were collected by filtering 10 to 20 mL per filter (depending on culture density). Samples were placed into an oven at 60°C overnight. The filters with biomass were weighed to determine dry cell weight and then ashed at 500°C for 4 hours to determine the AFDW and ash content of the biomass.

5.2.3.2. Nitrogen

Nitrogen levels for nitrate and ammonium were monitored using flow-injection analysis with a Lachat QuikChem 8500 with samples being analyzed in duplicate.

5.3. Results

Experimentation was completed during two periods: February 17th to March 27th, 2015 and April 2nd to May 7th, 2015. During these times, flat-panel photobioreactors (panels) and open raceway ponds (raceways) were operated under semi-continuous growth conditions with different variables being changed as highlighted in Table 5.1 for February 17th to March 27th and Table 5.3 for April 2nd to May 7th.

5.3.1. February 17th to March 27th Experimentation

Initial experimentation was completed in panels and raceways during February 17th to March 27th, 2015. During this timeframe, cultivation was accomplished using *Scenedesmus acutus* strain 0414 (strain 0414). Figure 5.1 shows the light intensity (Fig. 5.1a), ambient and culture temperature (Fig. 5.1b), nitrogen concentration (Fig. 5.1c), and volumetric ash free dry weight (AFDW) for cultures in panels (Fig. 5.1d) and raceways (Fig. 5.1e) throughout the cultivation timeframe. For both panels and raceways, cultures were operated in a semi-continuous manner, at the first point of harvest indicated by points 1 (Fig. 5.1d) and 9 (Fig. 5.1e), respectively, nutrients were added back to provide for the volume of culture removed. For panels, this caused significant nutrient limitation

Table 5.1: Description of changes that occurred during experimentation from February 17th to March 27th. Numbers correlate to key points of change in Figures 1.

Key Points	Date	Description of changed that occurred
0	2/18/15	Panels: experiment started
1	2/21/15	Panels: added 1/4 BG-11 for the volume removed
2	2/26/15	Panels: added 1/4 BG-11 for full volume due to stressing
3	3/3/15	Panels: added 1/4 BG-11 for full volume after harvest
4	3/8/15	Panels: shutoff night aeration until 3/17/15
5	3/17/15	Panels: changed to 1/6 BG-11
6	3/24/15	Panels: changed to 1/4 BG-11
7	3/27/15	Panels: experiment completed
8	2/17/15	Raceways: experiment started
9	2/22/15	Raceways[a]: added 1/6 BG-11 for volume removed; no micronutrients
10	2/27/15	Raceways: added 1/6 BG-11 for full volume; no micronutrients
11	3/3/15	Raceways: added micronutrients at 1/50 BG-11
12	3/5/15	Raceways[b]: did not harvest, but added nutrients to prevent further stressing
13	3/8/15	Raceways: experiment shutdown to start with new log phase culture
14	3/10/15	Raceways[b]: second experiment started
15	3/19/15	Raceways: culture temperature too high causing cell lysis
16	3/23/15	Raceways: experiment ended

[a]1/6 BG-11 in raceways is limited to nitrate, phosphate, and iron concentrations.
[b]Added 1/6 BG-11 plus the 1/50 BG-11 micronutrients for full volume

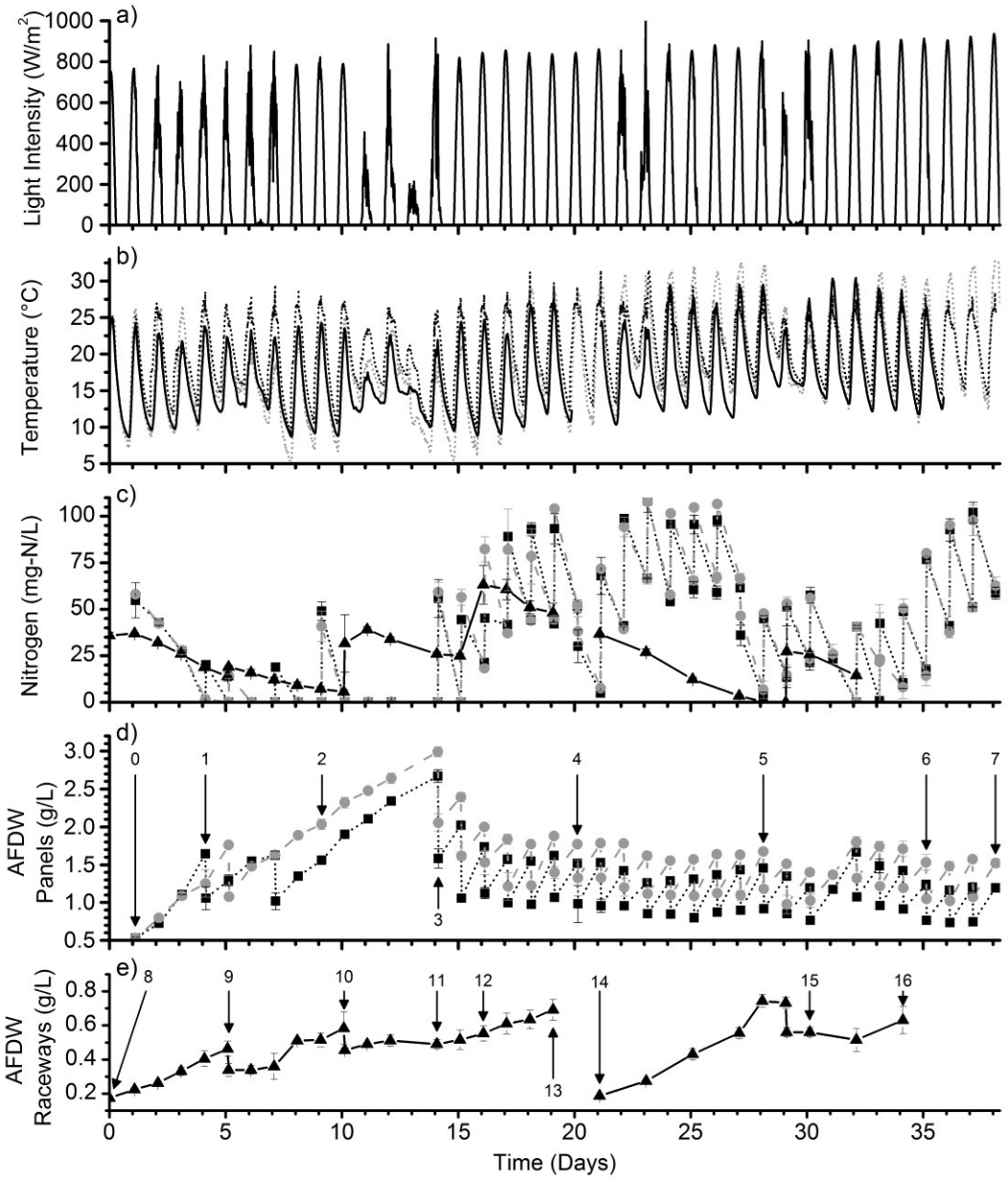

Figure 5.1: Light intensity (a), temperature (b), nitrogen concentration (c) and AFDW (d, e) for cultivation occurring between February 17th and March 27th, 2015 using Strain LB 0414. Average ± S.D. for raceway ponds (black solid line/filled triangle) are reported for two separate experiments. Average ± S.D. for panels are reported for removing 29% of culture (gray dash line/filled circle) and 36% of culture (black dot line/filled square). Numerical identification of key changes are indicated in the figure and follow the descriptions found in Table 5.1.

and reduced culture productivity (Table 5.2). This resulted in an average productivity of 10.9 ± 4.9 g/m^2/day by removing 36% of the culture volume and 10.2 ± 4.7 g/m^2/day by removing 29% of the culture, with nutrients consumed within 24 hours (Fig. 5.1c). This is

significantly lower than when providing nitrogen sufficient cultures by adding nutrients based on the entire cultivation volume and not the volume being removed, which was 19.7 ± 3.9 and 18.7 ± 3.7 g/m^2/day for 36% and 29% being removed, respectively. In raceways, cultivation was initially tested to determine if tap water would provide enough micronutrients for growth. Initial growth showed minimal effects from not adding the micronutrients (Fig. 5.1e), but this is attributed to the high levels of micronutrients found in the inoculum. This is based on the lack of growth between points 10 and 11 (Fig. 5.1e), which was from the second harvest without the addition of micronutrients thru when micronutrients were added at a concentration of 1/50 bg-11 and started to show growth. This was corrected in later experimentation by adding micronutrients when adding nitrogen, phosphorus and iron. In the raceways, the macronutrients were added at a concentration of 1/6 BG-11 Medium to prevent nutrient limitation, while the

Table 5.2: Average ± S.D. for solar irradiance, biomass productivity, and photosynthetic efficiency for experiments completed in February and March. Values are for periods between key points of change to highlight differences that occurred. Key points are identified in Table 5.1.

Key Points[a]	Dates	Irradiance	Biomass Productivity		Photosynthetic Efficiency[b]	
		kWh/m^2/d	g/m^2/d		%	
			Panels			
			36% removed	29% removed	36% removed	29% removed
0-3	2/18/15- 3/3/15	4.2 ± 0.8	10.9 ± 4.9	10.2 ± 4.7	1.47 ± 0.69	1.38 ± 0.70
3-4	3/3/15-3/9/15	5.5 ± 0.5	22.5 ± 4.5	18.2 ± 5.8	2.24 ± 0.40	1.82 ± 0.53
4-5	3/9/15-3/16/15	5.4 ± 0.8	20.0 ± 3.9	19.8 ± 1.9	2.03 ± 0.18	2.04 ± 0.26
5-6	3/17/15-3/24/15	5.5 ± 1.1	17.2 ± 2.5	16.6 ± 2.5	1.75 ± 0.39	1.70 ± 0.44
6-7	3/24/15-3/27/15	6.5 ± 0.2	18.3 ± 1.4	20.1 ± 2.6	1.54 ± 0.49	1.70 ± 0.53
3-7	3/3/15-3/27/15	5.6 ± 0.8	19.7 ± 3.9	18.7 ± 3.7	1.95 ± 0.38	1.85 ± 0.40
Raceways						
8-13	2/17/15- 3/8/15	4.6 ± 0.9	3.5 ± 2.7		0.42 ± 0.32	
14-16	3/10/15-3/23/15	5.2 ± 0.9	4.1 ± 4.8		0.30 ± 0.44	

[a]Key points identified in Table 1
[b]Assumes energy value of 5.5 kWh/kg of biomass

micronutrients were adjusted to 1/50 BG-11 Medium for the entire volume, since the concentration found in BG-11 Medium contained excessive levels of micronutrients.

Due to culture temperature issues in raceways, highlighted as point 15 on Figure 5.1e, the culture exhibited significant amounts of cell lysis as peak culture temperatures reached above 30°C (Fig. 5.1b). This caused significant culture loss and growth inhibition and experimentation shutdown at point 16 (Fig. 5.1e).

Once panels were provided with sufficient nutrient levels at point 3 (Fig. 5.1d), semi-continuous cultivation was accomplished by removing either 29% or 36% of the culture volume daily. In an attempt to reduce energy consumption, aeration was turned off at night starting at point 4 and ending at point 5. Figure 5.1d shows that cultivation had a drop in the peak culture density; this may be attributed to cloudy weather as shown by the lower light intensity on days 22 and 23 (Fig 5.1a). Furthermore, Table 5.2 shows that productivity without aeration was not significantly different, which showed that with aeration cultures had a biomass productivity of 22.5 ± 4.5 g/m^2/day and 18.2 ± 5.8 g/m^2/day for 29% and 36% removed, respectively, and a productivity of 20.0 ± 3.9 g/m^2/day and 19.8 ± 1.9 g/m^2/day without aeration. Due to operating under outdoor conditions, environmental factors including temperature and light intensity have a significant impact on productivity and account for the large standard deviation values being reported as shown in Figure 5.2.

To further evaluate nutrient requirements being added to panels, the concentration of BG-11 Medium being added was reduced from 1/4 to 1/6 between the points of 5 and 6 on Figure 5.1d. As shown by the drop in daily peak AFDW, the culture was not

harvested on day 31 to allow the operating density to increase and prevent photoinhibition from occurring. Table 5.2 shows that the average productivity during this timeframe dropped to 17.2 ± 2.5 and 16.6 ± 2.5 g/m^2/day for harvesting volumes of 36% and 29%, respectively.

