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Removal and Utilization of Wastewater Nutrients for Algae Biomass and Biofuels

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REMOVAL AND UTILIZATION OF WASTEWATER NUTRIENTS FOR ALGAE BIOMASS AND BIOFUELS

by

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ABSTRACT

Removal and Utilization of Wastewater Nutrients

for Algae Biomass and Biofuels

by

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Utah State University, 2010

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The Logan City Environmental Department operates a facility that consists of 460 acres of fairly shallow lagoons (~ 5'deep) for biological wastewater treatment that meets targets for primary and secondary treatments (solids, biological oxygen demand (BOD), and pathogen removal). Significant natural algal growth occurs in these lagoons, which improves BOD removal through oxygenation and also facilitates N removal through volatilization as ammonia under high pH conditions created by algal growth. Phosphorus, however, is non-volatile and stays in the water and likely cycles in and out of algal cells as they grow and die in the lagoons. In the near future, the regulatory limits on phosphorus released from the Logan wastewater treatment facility are likely to become significantly lower to counter potential downstream eutrophication. One way to potentially lower phosphorus levels in the wastewater effluent is through management of

algal growth in the lagoons. As mentioned above, algae growth naturally occurs in the treatment lagoons and if the algal biomass is harvested when growth yields are highest, the phosphorus contained in the cells could be removed to obtain phosphorus-free water. The algal biomass could then be used for production of biofuels. This research focuses on laboratory and pilot assessments to show the ability of algae indigenous to the Logan lagoons to uptake phosphorus and produce biomass that can be used for biofuel production.

(71 pages)

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Erick W. Griffiths

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LIST OF SYMBOLS, NOTATIONS, AND DEFINITIONS

Abbreviation Key

BOD	Biological Oxygen Demand
CO_2	Carbon dioxide
DO	Dissolved Oxygen
FAME	Fatty acid methyl ester
MGD	Million gallons per day
mg/l	Milligrams per liter
$NaNO_3$	Sodium nitrate
NH_3	Ammonia
NH_4Cl	Ammonium Chloride
NH_2CONH_2	Urea
N	Nitrogen
P	Phosphorus
PAO	Phosphorus accumulating organism
TAG	Triacylglycerol
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The City of Logan treatment facility (see Figure 1-1A) processes wastewater from several local communities: Logan, Smithfield, Hyde Park, North Logan, River Heights, Providence, Nibley, and Utah State University. Overall, an average of 15 million gallons per day (MGD) of wastewater is processed through the treatment plant. The wastewater is treated through a 460 acre facultative lagoon system consisting of 7 ponds: A1, A2, B1, B2, C, D, and E. Upon entering the treatment facility, the wastewater is distributed between 2 sets of parallel ponds, A and B (see Figure 1-1B), where the majority of solids are removed. The waters are then recombined in pond C, which, along with ponds D and E, further polish the water by lowering the solids, biological oxygen demand (BOD), and pathogen levels to below regulatory limits.

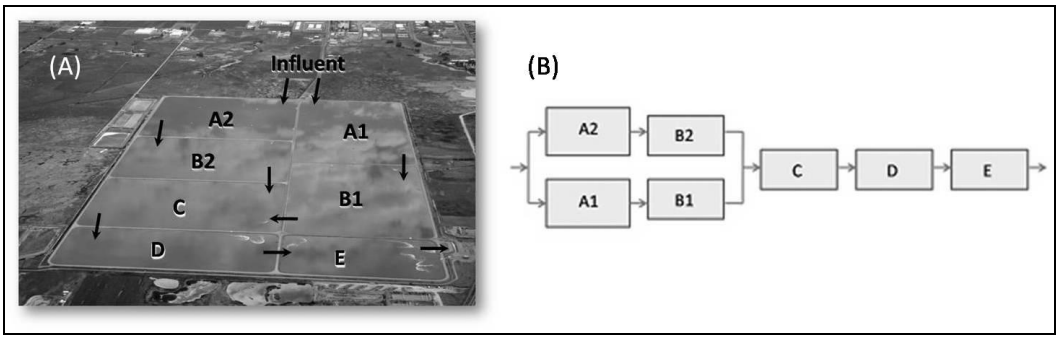

Figure 1-1. (A) Photograph of the Logan city wastewater lagoons with the ponds labeled. Arrows indicate direction of flow. (B) Schematic representation of the Logan wastewater lagoons system (not to scale).

Depending on the concentrations of the various constituents, wastewater is classified as being of strong, medium, or weak strength (Metcalf and Eddy, 1991). Table 1-1 shows the concentrations of the constituents in the Logan city wastewater treatment facilities influent and effluent streams over the four seasons: spring, summer, fall, and winter. The wastewater in the Logan facility is comparable to typical weak strength wastewater (see Table 1-1).

Table 1-1. Characteristics of the wastewater at the Logan treatment facility.

Constituent	Typical Weak	Spring (March - May)		Summer (June - August)		Fall (September - November)		Winter (December - February)	
(mg/l)	Wastewater†	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
BOD_5	110	117±53	24±8	115±103	40±18	130±125	27±24	163±108	25±7
NH_3	12	16±4	12±5	14±3	1±1	20±6	5±3	20±4	13±5
TP	6	6±2	5±1	4±1	4±0.5	5±1	4±1	7±2	5±1
TSS	100	117±33	33±11	99±48	37±18	120±44	24±15	154±109	32±9

†(Metcalf and Eddy 1991)

Upon completion of the treatment process, the water eventually discharges into Cutler Reservoir, which is used for fishing and other recreational purposes. Although the City of Logan currently meets the regulatory standards on effluent phosphorus (P) levels, the limits are likely to be significantly lowered in the near future to counter potential downstream eutrophication in the reservoir (DEQ, 2009). Therefore, the city of Logan will need to implement a P removal processes.

One way to potentially lower the P level in the effluent is through managing the growth of the naturally occurring algae in the lagoons system. Algae uptake P during growth and therefore removal of algae after completion of the growth cycle under phosphorus limitations can eliminate P from the released wastewater. After harvesting, the recovered algae could be further used to produce biofuels. Thus this process would

meet the needs for tertiary treatment of wastewater (nutrient removal) while adding value to the overall process by producing biofuels.

The recent increases in fuel prices coupled with concerns over atmospheric greenhouse gas levels and national security have sparked renewed interest in non-petroleum based fuels. Algae are a promising feedstock for generation of such biofuels since they can be grown on marginal lands using poor quality water and nutrients and thereby do not compete with traditional agricultural resources required for food production. In addition, algae have the potential to produce more biomass and per land area than conventional agricultural crops (Chisti, 2007). However, in order for biofuels to compete with fossil fuels, production costs for the biofuels must be significantly lowered. Because the algae naturally grow in the City of Logan's wastewater lagoons, the opportunity for an integrated system that lowers overall cost of fuel production is economically attractive for the local community (Benemann, 1997).

Research Objectives

This research is based on the overarching hypothesis that algae indigenous to the Logan lagoons treatment plant can be stimulated and managed to simultaneously achieve goals of tertiary treatment (reduction of P and N concentrations to below regulatory limits) and biofuel production. However, to achieve this overall goal, relevant bioprocess design parameters that describe rates of algae growth, nutrient uptake as well as biomass yields and quality must be determined. This research focuses on laboratory and pilot assessments to generate scale-relevant kinetic data. Since temperatures in Logan vary widely with season (below freezing in the winter to over 30 °C in the summer), temperature is likely to be a major factor affecting algal process at a future large-scale

algae-based treatment facility. Also, due to low strength of Logan's wastewater (Table 1-1), algal growth will probably be limited by availability of nutrients, rather than availability (or penetration) of light – a scenario more likely in higher strength wastewaters (Larsdotter, 2006). Also it is possible that the composition of algal species in the wastewaters varies with seasons as the population adjusts to changes in temperature. The effects of N type and temperature on growth and nutrient removal will be evaluated in this study using inocula from different seasons to quantify likely seasonal variations in performance. Specifically, the objectives of this study are:

1. Evaluation of temperature and N sources on algae growth: Temperature will be varied between near freezing (4 C) and 30 C a range that is typically expected at the lagoons. Effects of N supplement on algae growth and P uptake will also be evaluated. N compounds expected to be major components of commercial fertilizer - ammonia, urea and nitrate will be tested.
2. Evaluation growth parameters with natural algae populations in different seasons
3. Pilot scale validation of laboratory results

Literature Review

Eutrophication of surface water bodies (such as lakes or other slow moving streams) occurs when nutrient rich conditions in these systems result in algal blooms. Excess algae growth severely effects water quality resulting in decreased water transparency, odor, oxygen depletion, and possible fish kills (Carpenter et al., 1998; Vymazal, 1995). Some species of algae (blue-green algae) can also produce biotoxins that can cause illness in animals and humans (Hoyle et al., 2003). N and P are generally accepted to be the critical nutrients leading to eutrophication (Vymazal, 1995) and their

presence in water bodies is most often a result of human activity. Possible sources of nutrients include sewage treatment plants, household detergents, septic systems, sediment, animal manure, and commercial fertilizer (Hoyle et al., 2003; Lindberg, 2003).

For most inland waters, P is the limiting nutrient that determines algal productivity (Correll, 1998; Stumm and Morgan, 1981). Municipal wastewaters, a potential source of P, contain both organic and inorganic P species present in soluble and insoluble forms (Nesbitt, 1973). However, most of the organic P and some of the complex phosphates are converted to soluble inorganic phosphate (PO_4^{3-}), also commonly referred to as orthophosphate or reactive phosphate) during the treatment process (Nesbitt, 1973). Thus, in biologically-treated wastewater, most of the P will likely be soluble although a small amount of insoluble organic P may be present. Coincidentally, phosphate is the form of P that is most readily assimilated by algae (Correll, 1998; Nesbitt, 1973; Vymazal, 1995) and significant amounts of P can be released back into the aqueous phase as phosphate following cell death (Vymazal, 1995). For regulatory monitoring, total phosphorus (TP), which includes all phosphorus forms (dissolved and particulate-bound) in a sample, is commonly measured although soluble phosphorus (primarily orthophosphate) is also sometimes reported.

Treatment methods for removing P from wastewater can be separated into three categories: physical, chemical, and biological. Physical treatment includes sedimentation, floatation, and filtration methods, which will only remove insoluble (organic and inorganic) P compounds (Morse et al., 1998; Wang et al., 2006). Chemical treatment includes chemical precipitation, chemical-biological precipitation, and ion exchange all of which primarily target removal of dissolved P forms (usually

orthophosphate) from wastewater. Chemical treatment is generally followed by physical treatment to remove the P-precipitates. Chemical precipitation, originally developed in the 1950's, is carried out by the addition of divalent or trivalent metal salts to wastewater, causing the precipitation of insoluble metal phosphates. The metals most suitable for precipitation are iron and aluminum, added as chloride or sulphate salts. Lime is also used during chemical precipitation, either individually or in combination with other metals and assists the P removal process by increasing the pH to lower the solubility of metal phosphates and by precipitating P as calcium phosphate. However, the precipitation processes produce large quantities of waste sludge containing the P bound metal salt that have to be often disposed off in landfills (Morse et al., 1998).

Phosphorus removal from municipal wastewater is possible without chemical precipitation, through use of biological methods. Conventional biological treatment includes activated sludge and oxidation ponds that primarily remove organic carbon (BOD) and nitrogen by metabolic transformation to carbon dioxide and ammonia (Nesbitt, 1973). Sludge removal from these systems can result in some P removal as well. However, biological P-removal is usually carried out using a process commonly referred to as Enhanced Biological Phosphorus Removal (EBPR). In this method, phosphate accumulating organisms (PAOs, primarily bacteria) that uptake P at levels significantly higher than their normal stiochiometric growth requirements are enriched using cyclic anaerobic and aerobic conditions. After the biological P accumulation is complete, PAOs can be separated through coagulation or flocculation leaving the treated effluent low in phosphorous (Mino et al., 1998). Excess sludge production and the use of chemicals is avoided by this process (Morse et al., 1998).

An alternate biological method for wastewater treatment is through growth of algae. There are primarily two designs that incorporate algae based treatments – (1) facultative waste stabilization ponds (also called facultative lagoons or ponds), and (2) high rate algae ponds (HRAP, also known as raceway ponds (EPA, 2002; Larsdotter et al., 2007)). In the US, facultative lagoon technology for wastewater treatment has been in use for about a century and in 2002, about 7000 lagoons were reported to be operational (EPA, 2002). In general, facultative lagoons are shallow ponds (4-8 feet deep) with minimal mixing, if any. Natural atmospheric aeration and algal photosynthesis at the water surface provides oxygen to lagoons that is then utilized by bacteria to assimilate carbon and reduce wastewater BOD. Further, pH increase due to algae growth volatilizes wastewater ammonia and removes (Larsdotter et al., 2007) N in facultative lagoons. Due to limited operational costs, lagoon systems are economically beneficial in the long run, but are land intensive and are therefore beneficial for treatment of wastewater from small, rural communities (EPA, 2002). The City of Logan uses a facultative lagoon system for wastewater treatment that will likely not be able to meet tertiary treatment regulations without modifications to the existing system.

Another algae based wastewater treatment method is through use of raceway ponds. This wastewater treatment method was first proposed by Oswald in the 1950's (Oswald and Gotass, 1957; Oswald, 1963) and the concept was later expanded to propose use of this system for energy production through harvesting and utilization of algal biomass (Benemann et al., 1977). The basic design of algae raceway ponds includes shallow ponds that are less than 1' deep and continuously mixed using paddle wheels (Weissman and Goebel, 1987). Since these systems are well mixed, controlled algae

growth can be achieved in comparison to open lagoons. As a result, treatment is more efficient and thereby less land intense. Further, better quality control on the algal biomass product can be achieved.

