THE POTENTIAL OF FRESHWATER MACROALGAE AS A BIOFUELS FEEDSTOCK AND THE INFLUENCE OF NUTRIENT AVAILABILITY ON FRESHWATER MACROALGAL BIOMASS PRODUCTION

By

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Submitted to the graduate degree program in Ecology and Evolutionary Biology and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.

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Date Defended: 24 November 2014

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ABSTRACT

Extensive efforts have been made to evaluate the potential of microalgae as a biofuel feedstock during the past 4-5 decades. However, filamentous freshwater macroalgae have numerous characteristics that favor their potential use as an alternative algal feedstock for biofuels production. Freshwater macroalgae exhibit high rates of areal productivity, and their tendency to form dense floating mats on the water surface imply significant reductions in harvesting and dewater costs compared to microalgae.

In Chapter 1, I reviewed the published literature on the elemental composition and energy content of five genera of freshwater macroalgae. This review suggested that freshwater macroalgae compare favorably with traditional bio-based energy sources, including terrestrial residues, wood, and coal. In addition, I performed a semi-continuous culture experiment using the common Chlorophyte genus *Oedogonium* to investigate whether nutrient availability can influence its higher heating value (HHV), productivity, and proximate analysis. The experimental study suggested that the most nutrient-limited growth conditions resulted in a significant increase in the HHV of the *Oedogonium* biomass (14.4 MJ/kg to 16.1 MJ/kg). Although there was no significant difference in productivity between the treatments, the average dry weight productivity of *Oedogonium* (3.37 $g/m^2/day$) was found to be much higher than is achievable with common terrestrial plant crops.

Although filamentous freshwater macroalgae, therefore, have significant potential as a renewable source of bioenergy, the ultimate success of freshwater macroalgae as a biofuel feedstock will depend upon the ability to produce biomass at the commercial-scale in a cost-effective and sustainable manner. Aquatic ecology can play an important role to achieve the scale-up of algal crop production by informing the supply rates of nutrients to the cultivation systems, and by helping to create adaptive production systems that are resilient to environmental

change. In Chapter 2, I performed a review and an analysis of data from the published literature on the large-cultivation of freshwater macroalgae. This study revealed that the large-scale cultivation of freshwater macroalgae is feasible at relatively low cost using currently available technologies such as the Algal Turf Scrubber system (ATS). In addition, graphical analyses of published data obtained from ATS systems of varying sizes in operation worldwide revealed that both macroalgal biomass productivity and nutrient removal rates are hyperbolically related to the areal loading rates of both total nitrogen and total phosphorus. An assessment of the limited existing literature on carbon dioxide amendments suggested that the effectiveness and need for CO_2 supplementation of macroalgal production systems like the ATS has not yet been conclusively demonstrated.

Overall, this thesis demonstrates that filamentous freshwater macroalgae have great potential as a feedstock for both liquid and solid fuels, especially if nutrient-rich wastewater can be used as the supply of water and mineral nutrients. In addition, this thesis highlights the importance of studying the algal cultivation conditions that influence trade-offs between nutrient loading, biomass productivity, and biomass energy content. In particular, the hyperbolic relationship between algal biomass productivity and the areal loading rates of both total nitrogen and total phosphorus should provide critical insight when considering the production costs of macroalgal biomass at the commercial-scale.

ACKNOWLEDGEMENTS

First of all, I would like to thank my advisor, Dr. Val Smith, for his generous support and infinite patience during my time in his lab. Without his guidance, it would be impossible for me to complete this thesis. My experiences in the Smith lab not only provided me a unique chance to explore multiple disciplines regarding algal biofuels research, but also inspired me to pursue this research further in the future. I also would like to thank my committee members, Dr. Jerry deNoyelles and Dr. Christopher Depcik. Dr. deNoyelles always shared his great expertise in aquatic ecology and phycology, and his insight helped me to overcome many difficulties in this project. In addition, Dr. Depcik provided me a chance to have hands-on experience in engineering project about testing the combustion characteristics of algal biomass. His encouragement made my interaction with the KU engineering department more valuable.

I thank many colleagues and mentors for their help. I thank Griffin Roberts and Dr. Susan Williams for providing the HHV and proximate analysis data of freshwater macroalgal biomass presented in Chapter 1. I also thank Taylor Patterson for his help in performing the experiment and literature review in Chapter 1. In addition, Troy Jensen kindly helped me in fieldwork. I thank Scott Campbell, Dean Kettle, Bruce Johanning, and Vaughn Salisbury for their support in my work at KUFS. I also benefited from the interaction with teachers and students involved in the NSF-sponsored RET program during the summers of 2012 and 2014. Working with these outstanding teachers and students provided me an opportunity to learn how to work as a group.

Financial support during my time at KU has come from the KU Field Station Small Grants and Kansas EPSCoR (Award Number: EPS-0903806). Especially, I am grateful to Dr. Judy Wu and the Kansas EPSCoR for generous GRA support.

Finally, I would like to thank my family, Mr. Jeong-Gu Yun, Mrs. Kyung-Ae Park, and Mr. Jin-Sung Yun. Their selfless support and love are the reasons I can stand where I am.

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GENERAL INTRODUCTION

Algae have long been viewed as a promising source for biofuels feedstock with the capacity for higher productivity per unit of land area than conventional terrestrial crops (Sheehan et al., 1998; Chisti, 2007; United States Department of Energy, 2010; Pate et al., 2011; Yun et al., 2014). Both marine and freshwater microalgae have great potential as a feedstock for biofuels production because of their rapid growth rates and high aerial productivity; their ability to sequester waste CO_2; and their strong potential for mass cultivation on marginal lands that do not compete with agriculture (Smith et al., 2010; Liu, 2014). Moreover, the use of nutrient-rich wastewater as a nutrient source for algal cultivation could increase the environmental sustainability of this process and reduce wastewater discharges of nitrogen and phosphorus (Sturm et al. 2012).

While the majority of algal biofuel research to date has focused on strain selection and optimizing the productivity of microalgae, macroalgae also have been proposed both as feedstocks for diverse biomass applications and as targets for liquid and solid fuels production (Yun et al., 2014; Chisti and Yan, 2011; Grayburn et al., 2013; Lawton et al., 2013). However, these efforts have primarily involved marine seaweeds and filamentous cyanobacteria, with a much lesser focus on the use of freshwater macroalgae (primarily members of the eukaryotic Chlorophyta) (Yun et al., 2014; Grayburn et al., 2013; Lawton et al., 2013; Demirbas, 2010; Khola and Ghazala, 2012). Nonetheless, freshwater macroalgae may have significant potential for liquid and solid biofuels that can be combusted directly, or co-combusted with more traditional energy sources (Grayburn et al., 2013; Tumuluru et al., 2012). In particular, their tendency to form dense floating mats on the water surface potentially makes biomass harvesting much more cost-efficient than dewatering an equivalent biomass of suspended microalgae (Grayburn et al., 2013; Hillebrand, 1983). Moreover, the large-scale cultivation of freshwater macroalgae is also feasible at relatively low cost using currently available technologies, such as the algal turf scrubber (ATS).

In this thesis, I explore the potential of freshwater macroalgae as a biofuels feedstock and the importance of nutrient supply on macroalgal biomass production. I first review the biofuels potential of multiple freshwater macroalgae species (Chapter 1). Second, I explore how nutrient availability during cultivation influences macroalgal biomass productivity ($g/m^2/day$) and energy content (expressed as the higher heating value, HHV), as well as the biomass parameters of proximate analysis [moisture (wt%), volatiles (wt%), combustibles (wt%), and ash (wt%)] (Chapter 1). Finally, I critically explore the pivotal role of inorganic nutrients (nitrogen, phosphorus, and carbon dioxide) in determining algal biomass productivity by freshwater macroalgae (Chapter 2).

CHAPTER 1: Freshwater macroalgae as a biofuels feedstock: mini-review and assessment of their bioenergy potential

INTRODUCTION

The urgencies of lowering greenhouse gas emissions and reducing the risk of critical disruptions in the world's energy supplies have spurred research and investments in the development of alternatives to fossil fuels (U.S. Department of Energy, 2013; National Research Council of the National Academies, 2012). Both marine and freshwater microalgae have great potential as a feedstock for biofuels production because of their rapid growth rates and high aerial productivity; their ability to sequester waste CO_2; and their strong potential for mass cultivation on marginal lands that do not compete with agriculture (Smith et al., 2010; Liu, 2014). Moreover, the use of nutrient-rich wastewater as a nutrient source for algal cultivation could increase the environmental sustainability of this process and reduce wastewater discharges of nitrogen and phosphorus (Sturm et al. 2012).

Although much of algal biofuel research has focused upon strain selection and optimizing the productivity of microalgae, macroalgae also can be used as a bioenergy feedstock, and they have been proposed for diverse biomass applications and as targets for a broad range of liquid biofuels (Chisti and Yan, 2011; Grayburn et al., 2013; Lawton et al., 2013). However, these liquid biofuels efforts have primarily involved marine seaweeds and filamentous cyanobacteria, with a much lesser focus upon the use of filamentous freshwater macroalgae (Division Chlorophyta) (Grayburn et al., 2013; Lawton et al., 2013; Demirbas, 2010; Khola and Ghazala, 2012).

In addition, biomass stands as the third largest energy resource in the world, behind only coal and oil (Tumuluru et al., 2011; Bapat et al., 1997). There is growing international interest in the use of biomass for power generation, because of its potential environmental benefits and long-term sustainability. Moreover, because newly-created biomass is considered to be carbon neutral, it can significantly reduce net carbon emissions and negative environmental impacts when it replaces coal or other fossil fuels (Tumuluru et al., 2012). For example, a recent analysis indicated that electricity generation from plant biomass-derived bioenergy sources increased 71.6% between 2011 and 2013 in the United Kingdom alone (Department of Energy and Climate Change, 2013). We suggest that in addition to serving as a feedstock for liquid fuels, macroalgae also have significant potential for the production of solid biofuels that can be combusted directly, or co-combusted with more traditional energy sources such as wood or coal.

Several authors have recently promoted the potential of freshwater macroalgae as a biofuels feedstock. For example, Grayburn et al. produced biodiesel from a mixed-species assemblage of *Cladophora* and *Rhizoclonium*, and a B5 blend of this algal biodiesel with commercial petrodiesel revealed fuel efficiency and exhaust emissions characteristics that were similar to 100% petrodiesel (Grayburn et al., 2013). In addition, Lawton et al. measured the productivity and higher heating value (HHV) of three common freshwater macroalgal species, and concluded that their high bioenergy potential makes them an under-utilized biomass source (Lawton et al., 2013).

Freshwater macroalgae, thus, may have significant potential as a biofuels feedstock. In particular, their tendency to form dense floating mats on the water surface potentially makes biomass harvesting much more cost-efficient than dewatering an equivalent biomass of suspended microalgae (Grayburn et al., 2013; Hillebrand, 1983). Moreover, we suggest that the large-scale cultivation of freshwater macroalgae is also feasible at relatively low cost using currently available technologies such as the algal turf scrubber (ATS). The ATS is an engineered system for wastewater nutrient removal that uses sloped flow-ways seeded with multiple filamentous macroalgae taxa, including the common freshwater genera *Oedogonium,* *Rhizoclonium, Ulothrix*, and *Microspora* (*Figure 1)* (Adey et al., 2011; Kebede-Westhead et al., 2003; Mulbry et al., 2008b; Pizarro et al., 2006).