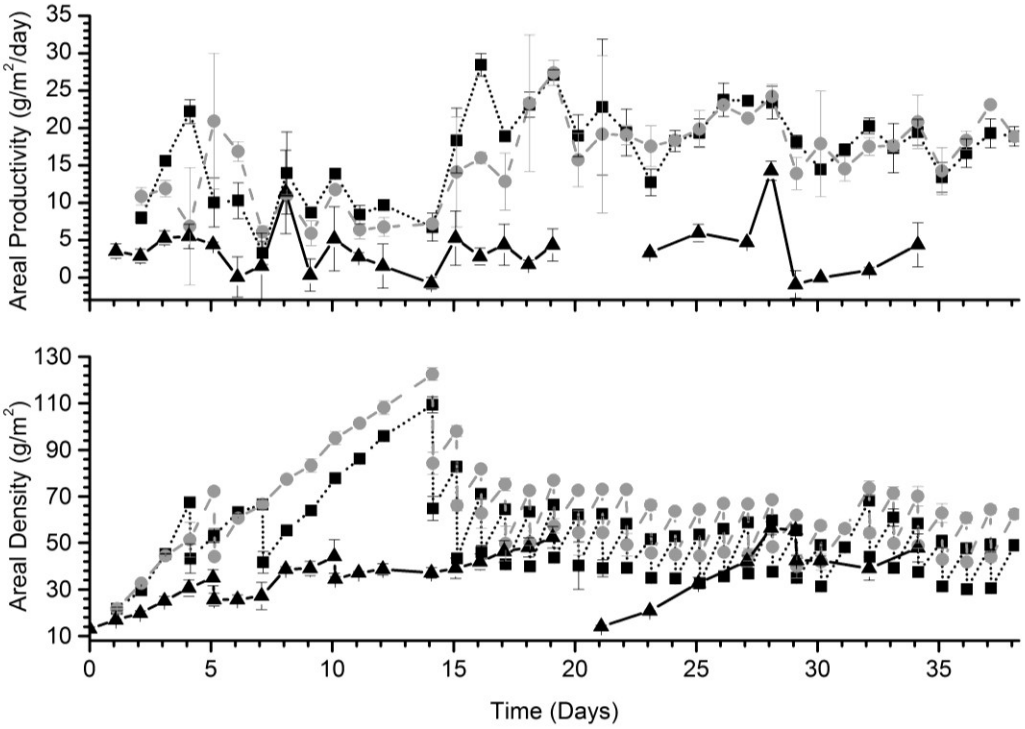

Figure 5.2: Areal density and areal productivity for experiments completed between February 17th and March 27th, 2015 using Strain LB 0414. Average ± S.D. for raceway ponds (black solid line/filled triangle) are reported for two separate experiments. Average ± S.D. for panels are reported for removing 29% of culture (gray dash line/filled circle) and 36% of culture (black dot line/filled square)

5.3.2. April 2nd to May 7th Experimentation

From April 2nd to May 7th a second set of semi-continuous experiments was completed in panels and raceways. However, the organism utilized was switched from strain 0414 to strain 0424, an organism with a higher peak temperature to accommodate warmer spring temperatures. In panels, condition 1 (Cond 1) and condition 2 (Cond 2)

Table 5.3: Description of changes that occurred during experimentation from April 2nd to May 7th. Numbers correlate to key points of change in Figures 5.3.

Key Points	Date	Description of changed that occurred
17	4/4/15	Panels: experiment started with Cond 1 peak temp set to 34°C, Cond 2 peak temp set to 38°C, Cond 3 peak temp set to 27°C
18	4/7/15	Panels: all conditions set to remove 29% of culture
19	4/13/15	Panels: Cond 3 set to remove 18% of culture
20	4/16/15	Panels: Cond 1 and 2 switched from $NaNO_3$ to NH_4HCO_3
21	4/19/15	Panels: Cond 3 switched from $NaNO_3$ to NH_4HCO_3
22	4/22/15	Panels[c]: all conditions intermittent sparging at night 1 min on: 30 min
23	4/26/15	Panels: Cond 1 36% of culture removed, Cond 2 peak temp set at 30°C, Cond 3: 29% of culture removed
24	5/1/15	Panels: Cond 1 peak temp set at 42°C
25	5/7/15	Panels: experiment ended
26	4/2/15	Raceways[b]: experiment started
27	4/8/15	Raceways[b]: removed 43% of culture
28	4/18/15	Raceways[b]: removed 65% of culture
29	4/22/15	Raceways: lost 1 of three ponds due to paddlewheel failure
30	4/23/15	Raceways[b]: removed 50% of culture
31	5/7/15	Raceways: experiment ended

[b]Added 1/6 BG-11 plus the 1/50 BG-11 micronutrients for full volume
[c]Remained this way until end of experiment to minimize ammonia volatility and toxicity

utilized strain 0424, while condition 3 (Cond 3) utilized strain 0414. Raceways, which lacked temperature control, used strain 0424 to accommodate higher peak temperatures.

Figure 5.3 shows the light intensity (Fig. 5.3a), ambient and culture temperature (Fig. 5.3b), nitrogen concentration (Fig. 5.3c), and AFDW for cultures in panels (Fig. 5.3d) and raceways (Fig. 5.3e) throughout the cultivation timeframe. The initial conditions for the panels were set with all panels receiving 1/4 BG-11 for the full culture volume and harvesting 29% of the culture volume every day once the desired culture density was achieved. In addition, Cond 1 had a peak operating temperature of 34°C, Cond 2 had a peak operating temperature of 38°C, and Cond 3 had a peak operating temperature of 27°C. The results show that both strains had nearly identical operating

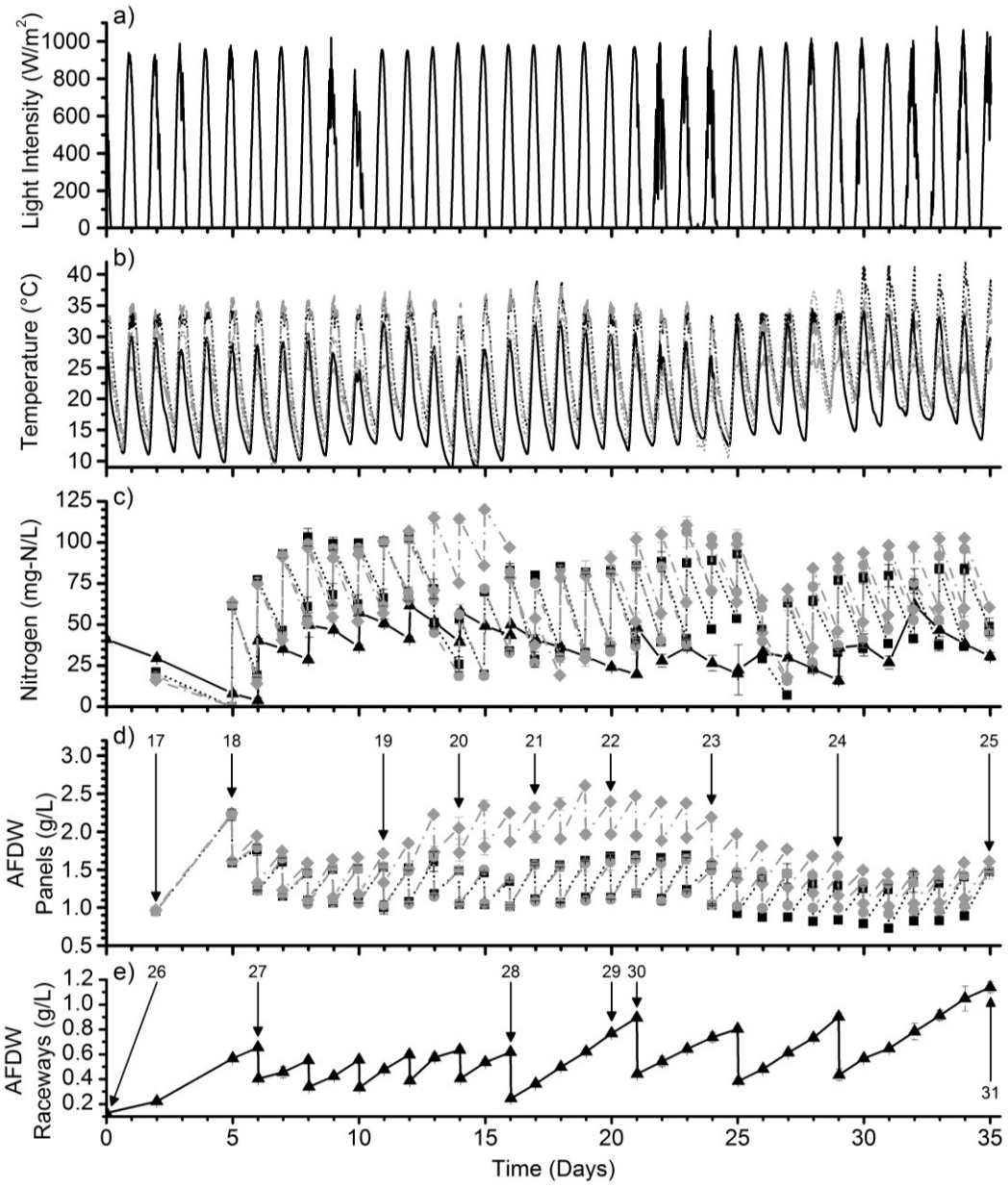

Figure 5.3: Light intensity (a), temperature (b), nitrogen concentration (c) and AFDW (d, e) for cultivation occurring between April 2nd and May 7th, 2015 using Strain LB-0414 and Strain LB-0424. Average ± S.D. for raceway ponds (black solid line/filled triangle) are reported for growth with Strain LB 0424. Average ± S.D. for panels are reported for Strain LB 0424 in Condition 1 (gray dash line/filled circle) and Condition 2 (black dot line/filled square), with Strain 0414 grown in Condition 3 (gray dash dot line/ filled diamonds). Numerical identification of key changes are indicated in the figure and follow the descriptions found in Table 5.3.

densities. Furthermore, strain 0414 in Cond 3 showed a productivity of 18.6 ± 3.7 g/m^2/day (Table 5.4), which was comparable to the overall productivity for strain 0414 operated during experimentation in February and March with 18.7 ± 3.7 g/m^2/day.

To determine the effect of reducing daily harvesting volume, Cond 3 at point 19 reduced the volume of culture being removed from 29% to 18% and was maintained at that removal rate until point 23 (Fig. 5.3d). During this timeframe, the peak culture density achieved increased from 1.63 ± 0.13 g/L to 2.33 ± 0.14 g/L and did not show a significant change in culture productivity (Table 5.4).

Table 5.4: Average ± S.D. for solar irradiance, biomass productivity, and photosynthetic efficiency for experiments completed in April and May. Values are for periods between key points of change to highlight differences that occurred.

Key Points[a]	Dates	Irradiance	Biomass Productivity			Photosynthetic Efficiency[b]		
		kWh/m^2/d	g/m^2/d			%		
			Panels					
			Cond 1	Cond 2	Cond 3	Cond 1	Cond 2	Cond 3
17-19	4/4/15-4/13/15	6.6 ± 0.7	-	-	18.6 ± 3.7	-	-	1.45 ± 0.62
17-20	4/4/15-4/16/15	6.8 ± 0.6	16.4 ± 4.6	17.3 ± 4.9	-	1.26 ± 0.52	1.33 ± 0.54	-
19-21	4/13/15-4/19/15	7.3 ± 0.2	-	-	21.3 ± 5.9	-	-	1.61 ± 0.47
20-22	4/16/15-4/22/15	7.4 ± 0.1	19.9 ± 4.2	19.6 ± 3.3	-	1.50 ± 0.32	1.51 ± 0.31	-
21-22	4/19/15-4/22/15	7.4 ± 0.1	-	-	21.8 ± 6.5	-	-	1.62 ± 0.46
22-23	4/22/15-4/26/15	6.3 ± 0.9	20.2 ± 4.9	18.5 ± 5.1	20.0 ± 1.5	1.75 ± 0.29	1.60 ± 0.32	1.65 ± 0.14
23-24	4/26/15-4/30/15	7.2 ± 0.8	19.8 ± 3.1	18.1 ± 1.1	16.3 ± 3.2	1.52 ± 0.17	1.40 ± 0.19	1.32 ± 0.11
24-25	5/1/15-5/7/15	7.1 ± 0.4	21.9 ± 3.4	17.3 ± 2.1	18.3 ± 2.9	1.69 ± 0.27	1.34 ± 0.18	1.41 ± 0.23
17-25	4/4/15-5/7/15	7.0 ± 0.7	19.2 ± 4.3	18.0 ± 3.7	19.2 ± 4.2	1.49 ± 0.39	1.40 ± 0.35	1.49 ± 0.39
			Raceways					
26-28	4/2/15-4/19/15	6.9 ± 0.6	8.2 ± 3.0			0.63 ± 0.29		
30-31	4/24/15-5/4/15	6.9 ± 0.8	8.6 ± 2.1			0.68 ± 0.14		
26-31	4/2/15-5/7/15	7.0 ± 0.7	8.7 ± 2.3			0.67 ± 0.21		

[a]Key points described in Table 5.3
[b]Assumes energy value of 5.5 kWh/kg of biomass

At point 20 for Cond 1 and 2 and point 21 for Cond 3, the cultures were switched from $NaNO_3$ to NH_4HCO_3 to evaluate if culture productivity changed with the different nitrogen source (Fig. 5.3d). Based on the data in Table 5.4, neither culture showed any significant change in culture productivity. At point 22, all three conditions were switched from continuous aeration at night to intermittent sparging at a frequency of 1 minute per 30 minutes. This was done to minimize ammonia toxicity and volatility at night when culture pH values increased due to the external CO_2 source being turned off as was shown in previous research (Eustance et al. 2015). This showed no effect on the cultures productivity.

At point 23 (Fig. 5.3d), all three conditions changed. Cond 1 had the culture volume being removed increased from 29% to 36%, Cond 2 had the peak culture temperature decreased from 38°C to 30°C, and Cond 3 had the culture volume being removed increased from 18% to 29%. By reducing the peak operating temperature in Cond 2, strain 0424 showed a small decrease in the biomass productivity being achieved from points 24 to 25 (Table 5.4). Furthermore, at point 24 (Fig. 5.3d), Cond 1 had the peak temperature increased from 34°C to 42°C and showed a small increase in productivity, which highlights this strain's capability to grow over a very large range of temperatures without having a significant change in culture productivity.

The raceways operated between April 2nd and May 7th, 2015 utilized strain 0424. At point 27 (Fig. 5.3e), 43% of the culture was removed every other day until point 28, where 65% of the culture was removed. This was accomplished once, since removing over 50% of the culture was challenging due to the shallow depth of the raceways. At

point 30, the cultures had 50% of the culture removed every 4 days. This provided a large operating range of areal densities to determine if there was a peak productivity associated with any of the culture densities. Based on Figure 5.4 and Table 5.4, the range in which the culture was operated showed no drastic effect on the biomass productivity, which averaged 8.7 ± 2.3 g/m^2/day.

