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INVESTIGATION OF MEDIA INGREDIENTS AND WATER SOURCES FOR ALGAE CO₂ CAPTURE AT DIFFERENT SCALES TO DEMONSTRATE THE CORRELATIONS BETWEEN LAB-SCALE AND LARGE-SCALE GROWTH

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INVESTIGATION OF MEDIA INGREDIENTS AND WATER SOURCES FOR
ALGAE CO₂ CAPTURE AT DIFFERENT SCALES TO DEMONSTRATE THE
CORRELATIONS BETWEEN LAB-SCALE AND LARGE-SCALE GROWTH

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in
Biosystems and Agricultural Engineering
in the College of Engineering at the University of Kentucky

By

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Lexington, Kentucky

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Lexington, Kentucky

2013

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ABSTRACT OF THESIS

INVESTIGATION OF MEDIA INGREDIENTS AND WATER SOURCES FOR ALGAE CO₂ CAPTURE AT DIFFERENT SCALES TO DEMONSTRATE THE CORRELATIONS BETWEEN LAB-SCALE AND LARGE-SCALE GROWTH

As energy use increases globally the environmental burdens increase alike. Many accusations have been made that carbon dioxide is a culprit of climate change. The University of Kentucky and Duke Energy Power have partnered to test carbon capture technology in a large scale project. To this end, the objective of this thesis is to investigate potential water media sources and nutrient sources at different volume scales for algae cultivation to help create a more environmentally viable and economically feasible solution and gain understanding in the upscaling of this process. As result of this research, lab grade urea with no EDTA had the greatest algae growth and pond water was the most viable alternative water source. Through a lifecycle assessment, pond water was found to be the most economical and environmentally friendly option. Algae growth decreased as the cultivation volume increased, due to light and CO₂ availability.

KEYWORDS: algae, carbon dioxide mitigation, media, climate change, nutrients

Tabitha Graham

July 19, 2013

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Chapter 1. Introduction

It has been estimated that the United States produces 5.56 billion tons of CO₂ per year, approximately 21% of the world's CO₂ emissions, from coal-burning power plants alone (Jeong et al., 2003). These numbers are expected to persistently rise if more stringent management practices are not promptly established. The United States' Environmental Protection Agency (EPA) has enacted regulations, such as the Greenhouse Tailoring rule, to force coal-fired plants to take steps toward decreasing carbon emissions (Bell, 2012). These efforts coupled with other research quests for carbon capture technology are speeding up the process and desire for finding a way to keep coal as a feasible energy source.

Coal is an energy resource that is available and processed in the United States, which creates jobs and energy security. Coal is not only a resource popularly utilized in the U.S., but it also serves as a valuable international energy supply. Approximately 41 % of the world's electricity is generated from coal (Association, 2009). Our world's dependence on coal creates a difficult balance between energy needs and environmental implications resulting in this issue being a major research priority.

The Commonwealth of Kentucky is economically dependent on coal production. Kentucky has been one of the top three coal producing states in the U.S. for the last 50 years (Association, 2011). In 2009, Kentucky had the lowest electrical cost in the nation at 0.0603 \$/kWh (Association, 2011). In 2009, approximately 93% of Kentucky's net electricity was produced from coal (Waddell, 2011).

CO₂ is taken out of the atmosphere naturally via plant photosynthesis and uptake by the oceans. The amount of natural CO₂ uptake cannot keep up with the amount of CO₂ that is released due to the amount of fossil fuels that are being used. Increasing CO₂ levels have escalated the need to develop systems to sequester CO₂, completely removing it from the carbon cycle. A second option is to develop methods to recycle CO₂, creating other methods to better utilize it and reduce the amount of fossil fuels used. Figure 1.1 shows the relationship between the natural carbon cycle and the addition of new cycles to address current needs.

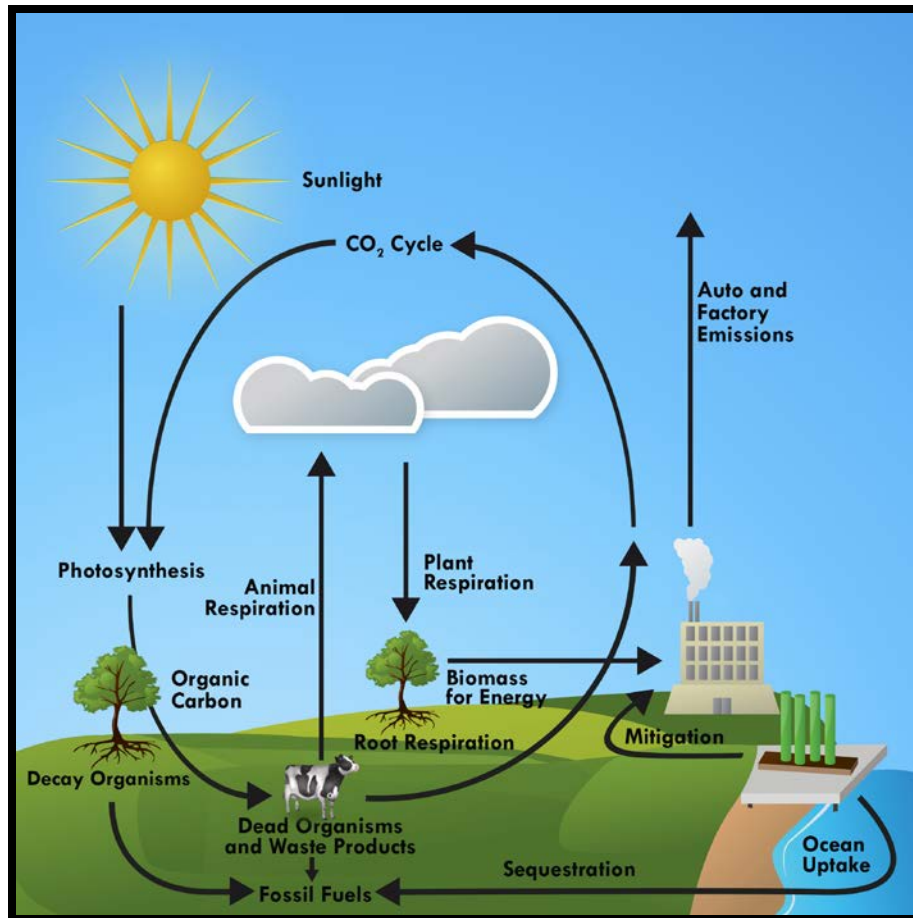


Figure 1.1: Flow diagram of the carbon cycle including the algae CO₂ mitigation system.

1.1 Sequestration Processes

Sequestration occurs naturally through plant growth. Forests in the northern hemisphere have been estimated to store about half of the human generated CO₂ emissions that do not appear in the atmosphere (Hughes and Benemann, 1997). Practices have been considered to help create more sequestration potential through plants, including preventing deforestation, increasing forest productivities, foresting non-forested areas and improving agricultural management practices (Hughes and Benemann, 1997). These natural means of CO₂ mitigation are attractive yet limiting. Forest protection and restoration have a threshold level that can be approached, but once reached, there is not additional room for CO₂ storage, and therefore other methods to manage CO₂ levels are needed.

Oceanic sequestration also occurs naturally in the environment. The ocean is estimated to annually store approximately two giga-tons of CO₂ (Sundquist et al., 2008). Yet, this atmospheric induced carbon capture may be causing negative effects on the oceans' pH level. There may be correlations between high acidity and negative effects on marine wildlife, which reinforces the need for other feasible carbon capture options (Sundquist et al., 2008).

Geological sequestration is another method that is being studied to reduce CO₂ emissions. This method prevents CO₂ pollution by condensing and burying emissions in underground reservoirs. The major motivation for using geologic sequestration is its allowance to continue using current energy generation techniques (Stuart, 2011). The volume required to sequester 50% of the CO₂ released is twice as large as the volume of crude oil removed from the ground. Also, the high energy and price demand for the sequestration process makes it an unattractive option. Injecting CO₂ in the deep ocean, geological strata, old coal mines and oil wells, and saline aquifers often results in leakage which makes it an ineffective option (Lal, 2008).

1.2 Mitigation Processes

Biological mitigation utilizes photosynthesis of a biological agent that can consume CO₂ in order to decrease carbon emissions. Biological mitigation also provides the incentive of the production of the biomass, a potentially valuable by-product. Biomass usage as an alternative to fossil fuels would also result in decreasing CO₂ emissions. One reasonable biological agent for CO₂ mitigation is algae (Stuart, 2011), which will be the primary focus of this project.

In locations where coal is a necessity, CO₂ capture by algae would provide an additional route to clean energy production. Currently, algae production is being executed in a variety of apparatuses to find the potential for mass cultivation using flue gas from coal-powered plants. Due to success at the smaller scale, algae growth systems are being up-scaled for commercial applications such as utilization at coal-fired power plants. The upscaling step brings attention to a variety of system factors to consider for optimal operation.

Water is a vital ingredient necessary for the utilization of algae production systems. However, water scarcity is an issue in within an energy system because of its functional reliance on water. Water is a key component specifically in energy production systems utilizing coal. The steps, including mining, transport, storage and refining coal, all require water usage. Also, water is used to reclaim the land and process the fuel (Younos, 2012). Due to water use in other areas of production, it is critical to consider the most responsible resource of water for media formulation to accomplish a sustainable carbon dioxide mitigation method. Ideally a non-potable water source would meet the criteria desired for this process. A few alternate water sources have been studied to determine the effects on a variety of algae species. These include natural, waste and industrial process water sources. In comparison to a collection of other biomass feed stocks, algae have much less of an impact on fresh water sources because of its ability to grow in non-potable water (Subhadra and Edwards, 2011).

Another reason the use of algae for carbon capture is thought to trump other plant sources is because of its high tolerance to extreme environments (Keffer and Kleinheinz, 2002). Using algae has also become an attractive feedstock option because it provides the useful by-product of biomass (Stuart, 2011). Algae appears to be a viable option because it grows at rapid rates and can produce useful by-products in the industries of agriculture, food, fuels and pharmaceuticals.

This research examines microalgae's behavior in an array of water and nutrient sources available for media formulation and upscaling reliability. In addition, a water-sourcing lifecycle assessment was performed. Attaining a more viable media water source would decrease resources needed for the system, which would reduce spending and minimize the environmental impacts of the process. Also, testing different sources of lab and industrial grade nutrients and cultivating algae in an airlift reactor and trailer-scale bioreactor will provide support for upscaling the process for large-scale coal-fired power plant use.

1.3 Research Objectives

Research is showing that algae can be used by coal-fired plants to decrease the amount of carbon dioxide they release from their operation. Testing carbon capture technology in a large scale project can be successful with the application of a lab scale setting to refine the process. The objectives of this research were to:

1. Test algae growth in potential nutrient and water sources for the media recipe. The nutrients investigated were combinations of lab grade and industrial grade. A variety of on and off site water sources were studied including: on-site; river water, well water and coolant water, and off-site; pond water, urban stream water and agricultural stream water. Tap water was used as the control. This research expanded on the use of a potable water source to grow algae for CO₂ capture.
2. Perform a lifecycle assessment on potential water sources for algae growth. The nutrients and water treatments were taken into account.
3. Identify and control the growth differences between a lab scale and up-scaled setting. The scaled environments that were investigated were laboratory flasks, an airlift reactor and a portable trailer unit. Looking at up-scale versions helped to provide the feasibility and operational assurance at an industrial setting.

Chapter 2. Background

2.1 Algae

Algae are photosynthetic microorganisms that are typically anaerobic. There are a vast number of algae species that vary in size and biological characteristics. The main ingredients for algae to grow are water, sunlight and carbon dioxide. There are several different kinds of algae that can be classified based on color and include: Cyanophyta, blue-green algae; Rhodophyta, red algae; Chrysophyceae, golden algae; Phaeophyceae, brown algae; and Chlorophyta, green algae (Van den Hoek et al., 1996). Due to increasing interest in biofuels, algae are appearing to be a more sensible feedstock because they are not used as a major agricultural crop for food production. Algae also encompass the advantageous trait of the ability to be grown in fresh and salt water, providing a range of feasible geographical locations for mass production. Therefore, it is important to consider the environments algae can thrive in and which are not resourceful.

Main factors that influence algae growth are temperature, light, nutrient levels and pH. Different levels of application of each factor can cause changes in the algae growth. The requirements for each necessary factor fluctuate depending on the algae species. Many researchers are focusing their efforts on the investigation of single factors, such as light levels, to obtain optimal conditions and eventually collaboratively design a setup optimal for all factors.

Typically microalgae's optimal temperature range is between 20 and 30°C. (Wang et al., 2008). Flue gas from coal fired power plant is released at a high temperature; therefore, it is important to consider the algae's tolerance to high temperatures because it can help reduce cooling costs (Wang et al., 2008). Temperature is also important in choosing algae growth climates because of variability in weather conditions based on geographic location.

Light is an important driving factor in photosynthesis, as represented by the equation of photosynthesis (Freeman et al., 2008):



Algae are typically grown on a light:dark cycle to simulate their natural environment. In the 1950s, researchers Tamiya, Iwamura, Shibata, Has and Nihei suggested that two reactions took place: one light dependent and the other light independent (Foy et al., 1976). Latter research conducted with blue-green algae supported this hypothesis. There is also a correlation between light, nitrogen and culture color. Research performed on a variety of blue-green algae compared a 24 hour light cycle to a 6:18, light:dark cycle (Foy et al., 1976). The resulting culture using 24 hour light was yellow-brown while the light:dark treatment was deep green. This suggests that the dark cycle is needed to produce the nitrogen, otherwise protein synthesis continues promoting a nitrogen deficiency (Foy et al., 1976).

There are specific nutrients essential in the formulation of growth media for algae to cultivate efficiently including nitrogen, phosphorous, sulfur, magnesium, iron and carbon. Nitrogen is an element that can be found in many different forms. The most common are nitrate, ammonia and urea (Wang et al., 2008). One way nitrogen could be easily and affordably implemented into the media is by using a wastewater source. Phosphorous also affects the growth of algae cells because it is a component of so many of the driving biological agents. Many proteins are needed for photosynthesis to occur; proteins synthesized by ribosomes are rich in phosphorous (Wang et al., 2008). Sulfur is another vital element for the growth of algae. Depriving the algae system of sulfur has been shown to slightly decrease the growth rate by less than five to 10 % of its initial rate (Zhang et al., 2002). Magnesium is critical as it is the central atom in the chlorophyll molecule used for the conversion of light to energy via photosynthesis. Iron enables nitrogen assimilation, facilitates metabolism and fosters the synthesis of chlorophyll. Finally, carbon dioxide is essential for the cultivation of algae. The carbon dioxide source being considered for algae production is flue gas, which is a by-product of coal combustion and in the Commonwealth of Kentucky, it is readily available.

While maintaining the appropriate amount of CO₂ keeps algae growing as quickly as possible, CO₂ levels can also affect the algae pH level. There is an inverse relationship between CO₂ saturation levels and pH: as the saturation of CO₂ increases, the algae's pH decreases. Algae are typically resistant to growth at a low pH; therefore, pH is necessary

to consider when optimizing the growth environment. Furthermore, if saturation levels are reached, the pH levels decrease to a level that is lethal to the algae.

2.2 Strain Selection

A variety of objectives are necessary to consider when choosing an algae species for the purpose of emissions remediation. One main concern is economic practicability; it is important to evaluate if valuable co-products can come from mass cultivation of algae. Also, the level of tolerance to CO₂ is necessary to consider with the ultimate goal being to maximize CO₂ mitigation. Temperature tolerance is another limiting variable that must be considered. Most thermal power stations discharge gases at 120°C (Hanagata, 1992). Therefore, it is important to identify which algae strains can tolerate high temperatures to decrease cooling costs. These variables have been considered for many different types of algae including: *Chlorella*, *Dunaliella*, *Spirulina* and *Scenedesmus* (Ono and Cuello, 2003).

Chlorella is an alga that has shown possibilities for CO₂ mitigation. The optimal temperature for the algae species *Chlorella* is between 20 and 25°C (Neish, 1951). *Chlorella* has a 40 % maximum CO₂ tolerance, yet in a study conducted by Hanagata (1992), the maximum growth rate occurred at a level of 10 % CO₂. *Chlorella* is also a viable selection species because it has the ability to thrive in a variety of environments. Research shows it has been successfully grown in an assortment of concentrations and trace elements (Ono and Cuello, 2003). This species also has the potential to be used as a valued product in high protein health foods (Ono and Cuello, 2003).

