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Assessing the Feasibility of Wastewater Effluent as a Nutrient Source for Algae in the Context of Biofuel Production

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Assessing the feasibility of wastewater effluent as a nutrient source for algae in the context of biofuel production

by

Justin Ryan Dore

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

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2016

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ABSTRACT

Algae have the potential to support the production of biodiesel due to their ability to produce large volumes of oils in relatively small footprints. However, algae production for biodiesel is hampered by the expenses associated with water and nutrients required for sufficient growth. A possible source of inexpensive and nutrient-rich water is the effluent of traditional wastewater treatment facilities. If wastewater effluent can support algae growth, the added benefit of reducing high nutrient loads to natural water systems can be realized. The overarching goal of the current research was to explore the feasibility of growing algae (Scenedesmus) using wastewater effluent from a local treatment facility. The algae growth in wastewater was comparable to the nutrient-rich Bristol's solution. Additionally, when a proposed limiting nutrient (bicarbonate) was raised, growth on wastewater effluent showed an increasing trend. However, this increase in growth rate was observed after the first week of growth. Similarly, when another typical limiting nutrient (iron) was added to the effluent, growth parameters were substantially increased compared to effluent alone. The fluorometry data also indicate that the effects of iron on algae growth are not realized until after the first week of growth. However, the normal turnover rate for wastewater in a sanitation plant is typically 1-3 days. The results of this experiment therefore suggest that carbonate and iron may not be beneficial supplements to algae grown using continuous-stream effluent from a wastewater facility. Yet, if the algae are grown in batch cultures for longer than one week, the addition of these limiting nutrients may result in elevated growth rates. Nevertheless, the data from the current research indicate that wastewater effluent may be an inexpensive and nutrient-rich medium for algae growth.

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1. INTRODUCTION

Peak oil is the point in time when the maximum rate of global petroleum extraction is reached, after which the rate of production enters terminal decline (Hubbert, 1956). This is a grim reality because oil is the center of modern life. Oil is the key factor for transportation, plastics and many forms of energy. Unfortunately, global oil production has dropped around the world indicating that our production is already declining. US domestic oil production peaked in 1970 and global production of conventional crude oil plateaued in 2005 at 74 million barrels per day (Berdellé, 2012). In the near future, there is likely to be a significant shortfall in energy supply, resulting in high energy prices and a reversal of many of the aspects of globalization that are currently taken for granted (Hanlon and McCartney, 2008).

If we continue at our global oil consumption rate and do not take advantage of alternative fuel, oil prices will continually rise, which will further burden the global economy. It is obvious that time is an issue and we need to find solutions to peak oil so we can continue our modern way of life. Finding and using alternative fuels will reduce the strain on the consumption of oil and also major environmental hazards that are associated with oil production would likely decrease with decreased global consumption. As old oil fields are depleted, oil companies are forced to drill in more remote and harsh areas, which will increase environmental risk. There are many options to choose from for an alternate fuel source; hydrogen, electricity, methanol, ethanol and biodiesel are all viable options.

It will probably take a combination of these alternate fuel sources to supply future energy demands. However, of these fuel sources the one that already has infrastructure set is biodiesel. Which means it will be the easiest and fastest to implement, because of the infrastructure in place. Additional benefits are that it can be produced from a variety of ways, like our waste products, and it is biodegradable. Biodiesel has the potential to, at minimum, reduce the use of fossil fuels, which would prolong our current way of life.

1.2 Biodiesel

Biodiesel is a renewable fuel manufactured from methanol and vegetable oil, animal fats, and recycled cooking fats (U.S. Department of Energy, 2006). The technical definition of biodiesel is: "The mono alkyl esters of long fatty acids derived from renewable lipid feedstock, such as vegetable oils or animal fats, for use in compression ignition (diesel) engines" (National Biodiesel Board, 1996).

Biodiesel can be produced from used grease or oil. Fats and oils are composed of triglycerides, three fatty acid molecules attached to a glycerol molecule. Some of the triglycerides separate from the glycerol molecule in heavily used oils and grease, which forms free fatty acids (FFA). FFAs react with the alkali catalyst used in the biodiesel process to form soap instead of biodiesel. High levels of FFAs require more soap to be removed, which results in less biodiesel. This happens in every batch to some degree, but if there is a high level of FFAs then the speed of the transesterification will be reduced by inhibiting the separation of the ester from the glycerin. Transesterification is the main reaction for converting oil to biodiesel. The transesterification process reacts an alcohol with the triglyceride oils contained in vegetable oils, animal fats, or recycled greases, forming fatty acid alkyl esters (biodiesel) and glycerin. The reaction requires heat and a strong base catalyst. Nevertheless, this is a very viable option to supplement petroleum diesel with biodiesel. The popularity of this process is growing. There are small programs up and running in which schools and universities use waste grease from their cafeterias and convert that into biodiesel for university vehicles. Right here at EIU we had a program that utilized oil from Thomas hall to power a university vehicle and at University of Rochester a group of students fueled a campus bus. Also, thousands of individual consumers have private systems in which they obtain used grease from local restaurants and use the resultant biodiesel for personal use. While these movements are promising and shows public awareness, realistically it is all a drop in the bucket when compared to the big picture. An option to propel this to the mainstream is by setting up an infrastructure using algae as a feedstock for biodiesel, in combination with waste bio-grease and bio-oil. Biodiesel production is currently only a small fraction of the estimated 50 billion gallons of distillate fuel and 128 billion gallons of all other transportation fuels that the U.S. consumes annually (EIA, 2005). Specifically, in 2014 biofuel accounted for 5% of our transportation energy (U.S. Energy Administration, 2015).

1.3 Impact of Biofuel and Renewable Energy

Biofuel is a term that covers a wide range of fuels. It is a source of fuel that is in some way derived from biomass, which includes solid biomass, liquid fuels and biogases. High gas prices, importing a majority of our oil from overseas, and low biofuel costs, are major reasons why biofuels are gaining public attention. Two common types of biofuels are bioethanol and biodiesel. Bioethanol is an alcohol made by fermenting sugar and starch crops using their carbohydrate components as a feedstock. Biodiesel is made from animal fats, vegetable oils, or recycled greases. Both can be used as a fuel for vehicles in their pure form and both are usually used as an additive to reduce emissions and consumption of oil reserves.

The energy distribution by sector in the U.S. is as follows: residential (22%), transportation (28%), industrial (32%), and commercial (18%) (EIA, 2005). Among these sectors, transportation is a likely target for biofuel use, where it is possible to lower the petroleum energy used in the transportation sector by using different types of biofuels. Biofuels provided only 1.8% of the world's transport fuel in 2008. However, investment into biofuels production capacity exceeded $4 billion worldwide in 2007 and is growing (Bringezu, 2009). Hopefully, this number will only increase and there will be more implementation into everyday life. Also, the total amount of renewable energy produced is only 6% of total energy production in the U.S. (EIA, 2005), compared to petroleum at 40% of total energy produced. The largest portion of renewable energy is hydroelectric power. There needs to be an increased concentration on renewable energy as a whole if we expect to keep up our way of life for continuing generations.

1.4 Current Biofuel Production

Biofuel from terrestrial oilseed crops like canola and soy is already in production, but it is in its infancy and there is always room for improvements. Some problems with the current production situation are agricultural runoff, rising food prices, low yield and the concern that the use of food crops for biodiesel may be uneconomic as a long term solution (Pimentel and Patzek, 2005). The use of our arable land for the production of terrestrial crops for biofuel has raised prices for food. Also, if we tried to satisfy current energy demand with just biofuel from food sources, we do not have enough agriculture land to produce enough biofuel from terrestrial crops. The arable land available for growing terrestrial crops for biofuel can only satisfy a small fraction of the demand for transportation fuel (Tyson et al., 2004). Algae require far less space and can be grown on nonagricultural land. If algae-derived biodiesel were to replace the annual global production of 1.1 billion tons of conventional diesel, then a land mass of 57.3 million hectares would be required, which would be highly favorable compared to other biofuels (NREL, 1998).

There are discrepancies whether net bioenergy from terrestrial crops is a gain or loss. The production of biodiesel from soy and ethanol from corn represents a net energy loss due to the energy required for crop production (Pimentel and Patzek, 2005, Talens et al., 2007). Other studies show there is a small gain in net energy for biofuels, the largest being 40% for corn ethanol biodiesel (Adler et al., 2007). It is difficult to obtain a concrete answer due to variables that change from case to case, like transportation costs for feedstocks, the value of byproducts, weather, location, and fertilizer source. Further research is needed to obtain concrete data.

