ADDRESSING SUSTAINABILITY ISSUES IN ALGAL PRODUCTION SYSTEMS

by

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(Under the Direction of Keshav Das)

ABSTRACT

Algae based production systems have the potential to supply copious amounts of feedstock for bioproducts such as fuel, feed, plastics and even food. It has been suggested that algae are capable of out-producing terrestrial plants in terms of sheer biomass per unit of land area. The promise of algae is great, however the potential of these aquatic organisms has yet to be fully realized in large-scale applications. This is due in large part to engineering challenges that confront this developing technology. These challenges exist throughout the production chain. Recently, concerns over these systems have surfaced in response to new regulations being enacted throughout the world, which have brought into question the sustainability of algal systems. This study seeks to address critical issues to enhance sustainability of algal production in environmental and economic aspects. These issues include water usage, carbon footprint, nutrient requirements, and cost of supply chain.

This study examines snow algae, a group of algae known to thrive under low temperature regimes. This study revealed that these psychrophilic algae may also be adapted to low light levels as well. Snow algae were shown to grow and function better under low light conditions. Likewise, final lipid concentration appeared to be enhanced at low light levels in one of the snow algal species. It is anticipated that these algae may enhance sustainability by allowing algae to be grown year-round in certain climates using a seasonal cropping system similar to those employed in terrestrial agricultural systems. Currently, much proposed and demonstration phase algae production is limited to warm season operations. Extending algae growth into the cool-season could enable year-round production in a greater number of locations. This study also revealed that for any algal system to achieve sustainability, the processes used throughout the value-chain must be carefully selected to complement one another. Individual unit operations cannot be selected in a void and a systems approach must be used to insure maximum sustainability. Such a systems approach could enhance economic viability and reduce environmental impacts associated with the large-scale production of algae bringing these systems closer to sustainable operation.

INDEX WORDS: Algae, Sustainability, Bioenergy, Life Cycle Analysis

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DEDICATION

This work is dedicated to Lindsay Leigh and my loving parents for never giving up on me and making sure this thing was finished even if it took forever.

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TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Background

Algae based production systems have the potential to supply copious amounts of feedstock for bioproducts such as fuel, feed, plastics and even food. It has been suggested that algae hold the potential to out-produce terrestrial plants in terms of sheer biomass per unit of land area. For example, the current U.S. Department of Energy (DOE) Bioenergy Technology Office (BETO) algae biomass productivity target for 2022 is 30 $g/m^2/d$ according to their March 2014 report (Jones et al., 2014). This is considered a conservative target while other sources have cited productivity of up to 146 $g/m^2/d$ under specific production and cultivation regimes (Laws et al., 1986). Generally speaking, yields of 15-30 $g/m^2/d$ are considered realistic as annual averages with studies suggesting upper limits in the range of 100-196 $g/m^2/d$ due to photosynthetic limitations (Darzins et al., 2010; Weyer et al., 2010). Regardless of the projections for yield, it is clear that compared to traditional and emerging terrestrial crop numbers shown in Table 1.1 algae hold the potential to out-produce many current terrestrial plant based production systems.

The promise of algae is great, however the potential of these autotrophic, aquatic organisms has yet to be fully realized in large-scale applications. This is due in large part to engineering challenges that confront this developing technology. Challenges have been identified along the entire production chain. These challenges include: species selection

Table 1.1 Potential biomass yields of common terrestrial crops

Crop	Productivity ($g/m^2/d$)
Algae	100 - 196[1]
Corn (including stover)	13.53 - 20.97 [2]
Sugarcane	49.39 - 58.86[2]
Grasses	2.44 - 13.53[2,3]
Pine	6.13 - 7.85[2,4]
Rapeseed	2.7[3]
Sweet Sorghum	29.63[2]
Eucalyptus	27.06[2]

[1]Darzins et al., 2010, Weyer et al., 2010, [2]Huber et al., 2006, [3]Dismuke, et al., 2008, [4]Munsell et al., 2010

and acclimatization, competition in cultivation, energy consumption and nutrient use throughout the production chain, efficient algae harvest and dewatering, cell disruption, lipid extraction, lipid purification, and a host of issues with downstream processing and conversion of biomass and macromolecules produced by algae into useable biofuels (Pienkos et al., 2009; Scott, 2010). Other concerns over these systems have surfaced in response to the Renewable Fuel Standard 2 (RFS2) passed into law in the United States in 2010. These rules and others being enacted throughout the world have brought into question the sustainability of algal systems as there are now legal definitions of renewable and advanced biofuels and defined benefits for the producers of these fuels.

While these policies define important concepts concerning key attributes of biofuels, U.S. policy does not provide clear sustainability criteria for these systems. This study examined the economic, environmental and social sustainability of algal production with a focus on environmental and economic criteria. As such, it is important to understand how each of these elements will be addressed. As the products that the algal systems examined here will replace are generally derived from petroleum, it is critical that these systems are competitive with petroleum. This analysis can be applied to the three elements of sustainability outlined above and these facets should be considered together when evaluating the overall sustainability of these systems. This requires analyses of the complete systems which both algae and petroleum encompass.

Economic sustainability in this study was determined by examining existing tea Analyses (TEAs) with the understanding that products provided from any system competing with petroleum must be cost competitive with oil prices (Amer et al., 2011, Davis et al., 2011, Jones et al. 2014, Klein-Marcuschamer et al., 2013, Nagarajan et al., 2013, Quinn and Davis 2015, Richardson et al., 2012, Xin et al., 2016) As such, it was expected that the feedstocks provided by algal systems must be priced near those derived from petroleum. At the very least, economically sustainable algal systems must be able to generate more revenues than it costs to operate them. As algal systems are compared to petroleum based systems it is necessary to consider the legacy of the petroleum industry which has been in development for over 100 years while biofuels from algae have yet to see success in industrial settings. It is also important to consider that petroleum is a finite resource that will, one day, run out regardless of the development of new technologies allowing access to more difficult to reach reserves. Biomass from organisms such as algae has the potential to provide an endless source of fuels and chemicals and as such, the cost of the environmental and social impacts should also be factored into this analysis as the economics of remediating damage to these environmental and social systems could far outweigh the economic benefits associated with algae production systems.

Environmental sustainability of the algal systems investigated was determined by examining existing life-cycle analyses (LCAs) (Clarens et al., 2012, Frank et al., 2011b, Quinn and Davis, 2015, Sander and Murthy, 2010, Singh and Olsen, 2010). These studies examined the life cycle of algal systems from the perspective of overall carbon emissions.

The working definition of environmental sustainability applied in this study regarded systems that are carbon neutral. That is, those systems where growing algal cells absorb carbon that would later be released during the combustion or use of end-of-life fuels and bioproducts. By comparison, petroleum-based systems release long-stored carbon back into the atmosphere with no method of recovering the emissions. Compared to petroleum, algal systems have a great advantage as they are fueled by photosynthesis which removes carbon from the atmosphere.

Social sustainability is much more difficult to define than economic and environmental elements as it is extremely difficult to quantify this concept. In broad terms, social sustainability defines the impact of systems on the communities in which they exist in terms of the ability of these communities to meet the needs of all members. These communities can range in scale from individuals to the entire planet and include all social structures within this scope. Social sustainability includes many complex social concepts including equal access to key services, equity among cultures, equity for future generations, and the ability of communities to meet their own needs. Further, definitions of social sustainability often refer to it as a process more than a state of being which makes quantifying this element even more difficult (McKenzie, 2004). Newer attempts to define this concept have used risk as a method for measuring social sustainability. In this framework, lower risk to communities correlates to higher levels of social sustainability (Eizenberg and Jabareen, 2017). This is perhaps the most quantifiable definition available in the literature although it requires extensive analysis and diverse skills to perform such an evaluation. Considering that social science skills are required to accurately perform these analyses, this study considered social sustainability at the basic level but concentrated on the economic and environmental elements. However overall, these three dimensions, economic, environmental and social comprised the envelope in which this study examined algal system sustainability.

Further discussions of sustainability specific to algae have been provided by recent, large-scale studies on these systems which involved the entire production chain and evaluation of their impacts. The National Renewable Energy Laboratory (NREL), the Pacific Northwest National Laboratory (PNNL), and Argonne National Laboratory (ANL) have recently released reports commissioned by the DOE. These reports have established accepted baseline values in terms of cost, technology and sustainability for the production of specific biofuels from algae. The first report (Davis et al., 2012) focused on the production of renewable diesel through algal lipid upgrading (ALU) while the second report (Jones et al., 2014) was concerned with whole algae hydrothermal liquefaction and upgrading (AHTL). These reports outline the consensus baseline values for these two pathways. While the two pathways have much in common, there are also significant differences concerning downstream processing and upstream biomass production. The ALU pathway is necessarily dependent upon lipids and as such production is enhanced through increasing lipid content in the algal biomass whereas the AHTL pathway analysis is concerned with maximum biomass productivity. These goals are inherently in conflict with one another as enhancing lipid production can reduce overall productivity due to stress on the metabolism of the organism. As such, previous literature that examines the ALU pathway generally cites increasing lipid yield as the most effective method for increasing productivity in these systems (Davis et al., 2011). Most studies in this area confirm that greater economic and environmental benefits are associated with maximizing lipid production than biomass productivity in these systems (Scott et al., 2010). In contrast, the AHTL process is not solely dependent upon lipids and utilizes all the biomass. Thus, it is predicted that AHTL pathway efficiency can be improved by maximizing total biomass productivity. In order to assure that AHTL processing remains effective it is necessary that this increase in biomass must maintain an overall chemical composition that is favorable to the liquefaction process (Jones et al., 2014).

Regardless of the conversion pathway chosen, sustainability will play a major role in the success of all algae based biofuel systems. While environmental sustainability is an important factor to consider, much of the impact from sustainable production will be realized in the economics of the processes. For example, it is currently economically advantageous for algae based biofuels to meet greenhouse gas (GHG) based sustainability criteria necessary to classify algal biofuels as advanced biofuels. In the United States, this includes meeting the required 50% reduction in GHG emissions as compared to petroleum fuels (Schnepf and Yacobucci, 2013). This certification qualifies the fuel as an advanced biofuel elevating it above renewable fuel status and opening the door to additional financial incentives not available to all biofuels. The current language of RFS2 mandates consumption of biofuels at different thresholds based on 4 categories: renewable fuels, advanced biofuels, cellulosic and agricultural waste-based biofuel, and biomass-based biodiesel. The advanced biofuel category started with a 1-billion-gallon threshold in 2010 which will escalate to 21 billion gallons by 2022. In order to enforce these mandates, the United States has developed a system using Renewable Identification Numbers (RINs). These RINs are assigned to every gallon of qualifying renewable fuel made in the United States. These numbers can be separated from the after production and sold to those deemed obligated parties by the legislation. This group of obligated parties includes most fuel producers and large consumers of fuel. These parties are required by law to accumulate a defined volume of RINs. Different categories of fuel result in RINs of different value and the RINs are traded on an open market based on supply from alternative fuel producers and demand by obligated parties. Ultimately, producers would prefer the Advanced Biofuels designation as the RIN value attached to these fuels are higher per gallon than the RINs for more common renewable designated biofuels such as corn based ethanol.

Cool-season Optimization

An apparent conflict arising in sustainability analyses of algal systems is the question of the efficacy of cool-season operation. This question illuminates a gap in the existing literature which provided an excellent opportunity for this study to examine. From a GHG-based LCA perspective, the literature suggests that cool-season yields are not sufficient to maintain sustainability criteria necessary to meet critical metrics such as those required for advanced biofuels designation in the United States. Specifically, it has been suggested that systems that incorporate cool-season growth using current accepted yields under these conditions are unable to meet the required 50% reduction in GHG emissions as compared to petroleum diesel necessary to achieve advanced biofuel status (Schnepf and Yacobucci, 2013; Clarens et al., 2010). In contrast, when one examines the systems using TEA it is predicted that it is necessary to run algal systems continuously throughout the year as consistent operation and production is necessary to maintain steady revenues required for financial sustainability. The metrics used to measure economic sustainability are the costs of finished products. There are two distinct goals for these metrics outlined in recent critical reports: biocrude cost of \$2.00/gallon for the HTL pathway (NAABB, 2014) and \$3.00 gasoline gallon equivalent (gge) for finished biofuels from any pathway (Jones et al., 2014). A widely regarded TEA analysis suggests that the number of days of operation is one of the most influential parameters on the economic success of algae production operations and achieving these goals (Davis et al., 2011).

The inherent conflict between environmental and economic sustainability goals in algae production provides an opportunity for an engineering solution. The literature suggests that research to explore the enhancement of algal productivity in the cool-season could have significant positive impact on both economic and environmental sustainability. In addition, the development of cool-season rotation would have social impacts as it would extend the operational cycle of algae cultivation facilities thus providing steady, highly-skilled jobs for local communities. The positive social impacts are extended in that the algae farms will also be able to provide feedstock for end-use facilities insuring their steady and continuous operation leading to more of the high-paying jobs associated with the bioeconomy. This issue is significant enough that variation in seasonal growth and its effect on viability of industrial scale algal production has been cited as a key, critical issue in the U.S. Algal Biofuels Strategy (Department of Energy, 2014).

Despite the apparent importance of this topic, there is very little evidence of this type of work in the literature. Cold-tolerant and psychrophilic algae have been identified and categorized but very little work about their biomass composition and productivity has been published. The only recent work in this area appears to be from Nelson et al. (2013)

which examines a species under the group Xanthophyceae, which is a cold-tolerant species of yellow-green algae that is capable of high lipid productivity. This species was identified as part of a larger bioprospecting study and was chosen due to its high lipid accumulation based on Nile Red Fluorescence Microscopy. The study suggests there is potential for this algal species to be a candidate for biofuel production in colder climates. However, upon contacting the authors of this study it was discovered that this species had many challenges with growth regimes as it has low productivity and prefers to grow as a filamentous algae on a substrate. Thus, work towards commercial production of this species has stalled (Personal Communication, April 2014).

While this recent study both characterized and explored the biofuel potential of *Xanthophyceae*, *Heterokonta*, there is much prior work dating back decades in the study of cold-tolerant and psychrotrophic algae. Since most of these algae were discovered in colored snow melts of arctic, Antarctic and mountain regions, they are commonly referred to as “snow algae”. There was much effort in bioprospecting for snow algae throughout the 1970s-1990s. Much of the published work on snow algae is concerned with the characterization and classification of these species and it does not address biofuel production or biomass productivity potential of the identified algae. This work includes a study by Hoham (1975) that provides valuable insight into the temperature tolerance of snow algae. This study identified optimal temperatures for the growth of several species including *Raphidonema nivale* (5°C), *Chloromonas pichinchae* (1°C), and *Cylindrocystis brebissonii* (10°C). This study also demonstrated that *C. pichinchae* was not viable at 20°C. These are useful data for species selection when bioprospecting for cold tolerant algae. As a follow up to this work, a study by Hoham et al. (1979) examined the development of the snow algae *Chloromonas brevispina* (Chlorophyta, Volvocales). Several studies by Ling and Seppelt (1990, 1993, 1998) examined *Chloromonas rubroleosa* (Volvocales, Chlorophyta), a red snow alga that cannot survive above 10°C, *Mesotaenium berggrenii* (Zygnematales, Chlorophyta) an alga that was found in grey snow and showed viability after being frozen at temperatures as low as -25°C, and *Chloromonas polyptera* (Volvocales, Chlorophyta) a snow alga that thrives at much lower pH than many of the other identified snow algae.

More recently, Remias et al. (2013) examined the biology of *Chloromonas polyptera* (Chlorophyta) and found that photosynthesis in this species is adapted to temperatures of about 1°C. Ling (2001) also reported on *Desmotetra aureospora*, Sp. Nov. and *Desmotetra antartica*, *Comb. Nov.* (Chlorophyta) two Antarctic snow algae that could not grow above 15°C. In fact, many snow algae species are incapable of growth at moderate temperatures (Hindák and Komárek, 1967). This information is key to selecting cold-tolerant species that can be grown in cool weather where temporary temperature swings into moderate temperatures (10-15°C) are possible. A study by Konopka and Brock (1978) observed that natural consortia of algae including *Aphanizomenon, Microcystis, and Anabaena* showed active photosynthesis at low temperatures in a natural setting but investigators were unable to duplicate this activity in the laboratory. This suggests some sort of conditioning is likely necessary to stimulate cold-tolerance in native algal species. These temperature range tolerance data are useful information for species selection as an ideal cold-weather algal candidate will thrive in colder temperatures with high biomass productivity and also survive moderate temperatures.

There are also studies which examine the growth of cold-tolerant and psychrotrophic algae. These studies are diverse and are concerned with the investigation of algae in natural systems. Within this body of work are studies by Takeuchi (2001, 2013) which examined the succession of snow algae on the Gulkana Glacier in the Alaskan Range throughout the year and at different altitudes. This work discovered several different optimal algal consortia rising to dominance throughout the year based on snow melt, pH and temperature. Likewise, a stratification of dominant species was discovered through altitudinal studies. The consortia were dominated by *Chlamydomonas nivalis* on the snow surface, and by *Ancylonema nordenskiöldii* and *Mesotaenium berggrenii* on the ice surface throughout the melting season. These species were also spatially distributed based on altitude with *C. nivalis* in higher altitudes, *M. berggrenii*; *A. nordenskiöldii*, and one Oscillatoriaceae cyanobacterium on the in the higher-middle altitudes; *Raphidonema* sp. in the middle altitudes; and one Oscillatoriaceae cyanobacterium in the lowest altitudes.

An expansive temperature related study by Goldman and Carpenter (1974) led to development of models for the temperature dependent growth of several algal species. This study projected temperatures down to 1°C and shows recorded data for algal growth at this temperature. However, this study examined a wide variety of algal species and species and did not define all the data points in the regression used to develop the kinetic model. The lowest growth temperature linked to a specific species in this study is 13.5°C which facilitated minimal growth of *Thalassiosira pseudonana* as reported in a study by Fuhs (1969). While this study offers insight into growth rates at low temperatures, it does not identify any species with substantial growth below 20°C or an adaptation to cooler temperatures. Similar work was conducted by Teoh et al. (2012) in a study that examined the potential effects of climate change on the fate of tropical algae and Antarctic snow algae. This study determined that some snow algae species (*Chlamydomonas-Ant; Chlorella-Ant*) were viable under increasing temperature regimes suggesting that snow algae could provide a robust consortium for sustained production in the cool-season that can tolerate modest swings in seasonal temperatures. A similar study by Stibal and Elster (2005) examined the effect of changing environmental conditions on *Raphidonema nivale and Raphidonema sempervirens*. This study showed *R. nivale* had optimum growth at 12°C and 200 μmol/m^2/s light intensity, while *R. sempervirens* grew optimally at 18°C and 50 μmol/m^2/s. The effect of light intensity combined with cold temperature growth in this study is particularly useful as low light incidence often accompanies the cool-season and optimizing growth under both low light and low temperature regimes will be critical for sustainable production throughout the year. The study by Konopka and Brock (1978) discussed earlier also considered light intensity and its combinatorial effect with temperature. The synergistic effects of these two factors appear to have a significant effect on photosynthetic activity allowing algae to adjust to changing environmental conditions again suggesting that some sort of conditioning is necessary for maximum growth in cool conditions with lower available solar radiation. Thus, the results of these studies will provide a starting point for the selection of potential cool-season growth algae candidates.

Further work into the compositional characterization of psychrotrophic algae has resulted in the identification of uncommon fatty acids synthesized in these algae grown at cold temperatures. Řezanka et al. (2008) report on the presence of medium-chain polyunsaturated fatty acids (PUFAs), e.g., 5,8,11- tetradecatrienoic and 6,9,12-pentadecatrienoic acids, in *Chloromonas brevispina* collected in a snow field of the Czech Republic in 2006. It is likely these PUFAs were synthesized in response to low temperature growth as unsaturation leads to increased mobility of lipids in the membrane and elsewhere allowing the organisms to maintain cellular function at reduced temperatures (Bell et al., 1986). This phenomenon was corroborated in work by Van Wagenen et al. (2012) where lower growth temperatures resulted in increased unsaturation in the lipids produced by *Nannochloropsis Salina*. The climate change study conducted by Teoh et al. (2012) discussed earlier also reported a decrease in PUFAs as growth temperature increased. Such lipids have favorable rheological and fuel properties for the production of high quality biodiesel with excellent cold flow properties (Knothe, 2005). This suggests that cool-season algae crops may be used for winter blend biodiesel production. This could result in a well-organized just-in-time production system for meeting the demand for cold-weather fuels. Similar results were discovered by Morgan-Kiss et al. (2002) in *Chlamydomonas subcaudata* and associated emergence of uncommon medium-chain (14 carbon) fatty acids. This study went further to identify a link between temperature and function of the photosynthetic apparatus. Light intensity was also shown to have an influence on lipid composition. Exposure to elevated light intensity (up to 850 μmol/m^2/s) was shown to result in the accumulation of lipids and the production of uncommon fatty acids such as eicosapentaenoic acid in *Nannochloropsis Salina* (Wagenen et al., 2012). These examples suggest regulation of lipid production through growth conditions is possible but complex involving many factors including growth temperature.

Braunegg et al. (2015) have suggested another sustainability advantage associated with the use of psychrophilic algae in production systems. As these organisms are able to grow at relatively extreme temperatures, they reduce the threat of invasive or contaminating species disrupting production in outdoor systems. While the snow algae system proposed here may not be an effective mechanism of crop protection during the warm season, it will provide some level of protection during the cool-season as competing organisms will likely have slower growth rates at reduced temperature and solar incidence. System collapse and contamination are major threats to sustainable algae production and research in this area is also a major part of the NIFA Algae Roadmap. Proposed crop protection methods can require costly chemical treatments or engineering of algal species. These methodologies can have economic costs in terms of raw materials, licensing fees, and labor. They also carry environmental costs such as GMO concerns, and potential contamination effects of chemical controls. Thus, the crop protection benefit associated with snow algae may provide both economic and environmental sustainability advantages due to the inherent robust nature of these organisms under less than ideal conditions for competitor and predator organisms.

While the studies identified here provide essential background for the identification of effective cool-season algae and algal consortia, there are no studies that have attempted to optimize algal biomass production in cold temperatures. Evidence for a compounding effect between low temperature and decreased solar radiation also appears to emerge when examining the prior literature in this area. As such most operating outdoor algal facilities show a dramatic decrease in biomass production in the cool-season which is generally accompanied by lower levels of solar radiation. This has a negative effect on sustainability from economic and environmental perspectives. Furthermore, the evidence cited here suggests that temperature and light level conditioning may be necessary for the optimization of cool-season/low solar radiation growth in algal systems.

Algae Biofuel Pathways

As presented in the NAABB report (2014), HTL has recently been identified as a game changing technology in the pursuit of sustainable algal biofuels. The HTL conversion pathway provides several advantages over the Algal Lipid Upgrading (ALU) pathway, which has historically been the guiding technology for algal biofuel development. This advance is predicted to lead to a fundamental shift in the way research is conducted in this field. The major advantage of HTL behind this shift is its relative robust performance over a wide range of biomass compositions. Specifically, HTL is an effective conversion technology over a wide range of macro-molecular (lipid, carbohydrate, protein) ratios in algal biomass. This is the key to answering the question presented here as it reduces the importance of engineering high-lipid producing species and systems of algae necessary for improving the performance and sustainability of the ALU pathway. It also eliminates the need for isolation of lipids from whole cell biomass. With the need for maximization of specific macromolecules (lipids) eliminated, research has now turned to the maximization of biomass as the primary goal for cultivation improvements. Recent advances in HTL have also resulted in increased yields with conversion rates ranging as high as 63.6%. This provides an average of 37% more fuel per unit of biomass than the ALU pathway (Elliott et al., 2013).

There are several other advantages associated with HTL that have helped shift the focus in this field from ALU to AHTL. As HTL requires water as a catalyst, it has eliminated the need to produce dry algal biomass before conversion. HTL requires a slurry of only 20% solids which can be achieved through inexpensive, low-energy methods (Elliott et al., 2013). HTL is also more tolerant of contamination in algal biomass. Contamination has posed a huge challenge to the conversion of algal lipids to biodiesel and advanced biofuels. Complications arising from contamination in ALU systems include catalyst poisoning and corrosion of reactors. HTL also avoids the challenges in meeting ASTM specifications for liquid fuels that are associated with the ALU pathway as oils produced from algae are often difficult to convert to methyl ester (biodiesel). The HTL process is producing a product that will be further refined using standard methods that produce fuels identical to their petroleum counterparts ensuring their compliance with existing specifications (NAABB, 2014).

Ultimately, new advances in HTL technology have provided a highly efficient pathway for conversion of algae to liquid fuels that is tolerant of diverse, whole-cell biomass compositions. The ALU pathway, which has been the focus of much of the past work in this field, faces many challenges that have yet to be solved in the laboratory. Many of these challenges stem from the requirement for purified lipids as the sole feedstock for this process. The recommended shift to HTL as the primary conversion technology for algal biomass allows an approach focused on biomass maximization without concern for lipid content or lipid extraction opening new options for cultivation and harvest technologies in algal systems.

While these advances in HTL are extremely important for the advancement of algal biofuels, significant progress on the ALU pathway already has been accomplished and should not be ignored (Davis et al., 2012). As such, it is important for any study that is considering changes in algal cultivation systems to consider the effects of such changes on the production of lipids in these systems. Increased biomass growth doesn't necessarily lead to increased lipid yields as lipid content per cell could decrease in altered systems. Likewise, it is possible that extending the algae growing season into cool and cold temperatures could lead to increased lipid production due to biochemical mechanisms within algal cells. Therefore, while this study will focus on the AHTL pathway, it will also consider changes to the ALU pathway induced by the proposed alterations to algal cultivation systems.

Nutrient Uptake in Algal Systems

Another major hurdle in achieving sustainability in algae based systems is the management of nutrients within these systems. It has been shown in several studies dating back decades that algae are capable of effective assimilation of nitrogen and phosphorus from wastewater (Azad and Borchardt, 1970; Healy, 1977). Furthermore, algae are capable of concentrating dilute sources of these nutrients to higher concentrations at the level required for crop fertilization or reuse in algae production (Chinnasamy et al., 2010). The recycling of nutrients has impacts across the scope of sustainability discussed here. The economics of these systems are improved through the use of low-cost wastewater as a nutrient source. This is coupled with the potential to recover these nutrients in a usable, high-valuable form as fertilizer. Environmental impacts are apparent, as removing and recycling these nutrients is key to environmental sustainability on a global scale. The social impacts are also important to consider as removal of these nutrients from the environment has massive impacts on the quality of life including reducing algal blooms and eutrophication in waterways, improving drinking water quality and extending the supply of agricultural nutrients needed to sustain the world's growing population.

