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Rotating Algal Biofilm Reactors: Mathematical Modeling and Lipid Production

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ROTATING ALGAL BIOFILM REACTORS: MATHEMATICAL MODELING AND LIPID PRODUCTION

By

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of

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ABSTRACT

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by

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Harvesting of algal biomass presents a large barrier to the success of biofuels made from algae feedstock. Small cell sizes coupled with dilute concentrations of biomass in lagoon systems make separation an expensive and energy intense-process. The rotating algal biofilm reactor (RABR) has been developed at USU to provide a sustainable technology solution to this issue. Algae cells grown as a biofilm are concentrated in one location for ease of harvesting of high density biomass. A mathematical model of this biofilm system was developed based on data generated from three pilot scale reactors at the City of Logan, Utah wastewater reclamation plant. The data were fit using nonlinear regression to a modified logistic growth equation. The logistic growth equation was used to estimate nitrogen and phosphorus removal from the system, and to find the best harvesting time for the reactors. These values were extrapolated to determine yields of methane and biodiesel from algae biomass that could be used to provide energy to the City of Logan if these reactors were implemented at full scale. For transesterification into biodiesel, algae need to have high lipid content. Algae

biofilms have been relatively unexplored in terms of cell lipid composition accumulation and changes with regard to environmental stressors. Results indicated that biofilm biomass was largely unaffected by nutrient stresses. Neither nitrogen limitation nor excess inorganic carbon triggered a significant change in lipid content. Biofilm algae grown with indoor lighting produced an average of 4.2% lipid content by dry weight. Biofilm algae gown outdoors yielded an average of 6.2% lipid content by dry weight.

(108 pages)

PUBLIC ABSTRACT

Creating renewable biofuels from algal biomass has the potential both to replace fossil fuels as an energy source and remediate environmental issues. Harvesting this biomass for use as biofuel feedstock presents a large barrier to large scale implementation of this solution. Growing the biomass in the form of a film attached to a surface could solve this harvesting issue. This work seeks to better understand both the biomass production and environmental remediation of a novel biofilm cultivation system through mathematical modeling. Mathematical models will help predict how much biomass can be grown, how much nutrients can be removed, and potential inhibitors to system performance. In addition this work also explores ways to increase the biofuel potential of this system by manipulating nutrient concentrations in order to obtain a more desirable feedstock. Through better understanding of biofilm systems in addition to developing ways to produce a better feedstock these systems can be better implemented for both purposes.

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Paul Woolsey

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INTRODUCTION AND NEED FOR STUDY

Need for Renewable Energy

Renewable and clean sources of energy are now at the forefront of engineering challenges due to future and current problems of climate change, environmental harm, and finite energy sources. The Energy Information Association estimates that there are approximately 1,300 billion barrels of oil and 6,300 trillion cubic feet of natural gas left in proven oil reserves. If consumption does not change from 2009 levels, this means that petroleum supplies will be exhausted in approximately 2051, and natural gas will be exhausted in 2065 (EIA, 2009). Measures should be taken to ensure that a sustainable form of energy is in place before sources are depleted. Not only are our energy sources running out, but they are also having a negative effect on the environment. In 2008 over 30 gigatons of carbon emissions were released into the air, and the latest decade has been the warmest ever recorded (EIA, 2010). This is in addition to damage caused by nitrous and sulfur oxides, as well as particulate matter. For these reasons there is a need for more suitable forms of energy generation.

Benefits of Algae

Transesterification of plant oils into fatty acid methyl esters (FAME) has been shown to be a viable source of biofuel (Chisti, 2007). The reduction of several important air pollutants, compatibility with current engine technology, and the ability to be mixed with petrodiesel all make FAME based biodiesel an attractive transportation energy solution. However problems arise when considering that a substantial land investment must be made for the cultivation of crops for fuel. Studies have indicated that 1% of the worlds cropland is used for biodiesel production which is supplanting 1% of petroleum

based fuels. Extrapolating these numbers out creates a severe problem when considering that valuable arable land also needs to produce food (Brennan and Owende, 2010). There is also a high energy investment growing crops for fuel in operating machinery and using fertilizers and chemicals.

Algae solve these critical issues in using biodiesel as a source of liquid fuel. Cultivation of algae does not require precious cropland. Two of the largest requirements for algae growth are water and sunlight, which are far more ubiquitous. Algae also grow faster than biofuel feedstock crops and can be harvested year round rather than once or possibly twice per year. Many species double their mass on the order of days leading to a higher productivity per unit land purposed for biofuel production (Chisti, 2007). Algae can also have a high percentage of their biomass in the form of lipids and fatty acids. Some species can reach as high as nearly 80% of their biomass by weight (Chisti, 2007). Such high lipid content for many algal species makes it an ideal feedstock for biodiesel.

Algae also have the added benefit of having several different co-products and co-processes that can occur during cultivation. Wastewater treatment, carbon dioxide sequestration, and production of co-products such as animal feed and fertilizer, all have the possibility to be run in conjunction with production of biodiesel or other biofuels (Brennan and Owende, 2010). Of primary interest to this study is the removal of nitrogen and phosphorus from domestic wastewater at the Logan, Utah municipal waste water treatment plant that uses an open pond lagoon system. Influent water provides nutrients for the algae to grow, thus creating biomass from free nutrients thus drastically decreasing the cost of production.

This project took place at the Logan Utah Wastewater Treatment plant. The plant consists of seven open ponds over an area of 460 acres with a capacity of 900 million gallons, a picture of the facility can be seen in Figure 1. An average of 15 million gallons of characteristically weak wastewater enters the plant per day (Griffiths, 2009). These facultative treatment ponds serve Logan, Utah and several smaller surrounding communities. Influent enters the headworks and flows in parallel through ponds A1 and B1, and A2 and B2, then combine in series through ponds C, D, and E. Retention time in the Lagoon system is approximately 90 days before the water is chlorinated and then discharged into Cutler Reservoir.

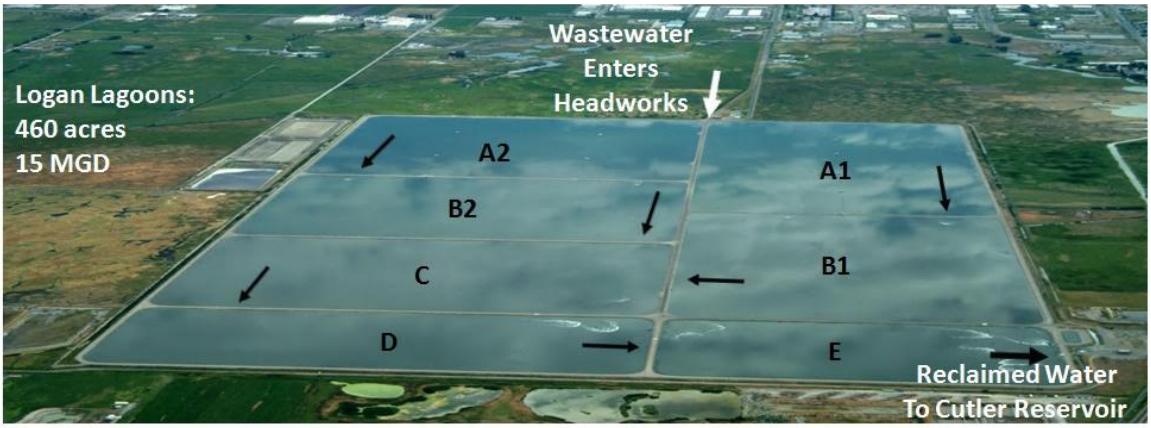

Figure 1: Logan Utah Wastewater Treatment Plant

Benefits of Biofilms

Using algae as a biofuel feedstock presents its own unique engineering and scientific challenges. One large inhibitor to the success of algae biofuel is simply the removal of algae from the water. Energy costs alone, with current methods, have been projected to be nearly 20-30% of the total cost of producing fuel (Brennan and Owende,

2010). Low cell densities of open pond systems compound the issue in that larger quantities of water must be treated to yield similar quantities of biomass as a closed reactor. A solution to the harvesting problem is using the biology of the algae to grow them in such a way that harvesting is easier. Algae can grow naturally as biofilms (Barranguet et al., 2005; Johnson and Wen, 2010; Liu et al., 1993; Roeselers et al., 2008). In this manner of growth, the algae stick to and grow on surfaces collectively. This makes for a highly concentrated source of biomass that facilitates harvesting (Christenson, 2011;Shen et al., 2009). Economic projections have not been made, but it is easy to see that harvesting an algal biofilm would be a fraction of the cost of harvesting suspended algae, because the harvesting process is projected to be a high cost for production of fuel from suspended algae (Brennan and Owende, 2010).

The Rotating Algal Biofilm Reactor

The rotating algal biofilm reactor (RABR) and spool harvester is an algae culturing and harvesting device developed by Logan Christenson at Utah State University (Christenson, 2011). The difference between a RABR and a rotating biological contactor (RBC) is that the RABR grows phototrophic biomass for nitrogen and phosphorus removal, whereas an RBC grows heterotrophic biomass for the treatment of BOD (Metcalf and Eddy, 2007). The RABR is a mechanically simple device. The RABR consists of a cylinder attached to a motor via a central shaft, a motor is attached to the shaft causing the device to rotate. The RABR is then submerged in water up to 40% of its height allowing for exposure to nutrients and the atmosphere (Christenson, 2011; Jackson and Jackson, 1972; Przytockajusiak et al., 1984). The rotating photobioreactor, and other algae biofilm reactors, are thought to have several benefits over more traditional algae

culturing techniques (Christenson, 2011; Johnson and Wen, 2010). The RABR grows algae by way of a biofilm. A photograph of a rotating algal biofilm reactor can be seen in Figure 2.

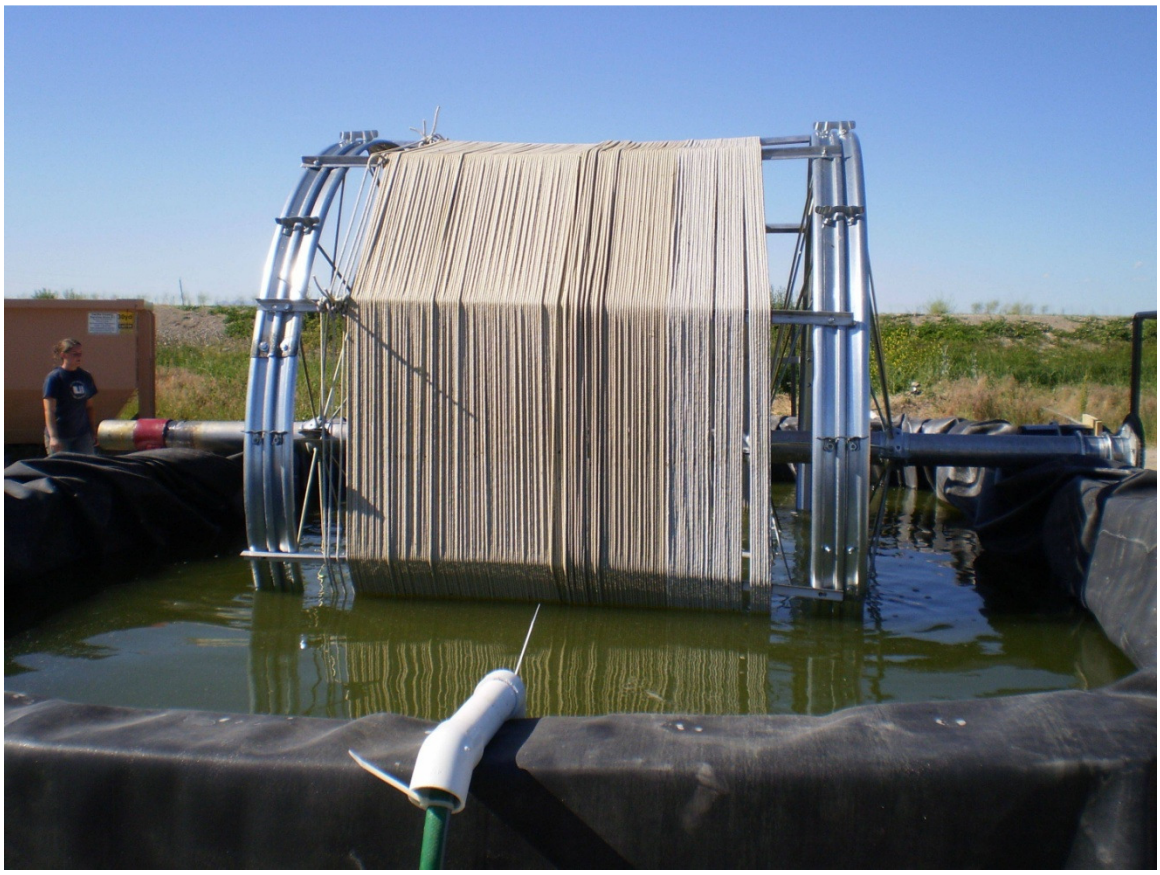

Figure 2: A Pilot Scale RABR Unit.
(Logan, UT Municipal Wastewater Treatment Plant)

The cells attach to a substratum wrapped around the cylinder and then grow on the surface of the device. A centralized and thick algal culture has implication for the harvesting of the algae, as it can now be scraped off the surface instead of processed through costly centrifuges or filters. Other advantages of the RABR include good gas exchange due to exposure to the air, possible benefits from light/dark cycling of the

algae, and wastewater nutrient removal due to a high quantity of centralized biomass (Christenson, 2011).

Modeling of Biofilms

There is little refereed literature concerning the modeling of algae biofilms, and none concerning the growth of biomass on a rotating cylinder such as the Rotating Algal Biofilm Reactor. There has been some research into the modeling of biofilm production, however like most topics concerning algae there are far more papers considering its growth as suspended particles (Celekli et al., 2009; Liehr et al., 1989; Mesple et al., 1995; Wang and Zhang, 2010). The Rotating Biological Contactor has also been studied, however the focus is on heterotrophic growth and not autotrophic growth (Baban et al., 2010). Much of the literature concerning algal biofilm biomass focuses on the fate of one substance, such as inorganic carbon, or stream periphyton. Often these models assume the algae grow on a fixed bed and not in the form of the RABR (Buzzelli et al., 2000; Duong Hong SonFujino, 2003).

From the lack of refereed publication concerning specifically growth of algal biofilms on a rotating drum, and the lack of biofilm algae models when compared to heterotrophic systems, developing mathematical models to provide insight into these reactors would be beneficial. Improving design and performance can be greatly facilitated through mathematical model development. Mathematical models can estimate system behavior in response to different levels of key factors and provide valuable feedback. Models also allow us to determine what parameters the system is most sensitive to. Making changes with regard to some parameters my yield less of the desired result when compared to others. This allows the engineer to gain insight into how the system

performs, and what parameters would be most crucial when making improvements to the system.

Biofilms as a Source of Fatty Acid Methyl Esters

Biofilms have been explored intensively in the realm of secondary and tertiary wastewater treatment. However there are minimal reports on the biomass yields of algae cultivated as biofilms, and few papers were found that explicitly focused on biomass production in biofilms. In consulting several papers concerning a review of current technology concerning algae based biofuels, each focused on open ponds and closed photobioreactors as primary cultivation (Brennan and Owende, 2010; Sander and Murthy, 2010; Shen et al., 2009), only one paper mentioned algal biofilms (Shen et al., 2009). Considering the large variety of ways that algal biomass can be converted to fuel and biofilm growth provides a cheap and elegant solution to the problem of costly harvesting, growing algae as biofilms could provide a solution. If biofilms are shown to perform as well as their suspended growth counterparts or augment production of existing open pond systems, they will have wide application as a biomass source. For these reasons biofilms merit investigation as a source of biomass for not only producing biofuels, but potentially other applications as well. Not until recently has biofilm growth of algae been seen as a source of biomass, and the article suggests that the cultivation method still needs refinement (Shen et al., 2009).

Triacylglycerol (TAG) production in microalgae is the primary source of fatty acids for the production of FAME. There have been several studies addressing fatty acid profiles, lipid content, and increasing their content in microalgae (Chiu et al., 2009; Eichenberger, 1976; Li et al., 2008; Li et al., 2010; Lv et al., 2010; Tonon et al., 2002;

Widjaja et al., 2009). However these have all been for suspended growth in closed reactors or open ponds. Not a single article was found in the literature search for this thesis that combined the topics of algal or phototrophic biofilms and lipid or TAG production. If biofilms are to be used as a FAME source, then lipid and fatty acid production needs to be quantified. Considering the lipid content by weight of green algae varies immensely with nutrient and culture conditions the lipid potential of biofilms should be explored. If the RABR is to be a feasible method of cultivating algae biomass for the production of biodiesel, it must be competitive with other methods of cultivation. A recent study has identified lipid productivity as one of the key aspects for choosing the strains of algae to be used for biodiesel production, therefore this aspect needs to be investigated with immobilized algae (Griffiths and Harrison, 2009). RABRs should have the advantage of easy downstream processing, but have not yet been evaluated for lipid productivity and content.

LITERATURE REVIEW

Biofilm Modeling

Some literature exists concerning the mathematical modeling of biofilms (Flora et al., 1998; Lamotta, 1995; Liehr et al., 1998; Liehr et al., 1990; Wolf et al., 2007). While a majority of literature addresses bacterial biofilms, algal biofilms have received some attention. One observation from this literature review is that there are very few papers taking a multi-faceted approach to the modeling of algal biofilms, and even fewer taking into account a realistic scenario involving both bacteria and algae in the same biofilm (Wanner and Reichert, 1996; Wolf et al., 2007). Many papers focus on one or two key nutrients or parameters rather than considering the whole system. Often the targets of such focus include pH value, and potential carbon and phosphorus limitation in biofilms. The diffusion of nutrients into the biofilm, as well as the concentration boundary layer has also received attention from researchers (Lamotta, 1995; Larned et al., 2004; Liehr et al., 1989).