Only a relatively small number of studies have examined the potential of freshwater macroalgae as a biofuels feedstock to date, however, many groups of common freshwater macroalgae remain to be tested for the proximate analysis and elemental composition of their biomass (Lawton et al., 2013; Tumuluru et al., 2012). In addition, because industrial-scale biofuel applications will require large quantities of biomass, it is important to explore the degree to which the biomass productivity and energy contents of freshwater macroalgae are affected by their growth environment, especially with regards to key factors such as nutrient and light availability, temperature, and salinity (Smith et al., 2010; Sturm et al., 2012; Shurin et al., 2013). In particular, many eukaryotic algae accumulate lipids in their cells under conditions of nutrient limitation, but exhibit trade-offs between the yields and productivity of algal biomass versus its lipids content (Shurin et al., 2013; Subramanian et al., 2013). Moreover, because the higher energy densities of algal biomass are typically associated with higher lipid contents, there are general trade-offs between the yields and productivity of algal biomass versus energy content (Shurin et al., 2013; Subramanian et al., 2013). Studying the trade-offs between biomass productivity and energy content can provide additional new insights into optimizing net energy yield from freshwater macroalgal cultivation systems. In this study, we conducted both a literature review and preliminary laboratory experiments (1) to review the biofuels potential of multiple freshwater macroalgae species, and (2) to explore how nutrient availability during cultivation influenced their biomass productivity (g/m^2/day) and energy content (expressed as the higher heating value, HHV). In addition, we performed a thermal gravimetric analysis (TGA) for the assessment of moisture (wt%), volatiles (wt%), combustibles (wt%), and ash (wt%) contents of the freshwater macroalgal biomass to explore the effects of growth conditions on these additional biomass parameters.

MATERIALS AND METHODS

Proximate Analysis and Elemental Composition of Freshwater Macroalgae

Electronic databases including Science Direct, Springer Link, and JSTOR were consulted to locate publications on chemical composition of freshwater macroalgae. Search terms included *freshwater macroalgae*, *elemental composition*, *higher heating value* (HHV), and *proximate analysis*. As discussed by Lawton et al., HHV was used to estimate the biofuel potential of these biomass feedstocks (Lawton et al., 2013). Once suitable references were identified, all HHV values were converted to consistent units (MJ/kg), and the data obtained from proximate biomass analyses using ASTM-established procedures (moisture, ash, volatile matter, and fixed carbon) were expressed as a percent of the total biomass of the sample (wt%; see Table 1 legend). In addition, the elemental contents of C, H, O, N, and S were expressed as percent oven-dry weight (dw%, where the percent weight of CHONS and ash content sums to 100%).

Freshwater macroalgae are taxonomically diverse, and commonly observed species include representatives from the genera *Cladophora*, *Enteromorpha*, *Hydrodictyon, Microspora*, *Mougeotia*, *Oedogonium*, *Rhizoclonium*, *Spirogyra,Tribonema*, *Ulothrix*, *Vaucheria*, and *Zygnema* (Hillebrand, 1983). Among these 12 commonly observed genus of freshwater macroalgae, we were able to obtain data for the higher heating value (HHV) and ash contents of *Cladophora, Oedogonium, Spirogyra*, *Pithophora*, and *Hydrodictyon*. Although data for proximate analysis and CHONS elemental composition measurements were unfortunately not available for all 12 of these species, literature information for the moisture, fixed carbon, volatile matter, and elemental composition of *Cladophora* and *Spirogyra* was found. In addition, data for the elemental composition of *Oedogonium* were obtained. These pooled data were then compared to traditional energy sources summarized by Demirbas and Tumuluru et al., including agricultural and food processing residues, wood, and coal (Tumuluru et al., 2012; Demirbas, 2003).

Freshwater Macroalgae Cultivation under Different Nutrient Supply Rates

We performed three separate14-day experiments in order to examine the productivity and energy content of a natural freshwater macroalgae assemblage dominated by *Oedogonium* sp. in the summer of 2012. We cultivated these algae in 5 L volume shallow trays subjected to four different dilution rates using pre-chlorination effluent obtained from the City of Lawrence, KS, wastewater treatment plant (WWTP) as a growth medium. This pre-chlorination wastewater contained an average of 20.4 ± 4.6 mgN/L and 3.5 ± 0.9 mgP/L of total nitrogen and phosphorus, respectively (Sturm et al., 2012). The majority of the total nitrogen (TN) (96%) and total phosphorus (TP) (92%) in the wastewater supply was present in the dissolved form, and thus was bioavailable for the algal cells (Sturm et al., 2012).

At the beginning of each experiment, each of the four trays was inoculated with 10 g fresh weight of a mixed-species inoculum of freshwater macroalgae obtained from a wastewater effluent-fed stock tank maintained year-round in the University of Kansas greenhouse. Almost 90% of the biomass inoculum was composed of species from the genus *Oedogonium*, but representatives from the genera *Spirogyra*, *Ulothrix*, and *Vaucheria* were observed as well. Because we inoculated the experimental system with naturally-occurring macroalgae, bacteria were present, but these bacteria only represented a minor component of the total cultivated biomass. After inoculation, each 5L culture tray was assigned one (and only one) of four different wastewater dilution rate treatments (0.1 L/day, 0.5 L/day, 1.0 L/day, 2.5 L/day) to create a strong resource supply gradient; slower dilution rates correspond to lower nutrient supply rates, and thus to a stronger degree of nutrient limitation. Each day the specified water volume of used growth medium was carefully removed from each tray, and was replaced with an equal volume of fresh pre-chlorination wastewater from the Lawrence, KS, WWTP. No macroalgal biomass was removed during the medium replacement process, and no significant growth of contaminating microalgae or other microbes was observed.

All four tray cultures were maintained at an operating volume of 5 L with small daily additions of distilled water to replace evaporative losses, and were illuminated at 1520 μE m^{-2} s^{-1} with a LED light on a 12:12 light cycle. After 14 days of cultivation, each of the four trays was separately harvested and the fresh weight (g FW) of the freshwater macroalgae was measured after gentle centrifugation for 3 minutes to remove extracellular water. The harvested biomass was then oven-dried at 65°C for 24 h to measure dry weight yields from the four macroalgae assemblages. The respective dry weight productivities from each tray were then calculated using the equation $P = (B_f - B_i)/A/T$, where B_f and B_i are the final and initial dry weight values (g dry wt.), A is the water surface area (0.06 m^2) of the culture trays, and T is the total number of days in culture (14 days). The higher heating value (HHV) of dried biomass subsamples from each tray was measured in triplicate with a Parr 6200 calorimeter, using decane (99+%; Fisher) as a combustion agent. In addition, thermal gravimetric analysis (TGA) was employed for the assessment of moisture, volatiles, combustibles, and ash content. The proximate analysis was conducted in triplicate with a SDT-Q600 (Module DSC- TGA) from TA Instruments. Samples of 10-15 mg were placed in a tared aluminum ceramic crucible, heated at a rate of 10 °C/min to 850 °C with a carrier flow rate of nitrogen at 100 mL/min, cooled to 120 °C, switched to air, and then heated again to 850 °C with the same gas flow and heating rates. The software method for the TGA instrument was programmed to suspend the heating ramp around 100 °C and 850 °C until the weight change was <0.01 %/min to ensure the accuracy of the proximate analysis. Sample moisture contents were determined at temperature of 110 °C ; volatiles (or pyrolysis content) were determined by moisture minus the end value of the nitrogen cycle; fixed carbon (or post pyrolysis combustibles) were determined by volatiles minus the end-value of the air cycle; and ash was determined as the percent weight remaining.

Two interval plots with 95% confidence interval for the mean were created to test for statistically significant differences between the HHV and productivity values obtained from the four different dilution treatments. Similarly, graphs were constructed to represent the weight and derivative of weight curves for the TGA. We then compared the dry weight productivity of these four macroalgal assemblages with productivity data for terrestrial crops obtained from U.S. Department of Agriculture records (United States Department of Agriculture, 2012).

RESULTS AND DISCUSSION

Proximate Analysis and Elemental Composition of Freshwater Macroalgae

Table 1 and *Table 2* provide the proximate analysis and elemental composition data for multiple species of filamentous freshwater macroalgae, terrestrial biomass residues, wood, and coal. The fixed carbon and volatile matter contents of *Spirogyra* were typically higher than wood, but were comparable to terrestrial biomass residues; however, the fixed carbon content of *Cladophora* (0.98 wt%) was the lowest among all of the biomass feedstocks. In contrast, the ash contents of freshwater macroalgae were highly variable. Our database suggested *Oedogonium* had the lowest ash content (3.7 wt%) among the filamentous freshwater macroalgae, whereas *Cladophora* and *Pithophora* had higher ash contents than the other species.

Differences in bioenergy potential of biomass feedstocks were reflected in their higher heating values (HHV), and *Figure 2* compares the HHVs of freshwater macroalgae, terrestrial biomass residues, typical wood, and coal. The HHVs of traditional biomass ranged from 10.73 MJ/kg to 28.17 MJ/kg, and overlapped with the HHVs for freshwater macroalgae, which ranged from 12.10 MJ/kg to 22.34 MJ/kg. *Spirogyra* had the highest HHV of 22.34 MJ/kg and *Oedogonium* had the second highest HHV of 20.10 MJ/kg. These values were almost twice as high as the typical HHV of wood (11.86 MJ/kg). The data in *Figure 2*, thus, suggest that filamentous freshwater macroalgae have HHV levels that are comparable to traditional biomass, and confirm that they have promising bioenergy potential.

It is usually expected that the biomass contains less carbon and more oxygen, and has a low heating value, in comparison with coal (Tumuluru et al., 2012). The review presented here suggests that *Spirogyra* and *Cladophora* had lower carbon and higher oxygen contents than agricultural and food processing biomass residues. However, the carbon and oxygen contents of freshwater macroalgae appear to be comparable to wood. Among the three genera of freshwater macroalgae studied here, *Oedogonium* had the highest carbon content (46.81 dw%), and *Spirogyra* had the lowest oxygen content (5.83 dw%).

Nitrogen, sulfur, and ash contents also varied significantly among the freshwater macroalgae studied. *Oedogonium* had the lowest ash content (3.7 wt%) and the lowest sulfur content (0.1 dw%); in contrast, *Cladophora* had the lowest nitrogen content, but the highest sulfur content (3.96 dw%). Although *Spirogyra* had low amount of sulfur (0.5 dw%), and an intermediate ash content (13.99 wt%), it had the highest nitrogen content (41.59 dw%). Both terrestrial biomass (all residues and wood) and the macroalgal species *Oedogonium* and *Spirogyra* had lower sulfur contents than coal; whereas, *Cladophora* had the highest sulfur contents among all reviewed feedstocks. Although most freshwater macroalgae contained lower sulfur contents than coal, freshwater macroalgae had higher nitrogen contents than both terrestrial biomass and coal.

When we consider direct combustion of filamentous freshwater macroalgae along with traditional solid biofuels, the initial nitrogen, sulfur, and ash contents of combusted biomass are directly related to the subsequent post-combustion nitrogen oxides (NO_x) and sulfur oxides (SO_x) emissions, corrosion, and ash deposition (Tumuluru et al., 2012). In addition, the ash and moisture contents of different biomass sources influence their ignition, flame stability, combustion, and deposition of fouling agents, such as alkaline and chlorine containing species, on boiler heat-transfer surfaces (Lawton et al., 2013; Tumuluru et al., 2012). In addition, although high ash contents appear to be advantageous for producing a biocrude with lower oxygen and larger HHV, high water and ash contents may negatively influence alternative biomass energy production processes, such as biogas production (Roberts et al., 2013; Lawton et al., 2013).