While the opportunities to capture and recycle environmentally dilute nutrients exist in algal systems, most commercial and pilot scale outdoor operations do not utilize nutrient recovery to enhance the environmental, social and economic sustainability of the process. However, recent LCA work on algal systems have identified nutrient delivery, reuse and recycle as crucial process targets for improving overall lifecycle emissions and costs for these systems (Clarens et al., 2010). In fact, Davis et al. (2011) also identified nutrient recycle (along with operating days per year) as one of the highest impact factors in sensitivity analysis in algal TEA. The published data suggests that the study of nutrient recycle in algal systems is an important factor in developing sustainability practices and criteria for large-scale algae production.

Traditional biological nutrient removal from wastewater uses a bacterial remediation. Recent studies have shown that algal systems produce nearly double the biomass and 20% more net energy than these legacy bacterially based systems (Selvaratnam et al., 2015). Several algal species have been studied for their nutrient removal efficiency but such a study has never been conducted at low temperatures or on psychrophilic algae. Therefore, this study will consider the effect of cool and cold season production on the ability of algae to remove phosphorus and nitrogen from aqueous systems.

Nitrogen in Algal Systems

Coupling algal biomass production with nitrogen removal has been studied extensively. The nitrogen uptake efficiencies of many algal species have been analyzed and reported in the literature. The existing data includes a diverse portfolio of algal species including thermo-tolerant and acidophilic organisms (Selvaratnam et al., 2015). Rates of nitrogen removal vary by species but are generally found to be on the order of ~1mg $L^{-1}d^{-1}$. However, it is important to note that these aggressive removal rates are often observed at optimal growth temperatures for the algal species of interest. A sample of removal rates can be found in Table 1.2.

Table 1.2 Observed Nitrogen (NH_4) Removal Rates of Various Algal Species

Algal Species	NH_4 Removal Rate (mg/L/d)	Source
Galdieria sulphraria	4.7-5.0	Selvaratnam et al., 2015
Chlorella vulgaris	5.44	Lau et al., 1998
Chlorella pyrenoidosa	3.4	Tam and Wong, 2014
Scenedesmus intermedius	83.67	Jimenez-Perez et al., 2004
Nannochloris sp.	56.06	Jimenez-Perez et al., 2004
Spirulina maxima	2.9	Cañizares et al., 1994
Chlorella vulgaris	10.5	Aslan and Kapdan, 2006

It has been demonstrated that nitrogen uptake is highly temperature dependent in both algal and bacterial systems with rates slowing at suboptimal temperatures (Reay et al., 1999). However, it is possible that psychrophilic algae may demonstrate higher nitrogen uptake rates than model laboratory algae at low temperatures as metabolic processes for the sustained growth at these temperatures would require sustained nitrogen uptake. This study will profile psychrophilic organisms with the potential to demonstrate sustained nitrogen uptake and will compare their nitrogen uptake efficiency with model, production algae that are selected based on their performance under optimal conditions.

A recent study by Roukaerts et al. (2015) has examined the natural phenomenon of nitrogen uptake in sea ice off East Antarctica. This is an extremely relevant study to the work presented here as this natural phenomenon may be used to remove nitrogen from wastewater in built systems at low temperatures such as those seen in the Antarctic ice pack. The rates in natural systems are low, observed uptake is on the order of ~1 nmol/L/d as opposed to 1 mg/L/d rates observed in controlled systems with optimal conditions. However, it is possible that such rates may be achieved in lower temperatures using engineered systems. This study will seek to determine if psychrophilic algae maintain reasonable nitrogen uptake capabilities in-line with the growth rates observed by these organisms at lower temperatures and lower light-incidence.

Phosphorus in Algal Systems

As much work is already being done on the removal of nitrogen using algal systems, it is of interest in this project to examine the uptake of phosphorus under varied environmental conditions. It would be incredibly advantageous to identify conditions under which specific algal species exhibit increased phosphorus uptake and remediation abilities. The literature does report some phosphorus removal rates for various algal species, a sample of which is shown in Table 1.3.

Table 1.3 Observed Phosphorus (PO_4) Removal Rates of Various Algal Species

Algal Species	PO_4 Removal Rate (mg/L/d)	Source
Galdieria sulphraria	1.5-1.7	Selvaratnam et al., 2015
Chlorella vulgaris	1.30	Lau et al., 1998
Chlorella pyrenoidosa	10.7	Tam and Wong, 2014
Scenedesmus intermedius	20.83	Jimenez-Perez et al., 2004
Nannochloris sp.	10.15	Jimenez-Perez et al., 2004
Spirulina maxima	3.0	Cañizares et al., 1994
Chlorella vulgaris	2.0	Aslan and Kapdan, 2006

Phosphorus is an essential element in important biological molecules such as DNA, RNA and phospholipids. It is also critical in energy storage and release in biological systems through the ATP molecule (Shock and Kathy, 2003). There is accelerating concern over the potential depletion of global phosphorus resources. As phosphorus exists in a finite amount, this depletion could lead to a shortage bringing with it many economic and ecological issues (Cordell and White, 2011). Conversely, excess phosphorus in the environment can have negative environmental consequences as excess presence of this element in water resources leads to eutrophication from high concentrations of algal growth. This phenomenon results in the depletion of oxygen from aquatic systems and the subsequent loss of wildlife through hypoxia. This balance between the need for phosphorus in commercial agriculture and the contaminating effects of this element from agricultural and industrial runoff is an important engineering challenge. Agricultural systems often employ fertilizers containing phosphorus in concentrations on the order of 1g/L while contaminating phosphorus is found in water bodies at concentrations in the 1µg/L to 1mg/L range (1ppb to 1ppm). Because of this exponential discrepancy between useable concentrations of phosphorus and concentrations of contaminating phosphorus, a system that can remove phosphorus from the environment and concentrate it would be both environmentally and economically advantageous.

As stated above, algae have been demonstrated to have a high capacity for phosphorus uptake and as such have been investigated as a mitigation technology for wastewater contaminated with high levels of phosphorus (Xu et al., 2014; Shilton et al., 2012; Chinnasamy et al., 2010). Previous work by Powell et al. (2008) has demonstrated that algal growth on media with similar composition has resulted in increased phosphorus uptake by *Scenedesmus* spp. It has also been demonstrated that under specific environmental conditions, algae are capable of increased phosphorus uptake by accumulating excess phosphorus as polyphosphate, a highly energetic storage molecule shown in Figure 1.1 (Azad and Borchardt, 1970; Eixler et al., 2006; Powell et al., 2008). This phenomenon is known as luxury uptake of phosphate (LUP).

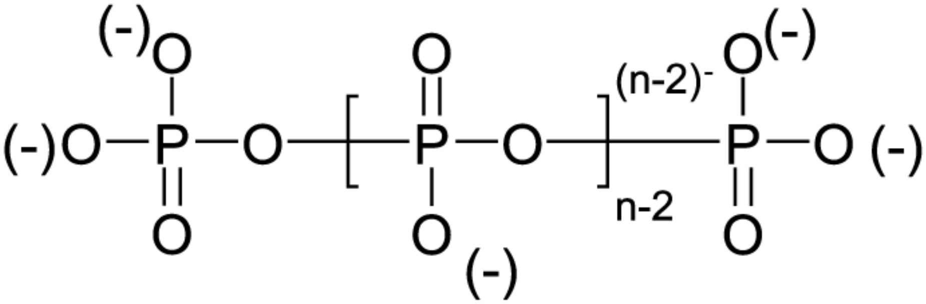

Figure 1.1 Polyphosphate Structure (Kalendová et al., 2004)

Under normal conditions, phosphorus content of algal biomass is approximately 1% of dry weight (Rösch et al., 2012). Under LUP facilitated by high phosphorus content in growth medium, levels of phosphorus in algal biomass have often been reported around 3% and there is evidence of phosphorus levels as high as 4% (Powell et al., 2011).

Additional work by Powell et al. (2008) suggests that high growth temperature and low light levels may enhance LUP. However, this work contrasts with work by Martinez Sancho et al. (1999) which shows high light intensity enhances phosphorus uptake. The discrepancy may be due to the fact that Martinez Sancho used a single organism (*Scenedesmus obliquus*) while Powell examined an algal consortium dominated by *Scenedesmus* spp. There is also evidence that phosphorus starvation before introduction of replete phosphorus can increase LUP (Eixler et al., 2006; Aitchison and Butt, 1973). Ultimately, it appears the factors influencing phosphorus uptake are complicated and are likely dependent upon the algal species itself. Thus, if phosphorus remediation is to be a principal function of algal systems, it is important to examine phosphorus uptake for the specific consortium or species found in the system. It is also important to consider that the nutrient uptake of algal systems is not necessarily only dependent upon high uptake rates of individual cells. High phosphorus content in growth media can also lead to accelerated growth rates and increased biomass production in the form of increased cell production. In this scenario, actual phosphorus removal and sequestration may be higher overall compared to a system where individual algal cells collect high concentrations of phosphorus. Simply put, algal systems with high biomass yield and average phosphorus uptake could be as or more effective at removal of phosphorus than those with high phosphorus content and average biomass production.

Seasonal Productivity

Of interest in this work is the maximization of biomass production on a seasonal basis. Recent developments in hydrothermal liquefaction (HTL) of algae have resulted in greater than 50% conversion of algal biomass to biocrude. This major enabling breakthrough has provided a viable alternative to the more conventional ALU pathway. In addition, it has been determined that when following the AHTL pathway, the ratio of carbohydrates, lipids and proteins is much less important than in the ALU pathway (DOE, 2014). This breakthrough has caused a fundamental shift in algae development toward maximizing overall biomass productivity and away from maximizing lipid production. Likewise, maximization of overall, annual biomass productivity will have sizeable benefits for systems utilizing anaerobic digestion conversion technology, whether it be in concert with lipid recovery, HTL or on whole algae cells. Operating under this assumption and considering the findings of Davis et al. (2011) described above, it can be derived that maximizing days of operation is the single most important factor in optimizing algae productivity for systems that are not dependent upon lipids or any other single macromolecule as a primary feedstock.

With the goal of biomass maximization in mind, it is apparent that cool-season production, or lack thereof, is a major impeding factor in achieving sustainable algae biomass/bioenergy systems. To address this issue, it is proposed that algal crop rotations be developed for seasonally and spatially optimized production for these systems. As with terrestrial crop producers, algae producers should be considering a portfolio of multiple options in terms of potential crops. Successful, terrestrial producers make critical decisions on a seasonal basis as to which crops will be grown based on forecasted weather and commodity market conditions. This flexibility offers these producers informed influence over the profitability and sustainability of their farms. Without similar choices algae based systems will face a competitive disadvantage to terrestrial crops in terms of efficient land utilization.

Species Selection

Algal cultivation system productivity is dependent upon many factors. Perhaps the most influential factor is the type of algae being cultivated in these systems. Two general approaches have been used to maximize production from these systems. The first uses native algal species from the environment in which the systems are to be deployed. The logic in this methodology is that the native algal flora is most likely to survive in the conditions in which the system is operated. The second species selection methodology seeks to identify algal species that will have increased production based on their genetic makeup. These species can be identified via prospecting for natural algae that show increased productivity and competitiveness or they may be engineered using modern genetic and molecular techniques. This method depends on the highly productive algal species maintaining productivity when transitioning from laboratory conditions to unpredictable and dynamic natural conditions.

Both of these approaches have drawbacks and advantages. Native flora systems insure robust growth of the target organisms and the ability to deal with local environmental pressures such as specific competing organisms, weather variability and predators. However, such organisms are generally not naturally high-productivity and likely focus metabolic resources on survival versus biomass production. High-productivity organisms generally exhibit the opposite characteristics; susceptibility to local environmental pressures and high biomass productivity. Due to the compromises faced in these systems much research has been conducted to develop hybrid systems which leverage the advantages and reduce the risk of these two approaches. An example of this hybrid approach is the bioprospecting of locally adapted algal species with relatively high productivity. This involves identifying several species that are found to be stable in the local environment and selecting the most productive based on biomass assays. This approach can be effective and will generally result in identifying organisms that have moderate biomass productivity and robust survival in local outdoor systems. A similar option is to use biological engineering to enhance the productivity of locally robust organisms. This approach will often result in producing organisms with high productivity and that are moderately robust in local environments.

Consortia

Another method that has been demonstrated to increase survival and productivity of algal systems is the use of algal consortia (Kazamia et al., 2012). These consortia are mixtures of two or more algal species that possess symbiotic traits that contribute to the overall robustness of the system. These consortia can be developed through experimental data using species with complementary traits which are predicted to result in optimized systems which are both robust under natural conditions and productive in terms of key metrics (lipid production, biomass production, etc.). Alternatively, these consortia can be isolated from the natural environment that provides a grouping of organisms chosen via natural selection to exhibit optimal growth under ambient conditions. The second method provides a rapid and reliable method for identification of effective consortia while the first method can be less efficient in terms of time but may result in superior attributes in terms of combined environmental resilience and productivity (Hena et al., 2015)

An example of an effective algal consortium was demonstrated by Chinnasamy et al. (2010). This system used a consortium of native algal species to treat carpet industry wastewater. The algal species were isolated from wastewater with extremely high contamination from a community with several carpet mills. This consortium contained at least 15 different algal species. This collection of algae proved to be both robust to natural conditions and extremely effective at removing nutrients from the industrial effluents they were isolated from. The algae were also very productive with the potential to produce 9.2–17.8 metric Mg/ha/year of biomass. The combined qualities of constituent algae have been shown to have synergistic effects on the health and productivity of algal consortia often leading to higher productivity systems than those employing algal monoculture.

Crop Protection

The result of algal species selection for open systems approaches is often a compromise between high production and viability of these organisms in the open environment. Algae are constantly under attack by various predatory organisms such as grazers, bacteria and viruses. To insure the survival of target organisms there are various methods that can be employed to improve viability of algal crops in open environments. These crop protection methods include chemical treatment, biological controls and physical barriers to the local environment (Hannon et al, 2010). Chemicals which can be employed for this purpose include antibiotics, ammonia, and other substances that prevent the reproduction of predators and competitive organisms (Passero et al, 2017). Biological controls include symbiotic organisms that control competitors while leaving the algal crop unharmed. Physical barriers can be employed to eliminate competitors and contamination of the system. These resulting closed systems have excellent crop health but increase the cost of production substantially, often requiring artificial sources of light as described above for photobioreactor systems.

Objectives

The overall goal of this work was to address outstanding issues in the sustainability of algal biofuel systems. Identification of pertinent issues was guided by The Algal Biofuels Strategy Workshop (DOE, 2014) at which the investigator was an attendee. Following the workshop, an extensive literature search was conducted to pinpoint specific gaps in algal research. Gaps were identified that have the potential to have marked positive impact on the performance of existing algal biofuel production systems. Using this analysis, two major objectives were selected for this study:

1. Optimizing seasonal growth of algae
2. Optimizing nutrient uptake under variable environmental conditions

As discussed earlier these two areas have been identified as having major impact potential on the overall sustainability and profitability of algal biomass systems (Davis et al., 2011). Both of these factors ultimately contribute to increases in biomass yield and reductions in costs of systems using the AHTL pathway. This study also examined the influence of variable environmental conditions on the production of lipids in these algal systems. While not a focus of this study, such analysis could lead to the identification of specific system conditions (algal species, temperature, light intensity) that would provide a valuable increase in lipid production for the ALU pathway.

In addition to addressing Objectives 1 and 2 above this study was also designed to provide guidance on the sustainable development of algal production systems. Thus, a third objective in this study was identified.

3. Identify specific recommendations for enhancing the sustainability of algal production systems

As such, this study includes an in-depth review of sustainable water and nutrient management in algal systems. This review used data collected in this study and existing literature to identify key factors in developing sustainable algal production with specific regard to water and nutrients.

Sub-Objectives

In pursuit of Objectives 1-3 listed above, several sub-objectives were necessary. These are presented here along with overview of the methodology used to achieve the sub-objectives.

Identifying potential cool-season algae crops

The first decision addressed in the context of biomass productivity optimization is the selection of optimal algal species or consortia based on historical, local seasonal conditions throughout the year. Two major contributing factors to this decision are temperature and light intensity. Seasonal variation is often such that lower temperatures are often accompanied by reduced light intensity. Traditional algae systems have often been developed assuming ideal light and temperature conditions. As such, it has been found that data from the laboratory is not useful when developing real-world, outdoor algae production systems (DOE, 2014, NAABB, 2014). Laboratory data representing real-world conditions will be extremely valuable in assisting in scale-up and deployment of industrial algal systems. Furthermore, collection of scientifically valid data reflecting dynamic environmental conditions will provide a basis by which producers can select seasonal algae crops to improve if not maximize biomass production under predicted weather conditions.

With this understanding, this study examined several algal species for growth rate, chemical composition and biomass yield under a variable temperature/light intensity regime. The species selected for this study are shown in Table 1.4 along with data on their temperature and light dependent growth where available.

Table 1.4 Selected algal species for temperature and light intensity studies

Species	Optimal Temp.	Known Growth Temperature Range	Source	Reference(s)
Scenedesmus bijuga	TBD	5^{1} - 40°C^{2}	UGA	[1]Our Preliminary Data [2]Bajaj and Srivastava 1985
Chlorella sorokiniana	38°C^{3}	10^{4}-38°C^{3}	UGA	[3]Franco et al., 2012 [4]Our Preliminary Data
Chlamydomonas yellowstonensi	16°C^{5}	0 - 25°C^{5}	UTEX SNO134	[5]Hoham 1975
Chlamydomonas augustae	11 - 18°C^{6}	4 - 32°C^{6}	UTEX B SNO155	[6]Teoh 2013

The species studied here were selected based on two sets of criteria. *Scenedesmus bijuga* and *Chlorella sorokiniana* were isolated from wastewater from Dalton, GA and are native species and have been demonstrated as well as adapted to growth in the Southeastern United States. *Chlamydomonas yellowstonensi* and *Chlamydomonas augustae* were identified based on literature review of “snow algae” which are algae often found in glaciers or snow deposits, which have specifically adapted to grow at lower temperatures near the freezing point of water. These particular species were found to be available from the UTEX algae repository and met initial screening criteria demonstrating growth across a wide range of temperatures in BG-11 growth media. Several other snow algae were screened for this study including: *Chodatia* *tetrallantiodea, Chlorommonas brespevina, Chlorosarcinopsis sempervirens and Raphidonema nivale. C. sempervirens* and *R. nivale* demonstrated irregular, low productivity growth under the screening protocol used here. *C. tetrallantiodea* and *C. brespevina* showed no growth at all in BG-11 media over a wide range of temperatures (5-20°C).

Light and temperature regulated growth of identified algal species

This study examined the effect of variable temperature and light regimes on the productivity of the algal species identified above. The species of interest were grown at temperatures ranging from 5- 20°C with light exposure of 50- 300 μmol/m^2/s. These conditions were selected to approximate cool-season growth in temperate climates in the continental United States. Growth models for cool-season growth based on these data were constructed and relationships between algal productivity and environmental conditions were identified.

Nutrient recovery potential based on seasonality

Previous analysis has suggested that biomass productivity and annual days of production have the greatest influence on sustainability of algal systems (Clarens et al., 2010). The second most influential parameter has been identified as nutrient delivery, recycle and reuse (Davis, et al. 2011). As algal systems require a significant amount of nutrient supplementation, the ability to recover and reuse these nutrients from the biomass and effluent can greatly enhance the economics and reduce the environmental impact of these systems. This study addressed this issue by extending the model developed above to include nutrient recovery and recycle based on data collected in the laboratory of the investigators.

The unique growth conditions developed for this study, which offered control over temperature and light exposure were leveraged to study the effect of seasonality on nutrient uptake by algae. Preliminary screening of the species of interest indicated that growth temperature has a correlation to the rate of nutrient removal from growth media. Many recent studies have demonstrated that linking algal biofuels to nutrient-rich wastewater remediation has a beneficial effect on the productivity and sustainability of these systems (Chinnasamy et al. 2010; DOE, 2014). Therefore, it follows that a realistic perspective on the seasonal effects on this process will assist in developing accurate models for algal growth on high-nutrient media. To this end, temperature dependent nutrient assays were performed on the species studied here. These analyses assayed for phosphate and nitrate removed from the media after ~14 days of algal growth. This data was compared across species and growth conditions set up for the productivity investigation.

Seasonal algae lipid regulation

Previous studies have shown strong links to seasonality and algal lipid production. However, these studies have shown inconsistent results with maximum lipid yield per gram biomass peaking in both Fall and Summer months (Olofsson et al., 2012). It follows that this regulation is species dependent. As part of this study includes the examination of snow algae (*C. yellowstonensis and C. augustae*), it was useful to examine the lipid production of these organisms under variable environmental conditions. This analysis of lipid production was also performed on two model laboratory/production species (*S. bijuga and C. sorokiniana).*

Sustainable algae production

A review of existing, published literature was conducted to examine the current state-of-the-art for sustainable algae production. The review was intended to provide a current, objective investigation into the potential for algae to provide a source of renewable biofuel that offers both economic and environmental advantages over current petroleum based liquid fuels. It addressed sustainability in terms of water and nutrient conservation and recycle. The overall goal of the review was to identify targets along the supply chain for improving sustainability of the overall system. The review also sought to identify the potential for these systems to both remediate polluted water sources and recover nutrients in a usable form from algae.

These objectives were selected to fill gaps identified in the existing literature. The objectives were also designed to address conflicts between the existing TEA and LCA studies on algal systems. As described earlier, TEA studies suggest that suggest algal production systems should not operate in the cool season when algal production is not optimal using current algal species and outdoor growth systems. On the other hand, LCA studies suggest that algal production must be extended to nearly continuous annual operation in order achieve Advanced Biofuel status. Advanced Biofuel status according to RFS2 requires greenhouse gas emissions reductions equal to 50% of those associated with fossil fuel production (Schnepf and Yacobucci, 2013). In order to meet the recommendations of both LCA and TEA studies it is clear that year-round production coupled with nutrient recovery are necessary improvements to existing algal production systems. This study attempted to provide guidance in reconciling these recommendations and improving the sustainability of algae production.

CHAPTER 2

BIOMASS PRODUCTIVITY OF SNOW ALGAE AND MODEL PRODUCTION ALGAE UNDER LOW TEMPERATURE AND LOW LIGHT CONDITIONS[1]

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[1] Geller, D.P., Das, K.C., Bagby-Moon, T., Singh, M., Hawkins, G., Kiepper, B. 2017. To be submitted to *Algal Research.*

Abstract

This study was designed to determine biomass productivities of specific algal species under low temperature and low light conditions. The algal species examined in this study included two psychrophilic algal species (*Chlamydomonas yellowstonensis* and *Chlamydomonas augustae*). These species are commonly known as “snow algae” due to their ability to grow in low temperature water bodies including ice and snow deposits. Additionally, two model production algal species used in high biomass productivity pilot studies (*Scenedesmus bijuga* and *Chlorella sorokiniana*) were evaluated. Currently, temperature dependent growth data within known optimal limits exists for these model production species but there is no detailed information about their biomass productivity under low temperatures. In addition, little information can be found about the potential for productivity of these species under limited light exposure. This study examined biomass productivity of these four species at four relatively low temperatures (5, 10, 15, and 20°C) with three relatively low light exposures (50, 100, and 300 μmol/m^{2}s). It was hypothesized that the two psychrophilic algae species would produce more biomass per day than model production algal species under these limiting conditions. This study found that both snow algae species performed better than their production counterparts at the lowest temperature (5°C) and two lower light intensities (50 and100 μmol/m^2/s). *C. augustae* growth rate was shown to have a positive correlation with temperature and a *negative* correlation with light intensity for the values observed in this study. This finding has significant implications for the use of *C. augustae* as a cool-season algal crop and a source of valuable genetic material for future engineering of algae. This could lead to the development of cool-season algal crops for sustainable, year-round, industrial production

of algae in temperate climates. Furthermore, both of the snow algae species studied here showed potentially inhibited growth at the highest light intensity studied here.

Introduction

Developing methods for sustainable, industrial production of algae is a complex challenge that involves a wide range of variables including: species selection and conditioning, competition in cultivation, energy consumption and nutrient use throughout the production chain, algae harvesting, algae dewatering, cell disruption, lipid extraction, lipid purification, and a host of issues with downstream processing and conversion of biomass and macromolecules produced by algae into useable biofuels (Pienkos et al., 2009; Scott et al., 2010).

An apparent conflict arising in sustainability analyses of algal systems is the question of the efficacy of cool-season operation. From a GHG-based LCA perspective, the literature suggests that cool-season yields are not sufficient to maintain sustainability criteria necessary to meet critical metrics such as those required for advanced biofuels designation in the United States. Specifically, it has been suggested that systems that incorporate cool-season growth using current accepted yields under these conditions are unable to meet the required 50% reduction in GHG emissions as compared to petroleum diesel necessary to achieve advanced biofuel status (Schnepf and Yacobucci, 2013; Clarens et al., 2010). In contrast, when one examines the systems using technoeconomic analysis (TEA) it is predicted that it is necessary to run algal systems continuously throughout the year as consistent operation and production is necessary to maintain steady revenues required for financial sustainability. A highly regarded TEA analysis suggested that the number of days of operation is one of the most influential parameters on the economic success of algal production operations and achieving these goals (Davis et al., 2011). Likewise, an analysis of over 40 algae system TEAs and LCAs conducted by Quinn and Davis (2015) suggests that seasonal and regional variability in productivity can have a profound effect on the overall economic viability of algal production systems.

Ultimately, the conflict faced when trying to achieve economic and environmental sustainability in algal systems will need to be addressed if production of biofuels from algae is to become a viable component of the global energy mix. Reconciling these issues will be achieved by increasing cool-season algal productivity to provide sustained biomass production leading to both the desired reduction in GHG emissions and economic viability. One pathway that may lead to resolving the inherent conflict between economic and environmental sustainability of algal systems is the development of cold-tolerant and psychrophilic algal species as cool-season crop alternatives to model production algal species.