Another species of interest is *Dunaliella*. Experiments at a variety of different temperatures and equal cell densities found *Dunaliella*'s optimal cell division temperature to be 20°C (Wegmann and Metzner, 1971). Using both Soerensen and McIlvain buffers, results supported the finding that *Dunaliella*'s optimal pH is in the range of 6.0 and 6.2 (Wegmann and Metzner, 1971). The approximate maximum CO₂ concentration in this species is 15 % (Nagase, 1998). *Dunaliella* also proves to be suitable for more than just CO₂ mitigation. Depending on growth conditions *Dunaliella tertiolecta* can remove between 51 and 96 % of nitric acid present at 15 % CO₂ and 1000

ppm of NO (Ono and Cuello, 2003). *Dunaliella* also provides useful by-products, such as β -carotene, which help with economic barriers for mass production (Ono and Cuello, 2003).

Spirulina is another species that has demonstrated the potential for carbon capture. *Spirulina* has an optimal temperature range between 30 and 35°C (Oliveira et al., 1999). The optimal pH for *Spirulina* was found to be in a wide range between 8 and 11 (Oliveira et al., 1999). *Spirulina* is not just used for CO₂ mitigation: as a result of its high protein content, *Spirulina* has been used for the production of food products and aquaculture nutrition (Oliveira et al., 1999).

Finally, *Scenedesmus* is a strain that appears to be a reasonable option for alleviating large amounts of CO₂ emissions. Recent testing showed that *Scenedesmus sp.* has an optimal temperature of 27°C with a growth rate of 0.0284 1/hr (Cassidy, 2011). *Scenedesmus sp.* can also tolerate a high maximum CO₂ concentration, estimated to be 80 % (Hanagata, 1992). Based on previous results within the overall research project, *Scenedesmus* was selected as the algae to investigate further.

2.3 Growth Media

A successful algae growth media requires specific nutrients including nitrogen, phosphorous, sulfur and carbon (Wang et al., 2008). Many studies have been conducted to inspect the effects of different nutrient levels on algae growth.

There are many different nitrogen sources that can be used to grow algae such as animal wastes, urea (CO(NH₂)₂), potassium nitrate (KNO₃) and Ammonium Chloride (NH₄Cl). Urea is an attractive media source because it is relatively inexpensive and widely available. In a particular study, two strains of the algae *Scenedesmus*, *dimorphus* and *quadricauda*, were grown at varying nitrogen concentrations (urea concentrations included: 0.02 g/L, 0.04 g/L, 0.08 g/L, 0.1 g/L and 0.2 g/L) with urea as the nitrogen source (Goswami, 2011). The results revealed that the 0.1 g/L concentration of urea showed the greatest growth potential for biomass and CO₂ consumption for both species after 11 days (Goswami, 2011). Also, from the results of this study, it could be suggested

that too much nitrogen could actually inhibit growth rates. Another nitrogen concentration level study performed on *Scenedesmus obliquus* and *Chlorella vulgaris*, using KNO_3 and NH_4Cl as the nitrogen sources, also found a positive correlation between algal biomass and increasing nitrogen levels (Piorreck et al., 1984). Experiments have also been done to compare *Scenedesmus* and *Chlorella* in M-8 media and urea media; the results found that the difference between the growth rates was insignificant therefore making urea media the more feasible option because of its affordability (Crofcheck et al., 2013).

Phosphorous is a nutrient that is often discussed along with nitrogen. Typically nitrogen and phosphorous fertilizers are applied therefore resulting in agricultural waters with an increasing density of these nutrients. High levels of phosphorus and nitrogen can cause algae growth that consumes all of the oxygen which can result in fish death. While all of this may seem to negatively portray the effects of fertilizers and algae, it supports the fact that fertilizers are an excellent source for algae growth and could be an affordable media ingredient. Research has been performed on algae growth in fertilizers, and in one study for the species *Tetraselmis suecica*, the trade fertilizer Igromurtonik produced the best growth rate at a nitrogen to phosphorus level of 24:1 (Corsini and Karydis, 1990).

Sulfur nutrients have also been studied with regards to algae growth. The algae strain *Chlamydomonas reinhardtii* has demonstrated that in a sulfur deprived environment the efficiency of Photosystem II, the step in photosynthesis when excitation energy is converted to chemical energy, decreases (Zhang et al., 2002). Another study also looked at how altering levels of sulfate would change the growth abilities of algae. In that research, a variety of strains were grown in sulfate consisting of 850 ppm of sulfur (Wheeler et al., 1982). The algae strains showed diversity in their resulting behaviors. Some showed decreased growth with any reduction in sulfate, others maintained growth until sulfate levels reached 0.85 ppm, and another showed no changes with sulfate levels (Wheeler et al., 1982). Therefore, depending on the strain being used, sulfur addition could be variable in media recipes.

Carbon dioxide is the primary compound that needs to be biologically fixed by the algae. Therefore, the CO₂ concentration is important to consider the effects on algae growth. In the red alga species *Porphyra leucosticta*, high levels of inorganic carbon have been shown to decrease the amount of soluble protein and nitrogen content (Mercado et al., 1999). A study performed on *Chlorella* sp. and *Scenedesmus* sp. at different concentrations of CO₂ demonstrated that as CO₂ concentration increased biomass also increased. The greatest biomass was found to be at the highest tested level of 24 % CO₂ (Makareviciene et al., 2011). Yet there is still a vast amount of controversy about what the effects of CO₂ levels on algae growth because of the results of other studies. Another CO₂ concentration study with the algae strain *Nannochloropsis oculata* found that at 5 %, 10 % and 15 % CO₂ levels the algae growth was completely inhibited (Chiu et al., 2009). While there are a few different findings, many researchers suggest at turbulent flow conditions, CO₂ addition can result in growth being repressed (Makareviciene et al., 2011).

One of the main ingredients for algae media is water. It is essential to investigate water usage for algae systems to ensure that large scale growth operations for CO₂ mitigation are sustainable processes. This poses the idea that non-potable water sources would be a more reasonable option as a water supply. Many different water sources are being investigated for mass algae production. These include non-potable waters that come from natural, waste and industrial process sources.

Using a natural water source would be efficient for the media water makeup. This is a subject that has been researched in a few studies. National Chung-Hsing University investigated the potential use of the Green river's water as a medium (Ramaraj et al., 2010). This study found that algae was capable of growth using river water and was quantified by the average value of chlorophyll measured, 1.1 mg/L (Ramaraj et al., 2010). River water could be a more economical and less wasteful source than non-potable water. Another study researched the outcome of using forested river water compared to agricultural river water to grow marine dinoflagellates and diatoms (Graneli and Moreira, 1990). The results showed that for diatoms the growth rate was greater in the agricultural water, and for dinoflagellates the growth rate was higher in the forest river water (Graneli

and Moreira, 1990). These differences could have been due to variations in the different water's properties.

Wastewater has also been studied as a potential sustainable water source for algae media. There are many different sources available for wastewater collection that could be of interest for algae cultivation. One feasible source would be water from a municipal treatment plant. In a water treatment plant, an assortment of wastewater types are available including: wastewater before and after primary settling, wastewater after the activated sludge tank and wastewater from the sludge centrifuge (Wang et al., 2010). *Chlorella* sp. has the ability to grow in all four types of wastewater but thrives best in the wastewater from the sludge centrifuge because of the increased quantities of nutrients available (Wang et al., 2010). Another wastewater that has been researched for algae growth is wastewater from animal operations. Many researchers focus on the removal of nutrients as a wastewater treatment method. Yet another objective of interest is the effectiveness of wastewater from animal operations for the growth of algae. In a study using dairy wastewater, algae growth was found to be best in the less diluted media because of the extra nutrients available (Woertz et al., 2009). Swine waste has also shown capability as a media ingredient; media research performed on different additive levels of swine waste showed that growth occurs best at a composition of 3% treated swine urine (Kim et al., 2007). Another study, investigating algae growth in dairy, swine, beef and sheep manure mixed with *Chlorella vulgaris* and *Scenedesmus* sp., found that all of the manure samples peaked at 290 nm related to suspended solids and absorbance decreased steadily until 700 nm (Pecegueiro do Amaral, 2012). This demonstrates that algae have specific wavelengths of light that they absorb and grow better in.

Many different processes in industrial production require the use of water to create a product. Generating energy from non-renewable resources, such as oil, gas and coal, produces non-potable water as a by-product. A variety of process-resultant waters have been researched within the topic of the investigation of algae growth. One alternative water source that is being studied is "produced water," which is a result of generating oil and gas (Laur, 2012). An industrial member of the National Alliance for Advanced Biofuels and Bioproducts (NAABB) has successfully used algae grown in oil and gas

process water to produce biodiesel that meets the standards of the American Society for Testing and Materials (ASTM). Another process water that has been investigated for development of algae media is brewery effluent. In the study, *Chlorella Vulgaris* was grown in control media, diluted and complete effluent media (Raposo et al., 2010). The diluted effluent was found to have the highest growth rate as a result to having the appropriate amount of nutrients, not an over or under load (Raposo et al., 2010). This supports the idea that having too much of a nutrient can inhibit algae growth. Carpet effluent has also been researched as part of the media formula for algae growth. In these experiments, the treated and untreated carpet effluent was found to be a successful media sources for a variety of algae species (Chinnasamy et al., 2010).

This study concentrates on a variety of alternate water sources including; pond, stream, river, well and boiler waters. Experiments were performed with potential natural and process waters available on-site that could support a bioreactor at the power plant scale. Using non-potable water would be the most economically and environmentally alternative to tap or de-ionized water.

2.4 System Design

There are two methods being used for large-scale algae cultivation: open and closed systems. Raceway ponds are the most common open systems used. Closed systems are called photobioreactors (PBR), which are tubes, plate, or bags that can be made out of a variety of materials. Each design offers its own advantages and disadvantages to feasibility of mass scale algae production.

Open ponds for mass algae growth has been a topic for study since the 1950s (Tredici and Materassi, 1992). There are a variety of open system pond designs that are used for culturing algae, including large un-mixed ponds, deep aerated ponds, circular ponds with a rotating agitator, raceway ponds and sloped meandering ponds or shallow ponds with a circulating pump (Andersen, 2005). Open pond systems are less expensive than a closed system yet they are not as versatile for a large range of algae species (Andersen, 2005). Another factor important when determining the design type is the regional weather of where the system is being placed. This becomes of even greater importance if the system

is going to be an open system because of the vulnerability of the system to the outdoor environment. While temperature of the environment plays a role in the system's productivity, the rainfall can also have an effect. Rainfall can dilute the culture media and cause a decrease in algae growth rate (Andersen, 2005). Containments are also a concern for open systems and make scaling the system a difficult task. One attempted solution to this problem was to use plastic covers or green house over the open ponds. However, these methods were not believed to be extremely effective and thought to generate more operational issues (Chaumont, 1993). Measuring growth variables is essential to maintain a healthy culture. This is a more difficult task for an open system because it might not be as homogeneous and therefore, the testing might not be representative of the whole culture.

In the 1970s, closed reactors starting becoming a preference for research on algae cultivation (Tredici and Materassi, 1992). A closed photobioreactor can be made of plates, tubes, or bags made from glass, plastics, or other translucent material (Lehr and Posten, 2009). Closed reactors have numerous advantageous compared to open systems. A study performed with *Spirulina* comparing open and tubular closed system found that the closed system had higher daily productivities, better optimal temperature attainment and an extended cultivation time frame (Tredici and Materassi, 1992). With closed systems there is more control over variables that can affect algae growth such as temperature, species selection and contamination. Also, closed reactors have much lower dependence on weather compared to open reactors because they can be located in a temperature controlled environment, such as a greenhouse. The disadvantage to using a closed system is the cost to build and operate the reactor; economic feasibility is one of the most important factors to make closed bioreactors viable for mass algae production.

This research focuses on using closed photobioreactors in conjunction with a reservoir of similar volume to maximize volume and ensure the algae have a sufficient dark period. The current pilot-scale system is installed at Duke Energy's East Bend Station in Boone County, KY and has a total volume of 18,927 gallons. The majority of replicated experiments have been done in 400 mL volumes in flasks, including the water source

experiments proposed here. Hence, there is a need to correlate the flask results to expected results in the pilot-plant system and beyond.

2.5 Upscaling

Photobioreactor scale-up is still an area that has room for much improvement. The performance of a bioreactor is expected to decline when scaled-up unless the frequency of light-dark interchange of the fluid is maintained (Molina Grima et al., 1999). Mass and heat transfer analytical approaches have been suggested and studied to help understand and better predict upscaling behaviors (Molina Grima et al., 1999). Increasing the scale from the laboratory to an industrial environment has a variety of different system design changes that can alter algae growth. At a flask scale, the system would not be continuous as in a larger scale. Light is another factor that would be altered in the lab. Natural sunlight is going to produce different results than using artificial lights. Artificial light is able to be controlled and calibrated to the optimal conditions while natural light can be unpredictable.

Chapter 3. Materials and Methods for Experiments

3.1 Algae Culture

Scenedesmus acutus (UTEX 72) was purchased from the University of Texas' algae culture collection (Austin, Texas). *Scenedesmus* seed cultures were grown and maintained for five days in urea media. The urea media was prepared with city water that was dechlorinated by the addition of 0.06 g/L sodium thiosulfate and filtered using a 0.2 μm , 47 mm diameter Nalgene nylon membrane filter (Rochester, NY). To maintain the growth of the algae stock culture, they were transferred into new media for continued growth or used as inoculums for experiments. The inoculated flasks were incubated using a 16:8 hours light:dark cycle regime that consisted of warm (Philips F32T8/TL741 Alto, 32 Watts, Salida, KS) and cool white (Philips F32T8/TL735 Alto, 32 Watts, Salida, KS) fluorescent lights (70 $\mu\text{mol}/\text{m}^2$ per second). To use algae for inoculation of an experiment the algae and water were separated using the Thermo Scientific Sorvall Legend XTR centrifuge at 1800 rpm for 30 minutes. After the algae were centrifuged they were placed in de-ionized water to maintain consistency in the algae concentration. This process is used for all of the experiments in this research.

Using a laminar flow hood environment, 400 mL of fresh urea media and 2 mL of the algae stock culture were combined in a 500 mL Erlenmeyer flask. The freshly inoculated media stock culture flasks were positioned on a shaking table operating at 100 rpm and kept at approximately 22°C, room temperature. To saturate the flask with CO₂, 3% anaerobic grade cylinder CO₂ was bubbled into the cultures at approximately 0.14 L/min. Air was also bubbled in at 4.4 L/min of air for the system. The tubing was inserted through a foam stopper and placed into the opening of the 500 mL Erlenmeyer flask.

3.2 Dry Weight and Growth Rate

Whatman binder-free glass microfiber filters (type 934-AH, 24 mm diameter, Rochester, NY) were used to filter *Scenedesmus* biomass samples. The samples were dried at 105°C for 24 hours to determine the dry weights (DW) of the samples. The crucible was first weighed, and then the filters were moistened and dried before adding algae to help ensure

filters remained in the correct place. Algae was then added and dried and a final weight was recorded. Algae dry weight produced per liter (g/L) was used to estimate biomass content. Using basic biochemistry concepts we can use the slope of the growth curve as the growth rate (mg/L/hr) because the growth curves of cultivation time versus DW were linear (Shuler and Kargi, 1992).

3.3 Media Preparations

When upscaling a system it is important to consider economic feasibility. Nutrient supply is an essential factor that drives the growth of algae; therefore experiments are necessary to evaluate nutrients of different grades. Six different media were formulated with different sources for nitrogen, potassium, phosphorus and EDTA (iron source). M-8 media, which utilized lab grade EDTA, is what was used to gage the progress of changing the media compositions success. The lab grade urea recipe was tested and in addition the urea recipe was tested using KNO_3 as an alternative nitrogen source to urea, and both of these used lab-grade EDTA. EDTA is also being investigated because it is one of the more expensive nutrients used (Crofcheck, 2013). EDTA plays an essential role by dissolving iron ions from algae metabolism consumption (Crofcheck, 2013). This study also looked at three different EDTA regimes: commercial urea with commercial EDTA (Sprint 300 Na.EDTA.Fe(III)), commercial urea with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ replacing EDTA and lab grade urea with commercial EDTA (Sprint 300Na.EDTA.Fe(III)). The six media formulations are summarized in Table 3.1.

Table 3.1: Nutrient media formulations to compare lab-grade and commercial nutrients. Different sources of nitrogen, phosphorus, potassium and iron were used (where phosphorus and potassium are coupled). For the different formulations only one nutrient (nitrogen, phosphorus and potassium or iron) was altered from the control formulation (M-8).