There are several different approaches to modeling biofilms, which include both algal and bacterial. The first models proposed were one dimensional continuum models developed in the early to mid 1980's. While these methods are relatively simple compared to more sophisticated means of analysis, specifically in the interaction of the substrate and the biofilm, the approach has shown to be a useful means of modeling (Wanner and Reichert, 1996). Other model types exist including discrete continuum models, hybrid discrete continuum models, and multi-dimensional continuum models. Each of these model types increase overall model complexity (Chaudhry and Beg, 1998). In the case of phototrophic biofilms, one-dimensional modeling is still in use. It appears

as if phototrophic biofilm modeling is primarily being used for wastewater treatment (Buzzelli et al., 2000; Cerucci et al., 2010; Duong Hong SonFujino, 2003). The model goals are nutrient removal and biomass production in bulk. Other models appear to focus on the exact spread of the biofilm as it grows, resolution that is not needed in this case. Further complexity is added in that full fluid dynamic simulations come into play via the Navier-Stokes equation, with the fluid mechanics assumed to be pseudo-steady state as the biofilm grows (Wang and Zhang, 2010). These models are successful at predicting the specific shape of the biofilm, often providing unnecessary resolution. Bulk biomass, lipid concentration, and nutrient uptake can be modeled sufficiently with one-dimensional continuum analysis. Considering the 90 day retention time of the Logan Lagoons system advanced fluid dynamic techniques are likely unnecessary.

Carbon species and pH have been identified by researchers as areas of prime interest, due to the high pH values that occur in phototrophic biofilms, specifically in the deeper regions. The argument is that the only carbon sources bio-available to algae are carbon dioxide and bicarbonate, and high pH conditions transform these compounds into carbonate, thus causing a carbon limitation at the base of the biofilm near the substratum (Flora et al., 1998; Liehr et al., 1990; Liehr et al., 1998). Thus it becomes important to consider pH changes over time as it will likely rise due to uptake of carbon dioxide and bicarbonate due to the algae growth.

While most studies focus on one specific aspect of the biofilm for modeling, there has been one comprehensive approach to modeling mixed culture, but predominantly phototrophic biofilms. Researchers from the Netherlands proposed the PHOBIA model, a mathematical modeling including carbon speciation, light, light adaptation, nutrient

uptake, phototrophic and heterotrophic growth, and external poly-saccharide production (Wolf et al., 2007). While this model was not verified when compared to actual data, at least according to the article, it appears to be the most exhaustive and complete model in algal biofilm modeling. One interesting aspect of this model is the dynamic switching of limiting substrate. Limits were set on different limiting parameters, and the model switched to the proper limiting substrate. This model appears to be the most complete model to date (Wolf et al., 2007).

Wastewater Treatment and Biofilms

Algae, both suspended and biofilm, have been shown to be an effective method of treating municipal wastewater (Aslan and Kapdan, 2006; Craggs et al., 1996a; Craggs et al., 1996b; Garcia et al., 2008; Hosetti and Frost, 2009; Kelly, 2002; Kent et al., 2005; Kong et al., 2010; Larsdotter et al., 2007; Tarlan et al., 2002; Voltolina et al., 1999; Wang et al., 2010; Woertz et al., 2009) and livestock wastewater (de Godos et al., 2009; Gonzalez et al., 2008; Jimenez-Perez et al., 2004a; Wilkie and Mulbry, 2002; Woertz et al., 2009). Algae based wastewater treatment was first largely proposed in 1958 by Oswald and Gotaas (Oswald and Gotaas, 1955). Nitrogen and phosphorus are generally the primary contaminants that algae remove, but they have also been shown to take up heavy metals (Ahluwalia and Goyal, 2007; Garcia-Meza et al., 2005; Jacinto et al., 2009; Khoshmanesh et al., 1996; Mallick, 2002; Pascucci and Kowalak, 1999). Biofilms have been shown to be competent in these areas as well (Mallick, 2002). While algal biofilms have not been viewed as a source of biomass feedstock, they have been explored in terms of wastewater treatment, commonly either immobilized in alginate or as a trickling filter. Immobilized algae have been shown to be as effective as suspended algae at treating

wastewater. Under proper conditions many immobilized biofilms are capable of removing more than 80% of the total phosphorus and greater than 90% of the total nitrogen in a wastewater stream (Przytockajusiak et al., 1984; Shi et al., 2007; Zhang et al., 2008). Nutrient removal has been observed primarily in species of Scenedesmus and Chlorella. While algal biofilms have been shown to be a promising form of nutrient removal from wastewater streams, they are not without issue as discussed below.

Continuous operation of algal biofilm wastewater treatment methods shows lower removal of nutrients over time than suspended cultures (Ruiz-Marin et al., 2010). A study reported an initial high removal rate of nitrogen and phosphorus, which decreased over a time, removing less than 30% of total nitrogen and phosphorus after a 10 day period. This performance was attributed to culture age and collapse (Ruiz-Marin et al., 2010). If the biofilms were to be harvested for biomass, this issue would be alleviated, valuable product could be acquired, and wastewater could still be treated upon harvesting of the previous algae resulting in new growth and a hence a continuously operational system. While the research indicated that the nutritional value of the algae was poor, this condition could possibly be remedied by changes to the nutritional regime of the algae or possibly by timing the harvest of the algae to prevent a large decline of the culture.

Algal biofilms could also be useful for treating low strength wastewaters. Biofilms naturally have a nutrient gradient within the film itself. This drives a flux of nutrients into the film. Under low strength wastewaters it appears that this driving force causes more nutrient uptake and more biomass production in biofilm systems compared to suspended growth algae, where the nutrient flux would not be as strong (Kim, 1995). However, this phenomenon would likely only hold under laminar flow conditions in

which the metabolism of the biofilm drives the nutrient gradient. Researchers were able to increase glucose removal by a biofilm by increasing the speed of the flow past the film. It is possible however that this could cause shear forces that would result in the biofilm detaching from its substratum (Kim, 1995).

Treatment of wastewater through algal biofilms has also been employed in rotating discs. One of the first studies was performed in 1971, and while the study attempted to use rotating discs with triangular cross sections proper light prevented the apparatus from producing any meaningful results (Torpey et al., 1971). Work was later done with *Stichococcus bacillarus* growing on rotating Styrofoam discs half submerged in water (Przytockajusiak et al., 1984). While these disks were successful at removing ammonia from the water source, they were not capable of removing other nitrogen sources. They were also not able to remove all the nitrogen, but it was noted that this could be outweighed by the ease of harvesting the algae preventing effluent from containing biomass (Przytockajusiak et al., 1984). Another study used pilot scale rotating discs with attached algae growth and found that the removal rate of the algal biofilm was six times greater than that of the suspended algae (Jackson and Jackson, 1972). Additional rotating algal discs greatly increased performance of phosphorus removal as well, yielding a consistent 90% removal with an appropriate number of discs. The study recommended using rotating algal discs as an addition to typical wastewater treatment because it can significantly improve effluent quality (Jackson and Jackson, 1972). This design has also been employed for the successful removal of hydrocarbons by phototrophic biomass (Suzuki and Yamaya, 2005). RABRs seek to integrate the role of algal biofilms as a treatment medium with the mechanical design of a rotating biological

contactor, similar to those of the rotating algal discs previously discussed but with the added goal of biomass cultivation (Christenson, 2011).

There is also concern that most attached algae research for wastewater treatment has been at the benchtop scale. There have been a few studies concerning the large scale use of algal biofilms as a treatment method, and one was even a surprise as the treatment design facilitated the growth of algal biofilms instead of suspended algae (Hemens and Mason, 1968). A more recent study employed the use of an algal turf scrubber as a means of removing nutrients from a wastewater stream. This device achieved effluent phosphorus concentrations of less than 1mg/L. While data from an entire year of treatment showed higher effluent concentrations, this was likely due to experimentation by the researchers into plant operation during the year. It was also suggested that this particular device selected for cyanobacteria heavily in a mixed bacteria and green alga culture (Craggs et al., 1996b).

Lipid Production in Algae

The effect of various nutrient, energy, and environmental conditions on suspended growth microalgae has been quite extensive. Nitrogen deprivation in particular has shown an increase in the lipid and TAG content of microalgae. Phosphorus deprivation, an increase in carbon dioxide, and an increase in pH also show higher lipid and TAG values in literature. These factors are summarized in Table 1.

Table 1: Effect of Various Factors on Biomass Growth and Lipid Content in Microalgae

Factor	Biomass Growth	Lipid Content and Fatty Acid Profile
Sunlight	Light is the primary substrate for biomass growth and will cause an increase in algae growth, until photo-inhibition begins to occur. (Richmond, 2004)	Generally light spurs lipid synthesis, while dark periods see a decrease in tag content, likely due to synthesis of polar lipids for cell membranes. (Thompson, 1996)
Nitrogen	7-10% of algal biomass is comprised of Nitrogen, making it an essential nutrient. Higher concentrations increase biomass growth. (Richmond, 2004)	Low nitrogen spurs lipid synthesis in many strains of algae, some reaching as high as 40% by weight in *Chlorella* and *Scenedesmus*, which may be due to catabolism of chloroplast lipids for energy storage. (Thompson, 1996)
Phosphorus	Phosphorus is a second important nutrient for algae, and higher concentrations increase biomass	Phosphorus limitation has been shown to increase algae lipid content. Limitation also seems to encourage production of unsaturated fatty acids. (Guschina and Harwood, 2006)
Carbon Dioxide	Carbon dioxide along with bicarbonate form the primary carbon sources for algae. (Richmond, 2004)	Low Carbon Dioxide concentrations have been shown to repress fatty acid synthesis, which also may cause an increase in unsaturated fatty acids.(Guschina and Harwood, 2006)
pH	Little is found in literature concerning algae growth and pH.	In *Chlorella* species an alkaline pH stress resulted in higher lipid accumulation. (Guschina and Harwood, 2006)
Oxygen	Oxygen has shown to inhibit cell growth and become toxic to algae in very high concentrations. (Richmond, 2004)	Algae show a decrease in Poly Unsaturated Fatty Acid (PUFA) content under increasingly heterotrophic conditions. (Guschina and Harwood, 2006)

No refereed publications concerning lipid concentrations in green algae grown as biofilms have been found during work on this thesis. The one crucial aspect that will help decide the commercial success of algae derived biodiesel via transesterification is the lipid productivity. This has been an issue in algae cultivation methodology. Depriving the algae of nitrogen causes spikes in lipid content; however limiting the nitrogen also inhibits the growth of the biomass (Sialve et al., July). Thus either a small amount of biomass can be grown with high lipid content, or a larger amount of biomass can be grown with lower lipid content. One study shows the advantage of a large quantity of biomass with a low lipid content approach (Widjaja et al., 2009). Table 2 shows the lipid productivity of *Chlorella vulgaris* and *Scenedesmus obliquus* under different growth conditions.

Table 2: Lipid Productivity under Different Growth Conditions

Species	Conditions	Lipid Content (%w/w)	Lipid Productivity (mg/l-day)	Biomass Productivity (g/l-day)
Scenedesmus (Li et al., 2010)	2:1 N:P	30	8.7	0.03
	4:1 N:P	22	8.5	0.056
	8:1 N:P	21	11.3	0.056
	12:1 N:P	24	15.1	0.066
	20:1 N:P	25	20.3	0.083
Chlorella (Widjaja et al., 2009)	7 days -N	40	11	-
	14 days -N	52	12	-

It appears from Table 2 that even a two-stage growth approach wherein algae have plenty of nutrients and are then deprived of nitrogen shows a marginal gain over complete nitrogen starvation. However, when compared to algal growth under nutrient sufficient conditions the previously described two stage cultivation method seems to underperform in terms of lipid productivity. In suspended growth systems algae nutrient deprivation, while increasing overall lipid content of algal biomass, decreases lipid productivity.

Biofilm and Suspended Algal Growth and Lipid Production Rates

To be competitive with suspended growth algae, biomass yields must be similar for a biofilm based reactor. Any competitive gain obtained through decreasing expensive harvesting costs would be lost if algal biofilms yielded less biomass and lipid. Therefore comparisons of the biomass and lipid yields are important to see if biofilms yield similar results to suspended systems. The issue is that commonly reported units of g/l-day for conventional algae culturing reactors have little meaning when considering a fixed film system, which reports growth in terms of g/m^2-day. However, a comparison could be made to flat plate reactors that often report growth in terms of g/m^2-day.

There are limited reports concerning biomass productivity of algal biofilms, but the evidence indicates that the growth rate is similar across both modes of growth (Jimenez-Perez et al., 2004b; Johnson and Wen, 2010). A paper examining the treatment of swine effluent reported that both biofilm and suspended growth algae yielded nearly the same biomass growth (Jimenez-Perez et al., 2004a). This result would be expected because both types of algae were exposed to similar nutrient levels under the same light conditions. For more quantitative evidence, a study compared the yields of suspended and

fixed growth systems of the same volume and under the same conditions (Johnson and Wen, 2010). In 200mL reactors, the biofilm yielded 0.034 g/day, while the suspended growth system showed 0.027 g/day. The advantage clearly goes to the biofilm in this study, with over a 20% increased yield over the suspended growth system (Johnson and Wen, 2010). The fatty acid productivity for the biofilm reactor was similarly higher due to the same concentration of approximately 9% by weight for both samples (Johnson and Wen, 2010).

This comparison however is not ideal considering there are more advanced reactors to cultivate algae than a simple tub. Outdoor raceway ponds and indoor reactors have been optimized over several studies for both algal growth and lipid content (Garcia et al., 2008). While such optimization does not exist for biofilm based reactors, a comparison should be made to see how well even basic biofilm reactors compare to more technically advanced forms of cultivation. Table 3 compares biomass yields of various growth modes and culture conditions for species of the green alga genus Chlorella.

Table 3: Growth and Lipid Yields of Chlorella under Varying Cultivation Modes

Organism	Method of Growth	Growth Yield (g/m^2-day)	Fatty Acid %	Lipid Productivity (mg/l-day)
Chlorella sp. (Johnson and Wen, 2010)	Attached/Dairy Wastewater	25.7	9	231 mg/m^2-day
Chlorella sp. (Johnson and Wen, 2010)	Suspended/Dairy Wastewater	1.27 g/l	9	12.7
Chlorella vulgaris (Widjaja et al., 2009)	Suspended/Normal Nutrition	-	29.5	12.77
Chlorella Vulgaris (Widjaja et al., 2009)	Suspended/Increased CO2	-	25	13
Chlorella Vulgaris (Widjaja et al., 2009)	Suspended/Increased CO2 -N	-	52	12
Chlorella sorokiniana (Cuaresma et al., 2009)	Suspended-Flate Plate/Normal Nutrition	185	-	-
Chlorella sp. (Hase et al., 2002)	Raceway Pond/ Increased CO2	13.2	-	-

From Table 3 several things can be inferred. Compared to advanced flat plate reactor design, the biofilm reactor falls short in terms of biomass productivity. However, the biofilm reactor almost doubles the biomass productivity of the preferred economic method of algae cultivation, the outdoor raceway pond. While most advanced flat plate reactors are considered too expensive in capital costs for economically feasible algae production, the only difference in the study between the outdoor algae pond and the biofilm reactor was the addition of a substratum for the algae to attach to. This study used polystyrene for the biofilm substratum (Johnson and Wen, 2010). In terms of lipid productivity. the 12.7 mg/l-day of the suspended growth algae from the study could be used as a conservative estimate for the biofilm lipid productivity, considering the similar fatty acid concentration and higher yield (Johnson and Wen, 2010). Despite a low fatty acid percentage, the lipid productivity was similar to other methods of suspended cultivation. Overall the potential to nearly double algae growth yields and maintain lipid productivity with a modest cost increase is a very attractive combination that merits further investigation.

Potential Benefits of Biofilm Cultivation

Harvesting has recently been estimated at 20-30% the cost of producing biodiesel from algae through methods such as centrifugation, filtration, and dissolved air flotation, all energy and resource intensive processes (Brennan and Owende, 2010). Clearly this is an impediment to the commercial success of algae as a biofuel feedstock. A recent life cycle analysis has shown that algal dewatering is the biggest energy sink in the algal biodiesel process (Ruiz-Marin et al., 2010). A paper recently published stated that

dewatering and harvesting methods need to be judged on three criteria: 1) rate of water removal, 2) solid content of the recovered algae, and 3) efficiency/yield of the de-watering technique (Uduman et al., 2010). A biofilm reactor has an advantage in these three areas.

The first criterion for a biofilm based reactor is achieved as the algae are grown so there is no rate of water removal. By the nature of the biofilm itself the water is removed due to the dense concentration of algae, which makes algal biofilms a form of algae dewatering. Depending on the density of the biofilm and the percent solids by weight desired for the energy conversion process, a second de-watering step may be needed. Often for suspended cultures, a dewatering technique is used to increase the suspended culture to approximately 5% solids by weight, and then if required a second process is usually used to increase the concentration to 10-25% depending on the application (Uduman et al., 2010). Indoor biofilms have been shown to be approximately 6% solids by weight, enough to eliminate the first step, although some algal biofilms have achieved a range of 10-15% which may be sufficient to remove the need for all algae dewatering and harvesting processes (Christenson, 2011).

The solid content of recovered algae would be expected to vary from harvest to harvest as growth conditions, ambient conditions, and the proportion of species in the mixed culture growth changes. However, there is a lower bound for this value, which is much higher than suspended growth algae. A recent study showed that a biofilm of Chlorella *sp* achieved 6.25% solids dry weight with growth on dairy waste effluent (Johnson and Wen, 2010). This is lower when compared to 7.84% solids dry weight discharge of a centrifuge used in the same study, but the energy use for the centrifugation

method was higher. Other more recent work has shown that under outdoor growth conditions, a mixed culture algal biofilm achieved a range of 10-15% solid dry weight (Christenson, 2011). This is enough to bypass nearly all subsequent dewatering steps for conversion from biomass to energy.

The last criterion will be difficult to evaluate as a biofilm harvesting technique has not yet been tested. Wastewater treatment methods that grow algal biofilms such as trickling filters and tubular reactors have not been focused on biofilm removal. Assuming that harvesting the biofilm would be by a simple scraping mechanism, losses should be minimal and attributed mainly to biomass that remains after the scraping or leaks away from the scraping mechanism. Also after harvesting some seed algae should remain to grow biofilm in subsequent cycles. This is necessary to prevent another attachment phase and has been shown to increase production. A comparison of biofilms as a dewatering method to other common methods of algae separation and dewatering is shown in Table 4.