Unfortunately, none of the studies reviewed here provided complete lists of elemental content. However, the inorganic elemental contents in algae can be expected to vary greatly depending on local environmental conditions (Tumuluru et al., 2012; Shurin et al., 2013). In addition, genera from the family of Cladophoracea, including *Cladophora* and *Pithophora*, can be expected to have unusually high Si contents because they typically exhibit a dense surface coating of epiphytic diatoms, which are surrounded by a silica cell wall (Hardwick et al., 1992; Saunders et al., 2012). The concentrations of silica, alkali metals, and chlorine elements in biomass are important because they can form alkali silicates and alkali sulfates that can results in fouling and corrosion of the boiler heat-transfer surfaces, and lower ash-fusion temperatures (Tumuluru et al., 2012; Duong and Tillman, 2009).

In their comparative study of freshwater macroalgae for biofuels applications, Lawton et al. considered carbon content as a key criterion for selecting target species of freshwater macroalgae (Lawton et al., 2013). While we strongly agree with their argument, our review suggests that the contents and emissions of nitrogen, sulfur, and other inorganic elements must be considered as well when evaluating the biofuels potential of freshwater macroalgae biomass intended for direct combustion. Unfortunately, we were not able to evaluate either the atmospheric emissions characteristics or the energy return on investment (EROI) of the solid fuels examined in our study. However, we cautiously speculate that the EROI of the freshwater macroalgae may be greater than that of freshwater microalgae because fossil energy intensive factors, such as biomass drying and harvesting, are likely to require relatively higher energy inputs for the processing of freshwater microalgae (Zaimes and Khanna, 2013). While there is no significant difference in the higher heating value between freshwater macroalgae (12.1–22.34 MJ/kg) and freshwater microalgae (18.66 MJ/kg), the dense floating mat formation of freshwater macroalgae may reduce a substantial amount of non-renewable energy input for biomass drying and harvesting (Lawton et al., 2013; Chaiwong et al., 2012; Boyd, 1968; Zaimes and Khanna, 2013). Because the cultivation of macroalgae is still in its early stages of development, we cannot provide reliable estimates of its production costs at this time.

HHV, Productivity, and Proximate Analysis of Freshwater Macroalgae Grown under Different Dilution Rates

Our experiments revealed no significant effect of dilution rate on the dry weight productivity (DW, g/m^2/day) of the natural freshwater macroalgae species assemblage cultivated in this experiment (*Figure 3*). The average dry weight productivity of our freshwater macroalgae assemblage over the 14 day growth period was 3.37 g/m^2/day, and its comparison with terrestrial vegetation revealed that these cultured freshwater macroalgae exhibited higher areal productivities than common agricultural biomass crops (*Figure 4*). In addition, we note that the productivity of freshwater macroalgae measured in our laboratory study is at the lower end of the impressive range of dry weight productivities (2.5 g/m^2/day to 25 g/m^2/day) achieved by *Oedogonium*, *Ulothrix*, *Rhizoclonium*, and *Microspora* grown in a 30 m^2 outdoor pilot-scale ATS raceway (Mulbry et al., 2008b).

Although we were not able to find a significant relationship between macroalgal productivity and dilution rate, it is usually considered that the productivity of algae is limited by their supply of growth-limiting nutrients (Smith et al., 2010). For example, Mulbry et al. demonstrated that the mean algal productivity (DW, g/m^2/day) significantly increased from 2.5 g/m^2/day to 25 g/m^2/day when nutrients supplying rate increased (Mulbry et al., 2008b). It is possible that the small surface area of our experimental trays limited algal biomass accumulation.

However, it is important to note that the tray cultures displayed an increase of energy content under more nutrient-limited conditions (*Figure 5*). Although the trend of increasing energy content under more nutrient-limited conditions was not clear across four dilution rates as has been observed in cultures of microalgae (Rodolfi et al., 2009), the most nutrient-restricted treatment (0.1 L/day) produced the most energy-rich biomass (in MJ/kg dry wt.); in contrast, the highest dilution rate (2.5 L/day) was likely to be nutrient-saturated, and this experimental treatment yielded the lowest biomass energy contents of the filamentous freshwater macroalgae. This response of energy content to nutrient supply conditions is consistent with the evidence for strong plasticity in cellular nutrient content that has frequently been observed in microalgal cells (Shurin et al., 2013; Mandal and Mallick, 2009; Feng et al., 2011; Jiang et al., 2012). In stressful nutrient-limiting conditions, particularly under N- or P-limitation, algal cells activate lipid biosynthetic pathways that favor the formation and accumulation of cellular lipids, which will increase the energy content of the algal biomass (Shurin et al., 2013; Rodolfi et al., 2009). In addition, it has been observed that stress from high salinity and low pH triggers lipid accumulation in microalgal cells (Shurin et al., 2013; Damiani et al., 2010; Takagi et al., 2006).

The proximate analysis of macroalgae grown at the four different nutrient supply rates is shown in *Table 3*. In addition, the weight and derivative of weight curves for the thermal gravimetric analysis (TGA) of each freshwater macroalgae biomass are represented in *Figure 6*. These data suggest that the content of combustibles and volatiles were lowest at the highest dilution rate (2.5 L/day); whereas, the biomass moisture content was the lowest at the lowest dilution rate (0.1 L/day). It is also important to note that the ash contents of the freshwater macroalgae tended to increase with dilution rate, reflecting higher cellular contents of mineral elements.

These data indicate that utilization of thermal gravimetric analysis (TGA) for proximate analysis has allowed us to extract valuable information that is relevant to objective, quantitative assessments of the potential of freshwater macroalgae as a biofuel feedstock. The volatile content reported here is directly related to the amount of algae that could be converted through pyrolysis (a thermochemical conversion of typically biomass to condensable hydrocarbon vapors). Pyrolysis of biomass results in significant formation of residual char, which still retains a large HHV and can be burned for energy. The fixed carbon in this study represents the weight of the char that can be burned in any post-pyrolysis conversion. The derivative weight of the pyrolysis and post-pyrolysis combustion stages indicates the temperatures at which the peak weight loss occurs in each stage. During pyrolysis this happens at 300 °C and during the post-pyrolysis combustion this is around 450 °C, indicating that algae pyrolysis char could be well suited for co-firing with traditional energy sources, such as wood and coal.

In addition to the measurements of HHV and proximate analyses reported here, preliminary attempts to convert freshwater macroalgal biomass into green biocrude using the high temperature/high pressure hydrothermal liquefaction process (HTL) have been successful (data not shown). We note that the presence of alkali species during the HTL reaction may play a catalytic role to reduce the final biocrude oxygen content, and thus the higher ash contents of algal biomass thus could be advantageous for producing a biocrude with low oxygen content and large HHV (Roberts et al., 2013). Considering the wide range of environmental tolerance of freshwater macroalgae that is reported in the literature, we suggest that further studies investigating the influence of different growth conditions on the chemical properties and productivity of freshwater macroalgae will provide valuable insights into the optimization of net energy yields from macroalgal cultivation and biofuel production (Hillebrand, 1983). In addition, the emissions of algal biomass are likely to depend on a range of growth conditions, including the nutrients availability (Lane et al., 2014). For example, Lane et al. found the fraction of fuel N during the combustion of *Oedogonium* was reduced by 43-49% by cultivating the macroalgae under nutrients-limiting condition (Lane et al., 2014). Although the emissions during macroalgae combustion remain to be analyzed, biomass combustion-related flue gases, such as nitrogen oxides (NO_x) and sulfur oxides (SO_x), are considered as harmful pollutants causing smog and ocean acidification (Lane et al., 2014; Hunter et al., 2011). In addition, greenhouse gases (GHGs), such as carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), are associated with the combustion of biomass (Intergovernmental Panel on Climate Change, 2007). Therefore, the analysis of emissions during the combustion of macroalgal biomass should be performed to evaluate potential utilization and environmental impact. In particular, the direct combustion of macroalgal biomass will be only viable if the combustion products are either comparable to, or lower than, the currently-used fuels that they would replace.

Overall, the productivity and HHV data from our experiment are consistent with the conclusion by Lawton et al. that the high productivity and bioenergy potential of *Oedogonium* make this species an ideal freshwater macroalgal target for bioenergy applications (Lawton et al., 2013). In addition, the results from our review and experiment suggest that freshwater macroalgae are an under-utilized biomass feedstock with high potential for biofuels production (Lawton et al., 2013). We hope that this additional new biomass source can contribute to progress towards the creation of a robust and cost-competitive biomass-based biofuels industry in the US (Reed, 2012).

CONCLUSIONS

This study compares the productivity and biofuels potential of freshwater macroalgae with traditional biomass to explore the suitability of freshwater macroalgae as a biofuels feedstock. Our results suggest that filamentous freshwater macroalgae have strong potential as directly-combusted biofuels due to their high productivity, and due to the similarity of their HHVs and chemical composition to conventional bioenergy sources (terrestrial biomass residues and wood). We thus strongly agree with Lawton et al. that green freshwater macroalgae have much potential for biomass applications, but that they currently are an under-utilized feedstock (Lawton et al., 2013).

Although we did not evaluate the suitability of freshwater macroalgae for the production of liquid transportation fuels in our study, we stress that this is yet another exciting possibility. For example, Grayburn et al. obtained algal biodiesel fuel from lipids extracted from a mixed-species assemblage of macroalgae that was dominated by *Cladophora* and *Rhizoclonium* (Grayburn et al., 2013). A B5 blend of their algal biodiesel and petrodiesel was tested in a 13.4-kW internal combustion engine, and this fuel mixture exhibited a performance that was comparable to pure petrodiesel in terms of fuel efficiency and carbon dioxide and carbon monoxide exhaust emissions (Grayburn et al., 2013). Potentially more importantly, there was a reduction in exhaust emissions of NO_x from 209 ppm to 162 ppm for the petrodiesel and algal B5 fuels, respectively (Grayburn et al., 2013), however, further test is required to determine if different combustion conditions, such as combustion timing, influence this result. Moreover, Neveux et al. have recently examined the biocrude yield and productivity from the hydrothermal liquefaction of several species of marine and freshwater green macroalgae (Neveux et al., 2014). These species were identified as suitable feedstocks for scale-up and further HTL studies based on biocrude productivity, as a function of biomass productivity and the yield of biomass conversion to biocrude oil (Neveux et al., 2014).

Although our study suggests that the nutrient-limited growth condition is likely to cause an increase in the energy content of freshwater macroalgal biomass, many additional growth factors can potentially influence their net energy yields. We thus suggest that researchers should pay more attention to this under-utilized algal group with careful consideration of the abiotic and biotic conditions that will be needed to achieve cost-effective macroalgal cultivation at the commercial scale for the production of both solid and liquid biofuels.

Published as:

Yun, J.H., V.H Smith, F.J. deNoyelles, G.W. Roberts and S.M. Stagg-Williams. 2014. Freshwater macroalgae as a biofuels feedstock: Mini-review and assessment of their bioenergy potential. Industrial Biotechnology 10: 212-220. DOI: 10.1089/ind.2013.0033. The final publication is available at: http://online.liebertpub.com/doi/abs/10.1089/ind.2013.0033

Table 1. Proximate analysis of freshwater macroalgae, terrestrial biomass residues, typical wood, and coal. (Note: the absolute values summarized here reflect varying degrees of biomass drying that were not provided in the data source.)