Further complicating energy production from terrestrial crops is the harvesting limitations. For example, there is only a harvest once or twice a year for most crops, and the crops grow slowly, taking months to mature. By comparison, microalgae have much faster growth rates than terrestrial crops. The yield of oil from algae is estimated to be between 1,000 to 6,500 U.S. gallons per acre per year (4,700 to 18,000 $m^3/km^2 \cdot a$). This is 7 to 30 times greater than the next best crop, Chinese Tallow (700 U.S. gal/acre) (Shirvani et al., 2011). By comparison, corn averages 18 gal/acre and soybeans 48 gal/acre (Riesing, 2006). Research done by the National Renewable Energy Laboratory (NREL) states that algae are capable of producing up to 40 times the oil yield per unit area of land than terrestrial crops (Sheehan et al., 1998).

1.5 Algae

Algae comprise one of the most diverse and oldest groups. In evolutionary terms, algae have existed for 3.8 billion years (Bold and Wynne, 1985). A species range of 40,000 to 10 million has been estimated, with the majority belonging to the microalgae (Hawksworth and Mound, 1991). Algae are both eukaryotic and prokaryotic and they can range in size from giant kelps to prokaryotic cyanobacteria and unicellular microalgae. Algae are one of the earth's most important natural resources as well. They are the major O_2 producers and their role in the global food chain is essential. They contribute to approximately 50% of global photosynthetic activity (Wiessner et al., 1995) and form the basis of the food chain for over 70% of the world's biomass (Andersen, 1996). Algae occupy different habitat extremes extending from the tropical coral reefs to the polar regions. The ability to withstand environmental stress is matched by the capacity of algae to produce a vast array of secondary metabolites, which are of considerable value in biotechnology programs including the aquaculture, health, and food industries (Andersen, 1996).

Algae are great bioindicators, where they help humans assess the health of different environments and ecosystems. Algae microbial ecology is used to assist the study of industrial, urban, and agricultural environmental pollution. Assessments of microbial diversity are particularly important in environmental impact assessments, and the microalgae are sensitive indicators of ecological changes (Kelley et al., 1998). Algal populations are also sensitive to changes in nutrient status as is the case with phosphate enrichment (Gibson et al., 1996). It is obvious that algae make a significant contribution to the Earth’s environmental stability.

The most extensive research into the development of biofuels from algae was performed by the National Renewable Energy Laboratory (NREL) from 1978 to 1996 (Sheehan et al. 1998). The NREL concluded that a more practical approach for production of algae biodiesel is to utilize wastewater treatment for algae propagation (Sheehan et al. 1998), an already well developed technology (Oswald, 2003). Benemann (2007) concluded that algae to biodiesel will have a large impact with the combination of wastewater treatment. In the past, the conclusions were that large-scale algae cultivation for biofuel production was uneconomical. However, due to higher oil prices and improved biofuel technology, in combination with the use of waste-water effluent as a biofuel feedstock, the economics have improved and the process could be profitable as well as environmentally beneficial (Sheehan et al., 1998).

1.6 Lipid Chemistry and Production

Lipid type and quality is essential to biodiesel production. Lipids are fatty acids and their derivatives, as well as substances related biosynthetically or functionally to these compounds (Christie, 2003). Fatty acids generally contain even numbers of carbon atoms in straight chains normally in the range of C14 to C24, with a carboxyl group at one end. Very poor quality lipids with respect to bioenergy (high degree of unsaturation, high free fatty acid content, etc.) may require different processing (*i.e.* thermal depolymerization) to convert these lipids into biodiesel. Pure cultures of green algae contain primarily C18 and C16 fatty acids with a high degree of unsaturation (Thompson, 1996). This research will be conducted with Scenedesmus species because of the high yield and excellent quality of the lipids (Riesing, 2006).

1.7 Wastewater Treatment

Sanitary waste water treatment systems helps to keep our local communities and environments clean. Unfortunately, high energy and expensive processes are required to remove pollutants, turbidity, and nutrients from our waste water. There are several conventional ways to remove nutrients, mainly nitrogen and phosphorus, from waste water. The most common way of removing nitrogen is though denitrification leading to reduction of nitrate to nitrogen gas, which is released to the atmosphere (*e.g.* Metcalf, 2003). Heterotrophic bacteria use the nitrate as a replacement oxygen source under anoxic conditions to break down organic substances.

There are a variety of ways to remove phosphorus from wastewater. One of the most common ways to remove phosphorus is through chemical precipitation using $FeCl_3$. Both phosphorus and nitrogen can be removed by assimilation. Assimilation is the reduction of a nutrient in wastewater by incorporating the nutrient as an essential element in biomass. This is done through growth of photosynthetic organisms (plants, algae, some bacteria and cyanobacteria) and is achieved through treatment ponds containing these organisms.

The breakdown of organic matter occurs through microbial decomposition with the resulting elimination of organic carbon (OC) (Hynes, 1974). The technique of promoting algae growth for nutrient removal was first developed by Oswald et al. (1957). In general, moderate organic pollution derived from crudely treated wastewater causes benthic invertebrate density and biomass to increase, species diversity to decrease, and community composition to become dominated by Chironomidae and Oligochaeta (Wiederholm 1984, Seager and Abrahams 1990, Wright et al. 1995). In addition, organic pollution has negative effects on the biomass and diversity of algae and macrophytes (Hynes 1974, van Dam et al. 1994). In order to quantify organic pollution in running water, the “saprobic system" was proposed. The saprobic system is based on indicator species, which were mainly benthic invertebrates, but also included microbes and plants. The saprobic system provided a system to grade and analyze the pollution level of streams with varying levels of OC. Knowing certain species tolerance level of pollutants allows us to monitor and regulate water systems.

Our current waste water treatment system is efficient at removing OC from wastewater, but the technology and efficiency of removing inorganic nutrients is limited. This is especially true for developing countries and urban areas. Water-quality issues in densely populated areas have shifted from problems associated with organic pollution to problems with inorganic nutrients (Smith et al. 1987). This research proposes an option that would further remove nutrients from effluent, as well as cut costs through the sale of biodiesel.

Additionally, regulation of farm water pollution is becoming more intense in recent years (California Water Board, 2003). Factory farms with over 1,000 head of cattle can be major sources of water and air pollution (Centner, 2001). Farms that raise animals for slaughter or dairy produce a large amount of waste which becomes run-off and contaminates ground water. Utilizing an algal wastewater treatment system will cut down on pollution in this situation. The waste produced from the animals provides an economical feedstock for those algae in the same way sanitary effluent would.

1.8 Removal of Nutrients

Nutrients from wastewater effluent, industry waste, factory farms, and agriculture fields using fertilizers and pesticides are adding nutrients to our water systems. Which causes eutrophication, toxic algal blooms and dangerously low dissolved oxygen (DO) in the water systems. The problem only gets worse farther downstream a river. Nutrient cycling in headwater streams is particularly important in controlling nitrogen transport to downstream aquatic systems (Alexander et al. 2000). It has come to the unfortunate point that there are dead zones in our waters, where excess nutrients fuel algal blooms. These algal blooms deplete oxygen in the water that make the environment uninhabitable for fish and stationary bottom dwellers.

Agricultural run off and waste water treatments are key contributors to eutrophication and eventual dead zones. Nitrogen that runs off croplands into the Mississippi River and its tributaries has been implicated as a major cause of a "dead zone" in the Gulf of Mexico (Rabalais and Tuner, 1996). Increased nitrogen runoff can cause environmental problems in receiving waters, for example, the zone of decreased DO that develops seasonally in the Gulf of Mexico (Rabalais et al. 1998) and toxic algal blooms in lakes and coastal zones (Burkholder and Glasgow 1997). The amount of nitrogen transported by the world's rivers into the oceans has roughly doubled, and rates of nitrogen transport from developed areas have increased 10- to 50-fold as a result of human activities since the Industrial Revolution (Meybeck 1982). Humans have effectively doubled the global rate of nitrogen fixation (Vitousek et al. 1997). All this nitrogen is changing ecosystems around the world.

Modern agriculture has increased the use of fertilizers and pesticides worldwide, which contributed to raising nutrient concentrations in water systems. Between 1950 and 1998, worldwide use of fertilizers increased more than 10-fold overall and more than 4-fold per person (FAO, 1998). Unfortunately, agricultural plants do not use all the nutrients that they are given, so most of it is wasted. Tilman (1998) estimated that crops actually absorb only one-third to one-half of the nitrogen applied to farmland as fertilizer. Animal agriculture is by far more resource intensive than other forms of agriculture. It involves feeding grain to livestock and it generates additional pollution from the high concentration of animal waste. An algal wastewater system can remove additional inorganic nutrients from run off so less nutrients enter our waterways and therefore decrease eutrophication and dead zone impacts.