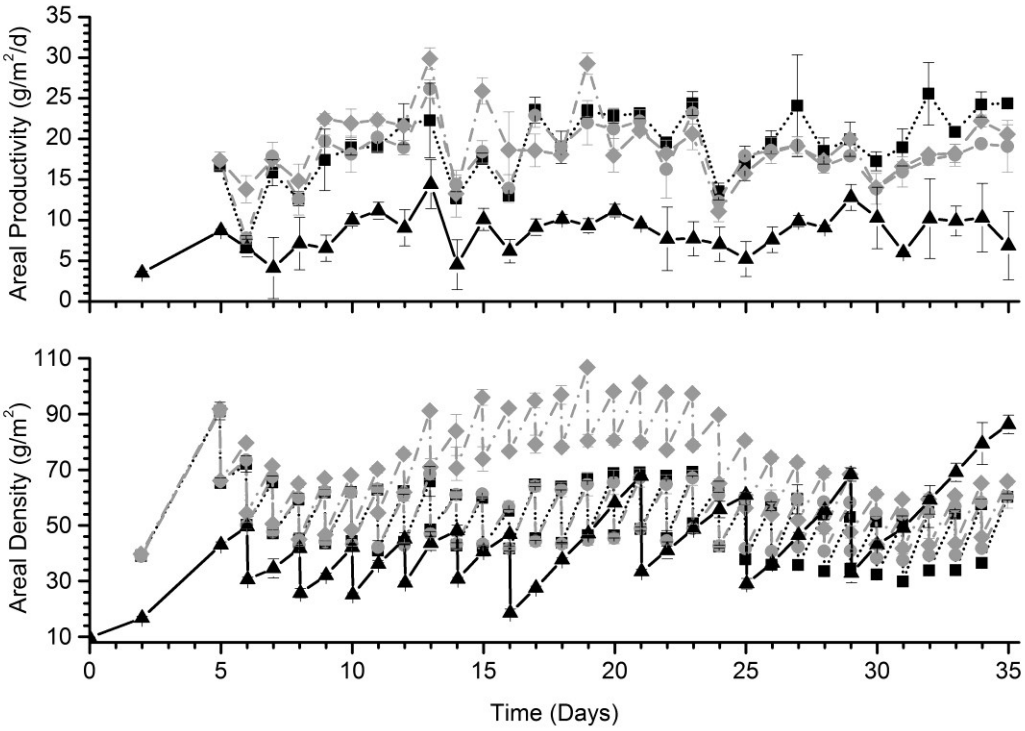

Figure 5.4: Areal productivity and areal density for experiments completed between April 2nd and May 7th, 2015 using Strain LB-0414 and Strain LB-0424. Average ± S.D. for raceway ponds (black solid line/filled triangle) are reported for growth with Strain LB-0414 Average ± S.D. for panels are reported for Strain LB 0424 in Condition 1 (gray dash line/filled circle) and Condition 2 (black dot line/filled square), with Strain LB-0414 grown in Condition 3 (gray dash dot line/ filled diamonds).

5.4. Discussion

5.4.1. Raceways vs Panels (Areal Density vs Volumetric)

Previous research has suggested that the optimal areal density for cultures is between 40 and 150 g/m^2 depending on culture depth and the species being cultivated

(Hartig et al. 1988; Grobbelaar et al. 1995). Hartig et al. (1988) concluded that the optimal areal density was between 40 and 45 g/m^2 for ponds operated at 15 to 30 cm. In addition, Grobbelaar et al. (1995) determined that by reducing the depth and with the high mixing rates, the cascade reactor increased the optimal areal density close to 150 g/m^2. This is similar to the result shown in the current study, where increasing the operating areal density above 100 g/m^2 (Fig. 5.2 and 5.4) in the flat-panel photobioreactors (panels) had little impact on the productivity suggesting that cultures with high levels of mixing can operate at higher culture densities. Comparatively, raceways operated at a depth of 7.5 cm have significantly less mixing, which contributed to the limited productivity independent of the areal density as shown in Figure 5.2 and 5.4. Both figures show no correlation between areal density and areal productivity. However, it is important to note that the cultures were operated within a range of 20 to 70 g/m^2 and may have needed an areal density greater than 100 g/m^2 to show a decrease in productivity.

The growth rate of raceways compared to panels for semi-continuous cultivation consistently showed that panels outperformed raceways with an average productivity of 19.0 ± 0.6 g/m^2/day compared to 6.62 ± 2.3 g/m^2/day, respectively. However, it is important to note that panels are used to provide an idea of the maximum productivity a culture may be able to achieve in raceways.

5.4.2. Temperature Concerns

One of the biggest concerns and reasons for a decrease in culture productivity in raceways compared to panels, or the value achieved in panels compared to the theoretical

limit of 77 g/m^2/day is the diurnal temperature fluctuation (Melis 2009). This is shown in Figure 5.5, which highlights two different days during April and provides culture and ambient temperature along with light intensity. Figure 5.5 shows that during the first few hours of the day the cultures are at sub-optimal temperatures. Previous research has

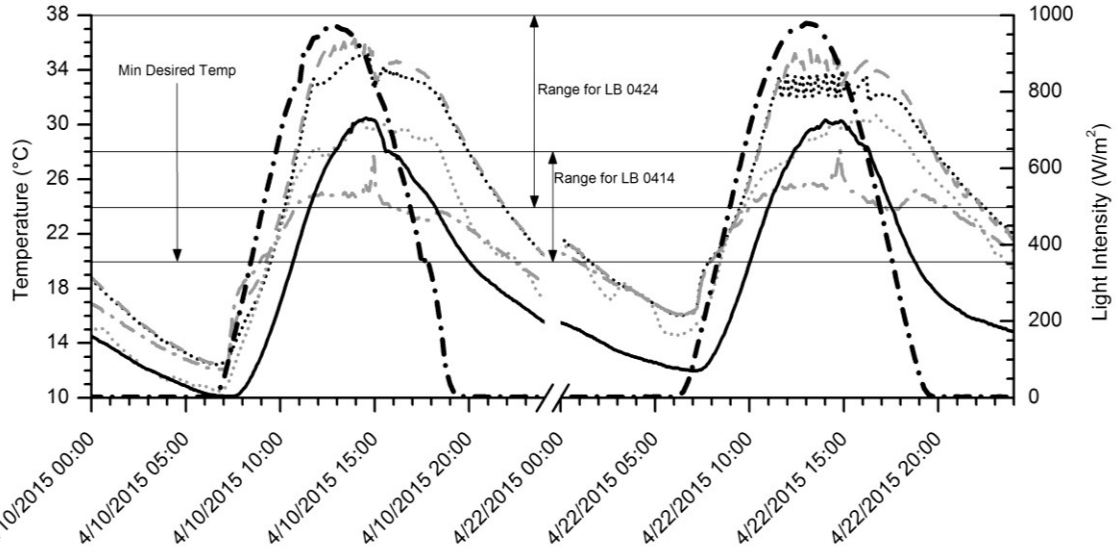

Figure 5.5: Actual and preferred temperature ranges for Strain LB-0414 and Strain LB-0424 at 2 different time points during cultivation in April 2015. Strain LB-0424 in raceways (black solid line) and panels in Condition 1 (gray dash line) and Condition 2 (black dot line/filled square), and Strain LB-0414 grown in Condition 3 (gray dash dot line). Ambient temperature (gray dotted line) and light intensity (black dash dot line) are shown for comparison.

shown that sub-optimal temperatures cause photoinhibition and reduced enzyme activity, which reduces culture productivity (Ras et al. 2013; Vonshak et al. 2001). Furthermore, Figure 5.5 showed that raceways required more time to reach the desired operating range compared to panels and helps explain the lower productivity achieved. Furthermore, the average morning temperature, defined as the time between 7 am and 10 am, was 11.2 ± 1.9°C in the raceways from February 17th to March 8th, while from April 2nd to May 7th the average was 14.8 ± 3.1°C. However, these values are both significantly lower than the values found in panels, which had averages of 18.0 ± 2.7°C and 20.3 ± 3.4°C for the

same timeframe. By decreasing the culture path length/depth, the system has a decreased heat capacity, and will therefore, increase in temperature faster, which minimizes time spent with sup-optimal morning conditions but will achieve higher peak temperatures. In panels, this is combatted by utilizing an evaporative cooler and stainless steel cooling lines in the culture media; however, the ponds did not and typically do not have temperature control. One of the main cited reasons for raceways to be operated at a depth of greater than 15 cm is to minimize temperature fluctuations and peak daytime temperatures, which can lead to culture crashes (Oswald 1988; Moheimani and Borowitzka 2007). This was shown in Figure 5.1e at point 15, where strain 0414 in raceways began to reach undesirable temperatures and the culture began to lyse and crash. Research has shown that cultures tend to have 4 to 5 °C range between the optimal temperature and the point where cultures begin to die from high temperatures (Ras et al. 2013; Buchanan et al. 2013). This has drastic effects on culture productivity if the temperature spikes unexpectedly, but may be minimized by rotating algal crops to accommodate the different temperature ranges as was done in this study by switching from strain 0414 to strain 0424.

5.4.3. Aeration at Night

Previous research has shown that limiting aeration at night can decrease lipid accumulation, but has little effect on log phase cultures (Eustance et al. 2015). To determine the long-term effect of limiting aeration at night, cultures were operated with no aeration from points to 4-5 (Fig. 5.1d), which showed no significant difference in the cultures biomass productivity (Table 5.2). Cultures were switched from nitrate to

ammonium at points 20 for Cond 1 and 2 and 21 for Cond 3 (Fig. 5.3d), and to avoid ammonia toxicity and volatility, were switched to an intermittent sparging frequency of 1 min on per 30 min for the remainder of experimentation at point 22 (Fig. 5.3d). Table 5.4 shows that the change to intermittent sparging at night had no significant impact on growth as was previously shown in Eustance et al. (2015). Furthermore, Cuello et al. (2014) showed that stopping the paddlewheel at night in semi-continuous raceways did not statistically change biomass productivity compared to constant mixing, which had the advantage of decreased energy consumption by 37 %.

5.4.4. Nitrogen Usage

Cultivation in panels for March showed an average nitrogen consumption rate of 36.4 ± 10.7 mg/L/day, while cultivation in April and May showed a consumption rate of 39.4 ± 7.2 mg/L/day. These values correspond to consuming between 77 and 87 mg-N/g-biomass. However, cultures required excess nitrogen to prevent a reduction in growth rate, as cultures that received approximately 60 mg-N/L/day had a higher growth rate than cultures that received approximately 40 mg-N/L/day. However, the consumption rate of nitrogen may be skewed as nitrogen was most likely removed during the time taken for a homogenous culture to mix. This is further exemplified in both Figure 5.1c and 5.3c, as the maximum nitrogen concentration did not continue to increase overtime, but reached a plateau that corresponded to the culture density. This suggests that the cultures were able to remove higher levels than that measured from the medium and were able to remove closer to 60 mg-N/L, as this was the amount being added to the cultures.

Cultivation in raceways showed lower nitrogen consumption rates due to lower biomass productivities. During cultivation in February and March, the average consumption rate was 3.8 ± 2.5 mg/L/day, while growth in April and May had a consumption rate of 7.1 ± 4.2 mg/L/day. This corresponded to a consumption rate between 64 and 76 mg-N/g-biomass, which is similar to the rate determined for panels.

5.4.5. Productivity and Photosynthetic Efficiency

Table 5.3 and 5.4 show productivity and photosynthetic efficiency values for panels and raceways operated between February 17th and May 7th, 2015. At the beginning of March, the average solar irradiance for cultivation was 5.5 ± 0.5 kWh/m^2/day and by the end of experimentation in May, the value had increased to 7.1 ± 0.7 kWh/m^2/day. However, during this timeframe the average biomass productivity in panels did not significantly change. This is attributed to a change in the angle of incidence of the sun, which increases in the summer and therefore provides a smaller fraction of the total light to a vertical surface. From March 11th to March 27th and April 23rd to May 7th PAR was measured on the front and backside of the panels. During these times, the average solar irradiance was 5.7 ± 1.0 kWh/m^2/day for March and 6.9 ± 0.8 kWh/m^2/day for April. When these values are converted to PAR based on the value found in Melis (2009) of 5 kWh/m^2/day is approximately 35 mol of photons/m^2/day, the average for March was 39.8 ± 6.7 mol of photons/m^2/day and for April was 48.5 ± 5.3 photons/m^2/day. However, the combined PAR for the front and backside of the panels was 36.2 ± 4.9 mol of photons/m^2/day in March and 30.8 ± 1.3 mol of photons/m^2/day in April. This shows that the panels were receiving less light at the surface and maintaining the same level of

productivity. However when calculating the photosynthetic efficiency (PE) as shown in Tables 5.2 and 5.4, PE decreased throughout experimentation for cultures grown in panels from 1.90 ± 0.07% (points 3-4) initially to 1.46 ± 0.05% for cultivation between points 17 and 25. Panels did not show an increase in PE, but this is due to the vertical orientation, which decreased the amount of usable light. When assessing PE based on the PAR reaching the surfaces of the panel, the PE increased from 2.0 ± 0.0% to 2.3 ± 0.2% for March 11-27th and April 22nd-May 7th, respectively, which indicates that more favorable culture conditions allowed increased daily productivity despite having less usable light. Assessing raceways, which utilize a horizontal surface, showed an increase in both biomass productivity and PE as experimentation proceeded towards higher sun angles. The average PE for raceways during April and May experimentation was 0.66 ± 0.02%, which increased from 0.36 ± 0.08% for cultivation in February and March. This also shows that factors other than solar irradiance alone increased biomass productivity and is most likely due to the increase in morning temperatures as was previously discussed. The values achieved for PE are significantly lower than the theoretical range of 8-10% suggested by Melis (2009). However, the values for panels are closer to the range suggested by Park et al. (2011) of 1.3 to 2.4% and suggested that the additional drop was due to cultures experiencing light saturation beyond levels of 200 μmol/m^2/s.