(Sources: Lawton et al., 2013; Tumuluru et al., 2012; Demirbas, 2003; Chaiwong et al., 2012; Boyd, 1968; Tillman et al., 2009)

Biomass	Moisture (wt%)	Ash (wt%)	Volatiles (wt%)	Fixed carbon (wt%)
Freshwater Macroalgae				
Cladophora	5.00	33.42	60.61	0.98
Hydrodictyon	-	11.90	-	-
Oedogonium	-	3.70	-	-
Pithophora	-	27.40	-	-
Spirogyra	8.45	13.99	65.48	12.08
Terrestrial biomass residues				
Almond shells	-	4.81	-	21.74
Corn cobs	15.00	1.40	76.60	7.00
Corn stover	35.00	3.25	54.60	7.15
Cotton gin	7.30	14.50	-	-
Hazelnut shells	9.20	3.50	60.10	27.20
Olive husk	33.00	1.60	-	-
Olive pits	-	3.16	-	18.19
Peach pits	-	1.03	-	19.85
Poplar	-	1.33	-	16.35
Rice husk	9.96	20.61	54.68	15.02
Sawdust	-	2.60	76.20	13.90
Sugarcane bagasse	-	11.27	-	14.95
Walnut shells	-	0.56	-	21.16
Wheat straw	-	8.90	-	19.80
Wood and coal				
Wood	42.00	2.31	47.79	7.90
Coal	7.16	11.52	31.23	50.09

Table 2. Elemental composition of freshwater macroalgae, terrestrial, biomass residues, typical wood, and coal, normalized to a dry weight basis.

(Sources: Lawton et al., 2013; Tumuluru et al., 2012; Demirbas, 2003; Chaiwong et al., 2012; Boyd, 1968; Tillman et al., 2009)

Biomass	C (dw%)	H (dw%)	O (dw%)	N (dw%)	S (dw%)
Freshwater Macroalgae					
Cladophora	21.57	3.01	44.11	2.29	3.96
Hydrodictyon	-	-	-	-	-
Oedogonium	46.81	7.10	38.48	3.70	0.10
Pithophora	-	-	-	-	-
Spirogyra	34.44	5.36	5.83	41.59	0.50
Terrestrial biomass residues					
Almond shells	45.34	6.02	42.61	1.17	0.02
Corn cobs	48.40	5.60	44.30	0.30	-
Corn stover	45.06	5.34	45.17	0.80	0.19
Cotton gin	42.97	5.42	35.14	1.41	0.50
Hazelnut shells	49.86	5.28	40.25	1.32	-
Olive husk	47.80	5.10	45.40	0.10	-
Olive pits	47.82	6.10	42.60	0.35	0.02
Peach pits	53.30	5.93	39.36	0.32	0.05
Poplar	48.55	5.86	43.78	0.47	0.01
Rice husk	34.95	5.46	38.87	0.11	-
Sawdust	50.63	5.61	40.80	0.11	0.04
Sugarcane bagasse	44.2	5.28	39.02	0.37	0.01
Walnut shells	50.07	5.72	43.43	0.21	0.01
Wheat straw	44.44	5.14	40.53	0.63	0.11
Wood and coal					
Wood	50.28	4.60	39.98	1.03	0.12
Coal	72.09	4.77	8.13	1.44	1.15

Table 3. Proximate Analysis (wt%) (± 1 S.D) of Freshwater Macroalgae Grown at Four Different Nutrient Supply Rates (see text).

	0.1 L/day	**0.5 L/day**	**1.0 L/day**	**2.5 L/day**
Moisture (wt%)	6.4 ± 0.1	7.0 ± 0.1	7.6 ± 0.1	7.0 ± 0.2
Volatiles (wt%)	67.6 ± 0.6	66.8 ± 1.1	66.0 ± 0.4	64.0 ± 0.7
Combustibles (wt%)	13.7 ± 0.1	13.9 ± 0.4	13.4 ± 0.0	12.8 ± 0.1
Ash (wt%)	12.3 ± 0.7	12.4 ± 1.5	14.0 ± 0.4	16.2 ± 0.7

Figure 1. An example of algal turf scrubber unit on a dock at the Patuxent River Park on the Patuxent River, MD. Adapted from “Toward Scrubbing the bay: Nutrient removal using small algal turf scrubbers on Chesapeake Bay tributaries” by W. Mulbry, P. Kangas and S. Kondrad. 2010. *Ecological Engineering* 36. p 537. Copyright 2010 by Elsevier B.V. Adapted with permission.

Figure 2. Higher heating value (HHV) comparison between freshwater macroalgae and traditional energy sources, including terrestrial residues, wood, and coal.

(Sources: Lawton et al., 2013; Tumuluru et al., 2012; Demirbas, 2003; Chaiwong et al., 2012; Boyd, 1968; Tillman et al., 2009)

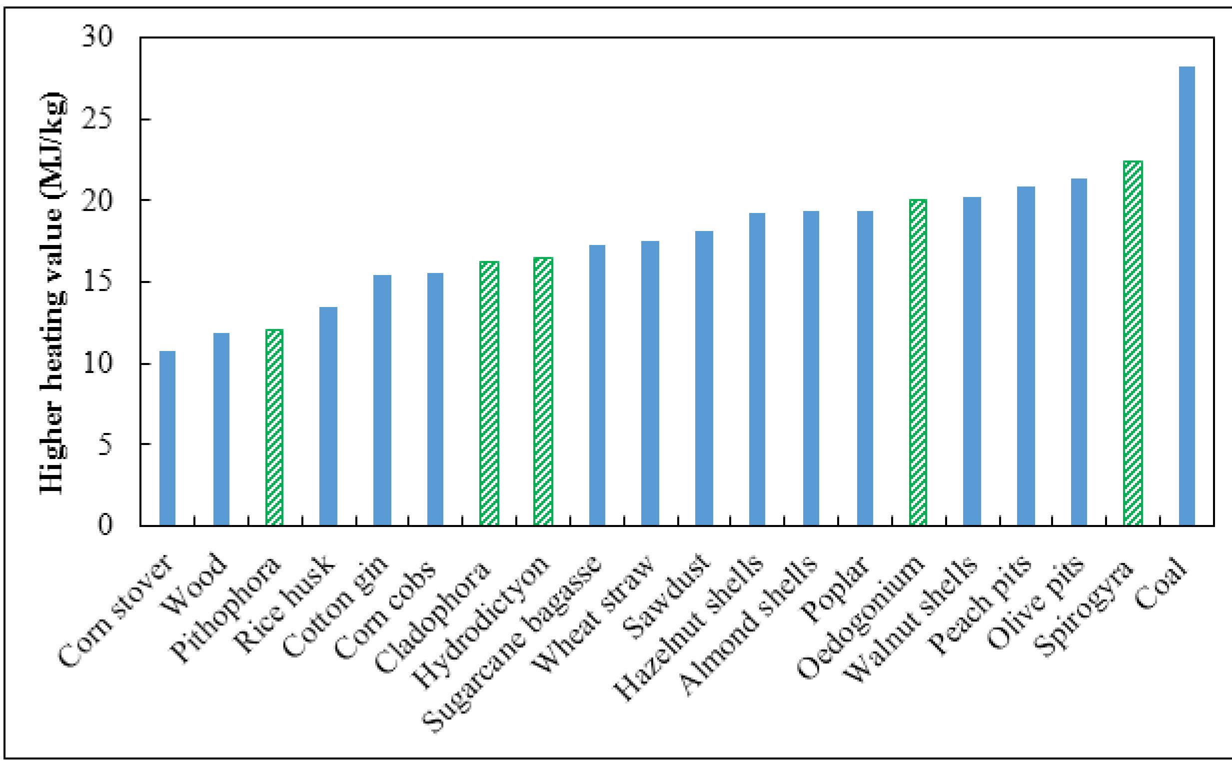


Figure 3. Dry weight productivity of freshwater macroalgae cultured at four different nutrient supply rates (see text), showing 95% confidence intervals for the mean.

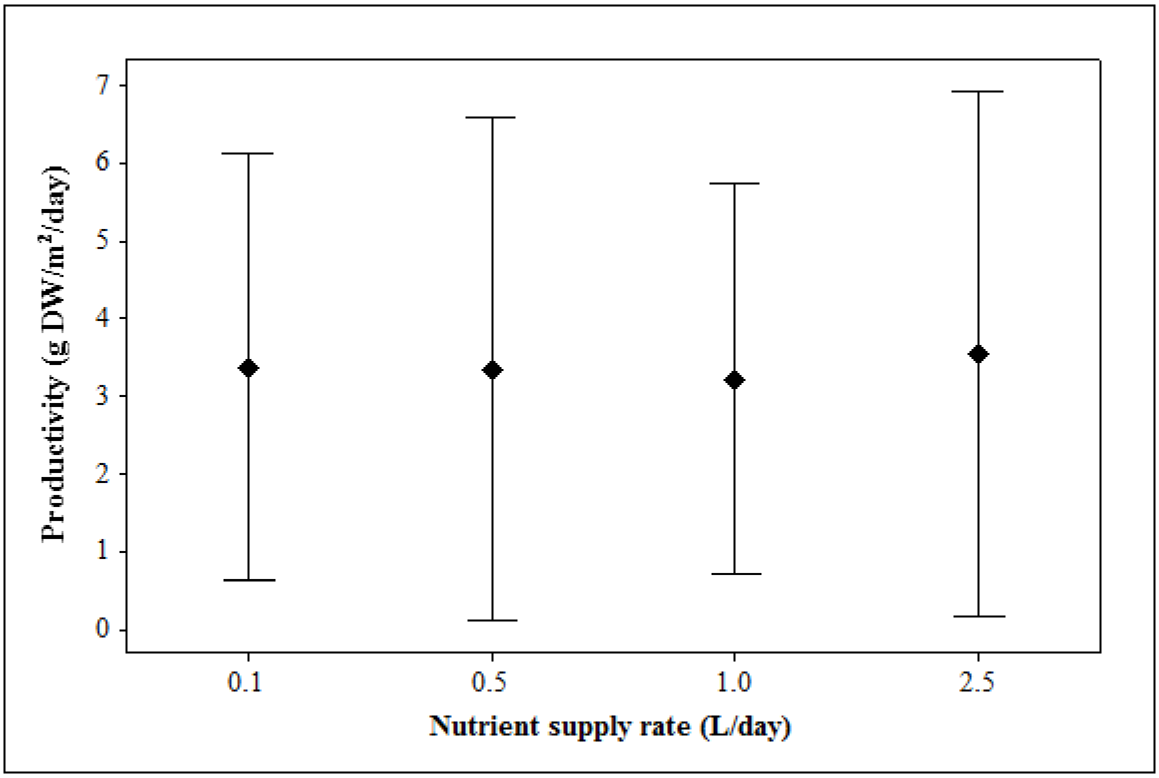


Figure 4. Productivity of freshwater macroalgae relative to common terrestrial plant crops.