Similar to facultative lagoons, N removal probably occurs through volatilization of ammonia at high pH in raceway ponds, although metabolic uptake of N by algae might also be significant in these systems due to generation of higher quantities of algal biomass under more optimal growth conditions. High pH conditions may also favor precipitation of phosphate with available cations (such as calcium or magnesium) to form metal phosphates that have low solublities at high pH (Larsdotter et al., 2007). These precipitation reactions are favored at high temperatures and in the presence of high concentrations of cations (e.g. in hard water) and P (e.g. in high strength wastes) (Larsdotter et al., 2007). However, presence of carbonates (also present in hard water) in such systems can inhibit precipitation of phosphates (Carlsson et al., 1997; Ferguson et al., 1973). Metabolic uptake by algae can also cause significant P removal, especially in systems with relatively low phosphate concentrations and therefore recovery of algal biomass can result in nutrient removal to meet tertiary treatment goals.

Soluble orthophosphate is easier to biologically uptake than other forms of P (such as insoluble P-precipitates or those associated with organic matter) and leads to more rapid algal growth. The relative amounts of soluble and insoluble P can provide information on the potential for algal growth within the system. If a high percentage of the TP is present as soluble orthophosphate it is more likely that rapid algal growth will occur than if the majority of the TP was mineral phosphorus incorporated in sediment, provided other conditions such as light and temperature are adequate (Correll, 1998;

Nesbitt, 1973; Vymazal, 1995). Also the amount of available P can provide an estimate of the potential biomass yields from the system, if it remains the limiting factor for algae growth.

Uptake of P by algae is not always stoichiometric and can be affected by algal physiology as well as P concentrations and its chemical forms, light intensity, pH, and temperature. There have been observations that show P uptake is inversely related to internal P concentrations of the cell (Cembella et al., 1984; Vymazal, 1995). Algae starting with low internal P concentrations have a higher maximum uptake rate than algae starting with high internal P concentrations (Vymazal, 1995). Therefore, the intracellular P concentrations could be a factor controlling the P uptake kinetics. Hernandes et al. (Hernandez et al., 2006) found that starvation enhanced algae P- removal from wastewater, because cells that have been starved of P tend to overshoot the necessary P uptake for cell growth. In most cases, however, P uptake kinetics can be described as a function of external P concentrations using the Michaelis-Menten model (Vymazal, 1995).

Some algae are also capable of luxury uptake, which is when a surplus of P is accumulated by the cell as polyphosphate. Phosphorus deficient cells are capable of incorporating P very rapidly and in amounts exceeding the normal requirements of the cell (Vymazal, 1995). (Powell et al., 2008) studied factors influencing luxury uptake of P by microalgae in wastewater stabilization ponds and they found luxury uptake to be a significant mechanism of P removal on a laboratory scale. Algal strains such as *Chlorella vulgaris* and *Scenedesmus dimorphus,* that are also present in facultative lagoons, have been found to be capable of significant amounts of P (>55%) when used

for treatment of high P containing dairy and pig wastes (González et al., 1997). Although the actual mechanism of P removal was not reported in this study, such a high fraction of P removal suggests luxury uptake.

Central to algal growth and the resulting P uptake is photosynthesis, which is the process of converting light energy to chemical energy (ATP and NADPH), while splitting water and producing oxygen. On average algae take up N and P together with carbon (CO_2) in the proportion C/N/P ≈ 106:16:1 during photosynthesis in a nutrient unlimited environment (Stumm and Morgan, 1981). Photosynthesis occurs on a diurnal basis with oxygen production during daylight and respiration during the night. Powell et al. (Powell et al., 2008) found that high light intensity can have a negative effect on P uptake such that algae exposed to lower light intensity (60 $\mu E/m^2$ s) contained a higher amount of P than those exposed to a high light intensity (150 $\mu E/m^2$ s).

pH has multiple effects on algal growth and P removal from wastewater. Increased algae growth raises the pH due to the use of CO_2 by algae during photosynthesis (Larsdotter et al., 2007). Besides the precipitation reactions of cations with P at high pH, algal growth rate and species composition may also be affected by pH, especially in mixed populations likely to be present in algae-based wastewater treatment systems. Optimal pH for growth of several algal species is reported to be in the range 7.5-8.5 (Acién Fernández et al., 2001; Chisti, 2008; Marcel et al., 2003; Molina et al., 2001), although there are species that can also grow at much higher pH (Ogbonna et al., 2000). pH values of beyond 10 can easily be reached in algal cultures in the absence of significant buffer or CO_2 supply and can lead to inhibition of growth (Qiang et al., 1996; Richmond and Cheng-Wu, 2001; Zhang and Richmond, 2003). N sources used for algae

growth can also affect the solution pH – when nitrate is assimilated by algae, pH can increase while assimilation of N from ammonia can decrease the growth solution pH to as low as 3 (Larsdotter, 2006).

Nitrogenous matter in wastewater is mainly composed of ammonium ions (NH_4^+), ammonia (NH_3), and organic nitrogen (urea, amino acids and organic compounds with an amino group) although oxidized forms of nitrogen, nitrite (NO_2^-) and nitrate (NO_3^-), may sometimes be present (Vymazal, 1995). At lower pH levels NH_4^+ ions are present, while at higher pH levels N is in the form of dissolved NH_3 gas (Crites et al., 2006). High pH levels results in higher NH_3 concentrations, which can be toxic to algae as well as to fish and other organisms (Lindberg, 2003). However, under high pH conditions, NH_3 gas can easily be volatilized into the atmosphere, especially beyond pH 10 (Pano and Middlebrooks, 1982). During the summer months, algae growth rates are higher due to higher temperatures, which causes a rapid increase in pH. Coupled with higher temperatures this increase in pH eventually results in significant N volatilization and cultures can become N limited. Chemostat studies have suggested that *Scenedesmus* sp. cells can be N limited up to an N:P ratio of 30 to 1 (Vymazal, 1995).

Temperatures between 15–25ºC is generally optimal for algae and lower temperatures result in decreased rate of metabolism and growth (Goldman and Carpenter, 1974). However, the specific effects of temperature are most likely different for individual species of algae. Although photosynthesis and P uptake might be expected to be lower at lower temperatures (Picot et al., 1993; Powell et al., 2008), use of algal strains that are adapted to temperate climates might still achieve treatment goals,

provided appropriate design and operating conditions (hydraulic residence times, mixing and light) are used.

Most current commercial algae operations use open-air systems. Closed culture systems, such as those operated indoors with artificial lighting or outdoors using optimized sunlight utilization strategies, are expensive and are difficult to scale up (Borowitzka, 1999). There are four major types of open-air systems currently in use: shallow big ponds, tanks, circular ponds, and raceway ponds. Raceway ponds, however, are most common due to the relatively easy and effective control possible with this design. Most paddlewheel raceway ponds are between 20 and 30 cm deep. In a report given by the Solar Energy Research Institute (SERI) (Weissman and Goebel, 1987), basic geometric parameters considered in constructing raceway pond design are pond size, number of channels, and length to width (L/W) ratio. L is the length of the center divider wall and W is single channel width. A large pond that has a low L/W ratio gives the most pond area with the least amount of wall length. Although open systems are less productive than photobioreactors due to inefficient utilization of sunlight or the presence of mixed algal populations and predators such as zooplankton, the cost benefits make them more suitable for use in wastewater treatment.

After P uptake, the algae must be removed from the wastewater prior to algal death in order to remove the P from the wastewater. There are many commercially available methods for removing algae from water, including dissolved air floatation (DAF), filtration, centrifugation, and sedimentation. Bare et al. (Bare et al., 1975) performed DAF studies on algae grown in the laboratory using seed taken from the primary ponds of the Logan City wastewater treatment plant and found that a 90% or

greater solid removal is possible given the right pressure, and coagulant dosage. Choice of separation processes for removal of algae can have a significant impact on the downstream processing choices for production of fuel or other co-products. Also, algae concentrations received from upstream growth reactors can also influence the economics of separation. Overall, the choice of separation method will depend on the process design of the integrated system.

The algal biomass recovered from the wastewater can be used as feedstock for several products including biodiesel, biomethane, fertilizer, and nutraceuticals. Several algal strains have been reported produce neutral lipids (triacylglycerides) that can be converted into biodiesel (Chisti, 2007; Hu et al., 2008; Olaizola, 2003; Schenk et al., 2008; Sheehan, 1998). These reports suggest that during nutrient (usually N or P) depletion, the lipid content of several algae can significantly increase. Table 2-1 shows lipid contents of some common wastewater algae.

In addition production of biodiesel or direct combustion for energy, algal biomass could also potentially be anaerobically digested, either by itself or in combination with other substrates to produce methane. Besides fuels, algae could potentially serve as fertilizer and supplement conventional mineral P sources that are likely to be largely depleted in less than 100 years (Abelson, 1999). High value nutraceuticals can also be extracted from algal biomass. Examples of such products include ω- fatty acids, β-carotene and other health care products. *Chlorella* sp., found commonly in wastewater (Table 2-1), is a source for β-1,3-glucan, which is an active immunostimulator and aids in lowering blood cholesterol (Laroche and Michaud, 2007).

Table 1-2. Lipid contents of algae reported in literature.

Alga	Lipid % (w/w algae)	Reference
Scenedesmus	16 – 40	(Thompson 1996)
Chlorella	28-32	(Chisti 2007)
Ankistrodesmus	18 – 73	(Williams and McMillan 1961)
Oocystis	15-20 20	(Richardson, Orcutt et al. 1969) (Aaronson, Berner et al. 1980)
Synura	13	(Cranwell, Creighton et al. 1988)

Based on estimates by Spolaore et al. (Spolaore et al., 2006) world annual sales of *Chlorella* sp. are in excess of US $38 billion. Species of *Chlorella* and *Arthrospira* also yield products such as anti-aging creams and regenerant care products used in the skin care market (Spolaore et al., 2006). Several algae species also produce poly unsaturated fatty acids (PUFAs) including ω-3 and ω-6 fatty acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) that are now components of infant dietary formulas and are believed to be essential for correct brain and eye development (Horrocks and Yeo, 1999). In adults, ω3 fatty acids also promote cardiovascular health and can act as breast and colon cancer chemopreventive agents (Barclay et al., 1994; Horrocks and Yeo, 1999; Senzaki et al., 1998). An analysis by Frost and Sullivan (Frost and Sullivan, 2005) showed that the US market revenue in 2004 for ω-3 (e.g. DHA and EPA) and ω-6 fatty acids (e.g. ARA) totaled $266 million, and is expected to reach a total of $671.9 million by the year 2011.

CHAPTER 2

PHOSPHORUS UPTAKE IN NATURAL WASTEWATER

Introduction

The Logan City Environmental Department in Logan, Utah operates a wastewater treatment facility that consists of 460 acres of open-air facultative lagoons for biological wastewater treatment. Significant natural algal growth occurs in the Logan lagoons, which facilitates nitrogen (N) removal through volatilization as ammonia (NH_3) under high pH conditions created by algae growth. Algae grow more rapidly at higher temperatures (Goldman and Carpenter, 1974) and as a result of the higher temperature and pH increase due to algae growth there is significant NH_3 volatilization during the summer months. However, phosphorus (P) is non-volatile and stays in the system and likely cycles in and out of algal cells as they grow and die in the lagoons.

In the near future, the regulatory limits on phosphorus released from the Logan wastewater treatment facility are likely to become significantly lower to counter potential downstream eutrophication (DEQ, 2009). One way to potentially lower P levels in the wastewater effluent is through management of algal growth in the lagoons. If the algal biomass is removed from the system when growth yields are highest, the P contained in the cells could also be removed to obtain phosphorus-free water. $C_{106}H_{181}O_{45}N_{16}P$ is a stoichiometic formula for the most common elements in an average algae cell (Stumm and Morgan, 1981). This formula shows that an N:P molar ratio of 16:1 is needed for optimal growth in an average algae cell. Therefore, N supplementation will be required

during the summer months to achieve sufficient N:P for optimal and sustained algae growth to uptake all of the phosphorus (P).

pH, dissolved oxygen (DO), temperature, total suspended solids (TSS), and nutrient data has been collected from the lagoon system over several years. Analysis of this data as it pertains to algal biomass productivity due to seasonal variation and treatment system operation will be presented.

Laboratory experiments have been conducted to determine potential N sources that can be used to enhance algae growth for optimal P uptake. Urea, nitrate, and ammonium, forms of N commonly used as fertilizers (Eaton et al., 2005; PotashCorp, 2007), were used as N supplements. Because temperature changes significantly from summer to winter at the Logan wastewater facility, algae were grown at 4, 10, 20, and 30 °C to determine the effect of temperature on algae growth.

Materials and Methods

Lagoons Sampling and Analysis

Periodic sampling of the lagoons was performed by the wastewater treatment system operators over the course of a 3 year period (2006-2009). On an average, sampling was done about once a month and included direct measurements of dissolved oxygen (DO), pH and temperature. Samples were also analyzed for ammonia nitrogen, total-P, and total suspended solids (TSS).

Lagoons Analytical Methods

DO, pH, and temperature were obtained by using an HQ40d Dual-Input Multi-parameter Meter Configurator with a PHC101 IntelliCAL Rugged Gel Filled pH Electrode and a LDO101 IntelliCAL Rugged Dissolved Oxygen Probe (Hach Company,

Loveland, CO). Chemical analysis was done by Chem-tech Ford Laboratories Murray, UT.

Shaker Flask Experimental Setup

Logan lagoons effluent was incubated in 250 ml Erlenmeyer flasks in the laboratory to stimulate growth of native algae populations. The flasks were placed on a shaker table to keep the samples well mixed and to prevent the algae from settling. Plant growth (Aqua Rays, General Electric, Fairfield, Connecticut) and natural sunshine (Natural Sunshine, Philips, Amsterdam, Netherlands) fluorescent lights were placed around the shaker table to promote photosynthesis. Tests for each N source were done in duplicate with one control. Analytical grade sodium nitrate ($NaNO_3$), and urea (NH_2CONH_2) were added to flasks at a concentration of 0.5 g/l.

Effects of temperature on algae growth was evaluated in these small volume growth systems by setting up the Erlenmeyer flasks in a refrigerated cold room for studies at 4 °C, a refrigerated incubator (Innova 4230 Refrigeratred Benchtop Incubator Shaker, New Brunswick Scientific, Edison, NJ) for studies at 10 °C, on the laboratory benchtop for studies at 20 °C, and finally a constant temperature room for studies at 30 °C.