Table 4: Comparison of Biofilms as a Harvesting and Dewatering Method to Other Processes (Uduman et al., 2010)

Process	Highest Possible Yield (TSS dw/w)	Energy Usage
Biofilm	6-15%	Low
Centrifugation	12%	Very High
Dissolved Air Flotation	1-6%	High
Gravity Sedimentation	0.5-1.5%	Low

The advantage of the algal biofilm comes into play when considering the material and operational cost of the process as a form of dewatering. If biofilms are being grown already in the form of a trickling filter or tubular reactor, the cost of the dewatering process is negligible, as it is incorporated into the cost of growing the algae. A more detailed analysis is required if reactors are to be designed and implemented specifically for the purpose of cultivating algal biofilms as a feedstock source. But considering algal biofilms supply a form of dewatering that is on par in terms of concentration and yield with other methods, takes no time for processing, and requires minimal energy, this biomass source merits further investigation. Dewatering of algae has been estimated to consist of 20-30% of the manufacturing costs for making biofuels (Brennan and Owende, 2010). Fixed growth algae could have the potential to eliminate a majority of that cost. The growth of algal biofilms should be looked at as a way to minimize dewatering costs in order to make algal biodiesel more economically attractive as a fuel source.

MODEL CONSTRUCTION, ASSUMPTIONS, AND CONSTRAINTS

Initial Aquasim Modeling

Initially a program called Aquasim was used for the modeling effort in this thesis. This software is based on biofilm modeling articles published by Wanner and Reichert in the late 1980's and early 1990's (Wanner and Reichert, 1996). This decision was primarily based on the fact that this software was one of the few packages that could handle biofilm biomass growth simply through user input. There was also evidence of phototrophic biomass being modeled within the software itself in the form of the PHOBIA model developed in the Netherlands (Wolf et al., 2007). However after working with the software for some time, the determination was made that several variables would be difficult to obtain and seemed overly complex for the data available. The software required values such as diffusivity of soluble nutrients through the biofilm, attachment velocities, and others to function. However, constraints on time and personnel limited the research that could be undertaken.

The values required could be determined from literature, or considered to be negligible in terms of the overall model. However it would be disingenuous to add extra complexity where it may not be warranted depending on the outcome of simpler models, and when the implementation of that complexity may be of questionable execution. Because this is a novel reactor and has not been explored numerically before, the Aquasim model was abandoned in favor of a simpler model, with fewer parameters that could feasibly be extrapolated from the data collected. The simpler model was more appropriate to RABR operation.

There is also something of a black box effect when using Aquasim. The algorithm used was referenced in a journal paper, but without access to the inner workings of the

program it becomes difficult to know exactly what is occurring. This may not be important; however not knowing what exactly is occurring within the code can be detrimental. This was another reason for the shift to a MATLAB model over using Aquasim.

Due to the precise way in which Aquasim wants the user to enter data and parameters it can also be inflexible in terms of the model approaches it can handle. Through the course of the modeling effort, Droop Kinetics were experimented with as a possible kinetic model for algae growth and nutrient uptake (Cerucci et al., 2010). This kinetic model was unusable within the scope of Aquasim. Due to these issues identified above, Aquasim was abandoned for one designed in MATLAB. Using MATLAB provides more control and flexibility. Therefore MATLAB was used for all subsequent modeling.

Matlab Model

Approach and Equations

Several different approaches and equations could be used to model this system. More complex biochemical type models have been used in terms of the rotating biological contactor (RBC) (Baban et al., 2010). However their usefulness is limited as they are concerned with heterotrophic as opposed to phototrophic biomass. Several other kinetic models exist for suspended growth algae as opposed to biofilm biomass. Internal cell quota kinetics have often been used in modeling of algae (Cerucci et al., 2010). However given the amount of data that could be collected and the parameters needed for analysis, this approach was also abandoned in favor of a simpler model.

Logistic growth equations have been used in population modeling, and have been used to model populations spanning from microbes to mammals (Sibly, 2005). The general logistic growth equation is shown in Equation 1.

Equation 1: General Logistic Growth

$$\frac{dN}{dt} = \mu N\left(1 - \frac{N}{K}\right)$$

In this equation N is the current population, K is the carrying capacity, the theoretical maximum number of organisms that can be grown in a given space, and μ is the maximum growth rate. Thus the actual population growth rate will be decline as the carry capacity is approached. However, higher growth rates will imply faster growth over the course of time despite being limited by the carrying capacity. The carrying capacity is the maximum number of organisms that can be grown. A higher carrying capacity not only implies that more biomass can be grown, but growth at lower biomass concentrations will be faster. Logistic growth has even recently produced good models for algal biomass growth (Chen et al., 2010; Yang et al., 2011).

A simple logistic growth equation could capture the behavior of the biomass population. However there is some inhibition inherent in the RABR system. Mutual shading is more obvious in a biofilm as opposed to a suspended growth culture. In fact a study has shown that only a portion of the biofilm near the surface is fully photosynthetically active. A study done on cyanobacterial mats found that only the exterior 0.5 mm of the film was available for light. This study also suggests that

inhibition occurs due to light limitation 0.1 mm of the film due to light attenuation by the upper layers of the film (Glud et al., 1990).

Inhibition has even more profound effects at higher photosynthetic photon flux densities experienced at the Logan Lagoons. Average mid-day sun energy often exceeded 2000 μmol/m^2-s. At a high light attenuation in algal biofilms and high photoinhibition, the maximum photosynthetic rate dropped drastically as the biofilm increased in thickness. Even at low attenuation and high photoinhibition, the maximum photosynthetic rate decreased with both biofilm thickness and increasing light intensity (Dodds et al., 1999). Taking this into consideration, there will be inhibition of growth in a biofilm such as this due to shading of biomass in deeper layers of the biofilm. Thus the logistic growth term will be raised to the term theta in order to represent some of this inhibition in the model.

The theta logistic model seeks to capture inhibitory effects of populations. Increasing numbers of the population have a greater effect on the growth of the population more than just by approaching carrying capacity as explained below. This is done by raising the logistic growth term to the power of theta. This is seen in Equation 2.

Equation 2: Theta-Logistic Growth

$$\frac{dN}{dt} = \mu N \left(1 - \frac{N}{K}\right)^{\theta}$$

This seems to be the instance in the case of the RABR. As algae grow they inhibit each other by shading as opposed to simply reaching a theoretical or observed maximum carrying capacity. While the value of theta does not correspond directly to any physical parameter in the system, it could provide a means of capturing this and other inhibitory

phenomenon occurring in the RABR. This population model has shown success in a large scope of applications (Sibly, 2005). Given that the logistic growth equation has shown to be successful in modeling algal biomass growth in recent literature, and the ability of this logistic growth variant to capture inhibition effects, these equations were employed in this model.

Having selected the theta-logistic growth model for this effort, it becomes prudent to discuss theta and its affect on the system. The value of theta can give important insights into the function of the system and how it responds to an increasing number of organisms. A theta value of one shows normal un-inhibited logistic growth. A value for theta greater than one implies that limitation is prevalent at the end of the growth cycle closer to the carrying capacity where it is inhibited the most. Theta values less than one imply that growth is initially very quick but the growth rate declines quickly and is slow to approach carrying capacity. A graph of per capita growth rate versus population size can be seen in Figure 3 taken from Sibly. In this figure pgr is the per capita growth rate, and N is the amount of organisms. All constants are the same except theta, which is labeled in the graph. By determining the fit of these equations to data, we can determine which of the types of inhibition described above are present in the algae, and learn more about how biomass production evolves over time.

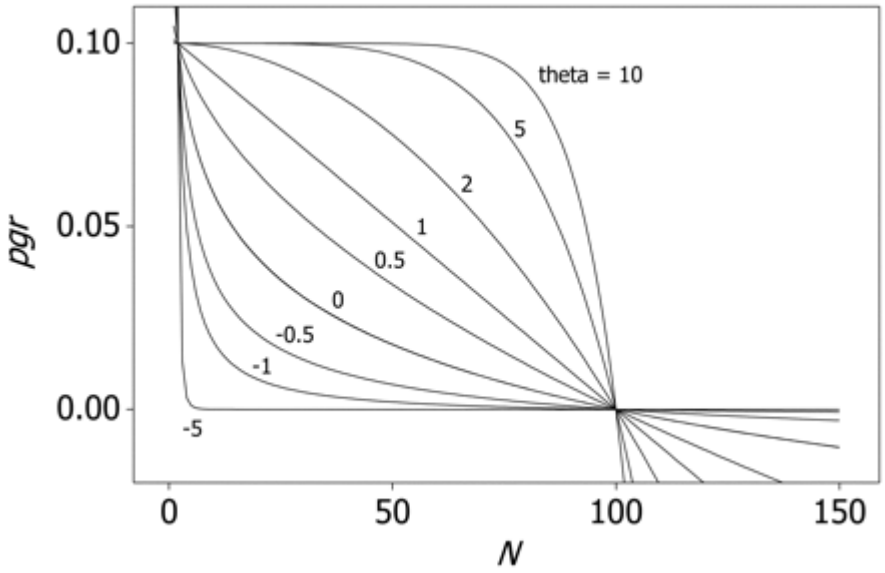

Figure 3: Effect of Theta on Logistic Growth Equation Per Capita Growth Rate (Sibly, 2005)

The equations used within this model are based on the previously described inhibited theta-logistic growth. Thus growth will slow as the population approaches a carrying capacity. The full set of equations can be seen in Equations three through five, and variables names are identified in Table 5.

Equation 3: Biomass Growth

$$\frac{dB}{dt} = \mu B\left(1 - \frac{B}{K}\right)^{\theta} - DB$$

Equation 4: Nitrogen Uptake

$$\frac{dN}{dt} = -\mu gNB * \frac{S}{V}\left(1 - \frac{B}{K}\right)^{\theta} + F(N_{in} - N)$$

Equation 5: Phosphorus Uptake

$$\frac{dP}{dt} = -\mu rPB * \frac{S}{V}\left(1 - \frac{B}{K}\right)^{\theta} + F(P_{in} - P)$$

Table 5: Modeling Variables

Variable	Identity	Units
t	Time	day
B	Biomass	g/m^2
N	Nitrogen	mg/L
P	Phosphorus	mg/L
μ	Growth Rate	1/day
K	Carrying Capacity	g/m^2
Θ	Inhibition Constant	dimensionless
F	Dilution Rate	1/day
g	Nitrogen Uptake	gN/gB
r	Phosphorus Uptake	gP/gB
D	Death Rate	1/day
S	Surface Area	m^3
V	Volume	L
A0	Initial Biomass	g/m^2

The state variables are biomass, nitrogen, and phosphorus. These variables will change in time. Volume, surface area, and dilution rate are constants based on the physical parameters of the system. Values for growth rate, carrying capacity, inhibition constant, death rate, phosphorus uptake, nitrogen uptake, and initial biomass are constant values based on data taken from RABR systems.

Assumptions

The model constructed in MATLAB is based on several assumptions. First is the assumption that growth is limited by nitrogen in the biofilm and Logan Lagoon system. Chemical analysis of Logan Lagoon biofilm algae grown on the pilot scale RABR by Logan Christensen in November of 2010 yielded a Carbon, Nitrogen, Phosphorus ratio of approximately 50:8:1 by mass, and 130: 18:1 molar ratio. Data collected during this study showed that the molar ratio of nitrogen to phosphorus in the Logan Lagoons wastewater

is frequently on the order of 1.35:1 this indicates heavily nitrogen limited growth because the ideal nitrogen to phosphorus ratio for non nutrient-limited growth is 16:1 (Stumm and Morgan, 1996). While phosphorus uptake by the biomass is considered in the model, it is not factored into growth of the algal biomass since it is present in excess. Considering the RABRs were grown in ponds fed by effluent from the Logan Lagoon system this assumption holds. Given the data described above, it was assumed that nitrogen would be the limiting nutrient for growth.

It was also assumed that within the biofilm diffusion of nitrogen through the biofilm was on such a quick time scale it is largely ignorable. Therefore diffusion of nitrogen through the bulk of the biofilm was ignored. In future iterations of the model this may be reconsidered. Excluding this aspect from the model was also due to difficulty in being able to measure the amount nitrogen through the thickness of the biofilm. The fact that the time scale of diffusion would be fast compared to the growth of biomass in the model and the difficult of accurately determining such a constant led to diffusion being excluded from model.

Within the process of creating various models, Droop Kinetics were considered as a possibility (Cerucci et al., 2010; Cherif and Loreau, 2010). After consideration, this complication was determined unnecessary. Droop kinetics assume that uptake is independent from nutrient assimilation into biomass, where as other kinetic equations assume assimilation and uptake are simultaneous. The idea of creating biomass from an internal reserve is important when considering luxury uptake of nutrients, thus allowing biomass to grow under otherwise unfavorable nutrient profiles until the internal quota is consumed. Droop kinetics have been employed in a wide variety of models concerning

algae for this reason. However with a relatively stable or slow changing nutrient profile within the Logan Lagoons system, an internal reserve of nutrient would likely reach an equilibrium with the external nutrient stream and thus be negligible. Until such time as the current model shows inability to predict biomass growth and nutrient uptake under a range of scenarios Droop Kinetics will not be used.

Construction

Constants were determined from non-linear regression of data collected from three pilot scale RABRs. The RABRs were operated starting in July of 2010. In each trial, data were collected for biomass on the rope surface and for influent and effluent nitrogen and phosphorus concentrations. The physical operational parameters can be seen in Table 6.

Table 6: Pilot Scale RABR Operational Parameters

Parameter	Value	Units
Diameter	6	ft
Pond Volume	10700	Liters
Influent Flow Rate	8	Liters/Minute
Rotational Speed	0.313	RPM
Length of Rope	4000	feet
Growth Surface Area	24.3	m^2
Installed Reactor Area (Plan View)	4.6	m^2

A diagram of how three pilot scale RABRs as used for data collection should theoretically work can be seen in Figure 4. Each system had independent influent and effluent streams, however all operational conditions were the same. Data were collected

every Monday, Wednesday, and Friday starting after the first week of construction to allow for an adequate amount of biomass to be grown.

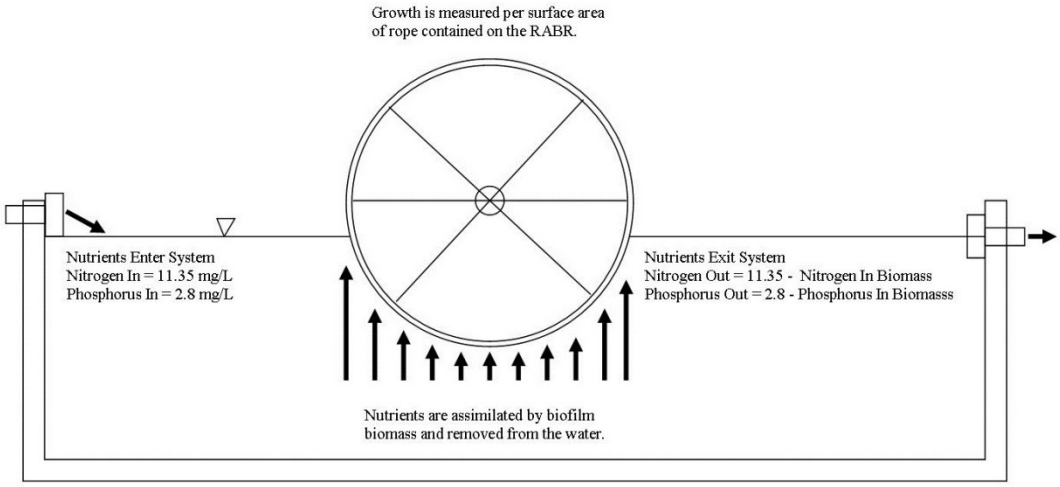

Figure 4: Diagram of Pilot Scale RABRs

The biomass data collected showed similar trends across all three reactors. While these reactors were operated under the same conditions, some variability could not be controlled because an open system was evaluated. The biomass production from each of these three reactors can be seen in Figure 5. Data shown was biomass per rope surface area, not plan view area.

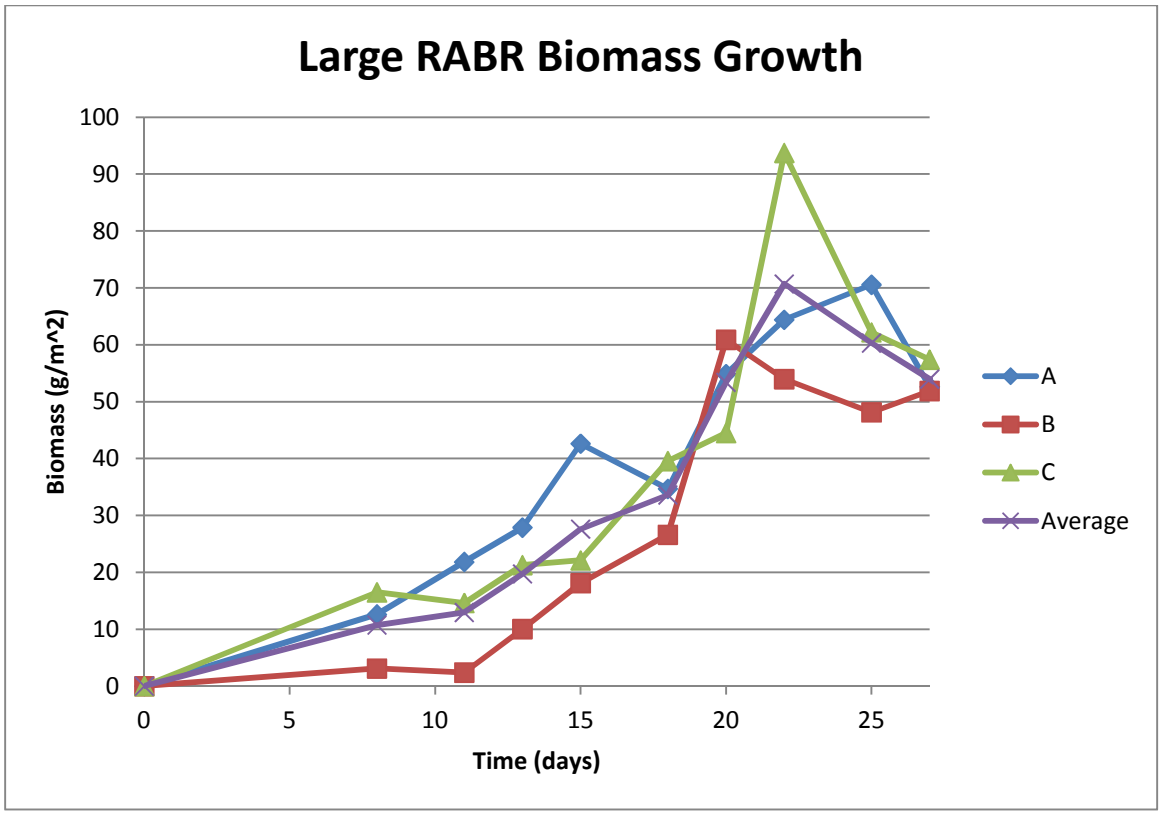

Figure 5: Large Outdoor RABR Biomass Growth

This data shows similar trends across all reactors. Some of the reactors were slow to start, that is likely because several of the reactors stopped turning after initial startup. However after these reactors were restarted they caught up quickly.