(Source: United States Department of Agriculture, 2012)

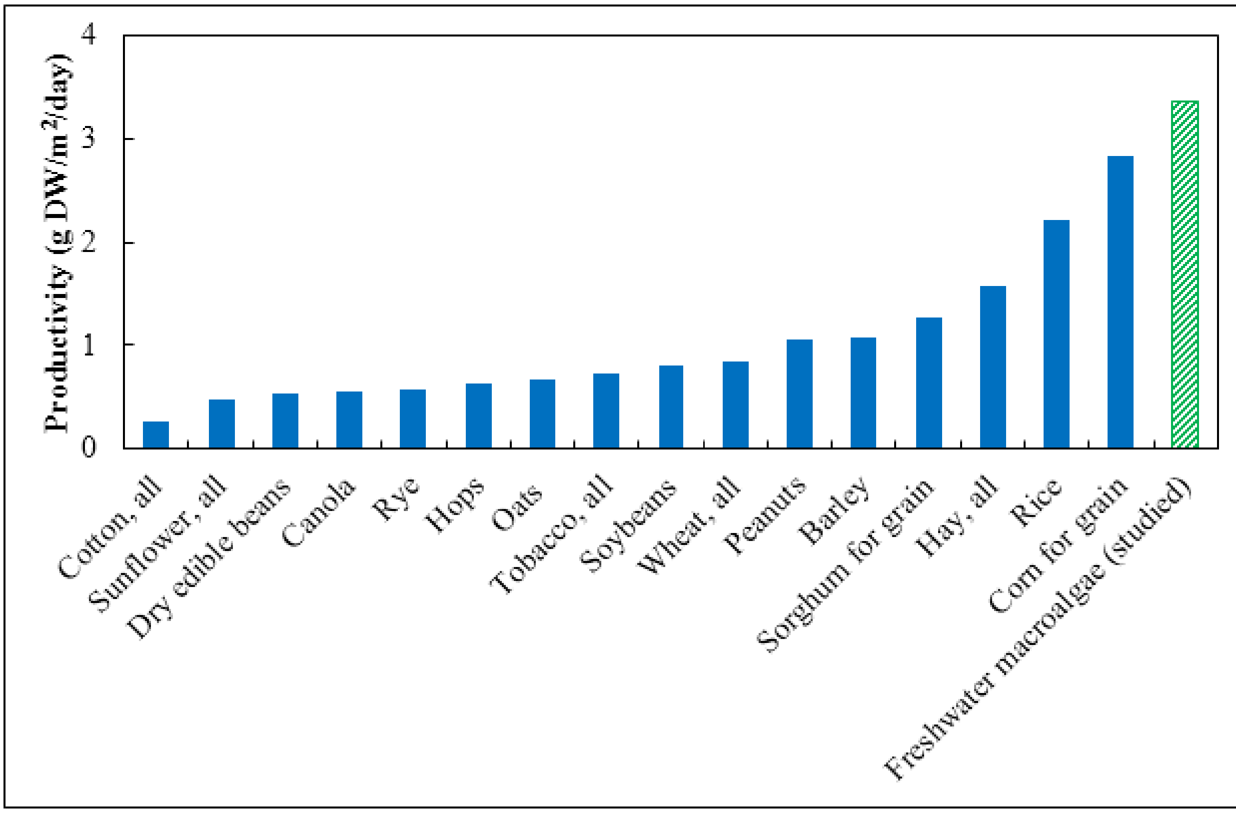


Figure 5. Higher heating values of dried freshwater macroalgae biomass cultured at four different nutrient supply rates, showing 95% confidence intervals for the mean.

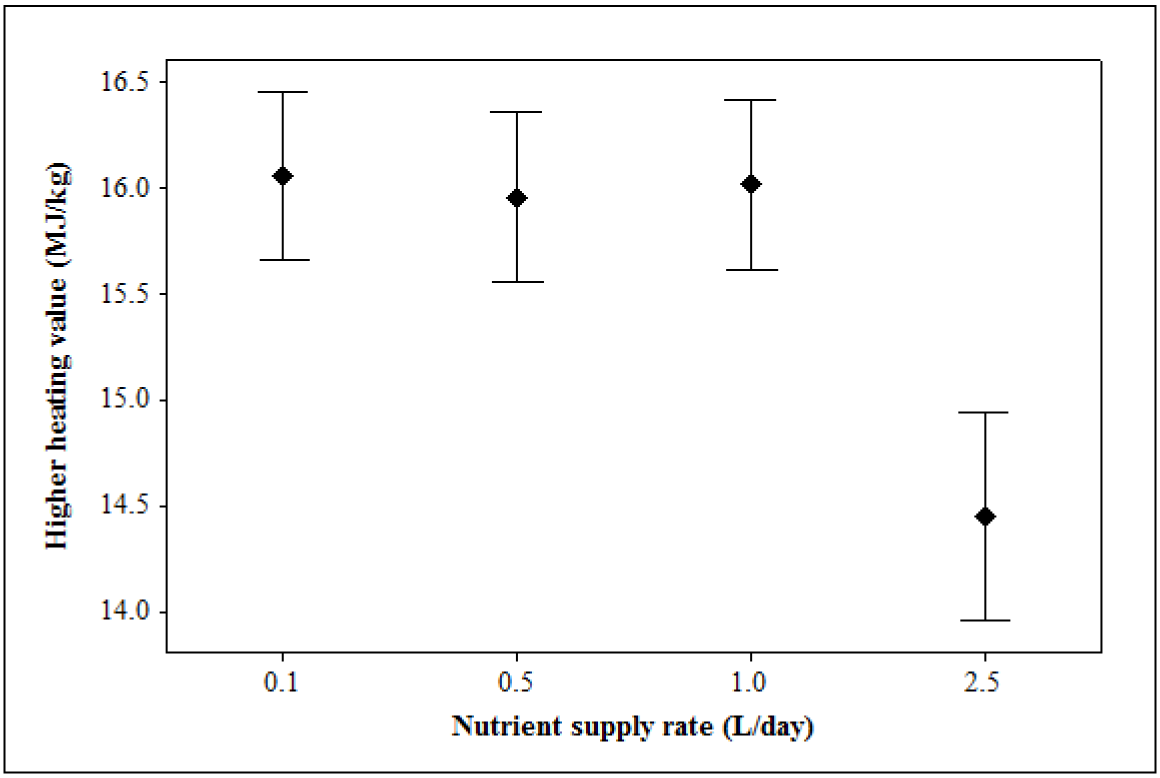
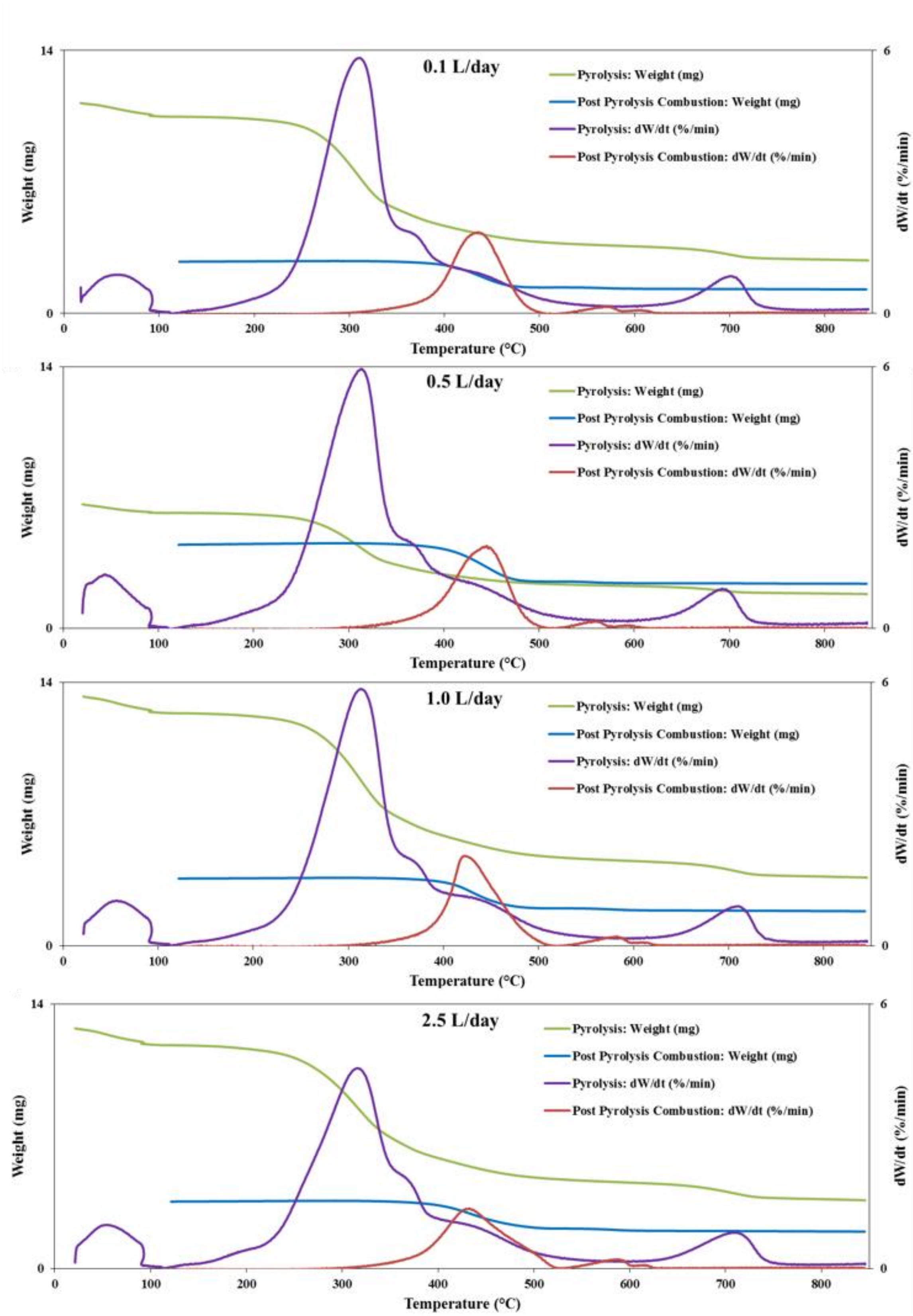


Figure 6. The weight and derivative of weight curves for the thermal gravimetric analysis (TGA) of macroalgae cultured at different nutrient supply rates.



CHAPTER 2: The influence of nutrient availability on freshwater macroalgal biomass production

INTRODUCTION

Algae have long been viewed as a promising source for biofuels feedstock with the capacity for higher productivity per unit of land area than conventional terrestrial crops (Sheehan et al., 1998; Chisti, 2007; United States Department of Energy, 2010; Pate et al., 2011; Yun et al., 2014). While the majority of algal biofuel research to date has focused on strain selection and optimizing the productivity of microalgae, macroalgae also have been proposed as feedstocks for diverse biomass applications, including liquid and solid fuels production (Yun et al., 2014; Chisti and Yan, 2011; Grayburn et al., 2013; Lawton et al., 2013). However, these efforts have primarily involved marine seaweeds and filamentous cyanobacteria, with a lesser focus on the use of freshwater macroalgae (primarily members of the eukaryotic Chlorophyta) (Yun et al., 2014; Grayburn et al., 2013; Lawton et al., 2013; Demirbas, 2010; Khola and Ghazala, 2012).

Although freshwater macroalgae represent a largely overlooked group of phototrophic organisms, they can exhibit high rates of areal productivity, and their tendency to form dense floating mats or substrate-attached turfs imply significant reductions in harvesting and dewatering costs relative to microalgae (Yun et al., 2014; Cole et al., 2013). In addition, freshwater macroalgae can play an important role in the removal of wastewater nutrients while simultaneously producing biomass for biofuels feedstocks (Cole et al., 2014). Moreover, the cultivation of freshwater macroalgae on non-arable land using wastewater as a nutrient feed would avoid the energy versus food debate associated with terrestrial bioenergy crops (Cole et al., 2013; Kraan, 2010; Nigam and Singh, 2011; Acien et al., 2012). Therefore, freshwater macroalgae cultivation is a promising component of large-scale biomass applications (Cole et al., 2013; Kraan, 2010; Jung et al., 2013).