Production algae perform best at optimal conditions, which often include warm temperatures (>20°C) and exposure to high levels of sunlight (>150 μmol/m^2/s) (Venteris, E.R. et al., 2014). Based on these conditions, ideal geography for the outdoor, open-pond based production of algae has often been identified as locations that maintain temperate conditions for most of the year, for example, the Desert Southwest of the United States. However, one factor, which needs to be considered in the siting of algal farms, is the availability of water. Open-pond systems require a tremendous amount of makeup water to compensate for rapid evaporation from the high surface area required for production of algae. This has resulted in a reconsideration of ideal sites for these facilities. A study by Venteris et al. (2014a) examined algal water demand as well as algae production and utilization infrastructure availability, water suitability (salinity), etc. This study produced a GIS based map that identified the Gulf of Mexico area as ideal for two model production species (*Anthrospira* and *Sphaeropleales*). The Florida peninsula was identified as the most suitable location in the U.S. Further analysis from Venteris et al. (2014b) identified a contiguous band from eastern Georgia and Florida to Southwestern Texas as a high productivity region for production of several algal species including *Nannochloropsis* and *Chlorella*. If cool-season adapted algae were identified that maintained modest biomass production, it is possible that this band could extend even further north into the United States to regions where other key resources such as water, suitable land and CO_2 are all sufficiently available.

This study was designed to identify algal species that have the potential for production in cooler months to provide the possibility of continuous production of algae for biomass at an industrial scale. This study specifically examined the biomass productivity of four species at four low temperatures (5, 10, 15, and 20°C) and three low light intensities (50, 100, and 300 $\mu mol/m^2/s$). Two of the species were selected due to their psychrophilic nature (*Chlamydomonas yellowstonensis* and *Chlamydomonas augustae*). Two species that serve as model laboratory organisms, *Scenedesmus bijuga* and *Chlorella sorokiniana*, were selected to serve as a baseline for comparison to mesophilic algae.

Materials and Methods

Species Selection

The species selected for this study are shown in Table 2.1 along with data on their temperature and light dependent growth where available.

Table 2.1 Snow algae and model laboratory algae used in temperature and light regulated growth studies.

Species	Optimal Temperature	Known Growth Temperature Range	Source	Reference(s)
Scenedesmus bijuga	TBD	5[1] - 40°C[2]	UGA[a]	[1]Our Preliminary Data [2]Bajaj and Srivastava 1985
Chlorella sorokiniana	38°C[3]	10[4]-38°C[3]	UGA[a]	[3]Franco et al., 2012 [4]Our Preliminary Data
Chlamydomonas yellowstonensis	16°C[5]	0 - 25°C[5]	UTEX[b] SNO134	[5]Hoham 1975
Chlamydomonas augustae	11 - 18°C[6]	4 - 32°C[6]	UTEX[b] SNO155	[6]Teoh 2013

[a]University of Georgia Bioconversion Research & Education Center
[b]Culture Collection of Algae at The University of Texas at Austin

The species were selected based on two sets of criteria. *S. bijuga* and *C. sorokiniana* were native species isolated from wastewater from Dalton, GA and have been demonstrated as well-adapted to growth in the Southeastern United States. These algae have served as model organisms for the production based algal research at the University of Georgia's Bioconversion Research and Education Center (Athens, GA). *C. yellowstonensis* and *C. augustae* were selected based on literature review of "snow algae" which are algae often found in glaciers or snow deposits. Many of these species have adapted to grow at lower temperatures near the freezing point of water. These particular species were found to be available from the UTEX algal repository and met initial screening criteria demonstrating growth across a wide range of temperatures in BG-11 growth media.

Several other snow algae were screened for this study including: *Chodatia tetrallantiodea, Chlorommonas brespevina, Chlorosarcinopsis sempervirens and Raphidonema nivale*. *C. sempervirens* and *R. nivale* demonstrated irregular, low productivity growth under the screening protocol used here. *C. tetrallantiodea* and *C. brespevina* showed no growth at all in BG-11 media at four relatively low temperatures (5, 10, 15, and 20°C).

Temperature and light controlled algae growth

Scenedesmus bijuga (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensis* (CY) and *Chlamydomonas augustae* (CA) were grown in 100 mL cultures of modified BG-11 media in 250-mL Erlenmeyer flasks which were placed in a fisher scientific 436 incubated orbital shaker operating at 100 rpm. The lid of the shaker was removed and the shaker was placed in a temperature and humidity controlled growth chamber with a constant humidity of 70-75%. Cultures were grown at 5, 10, 15, and 20°C with light exposure of 50, 100 and 300 μmol/m^2/s providing for a total of 12 treatments (conditions) per species with 3 replications (i.e., flasks) for each treatment, totaling 144 experimental units. Temperature was maintained within ±1°C for all treatments except 300 μmol/m^2/s treatments, which experienced a temperature drift of ±4°C due to heat generated from the lighting system. Light was cycled to provide 12 hours light and 12 hours dark conditions. Cultures were grown in triplicate for a minimum of 14 days. 500 μL of each culture was removed every 30 hours under sterile conditions and optical density (OD) @750nm was measured for each sample using BioTek Synergy H1 Hybrid Reader. OD readings were converted to algal cell concentrations (mg/L) using standard curves plotted from the OD of known biomass concentrations (mg/L) for each algal species. Growth curves (concentration vs. time) were plotted using the daily biomass data and biomass productivity rates (mg/L/day) which were determined as the slope of a linear regression of the linear phase of these growth curves. Figure 2.1 shows a representative plot and regression used to calculate the biomass productivity rate for *Scenedesmus bijuga* at 5°C and 50 µmol/m^2/s.

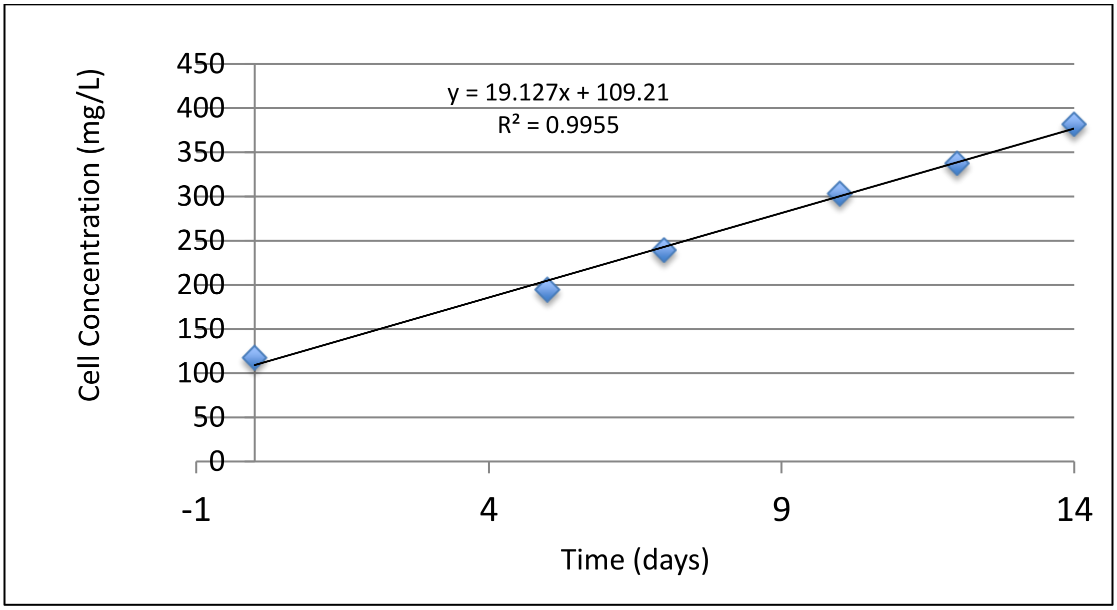

Figure 2.1 *Scenedesmus bijuga* cell concentration (y) versus time (x) at 5°C and 50 µmol/m^2/s. Linear regression for cell concentration was used to determine growth rate model.

The 144 data points (4 algal species x 4 temperatures x 3 light intensities x 3 replicates) collected above were then compared across the 4 species under each treatment regime to identify differences in growth patterns between snow algae and model production organisms. Growth models based on the treatment and species matrix were built using ANOVA and/or Linear Regression (where possible) using methodology described below and the most accurate models were identified. These models and the observed behavior of the algal species were used to identify differences between snow algae and production algae as well as between all four species examined here.

It should be noted that the purpose of this study was to examine the growth of the selected algal species under conditions of low light and low temperature. The equipment used in this study offered control of these parameters at the values specified above. The selection of these values was limited by equipment and resources and therefore the results obtained here must be considered within the limitations and context of this study.

Experimental design and data analyses

R Statistical Software was used for experimental design, regression and evaluation of the model (The R Foundation, 2015). Statistical analysis was performed in cooperation with Patrick Kriebel and ZiJun Xu of the University of Georgia Statistical Consulting Center under the Direction of Dr. Jaxk Reeves. Analysis was conducted in two steps. First, a linear regression was constructed in R, for each of the 144 treatments. Each of these linear regressions was based on 5-7 (X=Day, Y=Density) pairs, as described above. The slopes of these regressions were used to establish a growth rate for each treatment in mg/L/d. For example, in the 5°C and 50 μmol/m^2/s experiment, there is one regression equation for each replicate of SB, one for each replicate of CSO, one for each replicate of CA, and one for each replicate of CY. The same is true for each of the twelve (Light*Temperature) combinations.

Results and Discussion

Analysis

The average temperature and light dependent, observed growth rates for the species studied here are summarized in Table 2.2. Two of the CSO treatment regressions resulted in slightly negative growth rates. Since no growth was observed in these treatments a growth rate of 0 mg/L/d was assumed for CSO under these conditions.

Table 2.2 Average growth rates for SB, CSO, CY, and CA at 5, 10, 15, and 20°C and 50, 100 and 300 μmol/m^2/s.

		Species Growth Rate (mg/L/day)			
Temperature (°C)	Light Intensity (μmol/m^2/s)	*Scenedesmus bijuga* (SB)	*Chlorella sorokiniana* (CSO)	*Chlamydomonas yellowstonensis* (CY)	*Chlamydomonas augustae* (CA)
5	50	19.12	0	27.74	27.40
5	100	9.36	0	14.50	16.90
5	300	27.60	8.60	16.18	11.97
10	50	53.02	34.63	26.24	33.52
10	100	45.91	11.26	8.76	13.25
10	300	43.54	42.22	17.02	9.36
15	50	74.05	65.78	27.90	37.50
15	100	60.04	58.81	27.30	27.90
15	300	63.34	61.41	19.40	13.96
20	50	74.87	65.37	30.40	40.62
20	100	71.46	57.67	14.14	29.48
20	300	91.52	87.97	21.32	20.15

The growth rate estimates from the 144 linear regressions were used to build linear models for each species, predicting the growth rate, in mg/L/d, as a function of temperature (T) and light (L). This was done in two different ways, ANOVA in which both temperature and light were treated as class variables with fixed levels, and linear regression where both temperature and light were treated as continuous variables. Within each of the two methods, there is a 4-model hierarchy: 1) using both temperature and light as explanatory variables, 2) using only temperature, 3) using only light, or 4) using neither (null model).

Tables 2.3-2.6 below report all 6 linear regression and ANOVA models for each species, with the best of each highlighted. Intercept estimates for every regression model are given, however, these should not be taken literally as the models are only valid within the experimental parameters used here.

Scenedesmus bijuga

From the regression analysis conducted, using the best linear regression model from Table 2.3, temperature was shown to be the only statistically significant predictor of growth rate for SB in this study.

Table 2.3 Linear Regression and ANOVA parameters for *Scenedesmus bijuga* growth rate dependence on light intensity and temperature.

Model	**PARAM**	**Intercept Est.**	**Interc. P-Value**	**TEMP Est.**	**TEMP P-value**	**LIGHT Est.**	**LIGHT P-value**	**RMSE**	**RSQR**
LR(T,L)	**3**	0.164	0.982	4.002	<0.01	0.018	0.473	0.00876	0.8975
LR(T)	**2**	2.794	0.654	4.002	<0.01	———	———	0.00878	0.8911
LR(L)	**2**	5.018	<0.01	———	———	0.018	0.805	0.02788	0.0064
Model	**PARAM**			**TEMP F-Stat**	**TEMP P-value**	**LIGHT F-stat**	**LIGHT P-value**	**RMSE**	**RSQR**
ANOVA(T,L)	**6**			38.107	<0.01	2.110	0.2024	0.00736	0.9518
ANOVA(T)	**4**			29..83	<0.01	———	———	0.00832	0.9179
ANOVA(L)	**3**			———	———	0.158	0.8563	0.02690	0.0339

*Highlighted models represent best models for linear regression and ANOVA.

The P-value for the coefficient associated with temperature was several orders of magnitude less than the α-level of 0.05. The estimated regression coefficient for temperature is 0.004002, suggesting that for each one-degree increase in temperature (for temperatures ranging from 5° C to 20° C), the growth rate of the SB species will increase by 4.002 mg/L/day. The best linear regression for the growth rate of SB is shown in Equation 1 below, where Y_i is the growth rate for SB at temperature T_i and any light intensity within the parameters of this study and ε_i is the error associated with the regression.

$$Y_i = 2.794 + 4.002 \times T_i + \varepsilon_i \qquad (1)$$

The ANOVA for SB indicates that the growth rate is significantly different for the different levels of temperature (5° C, 10° C, 15° C, 20° C) and that the rate increases as temperature increases. According to both the ANOVA and linear regression models, there is not a significant difference in growth rate for the different levels of light. Figure 2.2 supports these conclusions, as there is no apparent trend related to light intensity but there is an apparent correlation to temperature.

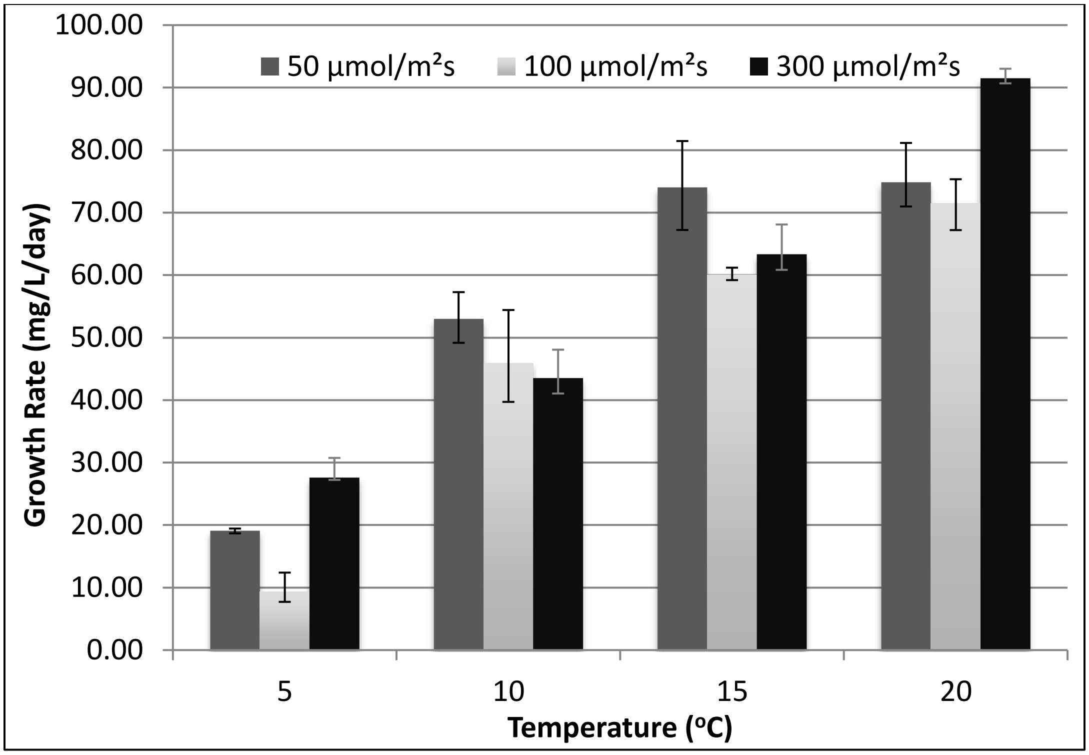

Figure 2.2 Growth rate vs. temperature and light intensity for *Scenedesmus bijuga*. Error bars represent observed growth rate range.

Chlorella sorokiniana

Based on the linear regression analysis conducted here, it was determined that temperature is a significant factor for predicting the growth rate of CSO. The parameters for both linear regression and ANOVA of CSO growth rates are shown in Table 2.4 and the best linear regression for the growth rate of CSO can be found in equation 2, where Y_i is the growth rate for CSO at temperature T_i and any light intensity within the parameters of this study and ε_i is the error associated with the regression.

$$Y_i = -20.726 + 4.888 \times T_i + \varepsilon_i \tag{2}$$

The p-value for the temperature coefficient is well below the α-level of 0.05, whereas the p-value of the coefficient for light exceeds 0.05 suggesting there is no statistical significance to the influence of light on growth rate of CSO within the parameters of this study.

Table 2.4 Linear regression and ANOVA parameters for *Chlorella sorokiniana* growth rate dependence on light intensity and temperature.

Model	**PARAM**	**Intercept Est.**	**Interc. P-value**	**TEMP Est.**	**TEMP P-value**	**LIGHT Est.**	**LIGHT P-value**	**RMSE**	**RSQR**
LR(T,L)	**3**	-27.192	0.0189	4.888	<0.01	0.044	0.2019	0.01172	0.8818
LR(T)	**2**	-20.726	0.0375	4.888	<0.01	———	———	0.01222	0.8427
LR(L)	**2**	33.902	0.0571	———	———	0.044	0.6244	0.03192	0.0249
Model	**PARAM**			**TEMP F-stat**	**TEMP P-value**	**LIGHT F-stat**	**LIGHT P-value**	**RMSE**	**RSQR**
ANOVA(T,L)	**6**			37.170	0.0003	3.184	0.1141	0.00918	0.9516
ANOVA(T)	**4**			24.042	<0.01	———	———	0.01142	0.9002
ANOVA(L)	**3**			———	———	0.244	0.7886	0.0332	0.0514

*Highlighted models represent best models for linear regression and ANOVA.

ANOVA resulted in a coefficient for temperature of 4.888. ANOVA also suggests that growth rate increases with increasing temperature although this relationship is likely not linear. ANOVA also suggested that there is no significant relationship between light intensity and growth rate of CSO within the parameters of this study as supported by Linear Regression and the plot in Figure 2.3 which shows a clear correlation between growth rate and temperature but unpredictable growth rates when considering light intensity.

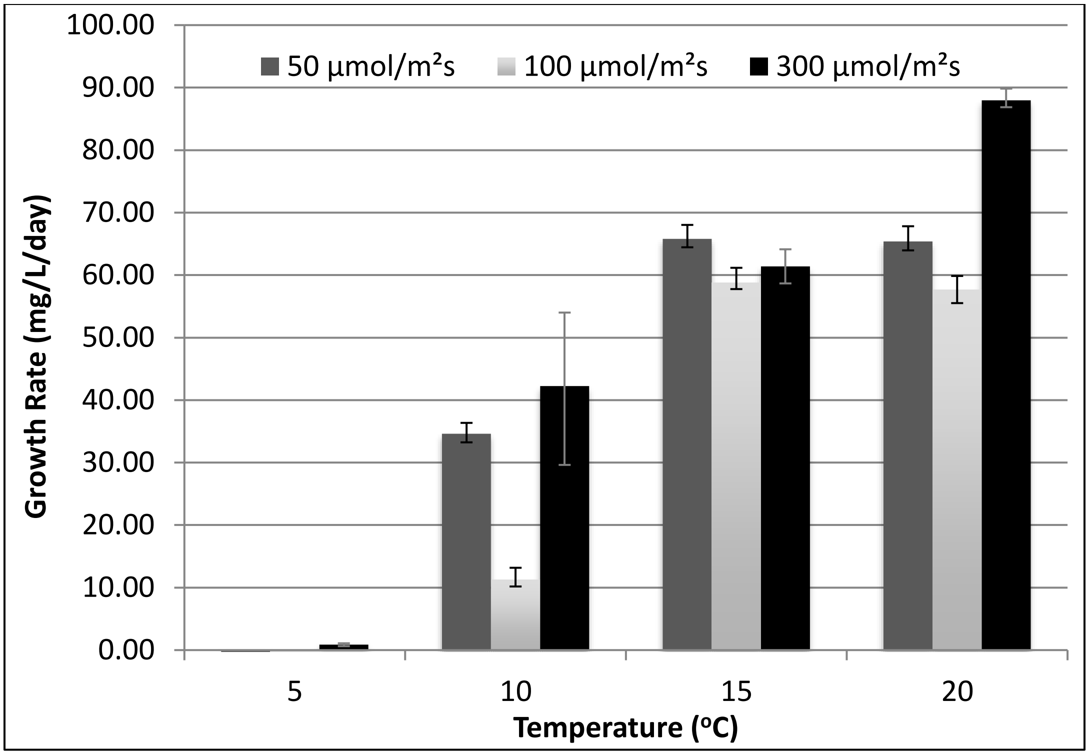

Figure 2.3 Growth rate vs. temperature and light intensity for *Chlorella sorokiniana*. Error bars represent observed growth rate range.

<u>*Chlamydomonas yellowstonensis*</u>

For CY, neither temperature nor light is a significant predictor of growth rate within the parameters of this study. As shown in Table 2.5, the p-values for both predictors are well above the α-level of 0.05 in the LR(T, L), LR(T), and LR(L) models for growth rate. This might indicate that the null model, which predicts the response variable under all conditions by the grand mean, gives the best fit. For this reason, an optimal linear regression equation for the growth rate of CY was not identified.

Table 2.5 Linear Regression and ANOVA parameters for *Chlamydomonas yellowstonensis* growth rate dependence on light intensity and temperature.

Model	**PARAM**	**Intercept Est.**	**Intercept P-value**	**TEMP Est.**	**TEMP P-value**	**LIGHT Est.**	**LIGHT P-value**	**RMSE**	**RSQR**
LR(T,L)	**3**	20.79	<0.01	0.298	0.4158	-0.024	0.2162	0.00678	0.2171
LR(T)	**2**	17.18	<0.01	0.298	0.4303	———	———	0.00704	0.0633
LR(L)	**2**	24.51	<0.01	———	———	-0.024	0.207	0.00668	0.1539
Model	**PARAM**			**TEMP F-stat**	**TEMP P-value**	**LIGHT F-Stat**	**LIGHT P-value**	**RMSE**	**RSQR**
ANOVA(T,L)	**6**			1.627	0.2797	8.223	0.0191	0.0044	0.7804
ANOVA(T)	**4**			0.580	0.6445	———	———	0.00736	0.1786
ANOVA(L)	**3**			———	———	1.3604	0.0159	0.00484	0.6018

*Highlighted models represent best models for linear regression and ANOVA.

The ANOVA(T, L) model for CY provides a better fit than linear regression; with an R-squared of 0.7804 for ANOVA compared to 0.2171 for linear regression. Furthermore, under the ANOVA(T, L) model, the expected value of growth rate is significantly different for the different levels of light (50, 100, and 300 μmol/m^2/s), but growth rate likely does not have a linear correlation to light intensity. However, under the ANOVA(T, L) model, as under the linear regression(T, L) model, the expected growth rate is not significantly different for the different levels of Temperature. This lack of linear correlation is apparent in Figure 2.4, however it should be noted that CY growth rates appear to be highest at the lowest light intensity (50 μmol/m^2/s) for every temperature in the scope of this study. Although this light effect was not determined to be statistically significant at 15°C, it was for the other 3 temperatures studied here.

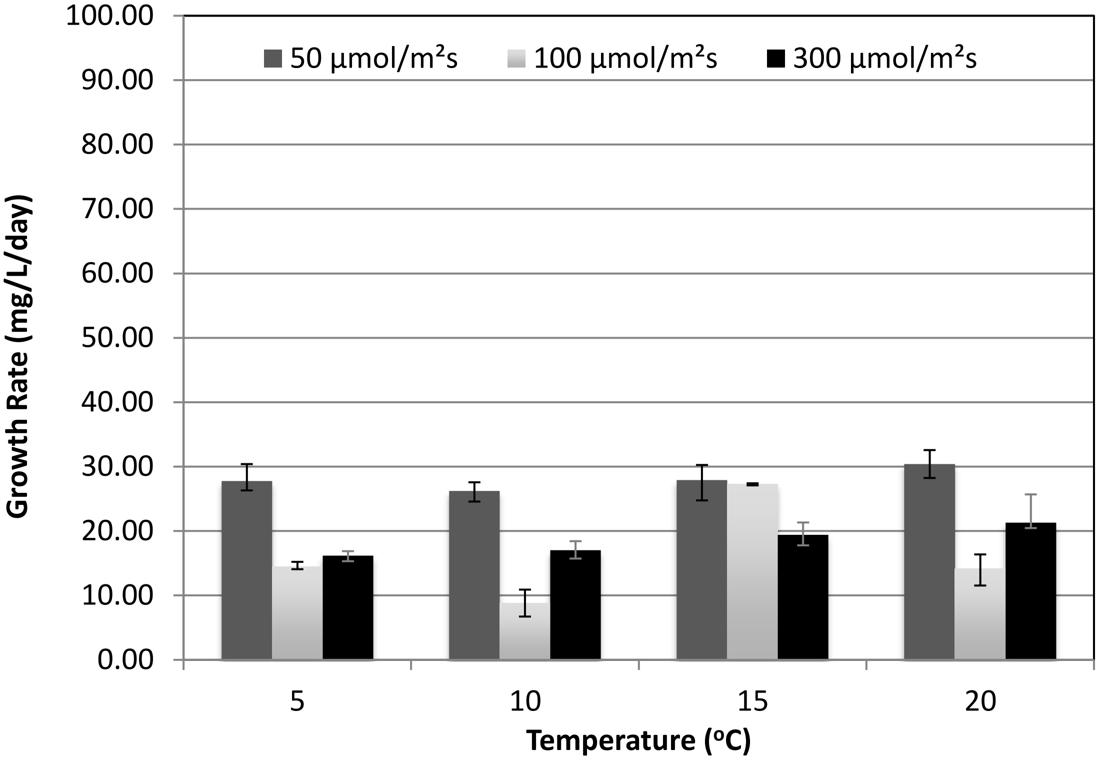

Figure 2.4 Growth rate vs. temperature and light intensity for *Chlamydomonas yellowstonensis*. Error bars represent observed growth rate range.

Chlamydomonas augustae

From the regression analysis conducted, both the temperature and light predictors appear significant for CA within the constraints of this study. Temperature is significant at an α-level of 0.05, while light is extremely significant, with a p-value far below 0.01. linear regression and ANOVA parameters are shown in Table 2.6 and the best linear regression is shown in Equation 3, where Y_i is the growth rate for CA at temperature T_i and Light Intensity L_i and ε_i is the error associated with the regression.