	Ingredient (g/L)	M-8	Urea (lab)	KNO ₃ (lab)	Urea (commercial)	Urea (commercial, no EDTA)	Urea (commercial, lab urea)
N source	Urea, lab		0.275				0.275
	Urea, commercial				0.55	0.55	
	KNO ₃	0.75		0.75			
P, K Source	KH ₂ PO ₄	0.185	0.1185	0.1185			
	Triple superphosphate (P source)				0.14	0.14	0.14
	Potash (K source)				0.068	0.068	0.068
	NaHPO ₄	0.065					
	MgSO ₄ ·7H ₂ O	0.1	0.109	0.109	0.109	0.109	0.109
	CaCl ₂ ·2H ₂ O	0.00325	0.055	0.055	0.055	0.055	0.055
EDTA, Fe Source	Na.EDTA.Fe	0.0025	0.02	0.02			
	FeSO ₄ ·7H ₂ O	0.0325				0.015	
	Sprint /300 Na.EDTA.Fe(III)				0.026		0.026

Media experiments were also conducted to investigate potential water sources available on and off-site of a specific coal-fired power plant site for use in a large-scale algae bioreactor. Duke Energy’s East Bend Station, located in Boone County, Kentucky, collaborated with this project by allowing a larger scale mitigation system to be implemented at their facility to further study the effectiveness of CO₂ mitigation through an algae bioreactor. There were a few on-site water sources that could serve as a substitute to using tap water, and included: well water, filtered well water, process water and boiler condensate water. The well water is a combination of waters extracted from a reservoir that is fed from seven different wells located around the property. There is also well and Ohio river water that is minimally filtered and pumped into the facility. Both of these water sources are used for non-potable duties, such as rinsing the floors and fire

prevention measures (sprinklers, hydrants, etc.). The boiler condensate is the water they use to create steam to power the turbine that condenses during the process, therefore it is essentially distilled water. There were also other off-site water sources being tested as potential media water because they have the potential of being present at power plants. The off-site testing waters included an urban stream, an agricultural stream and a pond. The urban stream water used was located in Lexington, KY in the Cane Run watershed; the sample was taken upstream from Lexmark (a local printer company) operation's discharge point. The agricultural stream was also located in the Cane Run watershed on the University of Kentucky Agricultural Experimentation Farm. This water was collected close to a horse grazing field. The pond water was collected from a pond located in front of the Gluck Equine Research Building on the University of Kentucky's campus.

Each of the water sources collected from a stream or river was sampled in a riffle. All of the water sources were collected in stackable 2-gallon high-density polyethylene pails. The research was divided into two experiments: off-site water sources and on-site water sources. Initially experiments were done without nutrient analysis and the water was only de-chlorinated. Next the water was taken to the University of Kentucky's Center for Applied Energy Research (CAER) for Total Organic Carbon (TOC) analysis and to The University of Kentucky's Environmental Research Teaching Lab to be analyzed for cations and anions.

Coupled Plasma Optical Emissions Spectrometer (ICP-OES) analysis was performed to identify and quantify the cations present in the water samples. The instrument used was a Varian Vista Pro ICP-OES under the following operating conditions; 1.2 kW power, 15 L/min plasma flow, 0.9 L/min nebulizer flow, 8 second replicate read time and 1 ppm Yttrium internal standard. The cations measured included aluminum, boron, calcium, copper, iron, potassium, magnesium, sodium, phosphorus and zinc.

The anions were identified and quantified using an Ion Chromatograph (IC) analysis. The instrument used was a Dionex ICS-2500. A combination of Na_2CO_3 and NaHCO_3 was used as the eluent at 1 mL/min with a suppressor current of 32 mA. The anions measured were nitrite, nitrate, phosphate, sulfate, chloride, fluoride and bromide.

The results from the analysis on all the water sources were used to calculate the nutrients needed in each water source. The nutrient additions to all water sources were calculated according to the urea media nutrient content to make the levels equivalent (Urea (lab) from Table 3.1). The nutrients needed for the on and off-site water sources are displayed in Table 3.2 and Table 3.3. The calculations can be found in Appendix A : Media Calculations.

Table 3.2: On-site nutrients to keep N, P, K, Mg and Fe constant at the level used in the urea recipe.

Nutrients	Tap Recipe (g/L)	Well Recipe (g/L)	Boiler Recipe (g/L)	Process Recipe (g/L)
Urea(NH ₂)CO	0.1771	0.17234	0.17875	0.17234
KH ₂ PO ₄	0.03718	0.03848	0.03848	0.03736
MgSO ₄ 7H ₂ O	0	0	0	0.03549
Na EDTA Fe	0.0065	0.0065	0.0065	0.0065

Table 3.3: Off-site nutrients to keep N, P, K, Mg and Fe constant at the level used in the urea recipe.

Nutrients	Tap Recipe (g/L)	Urban Recipe (g/L)	Ag Farm Recipe (g/L)	Pond Recipe (g/L)
Urea(NH ₂)CO	0.1771	0.1712	0.1698	0.17875
KH ₂ PO ₄	0.03718	0.0368	0.0362	0.03848
MgSO ₄ 7H ₂ O	0	0	0	0
Na EDTA Fe	0.0065	0.00599	0.0059	0.0065

3.4 Experimental Procedure

Lab vs. Commercial Media Ingredients

For the nutrient source experiments *Scenedesmus* was cultured on a shaking table (100 rpm) in triplicates in 6 different mediums at 22°C for 96 hours. The medias were prepared with city water (dechlorinated with 0.06 g/L sodium thiosulfate) and filtered through a 0.2 µm Nalgene nylon membrane filter (47 mm diameter). In the nutrient source experiments, 400 mL of each media and 15 mL of re-suspended algae were combined in a 500 mL flask and bubbled with 3% CO₂. For all of the cultures warm and

cool white fluorescent lights were used in a 16:8 hours light:dark period ($70 \mu\text{mol}/\text{m}^2$ per second). A flow meter (model VA20439, Dwyer Instrument, Inc., Michigan City, IN) and mass-flow controller (model 5850E, Brooks Instrument, Hatfield, PA) were used to regulate the CO_2 flow rate ($0.03 \text{ L gas}/\text{min}/\text{L liquid}$) and the air flow rate ($0.95 \text{ L gas}/\text{min}/\text{L liquid}$). For each flask 30 mL samples were taken at times 0, 24, 48, 72 and 96 hours (± 1 hour) to measure the dry weight and pH level. The experimental procedure for the nutrient media experiment is shown in Figure 3.1.

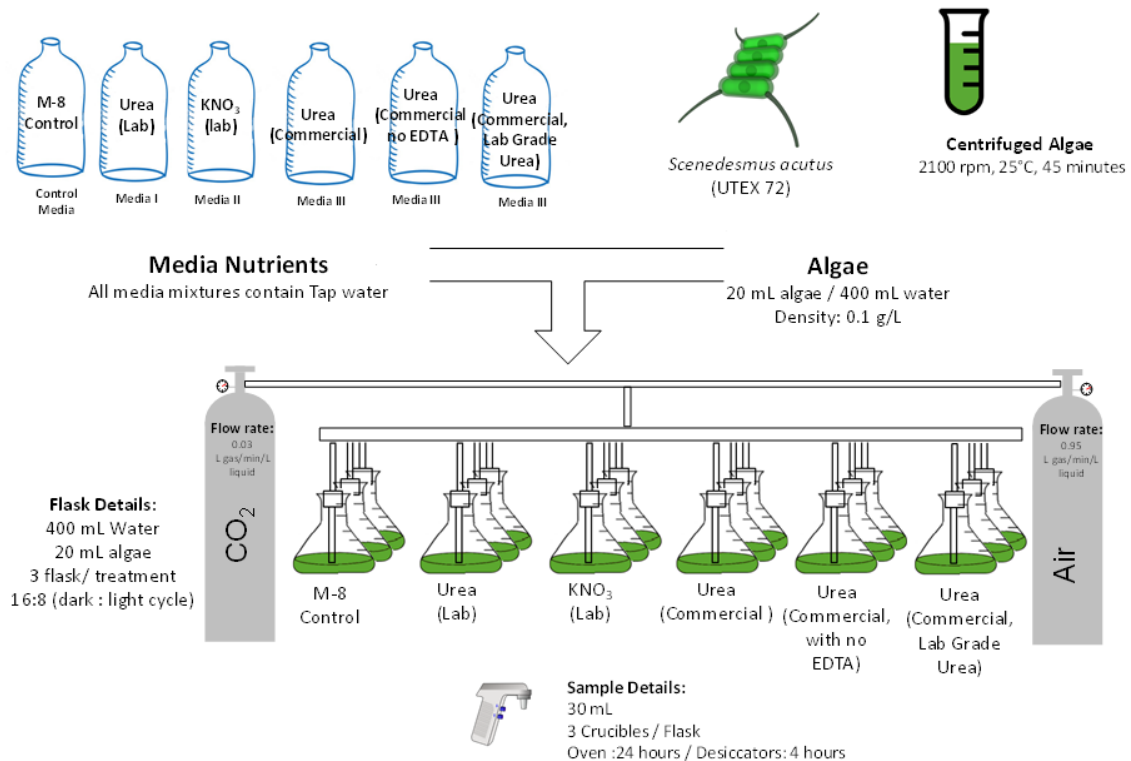


Figure 3.1: Schematic for the lab versus commercial scale nutrient experiment.

Lab vs Large Scale Water Sources

For the water source experiments, *Scenedesmus* was cultured on a shelf in triplicates in 8 different media at 22°C for 96 hours. The media were prepared with city tap-water (control), off-site water (agricultural stream water, natural stream water and pond) and on-site water sources (well water, process water and boiler condensate). One sterilization method used was dechlorinating each water with 0.06 g/L of sodium thiosulfate and

filtering the water through a 0.2 μm Whatman Nalgene nylon membrane filter (47 mm diameter, Little Chalfont, Buckinghamshire, UK). The alternative sterilization method used, in order to simulate the large-scale reactor methods, was the SteriPEN Freedom (Blue Hill, ME) handheld UV water purifier. To confirm the dependability and functionality of the SteriPEN Freedom, a preliminary test was conducted. The device's standards recommended that the SteriPEN be used for 48 seconds in a volume of 0.5 L. Based on the standard, the SteriPEN's success was tested in 400 mL samples with three replications for elapsing times including: 0 seconds, 30 seconds, 48 seconds, 90 seconds, 120 seconds, 150 seconds and 180 seconds. After sterilizing each sample, a volume of a 100 mL aliquot of each sample was transferred to a sterile bottle and colilert (Idexx colilert media, city, state) media added to each. Each sample was then poured into an IDEXX Quantitray 2000 and placed in the incubator for 24 hours. After the holding time had elapsed, the samples were examined in natural light for coliform bacteria and in UV-light for *E-coli*. The control sample that was not sterilized showed that there were bacteria present and the other samples had no bacteria present after the use of the pen thus demonstrating that the SteriPEN worked. The results for this bacteria water analysis can be found in Appendix B.

Utilizing the nutrient analysis results, nutrient additions to all water sources were calculated according to the urea media nutrient content to make the levels equivalent. In the flask-scale water source experiments, 400 mL of each media and 15 mL of re-suspended algae were combined in a 500 mL flask and bubbled with 3% CO_2 . For all of the cultures, warm and cool white fluorescent lights were used in a 16:8 hours light:dark period ($70 \mu\text{mol}/\text{m}^2$ per second). A flow meter (model VA20439, Dwyer Instrument, Inc., Michigan City, IN) and mass-flow controller (model 5850E, Brooks Instrument, Hatfield, PA) were used to regulate the CO_2 flow rate (0.03 L gas/min/L liquid) and the air flow rate (0.954 L gas/min/L liquid). For each flask, a 30 mL sample was taken at times 0, 24, 48, 72 and 96 hours (± 1 h) to measure the dry weight and pH level. After the growth tests were completed, 30 mL samples were taken from two flasks of each treatment. The alga was then spun out in the centrifuge at 2100 rpm for 45 minutes. The remaining water was analyzed for nutrients using ICP-OES and IC analysis. A schematic drawing of the water media experiment set ups are shown in Figure 3.2 and Figure 3.3.

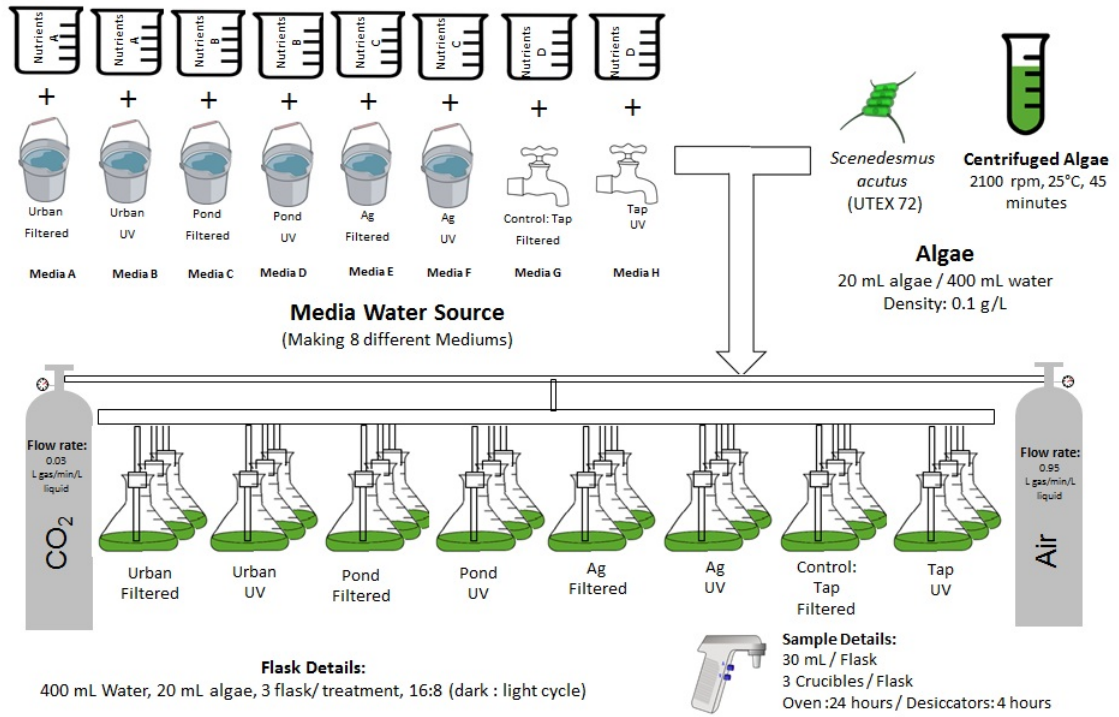


Figure 3.2: Schematic of the off-site water source media experiment.

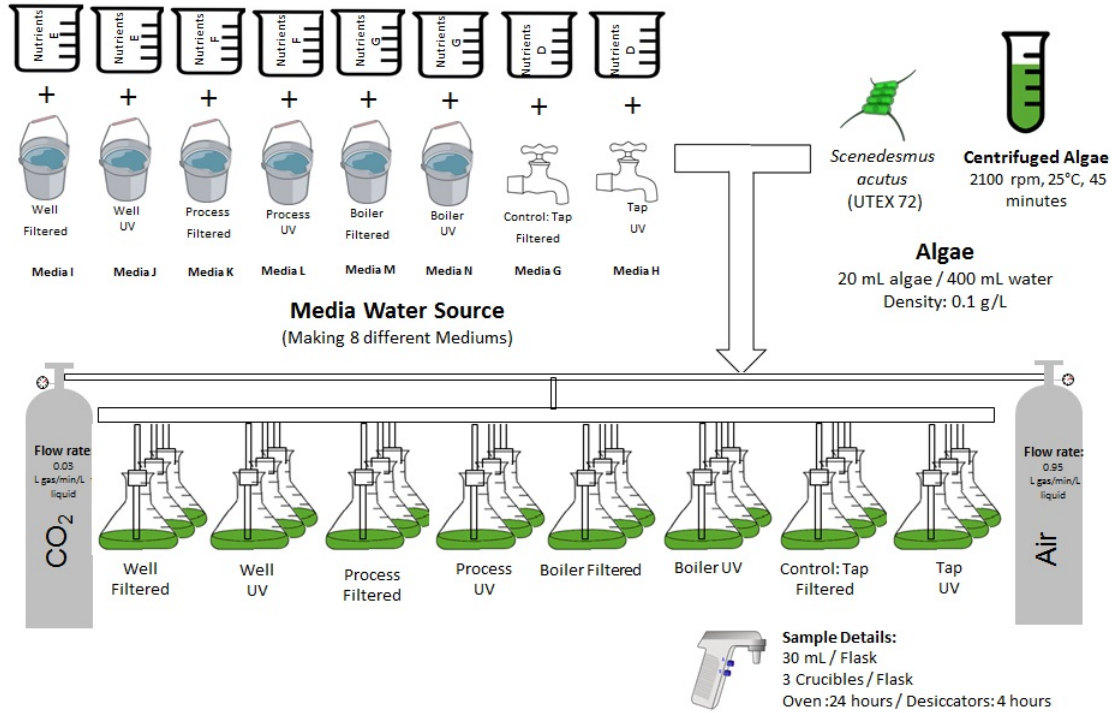


Figure 3.3: Schematic of the on-site water source media experiment.