With over 16 million dry tonnes produced annually, macroalgal cultivation is typically synonymous with the growth and harvesting of seaweeds (Cole et al., 2013; Kraan, 2010; Gao and McKinley, 1994, Rowbotham et al., 2012). However, several studies have explored the use of freshwater macroalgae for the bioremediation of high nutrient wastewater effluents derived from animal agriculture or human sewage (Cole et al., 2013; Adey et al., 1993; Craggs et al., 1996; Mulbry et al., 2008b). In addition, Cole et al. demonstrated the utilization of industrial flue gas as carbon source for freshwater macroalgae cultivation in large outdoor tanks, and further attempts to integrate freshwater macroalgae cultivation systems with agricultural, aquacultural, and industrial facilities to remove excessive nutrients is underway (Cole et al., 2013; Ray et al., 2014; Kangas and Mulbry, 2014).

The mass cultivation of algae for bioenergy applications has received considerable attention in the research community in recent years (Cole et al., 2013; Kazamia et al., 2014; Stephens et al., 2010). Nonetheless, the degree of scale-up that will be required to achieve the bulk growth of algae poses a significant number of challenges, ranging from the high energy costs of maintaining the cultivation and processing systems, to problems associated with biological contamination by pests and non-target organisms (Kazamia et al., 2014; Mata et al., 2010; Scott et al., 2010).

It has long been known that the photosynthetic conversion of sunlight energy into biomass in large-scale algal cultures is influenced by the availability of sunlight, water temperature, and nutrient availability (Smith and Crews, 2014; Goldman, 1979). Of these factors, more than 40 years of research in aquatic eutrophication science indicates that the availability of nitrogen and phosphorus will set the upper limit for algal production that can be attained in algal biomass cultivation facilities (Sakamoto, 1966; Vollenweider, 1976; Smith, 1979). A remarkably consistent response of surface waters to nutrient enrichment has, in fact, been observed worldwide (Smith, 2009). Moreover, measured rates of photosynthesis and the average growing season biomass of microalgae have both been demonstrated to be strongly and positively dependent upon water column concentrations of total nitrogen and total phosphorus (Sakamoto, 1966; Vollenweider, 1976; Smith, 1979).

Our goal in this review, thus, will be to explore the pivotal role of inorganic nutrients (nitrogen, phosphorus, and carbon dioxide) in determining algal biomass productivity by freshwater macroalgae. We will first briefly review recent experiences in the cultivation of freshwater macroalgae, and we will then examine how the supply rates of N and P to Algal Turf Scrubber (ATS) systems control both macroalgal biomass production and nutrient removal rates. In addition, we will briefly discuss the possible value of carbon dioxide supplementation.

MASS CULTIVATION PLATFORMS FOR FRESHWATER MACROALGAL BIOMASS PRODUCTION

Civilizations across the globe have harvested and consumed algae for centuries (McBride and Merrick, 2014). However, research on the mass cultivation of algal biomass successfully was not initiated until the late 1940s and early 1950s in the U.S., Germany, and Japan (McBride and Merrick, 2014; Mituya et al., 1953; Gummert et al., 1953; Cook, 1950). The two basic technological platforms currently used for producing algal biomass are open pond and closed systems (McBride and Merrick, 2014). Closed systems typically have higher productivity and are more stable, but they are typically more expensive to set up and operate than open systems (McBride and Merrick, 2014; Chisti, 2007). Although decades of effort have focused upon developing efficient and cost-effective closed systems for the cultivation of microalgae, closed systems for macroalgae cultivation have been much less frequently considered (Chen et al., 2011; Rorrer and Mullikin, 1999). We suggest that it is likely that the mass cultivation of macroalgal floating mats in closed systems will cause unacceptably high levels of light extinction, as well as heterogeneous light transmission through the cultures, which may hinder the establishment of dense macroalgal culture (Rorrer and Mullikin, 1999). Moreover, the algae-to-bioenergy pathway is more favorable when nutrients in wastewater effluents are used in place of commercial fertilizers (Adey et al., 2011). In addition, it seems unlikely that high volumes of wastewater can be efficiently used in a system requiring sterile conditions for macroalgal biomass production, and, therefore, the use of wastewater is unlikely to be a significant part of any successful large-scale closed system for macroalgal biomass production (Adey et al., 2011).

In contrast, open cultivation systems are much less expensive, and can be highly productive (up to 30 g $DW/m^2/day$) (Adey et al., 2011; Goh, 1986; Benemann and Oswald, 1996; Olguin, 2003; Craggs et al., 2003). Although the open systems are currently considered as the only feasible system to produce algal biomass competitively, they nonetheless present significant challenges stemming from the fact that they are exposed to unpredictable and uncontrollable metrological conditions; are at risk from both abiotic and biotic contaminants; and are often poorly mixed (McBride and Merrick, 2014; Adey et al., 2011; Waltz, 2009).

Extensive research effort has focused upon the identification of monocultures or polycultures of algal species that can thrive in outdoor cultivation systems despite the presence of environmental fluctuations and contamination pressures, while producing high biomass yields with a desirable energy and biochemical content (Nalley et al., 2014). Lawton et al. recently identified the cosmopolitan freshwater macroalgal genus *Oedogonium* as a target for biomass applications due to its high productivity, favorable biochemical composition, cosmopolitan distribution, and competitive dominance over other freshwater macroalgal genus (Lawton et al., 2013; Lawton et al., 2014). In addition, Cole et al. conducted experiments in large outdoor tanks measuring 10 m long and 3 m wide with an operation volume of 15000 L and demonstrated that the freshwater genus *Oedogonium*, and freshwater macroalgae in general, are key candidates for the large-scale culture and supply of feedstock biomass used for bioenergy applications (Cole et al., 2013). Interestingly, the average higher heating value (HHV, 18.5 MJ/kg) and dry weight productivity (5.85 g DW/m^2/day) of the pond-cultured *Oedogonium* biomass produced outdoors by Cole et al. exceeded the higher heating value (14.4-16.1 MJ/kg) and dry weight productivity (3.37 g DW/m^2/day) of same genus cultivated in 5-L laboratory tray cultures (Yun et al., 2014; Cole et al., 2013).

Although outdoor pond cultivation of macroalgae has been shown to be feasible by Cole et al. and others, we believe that Algal Turf Scrubber (ATS) systems provide a much more desirable alternative to outdoor ponds for the mass cultivation of freshwater macroalgae (Adey et al., 1993; Craggs et al., 1996). The ATS is an ecologically engineered system for wastewater nutrient removal that uses sloped flow-ways seeded with multiple filamentous macroalgae taxa, including the common freshwater genera *Oedogonium*, *Rhizoclonium*, *Ulothrix*, and *Microspora* (Yun et al., 2014; Mulbry et al., 2008b; Adey et al., 2011; Kebede-Westhead et al., 2003; Pizarro et al., 2006).

In ATS systems, nutrient-rich water is passed over a mixed-species assemblage of algae that is attached to a physical substrate, simulating the environments experienced by attached algae in moving water environments such as streams and rivers (Sandefur et al., 2011). The attached algae in ATS systems can be harvested by mechanical methods with relative ease (Sandefur et al., 2011; Hoffmann, 1998). In addition, ATS systems are less expensive to install and operate than closed systems (Sandefur et al., 2011).

We stress here that the measured biomass production rates of ATS systems are among the highest of any recorded values for either natural or managed ecosystems (Adey et al., 2011; Adey and Loveland, 2007). For example, Craggs et al. measured the yearly mean value for daily macroalgal biomass production (including trapped organic particulates) in their ATS as 35 g DW/m^2/day (Craggs et al., 1996; Adey et al., 2011). This value is more than ten times the average daily productivity measured in laboratory *Oedogonium* culture trays (3.37 g DW/m^2/day), and at least five times higher than the average daily productivity measured in large-volume pond systems (5.85 g DW/m^2/day) (Yun et al., 2014; Cole et al., 2013). Because of the fast growth rate of algae on ATS, this technology has been examined as a potential cost-effective technology for nutrient removal from municipal wastewater; aquaria; liquid waste from dairy and swine operations; and natural waters (Adey et al., 1993; Craggs et al., 1996; Kebede-Westhead et al., 2003; Pizarro et al., 2006; Mulbry and Wilkie, 2001; Wilkie and Mulbry 2002; Mulbry et al., 2005; Kebede-Westhead et al., 2006; Hydromentia, 2005; Mulbry et al., 2010; Anonymous, 1995; Blersch et al., 2013).

NUTRIENTS AVAILABILITY INFLUENCES FRESHWATER MACROALGAL BIOMASS PRODUCTION IN MASS CULTIVATION SYSTEMS

Because of the exceptional promise of ATS systems for algal biomass production, we compiled data from the refereed literature in order to perform comparative analyses of their real-world performance. In section below, we will explore how the supply rates of N and P influence the rates of productivity and nutrient removal exhibited by ATS systems. Although our focus will be on ATS systems, our discussion on the nutrient availability is expected to be relevant to other open pond freshwater macroalgae cultivation systems as well.

Empirical and theoretical background

It has been known since the classic work by the agricultural chemist Justus von Liebig that the supplies of mineral nutrients, especially nitrogen and phosphorus, can strongly limit crop production in any given climatic and hydrologic setting (von Liebig, 1855; Smith et al., 1999) and von Liebig's conclusions apply both to terrestrial and aquatic ecosystems (Smith et al., 1999). Algal biomass production, thus, should be determined primarily by the local availability of limiting nutrients: biomass yield should be directly proportional to the most growth-limiting nutrient, up to the point that this nutrient becomes non-limiting, and yield should then plateau (Berck et al., 1998).

Aquatic eutrophication science indicates that nitrogen and phosphorus are the primary limiting nutrients in aquatic ecosystems. Thus, the availability of N and P should set the upper limit for biomass production that can be attained in engineered algal cultivation facilities (Sakamoto, 1966; Vollenweider, 1976; Smith, 1979; Smith, 2009). For example, Krewer and Holm used a continuously flowing channel to explore the relationship between supply of growth-limiting phosphorus and the biomass yield of attached freshwater macroalgae (measured as areal concentrations of the photosynthetic pigment chlorophyll *a*) (Krewer and Holm, 1982). Using an outdoor flume that was 19.5 m long, 46 cm wide, and 51 cm deep, Krewer and Holm demonstrated that attached macroalgal biomass increased proportionally with the total dissolved phosphorus (TDP) level up to a supply rate of ~2 mg TDP/m^2/day, then leveled off as the TDP supply increased further and other resources became growth-limiting (*Figure 1*) (Krewer and Holm, 1982).

Studies of algae, therefore, suggest that the daily rate of biomass production in ATS systems should be hyperbolically related to their external nutrient supply rates. This hypothesized relationship is explored in the next section.

N and P supplies determine the upper limits of algal biomass productivity in ATS systems

In ATS systems, nitrogen and phosphorus have been regarded as the main controls on algal growth in ATS systems under most environmental conditions (Cole et al., 2014; Blersch et al., 2013). For example, Blersch et al. demonstrated a strongly positive relationship in the laboratory between the ammonia nitrogen loading rate (g NH_3-N m^2/day) and algal biomass productivity (g DW m^2/day) (Blersch et al., 2013). In addition, the results from lab-scale ATS units showed increasing levels of algal biomass productivity (up to 19 g DW/m^2/day) and biomass nutrient content with increasing nutrient loading rate (up to 2.4 g TN, 0.37 g TP/m^2/day) (Mulbry et al., 2008a).