The average of the data was then fit to the equations discussed in the previous section. The fitting was done through a Matlab function that takes the data, equations, and an initial guess for five parameters, growth rate, carrying capacity, theta, death rate, and the initial amount of algae and attempts to find the best values that fit the data. This function takes these initial guesses and determines a local minimum of the error between the data and the equations with the determined constants. The function also determines if the exit from the determination was due to convergence on a solution, or whether the determination could not reach a convergence in a given amount of iterations. The

function also returns the sum squared error between the data points and the values that the model predicts as another aid in helping identify a good fit. There can be several solutions that produce a similar sum square error and converge on a solution. Thus it is up to the operator to determine if the values of the fit reflect the data. For example a fit that converges may produce a carrying capacity value for biomass less than what was actually grown. Considering that the carrying capacity should be equal to or higher than the maximum algae grown in the physical system, this fit would be rejected by the end user, despite mathematical convergence.

The nutrient data collected in this experiment provided little insight into the nutrient uptake of the RABR, and uptake constants generated from the data produced poor results. Several issues led to this. There are some data gaps in the effluent nitrogen and phosphorus streams from the reactors due to lab equipment failure. Also the reactors were likely poorly mixed. The path taken by the water through the reactor pond is largely unknown. Due to design issues to making a pilot scale reactor in its own pond a reality, the media flow was not perfectly mixed. The influent concentration of nitrogen and phosphorus also change in time rather than remaining constant, further complicating issues. Due to having access only to grab samples, gaps due to lab equipment failure, and poor mixing, uptake constants could not be extrapolated or determined from this run.

Instead of using constants based on this data, they were interpreted from assays done by the USU soils laboratory on a sample of Logan Lagoon biofilm biomass. This analysis showed over triplicate data that 5.63% of the weight of the biomass was present as nitrogen and 0.68% was present as phosphorus. This corresponds to a molar ratio of nitrogen to phosphorus of 18:1, whereas the stoichiometric formula for algae biomass is

16:1 (Stumm and Morgan, 1996). With this data available it was assumed that, per gram of biomass grown, 0.00563 g of nitrogen was removed and 0.00068 grams of phosphorus were removed. This is also on a per gram basis, thus the biomass term in the uptake equations must be modified by the surface area for algae growth on the RABR. Given that the only constants to be extrapolated from the nitrogen and phosphorus data collected from within the RABR runs was a grams removed per biomass grown, I feel this is the best way to determine these parameters.

Model Constraints

After the model had been developed, it is beneficial to establish under what conditions useful in order to avoid misinterpreting results. Due to time constraints, only one set of data could be collected to use for modeling. Therefore the model constructed from this data has some constraints. Due to the model being calibrated for the nitrogen levels observed in the effluent of the Logan Lagoons, the model is likely only valid for ranges from 4.8-12 mg N/L that were measured throughout the course of the experiment and from 1.8 to 5 mg P/L that were measured. Operating the model under parameters different from these may produce unsatisfactory results.

This model was calibrated during the summer months. Irradiance is not directly considered within the model, yet it does have some effect over biomass yields and could affect model results. Fitting these parameters for various times of the year is recommended to either change them or ensure that they adequately match the values used in this thesis. The values used within this study were calibrated during the month of July, and are likely valid for similar summer months.

The model also appears valid only over a range of time before the culture crashes. From Figure 5 above it can be clearly seen that the biomass increases a peak yield, and

then quickly dies or sloughs off. The model however will predict the biomass to grow at a very slow rate until the biomass reaches the carrying capacity. Thus the model should not be employed past the time scale of that crash. Letting RABRs exceed peak biomass would cause a loss of product. For the purposes of predicting the highest amount of biomass in the minimum amount of time, the model is still useful.

Stated above was the assumption that growth within the Lagoons is limited by nitrogen. This also has an effect on when the model should be employed. If some other nutrient is limiting, then the model will not be valid. Should the phosphorus level decrease or the nitrogen level increase to the point where phosphorus is a limiting nutrient instead of nitrogen, the model will likely not produce satisfactory results. If both nutrients are present in the media stream in excess, the the model will likely not produce satisfactory results.

Model Applications

A population model can have several applications to engineering a biological system. Perhaps most importantly, it can help establish an average ideal harvesting time. If biomass is to be the main product from the RABR, then a mathematically ideal harvesting time can be computed from the model. Such an approach would not be ideal for every single RABR as slight differences will likely cause the optimal harvesting time to vary. Having a mathematical average will ensure the most biomass produced on average from an array of RABRs. If this model is calibrated over several different growing conditions either seasonally or monthly, ideal harvesting times can be found throughout the year in an effort to determine ideal harvesting time for each RABR individually.

The model can also help predict the amount of biomass that can be expected to be produced. If on average you can expect a certain amount of biomass to be harvested in a given time period, the amount of biodiesel and methane can be predicted. From these calculations the amount of currency made from biodiesel can be extrapolated. If the biodiesel is to be used to power the City of Logan, Utah municipal solid waste vehicles, the amount of excess or supplementation required to meet the need can be assessed at a better level then by assuming biomass production based on experimental data. As discussed previously the model would be most helpful if calibrated over several seasonal growing periods, which would expand the usefulness of the model.

Modeling can also help compare other methods of biofilm growth to the RABR system, and understand how fundamental design changes can affect system performance. If the same methods were used to fit data to the RABR under different scenarios or design changes, the implementation of this model can help determine how those changes affect growth. By examining the three most important constants for growth in the equations, μ, K, and Θ, comparisons can be made.

MODELING OF BIOMASS PRODUCTION

Model of Biomass Production

Using the Matlab script detailed in Appendix A the model parameters were fit to the average of the data collected from the three pilot scale RABRs. Initial guesses were varied in order to find the best fit. A few cases yielded similar sum squared errors and did converge on solutions. However some of these generated results that seemed infeasible, including carrying capacities below measured values from the reactor. One convergence was chosen as it returned a low sum square error, converged on a solution, and many initial guesses returned similar values. The values used in this model, determined by the Matlab script, are shown in Table 7. With these constants determined, they were used within the model to fit equations three, four, and five above to the biomass data.

Table 7: Constants used in Biomass Model

r **max growth rate (1/day)**	**d** **death rate (1/day)**	**K** **carrying capacity (g/m^2)**	**Theta** **inhibition constant (dimensionless)**	**A0** **initial biomass (g/m^2)**
0.38	0.05	262	5.67	0.5257

Within the constraints outlined in the section above including nitrogen limitation, summer growth, and the effect of nitrogen diffusing being negligible, the model provides a good estimation for the production of biomass when compared to the averaged data. Model results and the collected data are shown in Figure 6. The fitted curve in Figure 6 shows the major trend in biomass growth. On day 22 one data point yielded a clear excess over all the others despite being the same in operation, which appears to account

for the drastic rise in biomass in the data. The sum squared error for this fit was 45, among the lowest observed.

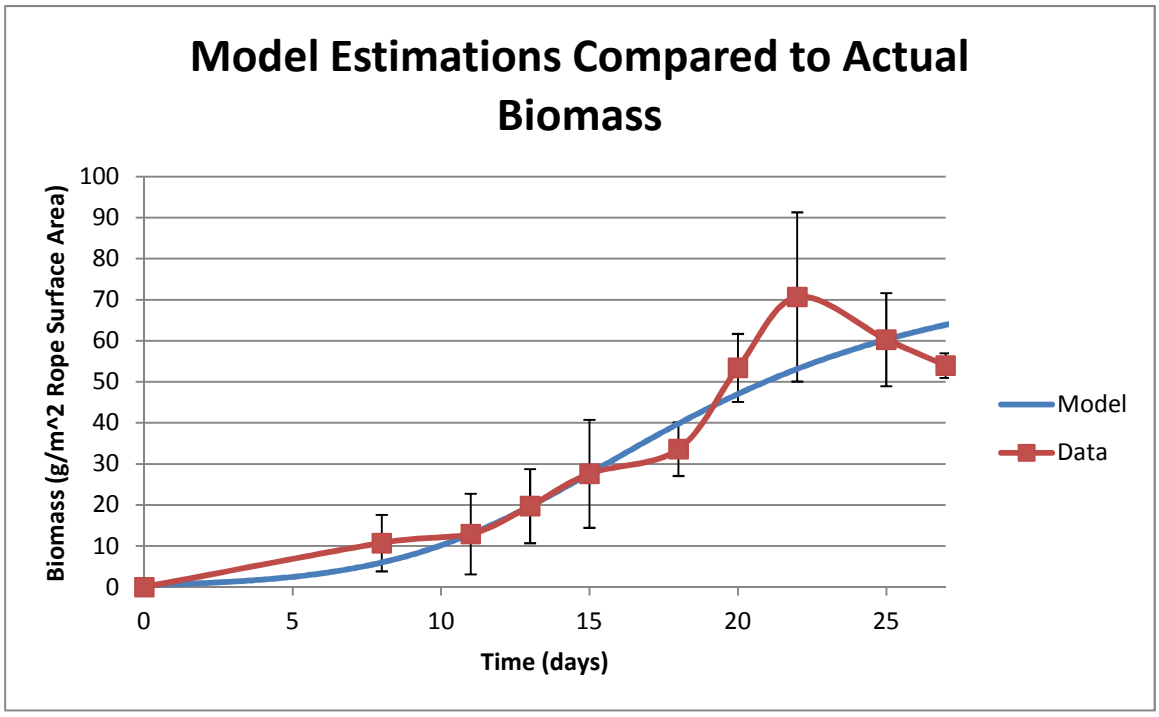

Figure 6: Model Estimations Compared to Actual Biomass

This fit makes sense within the realm of biofilm mechanisms regarding the value of theta. Research into biofilms has suggested that only a certain thickness of biomass on the outside of the film is photosynthetically active. A theta value greater than one inhibits the system more in the beginning of growth than at the end, a theta value less than one inhibits growth toward the end of the simulation time. It would make sense, in regard to the amount of biomass that is photosynthetically active, that as the biofilm grows it shades itself and inhibition starts closer to the beginning of the simulation. For this reason a theta value greater than one makes sense as the inhibition appears to occur early in phototrophic biofilms.

The model was also fit to Logan Christenson's data collected in November 2010 (Christenson, 2011). The same methods described above were employed to determine constants. The results are shown in Figure 7. While the data from November looks different from the data collected in July the model still produces a good fit. The growth observed in the Fall 2010 Season does not appear to show such a lag phase as the July data.

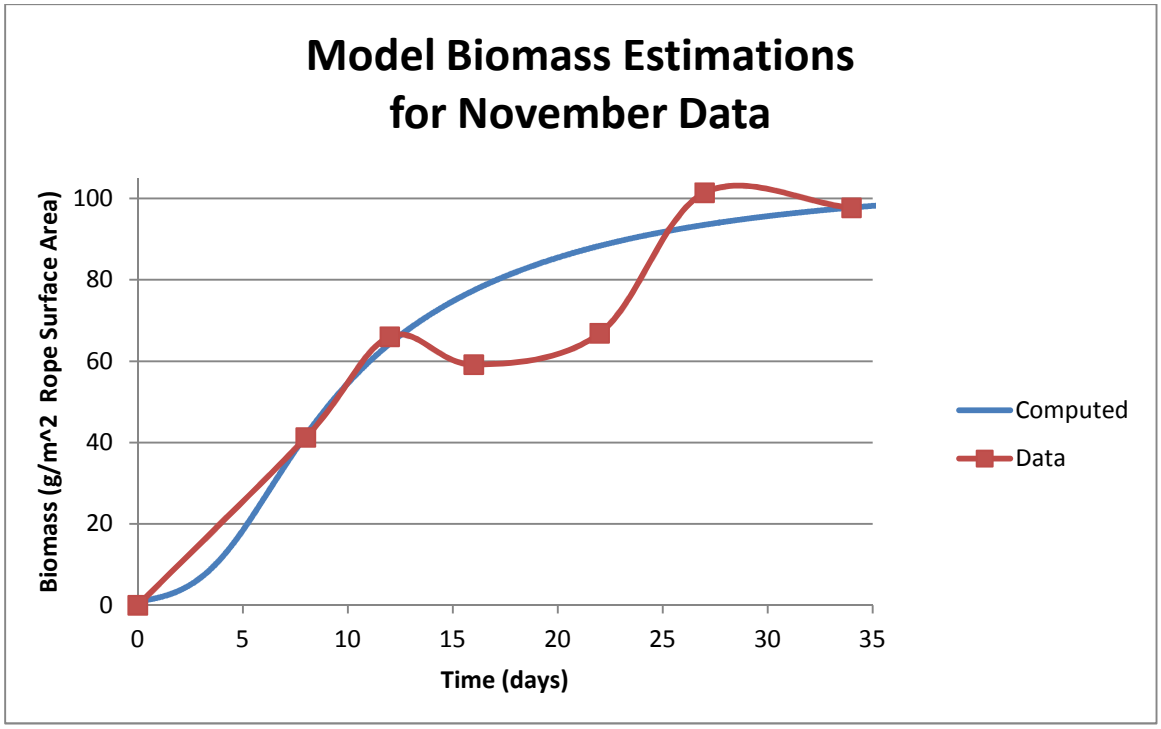

Figure 7: Model Biomass Estimations for November Data

Comparing constants from this fit to constants from the July data fit can show some differences in the two periods of growth. The set of constants for the two times of growth can be seen in Table 8.

Table 8: Comparison of July and November Model Constants

Month	r Maximum Growth Rate (1/day)	d Death Rate (1/day)	K Carrying Capacity (g/m^2)	Theta Inhibition Constant (dimensionless)	A0 Initial Biomass (g/m^2)
July	0.38	0.05	262	5.7	0.5
November	0.75	0.02	310	9.6	0.9

The November growth data seems to exhibit no lag phase, which is reflected by the higher growth rate. The faster initial growth observed in November could be attributed to lower sunlight not causing photoinhibition within the biofilm. Similarly a higher carrying capacity is observed. If the culture is experiencing little or no photoinhibition compared to the summer data, conditions would allow for more biomass to be grown, resulting in a higher carrying capacity. Theta is higher as would also be expected if more biomass were to be grown. More biomass implies more shading thus more inhibition. From this comparison it appears as if strong daytime sun could be inhibiting biomass growth of the RABR.

Ideal Harvesting Time and Biomass Production for a Full Scale RABR

Harvesting of a large scale RABR would require precise timing if using the proposed rope harvester device. If the rotational velocity is kept similar to the pilot scale units, the rotational speed would be 0.166 RPM. This correlates to a harvesting time of approximately 4.27 days. The ideal harvesting time can however be computed mathematically given the biomass model constructed above. The optimal productivity per cycle will occur where the derivative of the productivity is zero. This is shown in the equation below.

$$\frac{dP}{dt} = \frac{d}{dt}\left(\frac{B(t)}{t + h}\right) = 0$$

Equation 6: Optimization for Ideal Harvesting Time

Using the values obtained from the model it is possible to compute the optimal harvesting time numerically. This is included in the algae model script found in Appendix A. The optimal harvesting time was found to be 24.5 days into the cycle, and at the time of harvesting 59.7 grams of algae per meter squared of rope surface area was grown. For the pilot scale reactors, this corresponds to 1.4 kg of algae produced total. This is the maximum amount of biomass that can be achieved given the long harvesting time.

The data produced under these conditions may also be used to estimate what kind of biomass yields can be obtained from the proposed full scale RABR. When employed at full scale, the RABR will have a proposed size of 12 feet in diameter and 20 feet in length. Given these specifications and model estimates, we can see how much biomass can be produced by a RABR of full size under the caveat that the system will perform similarly, but scale for the increase in surface area. The scaled up reactor will have a surface area over 9 times the area of the pilot scale reactor with a total surface are of 220 m^2. Given that the optimal harvesting time is 24.5 days and harvesting requires 4.27 days, 59.7 grams of algae per square meter of surface can be produced. Thus a pilot scale reactor will produce 13.2 kg of algae in 29 days. This is a yield of 0.455 kg of algae per day per installed unit.

MODELING OF NUTRIENT REMEDIATION

Phosphorus Uptake

Phosphorus remediation is one the most crucial aspects of the Logan Lagoon wastewater treatment plant. The average nutrient concentration that was drawn into the RABR pond which was also the effluent from the plant was 2.8 mg P /l. The total maximum daily load (TMDL) study, although note in effect yet, implies two alternative limits on phosphorus discharge, 1 and 0.5 mg/L (Gaddis and Allred, 2009). While one RABR will not remediate the wastewater sufficiently to attain discharge limits from the pond constructed over the given amount of time, ponds in series will. This is of course assuming the reactor volume is continuously mixed. The model shows that after 23 days the concentration in the pond is 2.72 mg/L phosphorus. However given the size of the pond, the reactor contains nearly 30 grams of phosphorus.