1.9 Feasibility

There are several significant advantages and limitations to using biodiesel as a replacement for petroleum-based diesel (U.S. Department of Energy, 2006). The most important advantage is that it is a renewable domestic fuel source. This lowers demand for international fuel sources and creates domestic jobs. Another advantage is that biodiesel can be used in any diesel engine with little to no modification (National Biodiesel Board, 1996). This is a great benefit, because there is already a large infrastructure of diesel vehicles in America and even a larger number in Europe. An already established infrastructure makes change easier, rather than having to build new fueling stations or distribution centers. Biodiesel also emits less emissions than conventional diesel fuel. Compared with petroleum diesel fuel, biodiesel's tail pipe emissions of total hydrocarbons, particulates and carbon monoxide are reduced 55%, 53%, and 48%, respectively (Haas et al., 2001). Also, biodiesel is also more readily biodegradable than petroleum diesel (Zhang et al., 1998). A biodiesel spill would biodegrade into the Earth unlike petroleum-based fuels, which makes an area toxic, infects organisms, and creates an emergency clean-up operation.

There are further reasons to implement a biodiesel system. Algae biomass can be used as an environmentally safe fertilizer. It is a great fertilizer because it degrades slowly, thus it releases nutrients slowly, which can improve uptake by plants (Mulbry et al., 2005). The most popular microalgae that are grown for humans, animals, and cosmetics are *Spirulina* followed by *Chlorella* and *Dunaliella salina* (Spolaore et al. 2006). All three of those have high protein contents, as well as antioxidants, which make it a very healthy and popular supplement.

Despite the promise, two major problems that hold algae back as a major bioenergy resource are (1) the lack of an efficient and cost-effective method of harvesting and removing the water from the algae for it to be processed, and (2) finding an economical feedstock. It is still difficult and expensive to harvest algae. Different types of algae are harvested in different ways. One way of harvesting macroalgae is to simply install a net in the pond and raise it to bring the algae out of the water. The algae are then harvested using mechanical cutters similar to lawnmowers. However, this is time-consuming and will not retrieve all of the biomass. Other methods are settling, flotation, centrifugation and filtration. The larger the algae, the easier it is to harvest, but larger algae grow slower than microalgae, which are more difficult to harvest due to its smaller size. It is ideal to find a balance between speed of growth and size of algae to make it profitable and easier to harvest. The single largest energy cost for production of biofuels is the feedstock (Pimentel and Patzek, 2005). However, allowing waste water effluent to be used as a feedstock allows for a free renewable nutrient source for algae.

2. OBJECTIVES

1. Operate bench-scale algal growth experiments to determine the viability of wastewater as a nutrient source.
2. Explore whether algae is efficient at removing nutrients from sanitary effluent.
3. Determine the limiting nutrient in the effluent from the Charleston Waste Water Treatment Plant.
4. Raise the concentration of the limiting nutrient and observe whether it raises overall yield.

3. MATERIALS AND METHODS

3.1 Site Location

This research started in August of 2011 and was carried out until the middle of April 2012. Samples were taken from the sanitary district of Charleston, Illinois. Charleston is located 168 miles south of Chicago, IL, 81 miles southeast from Springfield, IL, with a latitude of 34.496 N and longitude of 88.176 W. The Charleston sanitary district has conventional methods of handling waste water, including a tertiary treatment facility that does not disinfect prior to discharge.

3.2 Sampling and Water Quality

Effluent was collected in plastic carboys from the effluent holding system of the Charleston Waste Water Treatment Facility (Figure 3.1). This is the final step before the wastewater is discharged into Cassell Creek. The water was transported to the Thut Greenhouse at Eastern Illinois University. The effluent was then analyzed to determine initial water quality.

Figure 3.1. Serpentine at the Charleston Waste Water Treatment Facility where water is collected prior to discharge into Cassell Creek.

3.3 Algal Growth and Identification

The stock Scenedesmus culture was purchased from UTEX algae culture collection (http://web.biosci.utexas.edu/utex/). Experiments were carried out in the Thut greenhouse under natural and artificial growing lights. Artificial lights were set at a 14 hour photoperiod from 6 am to 8 pm. Trials were conducted in batch cultures. A batch culture is a fixed volume system where the algae are grown under the same environmental conditions. Subsamples were taken periodically to identify the specific algae species (Figure 3.2) using a Nikon Labophot-2 microscope with an OptixCam Summit Series camera. Photos were taken and compared with catalogs of known genera.

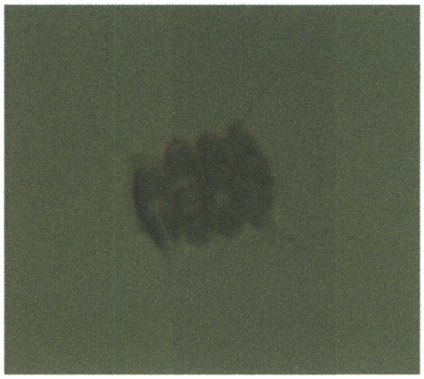

Figure 3.2. Microscopic image of Scenedesmus from batch cultures.

3.4 Experiment 1: Wastewater Effluent as a Nutrient Medium

This experiment was conducted from August 18 to September 22, 2011. The algal yield and nutrient usage was compared between four different nutrient solutions:

- 100% effluent
- 50% water/50% effluent
- Bristol's standard nutrient solution
- 50% Bristol's/50% water

After effluent was collected and initial water quality tests were conducted, 200 mL of one of the four solutions above was added to a 250 mL Erlenmeyer flask. This was followed by the addition of 25 mL of Scenedesmus suspension, which was prepared with Bristol's growth medium (Bold, 1949) in a 1 L Erlenmeyer flask with a retention time of at least two weeks. The solutions were placed in a Thermolyne Big Bill shaker (Figure 3.3) set at 200 rpm to keep the samples agitated throughout the trial. After a six day retention time, total phosphorous and ammonia water quality tests were conducted according to the 1985 Water Quality Stand Methods Handbook. Additionally, 30 mL of solution was used to determine the solids data. For dry weight analyses, samples were heated at 104°C in 30 mL crucibles overnight. To determine volatile solids, crucibles were heated in a furnace at 543°C for 20 minutes.

Figure 3.3. Shaker used to agitate the algal cultures.

3.5 Experiment 2: Alkalinity as a Limiting Factor for Algal Growth

This experiment occurred from November 21, 2011, to March 28, 2012. This batch culture experiment was conducted using 18 L carboys with the tops removed to maximize light intensity (Figure 3.4). Algae growth and nutrient removal was examined, as described with the first experiment, between four different alkalinity (sodium bicarbonate) concentrations:

- 100% effluent (130-220 mg/L)
- 100% effluent (adjusted to 300 mg/L)
- 100% effluent (adjusted to 400 mg/L)
- 100% effluent (adjusted to 500 mg/L)

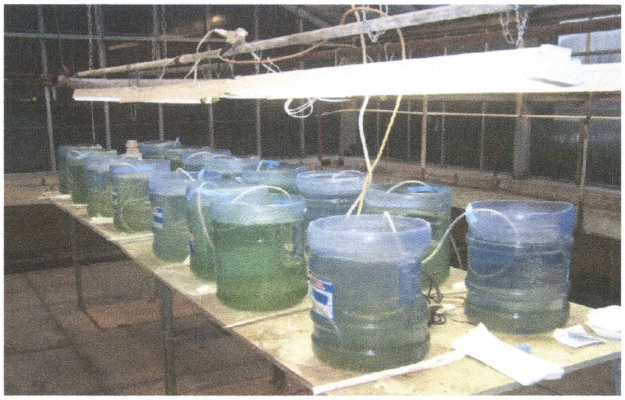

Figure 3.4. Carboys (18 L) used for algae growth.

Sodium bicarbonate was added directly to 16 L of effluent in each carboy to reach the desired concentration of alkalinity. One liter of algae inoculum was added to bring the total volume to of each carboy to 17 L. The algal inoculum was grown separately in Bristol's medium in an 18 L carboy. For all carboys, air pumps were used to keep the sample agitated and to supplement the CO_2 concentration over the three week growth period. Fluoroscence and pH values were measured from 20 mL subsamples each day to assess growth patterns using a Turner TD-700 fluorometer (Figure 3.5) and an Oakton pH 110 Series pH meter (Figure 3.5).

Figure 3.5. Fluorometer and pH meter used for assessing algal cultures.

Upon the completion of the experiments, water quality tests were performed to examine PO_4, TN, and NH_4 concentration using the Hach DR/890 Data Logging Colorimeter (Figure 3.6) using methods according to its handbook. Alkalinity and dry weight were measured according to 1985 Water Quality Stand Methods Handbook.

Figure 3.6. Portable colorimeter used to assess nutrient concentrations.