We used data compiled from the literature in order to explore the generalizability of these results. We performed a regression analysis on the experimental results from one large outdoor ATS and five laboratory-scale ATS studies that reported data on algal biomass productivity, as well as the total nitrogen (TN) and total phosphorus (TP) concentrations measured in the wastewater effluents supplied to the ATS units and in the harvested biomass (Mulbry et al., 2008a,b; Kebede-Westhead et al., 2003; Mulbry and Wilkie, 2001; Wilkie and Mulbry, 2002; Kebede-Westhead et al., 2006).

Our regression analysis (*Figure 2*) suggests there is a positive hyperbolic relationship between attached algal biomass productivity and the areal loading rates of nitrogen (r^2 = 0.6523) and phosphorus (r^2 = 0.6202). In particular, algal biomass productivity increases up to loadings of ~3 g TN/m^2/day and ~0.4-0.5 g TP/m^2/day, but then levels off. The apparent plateaus in freshwater macroalgal biomass productivity in Figure 2 suggest that consistently achieving mean areal total nitrogen and phosphorus loading rates of 3 g TN/m^2/day and 0.4-0.5 g TP/m^2/day will be necessary in order to maximize freshwater macroalgal biomass yields in ATS systems.

In addition, because the provision of nitrogen and phosphorus to algal cultivation systems can have significant capital costs if transporting waste nutrient source or the use of commercial

N and P fertilizer are required, Figure 2 suggests that increasing nutrients loading above 3 g TN/m^2/day and 0.5 g TP/m^2/day may not be necessary.

Nitrogen and phosphorus supply determines algal nutrients removal from ATS systems

The cultivation of freshwater macroalgae has been demonstrated to be effective in removing nitrogen and phosphorus from wastewater sources, and a saturating relationship between nutrient availability and algal nutrient removal has been reported (Jung et al., 2013; Vymazal, 1988). In general, nutrient removal from ATS is highly dependent on the quality of the supplied wastewater effluent, thus, increases in effluent nutrient concentrations in general result in higher N and P removal rates from the harvested algae (Kangas and Mulbry, 2014). For example, an ATS project treating diluted dairy manure effluent yielded nutrient removal values of approximately 1000 mg N/m^2/day and 150 mg P/m^2/day (Mulbry et al., 2008b). In contrast, an ATS project using agricultural drainage water (containing lower levels of influent N and P than that used in the manure ATS project) in southern Florida reported three-fold lower nutrient removal rates (300 mg N/m^2/day and 75 mg P/m^2/day) (Hydromentia, 2005).

Similar to the saturating relationship that we observed between nutrient availability and algal biomass productivity in ATS systems (*Figure 2*), analyses of our dataset revealed a hyperbolic relationship between nutrients loading rates and the areal removal rates of nitrogen (r^2 = 0.7864) and phosphorus (r^2 = 0.8940) in ATS systems. In particular, nitrogen and phosphorus removal rates increased up to ~3-3.5 g TN/m^2/day and ~0.5 g TP/m^2/day, and then leveled off. These relationships have implications for the optimization of the ATS process for algal production for water treatment through uptake and removal of dissolved pollutants, as optimization can be accomplished via strategic modifications of the nutrient loading rate (Blersch et al., 2013).

THE INFLUENCE OF CO_2 SUPPLEMENTATION ON FRESHAWTER MACROALGAL BIOMASS PRODUCTION

Although nitrogen and phosphorus supply is important factor determining algal biomass productivity and nutrients removal, the level of algal biomass that can be achieved at a fixed water column concentration of total phosphorus can vary by more than five-fold in hyper-enriched algal cultivation systems (Smith, 2003). One of the important factors that can potentially contribute to this observed variation in algal production include variability in the supplies of carbon dioxide that are available to support algal photosynthesis (Jansson et al., 2012). In this section, we will briefly summarize recent research on CO_2 supplementation in macroalgal biomass cultivation systems.

Pate et al. have recently suggested that along with nitrogen and phosphorus, CO_2 is likely to emerge as the dominant constraints for microalgal biofuels scale-up (Pate et al., 2011). In contrast, while some authors have suggested that enhancing the supply of CO_2 supply can promote the growth of filamentous freshwater macroalgae, the evidence for this need are not yet conclusive.

For instance, several studies have suggested that CO_2 supplementation may not have significant influence on the productivity of freshwater macroalgae (Lawton et al., 2013; Cole et al., 2013; Cole et al., 2014; Mulbry et al., 2008b; Roberts et al., 2013). Lawton et al. found no significant influence of CO_2 supplementation on the productivity of three common genera of freshwater macrolagae (Lawton et al., 2013). In addition, Roberts et al. cultured freshwater macroalgae *Oedogonium* sp. under several carbon addition treatments in outdoor open culture systems, and found that carbon-amended cultures experienced a decline in productivity over time (Roberts et al., 2013). Although maintaining culture pH was considered likely to contribute to decreases in the loss of added CO_2 to the atmosphere via off-gassing, Mulbry et al. found no significant difference between control and pH-maintained carbon supplemented ATS systems (Lawton et al., 2013; Cole et al., 2014; Mulbry et al., 2008b; Bidwell et al., 1985). In contrast, Cole et al. grew *Oedogonium* cultures at a constant pH of 7.5 through the addition of CO_2, and observed mean productivity levels of 8.33 g DW/m^2/day; these CO_2-supplemented systems were 2.5 times more productive than the corresponding unsupplemented controls (average productivity 3.37 g DW/m^2/day) (Cole et al., 2013). However, the presence of a CO_2 supplementation effect may depend primarily on the alkalinity of the water used during cultivation: Cole et al. suggested that higher alkalinity in culture water may contribute more in freshwater macroalgal productivity of freshwater macroalgae than the addition of CO_2 because higher alkalinity indicates higher dissolved inorganic carbon (DIC) readily available for the carbon absorption by macroalgal cells (Cole et al., 2014).

In addition, the formation of floating mats or thick algal turfs may hinder the effects of carbon supplementation on biomass productivity. In particular, diffusion of gases and nutrients to the algal cells becomes increasingly restricted as the macroalgal mat increases in thickness, so that even if biomass continues to accumulate, only the upper layer of the macroalgal mass is likely to be photosynthetically active (Krewer and Holm 1982; McIntyre and Phinney, 1964; Summer and Fisher, 1979). We suggest that careful analyses should be performed under a variety of environmental conditions and harvesting scenarios in order to further evaluate the desirability and cost-effectiveness of CO_2 supplementation in large-scale freshwater macroalgal cultivation (Pate et al., 2011).

CONCLUSIONS

This study explores the pivotal role of inorganic nutrients (nitrogen, phosphorus, and carbon dioxide) in determining algal biomass productivity by freshwater macroalgae. Because of the exceptional promise of ATS for algal biomass production, we complied data from the peer-reviewed literature to explore how the supply rates of N and P influence the rates of productivity and nutrient removal exhibited by ATS systems. Our results suggest that both macroalgal biomass productivity and nutrient removal rates are hyperbolically related to the areal loading rates of both total nitrogen and total phosphorus. This finding supports that the scale-up of freshwater macroalgal cultivation systems should consider optimizing the supply rates of N and P to achieve economically and environmentally sustainable operation of mass cultivation.

While nitrogen and phosphorus supply is important factor determining macroalgal biomass productivity and nutrients removal, carbon dioxide supply may influence the macroalgal biomass productivity as well. Although the effectiveness and need for CO_2 supplementation of macroalgal production systems like the ATS has not yet been conclusively demonstrated, our review suggest that maintaining optimal pH and dissolved inorganic carbon (DIC) are likely to be essential to achieve the effect of carbon dioxide supplementation on freshwater macroalgal growth (Lawton et al., 2013; Cole et al., 2013; Cole et al., 2014).

Although our study highlights the influence of N and P supply rates on macroalgal biomass productivity, the production of algal biomass is influenced by multiple abiotic and biotic factors. Thus, we suggest that researchers should pay more attention to the factors influencing the productivity of freshwater macroalgae to enhance algal biomass productivity and the commercial and environmental potential of large-scale cultivation.

Figure 1. A positive hyperbolic relationship between total dissolved phosphorus (TDP) loading rate and periphytic freshwater macroalgal chlorophyll *a*. The average data from two replicated channels in a divided chamber were used for a second order polynomial regression analysis (Krewer and Holm, 1982).

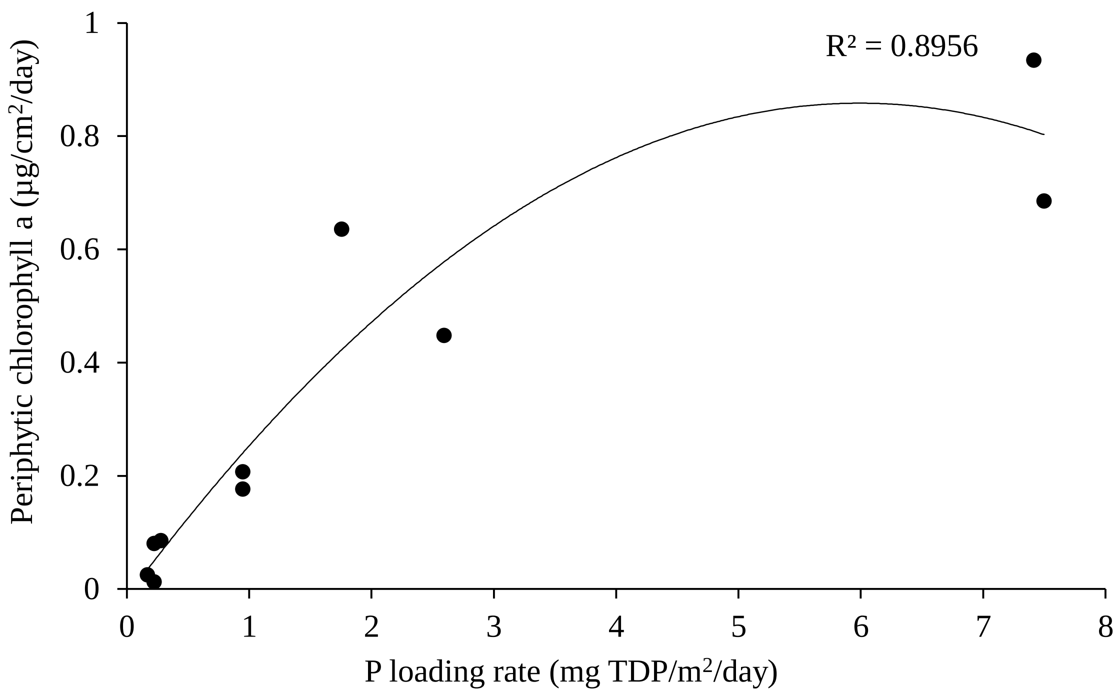
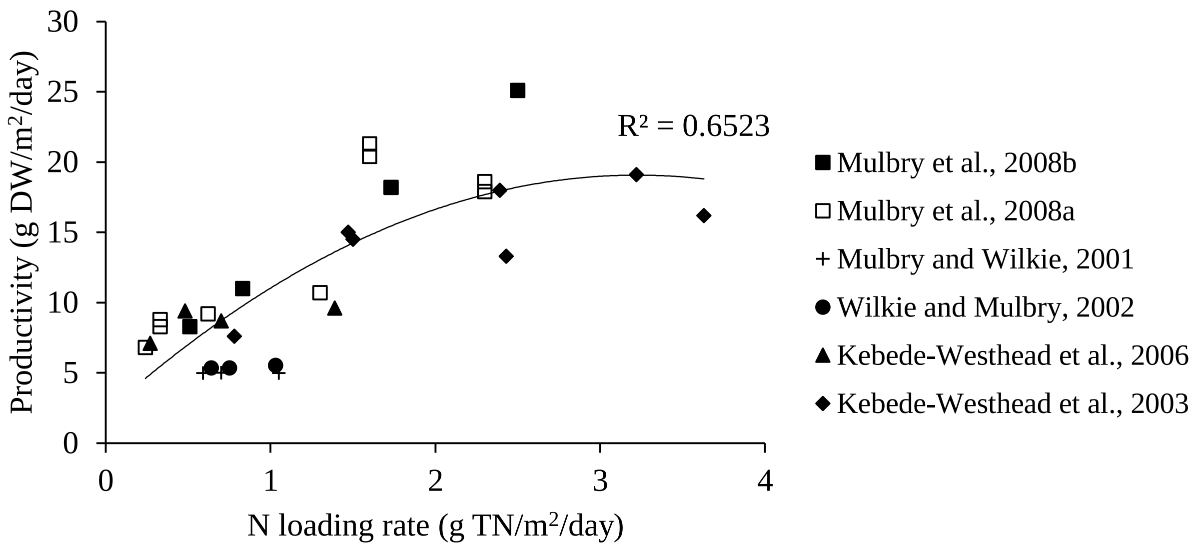


Figure 2. A positive hyperbolic relationship between the loading rate of total nitrogen and total phosphorus, and algal productivity in both indoor and outdoor ATS systems. Curves were generated using second order polynomial regression (Mulbry et al., 2008a,b; Kebede-Westhead et al., 2003; Mulbry and Wilkie, 2001; Wilkie and Mulbry, 2002; Kebede-Westhead et al., 2006).