From the ideal harvest time it is also possible to determine how much total phosphorus can be removed by a full scale unit. Assuming a full scale unit grows 59.7 g/m^2 of algae over 220 m^2 this represents 84 grams of total phosphorus removal over the course of 23 days per RABR. Estimating the average influent concentration of phosphorus is 5 mg/L and average flow into the ponds is 15 million gallons per day, over the course of the 24.5 day time, 5,600 kg of phosphorus needs to be removed to achieve an effluent concentration of 1 mg/L and 6,260 grams of phosphorus for an effluent concentration of 0.5 mg/L. Assuming 59.7 g/m^2 algae, 62,100 reactors are needed to remediate to 1 mg/L and 69,860 reactors are needed to achieve an effluent concentration of 0.5 mg/L. With influent to the RABR pond (Pond D) of 2.8 mgP/l, then 35,000 and 39,000 RABRs for 1.0 and 0.5 mg/L are needed , respectively. Increased yield or productivity obtained by Christenson (2011) would reduce the number of RABRs.

The amount of RABRs that could fit in Pond D is approximately 5,900, therefore filling Pond D with RABRs if it was converted into a raceway would be insufficient for total removal. For 5 mg/L phosphorus influent, remediation by RABRs would be 95.4% of the total area of the Logan Lagoons for remediation to 1 mg/L and 107% of the area for 0.5 mg/L. For 2.8 mg/L phosphorus influent, requirements would be 53.4% and 60% of the total Logan Lagoon area. Remediation from 5.0 to 0.5 mg/L by only RABRs would require more area than is available at the Logan Lagoons. Increased yield or productivity as obtained by Christenson (2011) would reduce the number of RABRs required.

Discussion

From the model there appears to be a large difference between the measured values for nitrogen and phosphorus actually exiting the system, and the removal effect of the RABR itself. Differences were obtained between the influent and the effluent however the modeling does not account for such a change. This appears to be due to the high dilution rate and the quantity of biomass grown. During the 27-day course of the experiment approximately 870 grams of phosphorus were pumped through the pond assuming an average influent of 2.8 mg P/L. A peak of 1700 grams of algae was grown in 22 days stoichiometrically removing only 12 grams of phosphorus total. This is only 1.3% removal of phosphorus.

Several mechanisms could be occurring to account for this difference. Luxury uptake could be occurring. The first could be the suspended growth in the system also removing nutrients. The effluent from the wastewater treatment plant contained suspended algae that could account for the change in nutrients from the influent to the effluent of the pond not reflected in the model. The average influent TSS concentration

was 34.2 mg/L. Given the volume of the pond there is approximately 366 grams of suspended algae in the pond not compensating for growth. This could be the reason for the difference in nutrients not observed in the model.

DISCUSSION AND INSIGHTS FROM SYSTEM MODELING

The model, while not able to provide changes in biomass yields based on influent nitrogen values, yields some interesting results and conclusions. Those come at the expensive of some caveats however given that this could only be constructed from one data set. Some of these are detailed in the constraints section, however some were not covered and requires further discussion. Several of these include transformational changes in order for proper dimensional analysis, where other techniques could be more appropriate.

The first of these issues is the transformations done to nutrient mass in order to achieve proper units. In equations four and five, the uptake rate was a stoichiometric ratio of nitrogen and phosphorus to algal biomass. This presents issues when algae are grown on a surface. Therefore the biomass produced had to be multiplied by the surface area of the reactor. In addition the amount of nutrient taken up by the biomass came from a volume of media. Thus the nutrients removed had to be scaled for both surface area and volume, which may not scale directly. Issues that include fluid flow within the reactor and duration of contact with the water per unit algae on the drum required future evaluation.

Complications also arose when attempting to establish the amount of rope surface area used for growing algae. Growth was observed on both the inside and the outside of the reactors, and recorded results include all growth. An additional complication is the approximation of the rope as a perfect cylinder and assuming all that surface area is available for growth. Anecdotal observance suggests that much of the rope surface area is

available for growth, however the rope contacts itself which may prevent growth or contain minimal growth compared to area exposed to the bulk volume of the reactor.

The optimal harvesting time for biomass was also determined. By fitting a mathematical model to the biomass production, the optimal average harvesting time could be computed numerically. This prediction is difficult when harvesting requires days, and is compounded by the fact that sampling would need to occur frequently and in replicate to determine ideal harvesting time experimentally. With a mathematical model available, the ideal harvesting time can be known computationally, and then it can be executed in the field with some confidence that the maximum yield is being achieved. Such long periods for algae harvesting impact system performance. Other ways to harvest the algae from the RABR, as opposed to the spool harvester, could be developed. Rotational speed could also be increased in order to facilitate faster harvesting. However additional speed may require an increase in motor size and power required to achieve the desired effect. The model shows that decreasing harvesting time will increase productivity significantly.

Insight was also gained into the performance of the RABR in terms of nutrient removal. Considering such low predicted removal in the ponds, any difference between influent and effluent nitrogen and phosphorus readings could either be an artifact of error within measurements or due to the high variability in influent nutrient concentrations. Thus any extrapolation performed from such data will likely be of questionable value. Assuming that the only source of nitrogen and phosphorus for the biomass is from the water, and that they remove from the water an amount comparable to what is found in the biomass, this can provide a better estimation. Knowing on average how much nitrogen

and phosphorus can be remediated per RABR can be a powerful tool in deploying them effectively, and give insight into how many are needed.

The value of theta can also provide feedback into the growth mechanics of the system. RABR performance data from both July and November that were fit to the model in Tables 7 and 8 yielded values of theta much greater than one. July and November data yielded theta values of approximately 5.7 and 9, respectively. These results imply that the per capita growth rate varies little until the culture reaches near carrying capacity and then drops off quickly. A higher value produced in the fall season implies that this affect is more pronounced under those conditions. This implication of inhibition towards the end of the growth cycle could lead to identification many factors for inhibition.

It is possible that light shading in the biofilm has a substantial affect only at the end of the growth cycle. If only a certain thickness of the biofilm is photosynthetically active, it could be that at a certain point this growth rate exceeds the photosynthetically active region, thus slowing growth towards the end of the cycle. It could also be simply capturing a slowing of growth as the biofilm enters the stationary phase. Values of theta greater than one imply a steady per capita growth rate until the biomass begins to approach the carrying capacity, as opposed to decreasing sharply after initial quick growth. This will allow for a more consistent growth until the point of inhibition is seen, during which biomass can be harvested.

FAME PRODUCTION OF ALGAL BIOFILM REACTORS

Experimental Design

Selection of Variables

In constructing an experiment to stimulate lipid synthesis in biofilms, literature was indentified that showed increases in lipid content within suspended cultures, this review can be seen in Table 2. From examining these potential variables, nitrogen and carbon were isolated as the most critical. Nitrogen starvation is well documented in literature as a technique for increasing lipid synthesis, as well as excess inorganic carbon nutrition (Chiu et al., 2009; Eichenberger, 1976; Lv et al., 2010; Piorreck et al., 1984; Widjaja et al., 2009). Light was excluded because it was shown to have a larger influence over fatty acid profile than it does actual lipid content (Solovchenko et al., 2008). Phosphorus was excluded as a variable because it had a smaller effect over lipid production than either nitrogen or carbon.

A full factorial design was chosen based on the selection of only two variables. Thus a high and low setting for both nitrogen and carbon was chosen thus indicating four cases needed to be explored as shown in Table 9.

Table 9: Experiment Scenarios

Scenario	N	C
1	+	+
2	+	-
3	-	+
4	-	-

For nitrogen, the high scenario was chosen to be 36 mg/L sodium nitrate as nitrogen, and this is based on the nitrogen to phosphorus ratio of algae biomass of 16:1 extrapolated

from an average of 5 mg/L phosphorus influent from the Logan Lagoons. The low nitrogen scenario was chosen to be 0 mg/L because many studies seeking to stimulate lipid synthesis by nitrogen starvation completely starve the culture of nitrogen. The high carbon scenario was chosen to be 1000 mg/L Sodium Bicarbonate. This value was chosen from a journal article citing that this concentration yielded excellent growth and lipid synthesis in *Chlorella sp.* a species known to be present in the Logan Lagoons and also forms biofilms (Yeh et al., 2010). The low carbon concentration scenario was chosen to be 100 mg/L to provide some buffering capacity to the media while still being an order of magnitude less than the high scenario. Within this experiment, eight reactors were operated, with two replicates for each scenario. After completion of the first replicate, the experiment was performed again in order to obtain quadruplicate data values.

Sodium nitrate was chosen as the nitrogen source because it is stable in water and will not volatilize as ammonia does at high pH. The use of nitrate will ensure that any uptake of nitrogen from the bulk volume is due to algae metabolism and not due to losses via other means.

The bio-availability of carbon dioxide gas, which would be sparged into the media, to the biofilm is largely unknown. Also constant sparging of carbon dioxide requires a strong buffer in order to mitigate large pH changes that could affect experimental results. The nature of this experiment excludes several prominent buffer systems. A phosphate buffer would interfere by increasing phosphorus levels. An organic buffer was evaluated with CO_2 sparging; however, this led to a large bacterial bloom which outcompeted the algae biofilm. Due to these issues, sodium bicarbonate was chosen as the inorganic carbon source.

Culture and Medium

The biofilm culture used was the mixed culture present at the Logan Lagoons Wastewater Treatment Plant. There is a large community of algae and bacteria. The culture was established by inoculating new reactors into Logan Lagoons wastewater from cell C, see Figure 1, where growth at the time was highest. A 2-week growth period in this medium allowed suspended algae to attach to the surface of the RABR. The reactors were then harvested, leaving a small quantity of seed algae on the rope to begin regrowth. This was the culture used throughout the rest of the experiment.

After the reactors were seeded using Logan Lagoons wastewater, the RABRs where then inoculated into sterile media developed by student Ashton Young (Young, 2011). This medium was made to emulate nutrient and ion levels present in the Logan Lagoons and is detailed in Table 10.

Table 10: Synthetic Media Composition for Phototrophic Biofilm Growth

Compound	Concentration (mg/L)
Ferric Citrate	9.96
Calcium Chloride Dihydrate	205
Magnesium Sulfate Anyhydrous	147.09
Potassium Chloride	5.6
Potassium Phosphate Dibasic	7.57
Potassium Phosphate Monobasic	17.8

Reactors were inoculated with sterile media to prohibit any suspended growth that would occur with natural Lagoon wastewater. This ensured a large portion of the nutrients will be assimilated by biofilm biomass, and nearly all metabolism can be attributed to it. Biomass yields were also more consistent by limiting the amount of

suspended growth. Large margins of error were seen when growing biofilms with suspended culture. The medium was adapted to fit the levels of nutrients required by this experiment. The culture was grown using a fed batch process. Continuous flow of medium was infeasible, thus every 2-3 days media was evaluated for nitrogen and phosphorus levels and then adjusted from the detected level to the desired values of 36 mg/L nitrogen, and 5 mg/L phosphorus.

Physical Setup

Each reactor was 9.5 inches in length, 6.5 wide, and 24 inches deep. This allowed for growth in 22L of media in order to mitigate large swings in nutrient levels and pH. Reactors themselves were 7 inches long consisting of 4-inch diameter ABS pipe. A photograph of the RABRs is shown in Figure 8.

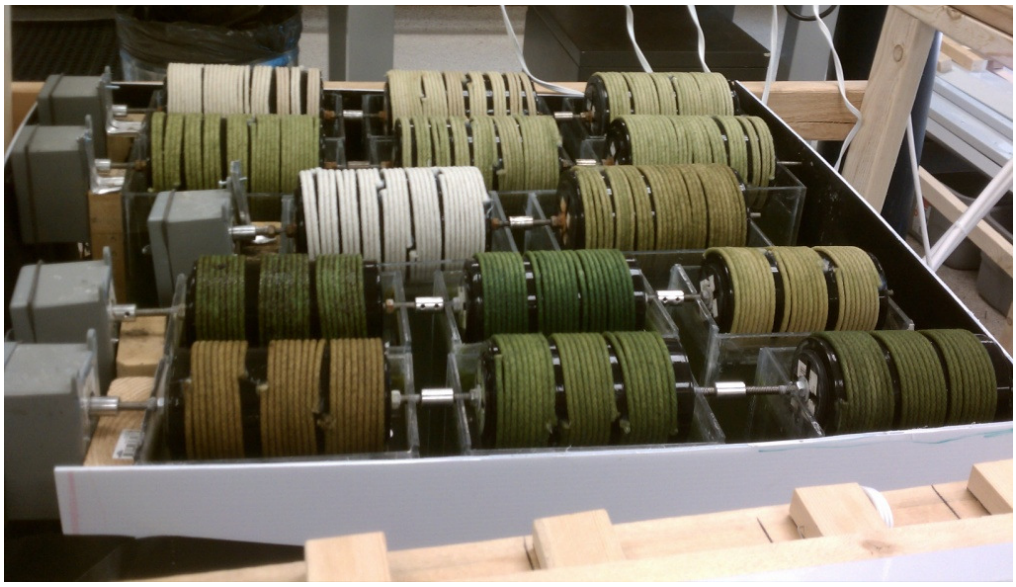

Figure 8: Experimental Setup of Laboratory Scale RABRs

This gives a total rope surface area of 0.2 meters squared for each RABR, in an installed reactor area of 0.04 meters squared. RABRs were then arranged in three rows, two rows

having three reactors, and one row containing two. Light bars were than suspended over the reactors emiting approximately 150 µmol/m2-s light at the reactor surface. Each light bar illuminated one bank of reactors and shields were placed between banks in order to ensure uniform light intensity between reactors. Banks of RABRs were connected to a motor via a central shaft and were rotated at 1.25 RPM. Data concerning FAME yield were also collected from the three large scale reactors used in the modeling section of this document. While no parameters were varied, the fame data from this experiment were collected and compared to the small scale RABR data.

Data Collection and Methods

Biomass and FAME concentration data were taken every three days starting the sixth day of the experiment and ending the twenty fourth day. This allowed the system six days in which to grow a harvestable quantity of biomass. Based on previous data collected by Logan Christenson, indoor biofilm cultures grown in this manner appeared to peak at approximately 20 days, which provides an adequate amount of time to show any potential decline in culture biomass. Due to the limited surface area of the RABR, different length rope segments were used in order to obtain multiple data points on the same replicate. Peak biomass yields from Logan Christenson's indoor data showed indoor cultures capable of producing approximately five grams of biomass per meter squared per day. Taking this value and reducing it to 2 g/m^2-d to compensate for lag growth at the beginning of the experiment and calculating the area of rope required for 100 mg of growth were computed and rounded to even increments in units of feet. The lengths and surface areas required can be seen in Table 11.

Table 11: Biomass Collection Setup

Day	Rope (ft)	SA Rope (m^2)	Scale Factor
6	10	0.014	356
9	7	0.010	509
12	5	0.007	712
15	4	0.005	891
18	4	0.005	891
21	3	0.004	1188
24	3	0.004	1188

Many studies report biomass growth per installed reactor area, or the areal footprint of land that the reactor occupies. Thus the advantage of the RABR system presents itself. In order to produce data that will be comparable, each data point must be amended. Thus each data point is scaled so that the growth obtained would be over the entire RABR surface area. This value is then divided by the surface area of the entire reactor. The scalar to be multiplied by can be seen in Table 11.

Biomass was harvested by removing the given rope segment from the reactor and pulling the rope through an orifice slightly smaller in diameter than the rope. This caused the biomass to slough off and be collected into a pre-weighed container. Samples were then lyophilized and weighed to determine dry weight. Transesterification of free fatty acids and neutral lipids was accomplished using the method developed by Daniel Nelson, and then each sample was analyzed by gas chromatography (Nelson, 2010). When possible samples were analyzed in duplicate for each reactor to ensure transesterification analysis precision. FAME percentages were then multiplied by biomass yields in order to determine lipid yield.

Effect of Nitrogen and Carbon on Fatty Acid Methyl Ester Yield

No statistical difference between any of the scenarios explored was found in this study. Neither nitrogen deprivation nor presence of extra inorganic carbon was correlated to with any amount of increase in lipid synthesis. The biofilms yielded approximately 3 to 5% lipid content by dry weight with little fluctuation either with time or nutrient regime. While the biofilm may not be as responsive to the environmental stresses that traditionally show increased lipid synthesis in suspended culture, the expectation was that at least some difference would be observed. A graph of FAME content over time can be seen in Figure 9.

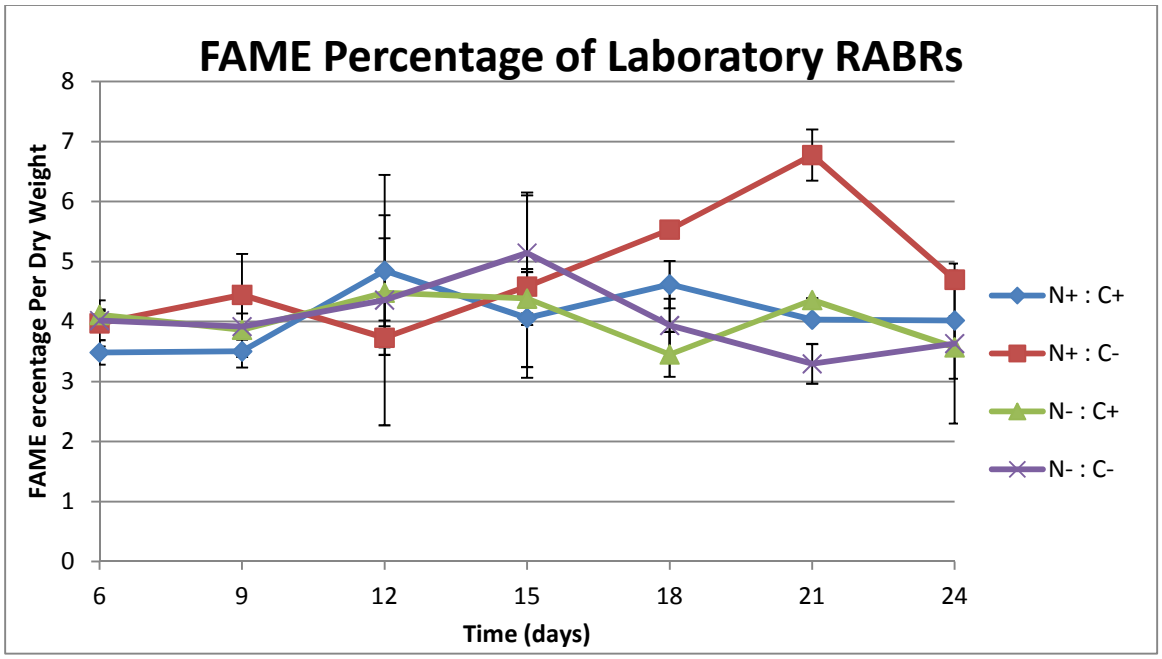

Figure 9: FAME Content over Time of Laboratory RABR Biomass Growth (Error bars represent one standard deviation)

Results presented in Figure 9 show that the measured fame content of the biofilm biomass was between 3.5 and 4.5%, with standard deviations showing the range at approximately 2-6.5%.