Table 2.6 Linear Regression and ANOVA parameters for *Chlamydomonas augustae* growth rate dependence on light intensity and temperature.

Model	**PARAM**	**Intercept Est.**	**Intercept P-value**	**TEMP Est.**	**TEMP P-value**	**LIGHT Est.**	**LIGHT P-value**	**RMSE**	**RSQR**
LR(T,L)	**3**	23.74	<0.01	0.834	0.0166	-0.072	<0.01	0.00552	0.7803
LR(T)	**2**	13.07	0.0918	0.834	0.134	———	———	0.00992	0.2101
LR(L)	**2**	34.18	<0.01	———	———	-0.072	<0.01	0.00732	0.5702
Model	**PARAM**			**TEMP F-stat**	**TEMP P-value**	**LIGHT F-stat**	**LIGHT P-value**	**RMSE**	**RSQR**
ANOVA(T,L)	**6**			9.467	0.0108	43.152	<0.01	0.00322	0.9503
ANOVA(T)	**4**			.0821	0.5182	———	———	0.0109	0.2353
ANOVA(L)	**3**			———	———	2.258	<0.01	0.00628	0.715

*Highlighted models represent best models for linear regression and ANOVA.

$$Y_i = 23.74 + 0.834\times T_i - 0.072\times L_i + \varepsilon_i \tag{3}$$

These results show a negative correlation between growth rate and light intensity. That is, as light intensity increases, the growth rate of CA appears to decrease within the parameters of this study. This phenomenon is apparent in Figure 2.5 which shows growth rates as a function of temperature and light intensity. Growth rates clearly shown to increase with increased temperature and decrease with increased light intensity. This behavior is statistically significant as the negative light coefficient has p-value <0.01. It is possible that this unique organism reaches saturation of photoreceptors at a low level of light (<50 μmol/m^2/s) and the levels of light observed here are inhibitory to the organism resulting in decreasing growth rates with increasing light level even at these low levels.

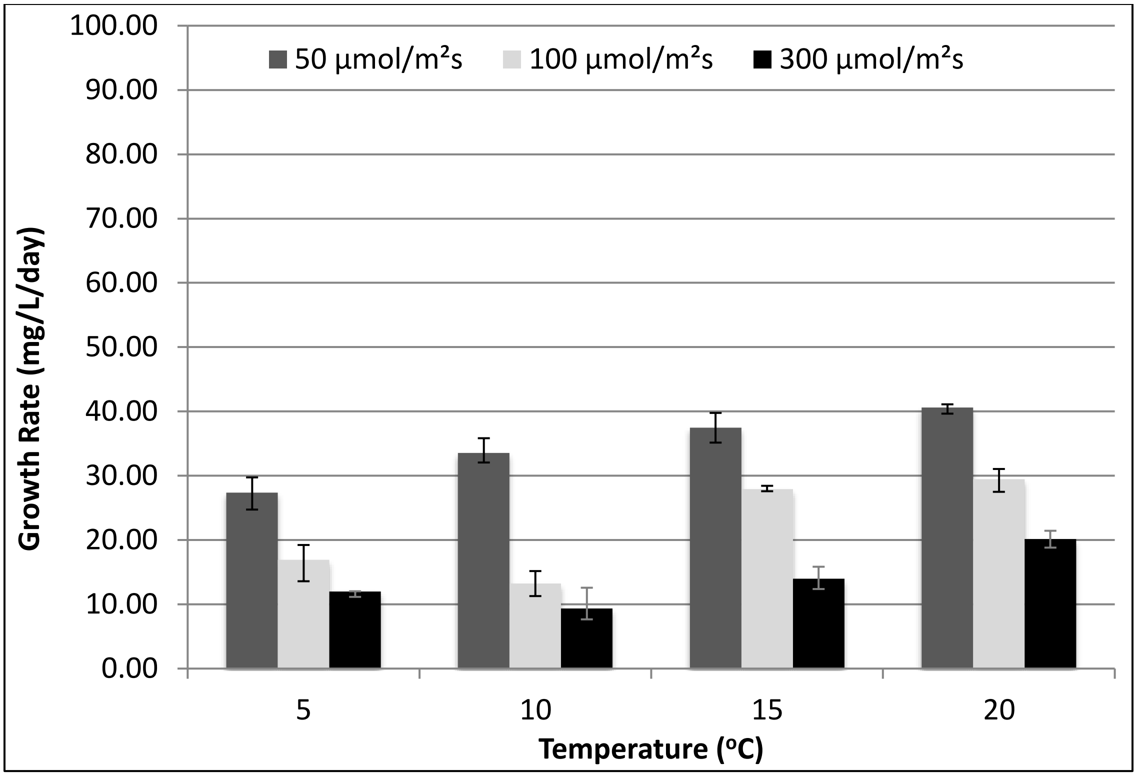

Figure 2.5 Growth rate vs. temperature and light intensity for *Chlamydomonas augustae.* Error bars represent observed growth rate range.

Light may appear to have a very small impact on the predicted growth rate, -0.072 mg/L/d for each µmol/m^2/s increase in light intensity, compared to a 0.834 mg/L/d change for each degree of temperature increase, but it should be noted that light is measured on the interval from 50 µmol/m^2/s to 300 µmol/m^2/s, while temperature is measured on a scale ranging from 0°C to 20°C. The ANOVA(T, L) model finds both temperature and light significant (in the same directions as the LR(T, L) model), but shows that the relationship is likely not linear.

Snow Algae vs. Model production Algae

Figures 2.6-2.8 compare the growth rates of the species studied here as temperature changes under constant light intensity.

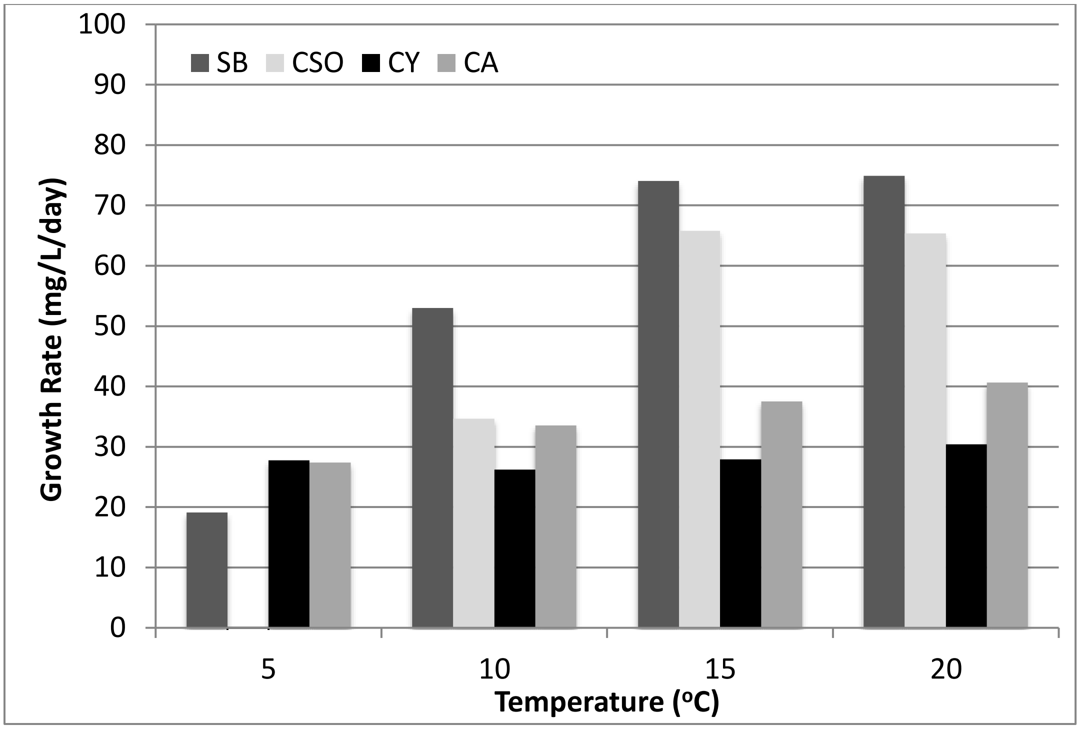

Figure 2.6 Growth rate vs. Temperature for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensis* (CY), and *Chlamydomonas augustae* (CA) grown under 50 µmol/m^2/s light. CSO was not viable at 5°C.

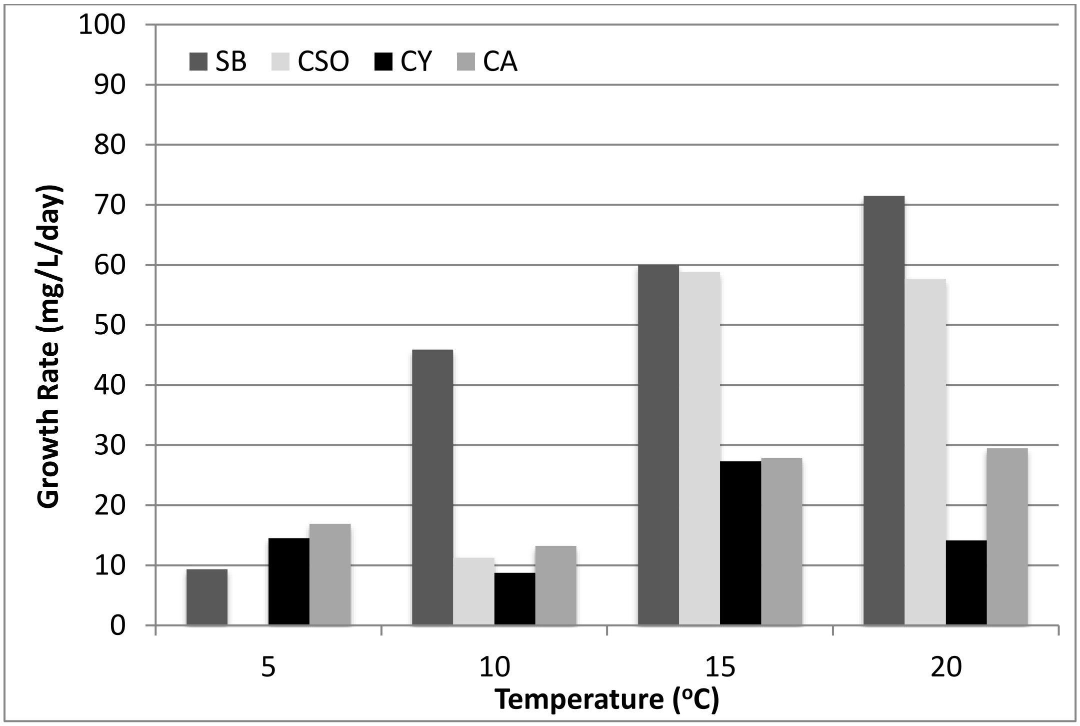

Figure 2.7 Growth rate vs. Temperature for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensis* (CY), and *Chlamydomonas augustae* (CA) grown under 100 µmol/m^2/s light. CSO was not viable at 5°C.

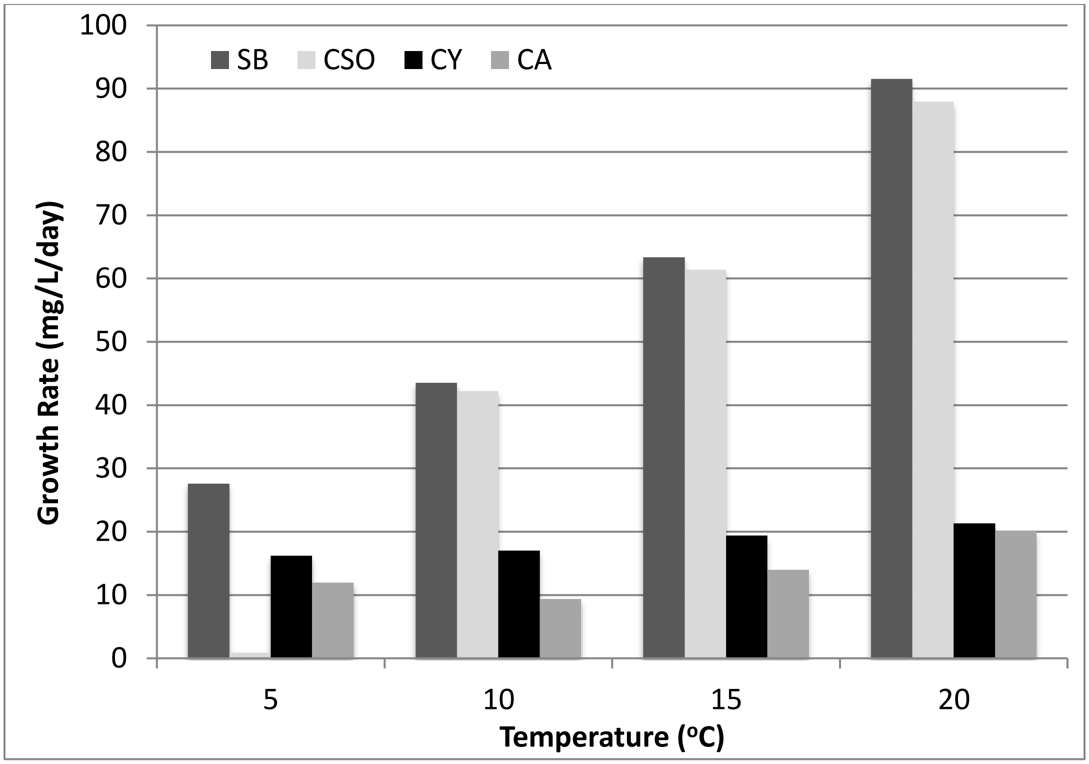

Figure 2.8 Growth rate vs. Temperature for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensis* (CY), and *Chlamydomonas augustae* (CA) grown under 300 μmol/m^2/s light. CSO was not viable at 5°C.

Snow algae (CY and CA) displayed higher productivity than the model production algae (SB and CSO) under the lowest light and temperature conditions studied here (5°C, 50 μmol/m^2/s). CSO essentially showed no growth at 5°C while SB biomass production was limited at 5°C until light levels reached 300 μmol/m^2/s. However, this behavior changed when temperatures were 10°C or higher. At these temperatures, SB and CSO begin to show higher productivities than the snow algae. SB and CSO maintained higher productivity than snow algae at all temperatures above 10°C for all light intensities studied here.

Productivities of snow algae in this study were observed to be the highest at the lowest light intensity for all temperatures studied here. It appears from the figures above and statistical analysis that light had a greater impact on productivity of CY and CA than temperature under the limitations of this study. At 300 μmol/m^2/s light intensity there was little statistical difference in productivity across the two snow algae species at the four temperatures studied here. This suggests there could have been an inhibitory effect caused by this relatively high light intensity for these species. The model production species (SB and CSO) did not show this type of behavior with the highest production for each species being observed at the highest light and temperature conditions (20°C, 300 μmol/m^2/s).

In order to assess the potential for crop rotations using a combination of model organisms and snow algae; the intersections of the predicted light and temperature dependent growth models were determined by solving the system of equations represented by the growth curves of SB, CSO and CA at the three discrete light intensities used in this study. CY was not considered as no statistically valid growth function was identified for this organism. The plots of the growth curves for SB, CSO and CA at 50, 100, and 300 μmol/m^2/s are shown in Figure 2.9.

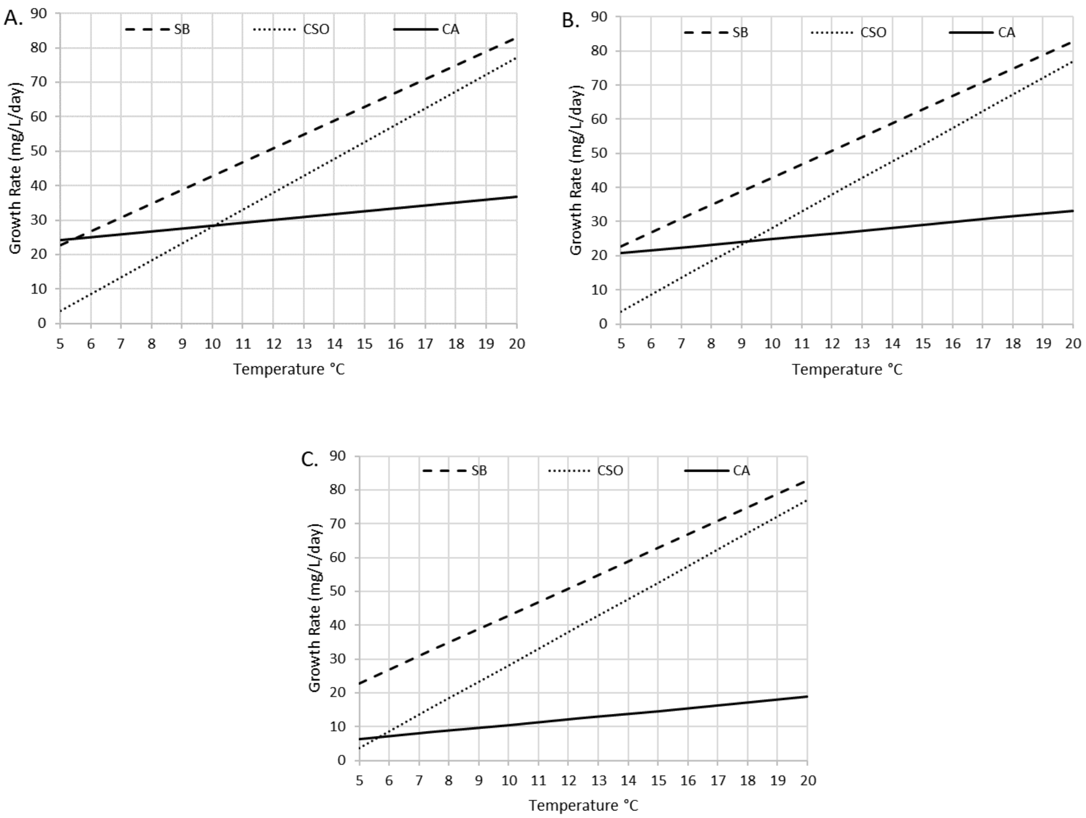

Figure 2.9 Predicted temperature dependent growth curves for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), and *Chlamydomonas augustae* (CA) for light intensities of 50 (A), 100 (B) and 300 (C) μmol/m^2/s.

50 μmol/m^2/s is the only light exposure level observed in this study where the snow alga CA was shown to produce more biomass than the model production organism SB. Using the growth models identified here at 50 μmol/m^2/s, CA is predicted to demonstrate higher productivity than SB at temperatures <5.48°C. SB demonstrated higher productivity than CA at all observed temperatures when light intensities were 100 and 300 μmol/m^2/s. The growth models suggested that CA productivity would exceed SB at temperatures <4.34°C under 100 μmol/m^2/s light exposure. Growth curves for CA and

SB are predicted to intersect at -0.21°C when exposed to 300 μmol/m^2/s light. Therefore, SB is expected to be more productive than CA in all viable systems at this level of light.

CA is more productive than CSO over a wider range of observed temperatures than CY at 50 μmol/m^2/s. Growth models suggested that at this light intensity CA produces more biomass than CSO at temperatures <10.08°C. CA also was observed to have higher productivity than CSO at the higher light exposures used in this study (100 and 300 μmol/m^2/s). The growth models predicted that CY would have higher productivity than CA at temperatures >9.19 and 5.64°C respectively for 100 and 300 μmol/m^2/s.

Table 2.7 Suggested algae crop rotation using *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), and *Chlamydomonas augustae* (CA). Temperature values are continuous and light exposure values are discrete at 50, 100, and 300 μmol/m^2/s.

		Light Intensity (μmol/m^2/s)		
		50	100	300
Temperature (°C)	<4.3	CA	CA	SB
	5.5-26.6	CA	SB	SB
	>26.6	SB	CSO	CSO

It should be noted that SB demonstrated higher productivity than CSO under all the temperature and light regimes observed in this study, but the growth curves predict that CSO would begin to outproduce SB at temperatures >26.55°C for all light intensities used in this study. Using the observed data and extrapolating these values outside of the conditions observed for this study, it was determined that an effective crop rotation could be designed as shown in Table 2.7 using continuous temperatures from 0 to >26.55°C and the discrete light exposure levels used in this study of 50, 100, and 300 μmol/m^2/s.

Summary and Conclusions

While the parameters of this study were restrictive in their temperature and light exposure levels, the data nonetheless revealed novel discoveries regarding the algal species examined here. At low temperature and light exposure, the model production species *Scenedesmus bijuga* and *Chlorella sorokiniana* both demonstrated limited growth rates with significant dependence upon temperature but with no statistical correlation to light exposure at the levels studied here. Average growth rates observed in these species under these conditions ranged from 9.35 to 91.52 mg/L/d for SB and from 0 to 87.97 mg/L/d for CSO. In this study, the lowest growth rates for these species correlated with the lowest light intensity (50 μmol/m^2/s) and temperature (5°C). Clearly, these species are experiencing environmental stress induced by the reduced temperature and light exposure examined in this study resulting in greatly reduced growth rates at low light and temperature levels. CSO was unable to grow at 5°C under any lighting scheme; whereas SB experienced greatly reduced growth rates at this temperature. As light had no statistically significant influence over the growth rates of these species under the conditions of this study it is assumed this effect was mainly due to the extremely low temperatures experienced here. This is expected, as most algal species do not demonstrate high productivity under low temperature conditions.

The results associated with CY and CA, the snow algae studied here provided insight into their growth behavior under low temperature and light conditions. CY growth rate had no statistical correlation with either light intensity or temperature under the parameters used in this study. It is possible that while CY can grow under low light and low temperature conditions, its metabolism is operating in a limited capacity maintaining a steady state growth rate that is not affected by changes in temperature or light in the values used in this study.

The results seen with CY are in great contrast to those observed with CA. CA growth rate showed significant correlation with temperature *and* light under the parameters used in this study. In addition, while there was a positive correlation between growth rate and temperature, a *negative* correlation was observed between growth rate and light intensity. This has implications for the value of CA as not only a potential candidate for cool-season growth in algal production systems but also as a genetic resource for algal engineering. The genes that control this apparent increase in low light productivity could be valuable when designing high-productivity, cool-season adapted algae. For example, a highly productive species could be transformed using genetic material from CA to provide sustained or even increased production under low-light conditions. It should also be noted that both snow algae species appeared to show inhibited growth when light levels reached 300 μmol/m^2/s where temperature appeared to have very little effect on growth suggesting a leveling of production at this light intensity.

As shown in Table 2.2, at extremely low light and temperature conditions (5°C, 50-100 μmol/m^2/s), snow algae, CY and CA, are shown to have higher biomass production rates than the production algae. In fact, CSO does not grow at 5°C. What is perhaps more significant is that SB does grow at this low temperature even under low light conditions. SB was also shown to have higher biomass productivity than snow algae at 5°C and 300 μmol/m^2/s. Thus, SB shows substantial productivity over a wide range of environmental conditions.

In general, as light intensity and temperature increase, laboratory algal species growth rates begin to overtake those of snow algae. At 10°C, SB outperforms both CY and CA at all light intensities, this behavior is consistent and significant for all observed temperatures above 10°C. CSO growth rates are statistically indistinguishable from those of the snow algae at 10°C for 50 and 100 μmol/m^2/s light exposure. However, CSO growth rate exceeds that of the snow algae at 300 μmol/m^2/s at 10°C. The growth rate exceeds that of both snow algae species for all observed temperatures above 10°C.

While many of the observed results were anticipated, some of the results were unexpected and provide new insight into the behavior of psychrophilic algae. The results associated with CA which showed a negative growth rate response to light intensity suggest that CA demonstrates higher biomass production under low light conditions. Based on these observations, CA could be well suited to growth in the cool-season of the Southeastern United States and other areas where lower average light intensities often accompany cooler temperatures and extended conditions below freezing are not observed. The observation regarding the growth rate of SB at lower temperatures was also notable as this species may be the best performing species of those studied here if the goal of an algal production system is to maximize biomass productivity under reduced light and temperature conditions assuming that light exposure is consistently near the 300 μmol/m^2/s value observed here.

CHAPTER 3

LIGHT AND TEMPERATURE DEPENDENT NUTRIENT UPTAKE AND LIPID ACCUMULATION IN SNOW ALGAE[1]

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[1] Geller, D.P., Das, K.C., Singh, M., Bagby-Moon, T., Hawkins, G., Kiepper, B. 2017. To be submitted to *Algal Research.*

Abstract

Psychrophilic algae commonly known as *snow algae* have been shown to exhibit novel growth patterns under variable light and temperature conditions. This study examines the nitrogen uptake, phosphorus uptake and lipid accumulation of two snow algae species, *Chlamydomonas yellowstonensis* (CY) and *Chlamydomonas augustae* (CA), under four temperatures (5, 10, 15, and 20°C) and three light intensities (50, 100 and 300 μmol/m^2/s). Two model production algae species, *Scenedesmus bijuga* (SB) and *Chlorella sorokiniana* (CSO) were grown under identical conditions and their lipid and nutrient uptake responses were also observed under identical light and temperature conditions.

Multiple regression analysis was used to identify statistically valid models for nutrient uptake and lipid accumulation in the algae studied here. Valid nutrient uptake models were identified for SB, CSO and CA. The nitrogen and phosphorus uptake models for SB and CSO showed a positive correlation with temperature and no correlation with light intensity. The snow alga CA showed a positive correlation with temperature for both nitrogen and phosphorus uptake. A negative correlation between light intensity and phosphorus uptake was also identified for CA. No correlation was identified between light and nitrogen uptake for this organism. The model production alga SB demonstrated consistently higher nutrient uptake rates than CSO and CA at all temperatures and light intensities studied here.

Only one statistically valid model for light and/or temperature dependent lipid accumulation was identified. The identified model for the snow alga species CY showed no correlation between temperature and lipid accumulation and a negative correlation

between light and lipid accumulation. The evidence that light has a negative correlation with lipid production and phosphorus accumulation in snow algae suggests that snow algae could be the source of useful genetic resources. These resources could assist in engineering algae that maintain productivity under low-light exposure growth conditions.

Introduction

Snow algae are algal species isolated from glacier and snow deposits that exhibit resilient growth at temperatures lower than 5°C. In Chapter 2, snow algae were shown to be more productive under reduced light intensity at these low temperatures. This was a significant discovery because it is thought that algae with similar characteristics could be used to extend the productive growing season for algal cultivation when producing algal biomass for fuels and products. The synergy of low light and low temperature to increase productivity in these species coincides with conditions observed in the cool-season in the Southern U.S. and other regions identified as ideal for algal production. Use of snow algae as a cool-season crop could lead to the extension of the growing season in outdoor algal production systems. This extension of annual production feasibility addresses one of the main obstacles identified in technoeconomic analysis (TEA) of algal cultivation as a widely-regarded TEA analysis suggests that the number of days of operation is one of the most influential parameters on the economic success of algal production operations and achieving these goals (Davis et al., 2011).