Scaling from the Lab to the Pilot Scale

The up-scale experimental procedures were studied at two larger scales including: an air lift reactor and a portable trailer scale reactor. The scale-up investigated was 400 ml to 8 L, and finally to 188 L in the portable trailer scale reactor. The airlift reactor was placed in a temperature controlled room maintained at 22°C for week long experimental runs. The airlift consisted of 4 tubes, each with a volume of 8 L. The waters used to make the media were tap water and pond water. These were chosen because they resulted in the best performance during the flask experiments. A UV-sterilizer (Emperor Aquatics Inc. 40 Watt SMART UV-sterilizer w/1.5" union ports, Model # 02040) was used to remove contaminations from the system's input water source. The water was pumped through the sterilizer within Emperor Aquatic's recommended rate range of 157 GPH- 262 GPH. In the initial inoculation of the airlift-scale water source experiment, 7.5 L of each media (sterilized tap and pond water) and 200 mL of re-suspended algae were combined in an 8 L capacity airlift and bubbled with 3% CO₂. For all of the airlifts, LED lights (Hort America - GreenPower LED production, module DR/B 120_110V) were used in a 16:8

hours light:dark period ($1016 \mu\text{mol}/\text{m}^2$ per second). A flow meter (model VA20439, Dwyer Instrument, Inc., Michigan City, IN) and mass-flow controller (model 5850E, Brooks Instrument, Hatfield, PA) were used to regulate the CO_2 flow rate ($0.007 \text{ L gas}/\text{min}/\text{L liquid}$) and the air flow rate ($0.177 \text{ L gas}/\text{min}/\text{L liquid}$). Sampling was done each day for each tube by draining 1 liter of the culture and taking a 30 mL well-mixed sample. Using this sample the dry weight and pH were measured. The remainder of the 1 L sample was returned to the air lift reactor.

The air-lifts and the trailer were illuminated with LEDs, while the flasks were illuminated with fluorescent lights. Hence, the size or the light source could result in a change in the growth rate. For this reason, a light testing experiment was performed to compare the growth rate of algae grown in the flasks with illumination from both the LEDs and the fluorescent lights. In each 500 mL flask, 400 mL of media and 50 mL of re-suspended algae were combined and bubbled with $0.03 \text{ L CO}_2/\text{min}/\text{L liquid}$. For half of the cultures LED lights ($1016 \mu\text{mol}/\text{m}^2$ per second) and the other half fluorescent lights. Both lighting types used a 16:8 hours light:dark period. The same flow meter and mass-flow controller as before were used to regulate the CO_2 flow rate ($0.03 \text{ L gas}/\text{min}/\text{L liquid}$) and the air flow rate ($0.95 \text{ L gas}/\text{min}/\text{L liquid}$). For each flask, 30 mL samples were taken at times 0, 24, 48, 72 and 96 hours ($\pm 1 \text{ h}$) to measure the dry weight and pH level.

The air-lifts and the trailer had an initial CO_2 flow rate of $0.007 \text{ L CO}_2/\text{min}/\text{L liquid}$, while the flasks' initial CO_2 flow rate was $0.03 \text{ L CO}_2/\text{min}/\text{L liquid}$. For the CO_2 flow rate testing experiment, 400 mL of media and 40 mL of re-suspended algae were combined in a 500 mL flask and bubbled with the larger scales CO_2 flow rate ($0.007 \text{ L CO}_2/\text{min}/\text{L liquid}$). The air flow rate was increased to maintain the flask overall flow rate (CO_2 flow rate + Air flow rate) in order ensure proper mixing. For all of the cultures, warm and cool fluorescent lights were used in a 16:8 hours light:dark period ($70 \mu\text{mol}/\text{m}^2$ per second). A flow meter and mass-flow controller were used to regulate the CO_2 flow rate ($0.007 \text{ L CO}_2/\text{min}/\text{L liquid}$) and the air flow rate ($0.973 \text{ L gas}/\text{min}/\text{L liquid}$). For each flask, 30 mL samples were taken at times 0, 24, 48, 72 and 96 hours ($\pm 1 \text{ h}$) to measure the dry weight and pH level.

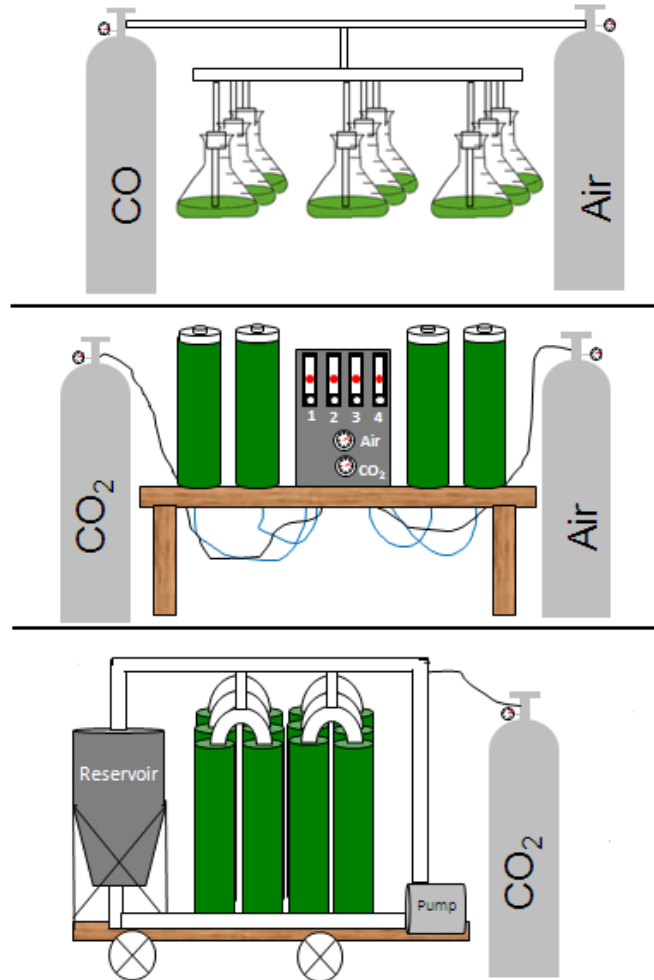


Figure 3.4: Schematic of the different experimental scales: lab, air-lift and trailer reactor.

The largest scale-up tested was the trailer algae bioreactor. The trailer reactor has a volume of approximately 188 L. Measurements of pH and dry weight were taken daily at each of these scales. Figure 3.4 shows the upscaling experiments schematics. As in the airlift reactor, the water was pumped through the UV-sterilizer (Emperor Aquatics Inc. 40 Watt SMART UV-sterilizer w/1.5" union ports, Model # 02040) to remove contamination.

In the initial inoculation of the trailer-scale water source experiment 7.5 L of each media (sterilized tap and pond water) 200 mL of re-suspended algae were combined in an 8 L capacity airlifts and bubbled with 3% CO₂. Three of the airlift inoculums were needed for

the trailer. The lab grade nutrient recipe was converted to the industrial grade fertilizers (conversion details can be found in Appendix A).

Table 3.4: Trailer reactor industrial grade nutrient recipe (for 188 L).

Nutrient	Amount (g)
Urea	28.02
Potash	3.67
Triple Super Phosphate	7.55
Sprint 330	0.24

LED lights (module DR/B 120_110V, Hort America - GreenPower LED production) were the light source used for the trailer reactor, operating at 16:8 hours light:dark period (1016 $\mu\text{mol}/\text{m}^2$ per second).

A flow meter (model VA20439, Dwyer Instrument, Inc., Michigan City, IN) was used to regulate the CO_2 flow rate (0.007 L CO_2 / min/ L fluid). In contrast to the smaller scales, air was not followed into the system; a pump (model 2ZWR6, Grainger, Minooka, IL) was used instead to prevent settling. The pump was connected to a variable frequency drive (GS1-10P2 model, Automation Direct) operating at 35 Hz. To sample, 2 L of the culture were drained, and from that a 30 mL sample was taken daily to measure the dry weight and pH level, then the remainder of the sample was returned back to the trailer culture.



Figure 3.5: Picture of the trailer scale reactor, 188 L total volume.

Chapter 4. Lifecycle Assessment

4.1 Introduction

In the United States, coal produces close to 56% of all utility provided electricity (Energy, July 1998). One of the resultants of coal-fired power plants is greenhouse gases, including CO₂. With a growing need for energy consumption and a large dependence on coal, interest is rising to investigate ways to mitigate CO₂ emissions to the atmosphere.

Microalgae reactor systems are one carbon capture method being explored to assist coal-fired power plants in decreasing their environmental impact. A variety of experiments have demonstrated algae's abilities to perform as a buffer for greenhouse gas emissions (GHGE). Although, an important factor to consider when designing this system is the environmental impacts of the inputs. Performing a life cycle assessment is required to identify the paramount inputs through the quantification of the emissions produced. This lifecycle analyzes the inputs related to the media needed for algae growth including water and nutrients.

4.2 Goal and Scope

The objective of this analysis is to examine the energy consumption necessary for media preparation as a component of an operating industrial scale algae CO₂ mitigation system. An evaluation was conducted of the net carbon dioxide emissions released for different media formulations being used in an algae-based system with the objective to mitigate CO₂ emissions from a coal-fired power plant.

The scope of this lifecycle assessment encompasses the energy and emissions for preparing the algae media. The boundaries of the system, shown in Figure 4.1, include the coal-fired power plant, algae photobioreactors (PBR), nutrient supply and water sourcing. One day of operation was selected as the functional unit because the power plant will function daily.

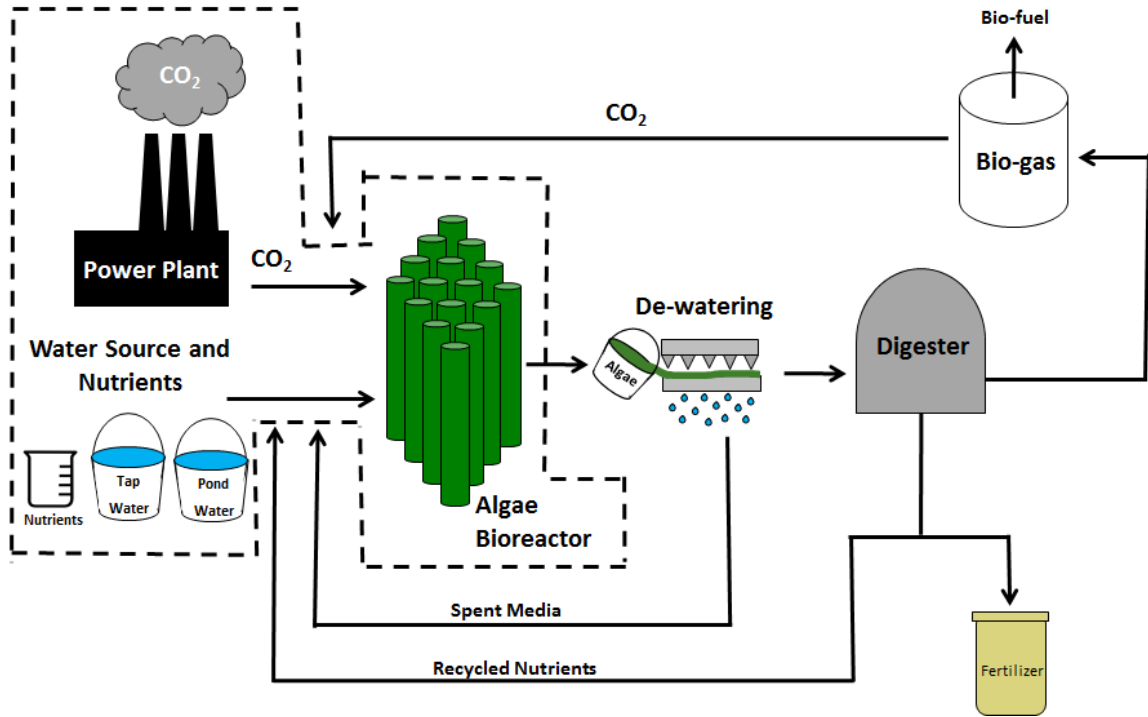


Figure 4.1: Drawing of the overall process being considered. The dotted line shows the boundary of the lifecycle assessment analysis.

In the dotted line represents the boundary line for this analysis. This study will be focused on the comparison of two different water sources, tap and pond water, for coal-fired power plant CO₂ mitigation. The diagram displays this study's areas of interest in the overall process.

4.3 Inventory

The reactor being considered in this LCA is structured as a closed environment multi-tube photobioreactor. This project's intention was to mitigate 5% of the power plant's CO₂ emissions, calculated to be approximately 36.4 metric tons/day. The volume of water needed to achieve this level of CO₂ mitigation was computed contingent upon the algae's (*Scenedesmus*) growth rate and the power plant's size. Research has shown that the algae strain *Scenedesmus* can grow at a rate of about 0.15 g/L/day (Crofcheck, 2013). This assessment's calculations were based on a small (30 MW capacity) Kentucky coal-fired power plant that is assumed to run 350 days per year 24 hours a day. From these parameters the needed yearly water volume was estimated to be 132,366 m³.

Nutrients are an essential component to maintain healthy algae growth. It was assumed that commercial fertilizers used in agriculture are the nutrient sources used for algae in a large scale photobioreactor. The fertilizers used to provide the algae with the necessary nutrients were urea, potash, Sprint 330 and Triple Super Phosphate (TSP). This analysis was performed based on using the media formulations in Table 4.1 and an algae growth test that showed when doubling the pond water nutrient recipe for the tap water the algae grew closer to the same rate for both water sources as shown in Figure 4.2.

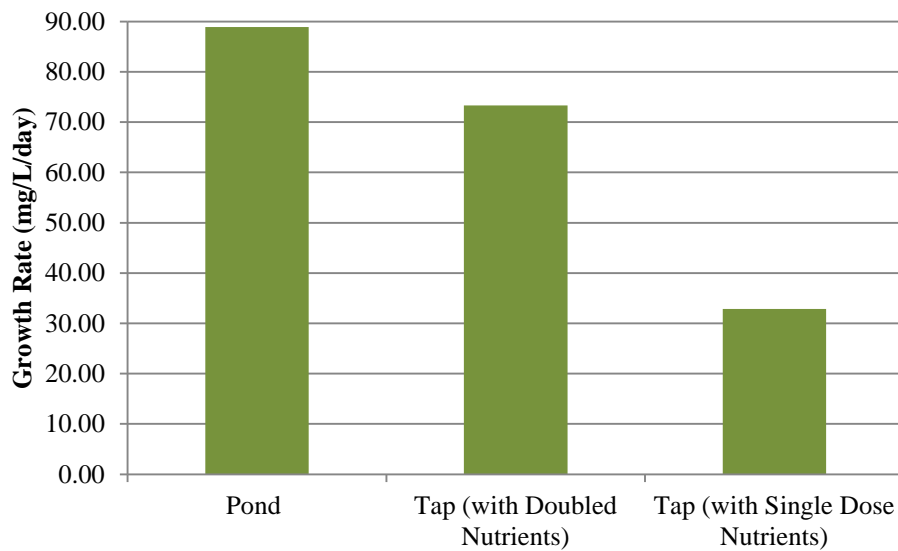


Figure 4.2: Growth rates for algae grown with pond and tap water, where nutrients levels were based on air-lift experiments.

Table 4.1: Commercial grade nutrient levels needed for algae growth in tap and pond water.

	Urea	Potash	Sprint 330	TSP
Tap (g/L)	0.296	0.036	0.00234	0.074
Pond (g/L)	0.148	0.018	0.00117	0.037

Manufacturing fertilizers is energy intensive therefore environmental pollutants must be considered. The energy needed and CO₂ emitted from the media make-ups were estimated in this study (Table 4.3). The energy and emissions of Sprint 330 were

neglected due to the negligible amount needed. Argonne National Lab’s GREET Model was used to estimate the other energy input and CO₂ output levels. Although Triple Super Phosphate’s levels were not listed in the GREET Model it did include Phosphate’s (P₂O₅) and CaCO₃’s. This information in combination with the fact that TSP is 45% P₂O₅ and 15% CaCO₃, the emissions and energy were approximated.