Shaker Flask Sampling and Analysis

Samples were taken daily in 2 ml volumes from each shaker flask and analyzed for soluble N and P. The Arrhenius equation ($\ln(k) = \frac{-Ea}{R}\frac{1}{T} + \ln(A)$) was used to determine the dependence of algal growth rate (k) on temperature (T).

Analytical Methods

Orthophosphate PO_4^{3-} was measured by the standard method based on the ascorbic acid method (method 4500 -P-D) (Eaton et al., 2005) using Hach PhosVer™ reagent (Hach Company, Loveland, CO). Ammonia (NH_3-N) was measured by the ammonia-salicylate method – a modification of the standard phenate method (Eaton et al., 2005), nitrate (NO_3-N) was measured by the standard method based on Cadmium reduction (Eaton et al., 2005). Urea was measured by the Diacetyl monoxime method – a modification o f the Diacetyl monoxime method recommended by the Standard Operating Procedures for Clinical Chemistry (Kanagasabapathy and Kumari, 2000). The soluble nitrogen and phosphorus measurements were made by first centrifuging (accuSpin Micro 17, Fisher Scientific) the sample. The total soluble nitrogen and phosphorus concentrations in the supernatant of the sample were then determined by the persulfate digestion methods (Eaton et al., 2005; Eaton et al., 2005; Eaton et al., 2005).

Results and Discussion

Alkaline conditions lead to the volatilization of ammonia (NH_3) since its solubility in water is a strong function of pH. Higher temperatures also facilitate volatilization of NH_3. Algae grow more rapidly at higher temperatures (Goldman and Carpenter, 1974) and as a result of the higher temperature and pH increase due to algal growth, NH_3 concentrations are very low in the treatment facilities effluent during the summer months (Figure 2-1A). P, however, is non-volatile and stays in the water and likely cycles in and out of algal cells as they grow and die in the lagoons. Because the algae are not removed from the system, the P remains in the water. Figure 2-1B shows the average total P concentrations at the treatment facility during the past few years in the

influent and effluent as approximately 5.3 mg/l and 4.1 mg/l respectively. The slight drop in P concentration through the treatment process could be due to solids settling in the lagoons.

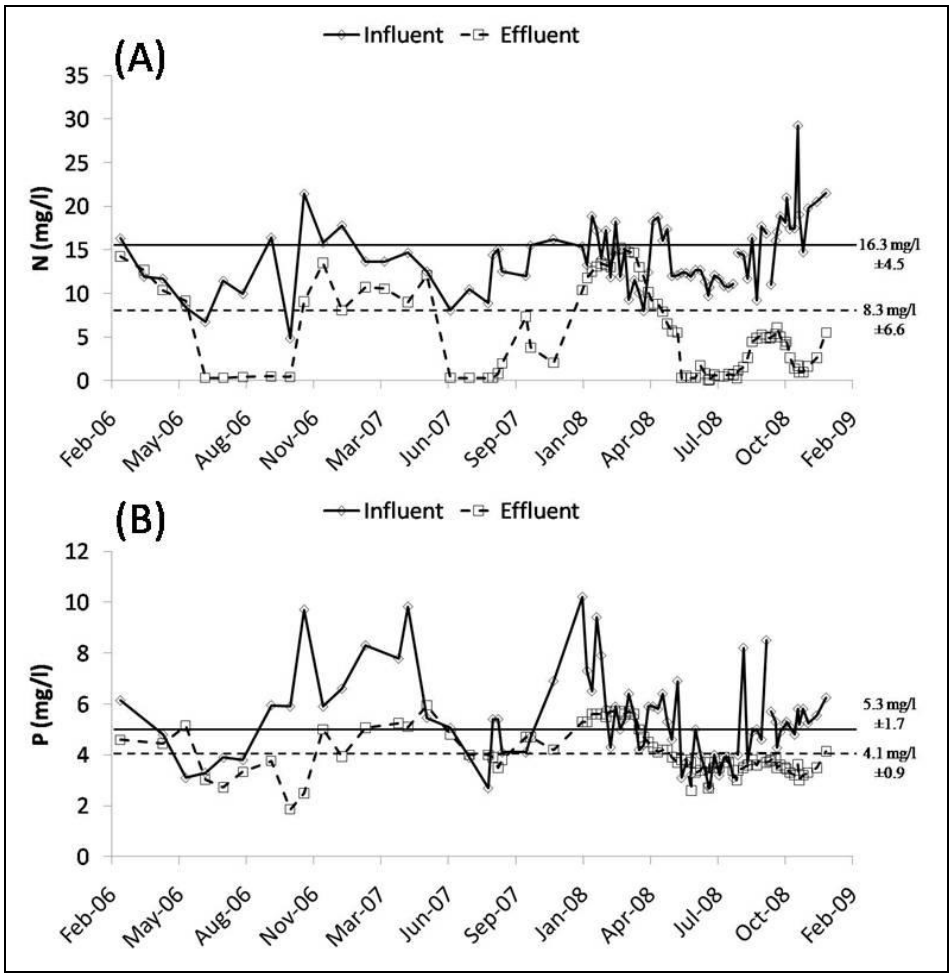

Figure 2-1. Seasonal (A) N concentrations and (B) P concencentrations in the influent and effluent of the treatment facility.

Important indicators of algal growth come from monitoring of measureable photosynthetic parameters. Increased algae growth raises the pH due to the use of CO_2 by algae during photosynthesis (Larsdotter et al., 2007). pH > 10 can easily be reached in

algal cultures in the absence of significant buffer or CO_2 (Qiang et al., 1996; Richmond and Cheng-Wu, 2001; Zhang and Richmond, 2003). Also, oxygen (O_2) is a product of photosynthesis and therefore release of oxygen into the water leads to higher dissolved oxygen (DO) levels. Therefore, a net increase in pH and DO levels indicates algae growth.

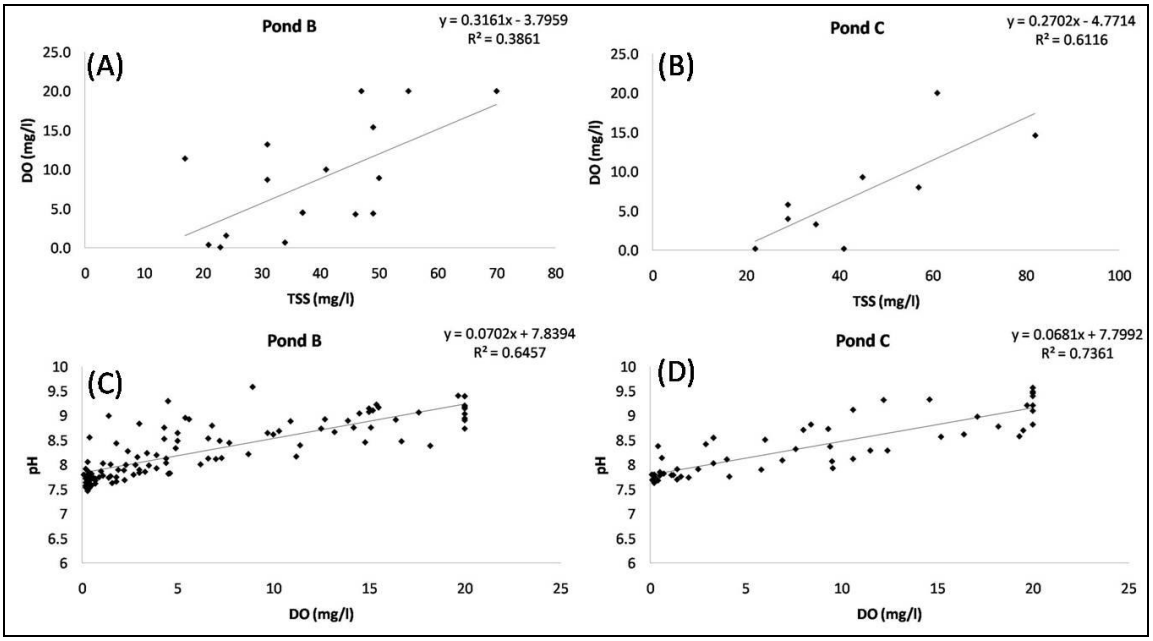

Figure 2-2. Correlation of (1) dissolved oxygen (DO) and TSS ((A) and (B)) and, (2) pH and DO ((C) and (D)) in B and C ponds, respectively. These data suggest that the biological activity in ponds B and C is related to algae growth.

The correlation as seen in Figure 2-2 (A, B) shows an increase in DO with an increase in algae biomass, reported as TSS. Figure 2-2 (C, D) shows the direct correlation of an increase of DO and pH, which is further evidence of algae growth. Since photosynthesis only occurs during the daylight hours, continuous monitoring of DO and pH is needed to characterize diurnal growth patterns. Since Logan is located at a

high latitudes, cold winter months might be expected to hamper or even stop algal productivity. However, our monitoring results of the algae relevant growth parameters (DO and pH) showed diurnal variations consistent with algae growth at cold temperatures (Figure 2-3B). Further, this activity was persistent for the 3 week monitoring period during December 2008 (see Figure 2-3A) that shows evidence of sustained photosynthesis and algae growth during winter.

Algae from the Logan treatment facility were grown at 4, 10, 20, and 30 °C to determine the effect of temperature on algae growth. Figure 2-4 shows a much faster P uptake rate at 30 °C during the first 5 days than at colder temperatures. The algae were also supplemented with an N source.

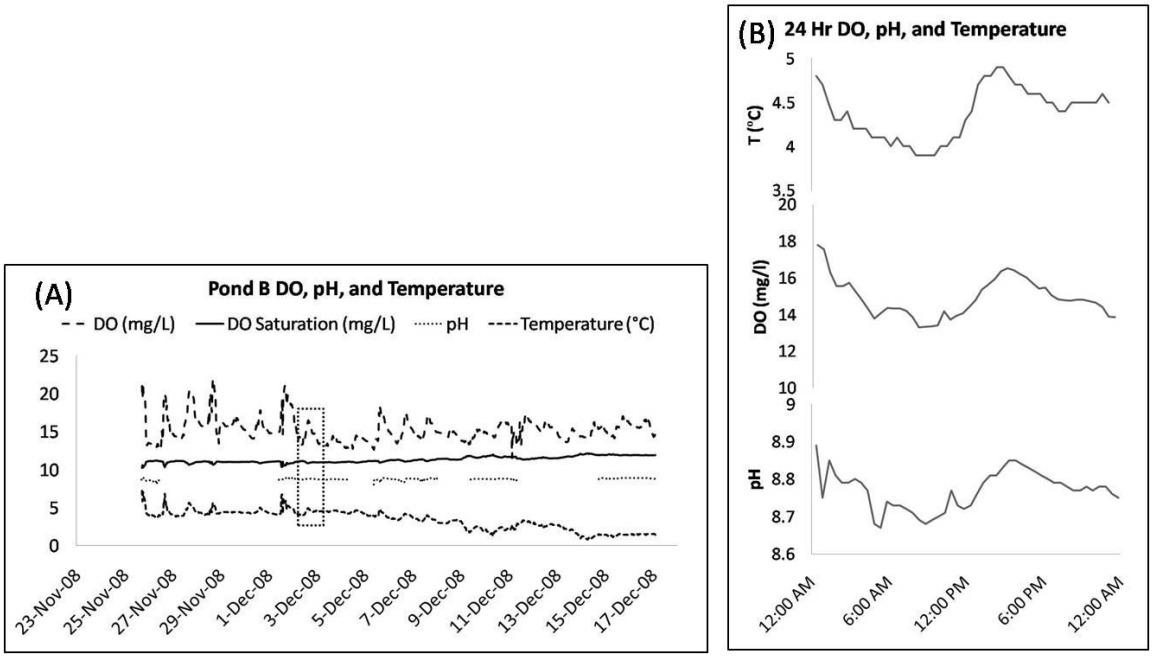

Figure 2-3. (A) Diurnal variation in pH, DO, and Temperature over a 3 week period. (B) Blow up of a 24 hr period indicated by the dotted box in (A).

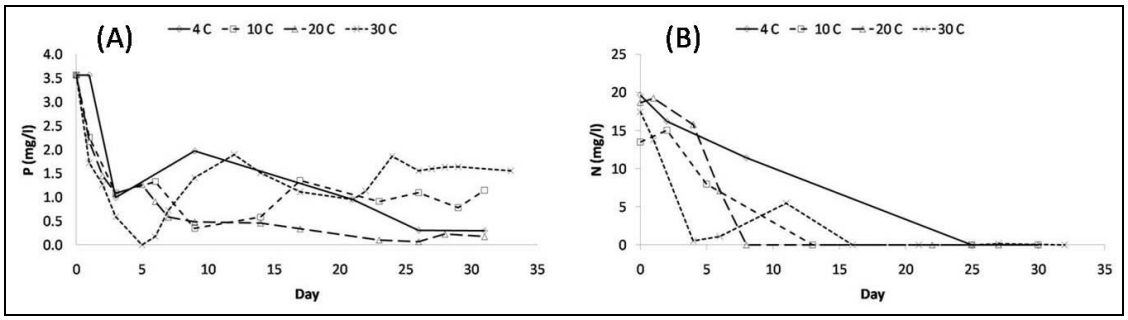

Figure 2-4. Soluble P uptake (A) and N uptake (B) by algae grown at 4, 10, 20, and 30 °C.

As previously mentioned, alkaline conditions lead to the volatilization of NH_3, which lowers the overall N:P molar ratio. In order for the algae to consume all of the P, N supplementation will be required especially during the summer months. Analytical grade sodium nitrate ($NaNO_3$), and urea (NH_2CONH_2) were added to flasks at a concentration of 0.5 g/l soluble P was then measured over time to monitor P uptake (Figure2-5).