Another factor that could positively affect the economic feasibility of algal systems is the coupling of algal production with environmental services, specifically wastewater remediation through nutrient uptake. Several studies have identified the removal of nitrogen (N) and phosphorus (P) from wastewater as a significant auxiliary benefit to the deployment of algal cultivation (Selvaratnam et al., 2015; Sturm and Lamer, 2010; Chinnasamy et al., 2010). Economic gains or savings created from this service increases the overall profitability of algal production systems and can increase total systemic sustainability. In addition, such synergy also improves the environmental and social sustainability of algal cultivation. As such, this study examines the uptake of N and P by snow algae and model production algae under variable light intensity (50, 100, and 300 μmol/m^2/s) and temperature (5, 10, 15, and 20°C).

Macronutrient composition could also affect the use of algal biomass to produce fuel and other products. In particular, lipid content is of special interest due to its utility in a number of conversion technologies (Davis et al., 2012; Foley et al., 2011). In fact, high lipid productivity is cited as a key characteristic for technoeconomic feasibility in systems that focus on this class of macromolecules (Griffiths and Harrison, 2009). To quantify the performance of snow algae as a possible rotational cool-season crop for algae cultivation this study explores the effects of light intensity and growth temperature on the concentration of lipids in algal biomass in both snow algae and model production algal species.

This study examines the combined effects of light intensity and temperature on the nutrient uptake and lipid production in *Chlamydomonas yellowstonensis* (CY) and *Chlamydomonas augustae* (CA), two species of snow algae and *Scenedesmus bijuga* (SB) and *Chlorella sorokiniana* (CSO), two model production algae species. This study focuses on light intensities that correlate with natural conditions associated with cool-season growth (50, 100, and 300 μmol/m^2/s). It also examines the effect of relatively low growth temperatures (5, 10, 15, and 20°C) which are also common during the cool-season in the Southern U.S.

Very few studies exist which examine the synergistic effect of these two growth parameters on lipids and nutrient uptake. A key study from Constantopoulos and Bloch (1967) examined the effects of light intensity on lipids production in *Euglena gracilis*. It was discovered that final lipid concentration decreased in this species with increasing light intensity from 120-610 footcandles (17.5-89.1 μmol/m^2/s). These cultures were grown at room temperature (~20-25°C) which is near the highest temperature used in this study (20°C). However, these light intensities are much lower than those used in this study (50-300 μmol/m^2/s), and are not very representative of outdoor ambient conditions, which can far exceed the highest intensity of this study (300 μmol/m^2/s).

Temperature dependent phosphorus uptake in algae has been explored in several studies using various algal species. A study by Martínez et al. (1999) showed that phosphorus uptake increased with increasing temperature for *Scenedesmus obliquus* in the temperature range 20-35°C which is outside the range evaluated in this study. Another study by Martínez Sancho et al. (1999) held temperature constant at 30°C and examined the impact of variable light intensity (11.7-89.9 μmol/m^2/s) on phosphorus uptake. A positive correlation between light intensity and phosphorus uptake was observed under these conditions.

Investigation into the impact of light and temperature on the uptake of nitrogen by algae including diatoms has been conducted. Key studies have examined the effects of light (MacIsaac and Dugdale, 1972) and temperature (Reay et al., 1999) on the uptake of inorganic nitrogen and have found that increasing light intensity and temperature generally results in increased nitrogen uptake. However, these studies approached the two factors independently and were limited by natural conditions for one of the variables in each of the studies. Furthermore, they were focused on inorganic nitrogen uptake and not total nitrogen as addressed in this study. The most relevant study to this work was conducted by Priscu et al. (1989) where temperature dependence of nitrogen uptake was studied in Antarctic sea algae consortia at temperatures ranging from 0.5-12.0°C. This study determined that for these consortia, nitrogen uptake was at a maximum at 0.5-3.0 °C. These extremely low temperatures coincide with the lower threshold for photosynthesis and it was determined that the metabolism of these organisms had been adapted to the cold conditions from which they were isolated. The low temperatures at which this study was conducted are below those examined here and could provide great insight into the results of this study. These observations were corroborated in a study by Lomas and Gilbert (1999) which examined specifically nitrate (NO_3^-) uptake by cool-water diatoms. They found that at temperatures between 7–25°C nitrate uptake decreased as temperature increased at constant light intensity.

If nutrient uptake and/or lipid production are enhanced under specific temperature/light configurations it will provide insight into optimizing algal production systems through species selection. The data collected here will provide critical engineering data for the design of extended production algal cultivation that seeks to take advantage of crop rotation using snow algae or similar species in the cool-season.

Materials and Methods

Species Selection

The species selected for this study are shown in Table 3.1 along with data on their temperature and light dependent growth where available.

Table 3.1 Selected algal species for temperature- and light-dependent nutrient uptake and lipid production studies.

Species	Optimal Temp.	Known Growth Temperature Range	Source	Reference(s)
Scenedesmus bijuga	TBD	5[1] - 40°C[2]	UGA	[1]Our Preliminary Data [2]Bajaj and Srivastava 1985
Chlorella sorokiniana	38°C[3]	10[4]-38°C[3]	UGA	[3]Franco, et al., 2012 [4]Our Preliminary Data
Chlamydomonas yellowstonensis	16°C[5]	0 - 25°C[5]	UTEX SNO134	[5]Hoham 1975
Chlamydomonas augustae	11 - 18°C[6]	4 - 32°C[6]	UTEX B SNO155	[6]Teoh 2013

The species were selected based on two sets of criteria. *S. bijuga* and *C. sorokiniana* were native species isolated from wastewater from Dalton, GA and have been demonstrated as well-adapted to growth in the Southeastern United States. These algae have served as model organisms for the production based algal research at the University of Georgia's Bioconversion Research and Education Center. *C. yellowstonensis* and *C. augustae* were selected based on literature review of "snow algae" which are algae often found in glaciers or snow deposits. Many of these species have specifically adapted to grow at lower temperatures near the freezing point of water. These particular species were found to be available from the UTEX algal repository and met initial screening criteria demonstrating growth across a wide range of temperatures in BG-11 growth media.

Several other snow algae were screened for this study including: *Chodatia tetrallantiodea, Chlorommonas brespevina, Chlorosarcinopsis sempervirens and*

Raphidonema nivale. *C. sempervirens* and *R. nivale* demonstrated irregular, low productivity growth under the screening protocol used here. *C. tetrallantiodea* and *C. brespevina* showed no growth at all in BG-11 media at the temperatures observed in this study (5, 10, 15, and 20°C).

Temperature and light controlled algae growth

Scenedesmus bijuga (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensi* (CY) and *Chlamydomonas augustae* (CA) were grown in 100 mL cultures of modified BG-11 media in 250-mL Erlenmeyer flasks in a shaker operating at 100 rpm. The shaker was placed in a temperature and humidity controlled growth chamber with a constant humidity of 70-75%. Cultures were grown at 5, 10, 15, and 20°C with light exposure of 50, 100 and 300 μmol/m^2/s providing for a total of 12 treatments (conditions) per species with 3 replications for each treatment, totaling 144 experimental units. Temperature was maintained within ±1°C for all treatments except 300 μmol/m^2/s treatments, which had temperature drift of ±4°C due to heat generated from the lighting system. Light was cycled to provide 12 hours light and 12 hours dark conditions. Cultures were grown in triplicate for a minimum of 14 days. Optical density (OD) @750nm was measured every 2-3 days. OD readings were converted to algal cell concentrations (mg/L) using standard curves plotted from the OD of known biomass concentrations (mg/L) for each algal species.

Nutrient analysis

Algal cultures were filtered through a pre-weighed 4.7-cm Whatman glass fiber filter and the filtrate was analyzed using two methods.

1. Total phosphorus (phosphate) was determined by the Molybdovanadate Method (Hach Method 10127), which has a range of 1.0 - 100.0 mg/L phosphate. A 10-mg/L standard was used to verify calibration during each set of tests.

2. Total nitrogen was determined by the Persulfate Digestion Method (Hach Method 10072), which has a range of 10 - 150 mg/L nitrogen. A 100-mg/L nitrogen standard was used to verify calibration during each set of tests. As some of the expected concentrations of nitrogen in this study exceeded this range; samples including standard were diluted 1:1 in deionized water before nitrogen analysis.

Final nitrogen and phosphorus concentrations ($[N_f]$ and $[P_f]$) in growth media were subtracted from the original concentrations in fresh media. Original concentrations were $[N_0]$ = 200 mg/L and $[P_0]$ = 10 mg/L. These values were verified by the above assays for each set of samples. The difference between final and initial nutrient concentrations was divided by the number of days each sample was grown (t) to yield a nutrient uptake rate (d[N]/dt or d[P]/dt) in mg/(L·day) as shown in Equation 1 for nitrogen and Equation 2 for phosphorus. This value was used for comparison of nutrient uptake efficiency between the treatments.

$$\frac{d[N]}{dt} = \frac{[N_0]-[N_t]}{t} \tag{1}$$

$$\frac{d[P]}{dt} = \frac{[P_0]-[P_t]}{t} \tag{2}$$

Lipid analysis

Lipid content was measured gravimetrically with Ankom XT10 (ANKOM Technology, Macedon NY) automated extraction system using hexane as solvent. 4.7-cm Whatman glass fiber filters were dried at 60°C overnight in a forced-air oven and cooled in a desiccator. The dried filters were weighed on a sensitive balance to 0.00001-g resolution (W_1). Algal cultures were filtered through the glass fiber filters and washed with ammonium formate and deionized water to remove any salt residues. The filters were dried at 60°C overnight in a forced-air oven and cooled in a desiccator. The cooled and dried filters and algae were weighed without an extraction bag (W_2). The filters were then inserted into the Ankom XT4 extraction bags and the bags were sealed with an impulse sealer. After drying using the method described above, the extraction bags were kept in a desiccant bag. Each individual bag was removed and carefully weighed providing a pre-extraction weight for the algal biomass, filter, and extraction bag (W_3). The extraction bags were then placed into the extractor and the extraction was performed for 1 h at 90°C with hexane as solvent. After extraction, the bags were then transferred to a forced-air oven and dried at 60°C overnight and cooled in a desiccator. The bags were then weighed providing a post-extraction weight for the algal biomass, filter, and extraction bag (W_4). Equation 2 was used to calculate the lipid content of the algae samples:

$$\text{Lipid }\% = \frac{(W_3 - W_4)}{(W_2 - W_1) x 100} \tag{2}$$

Where W_1 is the weight of the filter, W_2 is the weight of the algae and the filter, W_3 is the weight of the algae, filter, and bag pre-extraction, and W_4 is the weight of the algae, filter, and bag post-extraction.

Statistical Analysis

Multiple regression was performed in Microsoft Office Excel 2016 to determine model equations for temperature and light dependent nitrogen uptake rates, phosphorus uptake rates and lipid concentration for the four organisms of interest within the range of light exposure and growth temperatures observed here. If the overall P-value was determined to be $< \alpha$ (0.05) for the regression, the null hypothesis was rejected and the model was accepted as valid. Likewise, temperature and light coefficients were selected based on P-value from the regression using an $\alpha=0.05$. When only one of the temperature or light coefficients resulted in a P-value < 0.05, the regression was performed with the remaining variable to refine the model. For one model (CY/lipid concentration) the P-value for the light coefficient was determined to be ~0.06 and the P-value for the temperature coefficient was determined as 0.46. In this case the regression was run again using only light as the treatment variable and the P-value was found to be < 0.05 for the revised model and the light coefficient. This revised model was retained.

Results and Discussion

Multiple Regression

The results of multiple regression for temperature and light dependent nitrogen uptake rate, phosphorus uptake rate and final lipid concentration are shown in table 3.2 including the coefficient of determination (R^2), and P-values for the overall model, intercept, light, and temperature coefficients.

Table 3.2 Summary statistics of multiple regression for light and temperature dependent nitrogen uptake rate (d[N]/dt), phosphorus uptake rate (d[P]/dt), and final lipid concentration for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensis* (CY), and *Chlamydomonas augustae* (CA).

		P-value				
Model	Strain	Intercept	Temperature	Light	Model	R^2
d[N]/dt	SB	0.04	0.02	0.44	0.05	0.48
d[N]/dt	CSO	0.04	0.00	0.15	0.00	0.79
d[N]/dt	CY	0.84	0.10	0.12	0.09	0.41
d[N]/dt	CA	0.28	0.03	0.46	0.06	0.46
d[P]/dt	SB	0.00	0.05	0.48	0.12	0.38
d[P]/dt	CSO	0.26	0.00	0.46	0.00	0.72
d[P]/dt	CY	0.00	0.52	0.29	0.46	0.16
d[P]/dt	CA	0.00	0.01	0.04	0.01	0.63
% lipid	SB	0.06	0.49	0.10	0.19	0.31
% lipid	CSO	0.36	0.22	0.16	0.19	0.31
% lipid	CY	0.06	0.46	0.06	0.12	0.37
% lipid	CA	0.11	0.33	0.11	0.17	0.32

Table 3.3 shows the final accepted models for temperature and light dependent nitrogen uptake rate, phosphorus uptake rate and final lipid concentration based on statistical evaluation. This table does not include the models that were eliminated due to unacceptable p-value scores.

Table 3.3 Accepted models from multiple regression for light and temperature dependent nitrogen uptake rate (d[N]/dt), phosphorus uptake rate (d[P]/dt), and final lipid concentration for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensis* (CY), and *Chlamydomonas augustae* (CA).

Species	Model*	Units	P-value	R^2
SB	$d[N]/dt = 2.26 + 0.20T + \varepsilon$	mg/L/day	0.019	0.440
CSO	$d[N]/dt = -1.50 + 0.34T + \varepsilon$	mg/L/day	0.000	0.735
CA	$d[N]/dt = 1.51 + 0.17T + \varepsilon$	mg/L/day	0.023	0.421
SB	$d[P]/dt = 0.46 + 0.01T + \varepsilon$	mg/L/day	0.047	0.340
CSO	$d[P]/dt = -0.08 + 0.03T + \varepsilon$	mg/L/day	0.001	0.705
CA	$d[P]/dt = 0.43 + 0.01T - 0.0004L + \varepsilon$	mg/L/day	0.011	0.634
CY	$\%\ lipid = 13.57 - 0.04L + \varepsilon$	%	0.049	0.332

*T = temperature in °C, L = light intensity in $\mu mol/m^2/s$, ε = model error.

Nitrogen Uptake

Multiple regression provided statistically valid models for nitrogen uptake rates for SB, CSO and CA. No valid model was identified for CY. All three of the valid models showed positive correlation between nitrogen uptake rate and growth temperature. No significant correlation was determined for light and nitrogen uptake rate for any of the algal species examined here. R-squared values ranged from 0.421 to 0.725 suggesting the model fit CSO data better ($R^2 = 0.735$) than SB or CA ($R^2 = 0.440$ and 0.421 respectively). Figure 3.1 shows the plot of the three nitrogen uptake models over the range of temperatures studied here.

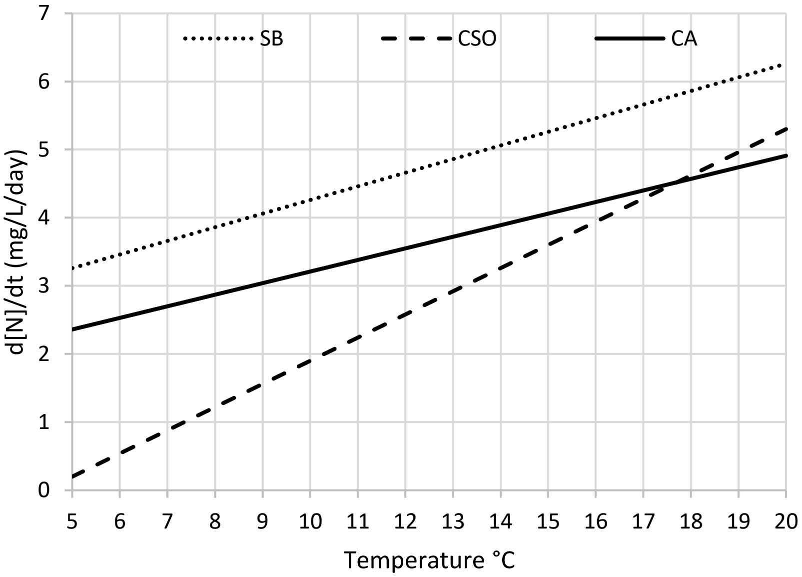

Figure 3.1 Temperature dependent nitrogen uptake rate models for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), and *Chlamydomonas augustae* (CA) over the range of growth temperatures used in this study (5 - 20°C).

The models displayed in Figure 3.1 show that the model laboratory organism SB showed higher rates of nitrogen assimilation than CA and CSO at all growth temperatures used in this study. The snow alga species CA exhibited higher nitrogen uptake rates than the model alga CSO for much of the temperature range studied here. The intersection of the curves for CSO and CA intersect at 17.7°C suggesting that CSO will start to show better nitrogen remediation performance than CA around this temperature.

It should be noted that CSO does not grow at 5°C and it can be assumed that d[N]/dt is very low if not zero at this temperature. It should also be noted that the slope of the CSO curve is larger than that of CY. The projected intersection of the CY and CSO curves is 26.9°C which is out of the scope of this study but is a realistic temperature for algal production. It is expected that CSO would exhibit better nitrogen uptake than CY at temperatures above this point.

The results observed for nitrogen uptake rates were consistent with the prior growth study (Chapter 2) for the model production organisms SB and CSO. Growth of both of these algal species showed a positive correlation with temperature and no correlation with light intensity. The nitrogen uptake rate model for the snow alga CA differed from the growth model for this organism as the nitrogen model did not correlate to light intensity. It only showed a positive correlation to temperature. This correlation with temperature was consistent with the growth model.

Phosphorus Uptake

As observed with nitrogen uptake rates, multiple regression identified statistically valid phosphorus uptake models for SB, CSO and CA. No valid model was identified for CY. The statistically valid models for the production algae SB and CSO reflected a positive correlation between phosphorus uptake rates and growth temperature. No correlation between phosphorus assimilation and light intensity was identified for these organisms. The phosphorus uptake model for snow alga CA showed a positive correlation with growth temperature and a negative correlation with light intensity. Temperature dependent phosphorus uptake models are shown in Figure 3.2.

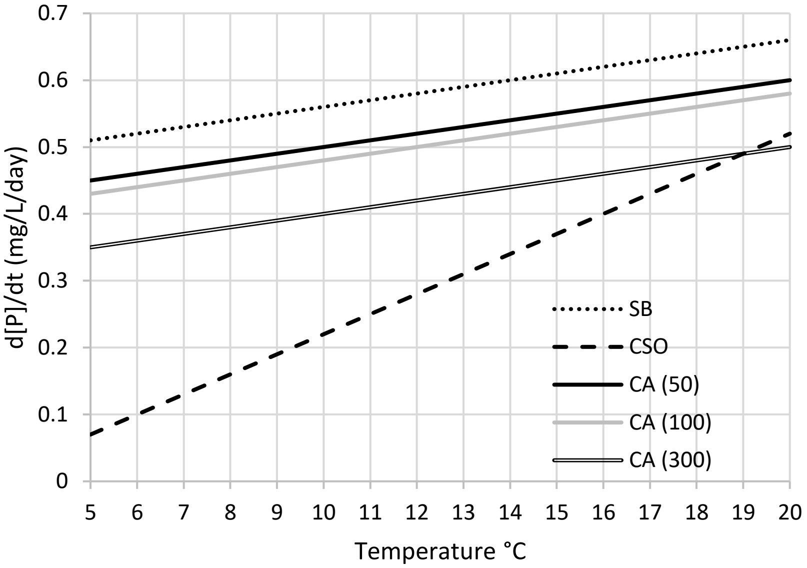

Figure 3.2 Temperature dependent phosphorus uptake rate models for *Scenedesmus bijuga* (SB), *Chlorella sorokiniana* (CSO), and *Chlamydomonas augustae* (CA) over the range of growth temperatures used in this study (5 - 20°C). Three models are shown for CA at the discrete light intensities observed in this study (50, 100, and 300 μmol/m^2/s).

As the model for CA suggested a correlation between light intensity and phosphorus uptake rate, three curves are shown for CA in Figure 3.2. These three curves reflect the phosphorus uptake performance of this algal species at the specific light intensities observed here (50, 100, and 300 μmol/m^2/s). Only one model is shown for SB and CSO because the models identified by multiple regression did not indicate any dependence on light for these species.

The models in Figure 3.2 show that the model organism SB had higher phosphorus uptake rates at all temperatures and light intensities observed in this study. The snow alga species CA generally performed better than the model organism CSO in terms of phosphorus uptake at the temperatures and light intensities studied here. The only exception is at the highest observed light intensity (300 μmol/m^2/s) where CA showed higher phosphorus uptake rates at temperatures above 19.5°C, the intersection of the CA model at 300 μmol/m^2/s and the CSO light intensity independent curve.

As observed with nitrogen uptake rates, the CSO model curve has a larger slope than the SB model suggesting intersection outside the range of temperatures observed in this study. The projected intersection of the SB and CSO curves is at 27.0°C. This is close to the 26.9°C projected for the intersection of the nitrogen uptake curves for SB and CSO shown in Figure 3.1 suggesting this may be the ideal temperature to succeed a SB seeded algal production pond with CSO if nutrient remediation is the goal of the system. These intersection temperature values were also consistent with the 26.6°C intersection observed in our previous work (Chapter 2) in growth models for SB and CSO.

The models determined for phosphorus uptake rate in SB and CSO were consistent with both growth models and nitrogen uptake models for these organisms. All three showed a positive correlation with temperature. SB also showed higher nutrient uptake and growth rate than CSO over the light intensities and temperatures used in this study. The phosphorus uptake model for CA was also consistent with the growth model for this organism reflecting a positive correlation between temperature and growth rate and the negative correlation between light intensity and growth rate.

Total Lipids

The multiple regression analysis for temperature and light dependent final lipid concentration did not produce statistically valid models for SB, CSO or CA. A valid model was only found for the snow alga CY. The lipid accumulation model for CY showed no correlation with growth temperature but it did show a negative correlation between lipid accumulation and light intensity. The light dependent lipid accumulation model for CY is shown in Figure 3.3.

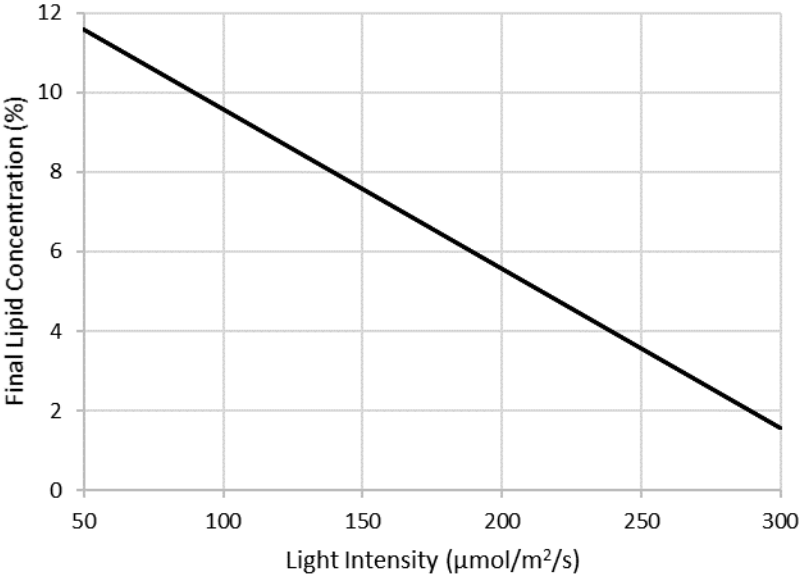

Figure 3.3 Light intensity dependent final lipid concentration model for *Chlamydomonas yellowstonensis* (CY) over the range of light intensities observed in this study (50- 300 μmol/m^2/s).

The negative correlation between lipid accumulation and light intensity observed for CY over the range of temperatures studied here was an unexpected result. As the lipid accumulation of other algal species studied here (SB, CSO and CA) did not produce statistically valid models with respect to light intensity and temperature, it was not possible to see if this effect was consistent with the other snow alga species (CA).

Likewise, our temperature and light dependent studies of nutrient uptake and growth rate (Chapter 2) did not produce valid models for CY.

Summary and Conclusion

The models for nitrogen and phosphorus uptake rates for the model organisms SB and CSO were consistent with previous research showing a positive correlation between growth rate and temperature and no correlation with light intensity for these algae grown under the conditions studied here. This suggests that temperature plays a major role in regulating metabolic processes for SB and CSO under the conditions observed here. An increase in temperature is followed by an upregulation of metabolism leading to increased growth and associated nutrient uptake. Growth of CSO is inhibited at 5°C and our results showed that nutrient uptake is also negligible at this temperature. This organism becomes viable between 5-10°C and it can also be assumed that observable nutrient uptake starts in this range which is consistent with the models determined here. Projection of the models for nitrogen uptake, phosphorus uptake to the intersection of SB and CSO was consistent suggesting CSO nutrient remediation capability begins to exceed that of SB at about 27°C under the light exposure levels observed here. This is consistent with the previously reported value of 26.6°C reported for the intersection of the growth rates of these two organisms.

The light and temperature dependent phosphorus uptake model for the snow alga CA was consistent with the previously reported growth model for this organism showing a positive correlation between d[P]/d[t] and temperature and a negative correlation between d[P]/d[t] and light. The nitrogen uptake model for this organism deviated slightly by showing no correlation to light intensity. However, there was a consistent positive correlation to temperature identified for d[N]/d[t] for CA. The overall performance of this organism suggests that it could serve as a useful source of genetic material for engineering algae for production in low-light growth regimes.

The snow alga CY was the only organism studied here for which a statistically valid model for light and/or temperature dependent lipid accumulation was identified. This organism showed a negative correlation between light intensity and lipid accumulation. No statistical correlation was identified for temperature and lipid accumulation in CY. The negative correlation to light could be a response to the excess energy provided by the high light intensity. The organism may be slowing lipid production as a response to the available excess environmental energy. It is possible that under these conditions, the cells have less need for lipid reserves as there is abundant light available for photosynthesis and other energetic processes. It is also possible that there are inhibitory effects associated with photosaturation under these conditions that reduce the accumulation of lipids in the algal cells.