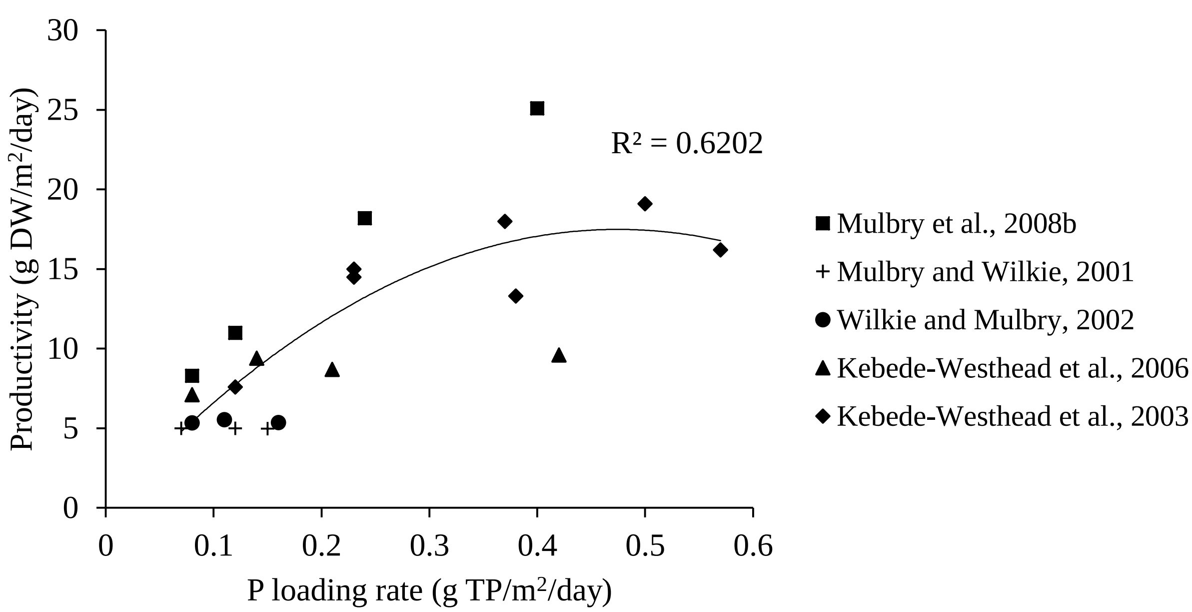
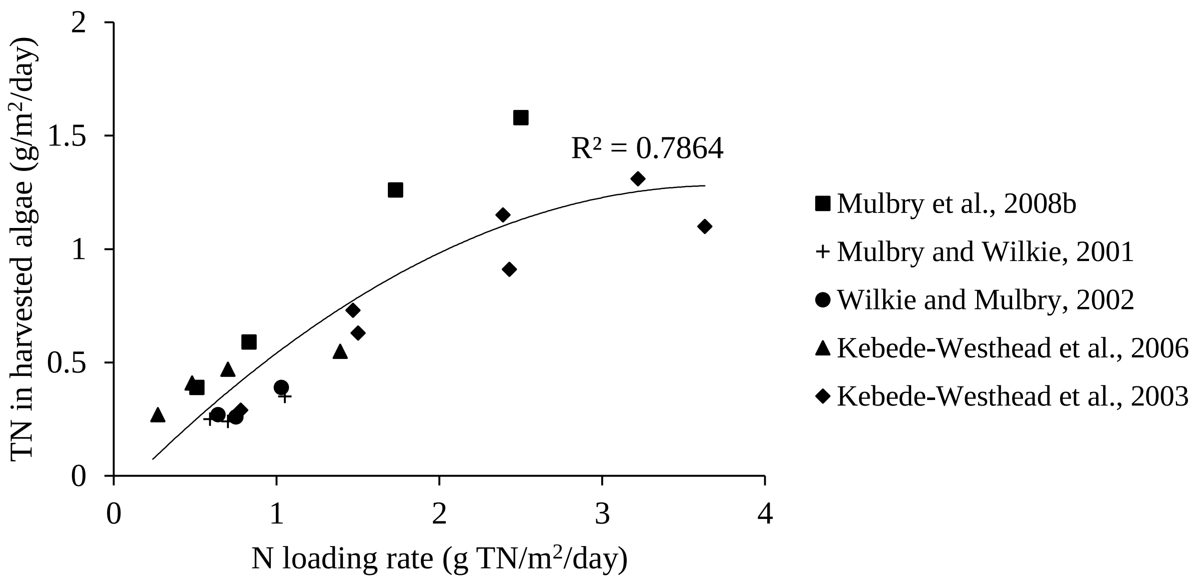
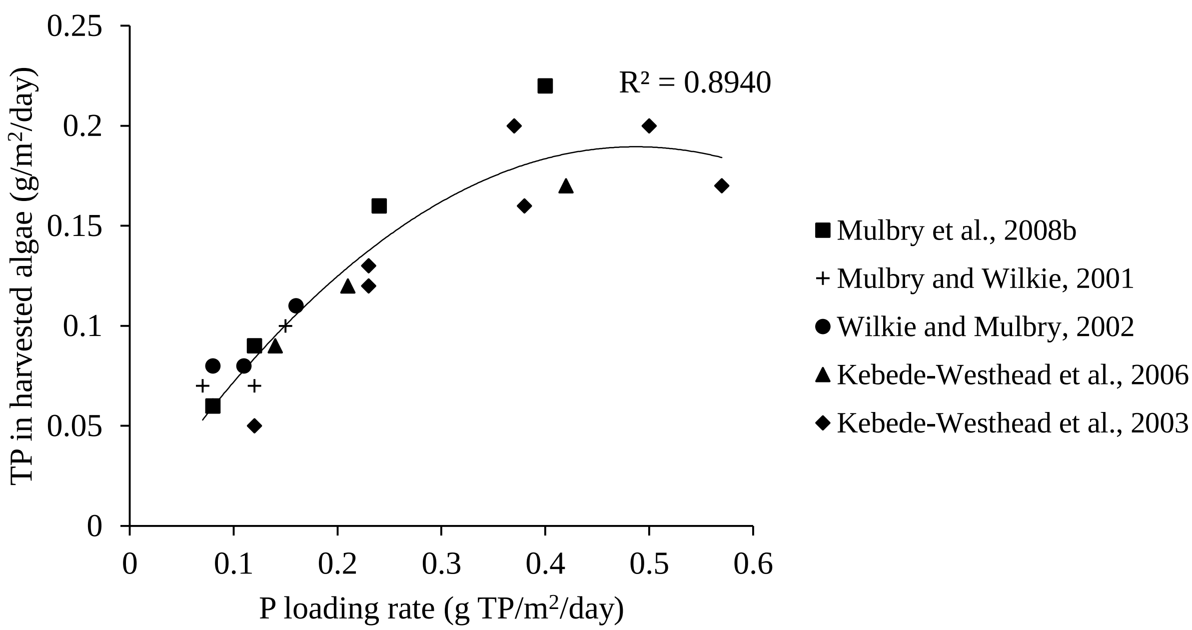


Figure 3. A positive hyperbolic relationship between the loading rate of total nitrogen and total phosphorus, and the total nitrogen and total phosphorus removal rates in harvested algae. Curves were generated using second order polynomial regression (Mulbry et al., 2008a,b; Kebede-Westhead et al., 2003; Mulbry and Wilkie, 2001; Wilkie and Mulbry, 2002; Kebede-Westhead et al., 2006).





GENERAL CONCLUSIONS

In this thesis, I have presented evidence for the potential of freshwater macroalgae as a biofuels feedstock and the critical role of inorganic nutrients in the mass cultivation of freshwater macroalgae.

The results from my first chapter highlight that filamentous freshwater macroalgae have strong potential as directly-combusted biofuels due to their high productivity, and due to the similarity of their HHVs and chemical composition to conventional bioenergy sources (terrestrial biomass residues and wood). Thus, I strongly agree with Lawton et al. that freshwater macroalgae have significant potential for biomass applications, but that they currently are an under-utilized feedstock (Lawton et al., 2013). Although this thesis suggests that the nutrient-limited growth conditions cause a statistically significant increase in the energy content of freshwater macroalgal biomass, many additional growth factors can potentially influence their net energy yields as well. I suggest that researchers should pay more attention to this under-utilized algal group with careful consideration of the abiotic and biotic conditions that will be needed to achieve cost-effective macroalgal cultivation at the commercial scale for the production of both solid and liquid biofuels.

While industrial-scale biofuel applications will require large quantities of biomass, the degree of scale-up that will be required to achieve the bulk growth of algae poses a significant number of challenges, ranging from the high energy costs of maintaining the cultivation and processing systems, to problems associated with biological contamination by pests and non-target organisms (Lawton et al., 2013; Cole et al., 2013; Kazamia et al., 2014; Mata et al., 2010; Scott et al., 2010). The review study presented in Chapter 2 examined how the supply rates of N and P to Algal Turf Scrubber (ATS) systems control both macroalgal biomass production and nutrient removal rates. The results suggest that algal biomass production and nutrient removal rates in engineered cultivation systems exhibit a positive hyperbolic relationship with both nitrogen and phosphorus supply. The results support that the scale-up of freshwater macroalgal cultivation systems should consider optimizing the supply rates of N and P to achieve economically and environmentally sustainable operation of mass cultivation. While there is a potential advantage of carbon dioxide supplementation in enhancing the productivity of freshwater macroalgae, the effectiveness and need for CO_2 supplementation of macroalgal production systems like the ATS has not yet been conclusively demonstrated.

In summary, this thesis demonstrates the potential of freshwater macroalgae as a biofuels feedstock and the pivotal role of nutrient supply rates in the productivity of freshwater macroalgae. Although this thesis highlights the importance of N and P supply in the production of macroalgal biomass, it is essential to understand the interaction of algal consortia under cultivation with other abiotic and biotic factors in the environment to enhance algal biomass productivity and the commercial and environmental potential of large-scale cultivation. By better understanding factors driving the net bioenergy yield of freshwater macroalgal biomass, we will get closer to realize algal cultivation at the commercial-scale.

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