Table 4.2: Energy and emissions levels associated with each of the nutrients considered in the analysis (Wang, 2008).

Ingredients	Energy (mmBTU/ ton)	CO₂ (g CO₂/ton)
Urea	46	1,327,641
Potash	8	600,559
Sprint 330 EDTA	Negligible	Negligible
TS Phosphate	8	620,610
Phosphate (P₂O₅)	12	891,170
CaCO₃	7	548,379

From these emissions estimates the nutrient emissions were calculated for each water type for this analysis, summarized in Table 4.4.

Table 4.3 : Energy and emissions for each of the nutrients based on water type.

Water Type	Pond (W-hr/day)	Tap* (W-hr/day)	Pond (kg CO₂ /day)	Tap (kg CO₂ /day)
Urea	797	1,594	79	158
Potash	16	32	4	9
TSP	36	72	9	18
TOTAL	849	1,698	92	185

* Twice the level of nutrients.

The individual water sources must also be considered for their environmental impacts, outside of the nutrients. The total water amount included the consideration of recycling water from the reactor harvest volume (approximately 17,264 m³). Also the evaporation volume of water was calculated to be 2,251 m³/day. The energy requirement to pump this volume was assumed to be the friction in the pipe. This value was calculated by the following equation (Daneshmand et al., 2012):

$$g = \frac{\Theta A(XS - X)}{3600}$$

Where,

g = amount of water evaporation

$\Theta = (25 + 19 V)$ = evaporation efficient (kg/m²h)

V = velocity of air above water surface (m/s)

XS = humidity ratio in sat.air at water surface temperature (kg/kg) (kg H₂O in kg dry air)

X = humidity ratio in air (kg/kg) (kg H₂O in kg dry air)

After calculating the values this volume of water had insignificant effects. Therefore, the emissions and energy required for the pond water being pumped in were disregarded.

The impacts for the tap water were considered. Tap water had an estimated 0.00521 kg CO₂/gallon CO₂ emission rate and an energy consumption rate of 0.00163 kWh/gallon (Dettore, 2009). From these estimates and the water needed, an approximation of the tap water daily energy and emissions were calculated. Tap water was estimated to emit 499 kg CO₂ per day and use approximately 6.9 kW of energy per day.

4.4 Financial Comparison

Capital assessment is an important subject of consideration in upscaling a process. For this analysis estimates for the costs of media ingredients and waters were calculated.

Examining the costs could help to decide if the environmental impacts in the assessment fell into a close range.

The media nutrients cost were estimated based on the price of the fertilizers. Each rate was recorded from a selling supplier (Table 4.4). The nutrients costs were approximated from these rates and the necessary nutrients needed for each of the two water sources.

Table 4.4: Cost summary and estimation source for each commercial grade nutrient based on the water type.

Fertilizer	Cost (\$/lb)	Tap (\$/day)	Pond (\$/day)	Source
TSP	0.47	30	15	Southern States
Potash	0.39	12	6	Southern States
Urea	0.36	88	44	Southern States
Sprint 330	6.6	12	6	Becker Underwood

Pond water was considered to be free while tap water was based on the assumption that the water company's price rate is \$2.00 per 1,000 gallons (EPA, 2009). The tap water cost was estimated to be \$191 per day.

4.5 Results and Discussion

After analyzing the individual pieces of this analysis it is evident that pond water has lower environmental and energy impacts. The total energy required with pond water was 1.5 kW/day, while the tap water required 9.5 kw/day. The total CO₂ emissions with pond water was 92 kg/day, while the tap water was 684 kg/day. In addition the cost of using tap water exceeds the pond water. This fact also makes the pond water the more viable option from a life-cycle assessment vantage. The resulting overall cost for using pond water was 71 \$/day, while the cost with tap water was 333 \$/day.

In summary, the pond water appears to be the more economically and environmentally sustainable option from the two water sources. This analysis provides support for further investigation of alternative water sources as the media ingredient to supply optimal results of the overall goal of minimizing CO₂ emissions. The use of pond water as a replacement to tap water could save money and reduce the environmental impact of growing algae at an industrial scale. Table 4.5 summarizes the energy, emissions and cost of the two water sources.

Table 4.5: Life cycle analysis results including energy, emissions, cost and CO₂ mitigation cost.

	Units	Tap	Pond
Total Energy Required	kW/s	13,680	2,160
Total CO₂ Emissions	kg of CO ₂ /day	684	92
Overall Cost	(\$/day)	333	71
CO₂ Mitigation Cost	\$/ton of CO ₂ Captured	9	2

Chapter 5. Results and Discussion

5.1 Lab and Industrial Grade Nutrients

On a commercial scale, the cost of media nutrients will be a significant portion of the overall costs. Hence, it's important to make sure that each component is necessary and cost effective. In the literature, M-8 is a popular media for lab-scale cultivation. M-8 utilizes KNO_3 as its nitrogen source, which can be expensive compared to other nitrogen sources such as urea. Therefore, a urea media was developed and shown to perform as well as the M-8 media on a laboratory scale with laboratory grade nutrients (Crofcheck et al. 2013). In this work, the performance of the urea media with commercial grade nutrients was evaluated, comparing different nitrogen, phosphate, potassium, EDTA and iron sources at the lab and commercial scales.

The growth curves, pH during cultivation, and growth rates for the six different media are shown in Figure 5.1, Figure 5.2 and Figure 5.3. When comparing the M-8 (lab) and Urea (lab), the growth rates are similar, as expected (Crofcheck et al. 2013). For comparing Urea (lab) and KNO_3 (lab), there was no effect of using KNO_3 in place of urea in the Urea recipe (P-value = 0.27). Comparing Urea (lab) and Urea (commercial) shows there is not a significant effect of using lab grade nutrients versus commercial grade (P-value = 0.051). Interestingly, when comparing M-8 (lab) to Urea (commercial), the Urea performed significantly better. One of the most expensive components of the media recipe is the EDTA, which is intended to make sure the iron in the media is biologically available. However, since EDTA doesn't always exist in natural waters, it is thought that the algae may be able to chelate the iron without the aid of EDTA. There was a significant difference between Urea (commercial) with and without EDTA (P-value = 0.181). Finally, in order to determine whether it was the change in nitrogen source or phosphorus and potassium (these are coupled in the commercial ingredient, such that the individual effects could not be tested) the lab grade urea was used with the rest of the Urea (commercial) ingredients. Comparing the two results, the media with the lab grade urea performed did not perform better than the media with the less expensive commercial urea (P-value = 0.07). Overall the results showed that urea commercial media performed best with no EDTA added.

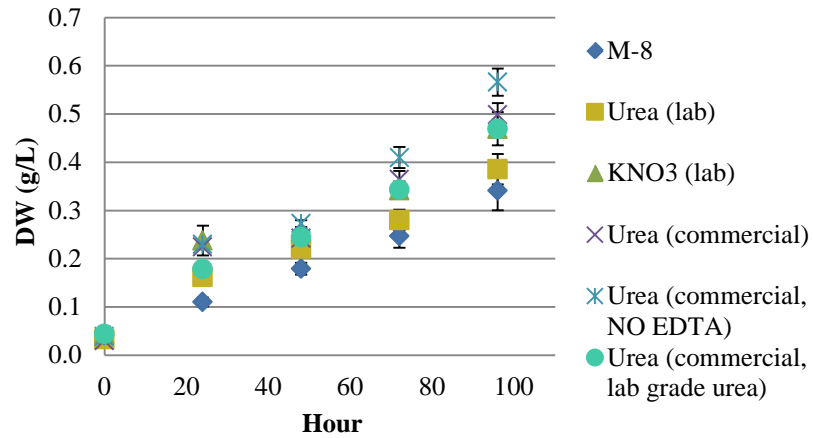


Figure 5.1: Dry weights during a 5-day cultivation using lab grade and commercial nutrients. Error bars represent standard error (n=3).

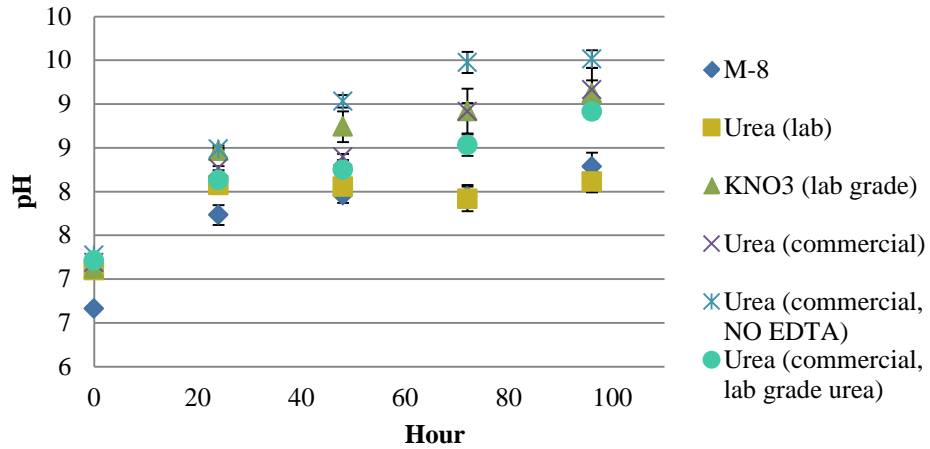


Figure 5.2: Cultivation pH measurements using lab grade and commercial nutrients. Error bars represent standard error (n=3).

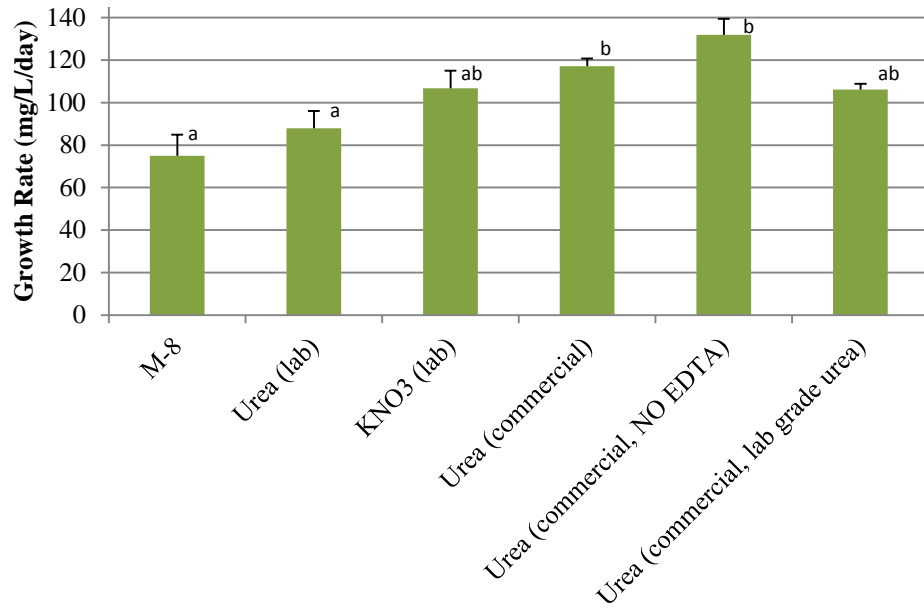


Figure 5.3: Growth rates using lab grade and commercial nutrients. Error bars represent standard error (n=3). Treatments with the same letter are not significantly different ($\alpha=0.05$).

The letters in Figure 5.3 represent the results of a pairwise t-test. The results show that the growth rates from M-8, Urea (lab), KNO₃ and Urea (commercial, lab grade urea) are considered statistically not different. Yet, Urea (commercial) and Urea (commercial, NO EDTA), are statistically not different from each other but statistically different from the other formulas. The results also show that the growth rates for KNO₃, Urea (commercial, lab grade urea), Urea (commercial) and Urea (commercial, NO EDTA) are not statistically different while, M-8 and Urea (lab) are statistically not different from each other but are statistically different from the other formulas.

5.2 Water Source and Sterilization Methods

5.3 On-site Water Sources

Initially on-site water source experiments were done with the full Urea recipe added to each source. One experiment compared the control Urea recipe, East Bend's well and condensed boiler waters. The well water performed the best based on dry weight content. The well water performed better because it contained nutrients that were not present in

the condensed boiler water. The condensed boiler water was essentially de-ionized water and therefore did not contain any nutrients that could help increase algae growth. Figure 5.4 shows the results graphical for dry weight of all water types over time.

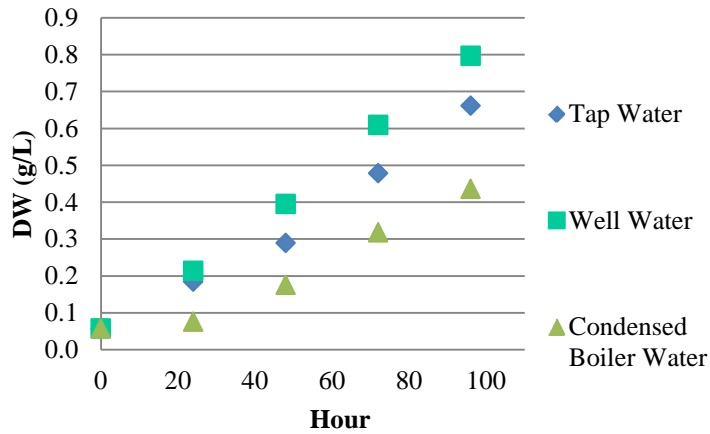


Figure 5.4: Dry weights for various on-site water sources (including tap, well and condensed boiler water), where the same amount of nutrients were added to each water type.

Also on-site well water and filtered well water sources were tested with full recipe nutrients added to compare their growth potential as media water. The filtered well water performed the best based on dry weight content; Figure 5.5 shows the results from this experiment.

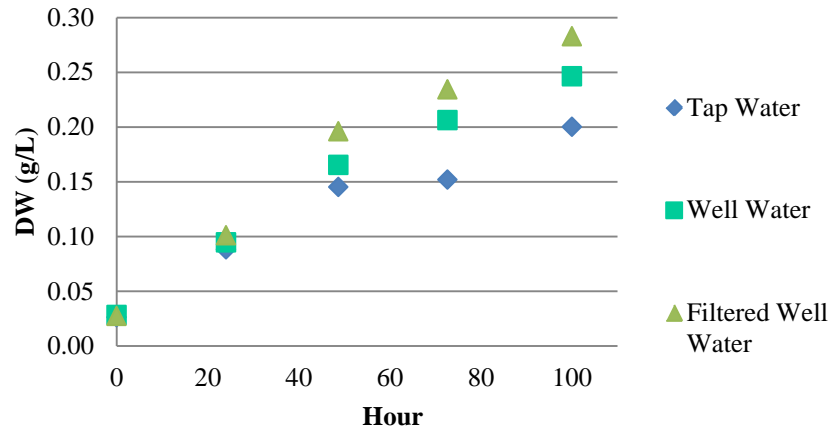


Figure 5.5: Dry weights for various on-site water sources (including tap, well and filtered well water), where the same amount of nutrients (full Urea recipe) were added to each water type.

After growth tests were performed with on-site water sources using the full Urea recipe the nutrient analysis was done on the available on-site waters including: tap, well, process and boiler. From this analysis nutrient additions were adjusted to the Urea media formula. In this way, the effect of the recipe ingredients should be the same for all treatments and the differences will be based on other ingredients in the waters.

Figure 5.6, Figure 5.7 and Figure 5.8 display the graphical results of dry weight, pH and growth rate comparisons.

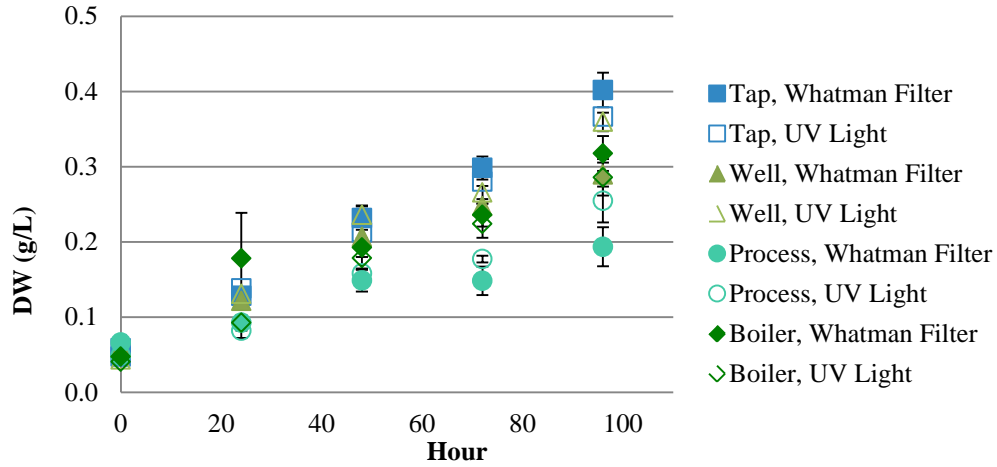


Figure 5.6: Dry weights during a 5-day cultivation for algae growth using on-site water sources with customized nutrients added based on the water type. Error bars represent standard error (n=3).