3.6 Experiment 3: Iron as a Limiting Factor for Algal Growth

This experiment was conducted from March 7 to 28, 2012. This batch culture experiment was conducted according to the methods discussed in section 3.5. Algae growth and nutrient removal was examined between four different solutions:

- 100% effluent
- 100% effluent with sodium bicarbonate (500 mg/L)
- 100% effluent with iron (III) chloride (0.625 μM)
- 100% effluent with 500 mg/L sodium bicarbonate and 0.625 μM of iron (III) chloride

4. RESULTS AND DISCUSSION

4.1 Experiment 1: Wastewater Effluent as a Nutrient Medium

This experiment focused on testing the viability of the effluent from the Charleston Waste Water Treatment Facility as a nutrient source to grow algae. The four solutions examined as nutrient sources were: 100% effluent, 1:1 effluent and water, 1:1 effluent and Bristol's medium, and 100% Bristol's medium. Total dissolved solids (TDS), volatile dissolved solids (VDS), total solids (TS), and total fixed solids (TFS) were determined at the conclusion of the experiment and compared with the initial values of 100% effluent. The results show that Bristol's medium had the highest TDS, TS, and TFS out of the four solutions (Figure 4.1), with 1:1 effluent and Bristol's medium having comparable TDS and TS values. The effluent alone led to values that were comparable to media containing Bristol's nutrients. The values for 1:1 effluent and water were negative because this solution was compared to the undiluted initial effluent.

Ammonia and total phosphorous concentrations were also measured to assess the effect of nutrient removal by the algae on the solutions tested. The media without Bristol's nutrients had the largest percentage changes to ammonia levels (between 50%-80%; Figure 4.2). This was expected because the algae without Bristol's nutrients would be forced to utilize the nitrogen from the effluent only. In contrast, the phosphorous removal rate was highest with Bristol's medium, although all the percentage removed was between 20-40% for all media.

The first objective of this project was to test the hypothesis that effluent is a viable nutrient source and would remove additional nutrients from wastewater. After the first experiment was conducted, it was clear that effluent could be used as a nutrient source to grow algae, which would be vital in the role of producing algae for the production of biofuel. In particular, the dry weight data were promising, because the effluent data were comparable to the Bristol's solutions. Bristol's growth standard created the highest dry weight values, which was expected because it is manufactured for the specific reason to grow algae efficiently (Bold, 1949). Effluent had the third highest value out of the four tested in each dry weight category. These data support that effluent is a viable nutrient source for the growth of algae. While it was not the best of the four solutions tested, it gave positive data that are comparable with Bristol's. Sanitary effluent would not be the best nutrient source available to grow algae, but what it lacks in optimum growth potential it makes up for in being freely available.

The second objective was to determine if algae could remove nutrients in the wastewater. Additional nutrients were indeed removed from the wastewater that otherwise would have been discharged in local water bodies. For example, algae reduced the total ammonia concentration from an average of 1.35 mg/L to 0.30 mg/L (77% removal efficiency), and phosphate levels went from 1.8 mg/L to 1.2 mg/L (30% removal efficiency) as shown in Figure 4.2. Overall, it was clear that algae have the potential to reduce the nutrient concentration in wastewater and, therefore, surrounding water systems. This could help reduce human impact on the environment.

4.2 Experiment 2: Alkalinity as a Limiting Factor for Algal Growth

We predicted that carbon to be the limiting nutrient due to the wastewater passing through an anaerobic digester as part of the sanitation process. Past experiments have helped strengthen this hypothesis. Studies by Allen (1955) on algal growth with sewage effluents demonstrated a marked increase in growth when five percent CO_2 was bubbled through the cultures. Both Witt and Borchardt (1960) and Gates and Borchardt (1964) in their studies on nitrogen and phosphorus removal from wastewater treatment plant effluents by the controlled culture of algae, realized the need for adding supplementary carbon dioxide. King (1970) also showed the necessity of carbon supplementation in wastewater oxidation ponds.

The second experiment focused on raising alkalinity to theoretically increase algae growth (Allan, 1955). The four solutions examined were effluent alone, or effluent supplemented with sodium bicarbonate to reach 300 mg/L, 400 mg/L or 500 mg/L. TDS, TFS, VDS and TS were determined at the conclusion of each trial to compare with the initial effluent (Figure 4.3). Results show a clear rise in all parameters from initial effluent through to the final alkalinity concentration of 500 mg/L, except with TFS where the medium with the highest alkalinity had an average value between pure effluent and that with 300 mg/L alkalinity.

In all cases, it was determined that the nutrient with the highest concentration was nitrogen, then phosphorous, followed by ammonia. Although ammonia had the lowest initial value out of the three nutrients tested, it had the highest removal percentage (Figure 4.4). Typically, the final concentration of ammonia was approximately 0.08 mg/L. Nitrogen and phosphorus showed similar percentage changes between media types, with approximately 40% of the nitrogen and 15-20% of the phosphorous removed during this experiment. Fluorometry was also used to measure growth for the four solutions tested (Figure 4.5). The fluorometry data indicate that algal growth was proportional to alkalinity, which is in agreement with the total solids data (Figure 4.3). As expected, the pH of the media as proportional to the amount of alkalinity (Figures 4.6 and

4.7). Algae can only grow until the limiting nutrient within the water system is depleted. The effect of alkalinity on temperature is also shown (Figure 4.8).

With retention times varying from hours to weeks with different sizes and types of wastewater treatment systems, the data from this experiment are important when thinking about implementing an algae retention step in the wastewater sanitation process. Realistically, an algae sanitation treatment system would not last nearly as long as 20 days (when alkalinity increases are noticed), but merely a few days, if that. The quick growth from the pure effluent samples is important to consider. This could show that alkalinity might not be the limiting nutrient or pure effluent with nothing added to it will produce a sufficient yield of biomass.

4.3 Experiment 3: Iron as a Limiting Factor for Algal Growth

The third experiment focused on raising an additional limiting nutrient (Fe) common to algal growth experiments. Four different media were examined in this experiment: pure effluent, effluent with 500 mg/L of sodium bicarbonate, effluent with 0.625 μM of iron (III) chloride, and effluent with both 500 mg/L of sodium bicarbonate and 0.625 μM of iron (III) chloride. TDS, TFS, VDS and TS were determined at the conclusion of each trial to compare with the initial effluent (Figure 4.9). The data clearly show that the addition of iron (III) chloride consistently increased the dry weight parameters, with the media containing only iron as a supplement having the highest values. This goes against the hypothesis that a combination of the iron and alkalinity inoculation would have an additive effect.

The nutrient with the highest concentration across the media was nitrate, followed by phosphorous, and then ammonia (Figure 4.10). With respect to removal, the combination of high alkalinity with iron led to the highest removal of ammonia (Figure 3.8). However, this same medium led to the lowest removal percentage of phosphate. Overall, the level of nitrate removal was similar across all media.

As with the second experiment, daily fluorometry and pH readings were conducted to measure algae growth patterns for the four solutions tested. The fluorometry data indicate that all three supplemented effluent media had increased algal growth compared to pure effluent (Figure 4.11). However, unlike the first experiment, the fluorometry data and solids data (Figure 4.9) showed some discrepancies. For example, the media with only iron had the highest solids data yet had the lowest fluorometry data of the three supplemented media. All media had relatively consistent pH values throughout the experiment, where the supplements added to the wastewater appeared to have negligible effects (Figure 4.12).