This study is consistent with our previous observations that there are differences in metabolic behaviors of psychrophilic snow alga CA and the mesophilic high productivity algal species SB and CSO used as model organisms. This primarily relates to increased metabolic activity in snow algae at low light intensity. The observations of CY lipid accumulation also suggest that high light intensity may shift metabolism away from lipid accumulation and toward protein production. This would make sense as at low light and temperature these organisms may wish to accumulate energy stores in the form of lipids as opposed to producing more cells when exposed to these abiotic stressors.

The overall results of this study are inconclusive as to the efficacy of snow algae for remediation of nutrients from wastewater as compared to model production species. The model species in this study (SB and CSO) have been shown to exhibit desirable remediation properties under favorable growth conditions (Chinnasamy et al., 2010). Based on statistical analysis most of the species exhibited similar nutrient uptake rates at most temperature/light combinations studied here. The one exception was SB which exhibited enhanced nitrogen and phosphorus uptake compared to the CSO and CA even at low temperature and light intensity. This observation does not suggest that the snow algae have any advantage in terms of nutrient remediation under low light and temperature conditions. The snow alga CA which displayed a negative correlation between light intensity and growth rate in our previous study also showed similar behavior in terms of phosphorus uptake rates which may have relevance if the mechanisms regulating this behavior were to be further explored.

The elevated final lipid concentration observed in the snow alga CY at low light exposure does suggest that there may be additional advantages to a crop rotation integrating this species into cool-season production especially when lipids are the macromolecule of interest in the production system.

This study identified several unexpected behaviors associated with snow algae and production algae grown under low light and low temperature conditions. More work is needed to determine the extent and details of these adaptive behaviors. Additionally, further research to identify genetic mechanisms for these unique properties of snow algae could provide useful genetic resources to develop specifically adapted algal species for cool-season production which could enhance the overall environmental and economic sustainability of algal biomass production systems.

CHAPTER 4

SUSTAINABLE WATER AND NUTRIENT MANAGEMENT IN ALGAL BIOMASS PRODUCTION SYSTEMS[1]

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[1] Geller, D.P., Das, K.C., Hawkins, G., Kiepper, B. 2017. To be submitted to *Algal Research.*

Abstract

Low market prices for petroleum, natural gas and their derivatives have created major economic challenges to the development of sustainable algal production systems for bioenergy and bioproducts. In addition; questions as to the environmental and social sustainability of algae have arisen during the study of life-cycle analysis (LCA) and public perception associated with these systems (Slade and Bauen, 2013). However, the clear potential for algae to rapidly generate large amounts of biomass suggests that algae could be a major contributor to a bio-based economy in both advanced and developing parts of the world.

This review examines the current state-of-the-art in algal production and looks at water and nutrient use, its efficiency and recycling as they influence the sustainability of this promising but immature technology. Also explored are specific technical bottlenecks and socioeconomic road-blocks to algal systems that have prevented the technology from reaching maturity, sustainability and success in the current political environment. Through this analysis the review identifies advanced algae-water separation technologies and the use of wastewater in algal systems as two high-impact opportunities to enhance the sustainability of algal systems.

Finally, this review looks at the future of industrial algal production, exploring its use as a source of food and feed as well as a source of fuel and bioproducts. This includes the role algal systems might play in an “all-of-the-above” approach to sustainable energy, water and food future.

Introduction

Many studies suggest that industrial algal biomass production systems have the potential to produce more biomass per square meter than almost all terrestrial plant systems (Darzins et al., 2010; Weyer et al., 2010; Ullah et al., 2014). Table 4.1 shows the impressive production potential of algal systems versus common and emerging crop and forest systems. Yet, despite this promise of algae, science and engineering have yet to sustainably unlock this productivity at an industrial scale. Due to this promising productivity, algal systems have been revisited many times throughout modern scientific research as a potential replacement for petroleum based fuels and products. Many major projects have been funded to explore the potential of algae as a catalyst for the development of a massive bioeconomy (Ryther, 1959; Sheehan et al., 1998; NAABB, 2014). However, it seems that just as this work begins to achieve new breakthroughs, the price of petroleum drops and interest in algal systems dissipates along with the financial support to keep this vital research progressing (Richardson et al., 2012).

Table 4.1. Potential biomass yield of common terrestrial crops.

Crop	Productivity (g/m^2/day)
Algae	100 - 196[1]
Corn (including stover)	13.53 - 20.97 [2]
Sugarcane	49.39 - 58.86[2]
Grasses	2.44 - 13.53[2,3]
Pine	6.13 - 7.85[2,4]
Rapeseed	2.7[3]
Sweet Sorghum	29.63[2]
Eucalyptus	27.06[2]

[1]Darzins et al., 2010; Weyer et al., 2010, [2]Huber et al., 2006, [3]Dismukes et al., 2008, [4]Munsell et al., 2010

The high yields often attributed to algae are usually theoretical or only achieved in laboratories under extremely controlled conditions. It has been shown that displacing the current 94 million barrel/day consumption of petroleum (EIA, 2016) would require large areas of dedicated algal production. Based on a report by Ullah et al. (2014) which assumes algal productivity of 10 g/m^2/day, it would take over 20% of arable land on the planet to meet the global demand for oil. Industrial productivity has still not reliably achieved this level of production at scale. Such production will likely be conducted outdoors and the algal systems will be exposed to atmospheric conditions which introduces high variability to yield using current state-of-the-art production methods.

Adding to the complexity of the situation is algal system demand for water and nutrients, specifically phosphorus (P) and nitrogen (N). As global population continues to increase so will agricultural demand for water, P and N. Water is, of course, vital to all living organisms and is becoming increasingly scarce in many parts of the world. As demand for water increases and stewardship of this resource decreases, potable water will become more valuable and its availability for non-human uses will dwindle. Likewise, N and P are critical to algal growth. Management of these elements in agricultural systems has not been effective in terms of conservation and environmental impact. Fertilizers containing N and P are often over-applied leading to run-off and leaching into waterways and subsequent dilution of N and P to concentrations unusable for fertilization of agricultural crops. This presents a great opportunity for conservation in algal farming. These systems can serve as an example through conservative use of these nutrients. Algae also can serve as effective concentrators of these nutrients which presents unique opportunities for recovery of nutrients.

It is important to understand that industrial algal biomass production systems will need to be economically competitive with existing systems providing raw materials to the food, fuel, fiber and chemical industries. Additionally, algal systems will need to deliver this biomass with little impact to the environment and communities in which algae is produced. These three dimensions, economic competitiveness, environmental impact and social acceptability comprise the envelope in which algal system sustainability is considered. This review examines algal system sustainability with a focus on water and nutrients. It also provides a brief overview on the state of the art for the industry to provide a context for the issues discussed here and presents conclusions based on the trends observed during the investigation into current scientific literature on the subject.

State-of-the-Art

Current state-of-the-art for algal production systems includes current and emerging technologies that maximize algal biomass production and harvest. The conversion technologies and pathways used to produce fuel or other valuable bioproducts from algae are not considered here.

Open Raceway Systems

The most commonly proposed method for producing algae at an industrial scale is the use of outdoor raceway ponds. As described by Christi (2016), these ponds are “oblong and shallow recirculating pond(s) with semicircular ends”. The general design of an algal raceway pond uses a slow-moving paddlewheel to move the water and algae around the pond resulting in mixing for gas exchange and insuring exposure of the growing algae cells to ambient light. These ponds are often designed to be less than 0.25

m deep to ensure exposure of the cells to the necessary level of light to maximize growth of the photosynthetic algae.

The traditional raceway design has been shown to be highly efficient assuming that there is adequate natural sunlight and moderate temperatures where these systems are deployed. The major inputs to this type of system are energy to run the paddlewheel, nutrients to insure high algal productivity, and additional water to makeup loss to evaporation. Because of these needs, researchers often couple raceway systems with wastewater treatment. This synergy provides several benefits including: adequate source of makeup water, nutrients from the wastewater and remediation of this wastewater from algal assimilation of polluting nutrients including nitrogen and phosphorus. When coupled with water treatment in this manner, it has been predicted that algal raceway ponds may provide low-input/high-output biomass production systems while addressing critical environmental issues by treating high-nutrient wastewater streams.

Photobioreactors

Efforts to develop industrial-scale enclosed photobioreactors (PBRs) for algae have been pursued in parallel with open pond system development. PBRs use physical barriers to isolate the algal crops from the threats of the natural environment. PBR systems are often in the shape of tubes or plates that achieve high surface area providing adequate exposure to light sources which can either be natural or artificial. The major advantage of these systems is increased stability due to the controlled nature of this type of production. Because these systems are closed, they can be sterilized and inoculated using proven technologies developed for the fermentation industry. This control can ensure that the selected algae will not face competition or threat from other microbes or predators which can lead to a highly stable production system. These systems can also employ artificial light, natural sunlight or a combination of both which also results in enhanced control over the system. Of course, using artificial light sources increases the cost of these systems which is a major disadvantage over outdoor production like raceway systems. Another major advantage of these systems is the ability to optimize the growth medium in which the algae grow. The combination of these factors leads to highly productive systems, but this productivity usually comes with an economic cost. The relative system stability provided by physical isolation results in closed systems which require supplementation of all necessary elements, including light, water, and nutrients, required for growth. They also require the constant removal of byproducts from algal growth which can threaten the viability of the organisms. This can be a costly and difficult process to implement and is often a major roadblock in the advancement of this technology.

Since PBR systems have robust crop protection, light control and nutrient management allowing for highly optimized production, they are well suited to the production of high-value chemicals which could offset the high cost associated with PBRs. Such products often require very specific environmental and physiochemical conditions which can only be achieved in a closed system. An example of this is the use of PBRs to produce omega-3 fatty acids for nutraceutical applications. Unlike biofuels, which are low-value, high-volume chemicals, omega-3 fatty acids are high value and this justifies the capital outlay required to produce algae at this scale. One of the most valuable components from algae is a carotenoid called astaxanthin which has excellent anti-oxidant properties. Because of the high demand for this compound, prices for astaxanthin are around $2500/kg (Milledge, 2010a).

An alternative to PBRs are bioreactors. Bioreactor (or fermenter) systems can be used for heterotrophic growth of algae. In such systems, the algae are fed a chemical source of carbon such as inexpensive industrial sugars usually captured from low-quality waste streams that are unfit for food or animal feed. These systems do not necessarily require light but PBRs and even open pond systems can be coupled with heterotrophic growth resulting in mixotrophic growth which includes both light and carbon supplementation. Several high-profile companies have pursued heterotrophic and mixotrophic systems for the production of extremely high value chemicals including cosmetics, personal care products and even specialty food production. However, these enterprises have struggled to achieve economic sustainability due to the high costs of maintaining PBR systems (Ferenbacher, 2016).

Algal Technoeconomic analysis (TEAs) have identified open pond systems as being the closest to commercialization due to their relatively low cost of operation (Amer et al., 2011). As such, this review will address sustainability in open pond production systems described earlier including the discussion of systems that have the flexibility to economically deliver feedstock for both fuel and chemical markets.

Sustainability in Algal Systems

When considering opportunities for sustainability in algal production, it is necessary to examine which issues will have the broadest impact on all facets of sustainability. To prioritize the components of algal production studies on technoeconomic analysis (TEA) and life-cycle analysis (LCA) are examined. The parameters of this analysis are very specific. As this review considers the optimization of overall biomass production without preference for macromolecule (protein, lipid, carbohydrate) composition or downstream processing; TEA and LCA will be considered with respect to maximizing biomass production. Likewise, this review will focus on processes within algal production systems and will not consider the engineering of organisms. Finally, as stated above, this review will assume systems are designed using raceway ponds producing biomass for end use as biofuels or bioproducts.

Technoeconomic Analysis

Numerous TEAs have been conducted on algal systems in recent years (Amer et al., 2011, Davis et al., 2011, Jones et al. 2014, Klein-Marcuschamer et al., 2013, Nagarajan et al., 2013, Quinn and Davis 2015, Richardson et al., 2012, Xin et al., 2016); and while not all studies agree, there is some consensus among them. In one of the most comprehensive studies on this subject, Quinn and Davis (2015), examined the results of over 20 TEAs for algal production systems and determined that there was incredible variability in production levels based on seasonality and location of production facilities. This uncertainty led to the identification of algal harvest as a key factor influencing the economic viability of algal production systems. Higher harvest cell densities result in lower costs for separations and smaller contribution to the overall economics while lower algal concentrations can result in excessive costs for harvest, but ultimately all the studies reported harvest of algae a major contributor to the overall cost of production.

Other studies on specific processes produced similar results in respect to economic viability of algal production. One prominent study was by Davis et al. (2011) that examined TEA with a specific emphasis on fuel production in the form of biodiesel from algal lipids. This study identified nutrient recycle and water supply as the most impactful factors on the cost of biomass production in the system. Other factors of note were nutrient demand and harvesting technologies. A study by Amer et al. (2011) that examined several different types of production systems found that in open pond systems over 90% of the cost of production is attributable to dewatering the algae. Nagarajan et al. (2013) identified algal harvesting and water and nutrient supply as the first and second most costly components of an open pond production system that used a novel process for producing biodiesel from algae. Klein-Marcuschamer (2013) identified harvesting as the single most expensive step in a facility producing aviation fuel from algal biomass.

Perhaps one of the most telling studies on the overall economics of algal production is that of Richardson et al. (2014). This study examined the impact of novel harvesting and extraction technologies on the economic viability of algal fuel systems. It demonstrated that the use of an electrocoagulation process as a harvest technology could increase the value of the products by 8.88% over centrifuge-based harvesting systems. In addition, the electrocoagulation system reduced costs of harvest by 4%. The combined effect of harvest cost reduction and increase of products using this new technology was 23%. This is a huge savings in overall production costs that could dramatically improve the economic viability of algal production systems.

The overall trend in all the TEAs discussed here is that algal harvesting plays a major, if not the most significant, role in the economics of algal cultivation. Quinn and Davis (2015) confirmed this in their sweeping evaluation of existing TEAs putting this factor at the top of their list of factors influencing the economics of algal production. In addition, water and nutrient supply in these systems are also very significant contributors to their economic viability.

Life Cycle Analyses

As with TEAs there are a multitude of comprehensive LCAs available for algal production systems (Clarens et al., 2012, Frank et al., 2011b, Quinn and Davis, 2015, Sander and Murthy, 2010, Singh and Olsen, 2010). These studies generally base their analysis on the accounting of greenhouse gases (GHGs) in the form of CO_2 equivalents within these systems effectively quantifying their impact on the environment. As such, these LCAs can guide the priorities of this review in terms of determining key factors in the environmental sustainability of algal production. Many of the major contributions to GHGs arise from downstream processing of algal biomass such as conversion to biofuels and bioproducts. However, there are some common themes that emerge as potential targets for GHG reduction in algal cultivation systems that are analyzed here. As with LCA, algal harvest is shown to have a major influence on environmental impact in these systems.

A key study by Quinn and Davis (2015) considered the findings of over 25 peer-reviewed LCAs. This is the same landmark study that contributed major themes to the analysis of TEA as well. This study considered downstream processing technologies in the overall evaluation of existing literature on LCAs and found that these processes had the greatest impact on LCA. However, their findings suggested that a novel hydrothermal liquefaction process (HTL) had the greatest potential to reduce GHG emissions associated with algal biofuel systems. Within the discussion of this technology it is apparent that the greatest advantage in terms of HTL on GHG impact is the ability of this technology to accept wet biomass reducing the energy required for harvest and thus lowering the GHGs associated with the overall process. This concept is reinforced by the fact that pyrolysis, a process with similar energy requirements to HTL that requires dry biomass was not environmentally favorable due to the energy required for drying in this conversion technology.

Another significant study in this area compared the LCA of algae to other bioenergy feedstocks (Clarens et al., 2010). They compared algal LCA to those of switchgrass, canola and corn and found that other feedstocks had lower environmental impacts than algal systems. The poor environmental performance was attributed to demands for carbon and fertilizers including N and P. They suggest that one method of mitigating this negative performance is the use of wastewater as a source of nutrients for algal cultivation. When using wastewater as a source of water and nutrients, they find that the GHG impacts of these systems were much more favorable than when using fresh water, thus improving performance in the algae-wastewater systems to better than terrestrial crops.

Aresta et al. (2005) and Singh and Olsen (2010) also identified water and nutrient recycle as methods to reduce the GHG contribution of algal biofuels. This was cited as one of the major advantages of algal systems, which allowed the offset of GHG generated in the harvesting and processing of algal biomass. Sander and Murthy (2010) examined the life cycle of algal biodiesel and found separation of the biomass into its constituent components (lipid, carbohydrate, protein) to be the major factor contributing to GHGs in this system. This processing step is outside the scope of this analysis. However, that study also linked the high GHG impact of constituent separation to the energy required to harvest and dry the algal biomass. In fact, they determined this step to consume 89% of the energy required for the entire system. As such harvesting energy was identified as a major contributor to the GHG footprint associated with this system.

Common themes emerging in a review of algal LCA literature are nutrient recycle and the use of wastewater as a source of nutrients. In addition, a common thread between LCA and TEA suggests that harvesting technologies can have a major positive impact on the results of both of these analyses. As such, these elements of algal production are analyzed as they relate to the overall sustainability of these systems.

Sustainable Water Use and Recovery

Water cost and availability is among the biggest challenges facing widespread adoption of algal systems as a source of biofuels and bioproducts. Not only do these systems require products that can compete on the low value markets occupied by petroleum products to be economically sustainable, they also need to minimize environmental impact and maintain social acceptability. Even though algae need relatively few resources (sunlight, water, air and nutrients), these systems can be very costly to operate. As such it is extremely important to consider economic factors in all facets of algal systems from growth through processing and transport. A key factor that has both positive and negative impacts on the economic sustainability of algal systems is water. The cost of providing enough water to sustain reasonable algal growth and ensuring the system can then concentrate the biomass at a reasonable concentration has been identified as a key obstacle in achieving positive economic return in algal production systems. In open pond systems, there is an additional requirement for sufficient water to make up for water loss to evaporation. There is also a need to remove a fraction of the water for treatment to reduce buildup of compounds in the pond that are toxic to algae. The water lost to treatment and evaporation is variable and based on meteorological conditions (Guieysse et al., 2013). However, it can have a profound effect on the economics as well as environmental and even social impacts of the entire system. However, algae have the capacity to thrive in municipal and agricultural wastewater and even heavily contaminated water. As such, the use of wastewater as a source of water for algal production systems is examined in this study.

This review will consider the value chain from growth through biomass harvest and will consider the interaction of the various technologies throughout this process.P is mined from phosphorus rock which is in finite supply. Thus, the earth has a finite supply of P, an element necessary to sustain life. Current agricultural practices often over apply N and P resulting in runoff and contamination of fresh water sources. This leads to degradation of water quality and dilutes N and P to concentrations that are not useful for agricultural application.

Opportunities for Sustainable Practices in Open Pond Algaculture

Through the analyses of water and nutrient issues associated with algal cultivation, it is clear that technologies that extend the use of water and nutrients in algal systems can have a positive effect on the overall sustainability of these systems. Likewise reducing energy impacts of managing these resources will also lead to improvements in sustainability.

Use of Wastewater in Algal Systems

An opportunity within algal systems that is beneficial to sustainability of both water and nutrient resources is the use of wastewater as the growth medium for algae.

Agricultural, industrial and municipal systems have employed algal wastewater treatment for decades. It is only in recent years that these systems have been considered a potential source of valuable chemicals, biofuels and bioproducts (Rawat et al., 2011; Pittman et al., 2011). However, since the main function of these systems is to remove nutrients and other contaminants from water, the economics can be favorable in these systems and could result in the cost-effective production of feedstock for bio-based products.

Wastewater Remediation

Studies have examined the efficacy of algal systems in removing specific contaminants from wastewater. For example, Ismail et al. (2017) have examined algal-bacterial consortia for removal of analgesics from water and the subsequent valorization of the biomass produced. This study found that the use of both algae and bacteria appears to have synergistic effects in the remediation process, and still result in the production of useful biomass for biofuel and bioproduct production. Algal consortia can also be effective in treating high nutrient wastewater from industrial processes such as mining, agriculture and anaerobic digestion. Palma et al. (2017) found that an algal consortium was effective at remediating nickel from mining tailings. The biomass derived from this process was determined to have several bioproduct applications due to its high carbohydrate content (~40%).

Nutrient Recycle

Algal systems have also been shown to be excellent at removing nutrients such as N and P from wastewaters. Management of these nutrients within agricultural systems will be critical in the future ability to produce enough food to meet the demand of a growing human population. Current agricultural systems often over apply these nutrients in the form of chemical and manure fertilizers, which lead to their deposition at very low concentrations in open water bodies and groundwater. As algae need these nutrients to survive and replicate the uptake of these nutrients by algal systems can be quite impressive (Pittman et al., 2011). It has been demonstrated that algae can concentrate these nutrients from mg/L levels to g/L concentrations, which are at the level required for crop fertilization or reuse in algal production (Chinnasamy et al., 2010). As such algal systems are excellent in treating municipal and agricultural waste waters and have the added benefit of providing a potential source of valuable fertilizer.

The behavior of algae grown in high P waters can enhance this remediation and concentration process. Singh and Das (2013) demonstrated the removal of 70-80% of the P in carpet processing wastewater that contained extremely high levels of P (20.31–35.10 mg/L as phosphate). The resulting algal biomass had 3% P by mass which far exceeds the ~1% P content normally seen in algal cells. This phenomenon was attributed to luxury uptake of P in which algae assimilate high levels of P as polyphosphates when subjected to an excess of the nutrient. This process has been characterized extensively by Powell et al. (2008 and 2011) and is a major advantage when using algae to remediate high P wastewater. It has been proposed that this type of system could be employed to concentrate P for use as fertilizer. This is one method of recovering this finite resource from the environment in a usable form.

Recent studies are revealing that the deliberate engineering of microbial communities containing algae, bacteria and other microbes can have a profound effect on the ability of these systems to remove nutrients and produce biomass. Higgens et al. (2016) demonstrated that an engineered consortium utilizing the algae *Auxenochlorella* *protothecoides* and the bacteria *Escherichia coli* have shown increased nutrient uptake compared to monoculture algal systems. These systems also show increased biomass production and increased lipid production. The study attributed these increases to symbiosis in the systems linked to cofactor synthesis where the bacteria were shown to synthesize components that increased the productivity of the algae in the consortia.

Studies have also examined the economics of integrated algal wastewater treatment and biofuel systems. These studies have produced mixed results based on the algae used and the biofuel product produced. Posadas et al. (2017) report that systems that incorporate algal nutrient removal from wastewater with biogas production are not economically viable due to the high cost of transportation of digestate solids produced in such systems. In contrast, a study by Xin et al. (2016) showed that a system employing algae for wastewater treatment and the production of bio-oil via pyrolysis had favorable economics with an internal rate of return of 18.7%. Such differences in economics show how crucial it is to match the correct technologies when designing this type of system.

Algae-Water Separation Technologies

One area where water plays a major role in algal operations occurs at harvest when the algae are separated from the water in which they are cultivated. A significant fraction of the cost associated with processing algae can be attributed to the fact that these production systems usually produce algae at very low concentrations in water and thus costly separations technologies must be used to recover the algal biomass at concentrations suitable to processing. A typical raceway pond produces algae at peak concentrations in the order of 1 g/L (1000 ppm) (Borowitzka, 2005). In practical terms, this means that for every unit of algae recovered from a peak raceway, there are nearly

1000 units of water to remove. Water removal is generally a very expensive process when traditional technologies are employed. Much research has been conducted seeking novel separations methods to address this issue. Many of the recovery processes that were examined leverage existing technologies used in other industries.

Centrifugation

Centrifugation is a common industrial separation processes that has been studied for the removal of algal biomass from the large quantities of water required for efficient algal production (Molina Grima et al., 2003). This process is generally the initial technology considered for laboratory and pilot scale algal harvesting since it has been proven effective in many cases and is a well-developed and characterized technology used in a wide variety of industrial processes (Mohn, 1988). However, the cost associated with operating the electric motors that drive industrial centrifuges can be high. For example, a study by Milledge and Heaven (2013) calculated that the energy required to harvest algae using a disc stack centrifuge is approximately 4 times that recovered if the algal biomass is used for biodiesel production. This calculation assumes that only algal lipids would be used for a viable product, but it still demonstrates the huge energy demand required in centrifugation. As such centrifugation is usually not appropriate as a separations technology in algal biomass systems.

The disparity in the cost of energy required to separate algae using centrifuges and the cost of products produced may be mitigated in several ways. The most direct is to capture more value from the products by using the whole cell as a profit center as opposed to just the lipids. Milledge (2010b) reports that the energy content of whole algal cells can be up to 3.16 times that of the lipid fraction. This vastly improves the economics of the system but still does not compensate for all the energy consumption required for centrifugation. Another method of optimizing the energy used in separations using centrifugation is to use other, less effective but more efficient systems as pretreatment before centrifugation. The technologies described below (filtration, flocculation, etc.) can be employed in tandem to reduce energy requirements of the final centrifugation while still reaching the high biomass concentrations associated with centrifugation (Milledge and Heaven, 2011).

Dissolved air flotation

Dissolved air flotation (DAF) is a promising technology being studied for algae-water separation. DAF is commonly used in many areas of food processing to remove fats, oils and greases from wastewater. In this process, flocculants are added to the algae-water mixture to concentrate the algae within the system. Then dispersed air is bubbled through the system to float algal cells to the surface of the system where they are collected using skimmers. This type of DAF harvesting was evaluated by Chen et al. (1998) using the alga *Scenedesmus quadricauda* and several different flocculants. They showed that DAF could work for algal harvesting, however the performance is dependent upon a variety of physio-chemical factors in the system such as pH, flocculant ionic strength and air flow rate. Likewise, Coward et al. (2013) reports the successful harvesting of several *Chlorella, Scenedesmus, and Chaetoceros* algal species using foaming DAF systems with removals ranging from 76-90%. Henderson et al. (2010) achieved biomass collection of 94-99% using DAF systems on algae and other microorganisms of varying morphology, including, *Microcystis aeruginosa* (cyanobacteria), *Chlorella vulgaris* (green alga), *Asterionella formosa* and *Melosira sp.*

(diatoms). Their study showed a correlation between the charge of the algal systems and control over the coagulation using various flocculants.