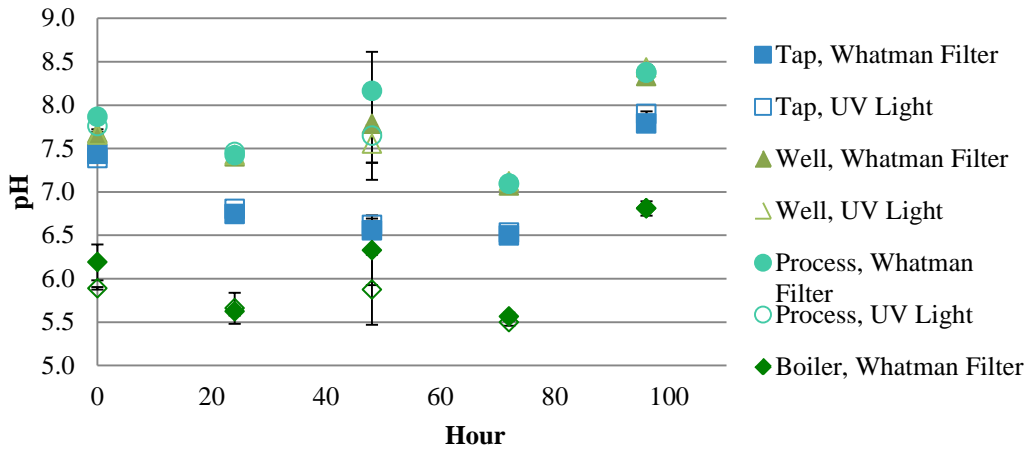


Figure 5.7: Cultivation pH measurements for algae growth using on-site water sources with customized nutrients added based on the water type. Error bars represent standard error (n=3).

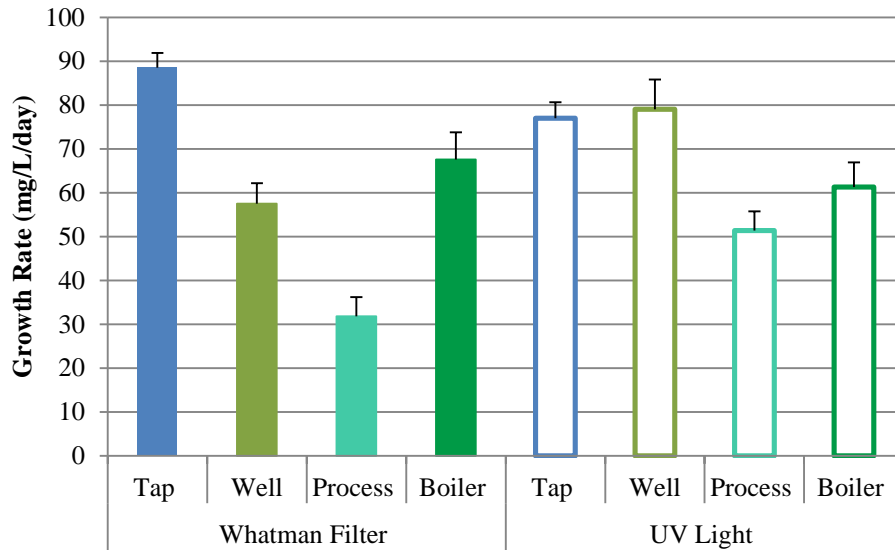


Figure 5.8: Growth rates for algae growth using on-site water sources with customized nutrients added based on the water type. Error bars represent standard error (n=3).

The tap water outperformed the on-site water sources when using the Whatman filter sterilization method. Both the well and process waters showed higher growth rates when using the UV light. These waters are likely to have other nutrients in them that increase algae growth, those nutrients get filtered out when using the Whatman filter. The process water is essentially de-ionized water and likely contained no additional nutrients. Therefore the process water demonstrated the lowest growth rate for both types of filtration. It was assumed based on testing that the UV light does kill present bacteria, yet the dead bacteria were still present and have a weight. After testing the UV light and recording dead bacteria weight differences the dead bacteria weights were considered insignificant. An area of further consideration would be if the dead bacteria were serving as a carbon source to the algae. This would be undesirable for the overall objective of mitigating CO₂ from coal-fired power plants.

The statistical analysis for this experiment was determined by performing a two-factor with replication ANOVA, shown in Table 5.1. The statistics provided evidence that there was not a significant difference between the on-site water sources. The ANOVA also projected that there was a statistical difference between the sterilization methods and the

interaction between the water type and sterilization was significant. It appeared that the UV sterilization allowed for the most growth.

Table 5.1: On-site sources ANOVA, based on growth rates (Figure 5.8), including tap water.

Source of Variation	SS	df	MS	F	P-value	F crit
Water Source	187	1	187	2.50	0.13	4.49
Sterilization	5202	3	1734	23.12	4.64E-06	3.24
W*S¹	1184	3	395	5.26	0.01	3.24
Error	1200	16	75			
Total	7774	23				

¹The interaction between the water source and sterilization method.

In addition, another two factor with replication ANOVA was performed on the on-site water sources, excluding the tap water. The results can be found in Table 5.2. Comparing these results to the first ANOVA above, the tap water made the small changes between the water sources not noticeable when included in the analysis. Therefore, when excluding the tap water, a significant difference exists between algae grown in the different on-site water sources. While there was no longer a significant interaction between the water source and sterilization method there still remained a significant effect of the sterilization method alone.

Table 5.2: On-site sources ANOVA, based on growth rates (Figure 5.8), excluding tap water.

Source of Variation	SS	df	MS	F	P-value	F crit
Water Source	552	1	552	6.28	0.03	4.75
Sterilization	2603	2	1302	14.81	5.75E -04	3.89
W*S¹	643	2	322	3.66	0.06	3.89
Error	1055	12	88			
Total	4853	17				

¹The interaction between the water source and sterilization method.

5.4 Off-site Water Sources

Initially the off-site sources with no additional nutrients added were tested. One experiment compared the control urea recipe, pond water, urban stream water and agricultural stream water. The urban stream performed the best based on dry weight content. Figure 5.9 shows the results graphical for dry weight of all water types over time.

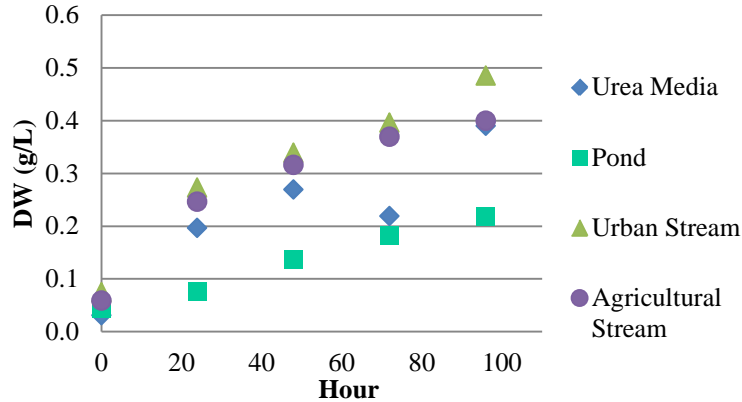


Figure 5.9: Dry weights for urea media and various off-site water sources (including pond, urban stream and agricultural stream), where no nutrients were added to the off-site waters.

After identifying that alga could prosper in the waters, an experiment with adjusted nutrients additions was conducted. The amount of nutrients added was based on water analysis performed. All waters' nutrients, including the tap water, were matched to match the full Urea recipe's nutrient loadings. The graphical results and growth rate comparisons are displayed in Figure 5.10, Figure 5.11 and Figure 5.12.

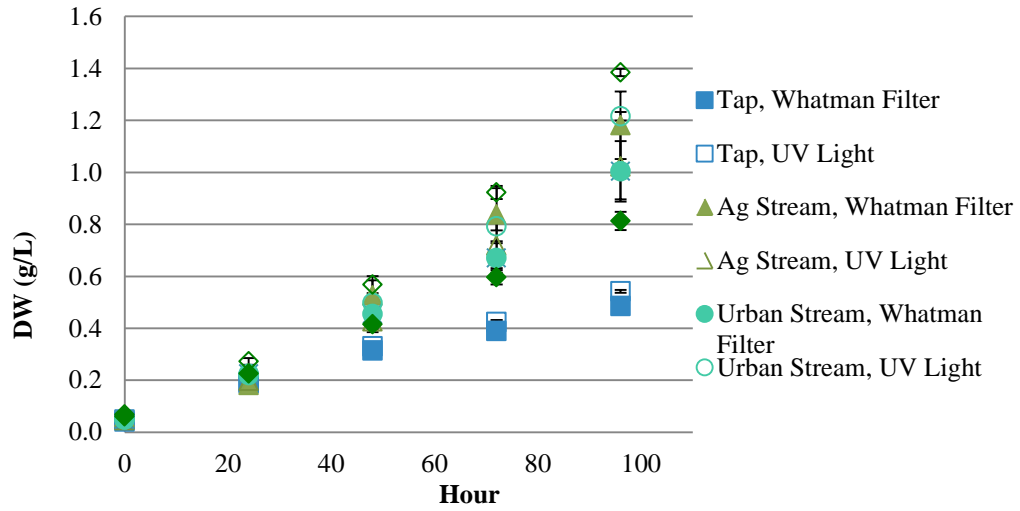


Figure 5.10: Dry weights during a 5-day cultivation using off-site water sources with nutrients added based on the water type. Error bars represent standard error (n=3).

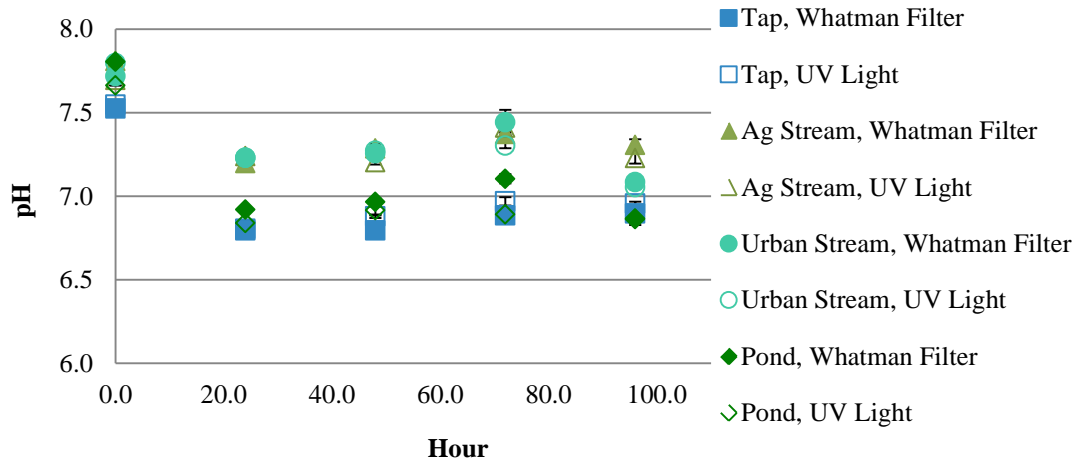


Figure 5.11: Cultivation pH measurements for algae growth using off-site water sources with customized nutrients added based on the water type. Error bars represent standard error (n=3).

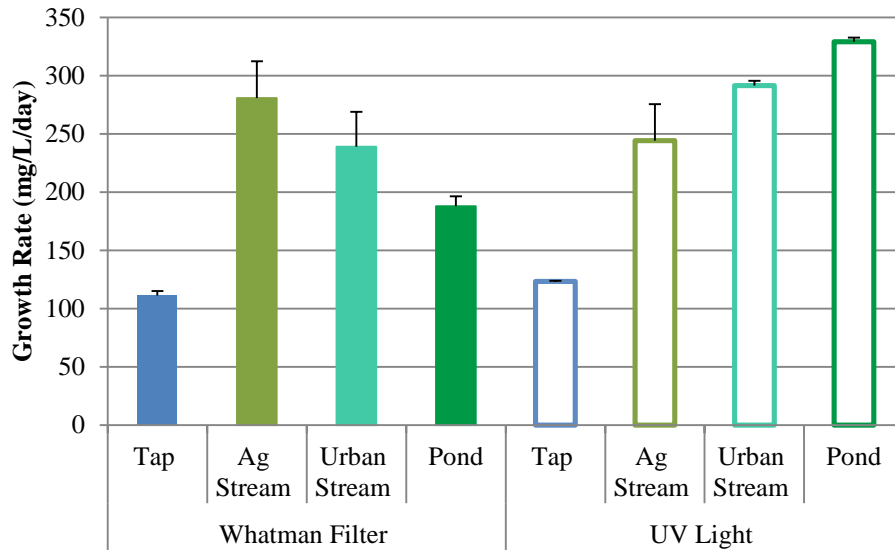


Figure 5.12: Growth rates for algae growth using off-site water sources with customized nutrients added based on the water type. Error bars represent standard error (n=3).

Figure 5.12 shows that the off-site water sources outperformed the tap water by both methods of sterilization, filtering and the UV light. The natural waters are likely to have other nutrients, such as micro-nutrients, in them that increase algae growth. The trend is different between the growth rates of the Whatman filtered off-sites sources and the UV light off-site sources. The urban stream and the pond water both had higher growth rates when the UV light was used instead of the filter. While the filter may have removed contaminants in addition it may have removed other growth promoting nutrients. Bacteria testing show that UV light does kill present bacteria, yet the dead bacteria were still present. . The dead bacteria weight was considered insignificant after testing the UV light and measuring the dead bacteria dry weight differences.

A two-factor with replication ANOVA statistical analysis was done for this experiment. The results are shown in Table 5.3. The results showed that there was a significant difference (P-value < 0.05) in the growth of algae by using different off-site water sources. All of the off-site waters appeared to work better than the tap water. There was also a significant difference found in the sterilization method used, making the UV sterilizer appear to work better. In addition the interaction was significant between the sterilization and water source used.

Table 5.3: Off-site sources ANOVA, based on growth rates (Figure 5.12), excluding tap water.

Source of Variation	SS	Df	MS	F	P-value	F crit
Water Source	10897	1	10897	9.69	6.69E -03	4.49
Sterilization	95240	3	31747	28.23	1.26E-06	3.24
W*S	26495	3	8832	7.85	1.91E -03	3.24
Error	17990	16	1124			
Total	150621	23				

¹ The interaction between the water source and sterilization method.

To better analyze the off-site sources another two factor with replication ANOVA was conducted on the off-site water sources, excluding the tap water. The results can be found in Table 5.4. In contrast to the first off-site water sources' ANOVA the effect of the sterilization was no longer significant (P-value > 0.05). The water sources were still significantly different, with the pond water performing the best. There also still remained a significant interaction between the water source and sterilization method interaction.

Table 5.4: Off-site sources ANOVA, based on growth rates (Figure 5.12), including tap water.

Source of Variation	SS	Df	MS	F	P-value	F crit
Water Source	12511	1	12511	8.39	0.01	4.75
Sterilization	94	2	47	0.03	0.97	3.89
W*S	24654	2	12327	8.27	5.53E -03	3.89
Error	17894	12	1491			
Total	55154	17				

¹ The interaction between the water source and sterilization method.

5.5 Upscale Comparisons

Review literature shows that algae bioreactor upscaling methodologies have not been well defined. There are several factors that can be variable when transitioning from a lab scale to a larger industrial scale. This research focused on three scales including; laboratory flask, airlift system and a portable trailer reactor (Table 5.5).