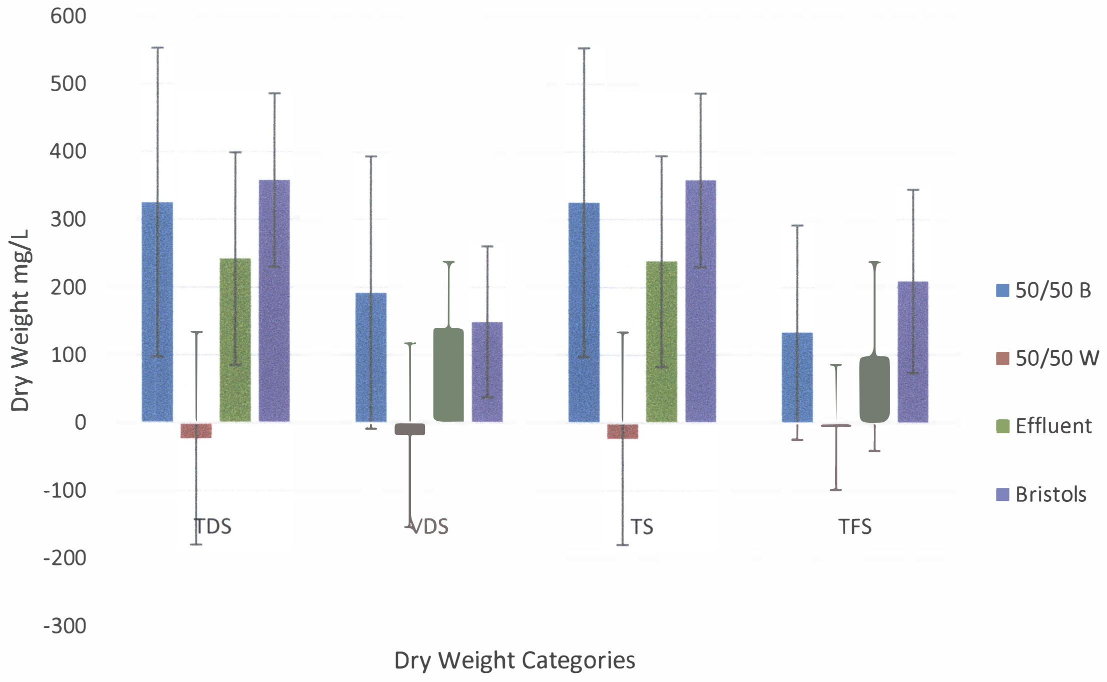

Figure 4.1. Average sample dry weight values calculated by subtracting the initial effluent dry weight from the final sample dry weight. TDS = Total Dissolved Solids, TFS = Total Fixed Solids, VDS = Volatile Dissolved Solids, TS = Total Solids. Legend: 50/50 W = 50% Effluent & 50% Water, 50/50 B = 50% Effluent & 50% Bristol's, Effluent = Pure effluent, Bristols = Bristol's growth medium.

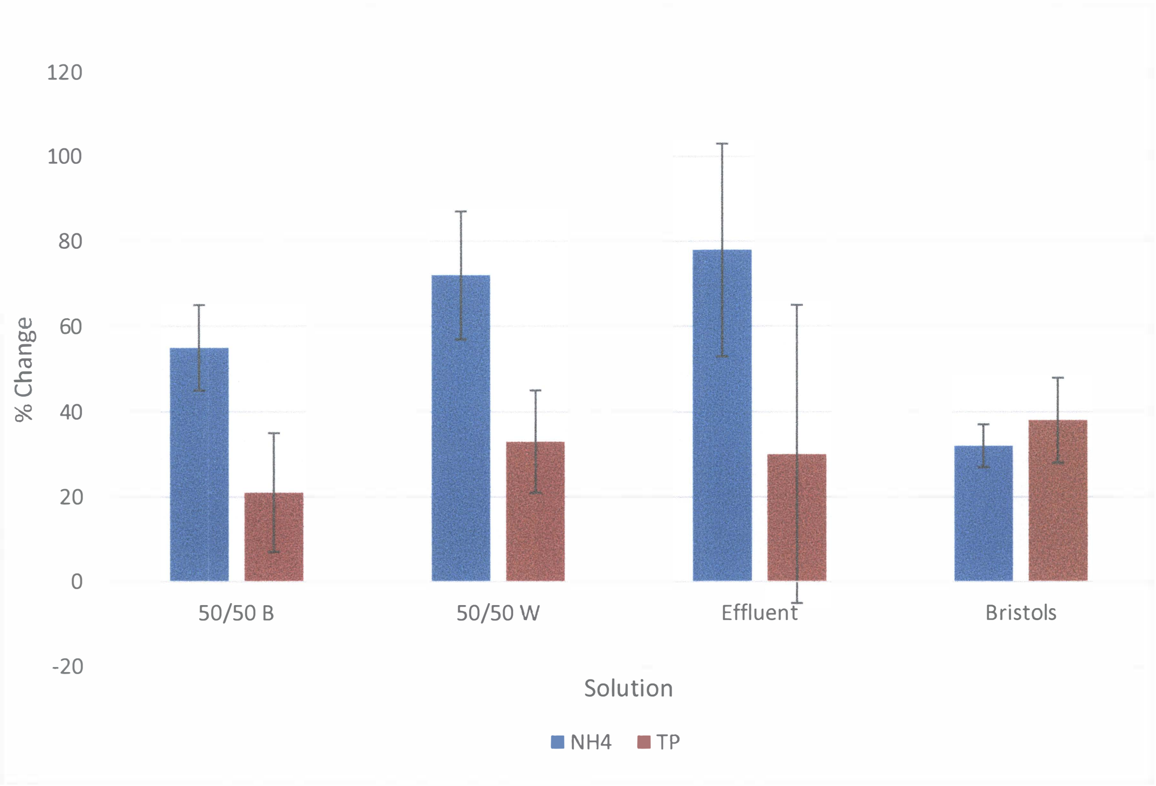

Figure 4.2. Average percent change for ammonia and phosphorous. Legend: 50/50 W = 50% Effluent & 50% Water, 50/50 B = 50% Effluent & 50% Bristol's, Effluent = Pure effluent, Bristols = Bristol's growth medium.

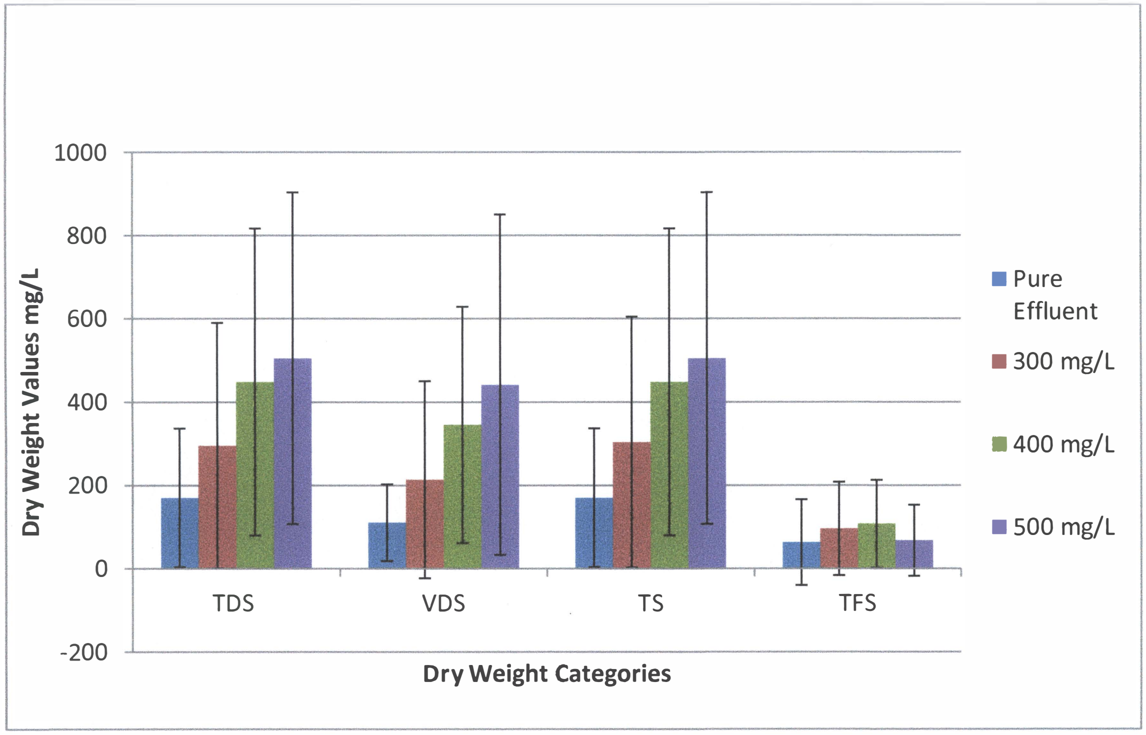

Figure 4.3. Average sample dry weight values calculated by subtracting the initial effluent dry weight from the final sample dry weight. TDS = Total Dissolved Solids, TFS=Total Fixed Solids, VDS = Volatile Dissolved Solids, TS = Total Solids. Legend: 300, 400 and 500 mg/L refers to concentration of calcium bicarbonate.