While this project proposed the modeling of lipid content within the biofilm as a function of different nutrient regimes, because no variation in lipid content in response to external stimuli was unobserved, there is nothing to model. The data indicated that, regardless of at least these two nutrient stresses, the biofilms will contain the same quantity of FAME. Whether or not these results can be extrapolated to other mono or mixed culture phototrophic biofilms is not yet known. Efforts would be better spent refining a model of biomass production, because growing large quantities of biofilm biomass would create more lipid yield than trying to increase lipid synthesis via nutritional manipulation based on results obtained from this research.

Since most of the scenarios produced statistically similar fame percentages save for the N+:C- case it would be prudent to focus on attaining the maximum amount of biomass possible to increase overall FAME yield. Considering the amount of lipids produced per surface area of reactor footprint, the high nutrient scenario, N+:C+ in Figure's 9 and 10, outperformed the others because it produced a larger quantity of biomass to procure lipids from. Results for yield of fatty acid methyl esters for each case over time are shown in Figure 10.

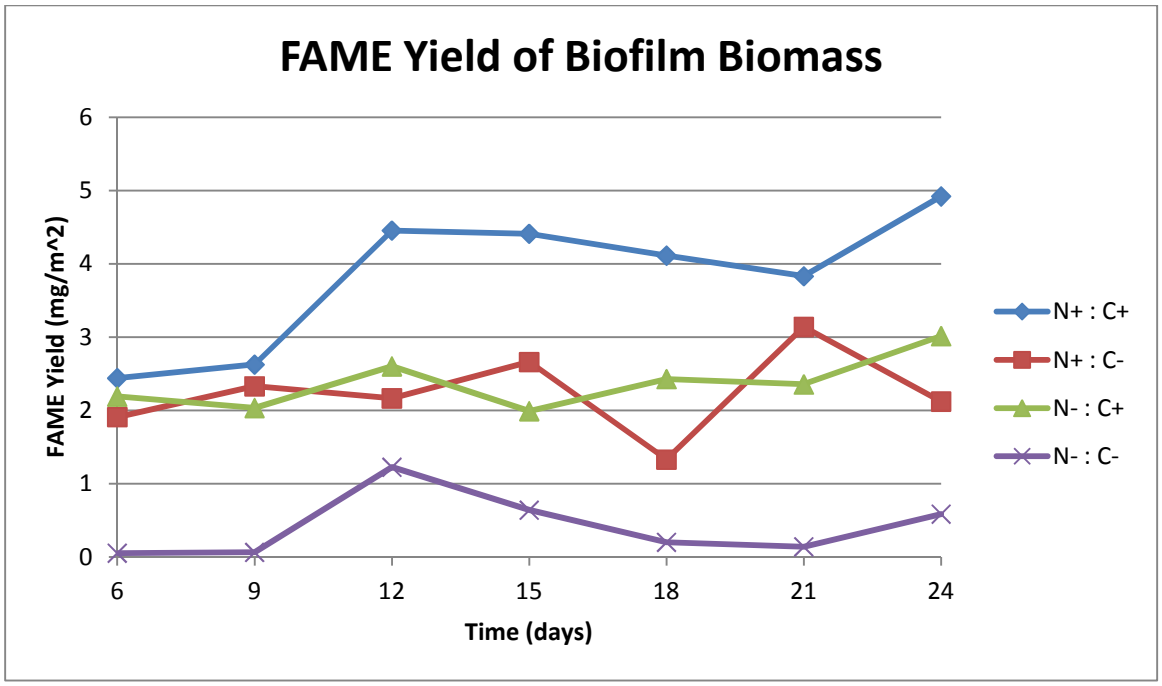

Figure 10: FAME Yield of Laboratory RABR's over Time for Different Nutrient Scenarios

From Figure 10 the FAME yields of biofilm biomass to be low. At most approximately 4.5 grams of FAME per installed reactor area is produced from these reactors. While the RABR does produce a substantial quantity of easily accessible biomass, it seems this biomass is less-suited for transesterification into biodiesel. Biofilm biomass however could be used in many other useful ways.

Biomass Yields

Biomass yields appeared to be similar across the range of nutrient parameters except for the high nutrient scenario. However, high variability was observed across all data points. Despite operation two reactors with the same two conditions over two different trials, margins of error were large. While standard deviations were large for one run of duplicates, they're in fact larger for the set of quadruplicates which represent two

reactors at the same conditions over the course of two experiment time periods. This implies that the history of the biofilm culture is important in reducing error, as well as biomass recovery methods.

The clearest trend that emerged from the biomass data is that the high nutrient treatment produced more biomass than the other scenarios. This result is expected as growth should be the best under optimal nutrient conditions. A graph for the first replicate of the experiment can be seen in Figure 11.

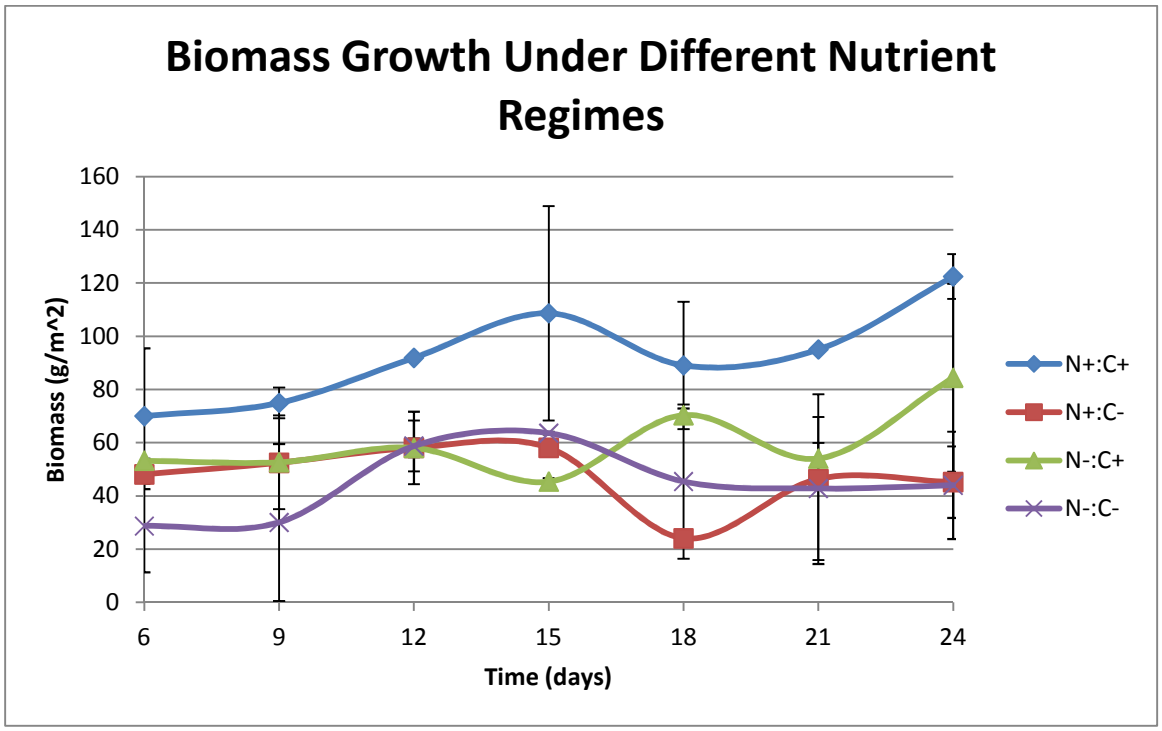

Figure 11: Biomass Yield of Laboratory RABR Units over Different Nutrient Regimes

(errors bars represent one standard deviation)

Secondly, it can be seen that in the indoor setting peak biomass growth was seen in three of the four scenarios at a time period of fifteen days. The only scenario not

exhibiting this behavior was the case with high carbon and low nitrogen. The data collected implies that the culture was still growing and had not acheived peak growth.

Comparison to Other Studies

Comparing the FAME yields of these reactors to others, it is observed that the laboratory scale RABRs perform poorly with regard to FAME production. For the indoor scale, results indicate that RABR performance yields are significantly lower than other studies. On average they produce an order of magnitude less lipids per unit time and area. Table 12 shows these comparisons below.

Table 12: Comparison of Biomass and FAME Yields to Other Studies

Organism	Method of Growth	Growth Yield (g/m^2-day)	FAME %	Lipid Productivity g/m^2-day
Mixed Culture Logan Lagoons (Christenson, 2011)	RABR – Lab Scale Modified Wastewater Outdoor	19.8	12	2.4
Mixed Culture (This Study)	RABR – Lab Scale/Synthetic Media Indoor	7.2	4.5	0.32
Mixed Culture (This Study)	RABR – Pilot Scale/Waste water Outdoor	18.3	6.8	1.244
Mixed Culture Logan Lagoons (Wahlen et al., 2011)	Suspended Growth Outdoor	-	10.7	-
Mixed Culture Logan Lagoons (Griffiths, 2009)	Suspended Increased CO_2 Outdoor	-	23.4	-

Lipid production in biofilm growth does vary, however it did not increase to an amount achievable by suspended growth algae. A FAME concentration higher than the 10.7% observed under normal lagoon conditions was observed in the biofilms, however this growth had a more favorable 12:1 nitrogen to phosphorus ratio, and in higher concentrations, compared with the average of 9:1 seen for the pilot scale RABRs. The value of 6.8% for FAME concentration may be more feasible and conservative value for what can be obtained with a full scale RABR as the experiment was more reflective of conditions that would be experienced.

Griffiths showed that suspended culture of Logan Lagoon algae showed a nearly 250% increase in lipid content with extra carbon addition in suspended cultures (Griffiths, 2009), however no detectable increase in content available for FAME production was seen with extra carbon nutrition in biofilms studied in this research. Results obtained by Griffiths indicate that Logan Lagoon biomass, in suspended form, is at least susceptible to this kind of nutrient function for an increase in lipid and fatty acid synthesis. While Logan Lagoon algae was used in both studies Griffths was grown in outdoor sun, while the laboratory scale RABR's were grown indoors at a light intensity of 150 $\mu mol/m^2$-second. This shows that either low light or the form of growth could be preventing increased biomass synthesis due to extra carbon nutrition.

Potential Reasons for Static Fame Yield

An essentially static FAME yield within the mixed phototrophic Logan Lagoons biofilm culture is contrary to what was expected. Biofilms often form in response to external stress, however having no response to nutrient depletion, of which the opposite is observed in suspended algae, is unexpected. Lipid content of Chlorella, a genera highly

represented in the mixed culture, often fluctuate from 10-40% under different environmental stresses (Illman et al., 2010; Lv et al., 2010). Thus it was hypothesized that while a biofilm would respond to these stresses, the response may potentially be more muted due to biofilms frequently forming in response to exterior stresses.

Results presented in the literature indicate that many biofilms may be carbon limited (Liehr et al., 1989; Liehr et al., 1990; Liehr et al., 1998). The theory is that in deeper depths of the biofilm the pH rises due to the high concentration of algae growth and carbon dioxide and bicarbonate species are transformed to carbonate, a form of inorganic carbon unusable by algae (Liehr et al., 1990; Liehr et al., 1998). This effect is compounded by the fact that the algae deepest in the biofilm is oldest, and is likely close to entering or being in stationary growth. Literature has shown that the stationary phase is when the cell makes most of its lipid content (Guschina and Harwood, 2006). So the biomass most likely to produce lipids sees no advantages from a high amount of carbon. This would seem to explain the higher amount of biomass growth as the exterior algae on the film would have access to the extra carbon, but low lipid yields, whereas the algae on the interior of the biofilm would have no such advantage.

Given the complex nature of the biofilm, a variety of factors could contribute to static lipid yields. However from examining these different potential reasons, it becomes clear that in a natural mixed culture setting it becomes difficult to determine ways to increase lipid synthesis within biofilms composed primarily of phototrophic biomass. The only identifiable difference is that higher outdoor light intensities appear to increase lipid production. This is converse to literature findings reported in Table 2. However, in this specific case the low energy received by the indoor laboratory cultures may be low

enough to harm lipid synthesis. Outdoor results in this study averaged 6.8% FAME by weight, while indoor cultures yielded 4.5%. Results obtained by Logan Christenson using outdoor light and ideal nutrient conditions produced 12% FAME by weight.

FAME YIELDS OF PILOT SCALE RABRS

While the yield of FAME from the pilot scale RABRs was not included in the modeling effort for reasons described above, the data were collected for comparison to the laboratory experiment. On average the pilot scale device did produce a greater fraction of biomass available as FAME than the laboratory RABRs. Across all three reactors in time, the average percentage of biomass by dry weight as fame was 6.8% with a standard deviation of 1.8. A chart of the FAME yields over time is presented in Figure 12.

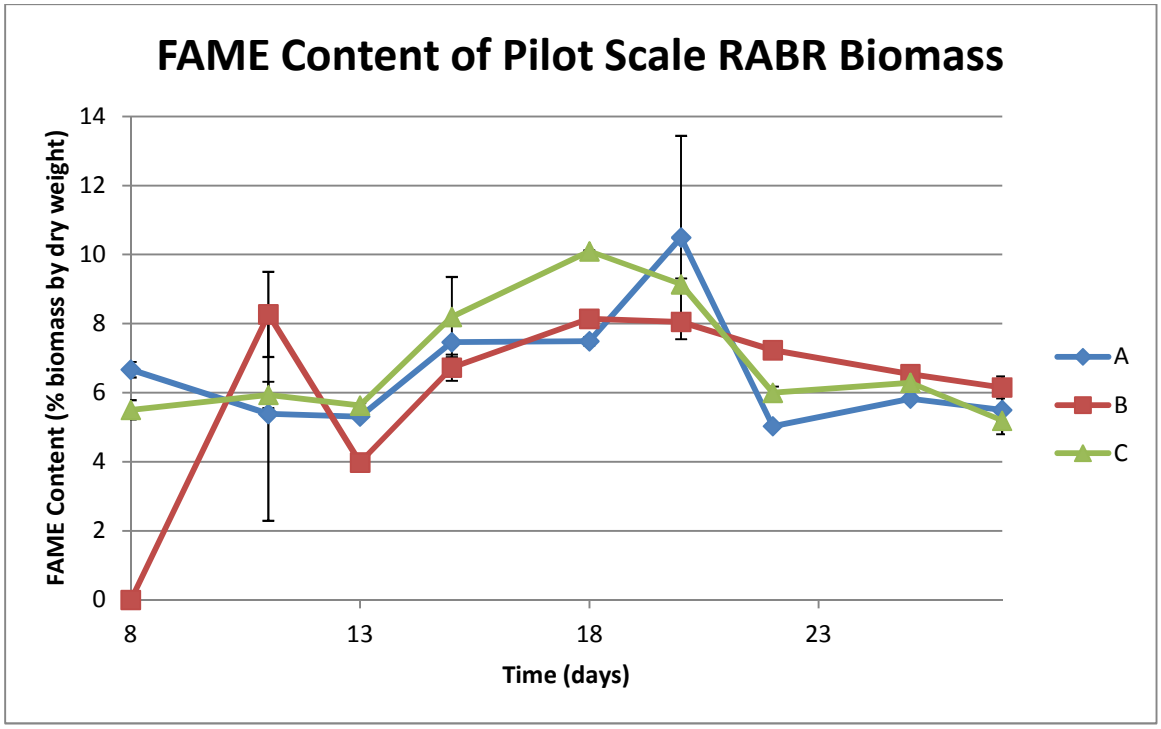

Figure 12: FAME Content of Pilot Scale RABR Biomass

(Error bars represent one Standard Deviation)

While the laboratory RABR scale experiment showed a static FAME content in time, the outdoor RABR biomass appears to exhibit a trend of increasing its FAME content until approximately day 20 and then shows a decline in content until the end of the experiment. This appears contrary to convention where typically algal biomass accumulates lipids in the stationary phase or in the transition to the stationary phase, as opposed to the growth phase which occurred in this study.

It is also important to examine FAME yield, the amount of FAME produced per unit land and time. FAME yield from the pilot scale RABRs can be seen in Figure 13. Peak biomass growth occurs at approximately 20-25 days (see Figure 5) within these units and peak FAME content occurs at approximately 18-20 days. However the biomass later in the growth cycle at 22 or 25 days actually yields more FAME per area than the biomass at day 18.