Phosphorus uptake rates were observed to be the greatest at higher temperatures. When the light intensity is held constant, maximum growth rate can be described as a function of temperature by applying the Arrhenius equation (Goldman and Carpenter, 1974) (see Figure 2-6 Equation 1). Taking the natural logarithm of the Arrhenius equation gives Equation 2 in Figure 2-6. When a reaction has a rate constant which obeys the Arrhenius equation, a plot of ln(k) versus 1/T gives a straight line. The P uptake rates in moles per day of all N sources grown at 4, 10, 20, and 30 °C were used in the Arrhenius equation to get the results shown in Figure 2-6. The results indicate that the algae growth rate based on P uptake is a function of temperature.

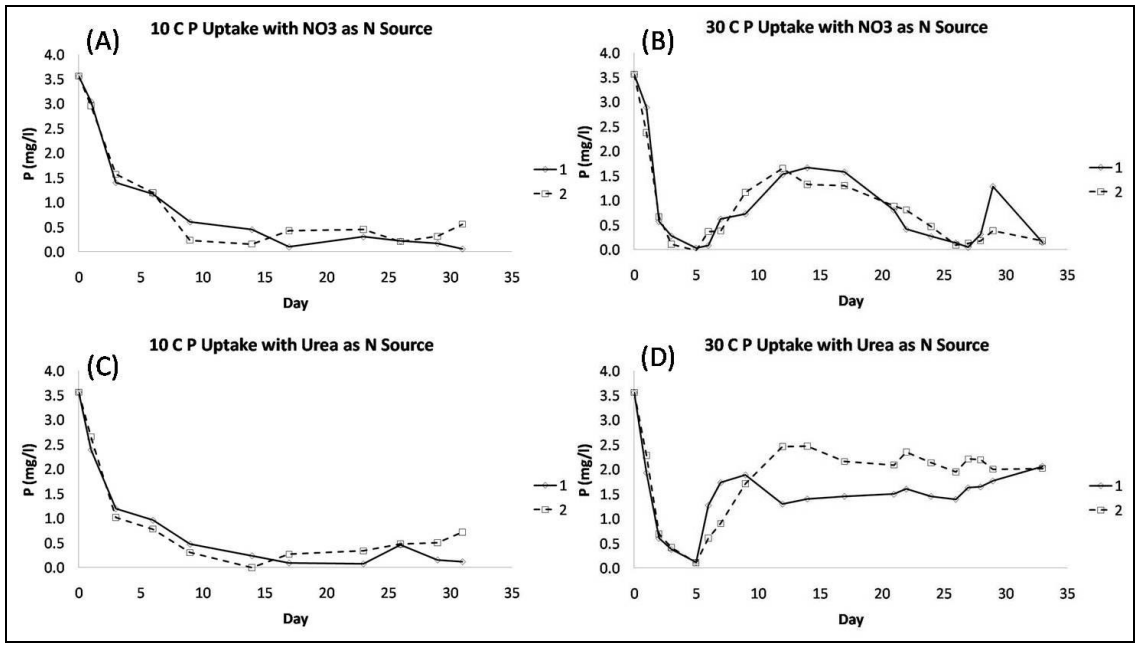

Figure 2-5. Algae grown with nitrate (A and B) and urea (C and D) as N sources at 10 C (A and C) and 30 C (B and D). 1 and 2 are replicates.

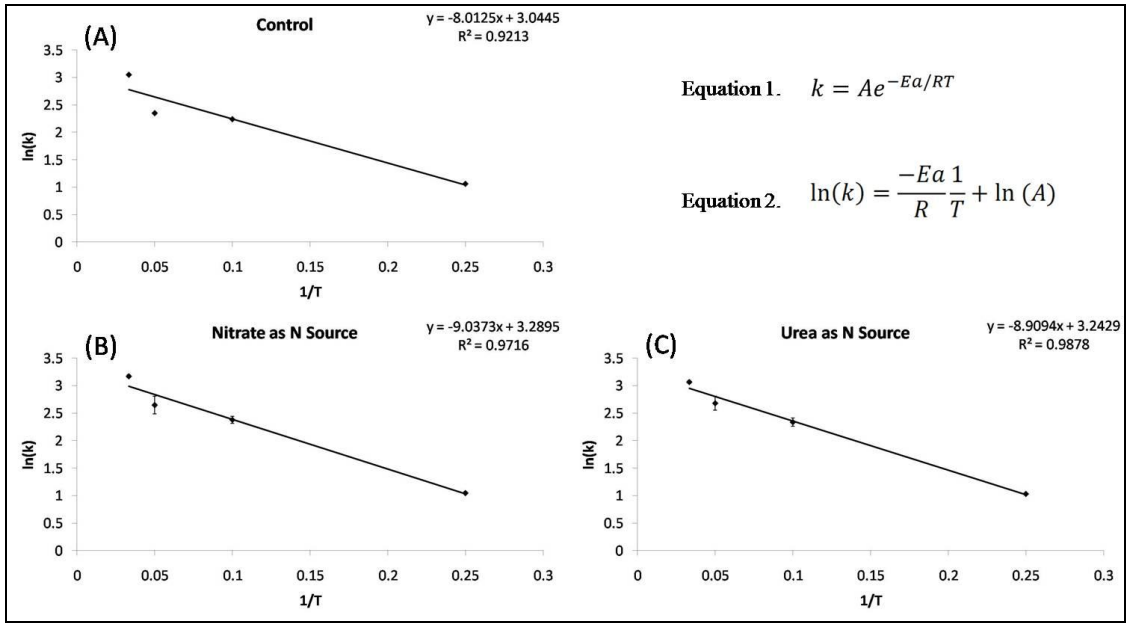

Figure 2-6. Arrhenius plots of algae grown at 4, 10, 20, and 30° C for control (A), nitrate (B), and urea (C) N sources.

Multiple algal strains that are known to be lipid accumulators have been identified in the Logan lagoons and are listed in Table 2-1. *Scenedesmus* and *Chlorella* have been the most dominant species observed in the Logan lagoons. These algal species have also been observed as the most dominant in the Logan lagoons by previous researchers at Utah State University (Bare et al., 1975).

Because the lipid content data for specific species given in Table 2-1 were obtained from pure culture studies in the laboratory, we can assume that the lipid content will not be as high in mixed populations under natural environmental conditions. Also, there will likely be losses during recovery of lipids from algal biomass. Therefore, a modest extractable lipid content of 10 % (w/dry algae wt) can be used to determine conservative potential biodiesel yields.

Table 2-1. Algae strains in the Logan lagoons and lipid contents reported in literature.

Alga	Lipid % (w/w algae)	Reference
Scenedesmus	16 – 40	(Thompson 1996)
Chlorella	28-32	(Chisti 2007)
Ankistrodesmus	18 – 73	(Williams and McMillan 1961)
Oocystis	15-20	(Richardson, Orcutt et al. 1969)
	20	(Aaronson, Berner et al. 1980)
Synura	13	(Cranwell, Creighton et al. 1988)

The Logan wastewater treatment facility treats 15 MGD and the water has an average of 4 mg/l total P (see Figure 2-1B). Again as a conservative estimate, we can assume only half of this P to be consumed due to algal growth in a wastewater system more optimized for algae growth. Using these assumptions we can estimate potential

biofuels production through two routes anaerobic digestion of algal slurries and biodiesel potential of harvested algae.

These conservative estimates of potential biofuel yields are listed in Table 2-2. If the estimated 13,050 kg of algal biomass grown per day in the lagoons were harvested, 120,000 gallons of biodiesel could potentially be produced per year.

Table 2-2. Conservative estimates of potential biofuel yields at the Logan lagoons.

	Yields	Reference
Biomass[1,3,5]	13,050 kg algae / day	
Biodiesel Potential[1,2,4,6]	***120,000 gallons / year***	
Methane Potential	76,000 moles CH_4 / day	(Yen and Brune 2007)
Energy Potential of Methane	6,028,200 kWh / year	(Ogden 2002)
Calculated Electrical Energy output *	***1,591,450 kWh / year***	(Bove and Lunghi 2006)

1. 15 MGD flow rate
2. Facility Operation 300 days/year
3. Available P = 2 mg/L
4. Lipid Content 10% (w/w) on a dry algae basis
5. Composition of algae $C_{106}H_{263}O_{110}N_{16}P$ (Stumm and Morgan 1981)
6. Density of Biodiesel 0.864 kg/l (Miao and Wu 2006)

* Assuming 33% Combustion Efficiency and 80% Generator Efficiency

Conclusion

Evidence of algae growth has been observed even during the cold winter months of the year. Laboratory results show that algae native to the Logan treatment facility still grow at colder temperatures just at a slower growth rate. Optimizing the algal growth in the Logan lagoons through utilization of available nutrients has the potential to remove P from the water.

Nutrient removal with algae is a promising treatment process that can be used to prevent downstream eutrophication and provide a renewable energy source for the city of

Logan. This integrated wastewater treatment and biofuel production system can thus benefit the community as well as the environment.

CHAPTER 3

ALGAE GROWTH IN A FIELD RELEVANT REACTOR DESIGN

Introduction

The Logan City Environmental Department in Logan, Utah operates a wastewater treatment facility that consists of 460 acres of open-air lagoons for biological wastewater treatment. Significant natural algal growth occurs in the Logan lagoons, which facilitates nitrogen (N) removal through volatilization as ammonia (NH_3) under high pH conditions created by algae growth. Algae grow more rapidly at higher temperatures (Goldman and Carpenter, 1974) and as a result of the higher temperature and pH increase due to algae growth there is significant NH_3 volatilization during the summer months. However, phosphorus (P) is non-volatile and stays in the system and likely cycles in and out of algal cells as they grow and die in the lagoons.

In the near future, the regulatory limits on phosphorus released from the Logan wastewater treatment facility are likely to become significantly lower to counter potential downstream eutrophication (DEQ, 2009). One way to potentially lower phosphorus levels in the wastewater effluent is through management of algal growth in raceway ponds. Raceway ponds are a less expensive alternative to closed system reactors, where algae can be cultivated under natural sunlight (Borowitzka, 1999). If the algal biomass is removed from the system when growth yields are highest, the P contained in the cells could also be removed to obtain phosphorus-free water.

$C_{106}H_{181}O_{45}N_{16}P$ is a stoichiometic formula for the most common elements in an average algae cell (Stumm and Morgan, 1981). This formula shows that an N:P molar ratio of 16:1 is needed for optimal algae growth. Therefore, N supplementation will be required during the summer months to achieve sufficient N:P for optimal and sustained algae growth to uptake all of the P. Urea, nitrate, and ammonium, commonly used as fertilizers (Eaton et al., 2005; PotashCorp, 2007), were used as N supplements to enhance algae growth for optimal P uptake. Algae were grown in laboratory scale raceway reactors as well as in outdoor pilot scale raceway reactors to evaluate growth and nutrient uptake using field relevant reactor designs. The elemental composition of the algae grown in the raceway reactors was also determined and will be presented.

Materials and Methods

Laboratory Raceway Experimental Setup

The lab scale raceway reactors were made of ¼“acrylic sheeting with the dimensions shown in Figure 3-1. The reactors have 2 channels and a length to width ratio of 2:1. Mixing was accomplished by a paddlewheel set-up. An electric motor (120

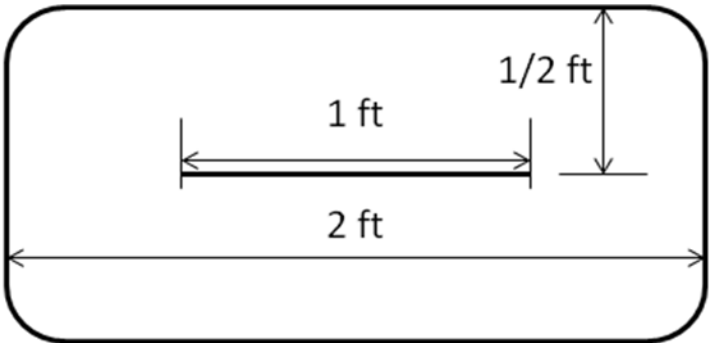

Figure 3-1. Shematic of laboratory raceway reactor dimensions.

V AC/DC Variable Low Speed Motor, Wondermotor, Walnut, CA) was used to rotate paddle wheels at approximately 10 rpm. Transparent acrylic was used to allow light penetration for photosynthesis to occur in a laboratory environment. Plant growth fluorescent bulbs (plant & aquarium F40, General Electric) were used to for the light source. The light spectrum of the plant growth bulbs is shown in Figure 3-1. The bulbs provided the red light necessary for photosynthesis around 680nm. Four plant growth fluorescent bulbs were mounted on the bottom and two on each 2 ft side for a total of eight bulbs around each reactor. The eight bulbs provide an average light intensity of 764 ± 104 umol m^{-2} s^{-1}in an empty reactor. Lights were on for 14 hours and off for 10 hours. Temperature was held at 21 ±3°C. The variation in temperature came from the lights warming the reactors followed by the reactors cooling after the lights were turned off.

Eight reactors were built to test a control and three potential N sources in duplicate. Analytical grade ammonium chloride (NH_4Cl), sodium nitrate ($NaNO_3$), and

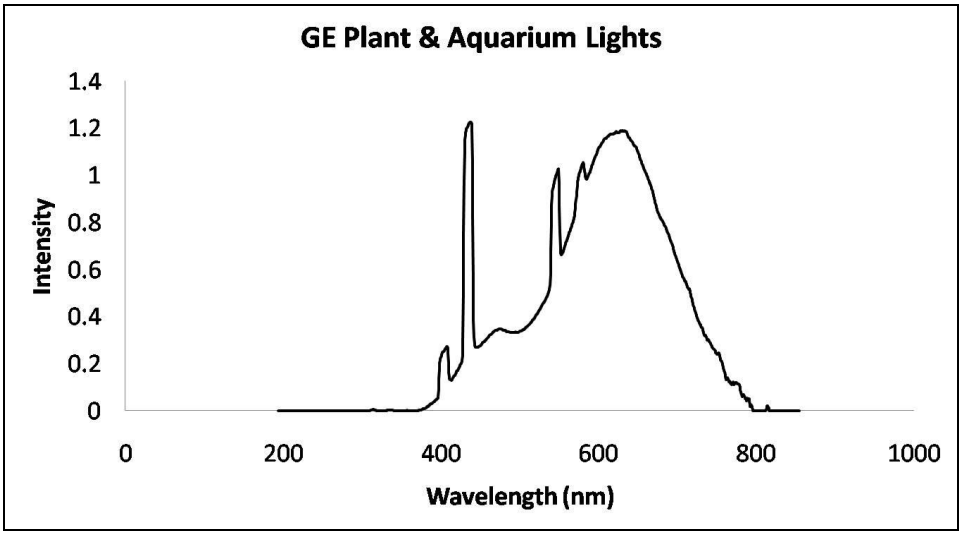

Figure 3-2. Light spectrum of GE plant growth fluorescent bulbs.

urea (NH_2CONH_2) were added to reactors at concentrations needed to bring the N:P ratio to 16:1 for optimal algae growth (Stumm and Morgan, 1981).