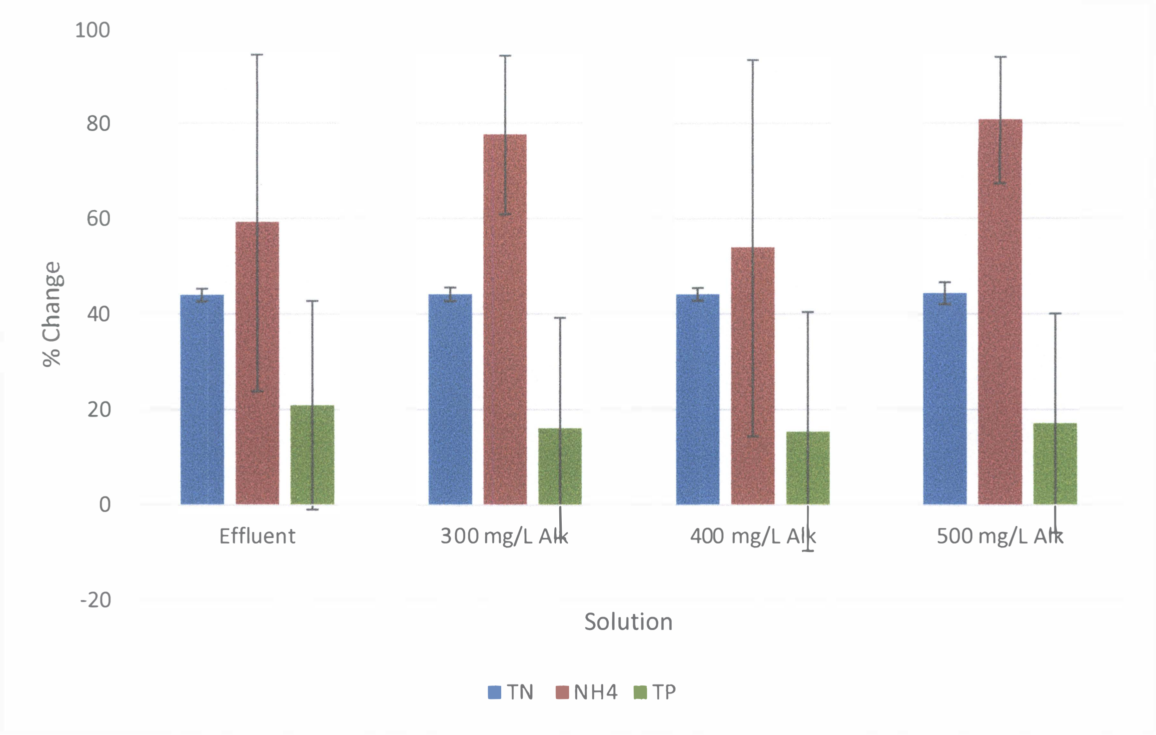

Figure 4.4. Percent change for total nitrogen (TN), ammonia (NH4), and total phosphorus (TP) under varying alkalinity (300, 400 and 500 mg/L of calcium bicarbonate).

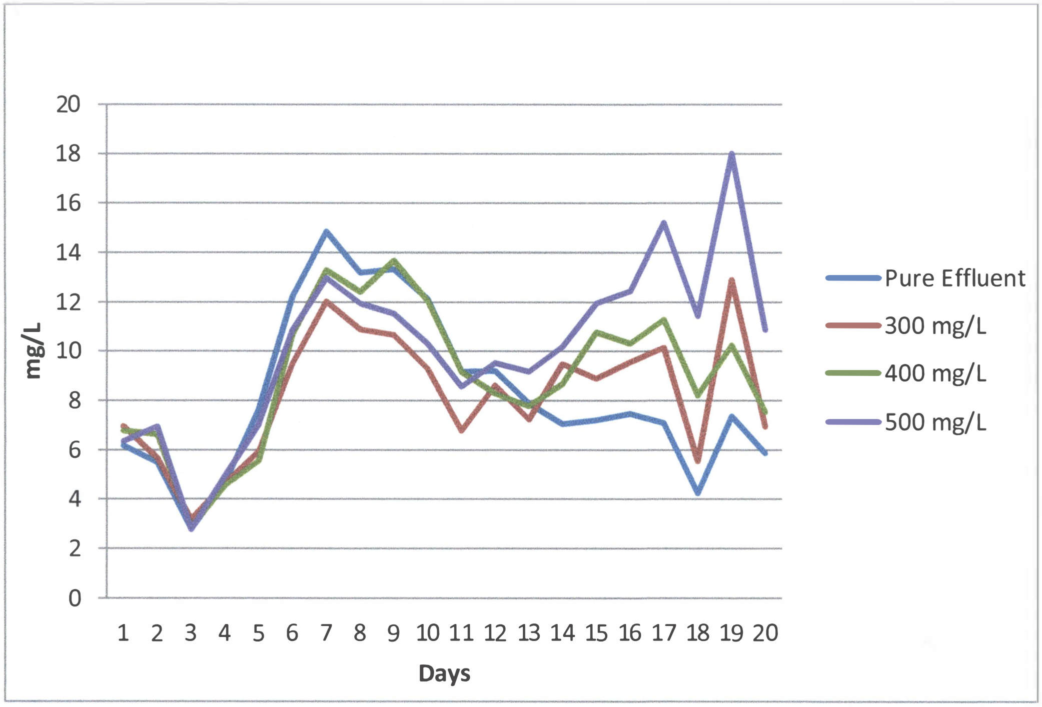

Figure 4.5. Average daily fluorometry reading under varying alkalinity (300, 400 and 500 mg/L of calcium bicarbonate).

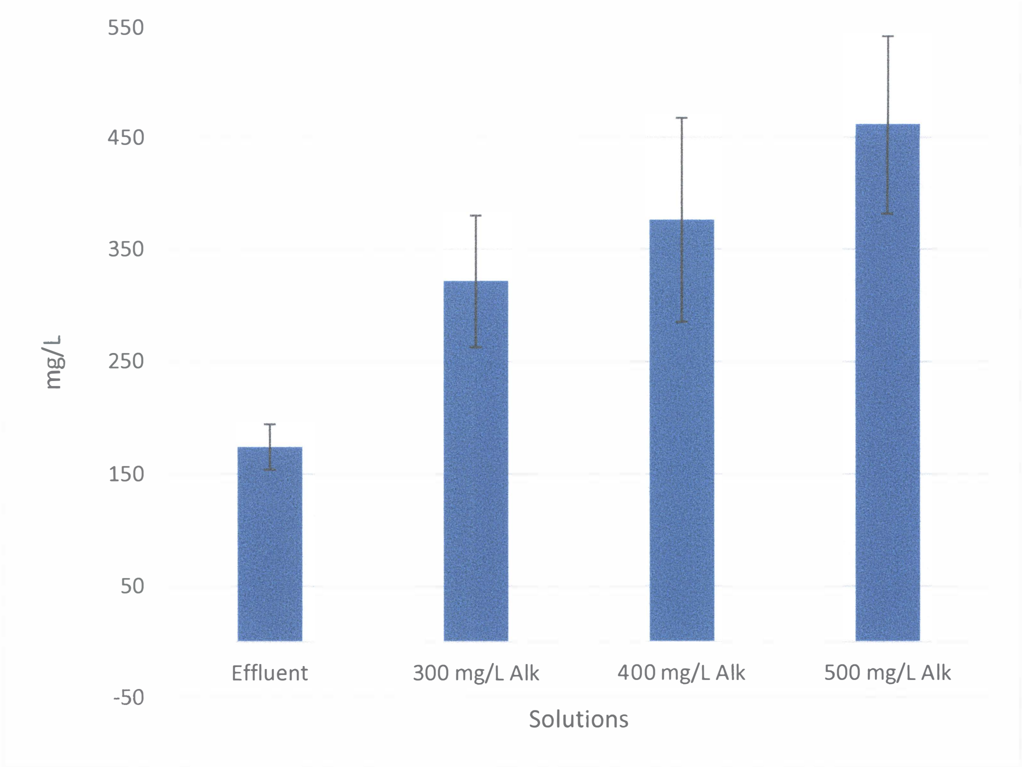

Figure 4.6. Average alkalinity through taken post experiment. Alkalinity ranged from natural (effluent) to adjusted (300-500 mg/L of calcium bicarbonate).