Table 5.5: Summary of various conditions for the upscaling studies. Warm and cool fluorescent lighting ($70 \mu\text{mol}/\text{m}^2$ per second) and LED lighting ($1016 \mu\text{mol}/\text{m}^2$ per second) was used in a 16:8 hours light:dark period

Reactor	Liquid Volume	CO ₂ flow rate (L gas/min /L liquid)	Air flow rate* (L gas/min /L liquid)	Lighting
Flasks	400 mL/flask	0.03	0.95*	Fluorescent
Flasks	400 mL/flask	0.03	0.95*	LED
Flasks	400 mL/flask	0.007	0.973*	Fluorescent
Airlift	8 L/tube	0.007	0.177*	LED
Trailer	188 L total	0.007	**	LED

* used for mixing, **mixing achieved due to pumping

5.6 Airlift Pond and Tap Water Experiment

The first scale up from the flasks was to an air lift system. The airlift reactor was built in a temperature controlled room maintained at 22°C and where the algae were cultivated for seven days before being harvested. The airlift consisted of 4 tubes, each with a volume of 8 L. The water media used, pond water, was chosen after the performance levels were observed for the various on and off-site sources selected. This experiment observed algae growth differences between pond water and tap water in an airlift system. The graphical results for dry weight and pH are displayed in Figure 5.13 and Figure 5.14.

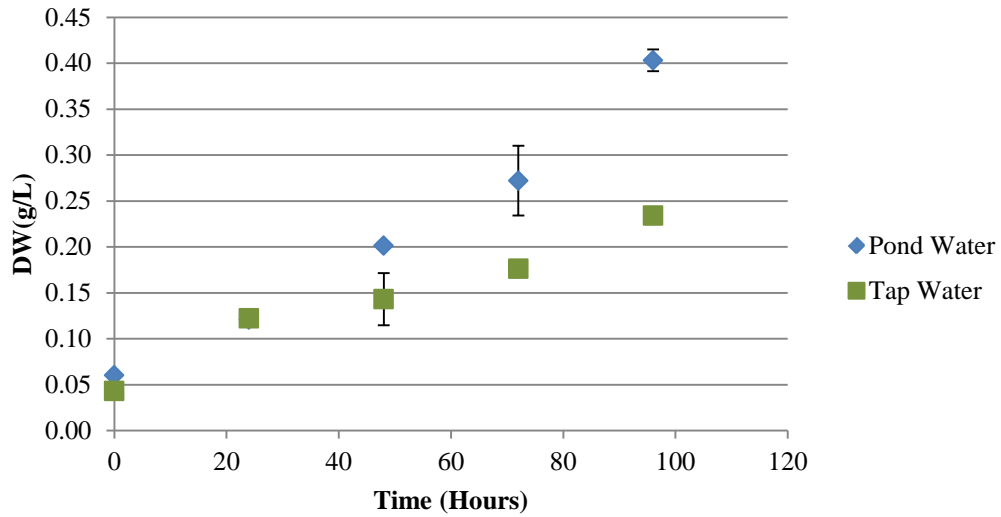


Figure 5.13: Dry weights during a 5-day cultivation using pond and tap at the airlift scale. Nutrients added were customized based on water type. Error bars represent standard error (n=3).

From the dry weight chart the pond water reached a higher biomass accumulation than the tap water during the 96 hours experiment.

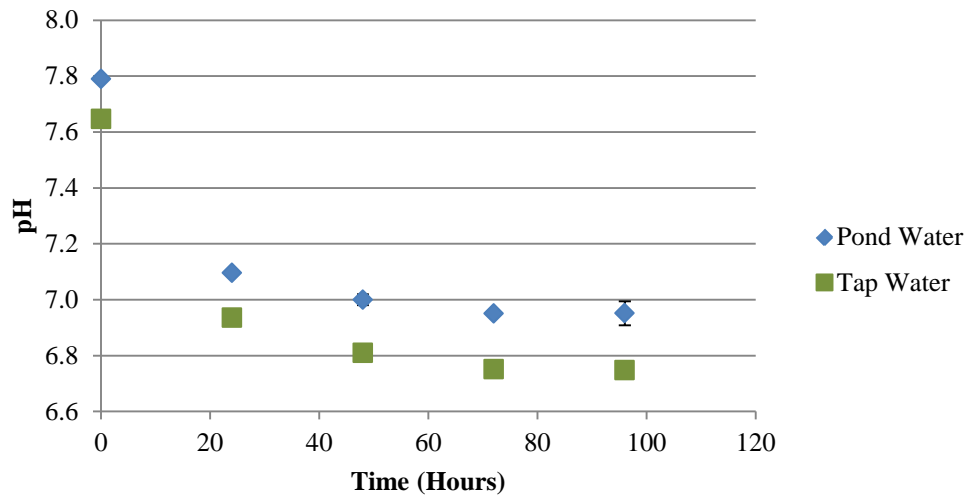


Figure 5.14: Cultivation pH measurements of algae grown in pond and tap water at the airlift scale. Error bars represent standard error (n=3).

The pH chart shows that for both water types the pH decreases and levels off at a stabilized range. This situation does not typically occur at the flask scale. Generally in the flask the pH increases and then levels off.

Reasoning for this is likely due to the upscaling size change. The algae are taking longer to consume all of the CO₂ because of scale differences, such as the light availability; therefore growth is not as quickly provoked.

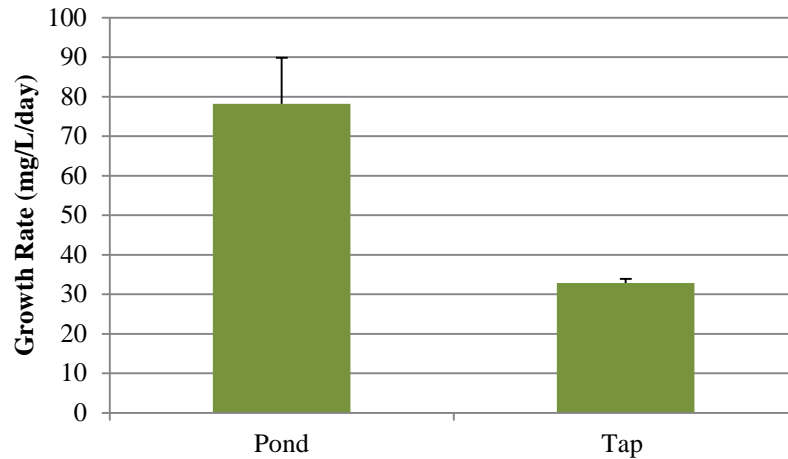


Figure 5.15: Growth rates of algae grown in pond and tap at the airlift scale. Error bars represent standard error (n=3).

A single factor ANOVA statistical analysis was done for this experiment. The results are shown in Table 5.6. The results showed that there was a significant difference (P-value < 0.05) in the growth rates of algae grown in pond versus tap water. The pond water showed greater growth than the tap water.

Table 5.6: Airlift pond and tap water ANOVA results based on algae growth rates (Figure 5.15).

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2720	1	2720	13.21	0.02	7.71
Within Groups	823	4	206			
Total	3543	5				

5.7 Flask LED and Fluorescent Light Experiment

Following the upscaling airlift testing the lighting (LED and fluorescent) regimes were studied. This test helped to clarify if growth variations were caused from a difference in

the lighting. Figure 5.16, Figure 5.17 and, Figure 5.18 display the dry weight, pH and growth rates for the lighting study.

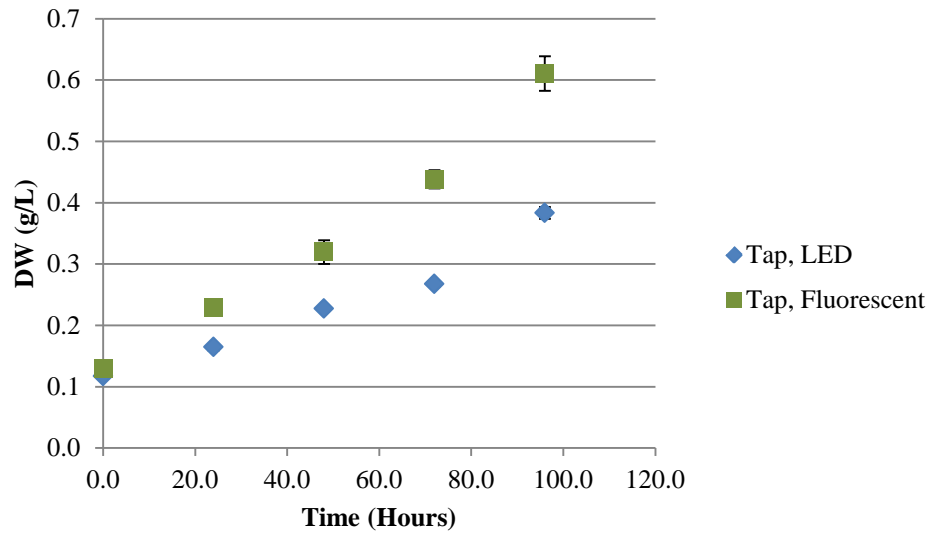


Figure 5.16: Dry weights during a 5-day cultivation in flask using fluorescent and LED lighting regimes. Error bars represent standard error (n=3).

The dry weight plot shows that the fluorescent lighting appears to have better algae growth over the 96 hour testing period.

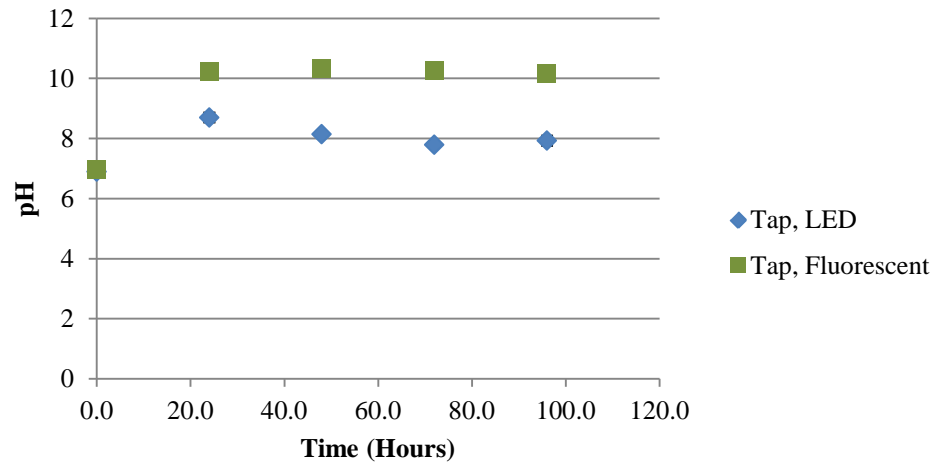


Figure 5.17: Cultivation pH values for flask cultivations done with the typical flask lighting (fluorescence) and with the lighting typically used with the larger airlift reactors (LEDs). Error bars represent standard error (n=3).

Compared to the past flask experiments the pH level is higher. In this experiment the fluorescent lighting resulted in a higher pH than the LED lighting. This may be due to the higher growth rate of the algae in fluorescent lighting. Overall the cause for the higher pH values in this experiment could be the starting density of the algae. In previous experiments initial algae density was 0.05 g/L, while in this experiment the initial algae density was 0.1 g/L. Having a higher density of algae in solution would increase the ability for CO₂ uptake.

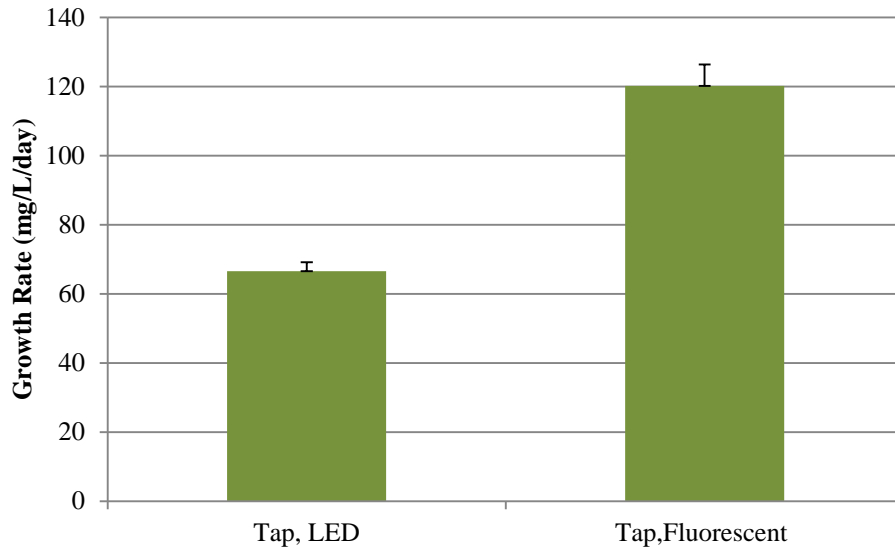


Figure 5.18: Growth rates for flask cultivations done with the typical flask lighting (fluorescence) and with the lighting typically used with the larger airlift reactors (LEDs). Error bars represent standard error (n=3).

The growth rate graph and statistical analysis, in addition to the dry weight plot, suggested that the fluorescent lighting had better growth. A single factor ANOVA statistical analysis was performed to analysis this experiment. The results are shown in Table 5.7. The ANOVA explained that there was a significant difference (P-value < 0.05) in the growth rates of algae grown using the fluorescent and LED lights. The fluorescent showed greater growth than the LED light.

Table 5.7: LED and fluorescent lighting ANOVA based on growth rates (Figure 5.18).

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4309	1	4309	65.00	1.28E -03	7.70
Within Groups	264	4	66			
Total	4573	5				

Although the fluorescent light may perform better for algae growth another important consideration is the cost and energy usage differences. For the light comparison experiment 1 LED strip was used (35W, 1016 $\mu\text{mol}/\text{m}^2$ per second) compared to two

fluorescent light strips (2 bulbs/strip, 128W total, $70 \mu\text{mol}/\text{m}^2$ per second). The resulting cost for the 5-day cultivation based on cost per g for algae produced was \$59 for the LEDs and \$135 for the fluorescent lighting. The electricity cost was assumed to be \$0.670/kw-hr based on the Duke Energy Kentucky's rate stated in the 2013 EIA sales, revenue, prices and customers report. Considering the cost and energy consumption, the LED lights seem to be a better option. Further analysis should be conducted to investigate the overall decision based on the significance of the lightings' growth differences. Choosing which light source depends on the overall goal of the project. If savings is the main objective, LED lighting would best serve the purpose. Yet, if the goal is algae growth fluorescent lighting would be the better choice. In addition, it would be interesting to study and compare LED, fluorescent and natural lighting for algae growth.

5.8 Flask and Airlift Flow Rate Comparison Experiment

After growing algae in the airlift reactor another detail of interest became the effects of flow rates on the alga's growth. This experiment observed algae growth differences between the CO_2 flow rates of the airlift reactor ($0.007 \text{ L CO}_2/\text{min}/\text{L liquid}$) and the flasks ($0.03 \text{ L CO}_2/\text{min}/\text{L liquid}$). This investigative study was conducted in the flasks with CO_2 flow rates equivalent to the previously tested airlift flow rate. To maintain the overall flow rate the air flow rate was increased to prevent settling. These results were compared with the growth of the algae grown in the flask at the typically used flask flow rate. Figure 5.19, Figure 5.20 and Figure 5.21 display the graphical results for dry weight, pH and growth rate.

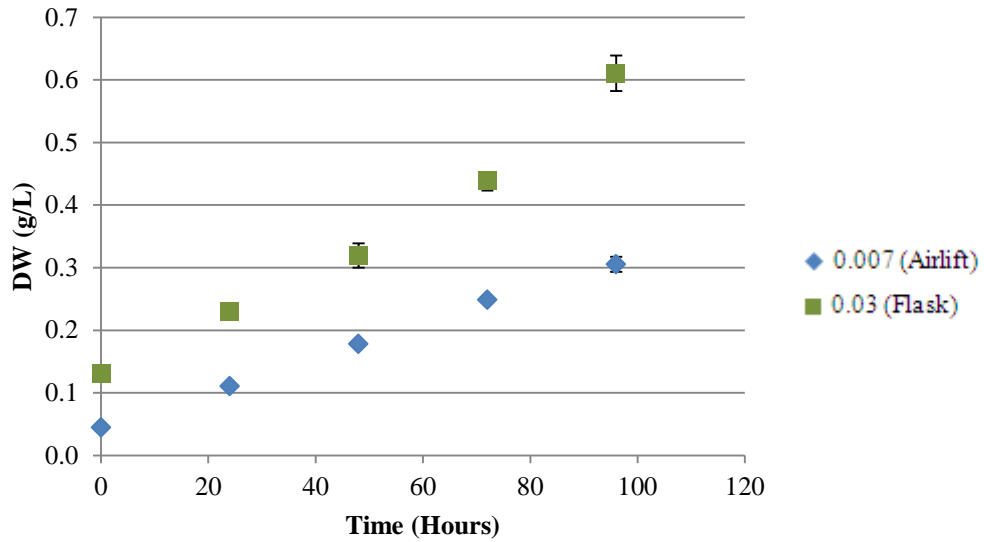


Figure 5.19: Dry weights for flask cultivations done with the typical flask CO₂ flow rate (0.03 L CO₂/min/L liquid) and with the flow rate typically used in the larger airlift reactors (0.007 L CO₂/min/L liquid).