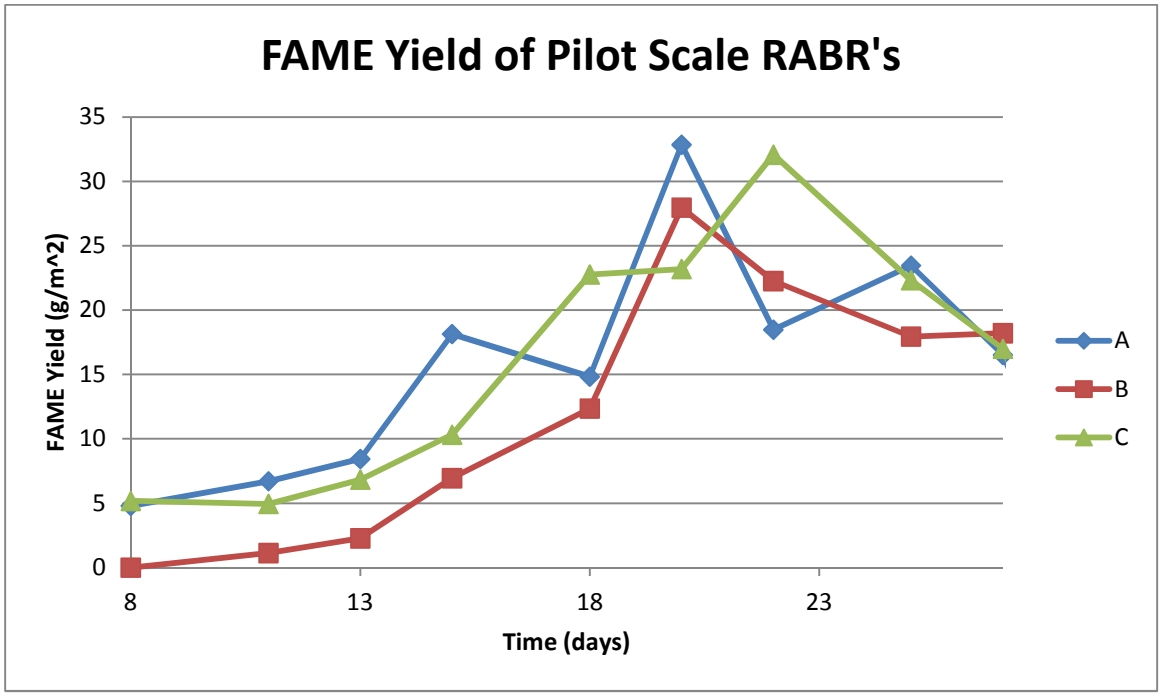

Figure 13: FAME Yield of Pilot Scale RABRs

The best strategy for increasing the amount of FAME that can be produced from these reactors would be to focus on an increase in the amount of biomass produced rather than on the amount of biomass present as FAME potential. From the FAME yield at the pilot scale, the maximum yield occurred when biomass was highest. From the C:N:P analysis discussed in the model above, it also appears that the Logan Lagoon algae is highly nitrogen limited. Increasing the amount of nitrogen in the water would likely stimulate an increase in biomass (Griffiths, 2009). In addition, an increase in inorganic carbon would also show an increase in biomass. Biofilms in this study appeared to be highly resistant to increases in lipid synthesis via nitrogen starvation and excess carbon nutrition based on the laboratory RABR data developed, and showed an increase in lipid production when exposed to stronger sunlight. Thus it appears that the most feasible way to increase lipid yield in biofilm biomass is through increasing biomass yield through nutrient addition, inorganic carbon supplementation, and outdoor sunlight exposure.

RABR PRODUCT RECOVERY

The goal of the city of Logan is to provide enough biodiesel for the operation of city garbage collection trucks throughout the year. Through the model and the lipid study it is possible to estimate whether this goal can be met. The average biomass content that can be recovered as FAME at day 25, which is close to the harvesting time of the reactor, is 6.2%. Under the assumption that the reactors are operational for 10 growth cycles per year out of a maximum 12 for weather or operational reasons, projections for the amount of biodiesel can be produced. These projections are presented in Table 13.

Table 13: Biodiesel Produced from RABR Implementation Scenarios

RABR Implementation	Biodiesel Produced Per Year (Gallons)
1mg/L Phosphorus Effluent	145,576
0.5 mg/L Phosphorus Effluent	163,767
Lagoon Pond D	13,831

The City of Logan currently has 36 garbage trucks that consume approximately 160,000 gallons of fuel every year (Griffths, 2011). Implementation of the 1 mg/L scenario does not quite meet this need, however it comes close. Small changes in seasonal biomass yields and lipid contents could increase production up to the required 160,000 gallons per year.

Increasing lipid content would be of great benefit in increasing the amount of fuel that can be produced. If remediation by RABRs down to 1 mg/L phosphorus is adopted, a 1% increase in FAME available content will increase the biodiesel yield by 23,480 gallons per year. This is enough fuel to power nearly three more municipal solid waste

trucks. However biofilms were stable with regard to environmental stresses that caused increased lipid synthesis in suspended systems. Effect of light limiting conditions should be evaluated to determine effects on lipid production.

If the average biomass FAME concentration is 6.8%, then a substantial quantity of biomass remains after extraction of intracellular lipids and fatty acids for FAME production. This biomass has value and could be used as feedstock for an anaerobic digester to recover energy costs and value from extra biomass. Literature has suggested that algae feedstock that has been through the transesterification process, though producing lower methane yields, is suitable for anaerobic digestion (Ehimen et al., 2011a). Based on the values produced in the aforementioned paper methane production per year was estimated, and is shown in Table 14. The amount of energy recovered from this process could be used to offset power use by the Logan Lagoons wastewater treatment plant, or be returned to the grid. If the energy is returned to the grid, approximately 420 homes could be powered on full scale implementation and 40 homes could be powered from implementation in pond D.

Table 14: Energy and Cost Recovery Via Anaerobic Digestion

RABR Implementation	Energy Produced (MWh)	Cost Offset Per Year
1mg/L - Phosphorus Discharge	4575	$21,639.03
0.5 mg/L - Nitrogen Discharge	5147	$24,343.04
Lagoon Pond D	435	$2,055.88

Calculations and assumptions used for this section can be seen in Appendix B. Physical constants and heating values for methane were determined from literature

(Cengel, 1998) as well as the recovery of methane from algal biomass (Ehimen et al., 2011)

COMPARISON TO PREVIOUS STUDY

Study Operation Differences

The large scale RABRs were previously studied under different conditions by Logan Christenson in October and November of 2010 (Christenson, 2011). Differences were seen in the studies for biomass yield, nutrient uptake, and FAME yield. Many notable differences between the two studies relate to the time of year the different studies were conducted. The October/November study occurred at a lower ambient air temperature, lower light intensity, and a shorter duration of light throughout the day. Operationally the only notable difference between the two scenarios was the retention time which was approximately 11.2 hours during the Fall 2010 Study, and 22.3 hours during the July 2011 study.

There was also a large difference in the amount of sunlight available to the biomass. The average photosynthetic photon flux density (PPFD) for the summer case was higher than the fall case, frequently on the average of three times higher. Duration of light was not quantified directly, but the period of irradiance would also be longer in the summer. Average PPFD for the two studies can be seen in Figure 14.

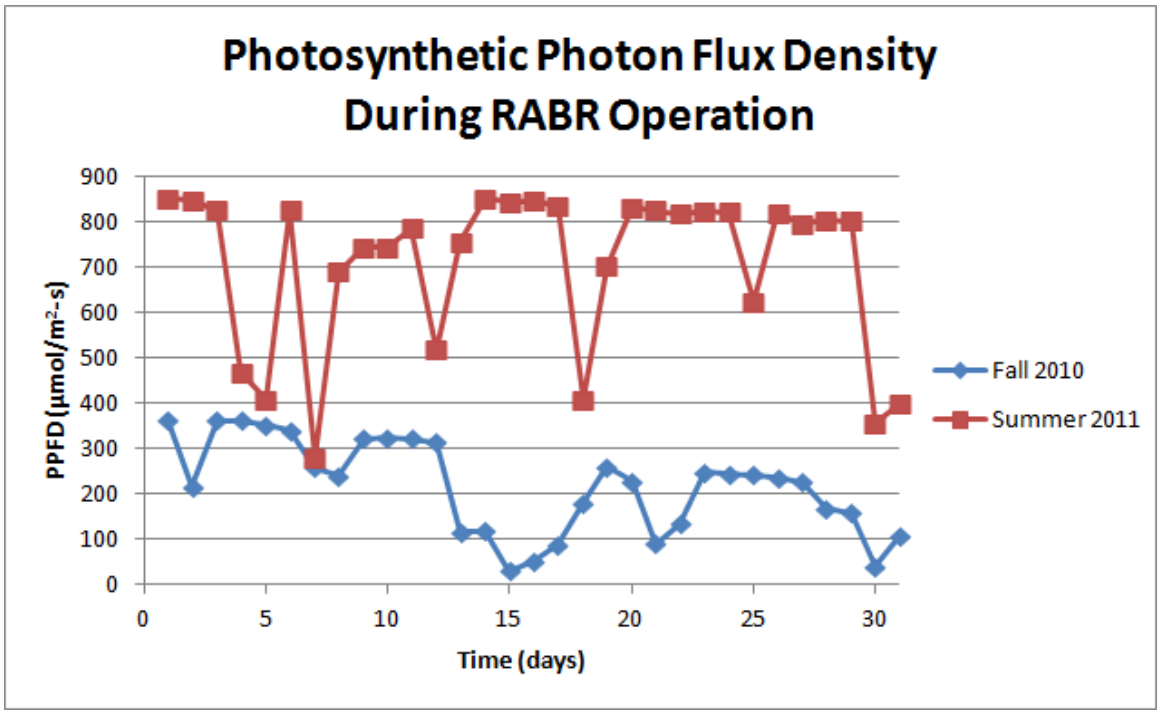

Figure 14: Photosynthetic Photon Flux Density During RABR Operation

Cloudy days had a grater affect on RABR operation, however strong mid-day sun often exceeded 2000 µmol/m^2-s. Such a large difference in PPFD will likely have an effect on biomass yields of the RABRs. At such high light intensities biomass growth is inhibited as cells must devote resources to quenching excess energy. This often takes the form of algae cells creating light blocking pigments. This expenditure by the cell impedes growth resulting in lower yields (Richmond, 2004).

The other primary environmental difference between the two scenarios is the average air temperature during the time of operation. Colder temperatures will not only affect the algae as it is in the air during the rotation of the RABR, but also the water temperature it is exposed to. The difference in average daily air temperature is seen in Figure 15.

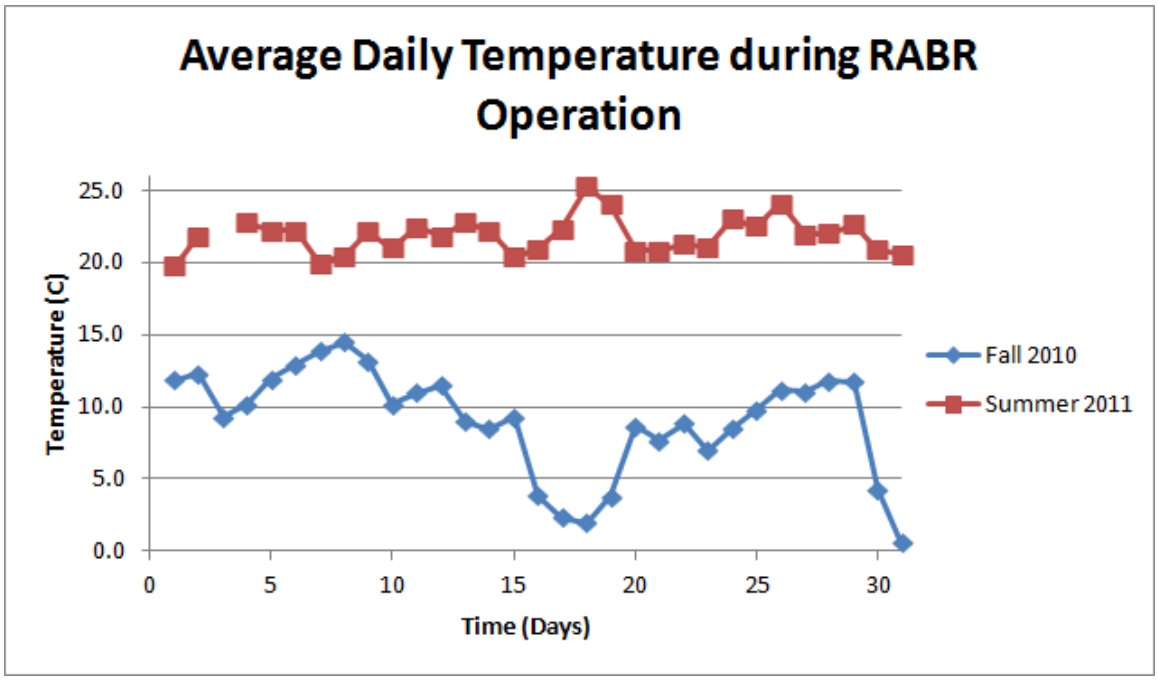

Figure 15: Average Daily Air Temperature During RABR Operation

The average air temperature during summer operation was between 20-25 degrees Celsius, and fall operation never exceeded 15 degrees. This difference in temperature will affect algal metabolic rates and likely have some effect on biomass yields.

Biomass Yield

Biomass yields and productivity for the two cases were different, however contrary to what would be expected given the previous differences. It would be expected that during the summer growth, stronger light and higher temperatures would increase biomass yields despite the lower dilution rate. However, as shown in Figure 16 Fall 2010 growth showed higher yields and quicker growth at the beginning of the experiment. As seen in Figure 16 summer growth showed a longer lag phase of growth, reached its peak earlier, and achieved a lower maximum than the Fall 2010 data. However, the Fall 2010 data were a set of single data points.

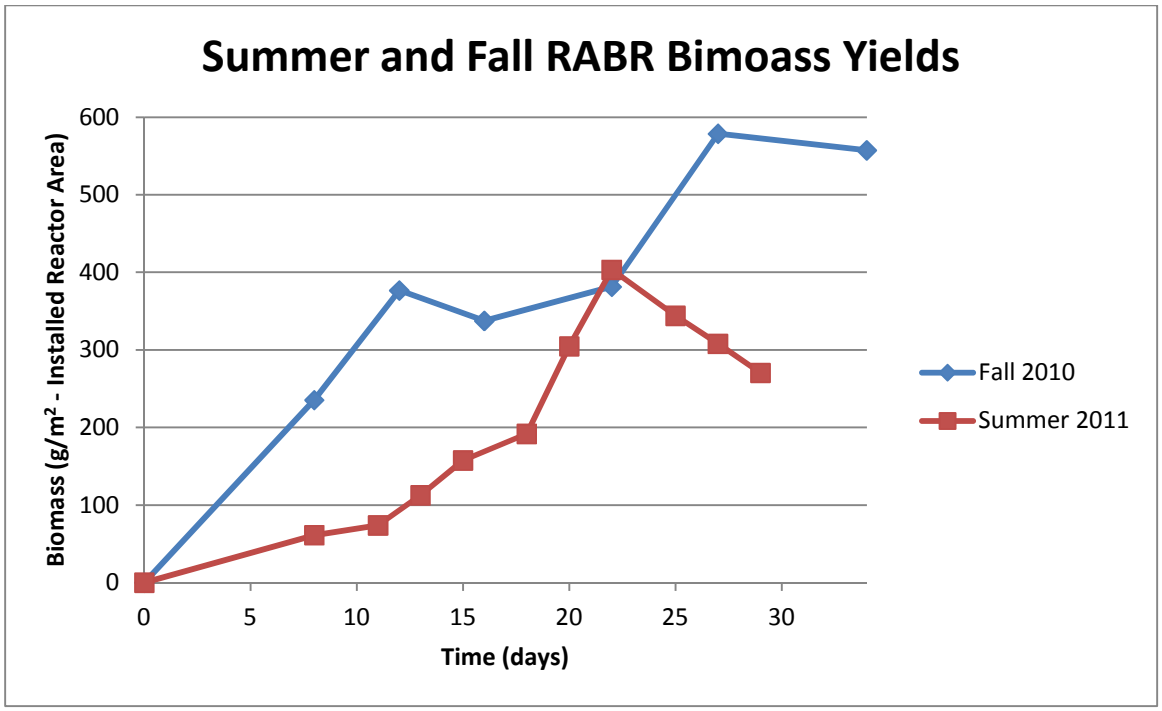

Figure 16: Summer 2011 and Fall 2010 RABR Biomass Yields

There could be several reasons for the decreased growth seen in the summer months and the quicker achievement of peak biomass growth. Strong sunlight is known to inhibit biomass production (Richmond, 2004). Cells divert resources from creating more biomass into creating intercellular compounds that shield them from the excessive sunlight they are receiving (Richmond, 2004). Strong daytime sun in the summer, frequently exceeding 2000 µmol/m^2-s, could potentially harm biomass yields. The earlier peak yield observed in the summer could be indicating that growth cycles are shorter in the summer.

A higher dilution rate in the Fall 2010 RABR system could also be part of the reason for the higher biomass growth and a longer growing time. The effect of dilution rate however may be negated by the fact that the nutrient levels observed between the two scenarios, explored further in the following section, are lower in the fall months than

they are in the summer. While the dilution rate in the summer is lower, the nutrient concentrations are higher. To further explore the importance of dilution rate, simultaneous testing under different dilution rates could provide insight as to how dilution rate affects biomass yields.

FAME Yield

FAME yields were quite different between experiments in July 2011 and Fall 2010. Yields for this study showed an average of 6.2% FAME content for outdoor reactors, and 4.5% for indoor reactors whereas Christenson's study yielded 12% FAME content (Christenson, 2011). Several noticeable differences between the two experiments may explain this difference. Data collected by Logan Christenson was for a closed system RABR enhanced raceway pond. This pond was operated in a fed batch mode with ideal nutrient concentrations outdoors. This setup had two large advantages, which were strong outdoor sun, coupled with nutrient replete conditions. Indoor scale reactors in this study were exposed to much lower PPFDs, of approximately 150 $\mu mol/m^2$-s, and ideal nutrient ratios. Such low light intensities might not have provided a suitable amount of energy for production of lipids and fatty acids for FAME production. The pilot scale outdoor reactors operated for this study were exposed to high light intensity as observed in Figure 16, however were exposed to less than ideal nutrient concentrations.

Nitrogen starvation should increase lipid synthesis, however this phenomenon only appears to be noticeable at very low nitrogen concentrations. Nitrogen levels experienced by the pilot scale reactors may not have been low enough to trigger the mechanism leading to increased lipid synthesis, thus resulting in lower yields. Previously seen in Table 3, lipid synthesis does not appear to increase dramatically until a nitrogen

to phosphorus ratio of 2:1 is acheived, so while growth is nitrogen limited the limitation does not appear to be enough to increase lipid yields. The data suggest that ideal nutrient concentrations would not only increase biomass yields but also increase lipid synthesis due to increased energy input and nutrition. This is observed through comparisons of data in Table 12.