Previous research that has assessed the productivity of raceways typically utilize smaller units with a surface area of 1 - 4 m^2 compared to the current study, which utilized raceways with a surface area of 30.37 m^2 (Moheimani and Borowitzka 2006; Raes et al. 2014; Torzillo et al. 2012). This is significant as it affects the distance the culture travels between paddlewheel points. With smaller raceways, the culture interacts with the

paddlewheel more frequently, which significantly increases the mixing rate and surface interactions. Raes et al. (2014) showed the change in the Reynolds number in a 1 m^2 raceway with a drop from 3250 to 1730 over the distance of 3 m, or the distance the culture traveled before encountering the paddlewheel again. This suggests a significant change in the Reynolds number over a small distance, which indicates that scaling information found in a 1 m^2 raceway may overestimate the productivity of larger systems by not accounting for decreased mixing and paddlewheel interaction. Located on the Arizona Center for Algae Technology and Innovation field site are raceways with a surface area of 4.22 m^2 and 30.37 m^2 with maximum linear path lengths of 9.4 m and 17.8 m, respectively, which highlights how a small change in area of a raceway can drastically change the distance between paddlewheels. This is critical as Oswald (1988) notably suggested that the distance between paddlewheels in large-scale cultivation will most likely be between 493 to 2120 m depending on the cultivation depth. This would drastically decrease the level of mixing and surface interaction, and will decrease productivity, which is rarely considered or discussed when reporting productivity values or in the estimation of large-scale cultivation productivity. Further work is needed to evaluate the effect of paddlewheel interactions and Reynolds number on productivity in larger systems.

5.5. Conclusions

- Flat-panel photobioreactors had an average biomass productivity of 19.0 ± 0.6 g/m^2/day compared to 6.62 ± 2.3 g/m^2/day for open raceway ponds.

- Cultivation in panels and raceways for March showed an average nitrogen consumption rate of 36.4 ± 10.7 and 3.8 ± 2.5 mg/L/day, respectively. Cultivation in April and May showed a consumption rate of 39.4 ± 7.2 and 7.1 ± 4.2 mg/L/day for panels and raceways, respectively. All of these numbers correspond to a nitrogen consumption rate of 64 to 87 mg-N/g-Biomass.
- Excess nutrients were required to prevent decreased biomass productivity
- Cultivation temperatures above 30°C caused strain LB-0414 to lyse and the culture to crash.
- Strain LB-0424 did not show any statistical difference in biomass productivity when the peak temperature reached 34, 38, or 42°C, but had lower productivity when the peak temperature was 30°C, which is the maximum temperature for strain LB-0414.
- The reduction in aeration or sparging of cultures to a frequency of 1 min per 30 min at night did not impact biomass productivity.
- Photosynthetic efficiency was between 1.40 and 2.24% for panels and between 0.30 and 0.68% for raceways.

5.6. Acknowledgments

The authors would like to acknowledge the Arizona Center for Algae Technology and Innovation for access to facilities and equipment, and its staff for their intellectual support.

5.7. References

Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70 (1-3):313-321

Buchanan AN, Bolton N, Moheimani N, Svodboda IF, Grant T, Batten D, Cheng NN, Borowitzka MA, Fallowfield HJ (2013) Algae for energy and feed: A wastewater solution. A review (trans: Pork C-oRCfHIA).

Crowe B, Attalah S, Agrawal S, Waller P, Ryan R, Van Wagenen J, Chavis A, Kyndt J, Kacira M, Ogden KL, Huesemann M (2012) A comparison of *Nannochloropsis salina* growth performance in two outdoor pond designs: conventional raceways versus the ARID pond with superior temperature management. International Journal of Chemical Engineering 2012:9

Cuello M, Cosgrove J, Randhir A, Vadiveloo A, Moheimani N (2014) Comparison of continuous and day time only mixing on *Tetraselmis suecica* (Chlorophyta) in outdoor raceway ponds. J Appl Phycol:1-9

Eustance E, Wray JT, Badvipour S, Sommerfeld MR (2015) The effects of limiting nighttime aeration on productivity and lipid accumulation in *Scenedesmus dimorphous*. Algal Research 10 (0):33-40

Eustance E, Wray JT, Badvipour S, Sommerfeld MR (Submitted) The effects of cultivation depth, areal density and nutrient level on lipid accumulation of *Scenedesmus acutus* in outdoor raceway ponds J Appl Phycol

Grobbelaar JU (2013) Mass production of microalgae at optimal photosynthetic rates. In: Dubinsky Z (ed) Photosynthesis. InTech, pp 357-371

Grobbelaar JU, Nedbal L, Tichy L, Setlik L (1995) Variation in some photosynthetic characteristics of microalgae cultured in outdoor thin-layered sloping reactors. J Appl Phycol 7 (2):175-184

Hartig P, Grobbelaar JU, Soeder CJ, Groeneweg J (1988) On the mass-culture of microalgae - areal density as an important factor for achieving maximal productivity. Biomass 15 (4):211-221

Hindersin S, Leupold M, Kerner M, Hanelt D (2013) Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors. Bioprocess Biosystems Eng 36 (3):345-355

Hu Q, Faiman D, Richmond A (1998a) Optimal tilt angles of enclosed reactors for growing photoautotrophic microorganisms outdoors. J Ferment Bioeng 85 (2):230-236

Hu Q, Kurano N, Kawachi M, Iwasaki I, Miyachi S (1998b) Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor. Appl Microbiol Biotechnol 49 (6):655-662

Melis A (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci 177 (4):272-280

Moheimani NR, Borowitzka MA (2006) The long-term culture of the coccolithophore *Pleurochrysis carterae* (Haptophyta) in outdoor raceway ponds. J Appl Phycol 18 (6):703-712

Moheimani NR, Borowitzka MA (2007) Limits to productivity of the alga *Pleurochrysis carterae* (Haptophyta) grown in outdoor raceway ponds. Biotechnol Bioeng 96 (1):27-36

Molina E, Fernandez J, Acien FG, Chisti Y (2001) Tubular photobioreactor design for algal cultures. J Biotechnol 92 (2):113-131

Oswald WJ (1988) Large-scale algal culture systems (engineering aspects). In: Borowitzka MA, Borowitzka LJ (eds) Micro-algal Biotechnology. Cambridge University Press, Cambridge, pp 357-394

Park JBK, Craggs RJ, Shilton AN (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 102 (1):35-42

Raes EJ, Isdepsky A, Muylaert K, Borowitzka MA, Moheimani NR (2014) Comparison of growth of *Tetraselmis* in a tubular photobioreactor (Biocoil) and a raceway pond. J Appl Phycol 26 (1):247-255

Ras M, Steyer J-P, Bernard O (2013) Temperature effect on microalgae: a crucial factor for outdoor production. Rev Environ Sci Biotechnol 12 (2):153-164

Richmond A, Cheng-Wu Z (2001) Optimization of a flat plate glass reactor for mass production of *Nannochloropsis sp*. outdoors. J Biotechnol 85 (3):259-269

Sánchez Mirón A, García Camacho F, Contreras Gómez A, Grima EM, Chisti Y (2000) Bubble-column and airlift photobioreactors for algal culture. AICHE J 46 (9):1872-1887

Slegers PM, Lösing MB, Wijffels RH, van Straten G, van Boxtel AJB (2013) Scenario evaluation of open pond microalgae production. Algal Research 2 (4):358-368

Torzillo G, Faraloni C, Silva AM, Kopecký J, Pilný J, Masojídek J (2012) Photoacclimation of *Phaeodactylum tricornutum* (Bacillariophyceae) cultures grown outdoors in photobioreactors and open ponds. Eur J Phycol 47 (2):169-181

Torzillo G, Pushparaj B, Masojidek J, Vonshak A (2003) Biological constraints in algal biotechnology. Biotechnology and Bioprocess Engineering : BBE 8 (6):338-348

Vonshak A, Torzillo G, Masojidek J, Boussiba S (2001) Sub-optimal morning temperature induces photoinhibition in dense outdoor cultures of the alga *Monodus subterraneus* (Eustigmatophyta). Plant, Cell Environ 24 (10):1113-1118

Waller P, Ryan R, Kacira M, Li P (2012) The algae raceway integrated design for optimal temperature management. Biomass Bioenergy 46 (0):702-709

Zemke P, Sommerfeld M, Hu Q (2013) Assessment of key biological and engineering design parameters for production of *Chlorella zofingiensis* (Chlorophyceae) in outdoor photobioreactors. Appl Microbiol Biotechnol 97 (12):5645-5655

6. SYNTHESIS CHAPTER: IMPROVING ALGAL CULTIVATION

Improving the feasibility of utilizing algae in the future for diverse products will require a significant change in how algae are cultivated. The purpose of this dissertation was to determine the effects of environmental and system changes on the biomass and/or lipid productivity in flat-panel photobioreactors (panels) and open raceway ponds (raceways). This was accomplished in the three preceding chapters by assessing and changing key variables for each system to track the impact on productivity.

The three research chapters each answer a specific question associated with the general concern outlined in the introduction. However, these chapters also provide a unique overview into how different parameters can have similar effects on the algal cell. Overall, the three chapters together are focused on three interrelated values: lipid productivity, lipid accumulation, and biomass productivity. Chapter 3 addressed the first general question of the dissertation, which utilized panels, focused on answering what affect will reducing aeration in cultures at night have on culture productivity and its biochemical composition? And will reducing aeration prevent ammonia volatility from occurring? Chapter 4 addressed the second general question of the dissertation, which utilized raceways, and focused on answering whether reducing the culture-depth increases biomass productivity and/or lipid productivity? Chapter 5 addressed the third general question of the dissertation, which utilized both panels and raceways, focused on answering which system has higher biomass productivity? and what may be limiting growth in the different systems? The results of these chapters and how they relate to each other with respect to lipid productivity, lipid accumulation, and biomass productivity is

discussed below and followed by a section on how the information found in this dissertation will impact the field of algal cultivation.

6.1. Lipid Accumulation Improves Lipid Productivity

Chapter 3 and 4 were focused on the accumulation of lipids in panels and raceways, respectively. In both systems, variables were changed to address challenges of energy usage. For panels this entailed assessing the requirement for aeration at night as eliminating nighttime aeration can significantly reduce the energy consumption for cultivation. For raceways, a majority of energy involved in cultivation is in harvesting the algae from an extremely dilute liquid culture.

In panels, the elimination of aeration at night reduced lipid accumulation by greater than 45.5 ± 9.1% during a ten-day growth/stress period. During the two separate experiments with no aeration completed in chapter 3, cultivation with continuous aeration showed that the average lipid productivity for batch experimentation was 0.10 ± 0.01 g-FAME/L/day, which corresponds to 4.0 ± 0.5 g-FAME/m^2/day, while cultivation without aeration had a lipid productivity of 0.06 ± 0.01 g-FAME/L/day or 2.2 ± 0.2 g-FAME/m^2/day. It is important to remember that these values are limited to batch experimentation, as the stress phase is required for increased lipid accumulation. If assessing the lipid productivity based on harvesting at late log phase, the lipid productivity would be 0.04 g-FAME/L/day, which corresponds to 1.7 g-FAME/m^2/day. It is important to note that nighttime aeration did not affect this productivity. Furthermore, if we utilize the information for semi-continuous growth found in chapter 5, we can estimate the lipid productivity based on the known biochemical composition for

Scenedesmus acutus strain 0414 and the areal biomass productivity of 19.2 g/m^2/day. This provides an estimated 1.9 g-FAME/m^2/day, which is significantly lower than that achieved when stressing the biomass with continuous aeration, but similar to the productivity achieved when eliminating aeration at night. The increase in both lipid accumulation and lipid yield are often found to be mutually exclusive; however, Rodolfi et al. (2009) showed similar results that in outdoor panels in which increasing lipid accumulation increased lipid productivity in 4 different strains. This is counterintuitive to the current dogma that originated from the Aquatic Species Program, which suggested that as lipid accumulation occurs total lipid productivity will decrease (Sheehan et al. 1998). Results from the raceways (Chapter 4) showed cultures struggled to accumulate lipids compared to panels (Chapter 5), with peak FAME levels of 21.4% vs 35.7%, respectively.

In raceways, despite the lack of high lipid accumulation, cultures had a lipid productivity of 0.5 ± 0.2 g-FAME/m^2/day if biomass was harvested in the log phase, and 0.8 ± 0.3 g-FAME/m^2/day if the culture was allowed time to accumulate lipids. The large standard deviation on the values is due to averaging different experiments, which had different productivities based on environmental conditions. Furthermore, based on data from Chapter 5, the estimated lipid productivity for semi-continuous cultures is approximately 0.4 g-FAME/m^2/d, which is within the range found using the log phase in batch experimentation.

Furthermore, cultivation showed that decreasing culture depth increased lipid productivity from 0.60 g-FAME/m^2/day to 0.82 g-FAME/m^2/day for cultivation in

February, 2015 for 20 cm and 7.5 cm cultivation depth, respectively. Productivity in December, 2014 was lower and the productivity for cultivation at 9 cm compared to 24 cm were 0.58 g-FAME/m^2/day and 0.36 g-FAME/m^2/day, respectively. This is an increase in lipid productivity of 61% in January and 38% in December. There has been no research in the area related to decreasing cultivation depth to increase lipid accumulation and productivity.

The ability to stress cultures to achieve peak lipid productivity also increases the lipid yield, which means less biomass is processed to extract the same amount of lipids. For strain 0414, the peak lipid accumulation was 35% FAME, compared to 10% FAME for log phase growth, which means downstream processing will need to harvest and extract approximately 1/3 of the biomass. This can significantly decrease the size and capital cost of downstream equipment for biodiesel production.

It is important to note that the measurement used for measuring lipid content is focused on FAMEs, which are the desired product for biodiesel. However, FAMEs do not represent the total lipids that can be measured gravimetrically, and represents only 30% to 80% of the total lipid content of the cell (Laurens et al. 2012; Doan et al. 2011; Wahlen et al. 2011), but are the portion that are utilized for biodiesel.