These studies provide substantial evidence that DAF systems can be used to successfully recover algal biomass from algal production systems with low cell concentrations. However, the economics of such systems can be problematic. DAF requires air pumps to achieve the air bubbles that float the coagulated biomass to the surface for collection. These pumps can be very expensive to run. In addition, these systems require expensive coagulants that can contaminate the algal biomass making it unusable in some downstream processes. In a review of algal process train options, Wiley et al. (2011) reported that the electricity for the pumps required to achieve acceptable concentration can account for over 50% of total processing costs in these systems. As an alternative, this study suggests the use of suspended air flotation (SAF). SAF uses chemical surfactants to produce the bubbles required for floatation. This process has been shown to be more efficient that DAF but the cost of the chemicals involved still may harm the overall economic sustainability of systems that would use this technology (Wiley et al., 2009).

Advanced Filtration

Filtration is another technology being explored for the collection of algal biomass from dilute systems. The most common of these methods is the use of a belt filter press. The belt filter press is a common piece of equipment that is used in many industries. These systems use a moving belt with pores that allow water to pass through. These presses can be economically favorable because they mainly rely on gravity to perform the dewatering. However, algal slurries processed in these systems can only achieve concentrations of 10-30% solids (Wiley et al., 2011; Sturm and Lamer, 2010) suggesting that downstream processes must be able to handle relatively high water content which may have an economic disadvantage on the processing side.

Other advanced algal filtration methods may provide a more effective alternative to the existing filtration technologies adopted from other industries. Membrane filtration also known as microfiltration or ultrafiltration is a rapidly developing technology that may be well suited for algal harvesting. This technology uses membranes with micrometer pore sizes that allow water to pass but retain the algal biomass. This results in extremely high recovery rates of algal biomass from dilute systems but the process is also subject to significant bottlenecks. Studies have demonstrated concentration of algae from growth media of up to 150X using this technology and these systems have shown essentially no algal presence in the permeate meaning all the valuable biomass was retained in the retentate (Zhang et al., 2010). However, these extremely high recovery rates are not without their drawbacks. The membranes used in these processes are susceptible to fouling which drastically reduces throughput and increases costs. Fouling is often attributed to algeogenic organic matter (AOM) and studies show that long-term use of this technology is not viable due to this issue (Babel and Takizawa, 2010). In response to this issue, methodologies are being studied to combat fouling using low pressure and parallel flow systems that do not allow the AOM to form a cake on the membrane (Rossignol et al., 1999) but no easy solution has yet been identified. Studies continue to examine the causes of this issue and to address the problems caused by AOM and the potential for its control or elimination (Lee et al., 2004).

Electrolytic Methods

One of the most promising harvesting technologies being investigated by the National Alliance for Advanced Biofuels and Bioproducts (NAABB) is the use of electricity to harvest algal cells. Richardson et al. (2014) summarized three methods for using electricity to harvest algal cells, namely, electrolytic flotation, electrolytic flocculation, and electrocoagulation. The most effective of the three technologies appears to be electrocoagulation (EC). This process uses low voltage electricity to generate a positively charged anode that attracts the negatively charged algae causing them to coagulate and sink. One major advantage of this technology is the high recovery rates that can be achieved. EC has been demonstrated to have a recovery rate of 98% whereas centrifugation has an effective recovery rate of 95%. This may not seem significant but the 3% increase in yield adds greatly to the economic return on these systems. In addition, EC is much less expensive to operate compared to centrifugation. Overall, EC was shown to cost 23% less than centrifugation as reported by Richardson et al. (2015).

Electrocoagulation technology is established for removing charged contaminants in wastewater treatment and has been demonstrated at scale in this industry. The major drawback to deployment of electrocoagulation is the amount of water left in the biomass after harvest with this technology. Final solids concentrations using this technology have only reached 4-6% for initial algal solids loads of 0.1% in field trials performed by NAABB. However, concentrations as high as 8% solids have been achieved in the laboratory (Richardson et al., 2014). Even at this level of concentration, the algal slurry is too wet to serve as an adequate feedstock for most processes. There are two pathways for using this relatively wet feedstock. The first is to further concentrate the biomass using a common technology such as centrifugation. At 5% solids over 95% of the water has already been removed from the initial 0.1% solids suspensions. This concentration of biomass greatly reduces the energy required to remove the remaining 5% water using more expensive technologies. The second pathway to utilization of this wet algal biomass is to implement conversion pathways that can utilize such feedstocks. Richardson and Johnson (2015) have identified Hydrothermal Liquefaction and Catalytic Hydrothermal Gasification as promising conversion pathways to produce liquid and gaseous fuels using feedstocks with high water content. Pursuit of technologies to utilize wet algal feedstocks continues and such processes could be major contributors to the economic and environmental sustainability of algal production systems.

Social Sustainability

Despite the wealth of LCA and TEA analyses available on algal production systems there is very little discussion in the literature about the social sustainability of algae as a crop. LCA and TEA provide useful information to guide discussion on the economic and environmental aspects of the overall sustainability of these systems but it appears that a comprehensive discussion on the social impacts is lacking. In order to have a complete understanding of the sustainability of this emerging technology it is important to consider its social impacts. One reason for the lack of analysis on the social implications of algae is likely the minimal deployment of these systems on a large scale. However, it is possible to analyze some of the projected impacts of these systems on social sustainability.

A study by Adenle et al. (2013) investigated the role of algae in developing countries and found that several factors make algal production in these regions risky. One is the lack of human capital to make these systems successful. This is likely an obstacle for the sustainable deployment of this technology in the developed world as well. Algaculture, while having much in common with traditional, terrestrial farming, is a new concept requiring specialized skills that are lacking in most populations. Due to the need for large land area for efficient algal production it is likely that algal production will occur in rural areas. As such it is necessary to include rural education and development as key components of plans for algal production system deployment. It is apparent, that algaculture systems have much in common with aquaculture systems. As such, there is the potential to integrate these systems into communities that already have experience and human capital in this area. For example, the catfish farms of rural Louisiana, Mississippi, Arkansas and Alabama exist in an impoverished area of the United States. This industry was hit hard by the economic downturn of the 2000s and has had difficulty recovering due to the recent influx of imported catfish into the U.S (Morgan, 2011). While the aquaculture industry is not as strong in this area as it once was, human capital, physical assets and cultural acceptance necessary for the sustainability of this type of industry are still in place in this region. Algaculture could provide much needed stimulus to rural economies that have seen their traditional industries disappear over the past 10 years.

Another social obstacle to the deployment of algaculture systems in developing countries identified by Adenle et al. (2013) is the use of genetic modification (GM) and genetically modified organisms (GMOs) in this industry. These concerns are universal and will affect the deployment of this technology in both developing and developed countries. Algae are microorganisms and can be easily modified using established and novel genetic manipulation (Radakovits et al., 2010). Recent studies show continued debate over the use of GMOs for food production (Fairfield-Sonn, 2016; Berning and Campbell, 2017). The attitudes and fears associated with GMOs also appear to be present with regard to bioproducts (Hasenheit et al., 2016). While the bioproducts and fuel GMO debate does not include the danger to human health associated with food products, concerns such as the establishment of monoculture and the perceived dangers of releasing GMOs into the natural environment persist. As algaculture is a relatively new concept in most countries the use of GMOs in this industry will likely face increased scrutiny. The obvious solution to this issue is to use non-GMO algae for these production systems. This is a possible option but GM is a powerful tool that may greatly improve the economic sustainability of many algal technologies. Education of the public about GMO safety and continued research into the effects of GMO algae may also alleviate some of this conflict but the issue will have to be addressed. It may ultimately be that GMO algae are only used in closed systems while open pond systems use algae developed from traditional breeding and selection techniques.

One area of social sustainability that has been the source of resistance to many first and second-generation biofuel and bioproduct technologies is the debate over food versus fuel. For clarification, first generation biofuels are those derived from food crops and second-generation technologies generally utilize non-food crops (Adenle et al., 2013). In both of these cases the feedstock is often grown on land that could be used for food production thus causing a debate over land use change and the ability to supply food for a growing population. In fact, it was reported by Mueller et al. (2011) that first generation biofuels may have been responsible for 3-30% of food price increases observed in the 2000s. This facet of social sustainability is one where algae systems may have an advantage over existing first and second-generation technologies. Although open pond algae cultivation does require extensive land areas, this land does not require high-quality soil that could be used to grow crops for consumption. Algae farms can be installed anywhere there is open land and adequate sunlight.

It is also important to understand that the potentially high productivity of algae reduces the overall demand for land resources to produce biomass as compared to terrestrial crops. Langholtz et al. (2016) have demonstrated that open pond aquaculture systems used for bioenergy would only utilize 8.6% of the land required by terrestrial crops producing equivalent biomass. Furthermore, this production would not compete directly with terrestrial crops for land resources and would likely displace pastures or other low value lands.

Additionally, if land resources were to become scarce algae can be cultivated on a much smaller footprint using photobioreactors. As photobioreactors can grow algae at much higher concentration than outdoor systems they have a much smaller demand for water and harvesting energy which eliminates arguments that algal bioproduct systems could siphon these resources away from food production (Ozkan et al., 2012). The advantage algal systems have over terrestrial systems in terms of the food versus fuel debate may be one of the strongest arguments for sustainability of this approach in all facets, economic, environmental and social, as the elimination of this controversy removes one of the major roadblocks in the development of a sustainable and successful bioeconomy.

Future Systems: Feed and Food

The advantages algae have over terrestrial systems in terms of land use may lead to an expansion of this crop into food and feed markets. As human population continues to increase there will be less resources available for the production of food and more demand from the expanding population. In fact, reports suggest there are enough food resources to sustain the human population until 2060-2080 (Sverdup et al., 2012). Likewise, human domestication of animals for food production requires massive amounts of feed to sustain these practices and it is becoming clear that the ecological impacts of this industry are having measurable effects on the environment (De Vries and De Boer, 2010). Algae offer a promising response to these issues of growing importance. It may be used to displace the inefficient production systems currently used to provide a fast-growing and reliable source of nutrition for both humans and livestock. Therefore, advances that are made in the pursuit of maximum algal biomass production for biofuels and bioproducts will directly translate to an algal-based food and feed industry leading to increased yields and better cultivation technologies. This potential coincident benefit makes research into this area even more critical and high-impact.

Currently, algal production is the third largest aquaculture crop producing 19,000,000 Mg of biomass per year with a projected value of $5.7 billion (Langholtz et al., 2016). Algae have use in many other industries outside the energy industry including food, food additives, nutraceuticals, fertilizers and feed for livestock and aquaculture (FAO, 2016). It should be noted that much of this demand is for multi-cellular macroalgae or seaweeds. The demand for these products mainly exist in Asian, South

American and African markets, but that could easily change if inexpensive protein becomes scarce due to increasing global command (Wikfors and Ohno, 2001).

Furthermore, microalgae based feed is critical to aquaculture markets worldwide and commands a significant premium where there is demand. These feed markets consume much of the cultivated algae and as such supply chain logistics already exist for moving commodity quantities of algae within this industry. This infrastructure could be applied to expand this industry into human consumption markets under the right conditions to supply copious amounts of high nutrition food. In fact, it has long been known that some algae can supply almost all nutrients required by the human diet including essential amino acids, fatty acids and vitamins (Krauss, 1962). This is also true for livestock nutrition making algae a promising option for feed . This leads to the conclusion that research pursuing the optimization of algal cultivation for biofuels and bioproducts could have the added benefit of preparing the scientific community and industry to embrace the expansion of this production into food markets for consumption by both humans and livestock.

Conclusion

It is clear that establishing sustainable methods for the cultivation of algae as a source of fuels and chemicals will still take considerable effort. The most formidable challenge in this area appears to be addressing economic issues associated with the cultivation and delivery of feedstock of suitable composition for currently available conversion technologies. The environmental impacts of these systems are still being determined but it is clear these effects will be highly dependent on production levels that can be achieved. As research explores using algal systems for nutrient recycle including nitrogen and phosphorus these impacts will be mitigated by the benefits inherent in the growth of algae. These benefits include the potential to remediate wastewater and remove harmful chemicals from polluted water bodies. This co-application of algal cultivation will also have a positive impact on economic and social sustainability as the cost and social benefits of efficient cleanup of contaminated water is apparent.

The interconnection between the economic, environmental, and social aspects of sustainability identified here lead to an important conclusion from this review. In order to capitalize on all the co-benefits and profit opportunity of algal production systems, it is extremely important to match appropriate technologies to the application and goals sought by these systems. For example, coupling wastewater treatment to the production of algae associated with feed and food production is not socially sustainable even though it may greatly enhance the economics of the system. However, when the end-use of the algal biomass is biofuels or bioproducts, remediation is a socially acceptable leverage of systems assets and will ultimately lead to improvements in both economic and environmental sustainability. Likewise, downstream conversion technologies must be carefully considered when looking at algal cultivation and harvesting. Specifically, technologies that can utilize feedstock with increased levels of water are very promising for enhancing the economic sustainability of algal production. This systems approach is of the utmost importance when designing algal biomass production systems and is crucial in establishing the sustainability of this emerging industry.

CHAPTER 5

CONCLUSION

This dissertation examined the current state of-the-art in algae production and identified several targets for improving sustainability of algal systems. This study identified the use of extremophiles to mitigate conflicts between recommendations in Life-Cycle Analyses (LCAs) and Technoeconomic Analyses (TEAs) of algal production systems. Specifically, psychrophilic algae known as snow algae were identified as potential tools in the extension of the algal growing season insuring year-round production recommended by TEAs and maintaining the greenhouse gas emissions required by LCAs to insure algal systems achieve the 50% reduction in GHG emissions needed for certification as advanced biofuels under Renewable Ruel Standard II. This work also included a review of algal production system sustainability in terms of water and nutrient conservation and recycle. This review attempted to identify further targets for improving sustainability of algae production on a systems level.

This study provided new insight into the behavior and biochemical processes of snow algae and expanded the understanding of well-characterized algae used as model production species. Specifically, this dissertation demonstrated that, as expected, the snow algae studied here (*Chlamydomonas yellowstonensis* and *Chlamydomonas augustae*) grew better at the lower light intensities (50 and 100 μmol/m^2/s) and lowest growth temperature (5°C) examined in this study than the model production algae included in this dissertation (*Scenedesmus bijuga* and *Chlorella sorokiniana*). The snow algae yielded more biomass at these conditions than SB and CSO which were unable to grow under these limiting conditions. In fact, CSO demonstrated no growth at 5°C under any of the light intensities studied here (50, 100, and 300 μmol/m^2/s).

Unanticipated results were observed when the growth temperature for these organisms was raised to 10°C. At this relatively low growth temperature, the average daily biomass productivity of SB, one of the model organisms, exceeded that of the snow algae CY and CA at the light intensities studied here. CSO, the other production species, demonstrated biomass production rates that were statistically indistinguishable from those of the snow algae under these conditions. The results suggest that at growth temperatures somewhere between 5-10°C, model laboratory organisms begin to acclimate to the reduced temperature and begin to perform better than the snow algae which have not been selected for optimized biomass production under any conditions.

The model organism SB demonstrated higher biomass production rates than the cold-adapted snow algae at 5°C and 300 μmol/m^2/s. These production rates were not anticipated for the production algae under these sub-optimal conditions. These results suggest that SB may be a suitable candidate for optimized cool-season production providing a basis for future studies to develop seasonally optimized algal crop rotations for biomass cultivation.

The production rate data from snow algae studied here also provided some interesting insight into the performance and behavior of these organisms under the conditions studied here. While CY demonstrated overall higher production rates, CA revealed a unique response to the conditions used in this study. As with all the species studied here, CA showed a positive correlation between biomass production rates and temperature. However, CA demonstrated a *negative* correlation between light intensity and growth rate for the experimental conditions studied here. This carries with it some unique implications that could impact future efforts to genetically engineer seasonally optimized production algae using genetic resources from snow algae. Or, for that matter, develop algae optimized to grow in sub-optimal geographies that lack generally adequate light exposure for algal production.

The growth models identified here for SB, CSO and CA provided a useful comparison of the productivity of these organisms across a range of low temperatures and low light exposures. These models were used to identify guidance for an algal crop rotation that would maximize biomass production over the range of conditions examined in this study. The models were also used to extrapolate the performance of these organisms under temperatures beyond the scope of this study. The results of this analysis provided the crop rotation guidance reported in Table 2.7. This rotation suggests use of the snow alga CA the lowest temperatures and light intensities with a transition to SB as temperatures rise and light increases ending with a final transition to CSO at high light intensity and temperature conditions.

Ultimately these discoveries have implications for the sustainability of algal production systems based on the results of the NAABB study (2014) and the U.S. Algal Biofuels Strategy (Department of Energy, 2014). As identified earlier in this dissertation, the identification of cool-season optimized algal production species could address issues highlighted by both the TEAs and LCAs that informed both aforementioned reports. Specifically, such seasonally optimized algal species could extend the production cycle of algal cultivation systems. This would increase economic sustainability of these systems through increased revenues derived from extended growth cycles. It would also coincidentally increase environmental sustainability of these systems. Specifically, it could lead to certification of biofuel systems using these organisms as a source of biomass as advanced biofuel systems. This certification would require that production levels of cool-season optimized algal crops are productive enough to sustain the 50% reduction in greenhouse gases as compared to fossil fuel systems. This is already achievable in warm season systems, but the potential to achieve this in the cool-season has been under scrutiny. The results of this study may illuminate a path to achieving this goal for extended growing seasons that will ultimately increase revenues and economic sustainability of these systems.

The examination of nutrient uptake and lipid accumulation reported here also provided data regarding the behavior of model and snow algae species under consideration here. Consistent with the production data reported in the growth study, the model production algae SB and CSO demonstrated a positive correlation between nutrient (nitrogen and phosphorus) uptake and growth temperature at under the light and temperature regime used here. No correlation between light intensity and nutrient uptake was determined for these organisms which was consistent with the growth study. The snow alga CA also demonstrated a positive correlation between nutrient uptake and temperature. However, CA showed a negative correlation between light intensity and phosphorus uptake. This was also consistent with the growth study. There was no correlation shown between light intensity and nutrient uptake for CA.

The lipid study did not provide any evidence of correlation between lipid accumulation and light or temperature for the model algae SB and CSO or the snow alga

CY. However, a negative correlation was determined for light intensity and lipid accumulation in this species. This was consistent with the negative correlation between light and growth/phosphorus uptake in CA, the other snow alga examined here. Taken together this evidence suggests that snow algae have unique metabolisms which may function more optimally at lower light intensity. This provides further insight into the value of snow algae as a source of genetic resources for the engineering of cool-season adapted algae crops.

The review of algal system sustainability presented here provides additional conclusions and observations that can help enhance sustainability of algal biomass production. These improvements to sustainability could be enhanced by the integration of cool-season crop rotations described in the earlier chapters of this dissertation. Likewise, proper management of water and nutrients is a key to the sustainability of algal systems. The data provided here on nutrient use by psychrophilic algae can provide a basis for evaluating sustainability of systems integrating these organisms into their operations in the context of the concepts discussed in the review. Water management can also be influenced by the snow algae studies as they provide key data in terms of remediation potential for these organisms in systems using high nutrient wastewater.

The most significant conclusion from the review on sustainability is the critical importance of selecting complementary processes throughout the production chain based on climate, water availability, nutrient source, and product portfolio. Selecting final products that lend themselves to maximizing and sustaining system productivity is key to maintaining economic, social and environmental sustainability. The selection of products guides the selection of target molecules from algal systems that in turn informs the selection of production methods. Availability of sunlight, adequate temperature, water and nutrients also must be considered throughout the value chain and must ultimately guide decision-making throughout the process.

In the context of the three elements of sustainability which guided this work (economic, environmental and social), there are several conclusions arising from this dissertation. First, the three elements are interconnected throughout the algal supply chain and must be considered together. Using a systems approach, the contribution of each element must be weighed when determining the sustainability of the process. This mandates that the results of LCAs, TEAs and social impact modeling must be considered when designing algal production systems. These tools can be used synergistically to shape systems that insure the three sustainability components are all being addressed leading to new technologies that improve on established processes used in the petroleum economy. This will help create a new bioeconomy that not only provides sufficient energy and materials for everyone, but does it in a way that preserves the environment and human quality of life.

This dissertation also addressed a key knowledge gap illuminated by the NAABB report (2014) and the U.S. Algal Biofuels Strategy (Department of Energy, 2014) where economic, social and environmental issues intersected. This involved providing data to help extend the algal growing season. The research presented here examined this intersection by considering the potential for cool-season crop rotations in algal cultivation. The successful implementation of this strategy would have a three-fold effect. First it would improve the economic sustainability of algal cultivation based on the analysis of existing TEAs. Extending the growing season would result in consistent income throughout the year improving economic viability of algal production.

Extended growing seasons would also reduce the environmental impacts based on the results of existing LCAs. These studies have shown that using current algal crops, cool-season productivity is too low and the resulting carbon balance does not meet the 50% reduction required for designation as an advanced biofuel (Schnepf and Yacobucci, 2013). Development of algal crops that could maintain productivity through the cool season would address this issue and allow year-long production simultaneously addressing economic and environmental sustainability issues.

The development of cool season algal crops would also improve social impacts by providing year-round jobs in rural communities where algal production might occur. It would also provide a consistent source of feedstock for processing operations providing economic incentives for their establishment. This would address the “chicken and egg” scenario common in emerging bioproduct technologies. In this scenario, it is difficult to convince producers to grow feedstocks without assurance that there will be facilities in place to process them when the crop is ready for harvest. Likewise, there is a reluctance in the business community to build bioprocessing facilities without a guarantee of consistent feedstock. The seasonality of most crops exacerbates this issue as it is difficult to justify constructing a facility that must slow or stop production due to periodic lack of feedstock. As algal rotations are short compared to terrestrial crops, extending production to an annual cycle would solve this issue by providing a consistent and reliable source of feedstock. This, in turn, would result in rural economic development and high-quality jobs improving the quality of life for these communities.

This dissertation also examined the nutrient remediation capabilities of potential candidates for cool-season crops. The organisms were also considered as a genetic resource for further development of high productivity, psychrophilic algae. While the data presented here is very early in the development process, there were interesting results that could provide the basis for further research to improve algal systems. The development of high productivity algal crops which also effectively remediate wastewater could have positive impacts on the overall sustainability of algae as a biomass feedstock. The sustainability benefits of algal nutrient remediation have already been discussed but when they are coupled with the ability to grow year-round, the impacts on social, environmental and economic sustainability are compounded.

Ultimately, this dissertation concluded that a systems approach is critical to insure sustainability of algal systems as each unit operation in the production chain influences all others. This concept translates to the sustainability of these systems as well when considering the three components of sustainability addressed here: economic, environmental, and social. Economic factors are often used to judge the sustainability of a system, but environmental and social impacts have similar influence on overall sustainability due to the interconnectivity of these three factors. For example, environmental policy is critical to maximize benefits through subsidies, grants and market controls that will in turn enhance economic sustainability. Social considerations are also influence the economics of projects such as algae production as local communities can ultimately affect the success of algae projects through support of or resistance to their progress. Likewise, environmental factors also greatly influence social acceptance of such systems. For example, pressures from community influencers can have impacts on economic considerations due to regulations such as waste disposal and other guidelines. Overall, these systems will require positive economic, social and environmental return for success and to truly achieve sustainability.

REFERENCES

1. Adenle, A.A., Haslam, G.E., Lee, L. 2013. Global assessment of research and development for algae biofuel production and its potential role for sustainable development in developing countries. *Energy Policy.* 61:182-185.
2. Aitchison, P.A., Butt, V.S. 1974. The Relation Between the Synthesis of Inorganic Polyphosphate and Phosphate Uptake by *Chlorella vulgaris. J. Exp. Bot.* 24(3):497-510.
3. Amer, L., Adhikari, B., Pellegrino, J. 2011. Technoeconomic analysis of five microalgae-to-biofuels processes of varying complexity. Bioresour. Technol. 102(20):9350-9359.
4. Aresta, M., Dibenedetto, A., Barberio, G. 2005. Utilization of macro-algae for enhanced CO_2 fixation and biofuels production: Development of a computing software for an LCA study. *Fuel Proc. Technol.* 86: 1679-1693.
5. Aslan, S., Kapdan, I.K. 2006. Batch Kinetics of Nitrogen and Phosphorus Removal From Synthetic Wastewater by Algae. Ecol. Eng. 28(1):64-70.
6. Azad, H.S., Borchardt, J.S. 1970. Variations in Phosphorus Uptake by Algae. *Envrion. Sci. Techol.* 4(9):737-743.
7. Babel, S., Takizawa, S. 2010. Microfiltration membrane fouling and cake behavior during algal filtration. *Desalination.* 261(1-2):46-51.
8. Bajaj, V., Srivastava, P. 1985. Morphological mutant of *Scenedesmus bijugatus (turp.) Kuetz. Curr. Sci.* 54(6):294-295.

9. Berning, J., Campbell, B. 2017. Consumer Knowledge, Perception and Acceptance of GMOs. Presented at the Southern Agricultural Economics Association 2017 Annual Meeting.
10. Borowitzka, M. A. 2005. Culturing microalgae in outdoor ponds. In I. R. A. Andersen (Ed.), *Algal culturing techniques* 205-218. New York: Elsevier.
11. Braunegg, G., Koller, M., Salerno, A., Schnitzer, H. 2015. “Microalgae for sustainable energy production?” *Energy Security and Development.* Ed. B. Sudhakara Reddy, Ed. Sergio Ulgiati. New Delhi: Springer India. 471-484.
12. Cañizares, R.O., Rivas, L., Montes, C., Domínguez, A.R., Travieso, L., Benítez, F. 1994 Aerated swine-wastewater treatment with K-carrageenan-immobilized Spirulina maxima *Bioresour. Technol.* 47:89–91.
13. Chen, Y.M., Liu, J.C., Ju, Y.H. 1998. Flotation removal of algae from water. *Colloids Surf., B.* 12(1):49-55.
14. Chinnasamy, S., Bhatnagar, A., Hunt, R.W., Das, K.C. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour. Technol.* 101(9): 3097-3105.
15. Christi, Y., 2016. Large-Scale Production of Algal Biomass: Raceway Ponds. In F. Bux and Y. Christi (Eds.) *Algae Biotechnology Products and Processes* (pp. 21-40). New York, NY: Springer.
16. Clarens, A.F., Resureccion, E.P., White, M., Colosi, L.M. 2010. Environmental Life Cycle Comparison of Algae to Other Bioenergy feedstocks. *Environ. Sci. Technol.* 44(5): 1813-1819.