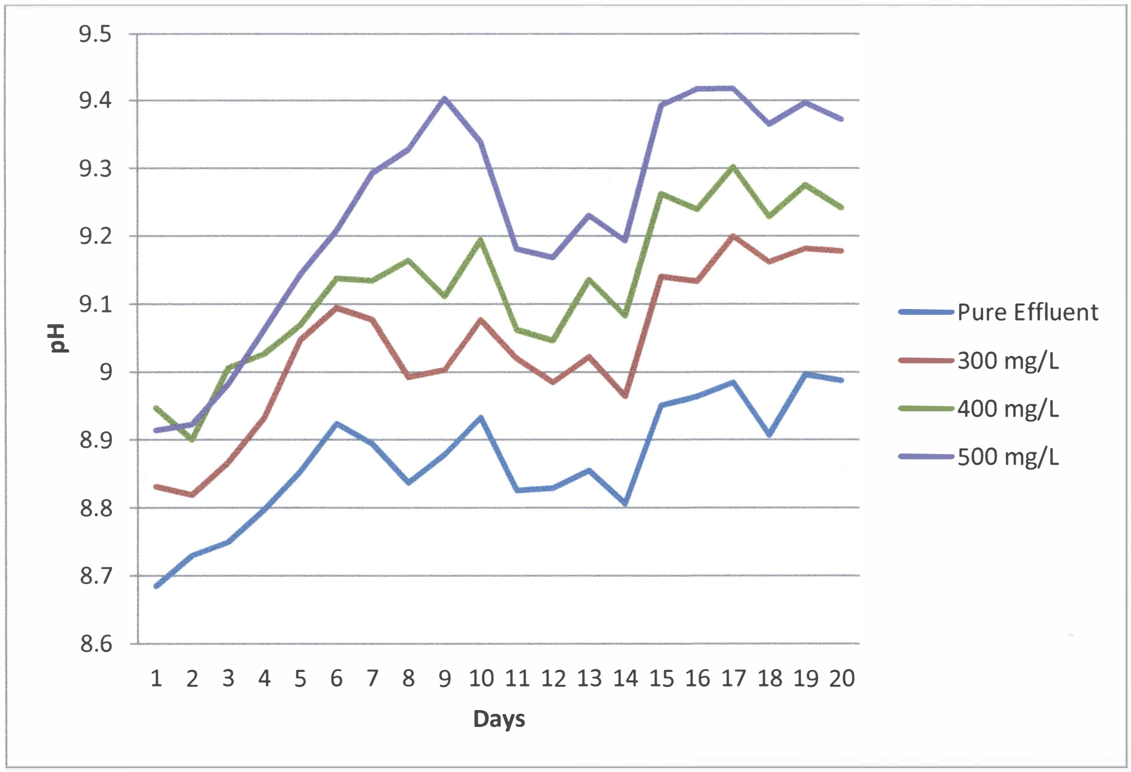

Figure 4.7. Average daily pH values under varying alkalinity (300, 400 and 500 mg/L of calcium bicarbonate).

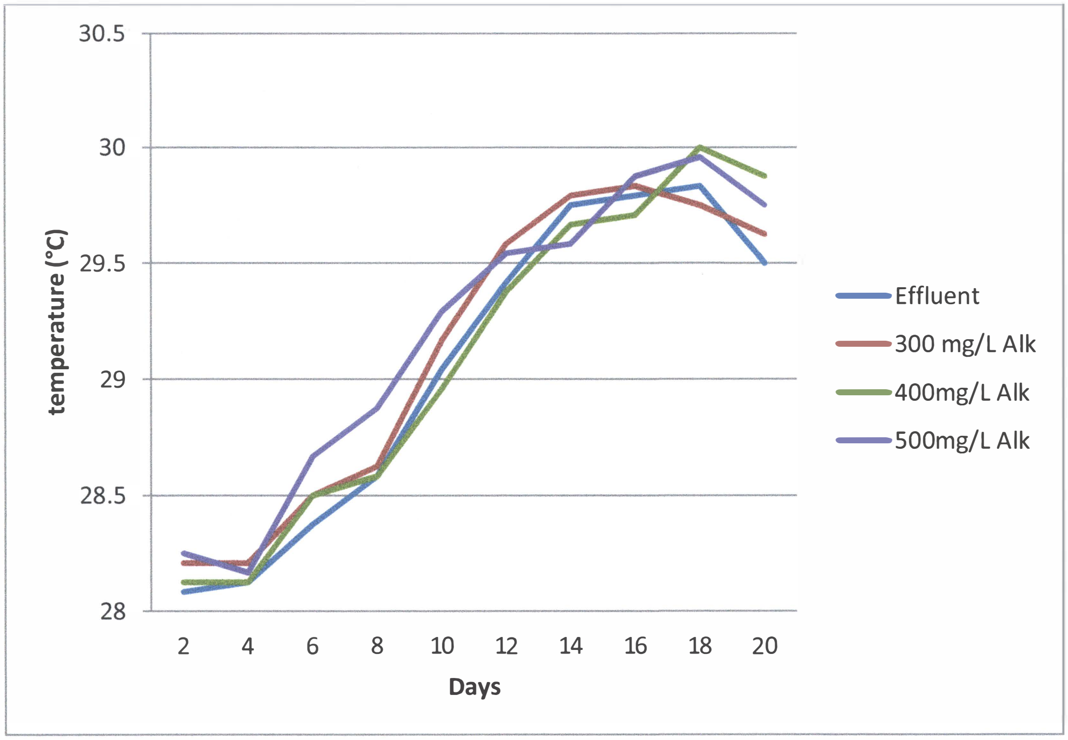

Figure 4.8. Average temperature values under varying alkalinity (300, 400 and 500 mg/L of calcium bicarbonate).

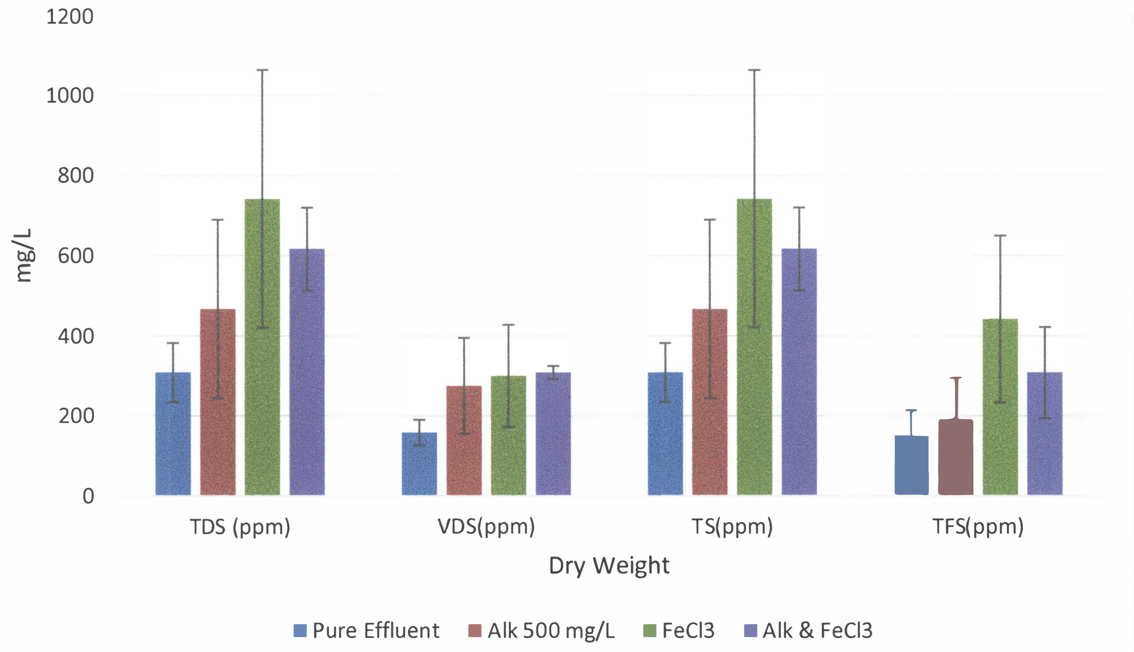

Figure 4.9. Average sample dry weight values calculated by subtracting the initial effluent dry weight from the final sample dry weight. TDS = Total Dissolved Solids, TFS = Total Fixed Solids, VDS = Volatile Dissolved Solids, TS = Total Solids. Alkalinity refers to concentration of calcium bicarbonate, while FeCl3 refers to iron (III) chloride at a concentration of 0.625 μM.