6.2. Key Variables that influence Biomass Productivity

In Chapters 3, 4 and 5 biomass productivity was reported, which provided a broad range of environmental conditions for biomass productivity. In all three chapters, the main variable that limited growth was cultivation temperature. In chapters 3 and 5, cultivation temperatures caused several concerns with strain 0414, since its growth ceases

above 30°C. In addition to high peak cultivation temperatures, during a majority of the year, nighttime temperatures drop below the desired operating range, which creates sub-optimal morning conditions. Previous research has shown that sub-optimal temperatures in the morning reduces enzyme activity and causes photoinhibition. This reduces the overall biomass productivity. In chapter 5, the average morning temperature, defined as the time between 7 am and 10 am, was 11.2 ± 1.9°C in the raceways from February 17th to March 8th, while from April 2nd to May 7th the average was 14.8 ± 3.1°C. However, these values are both significantly lower than the values found in panels, which had averages of 18.0 ± 2.7°C and 20.3 ± 3.4°C for the same timeframe. This is significant as Ras et al. (2013) discussed the theory that every 10°C increase will theoretical double the growth rate based on the Arrhenius function and can in part explain the decrease in productivity, along with the photoinhibition effect shown by Vonshak et al. (2001). Waller et al. (2012) introduced the concept of the algae raceway integrated design (ARID), which is designed to store the algal culture in a basin at night to minimize exposed surface area to prevent heat loss. This design showed a significant increase in the morning temperature of the culture and shows the possibility of centralized culture storage to reduce the effects of sub-optimal morning temperatures on cultures.

In many instances, it can be challenging to separate the effects of increased temperature and increased solar irradiance, which both increase from winter to summer. However, the increase in solar irradiance can be accounted for by comparing photosynthetic efficiencies (PE) because it utilizes measured biomass productivity and compares it against the total solar irradiance for the given day. However, chapter 3 showed that raceways had an increase in PE from the end of February to the beginning of

May. Panels did not show an increase in PE, but this is due to the vertical orientation, which decreases the amount of usable light from 36.2 ± 4.9 mol of photons/m^2/day for March 11-27th and 30.8 ± 1.3 mol of photons/m^2/day for April 22nd – May 7th. When assessing PE based on the PAR values at the surface of the panel, the PE increased from 2.0 ± 0.0% to 2.3 ± 0.2%, which indicates that more favorable culture conditions allowed increased daily productivity despite having less usable light. Similarly, in raceways, the average PE from February 17th to 27th was 0.49 ± 0.38%, while productivity from April 24th to May 4th was 0.68 ± 0.14%. This also shows that factors other than solar irradiance alone increased biomass productivity and is most likely due to the increase in morning temperatures as was previously discussed.

In algal raceways, one of the biggest concerns is not only the scalability of the cultivations systems, but also the scalability of the results from smaller raceways. Previous research that assessed the productivity of raceways utilized smaller units with a surface area of 1 m^2 compared to the studies completed in this dissertation, which utilized raceways with a surface area of 30.37 m^2 (Moheimani and Borowitzka 2006; Raes et al. 2014; Torzillo et al. 2012). This is significant as it affects the distance the culture travels between paddlewheel points. With smaller raceways, the culture interacts with the paddlewheel more frequently, which significantly increases the mixing rate and surface interactions. Raes et al. (2014) showed the change in the Reynolds number in a 1 m^2 raceway with a drop from 3250 to 1730 over the distance of 3 m, or the distance the culture traveled before coming into contact with the paddlewheel again. This suggests a significant change in the Reynolds number over a small distance, which suggests that scaling information found in a 1 m^2 raceway could overestimate the potential productivity

of larger systems due to the increased paddlewheel interaction. Located on the Arizona Center for Algae Technology and Innovation field site are raceways with a surface area of 4.22 m^2 and 30.37 m^2 with maximum linear path lengths of 9.4 m and 17.8 m, respectively, which highlights how a small change in area of a raceway can drastically change the distance between paddlewheels. This is critical as Oswald (1988) notably suggested that the distance between paddlewheels in large-scale cultivation will most likely have paddlewheels placed every 493 to 2120 m depending on the cultivation depth. This would drastically decrease the level of mixing and surface interaction, which would most likely decrease overall productivity and is something that is rarely considered when reporting values or in the estimation of large-scale cultivation productivity. This also limits the capability of lab-scale research to complete an accurate evaluation of implemented changes, as the results may not be scalable.

In the comparison of raceway productivity with panel productivity, limited research has occurred. Torzillo et al. (2012) cultivated *Phaeodactylum tricornutum* in an outdoor tubular reactor alongside a circular pond for comparison of the two systems. The results from the experiment showed that the open pond had a productivity of ~15 g/m^2/day compared to the photobioreactor with 12.5 g/m^2/day. Again, in this research, the pond utilized was 1 m^2 with a depth of 10 cm, but was designed as a circular system with a rotating arm that created a surface interaction every 5 second compared to raceways, which interact with the paddlewheel every 12 seconds for a similar surface area. Furthermore, it is important to note that the tubular photobioreactor utilized a centrifugal pump for culture flow and mixing, which has a significant amount of shear and can cause a drastic decrease in productivity (Torzillo et al. 2003). This is why many cultivation

systems utilize aeration for mixing or airlifting of the culture to minimize shear stress on the culture (Molina et al. 2001; Sánchez Mirón et al. 2000). Raes et al. (2014) compared *Tetraselmis sp*. in an open raceway pond and the Biocoil helical photobioreactor, which showed that the organism in semi-continuous growth achieved 3 g/m^2/day and 0.85 g/m^2/day, respectively. However, neither of the photobioreactors considered in the two research papers utilized flat-panel photobioreactors, which use aeration to mix the culture. This reduces concerns of shear stress compared to pumping the culture for mixing, which may explain why both papers showed better growth in open pond systems compared to photobioreactors that used pumps for mixing.

In chapter 3 and 4, the main goal was the accumulation of lipids by the algae. However, the main drawback to accumulating lipids is a decrease in biomass productivity. Lipid accumulation typically occurs when nitrogen depletion occurs, which stops cell-cycling and triggers the cell to accumulate lipids for later use as energy source (Eustance et al. 2013; Gardner et al. 2011). When looking at the average productivity during the log phase for batch cultures in panels, the average was approximately 15 g/m^2/day while growth in semi-continuous cultivation showed an average close to 20 g/m^2/day. This lower average is attributed to the culture's response to lower external nitrogen concentrations. This was reaffirmed in chapter 5, when the average nitrogen concentration being added to the culture was decreased from 60 mg-N/L/day to 40 mg-N/L/day, which decreased the biomass productivity from 20.4 ± 3.0 g/m^2/day to 16.9 ± 0.4 g/m^2/day. Furthermore, in both cases, the culture utilized only a portion (approximately half) of the nitrogen available, which suggests that to maintain high levels

of biomass productivity in the algal culture, significant levels of nitrogen must remain in the media to prevent a change in culture productivity.

6.3. Implications of the Research in the Field of Algal Cultivation

The implications of the above research on the field of algal cultivation are diverse. Chapter 3 focused on the elimination of aeration at night, which showed the potential to reduce energy costs at night. This is not only critical in photobioreactors, but in advanced systems that utilize centralized storage of culture at night. This includes the 2 designs that offer an alternative approach to traditional raceways. Both the ARID system and the Cascade reactor utilize gravity to circulate the culture and utilize storage basins for the point of pumping and collecting (Waller et al. 2012; Doucha and Lívanský 2009). This is critical, as these vessels will require significant amounts of aeration at night if the desired product is lipids for biodiesel. However, if the desired product is biomass for animal feed, which would benefit from the increased protein content of log phase biomass, then aeration at night is not required as was shown in chapter 5. The same concept can be applied to raceways, as previous research by Cuello et al. (2014) showed that turning the paddlewheel off at night had no impact on semi-continuous growth. However, the author did not assess the impact in batch cultures for the final goal of lipid accumulation.

Chapter 4 focused on the effects of changing cultivation depth in raceways. The results showed that decreasing cultivation depth increased areal lipid productivity and yield, which is vital in improving the feasibility of algal cultivation for increased productivity. One of the biggest hurdles in algal cultivation is harvesting the biomass. By increasing the culture density and lipid content by decreasing the cultivation depth can

significantly reduce the energy and size of equipment needed to harvest and process the biomass for lipid extraction. This knowledge is important as most papers discuss the use of raceways at depths between 15 and 30 cm (Sompech et al. 2012; Moheimani and Borowitzka 2007). There are two main reasons that have prevented many cultivation projects from operating at depths below this value. The first is due to the engineering principles outlined by Oswald (1988), which utilized the Manning equation to show that deeper ponds would require fewer paddlewheels per a given distance. The second reason is that shallower raceways have a smaller heat capacity, which allows raceways to have a larger diurnal temperature fluctuation. However, any diurnal temperature fluctuation in algal cultivation can cause lower biomass productivities. High temperatures in March 2015 caused strain 0414 to lyse and the culture quality to significantly decrease. The high temperature concerns in chapter 5 were addressed by changing from strain 0414 to strain 0424, which can tolerate higher cultivation temperatures. This highlights the need in the field to study multiple strains and identify organisms that can be used during different seasons to allow for crop rotation. Lower temperatures also limited productivity as it most likely slowed enzyme activity and caused photoinhibition (Ras et al. 2013). Low temperature concerns are currently being addressed in algal cultivation through the use of centralized storage to prevent heat loss at night, however, the scalability of these designs remain unknown and is an area that requires more study (Waller et al. 2012). However, further evaluation and investigation into systems like the ARID and Cascade reactor is required to evaluate concerns over higher culture temperatures.

Chapter 5 showed that the cultures needed excess nitrogen to remain in the media to prevent a decrease in biomass productivity. The levels remaining in the culture at the

point of daily harvest was typically between 40 and 60 mg-N/L, which was equivalent to the amount being added. This is significant, as prior research has not discussed the importance of nitrogen remaining in the media after harvesting in semi-continuous cultures. Furthermore, this indicates that recycling culture media will be required, and at a certain point, cultivation parameters must change to remove the additional nitrogen. In chapter 5, this was done by periodically harvesting without the addition of new nutrients. This slightly affected the biomass productivity the following 2 days, but would be a necessary requirement to remove the nitrogen in the media before being discharged from the cultivation facility.

7. SUMMARY CONCLUSION AND FUTURE RESEARCH

The purpose of this dissertation was to understand and improve algal cultivation by determining key variables that limit growth or lipid accumulation. This was accomplished in the three research chapters, which highlighted concerns of oxygen presence in cultures at night, culture path length for lipid accumulation, culture temperatures and nitrogen concentration limiting algal biomass productivity. These results were compared and discussed in relation to current literature and in the synthesis chapter to describe the overall conclusions that came from this dissertation. Finally, this dissertation highlights questions that still need further investigation to help improve the feasibility of algal cultivation.

7.1. Key Findings from the Research Chapters

Chapter 3 focused on the reduction and elimination of aeration in flat-panel photobioreactors (panels) to reduce energy consumption and to prevent ammonia volatility and toxicity. The key conclusions from this chapter are:

- Dissolved oxygen is required for algal biomass to accumulate lipids
- Lack of aeration of algae cultures at night resulted in anoxic conditions and anaerobic respiration, which reduced the rate of lipid accumulation and nitrogen uptake
- Cultures that were intermittently sparged with air at night showed a delay in lipid accumulation
- Intermittent sparging decreased aeration requirements by greater than 95% at night

- Cultures without aeration had a decrease in final BP content greater than 36.4 ± 3.7% compared to continuously aerated cultures
- Cultures with intermittent sparging had a decrease in final lipid content between 0 and 21.6 ± 0.8%, depending on how often the cultures were sparged
- Nighttime medium temperatures below 20°C in the intermittently sparged (1 min: 60 min and 1 min: 30 min) cultures had similar lipid accumulations to cultures with higher nighttime temperatures (~ 24°C) and reduced sparging timespans (0.5 min: 20 min to 0.5 min: 5 min)

Chapter 4 assessed the required nitrogen concentration and cultivation depth in open raceways for lipid accumulation to occur. The main conclusions from this chapter are:

- Decreasing cultivation depth from 20 cm to 7.5 cm and 24 cm to 9 cm affected overall biodiesel accumulation by 52% and 33%, respectively.
- A minimum operating culture density of 8.0 g/m^2 at 0.1 g/L was required to minimize initial photoinhibition of *Scenedesmus acutus*.
- Raceway cultures accumulating lipids had an average log-phase biomass productivity of 3.5 ± 0.9 g/m^2/day, with productivity during the stress-phase of 2.2 ± 1.4 g/m^2/day during the winter.
- Raceway cultivation showed peak productivity when cultures were at an areal density between 26.7 and 39.2 g/m^2.

- Biomass lipid accumulation in raceways was significantly lower than that achieved in previous research in outdoor flat-panel photobioreactors.

Chapter 5 focused on the comparison of biomass productivity in panels and raceways and attempted to determine what limited productivity. The main conclusions from this chapter are:

- Flat-panel photobioreactors had an average biomass productivity of 19.0 ± 0.6 g/m^2/day compared to 6.62 ± 2.3 g/m^2/day for open raceway ponds.
- Cultivation in panels and raceways for March, 2015 showed an average nitrogen consumption rate of 36.4 ± 10.7 and 3.8 ± 2.5 mg/L/day, respectively. Cultivation in April and May, 2015 showed a consumption rate of 39.4 ± 7.2 and 7.1 ± 4.2 mg/L/day for panels and raceways, respectively, corresponding to a nitrogen consumption rate between 64 and 87 mg-N/g-Biomass.
- High cultivation temperatures above 30°C caused strain 0414 to lyse and the culture to crash.
- Strain 0424 did not show any statistical difference in biomass productivity when the peak temperature reached 34, 38, or 42°C, but appeared to have better lower productivity when the peak temperature was 30°C.
- The lack of aeration or sparging at a frequency of 1 min per 30 min at night did not impact biomass productivity.
- Photosynthetic efficiency ranged from 1.40 and 2.24% for panels and from 0.30 and 0.68% for raceways.