17. Constantopoulous, G., Bloch, K. 1967. Effect of Light Intensity on the Lipid Composition of *Euglena gracilis*. *J. Biol. Chem.* 242(15):3538-3542.
18. Cordell, D., White, S. 2011. Peak Phosphorus: Clarifying the Key Issues of a Vigorous Debate about Long-Term Phosphorus Security. *Sustainability*. 3: 2027-2049.
19. Coward, T., Lee, J.G.M., Caldwell, G.S. 2013. Development of a foam flotation system for harvesting microalgae biomass. *Algal Res.* 2(2):135-144.
20. Darzins, A., Pienkos, P, Edye, L. 2010. Current Status and Potential for Algal Biofuels Production. NREL/T39-T2. Golden, CO: National Renewable Energy Laboratory.
21. Davis, R., Aden, A., Pienkos. P.T. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Appl. Energy* 88:3524–3531.
22. Davis, R., Fishman, D., Frank, E.D., Wigmosta, M.S., Aden, A., Coleman, A.M., Pienkos, P.T., Skaggs, R.J., Venteris, E.R., Wang, M.Q. 2012. *Renewable Diesel from Algal Lipids: An Integrated Baseline for Cost, Emissions, and Resource Potential from a Harmonized Model*. ANL/ESD/12-4; NREL/TP-5100-55431; PNNL-21437. Argonne, IL: Argonne National Laboratory; Golden, CO: National Renewable Energy Laboratory; Richland, WA: Pacific Northwest National Laboratory.
23. Department of Energy. 2013. Algal Biofuels Strategy: Proceedings from the November 19-20, 2013 Workshop, Phoenix, AZ. US Department of Energy, Washington D.C. http://www.energy.gov/eere/bioenergy/downloads/algal-biofuels-strategy-workshop-fall-event Accessed 12.01.2014.

24. Department of Energy. 2014. Algal Biofuels Strategy: Proceedings from the March 26-27, 2014 Workshop, Charleston, South Carolina. US Department of Energy, Washington D.C. http://energy.gov/sites/prod/files/2014/06/f16/march2014_abs_proceedings.pdf. Accessed 08.05.2014.
25. De Vries, M., De Boer, I.J.M. 2010. Comparing environmental impacts for livestock products: A review of life cycle assessments. *Livest. Sci.* 128(1-3):1-11.
26. Dismukes, G.C. , Carrieri, D., Bennette, N., Ananyev, G.M., Posewitz. M.C. 2008. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels, *Curr. Opin. Biotechnol.* 19(3):235-240.
27. Elliott, D.C., T.R. Hart, A.J. Schmidt, G.G. Neuenschwander, L.J. Rotness, M.V. Olarte, A.H. Zacher, K.O. Albrecht, R.T. Hallen, and J. E. Holladay. Process development for hydrothermal liquefaction of algae feedstocks in a continuous flow reactor. Algal Research 2 (2013): 445–454.
28. Eixler, S., Karsten, U., Selig, U. 2006. Phosphorus storage in *Chlorella vulgaris* (Trebouxiophyceae, Chlorophyta) cells and its dependence on phosphate supply. Phychologia. 45(1): 53-60.
29. Eixler, S., Selig, U., Karsten, U. 2005. Extraction and detection methods for polyphosphate storage in autotrophic planktonic organisms. *Hydrobiologia.* 533: 135–143.
30. Eizenberg, E., Jabareen, Y. 2017. Social Sustainability: A New Conceptual Framework. *Sustainability*. 9(1): 68-84.

31. Energy Information Administration (EIA). 2016. Short-term Energy Outlook. June 7, 2016. https://www.eia.gov/forecasts/steo/report/global_oil.cfm. Accessed, June 10, 2016.

32. Fairfield-Sonn, J.W. 2016. Political Economy of GMO Foods. *J. Man. Policy and Practice.* 17(1):60-70.

33. Fehrenbacher, K. 2016. Solazyme Ditches Biofuels (& Name) in a World of Cheap Oil. *Fortune*. http://fortune.com/2016/03/16/solazyme-terravia-ditches-biofuels/. Accessed April 19, 2017.

34. Foley, P.M., Beach, E.S., Zimmerman, J.B. 2011. Algae as a source of renewable chemicals: opportunities and challenges. *Green Chem.* 13:1399-1405.

35. Food and Agricultural Organization of the United Nations (FAO). 2016. The State of World Fisheries and Aquaculture 2016. FAO Fisheries and Aquaculture Department .

36. Franco, M.C., Buffing, M.F., Janssen, M., Lobato, C.V., Wijffels, R.H. 2012. Performance of *Chlorella sorokiniana* under simulated extreme winter conditions. *J. Appl. Phycol.* 24(4): 693-699.

37. Frank, E.D., Han, J., Palou-Rivera, I., Elgowainy, A., Wang, M.Q. 2011a.User Manual for Algae Life-Cycle Analysis with GREET. Argonne National Laboratory ANL/ESD/11-7. https://greet.es.anl.gov/files/algae-life-cycle-manual Accessed 12/20/2014.

38. Frank, E.D., Han, J., Palou-Rivera, I., Elgowainy, A., Wang, M.Q. 2011b. Life-Cycle Analysis of Algal Lipid Fuels with the GREET Model. Argonne National

Laboratory. ANL/ESD/11-5. https://greet.es.anl.gov/files/algal-lipid-fuels Accessed 12/20/2014.

39. Fuhs, G.W. 1969. Phosphorus content and rate of growth in the diatoms *Cyclotella nana* and *Thalassiosira fluviatilis. J. Phycol.* 5: 312-321.
40. Goldman, J. C., Carpenter, E.J. 1974. A kinetic approach to the effect of temperature on algal growth. *Liminol. Oceanogr.* 19(5): 756-766.
41. Griffiths, M.J., Harrison, S.T.L. 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol.* 21(5):493-507.
42. Guieysse, B., Béchet, Q., Shilton , A. 2012. Variability and uncertainty in water demand and water footprint assessments of fresh algae cultivation based on case studies from five climatic regions. *Bioresour. Technol.* 128:317-323.
43. Hach Company. 2014. HACH Method 10127: Phosphorus, Total - Molybdovanadate with Acid Persulfate Digestion Method. DOC316.53.01123.
44. Hach Company. 2015. HACH Method 10072: Nitrogen, Total - Persulfate Digestion Method. DOC316.53.01085.
45. Hannon, M., Gimpel, J., Tran, M., Rasala, B., Mayfield, S. 2010. Biofuels from algae: challenges and potential. *Biofuels.* 1(5): 763-784.
46. Hasenheit, M., Gerdes, H., Kiresiewa, Z., Beekman, V. 2016. Summary report on the social, economic and environmental impacts of the bioeconomy. BioSTEP, February 2016.
47. Healy, F.P. 1977. Ammonium and urea uptake by some freshwater algae. *Can. J. Bot.* 55(1): 61-69.

48. Henderson, R.K., Parsons, S.A., Jefferson, B. 2010. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. *Water Res.* 44(12):3617-3624.
49. Hena, S., Fatimaha, S., Tabassumb, S. 2015. Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. *Water Resour. and Industry*. 10:1-14.
50. Higgins, B.T., Gennity, I., Samra, S., Kind, T., Fiehn, O., VenderGheynst, J.S. 2016. Cofactor symbiosis for enhanced algal growth, biofuel production, and wastewater treatment. *Algal Res.* 17:308-315.
51. Hindák, F., Komárek, J. 1968. Cultivation of the Cryosestonic Alga *Koliella tatrae (Kol) Hind. Biol. Plantarum.* 10(2):95-97.
52. Hoham, R.W. 1975. Optimum temperatures and temperature ranges for growth of snow algae. *Arct. Alp. Res.* 7(1): 13-24.
53. Hoham, R.W., Roemer, S.C., Mullet, J.E. 1979. The life history and ecology of the snow alga *Chloromonas brevispina* comb. nov. (Chlorophyta, Volvocales). *Phycologia*. 18(1):55-70.
54. Huber, G.W. , Iborra, S., Corma, A. 2006. Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. Chem. Rev. 106: 4044–4098.
55. Ismail, M.M., Essam, T.M., Ragab, Y.M., El-khair, A., El-Sayed, B., Mourad, F.E. 2017. Remediation of a mixture of analgesics in a stirred-tank photobioreactor using microalgal-bacterial consortium coupled with attempt to valorise the harvested biomass. Bioresour. Technol. 232:346-371.

56. Jimenez-Perez, M.V., Sanches-Castillo, P., Romera, O., Fernandez-Moreno, D., Perez-Martinez, C. 2004. Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. *Enzyme Microbial Technol.* 34:392-398.
57. Jones, S., Zhu, Y., Anderson, D., Hallen, R.,Elliott, D., Schmidt, A., Albrecht, K., Hart, T., Butcher, M., Drennan, C., Snowden-Swan. 2014. *Process Design and Economics for the Conversion of Algal Biomass to Hydrocarbons: Whole Algae Hydrothermal Liquefaction and Upgrading.* PNNL-23227. Richland, WA: Pacific Northwest National Laboratory.
58. Kalendová, A., Veselý, D., Brodinová, J. 2004. Anticorrosive spinel-type pigments of the mixed metal oxides compared to metal polyphosphates. Anti-Corrosion Methods and Materials, 51(1): 6-17.
59. Kazamiaa, E., Aldridge, D.C., Smith, A.G. 2012. Synthetic ecology – A way forward for sustainable algal biofuel production? J. Biotechnol. 162(1):163-169.
60. Klein-Marcuschamer, D., Turner, C., Allen, M., Gray, P., Dietzgen, R.G., Gresshoff, P.M., Hankamer, B., Heimann, K., Scott, P.T., Stephens, E., Speight, R., Nielsen, L.K. 2013. Technoeconomic analysis of renewable aviation fuel from microalgae, Pongamia pinnata, and sugarcane. *Biofuels, Bioprod. Biorefin.* 7(4):416-428.
61. Knothe, G. 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process. Technol*. 86(10): 1059-1070.
62. Konopka, A., Brock, T.D. 1978. Effect of Temperature on Blue-Green Algae (Cyanobacteria) in Lake Mendota. *Environ. Microbiol.* 36(4): 572-576.

63. Krauss, R.W. 1962. Mass Culture of Algae for Food and Other Organic Compounds. *Am. J. Bot.* 49(4):425-435.

64. Langholtz, M.A., Coleman, A.M., Eaton, L.M., Wigmosta, M.S., Hellwinckel, C.M., Brandt, C.C. 2016. Potential land competition between open-pond microalgae production and terrestrial dedicated feedstock supply systems in the U.S. *Renew. Energy.* 93:201-214.

65. Lau, P.S., Tam, N.F.Y., Wong, Y.S. 1998. Effect of carrageenan immobilization on the physiological activities of *Chlorella vulgaris*. *Bioresour. Techol*. 63: 115-121.

66. Laws, E.A., Taguchi, S., Hirata, J., Pang, L. 1986. High algal production-rates achieved in a shallow outdoor flume. *Biotechnol. Bioeng*. 28:191–197.

67. Lee, N., Amy, G., Croué, J.P., Buisson, H. 2004. Identification and understanding of fouling in low-pressure membrane (MF/UF) filtration by natural organic matter (NOM). *Water. Res.* 38(20):4511-4523.

68. Ling, H.U. 2001. Snow algae of the windmill islands, continental Antarctica: Desmotetra Aureospora, Sp. Nov. and D. Antartica, Comb. Nov. (Chlorophyta). *J. Phycol.* 37(1):160–174.

69. Ling, H.U., Seppelt, R.D. 1990. Snow algae of the Windmill Islands, continental Antarctica. *Mesotaenium berggrenii* (Zygnematales, Chlorophyta) the alga of grey snow. *Antarct. Sci.* 2(2):43-48.

70. Ling, H.U., Seppelt, R.D. 1993. Snow algae of the Windmill Islands, continental Antarctica. 2. Chloromonas rubroleosa sp. nov. (Volvocales, Chlorophyta), an alga of red snow. *Eur. J. Phycol.* 28:77-84.

71. Ling, H.U., Seppelt, R.D. 1998. Snow algae of the Windmill Islands, continental Antarctica. 3. *Chloromonas polyptera* (Volvocales, Chlorophyta). *Polar Biol.* 20:320-324.
72. Lomas, M.W., Gilbert, P.M. 1999. Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. *Limnol. Oceanogr.* 44(3):556-572.
73. MacIsaac, J.J., Dugdale, R.C. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. *Deep-Sea Res.* 19:209-232.
74. Martínez, M.E., Jiménez, J.M., El Yousfi, F. 1999. Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga *Scenedesmus obliquus. Process Biochem.* 34(8):811-818.
75. Martínez Sancho, M.E., Jiménez Castillo, J.M., El Yousfi, F. 1999. Photoautotrophic consumption of phosphorus by *Scenedesmus obliquus* in a continuous culture. Influence of light intensity, *Process Biochem.* 34(8): 811-818.
76. McKenzie, Stephen. 2004. Social Sustainability: Towards Some Definitions. Hawke Research Institute, Magill, South Africa.
77. Milledge, J.J. 2010a. Commercial Production of microalgae other than as biofuels: a brief review. *Rev. Environ. Sci. Bio.* 10(1):31-41.
78. Milledge, J.J. 2010b. The potential yield of microalgal oil. *Biofuels. Int*. 4(2):44–45.
79. Milledge J.J., Heaven, S. 2011. Disc stack centrifugation separation and cell disruption of microalgae: a technical note. *Environ. Nat. Resour. Res*. 1(1):17–24.

80. Milledge, J.J., Heaven, S. 2013. A review of the harvesting of micro-algae for biofuel production. *Rev. Environ. Sci. Biotechnol.* 12:165-178.

81. Mohn, F. 1988. Harvesting of micro-algal biomass. In: Borowitzka LJ, Borowitzka MA (eds) *Micro-algal biotechnology*. Cambridge University Press, Cambridge

82. Molina Grima, E., Belarbi, E.H., Acien-Fernandez, F.G., Robles-Medina, A., Yusuf, C. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol. Adv.* 20(7–8):491–515.

83. Morgan, J. 2011. Economic downturn, imports hurt catfish industry. *Delta Farm Press.* March 25, 2011.

84. Morgan-Kiss, R., Ivanov, A.G., Williams, J., Mobashsher, K,. Huner, N.P. (2002) Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid mem- branes of a psychrophilic and a mesophilic alga. *Biochim. Biophys. Acta* 1561:251–265.

85. Mueller, S.A., Anderson, J.E., Wallington, T.J. 2011. Impact of biofuel production and other supply and demand factors on food price increases in 2008. *Biomass Bioenerg.* 35(5): 1623-1632.

86. Munsell, J.F., Fox, T.R. 2010. An analysis of the feasibility for increasing woody biomass production from pine plantations in the southern United States. *Biomass Bioenergy*. 34(12):1631-1642.

87. Nagarajan, S., Chou, S.K., Cao, S., Wu, C., Zhou, Z. 2013. An updated comprehensive techno-economic analysis of algae biodiesel. *Bioresour. Technol.* 145:150-156.

88. National Alliance for Advanced Biofuels and Bio-Products (NAABB). 2014. Full Final Report. U.S. Department of Energy, Office of Energy Efficiency & Renewable Energy. http://energy.gov/eere/bioenergy/downloads/national-alliance-advanced-biofuels-and-bioproducts-synopsis-naabb-final. Accessed 08.05.2014.
89. National Renewable Energy Laboratory. 2012. National Solar Radiation Database. http://rredc.nrel.gov/solar/old_data/nsrdb/. Accessed 09.28.2014.
90. Nelson, D.R., Mengistu, S., Ranum, P., Mashek, M., Mashek, D., Lefebvre, P.A. 2013. New Lipid-Producing, Cold-Tolerant Yellow-Green Alga Isolated from the Rocky Mountains of Colorado. *Biotechnol. Prog.* 29(4):853-861.
91. Olofsson, M. Lamela, T. Nilsson, E., Bergé, J.P., del Pino, V, Uronen, P. 2012. Seasonal Variation of Lipids and Fatty Acids of the Microalgae *Nannochloropsis oculata* Grown in Outdoor Large-Scale Photobioreactors. *Energies.* 5:1577-1592.
92. Ozkan, A., Kinney, K., Katz, L., Berberoglu, H. 2012. Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor. *Biorsour. Technol.* 114:542-548.
93. Palma, H., Killoran, E., Sheehan, M., Berner, F., Heimann. K. 2017. Assessment of microalga biofilms for simultaneous remediation and biofuel generation in mine tailings water. Bioresour. Technol. 234:327-335.
94. Passero, P.K., Dunn, G.P., Passero, M., Feris, K.P. 2017. Free ammonia offers algal crop protection from predators in dairy wastewater and ammonium-rich media. *Bioresour. Technol.* 243: 724-730.

95. Pienkos, P.T., Darzins, A. 2009. The promise and challenges of microalgal-derived biofuels. *Biofuels, Bioprod. Bioref.* 3:431–440.
96. Pittman, J.K., Dean, A.P., Osundeko, O. 2011. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol.* 102(1):17-25.
97. Posadas, E. Muñoz, R., Guieysse, B. 2017. Integrating nutrient removal and solid management restricts the feasibility of algal biofuel generation via wastewater treatment. *Algal Res.* 22:3946.
98. Powell, N., Shilton, A., Pratt, S., Christi, Y. 2008. Factors Influencing Luxury Uptake of Phosphorus by Microalgae in Waste Stabilization Ponds. *Environ. Sci. Technol.* 42: 5958–5962.
99. Powell, N., Shilton, A., Pratt, S., Christi, Y. 2011. Luxury uptake of phosphorus by microalgae in full-scale waste stabilisation ponds. *Water. Sci. Technol.* 63(4): 704-709.
100. Priscu, J.C., Palmisano, A.C., Priscu, L.R., Sullivan, C.W. 1989. Temperature dependence of inorganic nitrogen uptake and assimilation in Antarctic sea-ice. *Polar Biol*. 9(7):443-446.
101. Quinn, J.C., Davis, R. 2015. The potentials and challenges of algae based biofuels: A review of the techno-economic, life cycle, and resource assessment modeling. *Bioresour. Technol.* 184: 444-452.
102. R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

103. Radakovis, R., Jinkerson, R.E., Darzins, A., Posewitz, M.C. 2010. Genetic Engineering of Algae for Enhanced Biofuel Productions. *Eukaryot. Cell*. 9(4):486–501.

104. Rawat, I., Kumar, R.R., Mutanda, T., Bux, F. 2011. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Appl. Energy*. 88(10):3411-3424.

105. Reay, D.S., Nedwell, D.B., Priddle, J., Ellis-Evans, J.C. 1999. Temperature Dependence of Inorganic Nitrogen Uptake: reduced Affinity for Nitrate at Suboptimal Temperatures in Both Algae and Bacteria. Appl. Environ. Microbiol. 6:2577-2584.

106. Remias, D., Wastian, H., Lütz, C., Leya, T. 2013. Insights into the biology and phylogeny of Chloromonas polyptera (Chlorophyta), an alga causing orange snow in Maritime Antarctica. *Antarct. Sci.* 25(5):648-656.

107. Řezanka, T., Nedbalová, L., Sigler, K. 2008. Unusual medium-chain polyunsaturated fatty acids from the snow alga Chloromonas brevispina. *Microbiol. Res.* 163:373—379.

108. Rösch, C., J. Skarka, and N. Wegerer. 2012. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. *Bioresour. Technol.* 107:191-199.

109. Richardson, J.W. Johnson, M.D. 2015. Financial Feasibility analysis of NAABB developed technologies. *Algal Res.* 10:16-24.

110. Richardson, J.W., Johnson. M.D., Lacey, R., Oyler, J., Capareda, S. 2014. Harvesting and extraction technology contributions to algae biofuels economic viability. *Algal Res.* 5: 70-78.

111. Richardson, J.W., Johnson, M.D., Outlaw. J.L. 2012. Economic comparison of open pond raceways to photo bio-reactors for profitable production of algae for transportation fuels in the southwest. *Algal Res.* 1(1):93–100.

112. Rossignol, N., Vandajon, L., Jaouen, P., Quéméneur, F. 1999. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. *Aquacult. Eng.* 20(3):191-208.

113. Roukaerts, A., Cavagna, A.J., Fripat, F., Lannuzel, D., Meiners, K., Dehairs, F. 2015. Sea-ice algal primary production and nitrogen uptake rates off East Antarctica. Deep Sea Research Part II: Topical Studies in Oceanography. Elsevier, Amsterdam, Netherlands.

114. Ryther, J-H. 1959 Potential productivity of the sea. *Science.* 130:602–608.

115. Sander, K., Murthy, G.S. 2010. Life cycle analysis of algae biodiesel. *Int. J. Life Cycle Assess.* 15: 704-714.

116. Schnepf, R., Yacobucci, B.D. 2013. *Renewable Fuel Standard (RFS): Overview and Issues.* Congressional Research Service publication #R40155.

117. Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., Smith, A.G. 2010. Biodiesel from algae: challenges and prospects. *Curr Opin Biotechnol.* 21(3):277-86.

118. Selvaratnam, T., Pegallapati, A., Montelya, F., Rodriguez, G., Nirmalakhandan, N., Lammers, P.J., VanVoorhies, W. 2015. Feasibility of algal systems for sustainable wastewater treatment. *Renew. Energ.,* 82:71-76.

119. Sheehan, J., Dunahay, T., Benemann, J., Roessler, P. 1998. Look back at the U.S. Department of Energy's Aquatic Species Program: biodiesel from algae; Close-Out Report. NREL Report No. TP-580-24190.

120. Shilton, A.N., Powell, N., Guieysse, B. 2012. Plant based phosphorus recovery from wastewater via algae and macrophytes. *Curr. Opin. Biotechnol.* 23:884–889.

121. Shock, C., Kathy, P. 2003. Phosphorus Effects on Surface Water Quality and Phosphorus TMDL Development. Western Nutrient Management Conference 5:211.

122. Singh, A., Olsen, S.I. 2010. A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels. Appl. Energ. 88(10): 3548-3555.

123. Singh, M., Das, K.C. 2013a. Low Cost Nutrients for Algae Cultivation. In Bajpai, R., Prokop, A., Zappi, M. (eds.). *Algal Biorefineries Volume 1: Cultivation of Cells and Products*. (pp. 69-82). New York, NY: Springer.

124. Slade, R., Bauen, A. 2013. Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. *Biomass Bioenerg.* 53:29-38.

125. Stibal, M., Elster, J. 2005. Growth and morphology variation as a response to changing environmental factors in two Arctic species of *Raphidonema* (*Trebouxiophyceae*) from snow and soil. *Polar Biol.* 28:558-567.

126. Sturm, B.S.M., Lamer, S.L. 2010. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. *Appl. Energy.* 88(10):3499-3506.

127. Sverdup, H.U., Koca, D., Ragnarsdóttir. K.V. 2012. Peak Metals, Minerals, Energy, Wealth, Food and Population; Urgent Policy Considerations for A Sustainable Society. *J. Environ. Sci. Eng.* 5:499-533.

128. Takeuchi, N. 2001. The altitudinal distribution of snow algae on an Alaska glacier (Gulkana Glacier in the Alaska Range). *Hydrol. Process.* 15:3447– 459.

129. Takeuchi, N. 2013. Seasonal and altitudinal variations in snow algal communities on an Alaskan glacier (Gulkana glacier in the Alaska range). *Environ. Res. Lett.* 8(3):1-10.

130. Tam, N.F.Y., Wong, Y.S. 1994. Feasibility of using *Chlorella pyrenoidosa* in the removal of inorganic nutrients from primary settled sewage. Algal Biotechnology in the Aida-Pacific region. University of Malaya. pp. 291-299.

131. Teoh, M.L., Phang, S.M. Chu, W.L. 2013. Response of Antarctic, temperate, and tropical microalgae to temperature stress. *J. Appl. Phycol.* 25:285–297.

132. Ullah, K. Ahmad, M., Sofia, Sharma, V.K., Lu, P. Harvey, A., Zafar, M., Sultana, S., Anyanwu, C.N. 2014. Algal biomass as a global source of transport fuels: Overview and development perspectives. *Prog. Nat. Sci.* 24(4):329-339.

133. Van Wagenen, J., Miller, T.W., Hobbs, S., Hook, P., Crowe, B., Huesemann, M. 2012. Effects of Light and Temperature on Fatty Acid Production in *Nannochloropsis Salina.* Energies 5:731-740.
134. Venteris, E.R., McBride, R.C., Coleman, A.M., Skaggs, R.L., Wigmosta, M.S. 2014a. Siting Algae Cultivation Facilities for Biofuel Production in the United States: Trade-Offs between Growth Rate, Site Constructability, Water Availability, and Infrastructure. Environ. Sci. Techol. 48(6):2559-3566.
135. Venteris, E.R., Wigmosta, M.S., Coleman, A.M., Skaggs, R.L. 2014b. Strain selection, biomass to biofuel conversion, and resource colocation have strong impacts on the economic performance of algae cultivation sites. *Front. Ener. Res*. 2:37.
136. Wiley, P., Brenneman, K., Jacobson, A. 2009. Improved Algal Harvesting Using Suspended Air Flotation. *Water Environ. Res.* 81:702–8.
137. Wiley, P E., Campbell, J.E., McKuin, B. 2011. Production of Biodiesel and Biogas from Algae: A Review of Process Train Options. *Water Environ. Res.* 83(4):326-338.
138. Weyer, K.M., Bush, D.R., Darzins, A., Willson, B.D. 2010. Theoretical Maximum Algal Oil Production. *Bioenerg. Res.* 3:204–213.
139. Wikfors, G.H., Ohno, M. 2001. Impact of Algal Research in Aquaculture. *J. Phycol.* 37(6):968-974.
140. Wu, Y.H., Yu, Y., Li, X., Hu, H.Y., Su, Z.F. 2012. Biomass production of a Scenedesmus sp. under phosphorous-starvation cultivation condition, *Bioresour. Technol.* 112: 193-198.

141. Xin, C., Addy, M.M., Zhao, J., Cheng, Y., Cheng, S., Mu, D., Liu, Y., Dinga, R., Chen, P., Ruan, R. 2016. Comprehensive techno-economic analysis of wastewater-based algal biofuel production: A case study. *Bioresour. Technol.* 211:584-593.

142. Xu M., Bernards M., Hu Z. 2014. Algae-facilitated chemical phosphorus removal during high-density Chlorella emersonii cultivation in a membrane bioreactor. *Bioresour Technol.* 153:383-387.

143. Zhang, X., Hu, Q., Sommerfield, M., Puruhito, E., Chen, Y. 2010. Harvesting algal biomass for biofuels using ultrafiltration membranes. *Bioresour. Technol.* 101(14):5297-5304.