Pilot-Scale Raceway Experimental Setup

The outdoor pilot raceway reactors were made from water troughs (3X2X8 Tanks, Behlen Country, Columbus, Nebraska) that were lined on the inside with an acrylic coating (1K Waterborne Acrylic Clear Coat, Sher-Clear, Cleveland, OH) to prevent zinc from leaching into solution from the original galvanized metal. The raceways were mixed using paddle wheels designed to rotate at 5 rpm and are powered by 0.08 hp motors (Leeson Motors, Grafton, WI). The raceway ponds have two channels with a length to width ratio of 3.66:1 (Figure 3-3) and a volume of approximately 448 L.

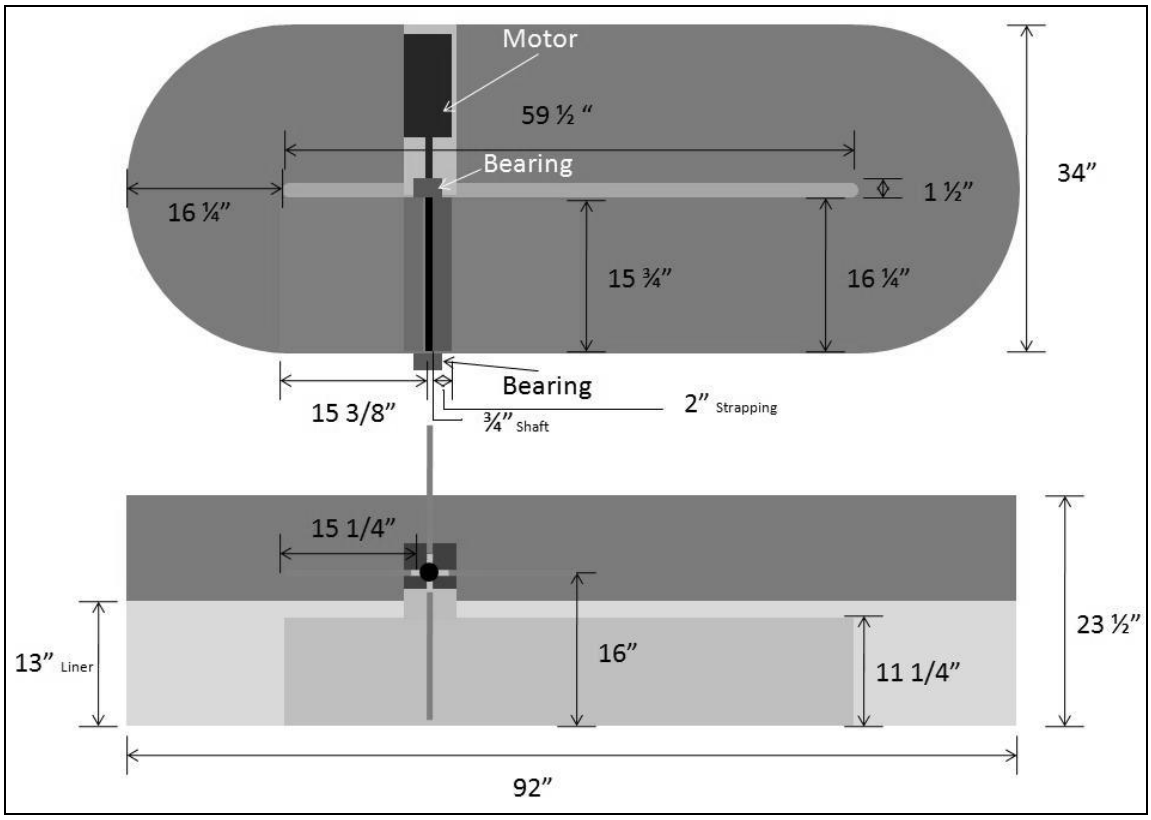

Figure 3-3. Schematic of the outdoor pilot raceway reactors.

Sampling and Analysis

Samples were taken daily in 50 mL volumes from each reactor and analyzed for soluble N, NO_3, NH_3, Urea, and P. Dissolved oxygen (DO), pH, and temperature were monitored continuously during the experiments. Suspended solids (TSS) were measured daily to directly quantify algae. Evaporation was compensated by the addition of nutrient-free water. The algae biomass was harvested at the end of the experiment with a Sharples T-1 continuous centrifuge and air dried in preparation for analysis of the elemental composition.

Analytical Methods

Orthophosphate PO_4^{3-} was measured by the standard method based on the ascorbic acid method (method 4500 -P-D)(Eaton et al., 2005) using Hach PhosVer™ reagent (Hach Company, Loveland, CO).

Ammonia (NH_3-N) was measured by the ammonia-salicylate method – a modification of the standard phenate method (Eaton et al., 2005), nitrate (NO_3-N) was measured by the standard method based on cadmium reduction (Eaton et al., 2005), and total phosphorus (TP) was measured by the persulfate digestion and ascorbic acid method (Eaton et al., 2005; Eaton et al., 2005). Urea was measured by the Diacetyl monoxime method – a modification o f the Diacetyl monoxime method recommended by the Standard Operating Procedures for Clinical Chemistry (Kanagasabapathy and Kumari, 2000).

The soluble nitrogen and phosphorus measurements were made by first filtering the sample through a 1.0 µm glass fiber filter to remove all suspended solids (Eaton et al., 2005). The total soluble N and P concentrations in the filtered sample were then

determined by the persulfate digestion methods (Eaton et al., 2005; Eaton et al., 2005; Eaton et al., 2005). Total suspended solids (TSS) was determined through the filter paper method (Eaton et al., 2005).

The laboratory reactors were monitored for pH continuously with pH meters (PHTX-92 Meter, OMEGA Engineering, Stamford, CT) and probes (OAKTON pH Electrode, Fisher Scientific) and DO was measured continuously with galvanic sensors (DO1200, SensoreX, Garden Grove, CA). Temperature was measured with T-type thermocouples (thermocouple, OMEGA Engineering, Stamford, CT).

The outdoor pilot-scale raceway ponds DO, pH, and temperature were measured by using an HQ40d Dual-Input Multi-parameter Meter Configurator with a PHC101 IntelliCAL Rugged Gel Filled pH Electrode and a LDO101 IntelliCAL Rugged Dissolved Oxygen Probe (Hach Company, Loveland, CO).

The dried biomass prepared at the end of the experiment was given to the Utah State University Analytical Laboratory for evaluation of the algae elemental composition. The percentage of elements carbon (C), nitrogen (N), and phosphorus (P) were determined using a Thermo Electron Iris Advantage Inductively-coupled Plasma Spectrophotometer (ICP) and a LECO TruSpec C/N.

Results and Discussion

The control did not have any additional N added to increase the N:P ratio to 16:1. The initial N:P for the control was 2.5:1. However, under optimal lighting and mixing conditions the algae was still able to grow to a concentration of approximately 100 mg/l from a starting point of 44 mg/l (see Figure 3-4). Although there was some P-uptake by the algae, the soluble P was not completely consumed in the control. The addition of N to

bring the N:P to 16:1 enhanced the algae growth enough to consume all of the soluble P available.

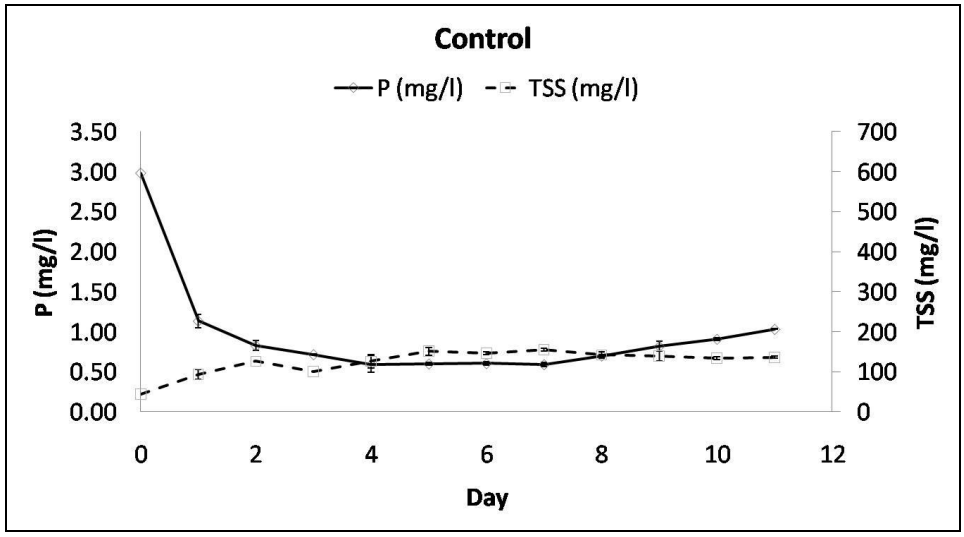

Figure 3-4. P uptake and algae growth (TSS) of controls.

N sources $NaNO_3$ and urea yielded comparable results (see Figure 3-5 B and C) enhancing the algae enough to consume all the soluble P and reach a TSS of approximately 500 mg/l. The N source NH_4Cl did not work as well as $NaNO_3$ or urea at enhancing algae growth with an overall biomass yield of approximately 300 mg/l TSS (see Figure 3-5 D). N sources $NaNO_3$ and urea performed the best overall promoting the algae growth enough to consume all of the soluble P and yield more biomass (see Figure 3-6). One possible explanation for the inferior performance of NH_4Cl could be that the N loss due to NH_3 volatilization is greater, which could explain the rapid loss of N in the NH_4Cl supplemented reactors. In addition, too much free NH_3 has been found to inhibit photosynthesis in some algal species (Azov and Goldman, 1982).

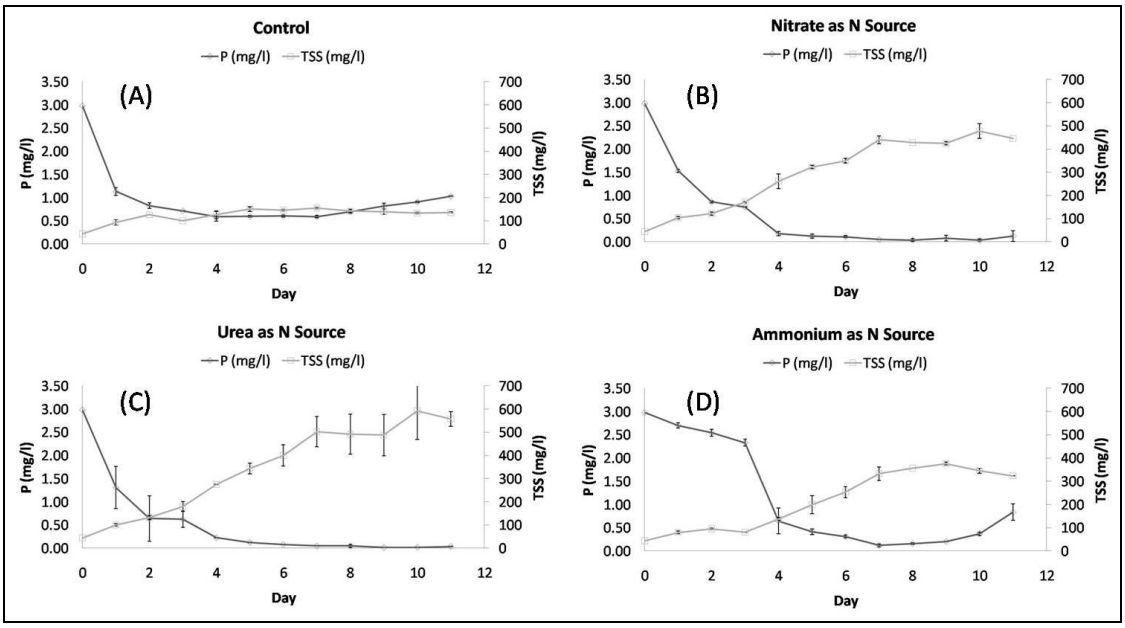

Figure 3-5. P uptake and algae growth for control (A), nitrate (B), urea (C), and ammonium (D) N sources.

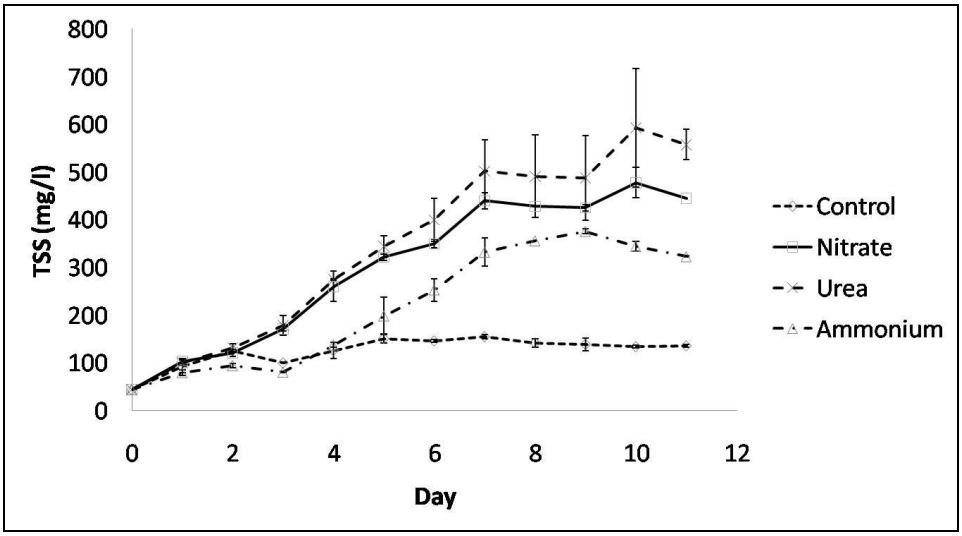

Figure 3-6. Comparison of algae growth reported as TSS over time.