7.2. General Conclusions and the Implications from Synthesis Chapter

The synthesis chapter provided an overview of how the different research chapters contributed to a cohesive goal to improving the feasibility of algal cultivation. This was described in three sections as follows: 1) lipid accumulation increases lipid productivity, 2) key variables that influence biomass productivity, and 3) the implications of the research on the field of algal cultivation. The results from chapter 3 and 4 concluded that lipid accumulation does increase lipid productivity, which corroborates the findings of Rodolfi et al. (2009) and goes against the central dogma created by the Aquatic Species Program (Sheehan et al. 1998). However, the increase in lipid productivity comes at the cost of biomass productivity, which decreases during the stressed phase. Chapter 5 concluded that panels have significantly better biomass productivity than raceways, which may in part be due to sub-optimal morning conditions as suggested by Vonshak et al. (2001) and Ras et al. (2013). Panels also tend to have better productivity associated with the increased mixing associated with aeration, which allows the cultures to operate at higher areal culture densities and increase culture efficiency. The implications of this research suggest that cultivation systems may need to operate in batch mode to provide better lipid productivity, and the need to identify methods of increasing early morning culture temperature. Previous research has shown that this may be possible by utilizing a deeper storage basin at night to minimize surface area and heat loss (Waller et al. 2012). Overall, the research in this dissertation provides valuable information and suggests that certain areas of research need further investigation to improve algal cultivation.

7.3. Future Research Recommendations

This dissertation highlighted important variables for improving both lipid and biomass productivity. However, further investigation is needed to continue to increase the feasibility of algal cultivation for low-value products.

7.3.1. Harvesting Rate and Areal Density

Research in this dissertation attempted to change harvesting rate and/or harvesting volume, both of which change the operating density. Previous and current research has shown that cultures operating with an areal density between 30 and 100 g/m^2 can provide the optimal productivity (Hartig et al. 1988). However, Grobbelaar et al. (1995) showed that the optimal areal density increases significantly with higher mixing rates and shorter path-lengths. The results from his work showed that in the cascade system, which operates at a depth of 0.7 to 1.7 cm has an optimal areal density closer to 150 g/m^2. In this dissertation, panels were operated at an areal density between 40 and 100 g/m^2 and showed no decrease in biomass productivity. In semi-continuous panels, operating density was determined by the volume of culture being removed daily. The range of volume being removed was between 18 and 36%, but the amount of biomass being removed was constant. This is critical for the downstream processing and removal of excess water. Further research needs to focus on investigating the effect of growing algae at higher areal densities, and accomplishing this by reducing the path length to prevent light limitation.

7.3.2. Raceway Scale-up, the Impact on Fluid Dynamics and the Reduced Interaction with the Paddlewheel

One of the biggest concerns in the field of algal cultivation research is the scalability of results. As previously discussed in the synthesis chapter, as raceways increase in size, the culture travels longer distances between interactions with the paddlewheel, this has a significant impact on the fluid dynamics and Reynolds number, which dictates surface interactions and mixing. Previous research has shown that over the distance of 3 m the Reynolds number decreased from 3250 to 1730 (Raes et al. 2014). This is significant as Oswald (1988) concluded that the desired distance between paddlewheels in a large facility would be between 500 and 2500 m. Based on the decrease in Reynolds number for a small raceway, a majority of the distance in a large facility would have sub-par mixing, which would significantly reduce biomass productivity. Arizona Center for Algae Technology and Innovation currently has five different sizes and orientations of raceways, which have an area ranging from less than 0.25 m^2 up to 30 m^2. These systems can be operated side-by-side to determine the effect of increased interactions with paddlewheels on overall productivity to provide valuable information for modeling the scale-up of raceways. However, one of the best ways to create a more consistent Reynolds number and mixing rate is to transition from flat raceways to inclined systems, which utilize the force of gravity to move the culture with a mixing rate that does not decrease over distance.

7.3.3. Inclined Systems

The focus of chapter 4 was reducing the cultivation depth in raceways. However, due to limitations in the flow of fluid over a flat surface, the minimum operating depth was kept at 7.5 cm. To overcome this limitation, it is suggested that further work investigate the use of inclined systems including the ARID and Cascade reactors. However, both current systems have significant limitations that need to be addressed. The ARID is designed to be operated at a depth between 7.5 and 15 cm, which as previously stated is deeper than desired. The system is designed for lower cost installation and use in agricultural setup, whereas the Cascade reactor was designed as a photobioreactor with culture flowing over smooth glass. This provides a significant challenge to minimizing cost in scaling the system. One of the biggest challenges with both of these systems is the requirement to lift the culture utilizing pumps. The shear utilized in most pumps causes a significant decrease in growth rate. Overall, further investigation needs to be accomplished to modify and combine the positive components of each system to improve the feasibility of utilizing an inclined system. This should include the assessment of different types of pumps or using airlift technology to minimize shear stress. In addition, a large-scale system will need a design that requires minimal material and construction to help reduce capital cost. Investigation into the appropriate materials, required inclination, and the determination of distance between CO_2 injection points are needed to provide a better assessment of the potential biomass productivity levels.

7.3.4. Wastewater

The work in this dissertation utilized lab-based fertilizer to eliminate variables that can arise from using different wastewaters. However, moving forward research needs to focus on the use of different wastewaters. Chapter 3 focused on the elimination of ammonia volatility and toxicity at night, which is crucial in highly concentrated wastewaters such as those from dairy and swine manure. However, further research is needed to investigate other considerations including the effect of turbidity and organic matter on the growth rate and possible contamination from other types of organisms. Furthermore, the question that needs answering and assessment is what are the uses for the biomass? This is important since there is the view that the biomass can be utilized as an animal feed to help close the food/waste cycle in dairies. However, in-depth assessment of biomass productivity and the effect on biomass quality when continually grown in dairy wastewater has not been addressed, which is critical in moving forward with attempting to utilize algae cultivation to bioremediate the wastewater with the resulting algal biomass providing a portion of the animal feed.

8. WORK CITED

Abeliovich A, Azov Y (1976) Toxicity of ammonia to algae in sewage oxidation ponds. Appl Environ Microbiol 31 (6):801-806

Acién Fernández FG, Fernández Sevilla JM, Molina Grima E (2013) Photobioreactors for the production of microalgae. Rev Environ Sci Biotechnol 12 (2):131-151

Amory AM, Vanlerberghe GC, Turpin DH (1991) Demonstration of both a photosynthetic and a nonphotosynthetic CO_2 requirement for NH_4^+ assimilation in the green alga *Selenastrum minutum*. Plant Physiol 95 (1):192-196

Atteia A, van Lis R, Tielens AGM, Martin WF (2013) Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochim Biophys Acta 1827 (2):210-223

Azov Y, Goldman JC (1982) Free ammonia inhibition of algal photosynthesis in intensive cultures. Appl Environ Microbiol 43 (4):735-739

Baird C, Cann M (2005) Environmental Chemistry. 3rd edn. W.H. Freeman, New York

Beardall J, Johnston A, Raven J (1998) Environmental regulation of CO2-concentrating mechanisms in microalgae. Canadian Journal of Botany-Revue Canadienne De Botanique 76 (6):1010-1017

Béchet Q, Shilton A, Park JBK, Craggs RJ, Guieysse B (2011) Universal temperature model for shallow algal ponds provides improved accuracy. Environ Sci Technol 45 (8):3702-3709

Bekheet IA, Syrett PJ (1977) Urea-degrading enzymes in algae. British Phycological Journal 12 (2):137-143

Blankenship RE (2002) Molecular Mechanisms of Photosynthesis. Blackwell Science,

Bongers LHJ (1956) Aspects of nitrogen assimilation by cultures of green algae. Mededel Landbouwhoogesch Wageningen 56 ((15)):1-52

Bonmati A, Flotats X (2003) Air stripping of ammonia from pig slurry: characterisation and feasibility as a pre- or post-treatment to mesophilic anaerobic digestion. Waste Manage 23 (3):261-272

Borowitzka M, Moheimani N (2013a) Open Pond Culture Systems. In: Borowitzka MA, Moheimani NR (eds) Algae for Biofuels and Energy, vol 5. Developments in Applied Phycology. Springer Netherlands, pp 133-152

Borowitzka MA (1997) Algae as food. In: Wood BB (ed) Microbiology of Fermented Foods. Springer US, pp 585-602

Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70 (1-3):313-321

Borowitzka MA, Moheimani NR (2013b) Sustainable biofuels from algae. Mitig Adapt Strateg Glob Change 18 (1):13-25

Boussiba S, Resch CM, Gibson J (1984) Ammonia uptake and retention in some cyanobacteria. Arch Microbiol 138 (4):287-292

BP (2014) BP Statistical Review of World Energy 2014. 63rd edn.,

Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH (2012) The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. Bioresour Technol 124 (0):217-226

Brune DE, Lundquist TJ, Benemann JR (2009) Microalgal biomass for greenhouse gas reductions: Potential for replacement of fossil fuels and animal feeds. J Environ Eng-ASCE 135 (11):1136-1144

Buchanan AN, Bolton N, Moheimani N, Svodboda IF, Grant T, Batten D, Cheng NN, Borowitzka MA, Fallowfield HJ (2013) Algae for energy and feed: A wastewater solution. A review (trans: Pork C-oRCfHIA).

Burris JE (1977) Photosynthesis, photorespiration, and dark respiration in eight species of algae. Mar Biol 39 (4):371-379

Cabanelas ITD, Ruiz J, Arbib Z, Chinalia FA, Garrido-Pérez C, Rogalla F, Nascimento IA, Perales JA (2013) Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresour Technol 131 (0):429-436

Cabije AH, Agapay RC, Tampus MV (2009) Carbon-nitrogen-phosphorus removal and biofilm growth characteristics in an integrated wastewater treatment system involving a rotating biological contactor. Asia-Pacific Journal of Chemical Engineering 4 (5):735-743

Chen R, Li R, Deitz L, Liu Y, Stevenson RJ, Liao W (2012) Freshwater algal cultivation with animal waste for nutrient removal and biomass production. Biomass Bioenergy 39 (0):128-138

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25 (3):294-306

Chisti Y (2008) Biodiesel from microalgae beats bioethanol. Trends Biotechnol 26 (3):126-131

Crowe B, Attalah S, Agrawal S, Waller P, Ryan R, Van Wagenen J, Chavis A, Kyndt J, Kacira M, Ogden KL, Huesemann M (2012) A comparison of *Nannochloropsis*

salina growth performance in two outdoor pond designs: conventional raceways versus the ARID pond with superior temperature management. International Journal of Chemical Engineering 2012:9

Cuello M, Cosgrove J, Randhir A, Vadiveloo A, Moheimani N (2014) Comparison of continuous and day time only mixing on *Tetraselmis suecica* (Chlorophyta) in outdoor raceway ponds. J Appl Phycol:1-9

de Boer K, Moheimani N, Borowitzka M, Bahri P (2012) Extraction and conversion pathways for microalgae to biodiesel: a review focused on energy consumption. J Appl Phycol:1-18

Doan TTY, Sivaloganathan B, Obbard JP (2011) Screening of marine microalgae for biodiesel feedstock. Biomass Bioenergy 35 (7):2534-2544

Dodd JC (1979) Algae production and harvesting from animal wastewaters. Agricultural Wastes 1 (1):23-37

Dorling M, McAuley PJ, Hodge H (1997) Effect of pH on Growth and Carbon Metabolism of Maltose-Releasing *Chlorella* (Chlorophyta). Eur J Phycol 32 (1):19-24

Dortch Q (1990) The interaction between ammonium and nitrate uptake in phytoplankton. Marine Ecology-Progress Series 61 (1-2):183-201

Doucha J, Lívanský K (2009) Outdoor open thin-layer microalgal photobioreactor: potential productivity. J Appl Phycol 21 (1):111-117

Doucha J, Lívanský K (2014) High density outdoor microalgal culture. In: Bajpai R, Prokop A, Zappi M (eds) Algal Biorefineries. Springer Netherlands, pp 147-173

Doucha J, Straka F, Lívanský K (2005) Utilization of flue gas for cultivation of microalgae (*Chlorella sp*.) in an outdoor open thin-layer photobioreactor. J Appl Phycol 17 (5):403-412

Doucha Jí, Lívanský K (1995) Novel outdoor thin-layer high density microalgal culture system: Productivity and operational parameters. Arch Hydrobiol Suppl 76:129-147

Douskova I, Doucha J, Livansky K, Machat J, Novak P, Umysova D, Zachleder V, Vitova M (2009) Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. Appl Microbiol Biotechnol 82 (1):179-185

Dvořáková-Hladká J (1971) A comparison of growth rate of algae as influenced by variation in nitrogen nutrition in *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Biol Plant 13 (1):1-11

Elrifi IR, Holmes JJ, Weger HG, Mayo WP, Turpin DH (1988) RuBP limitation of photosynthetic carbon fixation during NH_3 assimilation: Interactions between photosynthesis, respiration, and ammonium assimilation in N-limited green algae. Plant Physiol 87 (2):395-401

Emerson K, Russo RC, Lund RE, Thurston RV (1975) Aqueous ammonia equilibrium calculations: Effect of pH and temperature. J Fish Res Board Can, 32 (12):2379-2383

Eriksen N, Poulsen B, Lønsmann Iversen JJ (1998) Dual sparging laboratory-scale photobioreactor for continuous production of microalgae. J Appl Phycol 10 (4):377-382

Eustance E, Gardner RD, Moll KM, Menicucci J, Gerlach R, Peyton BM (2013) Growth, nitrogen utilization and biodiesel potential for two chlorophytes grown on ammonium, nitrate or urea. J Appl Phycol 25 (6):1663-1677