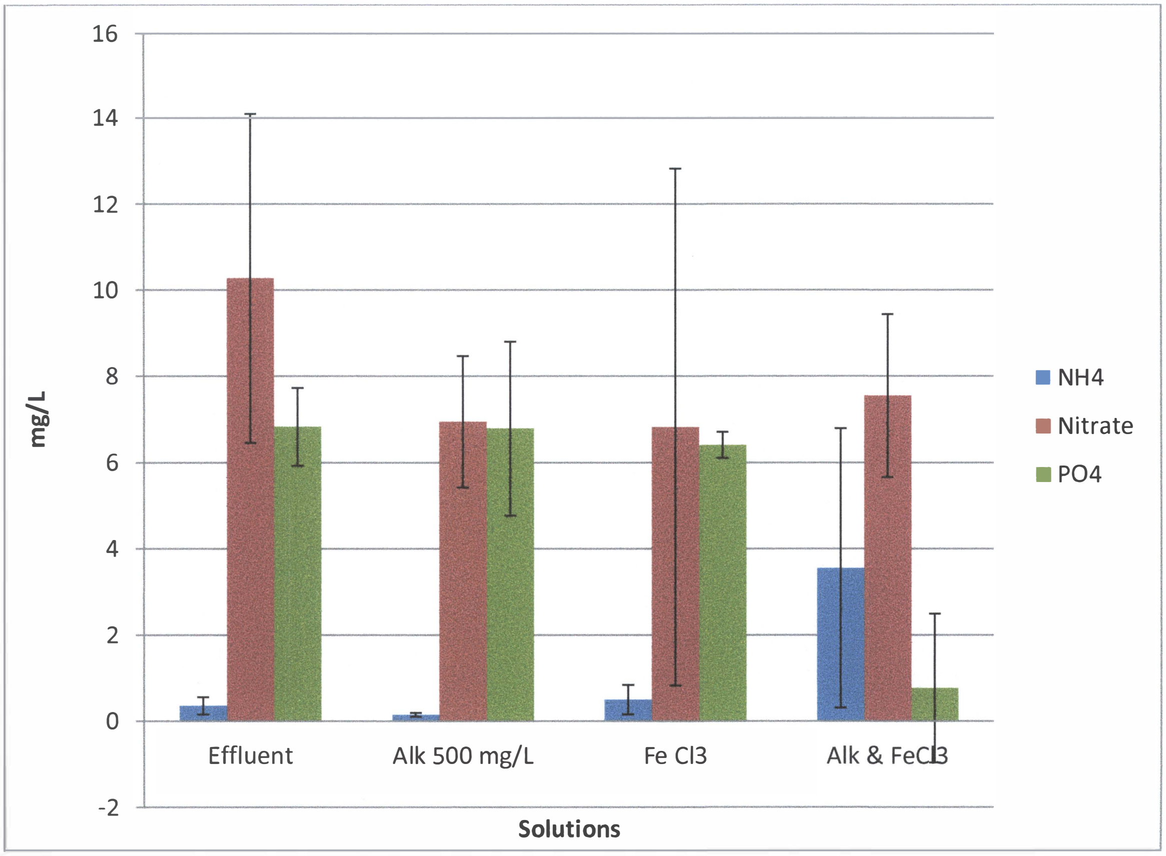

Figure 4.10. Percent change for nitrate, ammonia (NH4), and total phosphorus (PO4) under varying combinations of alkalinity (500 mg/L of calcium bicarbonate) and iron(III) chloride at 0.625 μM.

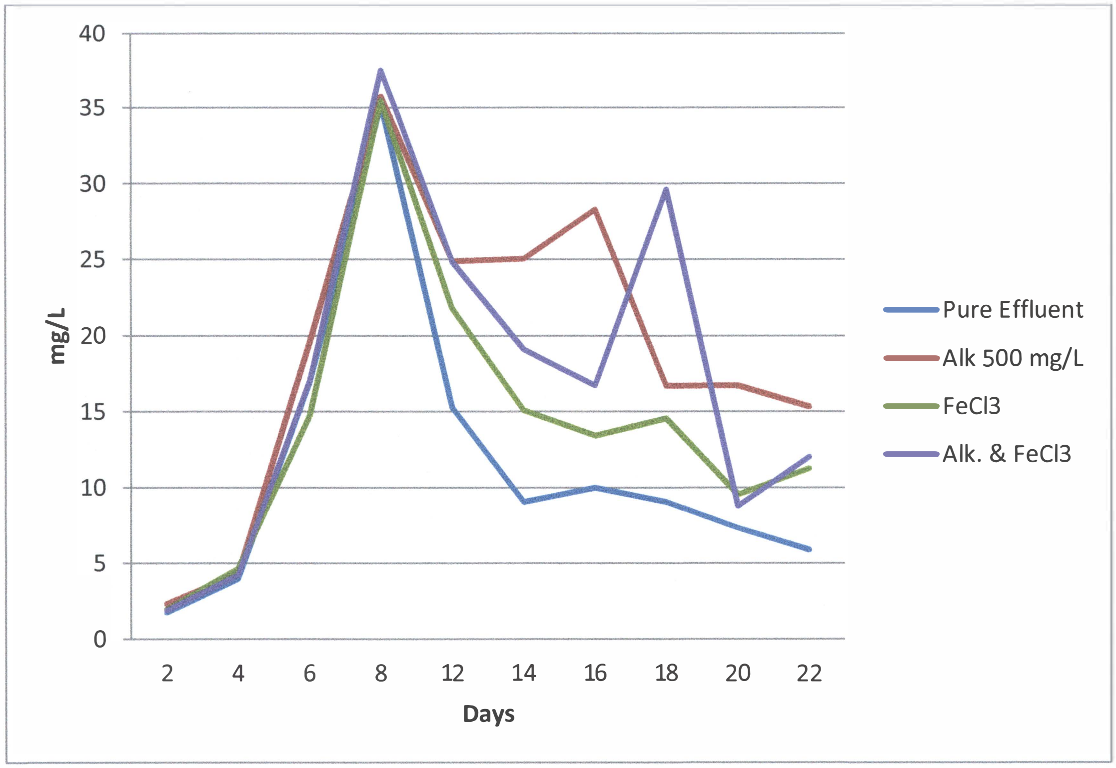

Figure 4.11. Average daily fluorometry reading under varying combinations of alkalinity (500 mg/L of calcium bicarbonate) and iron(III) chloride at 0.625 μM.

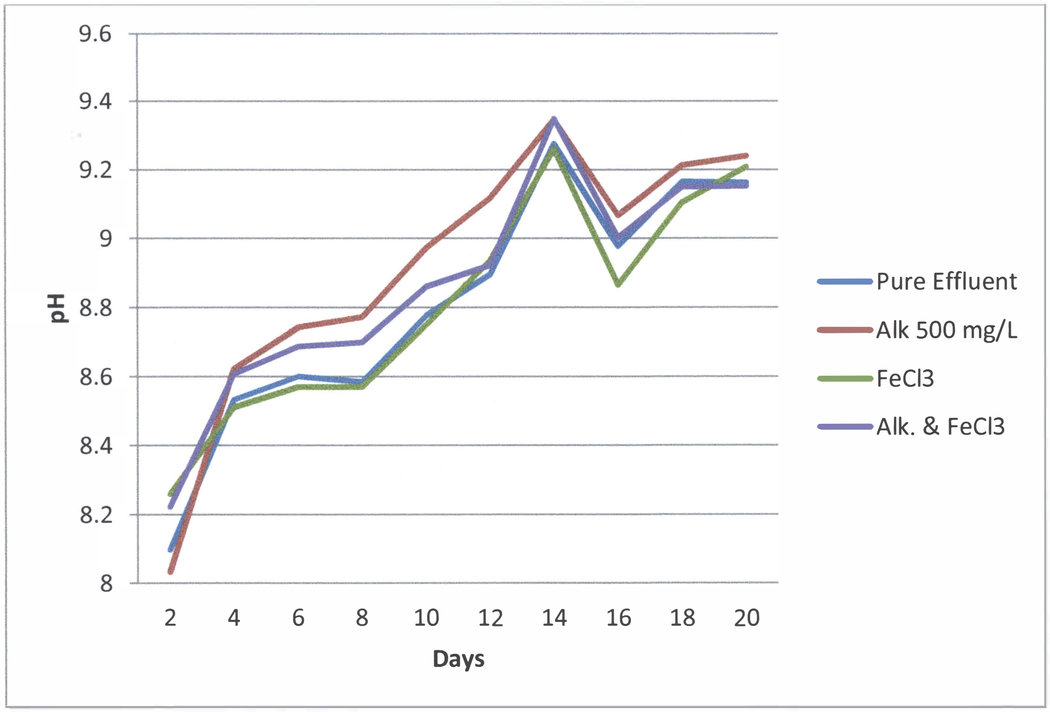

Figure 4.12. Average daily pH values under varying combinations of alkalinity (500 mg/L of calcium bicarbonate) and iron(III) chloride at 0.625 μM.

5. CONCLUSIONS

This thesis provided a positive step towards realizing the potential for biofuel production using wastewater as a free and renewable nutrient source. In the first experiment, the bench-scale trials demonstrated the usefulness of algae at nutrient removal from wastewater. This utilization of nutrients from wastewater suggests potential cost savings when compared to the purchase of fertilizers as a nutrient source. The second experiment showed that raising the alkalinity concentration would increase biomass yield (with a retention time over 10 days) and remove further nutrients. However, pure effluent produces the same initial growth rate as the solutions with raised alkalinity. Retention times for a sanitation plant would only be a few days at most, so increasing alkalinity may not provide any benefit to short retention time systems. Although, it is possible to raise alkalinity if an observed drop in growth rate occurs. If the algae are to be grown in batch cultures with wastewater effluent, the incubation time may be long enough to warrant addition of bicarbonate. Based on the data, the same conclusions can be applied to iron as a supplemental additive as well. Taken together, wastewater might only require an inoculation of a high lipid algae species to produce sufficient levels of oil for bioenergy, although this experiment would need to be replicated with a high lipid species to test this idea further.

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