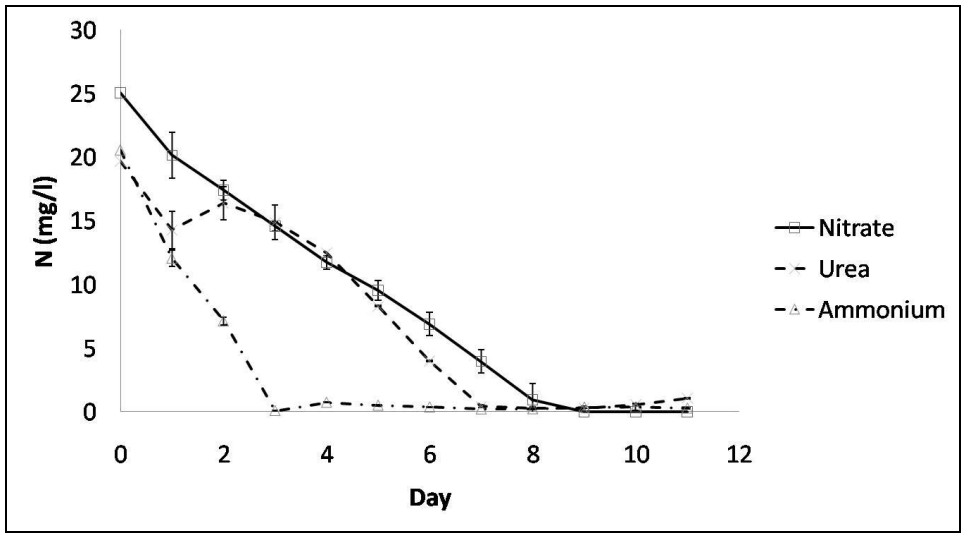

Figure 3-7. N consumption due to algae assimilation or volatilization.

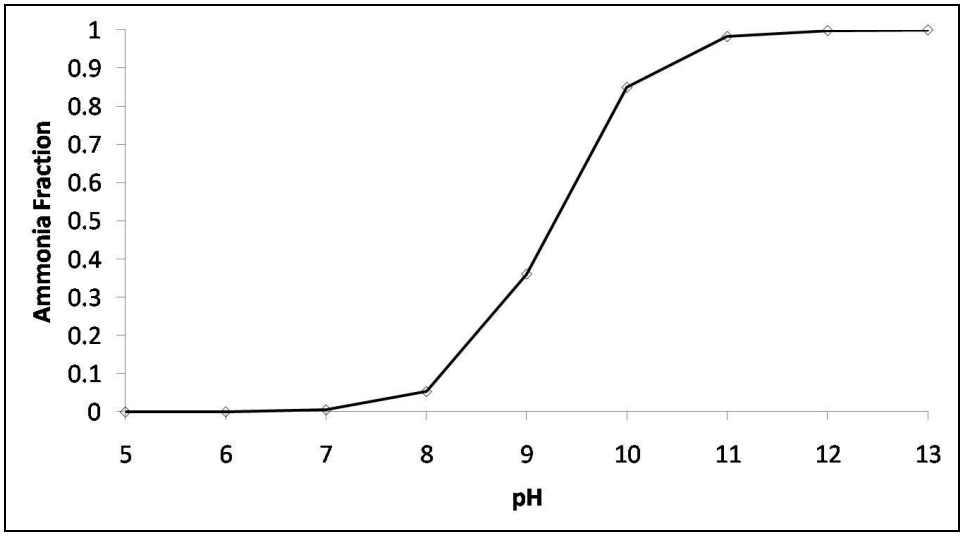

Figure 3-8. pH effect on ammonia fraction at 22 C calculated using equations presented by (Körner et al., 2001).

Ammonia can be present as molecular ammonia (NH_3) or as ammonium ions (NH_4^+). The equilibrium is dependent on temperature and pH. Only ammonium ions are present at pH 7 and ammonia at pH 12 (Crites et al., 2006; Körner et al., 2001), which

can be seen in Figure 3-8. Therefore, increased algae growth leads to high pH levels and in return NH_3 volatilization.

The pH was monitored continuously during the experiment (see Figure 3-9) and pH levels of above 10 were easily achieved because there was no additional CO_2 supplied. The diurnal variation seen in Figure 3-10 is due to photosynthesis during the day and respiration during the night. Higher overall pH was achieved by algae using N sources $NaNO_3$ and urea, which indicates more algae growth.

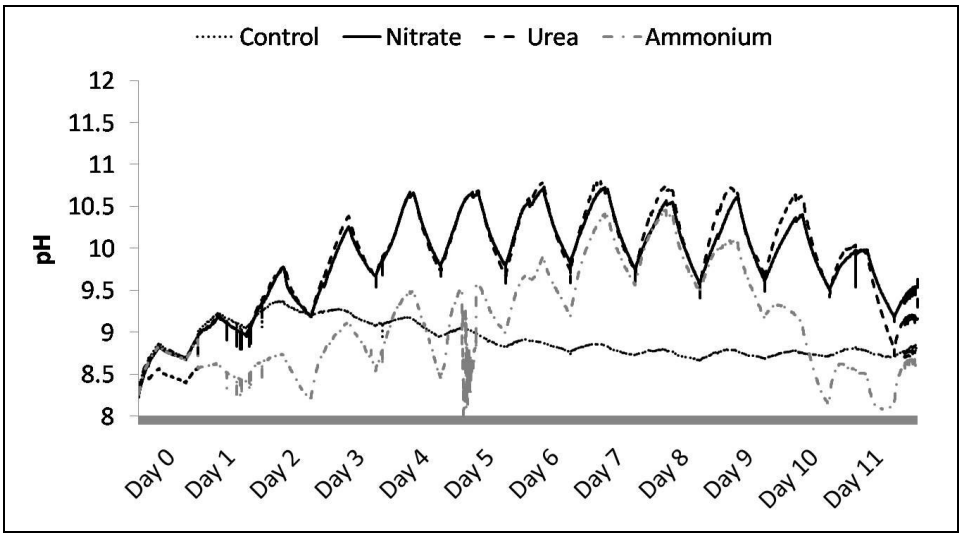

Figure 3-9. Diurnal variation of pH in reactors with control, nitrate, urea, and ammonium N sources.

In the presence of light, algae produce oxygen through photosynthesis and release the oxygen into the water and the DO level rises. Overall DO concentration increases with algae growth. The diurnal variation is a result of respiration at night and photosynthesis during the day. The production of oxygen by algae is seen in Figure 3-11 as an increase in DO. The DO saturation level at 21°C is approximately 8.9 mg/l. The

solid black line in Figure 3-11 indicates the water saturation level. The algae with N supplementation keep the water well above saturation for the majority of the time.

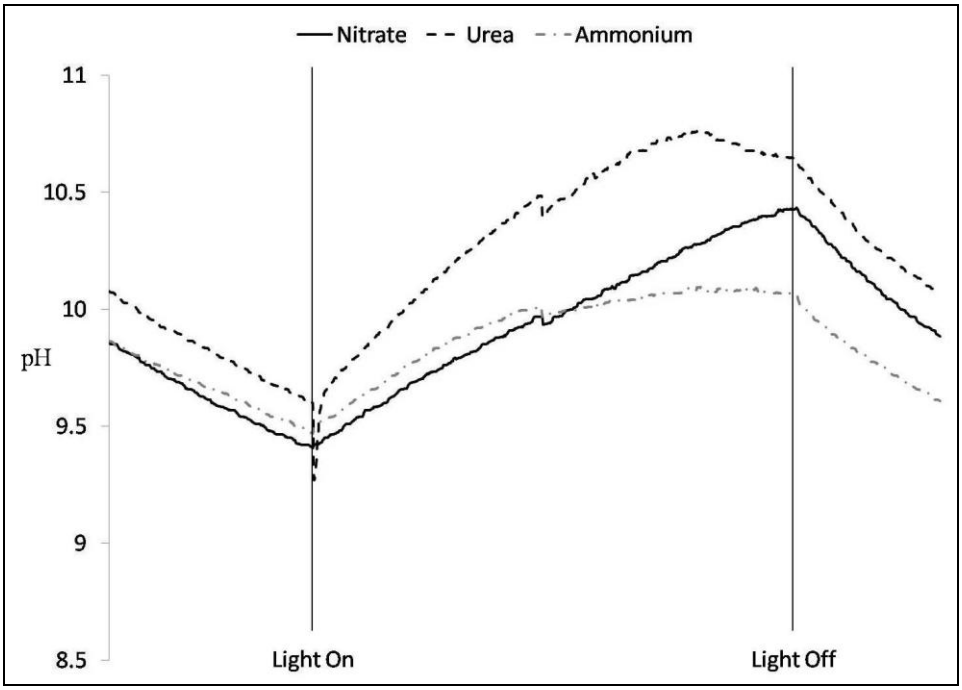

Figure 3-10. 24 hour diurnal variation of pH from day 8 to day 9.

In the outdoor pilot raceways, the N:P ratio was balanced to 16:1 for optimal algae growth with nitrate as the N source. Figure 3-12 (A) shows the P uptake and algae growth over time. The P was consumed down to low levels like what was observed in the laboratory raceway experiments. The initial TSS was 133 mg/l, which increased to a final TSS higher than 300 mg/l. Figure 3-12 (B) shows the nitrate uptake over time by the algae.

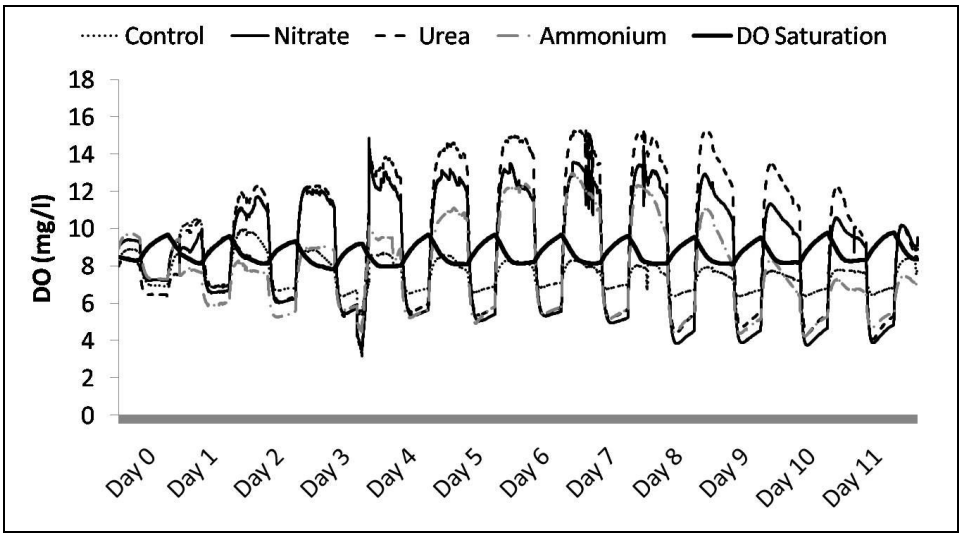

Figure 3-11. Diurnal variation of DO in reactors with control, nitrate, urea, and ammonium N sources.

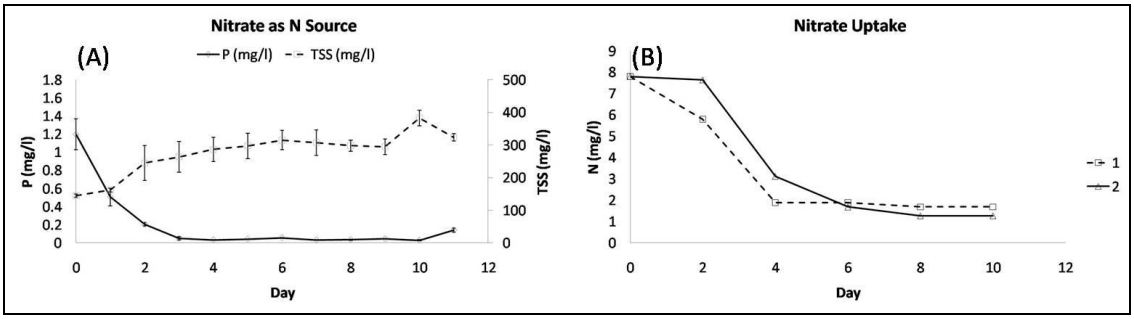

Figure 3-12.(A) P uptake with algae growth and nitrate uptake (B) over time in outdoor pilot raceway with nitrate as N source. 1 and 2 are replicates.

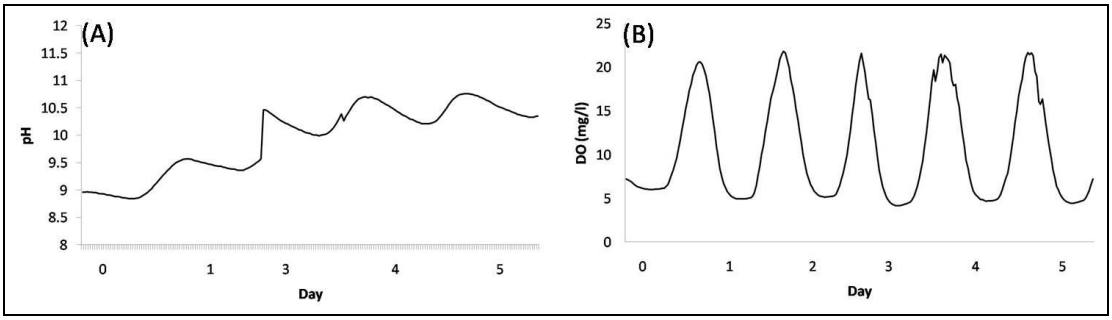

Figure 3-13. pH (A) and DO (B) in the outdoor pilot raceway with nitrate as N source.