Eustance E, Wray JT, Badvipour S, Sommerfeld MR (2015) The effects of limiting nighttime aeration on productivity and lipid accumulation in *Scenedesmus dimorphous*. Algal Research 10 (0):33-40

Eustance E, Wray JT, Badvipour S, Sommerfeld MR (Submitted) The effects of cultivation depth, areal density and nutrient level on lipid accumulation of *Scenedesmus acutus* in outdoor raceway ponds J Appl Phycol

Fernandes B, Teixeira J, Dragone G, Vicente AA, Kawano S, Bišová K, Přibyl P, Zachleder V, Vítová M (2013) Relationship between starch and lipid accumulation induced by nutrient depletion and replenishment in the microalga Parachlorella kessleri. Bioresour Technol 144 (0):268-274

Fernández E, Galván A, Quesada A (2004) Nitrogen Assimilation and its Regulation. In: Govindjee (ed) The Molecular Biology of Chloroplasts and Mitochondria in *Chlamydomonas*, vol 7. Advances in Photosynthesis and Respiration. Springer Netherlands, Boston, pp 637-659

Frank E, Elgowainy A, Han J, Wang Z (2013) Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae. Mitigation and Adaptation Strategies for Global Change 18 (1):137-158

Franklin ST, Martin KR, Baer RJ, Schingoethe DJ, Hippen AR (1999) Dietary marine algae (*Schizochytrium sp*.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. The Journal of Nutrition 129 (11):2048-2054

Fuggi A, Di Martino Rigano V, Vona V, Rigano C (1981) Nitrate and ammonium assimilation in algal cell-suspensions and related pH variations in the external medium, monitored by electrodes. Plant Science Letters 23 (2):129-138

Garcia J, Mujeriego R, Hernandez-Marine M (2000) High rate algal pond operating strategies for uurban wastewater nitrogen removal. J Appl Phycol 12 (3-5):331-339

Gardner R, Cooksey K, Mus F, Macur R, Moll K, Eustance E, Carlson R, Gerlach R, Fields M, Peyton B (2012) Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the Chlorophyte *Scenedesmus sp*. and the Diatom *Phaeodactylum tricornutum*. J Appl Phycol:1-10

Gardner R, Peters P, Peyton B, Cooksey K (2011) Medium pH and nitrate concentration effects on accumulation of triacylglycerol in two members of the Chlorophyta. J Appl Phycol 23 (6):1005-1016

Gardner RD, Lohman E, Gerlach R, Cooksey KE, Peyton BM (2013) Comparison of CO_2 and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. Biotechnol Bioeng 110 (1):87-96

Geider R (1992) Respiration: Taxation without representation? In: Falkowski P, Woodhead A, Vivirito K (eds) Primary Productivity and Biogeochemical Cycles in the Sea, vol 43. Environmental Science Research. Springer US, pp 333-360

Gendy TS, El-Temtamy SA (2013) Commercialization potential aspects of microalgae for biofuel production: An overview. Egypt J Pet 22 (1):43-51

Giordano M, Beardall J, Raven JA (2005) CO_2 concentrating mechanisms in algae: Mechanisms, environmental modulation, and evolution. Annu Rev Plant Biol 56:99-131

Godos Id, Blanco S, García-Encina PA, Becares E, Muñoz R (2009) Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. Bioresour Technol 100 (19):4332-4339

Gordon JM, Polle JEW (2007) Ultrahigh bioproductivity from algae. Appl Microbiol Biotechnol 76:969-975

Gowariker V, Krishnamurthy VN, Gowariker S, Dhanorkar M, Paranjape K, Borlaug N (2009) The Fertilizer Encyclopedia. Wiley, Hoboken, NJ

Griffiths MJ, Harrison STL (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 21 (5):493-507

Grobbelaar JU (2013) Mass production of microalgae at optimal photosynthetic rates. In: Dubinsky Z (ed) Photosynthesis. InTech, pp 357-371

Grobbelaar JU, Nedbal L, Tichy L, Setlik L (1995) Variation in some photosynthetic characteristics of microalgae cultured in outdoor thin-layered sloping reactors. J Appl Phycol 7 (2):175-184

Guckert JB, Cooksey KE (1990) Triglyceride accumulation and fatty-acid profile changes in *Chlorella* (Chlorophyta) during high pH induced cell-cycle inhibition. J Phycol 26 (1):72-79

Guerin M, Huntley ME, Olaizola M (2003) Haematococcus astaxanthin: applications for human health and nutrition. Trends Biotechnol 21 (5):210-216

Guštin S, Marinšek-Logar R (2011) Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. Process Saf Environ Prot 89 (1):61-66

Guy RD, Vanlerberghe GC, Turpin DH (1989) Significance of phosphoenolpyruvate carboxylase during ammonium assimilation: Carbon isotope discrimination in photosynthesis and respiration by the N-limited green alga *Selenastrum minutum*. Plant Physiol 89 (4):1150-1157

Hanagata N, Takeuchi T, Fukuju Y, Barnes DJ, Karube I (1992) Tolerance of microalgae to high CO2 and high temperature. Phytochemistry 31 (10):3345-3348

Hartig P, Grobbelaar JU, Soeder CJ, Groeneweg J (1988) On the mass-culture of microalgae - areal density as an important factor for achieving maximal productivity. Biomass 15 (4):211-221

Heggemann MH, Warnecke HJ, Viljoen HJ (2001) Removal of ammonia from aqueous systems in a semibatch reactor. Ind Eng Chem Res 40 (15):3361-3368

Hejazi MA, Holwerda E, Wijffels RH (2004) Milking microalga *Dunaliella salina* for beta-carotene production in two-phase bioreactors. Biotechnol Bioeng 85 (5):475-481

Heussler P (1985) Aspects of sloped algae pond engineering. Ergebnisse der Limnologie (20):71-84

Hindersin S, Leupold M, Kerner M, Hanelt D (2013) Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors. Bioprocess Biosystems Eng 36 (3):345-355

Hirano A, Ueda R, Hirayama S, Ogushi Y (1997) CO2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. Energy 22 (2–3):137-142

Hodson RC, Williams SK, Davidson WR (1975) Metabolic control of urea catabolism in *Chlamydomonas reinhardi* and *Chlorella pyrenoidosa*. J Bacteriol 121 (3):1022-1035

Hoffmann JP (1998) Wastewater treatment with suspended and nonsuspended algae. J Phycol 34 (5):757-763

Hu Q, Faiman D, Richmond A (1998a) Optimal tilt angles of enclosed reactors for growing photoautotrophic microorganisms outdoors. J Ferment Bioeng 85 (2):230-236

Hu Q, Kurano N, Kawachi M, Iwasaki I, Miyachi S (1998b) Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor. Appl Microbiol Biotechnol 49 (6):655-662

Hu Q, Zarmi Y, Richmond A (1998c) Combined effects of light intensity, light-path and culture density on output rate of *Spirulina platensis* (Cyanobacteria). Eur J Phycol 33 (02):165-171

Ingemarsson B, Oscarson P, Afugglas M, Larsson CM (1987) Nitrogen-utilization in *Lemna* III. Short-term effects of ammonium on nitrate uptake and nitrate reduction. Plant Physiol 85 (3):865-867

Johnson X, Alric J (2013) Central carbon metabolism and electron transport in *Chlamydomonas reinhardtii*: Metabolic constraints for carbon partitioning between oil and starch. Eukaryot Cell 12 (6):776-793

Kebede-Westhead E, Pizarro C, Mulbry W (2006) Treatment of swine manure effluent using freshwater algae: Production, nutrient recovery, and elemental composition of algal biomass at four effluent loading rates. J Appl Phycol 18 (1):41-46

Kumar K, Dasgupta CN, Nayak B, Lindblad P, Das D (2011) Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. Bioresour Technol 102 (8):4945-4953

Larsson CM, Larsson M, Guerrero MG (1985) Photosynthetic nitrogen metabolism in high and low CO_2-adapted *Scenedesmus* II. Effect of ammonium and methionine sulphoximine on nitrate utilization. J Exp Bot 36 (170):1387-1395

Laurens LL, Quinn M, Van Wychen S, Templeton D, Wolfrum E (2012) Accurate and reliable quantification of total microalgal fuel potential as fatty acid methyl esters by in situ transesterification. Anal Bioanal Chem 403 (1):167-178

Leftley JW, Syrett PJ (1973) Urease and ATP: Urea amidolyase activity in unicellular algae. J Gen Microbiol 77 (1):109-115

Leytem AB, Dungan RS, Bjorneberg DL, Koehn AC (2011) Emissions of ammonia, methane, carbon dioxide, and nitrous oxide from dairy cattle housing and manure management systems. J Environ Qual 40 (5):1383-1394

Li Y, Chen Y-F, Chen P, Min M, Zhou W, Martinez B, Zhu J, Ruan R (2011) Characterization of a microalga *Chlorella sp*. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour Technol 102 (8):5138-5144

Liang K, Zhang Q, Gu M, Cong W (2013) Effect of phosphorus on lipid accumulation in freshwater microalga Chlorella sp. J Appl Phycol 25 (1):311-318

Liao PH, Chen A, Lo KV (1995) Removal of nitrogen from swine manure wastewaters by ammonia stripping. Bioresour Technol 54 (1):17-20

Liu ZY, Wang GC, Zhou BC (2008) Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresour Technol 99 (11):4717-4722

Lourenco SO, Barbarino E, Mancini-Filho J, Schinke KP, Aidar E (2002) Effects of different nitrogen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: an evaluation for aquaculture. Phycologia 41 (2):158-168

Ludwig CA (1938) The availability of different forms of nitrogen to a green alga. Am J Bot 25 (6):448-458

Ludwig HF, Oswald WJ, Gotaas HB, Lynch V (1951) Algae symbiosis in oxiadation ponds: 1. Growth characteristics of *Euglena gracilis* cultured in sewage. Sewage and Industrial Wastes 23 (11):1337-1355

Lundquist T, Woertz I, Quinn NWT, Benemann JR (2010) A realistic technology and engineering assessment of algae biofuel production. Energy Biosciences Institute, Berkley, California

Maeda I, Miyashiro M, Hikawa H, Yagi K, Miura Y, Mizoguchi T (1996) Acceleration of starch degradation by suppression of H_2 evolution in *Chlamydomonas sp*. MGA161. Biosci, Biotechnol, Biochem 60 (6):975-978

Markou G, Nerantzis E (2013) Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. Biotechnol Adv 31 (8):1532-1542

Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen U-P (2006) A photobioreactor system for computer controlled cultivation of microalgae. J Appl Phycol 17 (6):535-549

Masojídek J, Kopecký J, Giannelli L, Torzillo G (2011) Productivity correlated to photobiochemical performance of Chlorella mass cultures grown outdoors in thin-layer cascades. J Ind Microbiol Biotechnol 38 (2):307-317

Matsumoto H, Hamasaki A, Sioji N, Ikuta Y (1997) Influence of CO_2, SO_2 and NO in Flue Gas on Microalgae Productivity. J Chem Eng Japan 30 (4):620-624

McAuley PJ, Dorling M, Hodge H (1996) Effect of maltose release on uptake and assimilation of ammonium by symbiotic *Chlorella* (Chlorophyta). J Phycol 32 (5):839-846

McGill SM (2012) 'Peak' phosphorus? The implications of phosphate scarcity for sustainable investors. Journal of Sustainable Finance & Investment 2 (3-4):222-239

Melis A (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci 177 (4):272-280

Miura Y, Yagi K, Shoga M, Miyamoto K (1982) Hydrogen production by a green alga, *Chlamydomonas reinhardtii*, in an alternating light/dark cycle. Biotechnol Bioeng 24 (7):1555-1563

Moheimani NR, Borowitzka MA (2006) The long-term culture of the coccolithophore *Pleurochrysis carterae* (Haptophyta) in outdoor raceway ponds. J Appl Phycol 18 (6):703-712

Moheimani NR, Borowitzka MA (2007) Limits to productivity of the alga *Pleurochrysis carterae* (Haptophyta) grown in outdoor raceway ponds. Biotechnol Bioeng 96 (1):27-36

Molina E, Fernandez J, Acien FG, Chisti Y (2001) Tubular photobioreactor design for algal cultures. J Biotechnol 92 (2):113-131

Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20 (7-8):491-515

Moll KM, Gardner RD, Eustance EO, Gerlach R, Peyton BM (2014) Combining multiple nutrient stresses and bicarbonate addition to promote lipid accumulation in the diatom RGd-1. Algal Research 5 (0):7-15

Molloy CJ, Syrett PJ (1988) Interrelationships between uptake of urea and uptake of ammonium by microalgae. J Exp Mar Biol Ecol 118 (2):85-95

Mulbry W, Kondrad S, Buyer J (2008) Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. J Appl Phycol 20 (6):1079-1085

Müller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu R-Y, van der Giezen M, Tielens AGM, Martin WF (2012) Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol Mol Biol Rev 76 (2):444-495

Nagase H, Eguchi K, Yoshihara K-I, Hirata K, Miyamoto K (1998) Improvement of microalgal NOx removal in bubble column and airlift reactors. J Ferment Bioeng 86 (4):421-423

Negoro M, Shioji N, Miyamoto K, Micira Y (1991) Growth of microalgae in high CO_2 gas and effects of SO_X and NO_X. Appl Biochem Biotechnol 28-29 (1):877-886

Norici A, Dalsass A, Giordano M (2002) Role of phosphoenolpyruvate carboxylase in anaplerosis in the green microalga *Dunaliella salina* cultured under different nitrogen regimes. Physiol Plant 116 (2):186

Norici A, Giordano M (2002) Anaplerosis in microalgae. Recent Res Devel Plant Physiol 3:153-164

Nunez VJ, Voltolina D, Nieves M, Pina P, Medina A, Guerrero M (2001) Nitrogen budget in *Scenedesmus obliquus* cultures with artificial wastewater. Bioresour Technol 78 (2):161-164