FUTURE WORK

Biofilm Fundamentals

For the diverse culture of algae present in the Logan Lagoons, it would be important to determine the response of the taxonomy of the biofilm algae with regard to seasonal and nutritional changes. This could be one potential reason for differences i006E biomass yields for the biofilms. As described earlier the range of organisms in the biofilm is diverse, spanning not only prokaryotic and eukaryotic algae, but different bacteria as well. Within algae specie, *Chlorella*, *Scenedesmus*, filamentous cyanobacteria, diatoms, and others are present. The image below shows the taxonomic diversity of the biofilms grown at the Logan Lagoons. The sample shown in Figure 17 was taken from an indoor RABR used for the lipid experiment detailed above. Even this small selection of biofilm shows a large diversity, which may have implications regarding the performance of the RABR system.

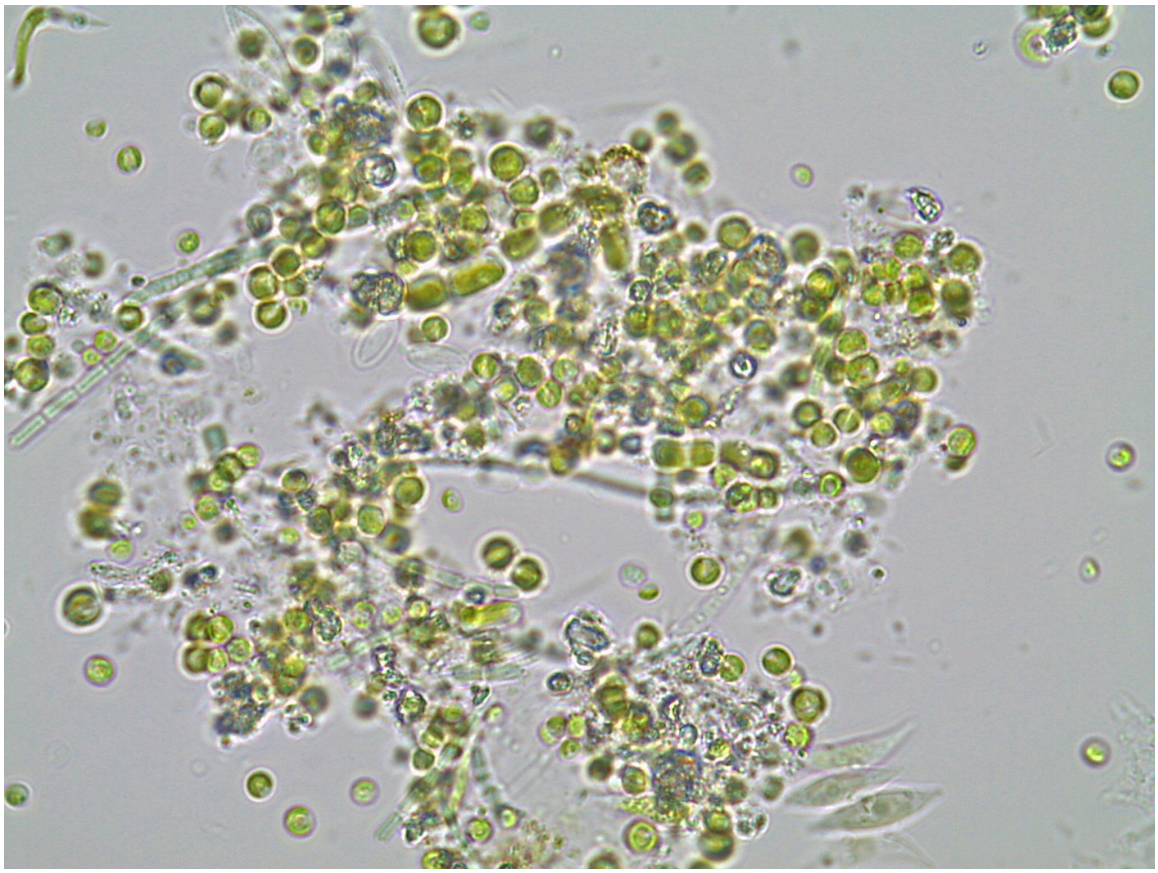

Figure 17: Logan Lagoons Biofilm (Photo taken by the author, May 2011)

By exploring the change in taxonomy over different nutritional regimes, dominating species in times of nutrient depletion and excess can be observed. This will allow the determination of what strains are most active depending on the influent to the Lagoons systems, and what will occur if any nutrient supplementation is needed. Observation of variation of algae taxonomy in time will demonstrate which species grow quickly, and which species have a longer lag phase.

During the course of this research, it became clear that the relationship between suspended and biofilm growth algae was quite complex and appeared to have a significant effect on the growth of both forms of biomass. Initially the previously

discussed lipid experiment was grown in actual Logan Lagoons wastewater and not synthetic media. This introduced a significant amount of suspended growth. Throughout the course of this initial experiment, it became clear that there were large error bars in the amount of biofilm biomass yields. While it cannot be said with any statistical certainty that this is the cause of the large error, the suspended biomass will remove nutrients from the bulk volume of the reactor, and thus hinder biofilm growth.

Because biomass will frequently grow in both suspended and biofilm modes given the presence of proper nutrition, the relationship between suspended and biofilm groth is important to explore in more detail. Biofilm biomass is not only easier to harvest, but more resilient to external stresses and thus potentially more desirable. In experiments for this thesis, all experiments in the laboratory study were conducted by inoculating seeded RABRs into reactors with initially sterile media, and were shielded on the sides from light penetration in order to discourage suspended growth. However the interaction between suspended and biofilm growth has exhibited itself in several ways. In laboratory scale raceways containing RABRs Logan Christenson observed that in a fed batch system the suspended algae largely settled out of solution and flocculated to the bottom (Christenson, 2011). In pilot scale reactors with a single RABR in a small pond TSS seemed to decrease, and in the laboratory scale reactors simultaneous suspended and biofilm growth yielded no real trend.

Understanding this interaction will be fundamental to the success of the RABR design should these be implemented at the Logan Lagoons wastewater treatment plant. Understanding how biofilm growth affects TSS will not only determine where a majority of the biomass will need to be harvested, but also impacts TSS discharge. It could be that

up to a certain amount of time the biofilm accumulates suspended algae, then as it grows to a critical depth it starts sloughing off. This growth pattern may also deal with partitioning of nutrients. Laboratory reactors, while designed to mitigate suspended growth through light control, still contained suspended growth and a large volume of water for growth.

Improving Existing Model

There are many ways the existing model could be improved to be more robust. The model does not predict biomass concentrations as a function of nitrogen due to the limited data that could be collected. If nitrogen is to be incorporated in future modeling efforts several technical challenges need to be overcome. Determination of Michaelis-Menten half saturation kinetic constants by varying dilution rate could be a possibility. There are many challenges, however, in obtaining reliable data. Using Logan Lagoon effluent would provide a medium with variation that would be difficult to control in order to gain valuable information, and indoor lighting solutions would not be optimal compared to sunlight that would be experienced outside.

One solution might be development of what could be called a RABR chemostat. Constant flux of the same media around the RABR at controlled conditions would be the best way to control as many variables as possible. Creating enough media to do this at the pilot scale would be expensive, so this would need to occur at the laboratory scale. To solve the light issue, the fiber optic cables available in USTAR Bioinnovations Building 620 could be used to bring light into the RABR to achieve an amount of light comparable to what might be experienced outside. Indoor controlled conditions and media coupled

with outdoor light may provide the most feasible way to explore the relationship between nutrients and biofilm growth.

Technical challenges in measuring the amount of nutrients exiting the system arise in the fact that real time detection of nitrogen and phosphorus is impossible, and sampling with any frequency would create a large quantity of samples for analysis. Given the duration of growth for the device is on the order of 25 days, this presents a large obstacle. Sampling methods and intervals would need to be developed in order to appropriately monitor nutrient effluent of the system. Analysis of a large amount of samples can occur with nutrient analysis instrumentation at the USTAR Bioinnovations Building 620; however, collection and storage of these samples presents the greatest challenge.

Focusing on ways to accurately measure and quantify biofilm properties would be useful to construct a more comprehensive model. Accurate quantification of biomass without disturbing the reactor would be of great importance. In the case of the RABR, quantification of photosynthetic activity in the form of oxygen evolution would also be rendered useless as oxygen can likely escape quickly into the air. Sampling small parts of the RABR may impede success of an indoor RABR chemostat, as any quantifiable amount may remove too much biomass for subsequent data to be useful. Modeling in such a way to gain predictive power into the RABR system would benefit greatly by developing new techniques to study these systems.

Additional Modeling Considerations

The model produced as a result of this thesis can also be expanded and improved upon. While the model does span many fundamental aspects of algae biofilm growth, it

only considers biomass growth and uptake of nitrogen and phosphorus. Now that large RABRs are constructed, data collection for future improvements in the model could occur. Several possibilities exist for proceeding, such as including in the influence of inorganic carbon, the relationship between suspended and biofilm biomass, and possibly interfacing with the algae and daphnia modeling being conducted by Dr. James Powell in the Mathematics and Statistics Department and Utah State University. These topics would provide the most benefit to future modeling efforts.

With the influence of inorganic carbon shown to be important with regard to the uptake of phosphorus in the Logan Lagoons (Griffiths, 2009), carbon dioxide would likely be another key variable to explore for the modeling of algal biofilms. To increase carbon dioxide concentration in the water, the use of high BOD waste to stimulate heterotrophic organisms could be evaluated. The evolution of this CO_2 production and uptake would be an interesting dynamic to explore both academically and for the feasibility of implementation of adding extra BOD into the Logan Lagoons system. Thie evaluation of carbon dioxide could provide insight into the saturation levels of inorganic carbon needed for optimal growth and nutrient uptake, but predict how much BOD would need to be added to cause that increase.

Interaction of suspended and biofilm biomass could also be important to the modeling. While Logan Christensen observed some settleability in a closed reactor system, this result did not seem to translate to the large ponds. While TSS data were not taken, anecdotal observation seemed to suggest a non-negligible amount of suspended biomass was present in the ponds for a continuous flow reactor. Considering a system including both suspended and biofilm growth would better emulate the Lagoons this

would be a desirable model. Retention time and flow speed, as well as other factors may contribute to this settleability. Exploring the behavior of suspended algae will also have implications concerning TSS discharges from the plant. If RABRs in series have a flocculating effect on algae, this effect could be quantified and ensure discharge limits could be met.

A third area where the model could potentially be expanded is to interface with the daphnia modeling already being developed on through Dr. James Powell. Currently it's relatively unknown whether daphnia can feed on biofilm biomass. Biofilms present a concentrated source of feed for daphnia. However, whether biofilm biomass is accessible to daphnia has yet to be evaluated. Biofilms are usually coated in a sticky slime called extracellular polymeric substance. Whether daphnia can overcome this barrier or even need a certain suspended algae concentration as food would be worth exploring. If daphnia cannot access biofilm biomass as a feed source, then the RABR may also be a way to control daphnia grazing, and including daphnia modeling may be relevant.

RABR Re-designs

During work with the RABR it become apparent that several alternate designs may benefit the operation of the device. This was not the primary focus of this work, however these design variations may be worth exploring. These potential designs are discussed in Appendix C.

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Appendix A: MATLAB Code

Algaemodel.m

```
% Run Solver
clear all
clc

% Declare Variables
r = 0.3808;            %Growth Rate
d=0.0533;              %Death Rate
K=262.0647;            %Carrying Capacity
theta=5.6694;          %Inhibition Constant
A0=0.5257;             %Initial Biomass
Area=24.3;             %Dilution Rate
Flow=8*24*60;
Volume=11468;
dil=Flow/Volume;


tmax=40;
N=4000;
dt=tmax/N;
time=linspace(0, tmax, N+1);

algae=0*time;
algae(1)=A0;  % set initial condition
nitrogen=0*time;
nitrogen(1)=0.01135;
phosphorus=0*time;
phosphorus(1)=0.0028;
bprime=0*time;
test=0*time;


   for n=1:N
      algae(n+1)=algae(n)+dt*(r*algae(n)*sign(1-algae(n)/K)*abs(1-algae(n)/K)^theta-
d*algae(n));
      nitrogen(n+1)=nitrogen(n)+dt*(-0.0534*r*nitrogen(n)*algae(n)*sign(1-
algae(n)/K)*abs(1-algae(n)/K)^theta*Area/Volume+dil*(0.01135-nitrogen(n)));
      phosphorus(n+1)=phosphorus(n)+dt*(-0.0068*r*phosphorus(n)*algae(n)*sign(1-
algae(n)/K)*abs(1-algae(n)/K)^theta*Area/Volume+(dil*(0.0028-phosphorus(n))));
      bprime(n)=(algae(n+1)-algae(n))/(dt);
   end

   for n=1:N
      test(n)=abs(bprime(n)-algae(n)/(n*dt+4.27));
   end
```

```
    for n=1:N-1
       test2(n)=test(n);
    end

    [C,indexopt]=min(test2);
    opttime=indexopt/1000;
    optalgae=algae(indexopt);


nitrogen=nitrogen*1000;
phosphorus=phosphorus*1000;

plot(time,algae,'b'), xlabel('time (days)'), ylabel('biomass (g/m^2)')
plot(time,nitrogen,'b'), xlabel('time (days)'), ylabel('nitrogen (mg/L)')
plot(time,phosphorus,'b'), xlabel('time (days)'), ylabel('phosphorus (mg/L)')
```

constant_determination.m

```
clear all
clc
%% Data Input
time = [0 8 11 13 15 18 20 22 25 27];
% days
algdat = [0      10.73059515   12.94612932   19.73540383   27.60842714   33.61634216
          53.41380156   70.70252293   60.30048708   53.97649174];                    %
g/m^2
%% Determine Constants
[params,fval,flag]=fminsearch(@(x) PWalgerror(time,algdat,x),[0.4, 0.05, 300, 5, 0.5]);

%% Declare Constants
r=params(1)                % growth rate/ resource
d=params(2)                % death rate of algae
K=params(3)                % carrying capacity
theta=params(4)            % rate of inhibition in theta system
A0=params(5)               % initial algae biomass

%% Run Solver
dt=.1;  % time step in Euler method

algae=0*time;
Nitrogen=0*time;
algae(1)=A0;  % set initial condition
Nitrogen(1)=2;

llike=sum(abs(algdat(1)-A0).^2);

for j=2:length(time)
   tspan=time(j)-time(j-1);   % time interval between current observations
   nspan=tspan/dt;            % Euler steps over this interval
   alg=algae(j-1);           % starting value for Euler on this interval
   N=Nitrogen(j-1);

   for n=1:nspan
      alg=alg+dt*(r*alg*sign(1-alg/K)*abs(1-alg/K)^theta-d*alg);   % Euler solver

   end
   algae(j)=alg;
   Nitrogen(j)=N;
end

%% Plot and output parameters
plot(time,algdat,'r*',time,algae,'b'), xlabel('time (days)'), ylabel('biomass (g/m^2)')
```

algaeerror.m

```
function llike=PWalgerror(time,algdat,params)
%
%   sum square errors for biomass growth model
%
%       B' = r B (1 - B/K)^theta - d B
%
%   time is a vector of times at which observations were made
%   algdat is a vector of biomass observations at those times
%   params are parameters to be estimated:
r=params(1);    % growth rate/ resource
d=params(2);    % death rate of algae
K=params(3);    % carrying capacity
theta=params(4); % rate of inhibition in theta system
A0=params(5);   % initial algae biomass

% running this  to find best parameters:
%     time = [0 8 12 16 22 27 34]; % days
%     algdat = [1 235.4 376.7 337.5 381.4 578.7 557.4]; % biomass observations
% now find hte minimum:
%     [x,fval,flag]=fminsearch(@(x) PWalgerror(time,algdat,x),[2.2, 0.1, 500, 10, 1])

% to see how this looks, use the parameters:
%      r=1.17; d=0; K=769; theta=4.98; A0=.916;
% run the code below and plot (commented below the loop)

dt=.1;  % time step in Euler method

algae=0*time;
algae(1)=A0;  % set initial condition

llike=sum(abs(algdat(1)-A0).^2);

for j=2:length(time)
   tspan=time(j)-time(j-1);   % time interval between current observations
   nspan=tspan/dt;            % Euler steps over this interval
   alg=algae(j-1);            % starting value for Euler on this interval

   for n=1:nspan
      alg=alg+dt*(r*alg*sign(1-alg/K)*abs(1-alg/K)^theta-d*alg);   % Euler solver
   end
   algae(j)=alg;
   llike=llike+sum(abs(algdat(j)-alg).^1);  % add to sum absolute error
end
```

```
% to plot, run parameters, F9 the loop above, and then
%    plot(time,algdat,'r*',time,algae,'b')
```

Appendix B: Calculations

$$Yearly\ Biomass\ Production = 29\ \frac{Growth\ Cycles}{Year} * 13\ \frac{kg\ biomass}{RABR} * N\ RABRs$$

$$Methane\ Produced = Yearly\ Biomass\ Production * 0.2\ \frac{m^3 CH_4}{kg\ biomass} *$$

$$(1 - 0.068 \frac{kg\ lipid}{kg\ biomass})$$

$$Energy\ Produced$$

$$= Methane\ Produced * 0.3\ Generator\ Efficiency$$

$$* 0.717\ \frac{kg\ methane}{m^3} * 50 \frac{MJ}{kg\ methane} * \frac{0.278\ kW * h}{1\ MJ}$$

Assumptions

- 29 growth cycles per year
- 13 kg Biomass per installed RABR
- 0.2 m^3 CH_4 per kg biomass
- 6.8% FAME content by weight which is the average FAME content achieved by the large scale RABRs in this study
- 30% generator efficiency
- 0.717 kg methane/m^3
- 50 MJ/kg CH_4 the lower heating value for methane

Sources

- 29 growth cycles per year
- 13 kg Biomass per installed