As also observed in the small scale laboratory experiments, an overall pH increase was recorded over time in the outdoor raceways (see Figure 3-13A), but DO (Figure 3-13B), for this experiment with nitrate as the N source, stayed fairly uniform most likely because the DO was already at supersaturation levels at the inception of the experiment. The outdoor water temperature during this time period stayed fairly uniform fluctuating between 15°C at night and 30°C during the day, which contributed to the uniform diurnal shifts in DO during this time period.

Figure 3-14 shows the nutrient uptake (Figure 3-14 A and C) along with the increase in algae concentration (Figure 3-14 C) reported as TSS, for the duplicates run in the outdoor pilot raceway reactors with urea as the N source. The TSS in this experiment began at 19 mg/l and finished at 250 mg/l, bringing the P concentration in the water below 1 mg/l. The algae growth was slower in this experiment than in previous ones. Slower growth rates during this time period were most likely due to the initial algae concentration being lower coupled with the fact the outdoor temperature was beginning to drop during the Fall months of the year (see Figure 3-15 C). As mentioned previously, lower temperatures slow algal growth rates.

The pH and DO levels in the outdoor reactors followed the overall trend of increasing with algae growth and algae concentration (see Figure 3-15 A and B). In the outdoor raceway reactor experiments the initial P levels were lower than the indoor laboratory experiments because no additional P was added to the reactors. The P level available in the Logan lagoons effluent at the time of the experiment was used.

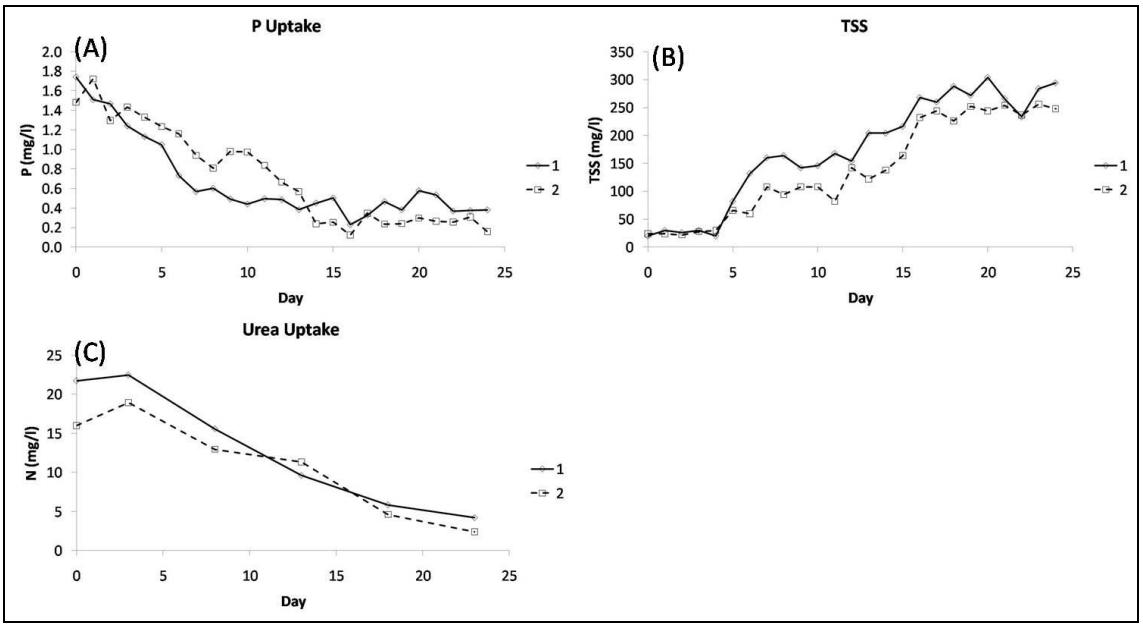

Figure 3-14. P Uptake (A), TSS (B), and Urea uptake (C) for outdoor pilot raceways with urea as N source. 1 and 2 are replicates.

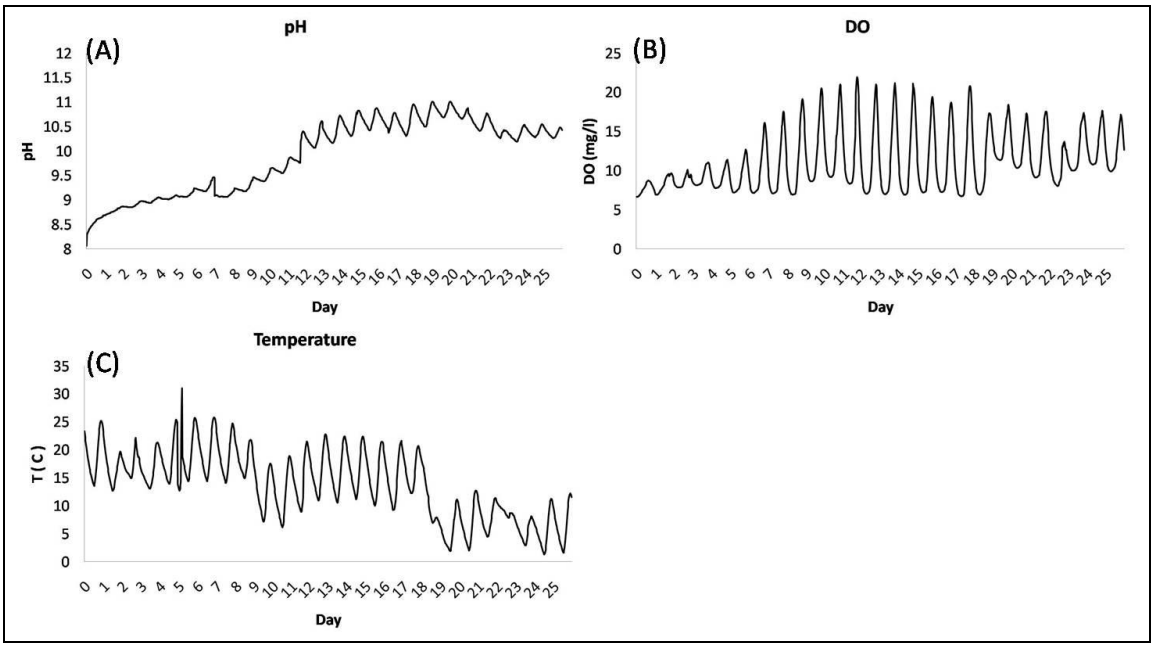

Figure 3-15. pH (A), DO (B), and Temperature (C) for outdoor pilot raceways with urea as N source.

The elemental composition of all harvested algae biomass samples taken at the end of the previous experiments was determined (see Table 3-1). The N:P of 4:1 in the control was significantly different than the other conditions. All other algae supplemented with some form of nitrogen had a much higher N:P ratio ranging from 12:1 to 14:1. The control algae seems to be more efficient at consuming P per mol of N, but not all the soluble P available was consumed by the algae.

Table 3-1. Elemental composition of Logan lagoon algae grown in raceway reactors.

N Source	C %	N %	P%	N:P
No N addition	33.8	4.0	2.1	4
$NaNO_3$	37.3	4.7	0.8	14
NH_4Cl	35.6	4.2	0.9	11
Urea	32.7	3.9	0.7	12
$NaNO_3$ Outdoors	30.0	3.7	0.7	12

Conclusion

The elemental composition of the harvested algae biomass from the raceways shows the N:P ratio (see Table 3-1) to be less than that of the average N:P ratio of 16:1 (Stumm and Morgan, 1981). This means that less N per mole of P is needed to maintain optimal algae growth. Balancing the N:P ratio closely would ensure that excess N is not leftover in the water after algae growth.

Nitrate and urea are suitable N sources for enhancing the algae growth for P uptake down to or below future potential regulatory levels. Ammonium did not perform as well as nitrate and urea as an N source most likely because of the high pH levels and

NH_3 volatilization. The outdoor raceway reactors did perform comparably to the laboratory raceways, but as would be expected temperature has a great effect on algae growth rates. However, P levels can be managed with algae if the algae are removed from the water during peak growth.

CHAPTER 4

THE EFFECT OF CO_2 ADDITION

Introduction

The Logan City Environmental Department in Logan, Utah operates a wastewater treatment facility that consists of 460 acres of open-air lagoons for biological wastewater treatment. In the near future, the regulatory limits on phosphorus released from the Logan wastewater treatment facility are likely to become significantly lower to counter potential downstream eutrophication (DEQ, 2009). One way to potentially lower phosphorus levels in the wastewater effluent is through management of algal growth in raceway ponds. Raceway ponds are a less expensive alternative to closed system reactors, where algae can be cultivated under natural sunlight (Borowitzka, 1999). If the algal biomass is removed from the system when growth yields are highest, the P contained in the cells could also be removed to obtain phosphorus-free water. The harvested algae can then be used to produce biofuels such as biodiesel.

Significant natural algal growth occurs in the Logan lagoons, which facilitates nitrogen (N) removal through volatilization as ammonia (NH_3) under high pH conditions created by algae growth. Algae grow more rapidly at higher temperatures (Goldman and Carpenter, 1974) and as a result of the higher temperature and pH increase due to algae growth there is significant NH_3 volatilization during the summer months. However, phosphorus (P) is non-volatile and stays in the system and likely cycles in and out of algal cells as they grow and die in the lagoons. $C_{106}H_{181}O_{45}N_{16}P$ is a stoichiometic

formula for the most common elements in an average algae cell (Stumm and Morgan, 1981). This formula shows that an N:P molar ratio of 16:1 is needed for optimal algae growth. Therefore, N supplementation will be required during the summer months to achieve sufficient N:P for optimal and sustained algae growth to uptake all of the P. Urea, and nitrate commonly used as fertilizers (Eaton et al., 2005; PotashCorp, 2007), were used as N supplements to enhance algae growth for optimal P uptake.

CO_2 is the exhaust compound produced when carbon (or a hydrocarbon) burns and combines with oxygen and is known to be a greenhouse gas. Like other plants, algae use CO_2 during photosynthesis. CO_2 can also be used to control the pH level in the water, because CO_2 reacts with water forming carbonic acid and lowering the pH (Drever, 1988). In this experiment the effects of CO_2 addition will be addressed. The total fatty acid methyl esters (FAME) from harvested biomass will also be presented.

Materials and Methods

Laboratory Raceway Experimental Setup

The lab scale raceway reactors were made of ¼" acrylic sheeting with the dimensions shown in Figure 3-1. The reactors have two channels and a length to width ratio of 2:1. Mixing was accomplished by a paddlewheel set-up. An electric motor (120 V AC/DC Variable Low Speed Motor, Wondermotor, Walnut, CA) was used to rotate paddle wheels at approximately 10 rpm.

Transparent acrylic was used to allow light penetration for photosynthesis to occur in a laboratory environment. Plant growth fluorescent bulbs (plant & aquarium F40, General Electric) were used to for the light source. The light spectrum of the plant growth bulbs is shown in Figure 3-1. The bulbs provided the red light necessary for

photosynthesis around 680 nm. Four plant growth fluorescent bulbs were mounted on the bottom and two on each 2 ft side for a total of eight bulbs around each reactor. The eight bulbs provide an average light intensity of 764 ± 104 umol m^{-2} s^{-1}in an empty reactor. Lights were on for 14 hours and off for 10 hours. Temperature was held at 21 ±3°C. The variation in temperature came from the lights warming the reactors followed by the reactors cooling after the lights were turned off.

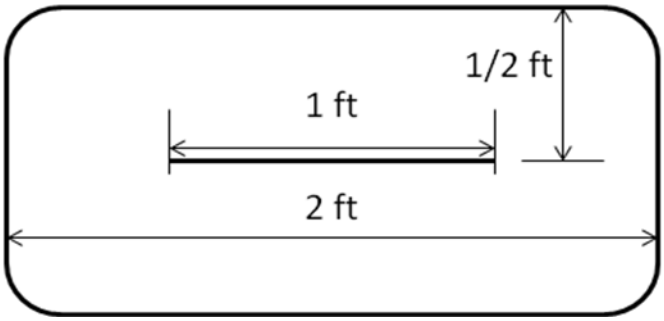

Figure 4-1. Shematic of laboratory raceway reactor dimensions.

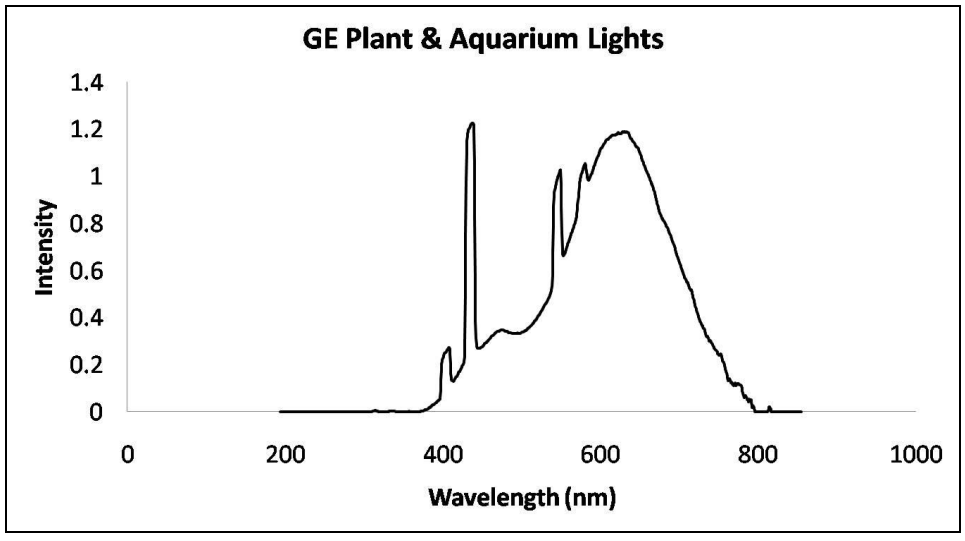

Figure 4-2. Light spectrum of GE plant growth fluorescent bulbs.