Ohta S, Miyamoto K, Miura Y (1987) Hydrogen evolution as a consumption mode of reducing equivalents in green algal fermentation. Plant Physiol 83 (4):1022-1026

Oswald WJ (1988) Large-scale algal culture systems (engineering aspects). In: Borowitzka MA, Borowitzka LJ (eds) Micro-algal Biotechnology. Cambridge University Press, Cambridge, pp 357-394

Oswald WJ, Gotaas HB, Golueke CG, Kellen WR (1957) Algae in waste treament. Sewage and Industrial Wastes 29 (4):437-455

Páez-Osuna F, Guerrero-Galván SR, Ruiz-Fernández AC, Espinoza-Angulo R (1997) Fluxes and mass balances of nutrients in a semi-intensive shrimp farm in north-western Mexico. Mar Pollut Bull 34 (5):290-297

Park J, Jin H-F, Lim B-R, Park K-Y, Lee K (2010) Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus sp.* Bioresour Technol 101 (22):8649-8657

Park JBK, Craggs RJ, Shilton AN (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 102 (1):35-42

Peavey DG, Gfeller RP, Gibbs M (1983) Presence of a Pasteur Effect in algae. Physiologie Vegetale 21 (3):337-342

Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman JB (2013) Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends Biotechnol 31 (3):134-138

Pérez-López P, González-García S, Jeffryes C, Agathos SN, McHugh E, Walsh D, Murray P, Moane S, Feijoo G, Moreira MT (2014) Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. Journal of Cleaner Production 64 (0):332-344

Picot B, Andrianarison T, Olijnyk DP, Wang XZ, Qiu JP, Brissaud F (2009) Nitrogen removal in wastewater stabilisation ponds. Desalination and Water Treatment 4 (1-3):103-110

Poulsen BR, Iversen JJL (1999) Membrane sparger in bubble column, airlift, and combined membrane–ring sparger bioreactors. Biotechnol Bioeng 64 (4):452-458

Powell N, Shilton A, Chisti Y, Pratt S (2009) Towards a luxury uptake process via microalgae – Defining the polyphosphate dynamics. Water Res 43 (17):4207-4213

Powell N, Shilton AN, Pratt S, Chisti Y (2008) Factors influencing luxury uptake of phosphorus by microalgae in waste stabilization ponds. Environ Sci Technol 42 (16):5958-5962

Přibyl P, Cepák V, Zachleder V (2012) Production of lipids in 10 strains of *Chlorella* and *Parachlorella* and enhanced lipid productivity in *Chlorella vulgaris*. Appl Microbiol Biotechnol 94 (2):549-561

Quan X, Wang F, Zhao Q, Zhao T, Xiang J (2009) Air stripping of ammonia in a water-sparged aerocyclone reactor. J Hazard Mater 170 (2–3):983-988

Raes EJ, Isdepsky A, Muylaert K, Borowitzka MA, Moheimani NR (2014) Comparison of growth of *Tetraselmis* in a tubular photobioreactor (Biocoil) and a raceway pond. J Appl Phycol 26 (1):247-255

Ramírez CA, Worrell E (2006) Feeding fossil fuels to the soil: An analysis of energy embedded and technological learning in the fertilizer industry. Resources, Conservation and Recycling 46 (1):75-93

Ras M, Steyer J-P, Bernard O (2013) Temperature effect on microalgae: a crucial factor for outdoor production. Rev Environ Sci Biotechnol 12 (2):153-164

Richmond A, Cheng-Wu Z (2001) Optimization of a flat plate glass reactor for mass production of *Nannochloropsis sp*. outdoors. J Biotechnol 85 (3):259-269

Rittmann BE, McCarty PL (2001) Environmental Biotechnology: Principles and Applications. MCGRAW-HILL Higher Education, San Francisco

Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 102 (1):100-112

Rollinson AN, Jones J, Dupont V, Twigg MV (2011) Urea as a hydrogen carrier: a perspective on its potential for safe, sustainable and long-term energy supply. Energy & Environmental Science 4 (4):1216-1224

Sánchez Mirón A, García Camacho F, Contreras Gómez A, Grima EM, Chisti Y (2000) Bubble-column and airlift photobioreactors for algal culture. AICHE J 46 (9):1872-1887

Sanudo-Wilhelmy SA, Tovar-Sanchez A, Fu F-X, Capone DG, Carpenter EJ, Hutchins DA (2004) The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry. Nature 432 (7019):897-901

Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: High-efficiency microalgae for biodiesel production. BioEnergy Research 1 (1):20-43

Schlögl R (2003) Catalytic synthesis of ammonia—a "Never-Ending Story"? Angewandte Chemie International Edition 42 (18):2004-2008

Scholz RW, Wellmer F-W (2013) Approaching a dynamic view on the availability of mineral resources: What we may learn from the case of phosphorus? Global Environ Change 23 (1):11-27

Sedlak R (ed) (1991) Phosphorus and Nitrogen Removal from Municipal Wastewater: Principles and Practice. 2nd edn. Lewis, New York

Selvaratnam T, Pegallapati AK, Montelya F, Rodriguez G, Nirmalakhandan N, Van Voorhies W, Lammers PJ (2014) Evaluation of a thermo-tolerant acidophilic alga, Galdieria sulphuraria, for nutrient removal from urban wastewaters. Bioresour Technol 156 (0):395-399

Sheehan J, Dunahay T, Benemann JR, Roessler P. Laboratory NRE (1998) A look back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from algae.

Shingfield KJ, Bonnet M, Scollan ND (2013) Recent developments in altering the fatty acid composition of ruminant-derived foods. animal 7 (Supplements1):132-162

Shiraiwa Y, Goyal A, Tolbert NE (1993) Alkalization of the medium by unicellular green-algae during uptake of dissolved inorganic carbon. Plant and Cell Physiology 34 (5):649-657

Sialve B, Bernet N, Bernard O (2009) Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 27 (4):409-416

Singh RN, Sharma S (2012) Development of suitable photobioreactor for algae production – A review. Renewable and Sustainable Energy Reviews 16 (4):2347-2353

Slegers PM, Lösing MB, Wijffels RH, van Straten G, van Boxtel AJB (2013) Scenario evaluation of open pond microalgae production. Algal Research 2 (4):358-368

Slegers PM, Wijffels RH, van Straten G, van Boxtel AJB (2011) Design scenarios for flat panel photobioreactors. Applied Energy 88 (10):3342-3353

Solomon CM, Collier JL, Berg GM, Glibert PM (2010) Role of urea in microbial metabolism in aquatic systems: a biochemical and molecular review. Aquat Microb Ecol 59 (1):67-88

Sompech K, Chisti Y, Srinophakun T (2012) Design of raceway ponds for producing microalgae. Biofuels 3 (4):387-397

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101 (2):87-96

Stephenson AL, Dennis JS, Howe CJ, Scott SA, Smith AG (2010) Influence of nitrogen-limitation regime on the production by Chlorella vulgaris of lipids for biodiesel feedstocks. Biofuels 1 (1):47-58

Thacker A, Syrett PJ (1972) The assimilation of nitrate and ammonium by *Chlamydomonas reinhardi*. New Phytol 71 (3):423-433

Torzillo G, Faraloni C, Silva AM, Kopecký J, Pilný J, Masojídek J (2012) Photoacclimation of *Phaeodactylum tricornutum* (Bacillariophyceae) cultures grown outdoors in photobioreactors and open ponds. Eur J Phycol 47 (2):169-181

Torzillo G, Pushparaj B, Masojidek J, Vonshak A (2003) Biological constraints in algal biotechnology. Biotechnology and Bioprocess Engineering : BBE 8 (6):338-348

Trentacoste EM, Martinez AM, Zenk T (2015) The place of algae in agriculture: policies for algal biomass production. Photosynthesis Res 123 (3):305-315

Turpin DH (1991) Effects of inorganic N availability on algal photosynthesis and carbon metabolism. J Phycol 27 (1):14-20

Turpin DH, Vanlerberghe GC, Amory AM, Guy RD (1991) The inorganic carbon requirements for nitrogen assimilation. Canadian Journal of Botany-Revue Canadienne De Botanique 69 (5):1139-1145

U.S. Department of Agriculture. National Agricultural Statistics Service (2013) Arizona Annual Crops.

Udert KM, Larsen TA, Biebow M, Gujer W (2003) Urea hydrolysis and precipitation dynamics in a urine-collecting system. Water Res 37 (11):2571-2582

Ueno Y, Kurano N, Miyachi S (1998) Ethanol production by dark fermentation in the marine green alga, *Chlorococcum littorale*. J Ferment Bioeng 86 (1):38-43

Vahmani P, Fredeen AH, Glover KE (2013) Effect of supplementation with fish oil or microalgae on fatty acid composition of milk from cows managed in confinement or pasture systems. J Dairy Sci 96 (10):6660-6670

Van Wychen S, Laurens LML. NREL (2013a) Determination of total carbohydrates in algal biomass.

Van Wychen S, Laurens LML. NREL (2013b) Determination of total lipids as fatty acid methyl esters (FAME) by *in situ* transesterification.

Vanlerberghe GC, Feil R, Turpin DH (1990a) Anaerobic metabolism in the N-limited green alga *Selenastrum minutum*: I. Regulation of carbon metabolism and succinate as a fermentation product. Plant Physiol 94 (3):1116-1123

Vanlerberghe GC, Joy KW, Turpin DH (1991) Anaerobic metabolism in the N-limited green alga *Selenastrum minutum*: III. Alanine Is the product of anaerobic ammonium assimilation. Plant Physiol 95 (2):655-658

Vanlerberghe GC, Schuller KA, Smith RG, Feil R, Plaxton WC, Turpin DH (1990b) Relationship between NH_4^+ assimilation rate and in vivo phosphoenolpyruvate carboxylase activity: Regulation of anaplerotic carbon flow in the green alga *Selenastrum minutum*. Plant Physiol 94 (1):284-290

Vanlerberghe GC, Turpin DH (1990) Anaerobic metabolism in the N-limited green alga *Selenastrum minutum*: II. Assimilation of ammonium by anaerobic cells. Plant Physiol 94 (3):1124-1130

Vonshak A, Torzillo G, Masojidek J, Boussiba S (2001) Sub-optimal morning temperature induces photoinhibition in dense outdoor cultures of the alga *Monodus subterraneus* (Eustigmatophyta). Plant, Cell Environ 24 (10):1113-1118

Wahlen BD, Willis RM, Seefeldt LC (2011) Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresour Technol 102 (3):2724-2730

Waller P, Ryan R, Kacira M, Li P (2012) The algae raceway integrated design for optimal temperature management. Biomass Bioenergy 46 (0):702-709

Weissman JC, Goebel RP, Benemann JR (1988) Photobioreactor design - mixing, carbon utilization, and oxygen accumulation. Biotechnol Bioeng 31 (4):336-344

Wensel P, Helms G, Hiscox B, Davis WC, Kirchhoff H, Bule M, Yu L, Chen S (2014) Isolation, characterization, and validation of oleaginous, multi-trophic, and haloalkaline-tolerant microalgae for two-stage cultivation. Algal Research 4 (0):2-11

Wett B, Rauch W (2003) The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. Water Res 37 (5):1100-1110

White DA, Pagarette A, Rooks P, Ali ST (2013) The effect of sodium bicarbonate supplementation on growth and biochemical composition of marine microalgae cultures. J Appl Phycol 25 (1):153-165

Williams PJlB, Laurens LML (2010) Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics. Energy & Environmental Science 3 (5):554-590

Woertz I, Feffer A, Lundquist T, Nelson Y (2009a) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng-ASCE 135 (11):1115-1122

Woertz I, Fulton L, Lundquist T (2009b) Nutrient removal & greenhouse gas abatement with CO_2 supplemented algal high rate ponds. Paper presented at the WEFTEC Annual Conference, Orlando, FL,

Xin L, Hong-ying H, Ke G, Jia Y (2010a) Growth and nutrient removal properties of a freshwater microalga *Scenedesmus sp*. LX1 under different kinds of nitrogen sources. Ecol Eng 36 (4):379-381

Xin L, Hong-ying H, Ke G, Ying-xue S (2010b) Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus sp.* Bioresour Technol 101 (14):5494-5500

Yao B, Xi B, Hu C, Huo S, Su J, Liu H (2011) A model and experimental study of phosphate uptake kinetics in algae: Considering surface adsorption and P-stress. Journal of Environmental Sciences 23 (2):189-198

Yoon H, Lim JH, Chung HK (2008) Ammonia removal model based on the equilibrium and mass transfer principles. B Kor Chem Soc 29 (3):555-561

Yoshihara K-I, Nagase H, Eguchi K, Hirata K, Miyamoto K (1996) Biological elimination of nitric oxide and carbon dioxide from flue gas by marine microalga NOA-113 cultivated in a long tubular photobioreactor. J Ferment Bioeng 82 (4):351-354

Zachleder V, Bišová K, Vítová M, Kubín Š, Hendrychová J (2002) Variety of cell cycle patterns in the alga Scenedesmus quadricauda (Chlorophyta) as revealed by application of illumination regimes and inhibitors. Eur J Phycol 37 (3):361-371

Zemke P, Sommerfeld M, Hu Q (2013) Assessment of key biological and engineering design parameters for production of *Chlorella zofingiensis* (Chlorophyceae) in outdoor photobioreactors. Appl Microbiol Biotechnol 97 (12):5645-5655

Zhang ED, Wang B, Wang QH, Zhang SB, Zhao BD (2008) Ammonia-nitrogen and orthophosphate removal by immobilized Scenedesmus sp isolated from municipal wastewater for potential use in tertiary treatment. Bioresour Technol 99 (9):3787-3793

Zhang K, Miyachi S, Kurano N (2001) Evaluation of a vertical flat-plate photobioreactor for outdoor biomass production and carbon dioxide bio-fixation: effects of reactor dimensions, irradiation and cell concentration on the biomass productivity and irradiation utilization efficiency. Appl Microbiol Biotechnol 55 (4):428-433