Eight reactors were used to test nitrate and urea with and without CO_2 addition in duplicate. Analytical grade sodium nitrate ($NaNO_3$), and urea (NH_2CONH_2) were added to reactors at concentrations needed to bring the N:P ratio to 16:1 for optimal algae growth (Stumm and Morgan, 1981). CO_2 addition was achieved through pumping pure CO_2 through spargers mounted to the bottom of the reactors. The amount of CO_2 added to the reactor depended on the pH level. A gas solenoid was placed in between the CO_2 source and reactor. The solenoid was controlled by a program written in Lab View based on pH level. PH 8 was chosen because the optimal pH level has been reported to be in the range 7.5-8.5 (Acién Fernández et al., 2001; Chisti, 2008; Marcel et al., 2003; Molina et al., 2001). In this experiment, if the pH level went above pH8, the solenoid would open allowing CO_2 to flow into the reactor until the pH level dropped below pH 8 again.

Sampling and Analysis

Samples were taken daily in 50 mL volumes from each reactor and analyzed for soluble N, NO_3, NH_3, Urea, and P. Dissolved oxygen (DO), pH, and temperature were monitored continuously during the experiments. Suspended solids (TSS) were measured daily to directly quantify algae. Evaporation was compensated by the addition of nutrient-free water.

Algae biomass was harvested at the end of the experiment using a Sharples T-1 continuous centrifuge and air dried in preparation for lipid analysis.

Analytical Methods

Orthophosphate PO_4^{3-} was measured by the standard method based on the ascorbic acid method (method 4500 -P-D) (Eaton et al., 2005) using Hach PhosVer™ reagent (Hach Company, Loveland, CO).

Ammonia (NH_3-N) was measured by the ammonia-salicylate method a modification of the standard phenate method (Eaton et al., 2005), nitrate (NO_3-N) was measured by the standard method based on cadmium reduction (Eaton et al., 2005), and total phosphorus (TP) was measured by the persulfate digestion and ascorbic acid method (Eaton et al., 2005; Eaton et al., 2005). Urea was measured by the Diacetyl monoxime method – a modification o f the Diacetyl monoxime method recommended by the Standard Operating Procedures for Clinical Chemistry(Kanagasabapathy and Kumari, 2000).

The soluble nitrogen and phosphorus measurements were made by first filtering the sample through a 1.0 µm glass fiber filter to remove all suspended solids (Eaton et al., 2005). The total soluble N and P concentrations in the filtered sample were then determined by the persulfate digestion methods (Eaton et al., 2005; Eaton et al., 2005; Eaton et al., 2005). Total suspended solids (TSS) was determined through the filter paper method (Eaton et al., 2005).

The laboratory reactors were monitored for pH continuously with pH meters (PHTX-92 Meter, OMEGA Engineering, Stamford, CT) and probes (OAKTON pH Electrode, Fisher Scientific) and DO was measured continuously with galvanic sensors (DO1200, SensoreX, Garden Grove, CA). Temperature was measured with T-type thermocouples (thermocouple, OMEGA Engineering, Stamford, CT).

The outdoor pilot-scale raceway ponds DO, pH, and temperature were measured by using an HQ40d Dual-Input Multi-parameter Meter Configurator with a PHC101 IntelliCAL Rugged Gel Filled pH Electrode and a LDO101 IntelliCAL Rugged Dissolved Oxygen Probe (Hach Company, Loveland, CO).

Percent FAME was determined through the use of a transesterification process to form methyl esters, which were quantified by gas chromatography using relevant methyl ester standards.

Results and Discussion

The addition of N to bring the N:P to 16:1 enhanced the algae growth enough to consume all of the soluble P. N sources nitrate and urea yielded comparable P uptake and biomass production results (see Figure 4-3 A and B). The addition of CO_2 seemed to increase P uptake and biomass production slightly. However, the overall biomass or TSS produced appeared to be lower in the reactors with CO_2 addition (see Figure 4-3).

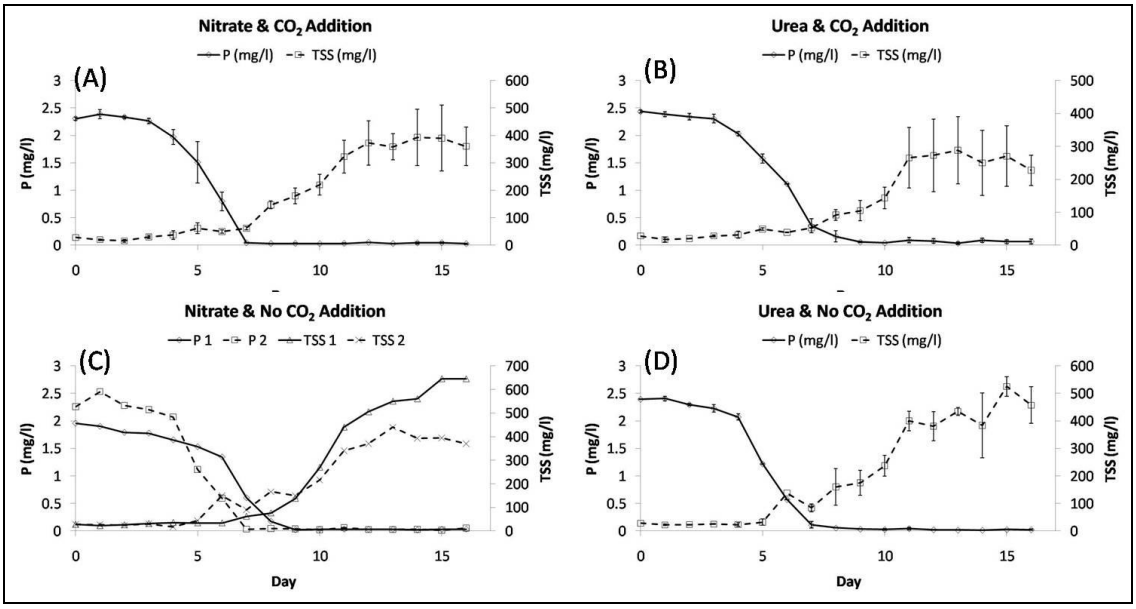

Figure 4-3. P uptake and algae growth for N source nitrate with CO_2 addition (A) and without (C), urea with (B) and without (D).

The pH in the reactors with CO_2 addition were held close to pH 8 (see Figure 4-4B. The pH in the reactors without CO_2 addition easily reached a pH higher than 10 (see

Figure 4-4 A). The DO in all the reactors was at supersaturation levels during the hours with the lights on (see Figure 4-4 C), but the DO in the reactors with nitrate and CO_2 addition had much higher DO levels than the others. The reactors supplemented with nitrate as the N source yielded a higher final algae concentration.

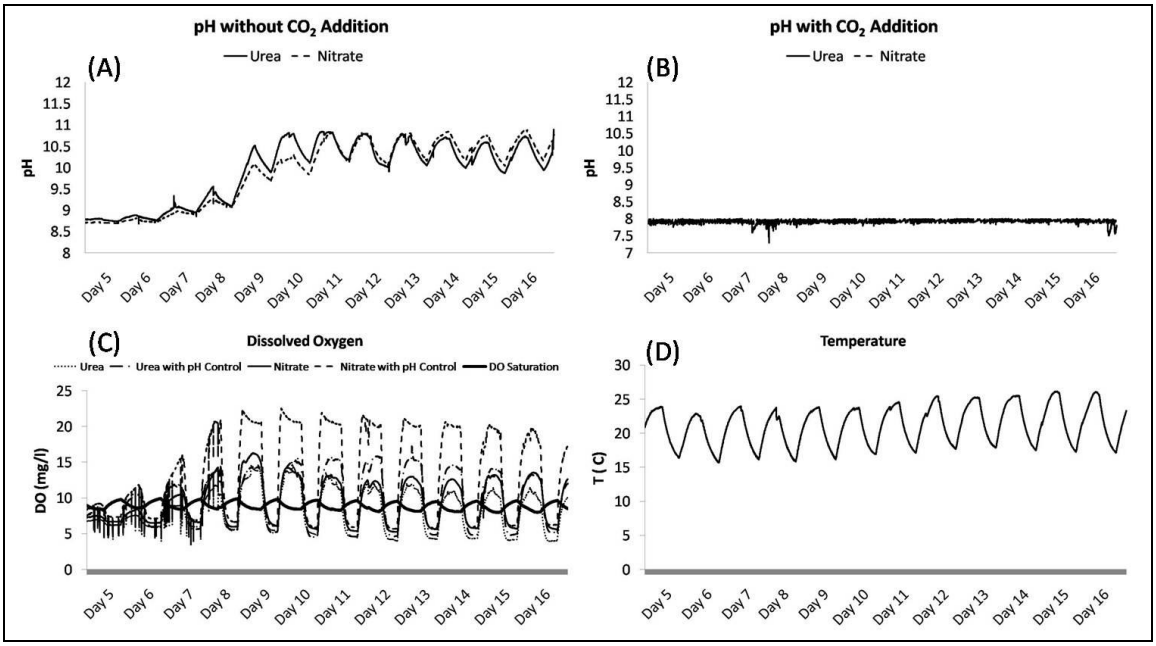

Figure 4-4. pH without CO_2 addition (A), with CO_2 addition (B), DO comparison of all (C), and temperature (D).

The greater benefit of CO_2 addition initially appears to be in the formation of lipids. The FAME percentage in the algae with CO_2 addition was approximately 3 to 4 times higher than in the algae without (see Table 4-1). This suggests that the algae may be carbon limited and CO_2 addition may be needed to increase lipid concentrations in the algae.

Table 4-1. % Fatty acid methyl esters

Treatment	Nitrate	Nitrate & CO_2	Urea	Urea & CO_2
% TAG	0.7	1.7	0.3	1.8
% FAME	7.3	23.4	5.6	18.8

Conclusion

The addition of CO_2 could be beneficial in increasing FAME concentrations in the Logan lagoon algae. The biodiesel could then be refined for use in city vehicles to help relieve financial burdens on the local community. If the algae biomass is digested via anaerobic digestion, the CO_2 produced from that process could potentially be pumped back into the water to be reused by new algae growing in the ponds, thus lowering the amount of CO_2 emissions into the atmosphere while increasing the FAME concentrations.

CHAPTER 5

SUMMARY

The Logan city wastewater treatment facility has a unique opportunity with their large open-pond treatment system to remove nitrogen (N) and phosphorus (P) and produce biofuels using algae grown in the wastewater. Removing the N and P from the water with algae provides a treatment method to meet potential state regulations and produce a product that will lower treatment costs.

The main objective of this research was to develop design parameters for an algae-based P and N removal system. The viability of the process was demonstrated in laboratory systems and key parameters associated with the process and performance under controlled laboratory conditions were determined. Pilot-scale performance was then compared with the laboratory results.

Laboratory results showed that algae native to the Logan treatment facility still grow at colder temperatures just at a slower growth rate and uptake P down to low concentrations. Optimizing the algal growth in the Logan lagoons through utilization of available nutrients has the potential to remove P from the water. If the system is N-limited, Nitrate and urea are suitable N sources for enhancing the algae growth for P uptake down to or below future potential regulatory levels.

Future work would include further testing to determine the performance of nutrient removal using algae on a large scale. The effects of water depth and mixing on algae growth need to be determined in order to treat multi-million gallons of water per day. Further experiments need to be done to enhance algal lipid production in the Logan

lagoons algae. Preliminary results in this study indicate that CO_2 addition may increase lipid concentrations.

Nutrient removal with algae is a promising treatment process that can be used to prevent downstream eutrophication and provide a renewable energy source for the city of Logan. This integrated wastewater treatment and biofuel production system can thus benefit the community as well as the environment.

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APPENDIX

CALCULATIONS FOR ESTIMATED BIODIESEL PRODUCTION

Given:

Parameter	Reference
Logan wastewater treatment facility flow rate is 15 MGD	(Logan City 2008)
Average total phosphorus concentration in effluent is 4.1 mg/l	(Logan City 2008)
Composition of an average alga is $C_{106}H_{263}O_{110}N_{16}P$	(Stumm and Morgan 1981)
Molecular weight of algae is 3553 g/mol	
Density of biodiesel is 0.864 kg/l	(Miao and Wu 2006)

Assumptions:

Parameter	Reason
Facility operation is 300 days/year	52 working weeks per year 6 working days per week 12 days off for holidays
Phosphorus available for algae growth is 2 mg/l	Half of the average total phosphorus of 4.1 mg/l for a conservative estimate

Calculations:

$$15{,}000{,}000\,\frac{gallons}{day} = 56{,}781{,}176\,\frac{liters}{day}$$

$$56{,}781{,}176\,\frac{liters}{day} \times 0.002\,\frac{grams}{liter}\,Phosphorus = 113{,}562\,\frac{grams}{day}\,Phosphorus$$

$$\frac{31\,\frac{grams}{mole}\,Phosphorus}{3{,}553\,\frac{grams}{mole}\,A\lg ae} = 0.008725\,\frac{gramPhosphorus}{gramA\lg ae}$$

$$\frac{113{,}562\,\frac{gram}{day}\,Phosphorus}{0.008725\,\frac{gramPhosphorus}{gramA\lg ae}} = 13{,}015{,}742\,\frac{gramA\lg ae}{day}$$

$$\left(13{,}015{,}742\,\frac{gramA\lg ae}{day} \times 10\%Lipids\right) \div 864\,\frac{gram}{liter}\,Biodiesel = 1{,}506.45\,\frac{litersBiodiesel}{day}$$

$$1{,}506.45\,\frac{litersBiodiesel}{day} \times 300\,\frac{days}{year} = 451{,}935.5\,\frac{litersBiodiesel}{year}\,or 119{,}388\,\frac{gallonsBiodiesel}{year}$$