The dry weight graph shows that the flask flow rate appeared to support better algae growth than the airlift flow rate. Using the airlift's lower CO₂ flow rate did not produce algae growth as well as the higher flask flow rate. Growth was not as prosperous at the lower CO₂ flow rate because the algae are able to take up more CO₂ than they are being supplied.

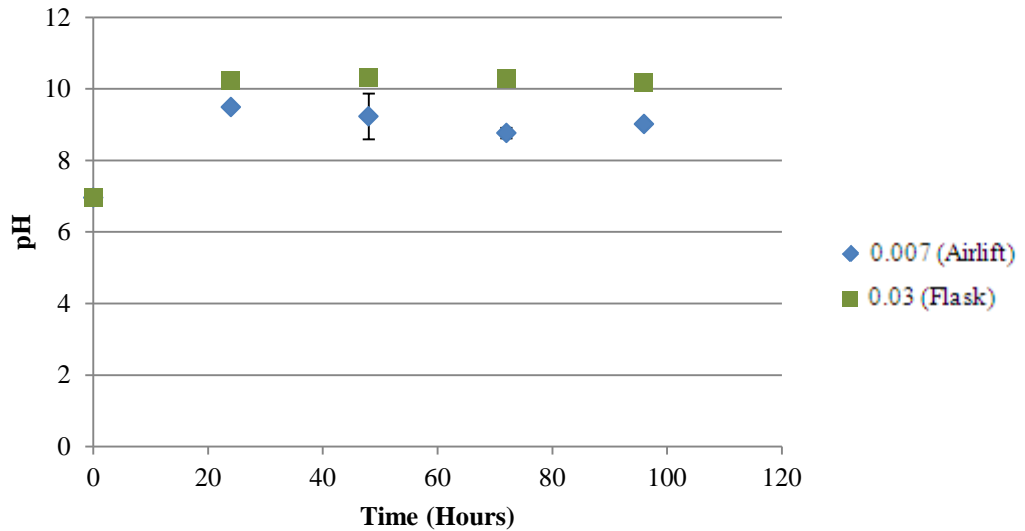


Figure 5.20: Culture pH values for flask cultivations done with the typical flask CO₂ flow rate (0.03 L CO₂/min/L liquid) and with the flow rate typically used in the larger airlift reactors (0.007 L CO₂/min/L liquid).

The pH graph shows that the algae's pH increases and then finds an equilibrium state. This suggests that the algae are using up all the CO₂ and thus neither level exceeds the algae CO₂ up-take abilities. Just as the lighting flask experiment, compared to the past flask experiments the pH level is higher. Although a higher pH is typically correlated with a lower CO₂ rate this experiment's results showed that the higher CO₂ rate (flask flow rate = 0.03 CO₂/ min/ L liquid) gave a higher pH. Overall the cause for the higher pH values in this CO₂ flow rate experiment and lighting experiment could be the starting density of the algae. In the past experiments the starting algae density was 0.05 g/L and in these experiments the starting algae density was 0.1 g/L. A higher density means there are more algae to consume the CO₂.

As expected the higher pH corresponds to the higher growth rate. Comparing growth at different CO₂ levels (0.03 and 0.007 L CO₂/ min/L liquid) suggest that growth is not directly related to an increase in CO₂. It appears that saturation is occurring somewhere between the two CO₂ levels. At the flask scale the amount of wasted CO₂ is not at an excessive level. Although when upscaling, if the CO₂ level is at point that is causing saturation, an unreasonable amount of CO₂ would be wasted. This emphasizes the need for finding the appropriate CO₂ input level.

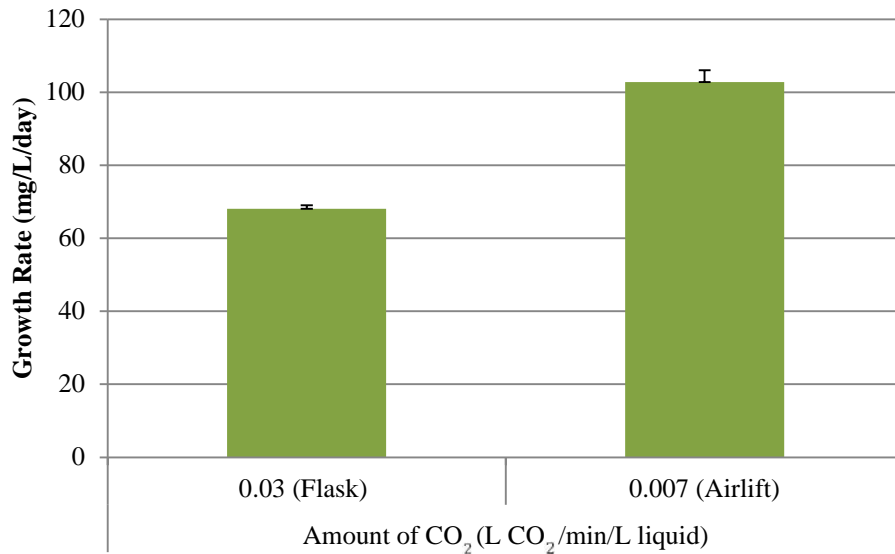


Figure 5.21: Growth rates for flask cultivations done with the typical flask CO₂ flow rate (0.03 L CO₂/min/L liquid) and with the flow rate typically used in the larger airlift reactors (0.007 L CO₂/min/L liquid).

A single factor ANOVA statistical analysis was done for this experiment. The results are shown in Table 5.8. The results showed that there was a significant difference (P -value < 0.05) in the growth rates of algae grown with the airlift flow rate and the flask flow rate. Using the flask flow rate showed greater growth than the airlift flow rate.

Table 5.8: CO₂ inputs (0.007 and 0.03 L CO₂/ min/L liquid) ANOVA based on algae growth rates (Figure 5.21).

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	456	1	456	25.42	7.30E -03	7.71
Within Groups	72	4	18			
Total	527	5				

5.9 Upscaling Growth Results

The largest scale tested was a trailer algae bioreactor at 188 L. Comparisons of the growths at the different scales (flask, airlift and trailer) can be seen in Figure 5.22, Figure 5.23 and Figure 5.24. Two different flask experiments were considered, one with the

same flow rate as the larger scales and one with the same lighting as the larger scales (the same data presented earlier, but now compared to the larger scale).

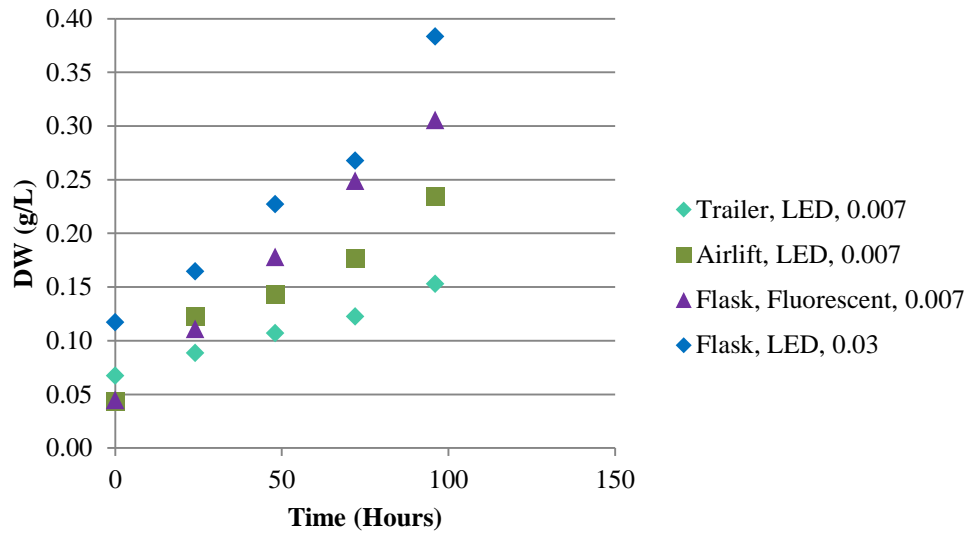


Figure 5.22: Dry weights over a 5-day cultivation for the three scales, the trailer, the airlift reactor and the flasks (with either the same flow rate or lighting as the larger scales). The legend follows the format: reactor, lighting, CO₂ flow rate (L CO₂/min/L liquid).

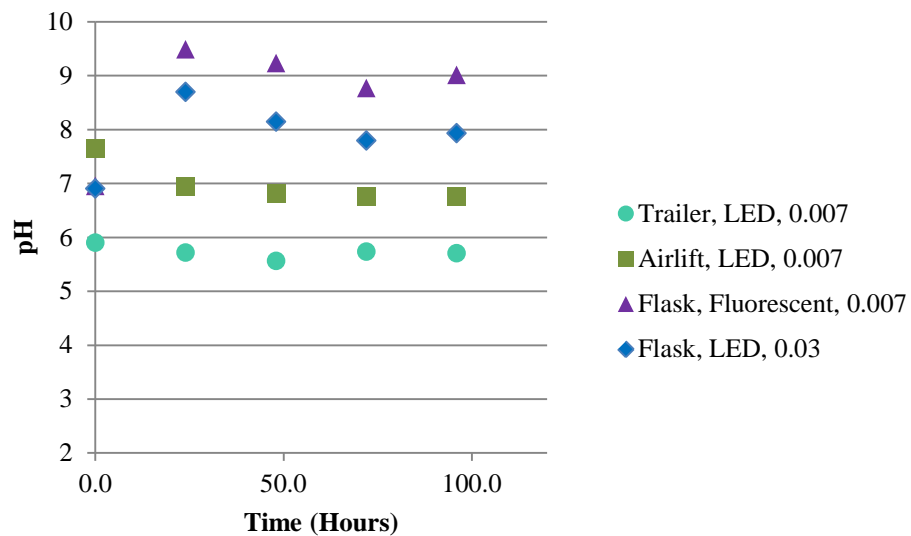


Figure 5.23: Cultivation pH values for the three scales, the trailer, the airlift reactor and the flasks (with either the same flow rate or lighting as the larger scales). The legend follows the format: reactor, lighting, CO₂ flow rate (L CO₂/min/L liquid).

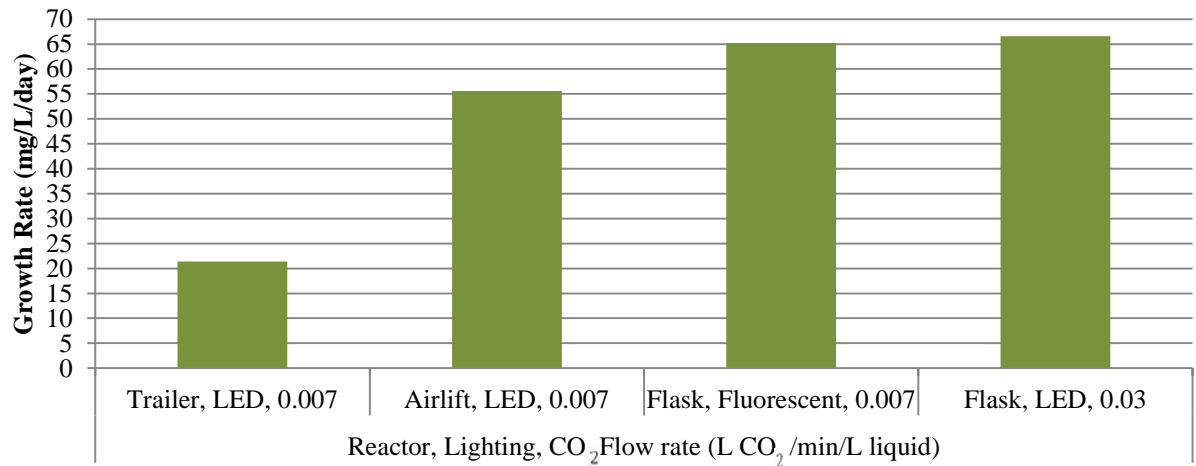


Figure 5.24: Growth rates for the three scales, the trailer, the airlift reactor and the flasks (with either the same flow rate or lighting as the larger scales).

An observation that can be made from the different growth rates (Figure 5.24) is that algae grew better in the flasks. Although the CO₂ flow rates are different for the two flasks experiments, the growth appears to remain similar possibly due to the increase in light with a decrease in CO₂. A statistical analysis could not be performed on this data because of the inability to do repetitions with the trailer due to time constraints. This demonstrates one of the difficulties with studying up-scaled reactors.

Beyond the water and nutrient type (both discussed earlier), there are noticeable differences between the flasks, airlifts and the trailer, including volume, geometry, lighting, and mixing. The volume and geometry contribute to differences in light penetration, which couples with changes in the light source being used. The mixing contributes to changes in the amount of CO₂ (unless properly controlled) and can also lead to the formation of a biofilm, which has been seen in other larger scale photobioreactors at the university. Another area of consideration is the amount of time the algae spend in the photo active part of the reactor. In both the airlifts and flasks, all of the algae are exposed to light during the light phase of the experiment (16 hours per day). In the trailer, only the algae in the photo active tubes are exposed to the light during the

light phase. Calculations show that the algae spend 45% of the time in the light and 55% of the time in the dark during the 16 hour light phase, resulting in 7.16 of light per day for the algae. It is well known that algae need a dark phase every day, but by using the dark tank to boost the total volume of the tank, the overall light exposure does suffer.

Chapter 6. Conclusions

This study focused on algae bioreactor upscaling for an algae-based CO₂ mitigation system for coal-fired plants. Two areas of interest in this study were water and nutrient sources, specifically related to needing a large quantity of both for the large commercial CO₂ mitigation system. Responsible sourcing of water and nutrients should save money and decrease the overall environmental impact of the process. Beyond the water and nutrient requirements, the study observed the behavior of algae growth at different scales (400 mL, 8 L and 188 L), where growth differences were due to volume, geometry, CO₂ availability, and lighting.

Lab and commercial sources for nutrients: nitrogen, phosphate, potassium, EDTA and iron sources were tested. The Urea media was shown to work as well as the literature based M-8 media. The nitrogen source did not seem to be the ingredient that caused the difference. When changing the other ingredients (potassium, phosphate, EDTA and Fe) to the commercial grade the growth rate improved. In addition, when EDTA was left out and only iron was added into the commercial urea the growth rate improve further. In summary, the results demonstrated that urea media with all commercial grade components without EDTA performed the best.

Waters readily available at a coal-fired power plant (referred to as on-site waters) and other waters available at a low cost (referred to as off-site waters) were used to make urea media. The on-site waters from a northern Kentucky coal-fired power plant, Duke Energy's East Bend, that were tested included: well, process and boiler waters. The off-site waters included: industrial stream, urban stream and pond water. The amount of nutrients added to each water type was adjusted based on the nutrient levels originally in the water. The testing showed that the pond water had the highest growth rate of the algae, even though the nutrient levels of N, P, K, and Fe were constant for each treatment.

To further consider the responsible sourcing of nutrients and different water sources a life cycle assessment (LCA) was conducted on the control (tap water) and the water that had the best growth (pond water). Each was evaluated for CO₂ emission levels, energy

requirement and cost. The pond water was found to be the more environmentally and economically viable option.

Due to the pond water having the most promising growth rate, the control (tap water) and pond water were grown in the larger airlift PBR. The growth rates displayed the same findings as in the flasks; the pond water media supported better algae growth. Yet, the pH behaved differently at the air-lift scale. The pH levels decrease and level when algae was grown in the airlift reactor. This occurrence is likely a result of upscaling the size of the system. In the air-lift it seems that the algae are taking more time to up-take the CO₂ present. This is likely due to upscaling variability in the design size change, such as the light availability.

The upscaling of algae was studied considering a variety of variables. After the first upscale, the air-lift reactor, two additional flask scale experiments were conducted to investigate if variation in growth between scales had a correlation with the lighting used (LED or fluorescent) or the CO₂ flow rate selected (flask or airlift flow rate). The lighting experiment results showed that algae had better growth in the fluorescent light. Although, the pricing and energy usage compared to the LED was much higher for the fluorescent light, which may make it the better option. When the CO₂ levels were compared the higher flow rate (flask flow rate, 0.03) showed better algae growth. Lastly, the three scales were graphically compared to see if there were differences in the growth rates from flask to airlift to trailer. Algae appeared to grow better in the flask in comparison to the airlift and trailer. The lighting alteration in the flask-scale seemed to level the growth rates for the flasks with two different CO₂ flow rates.

Chapter 7. Future Work

This study is one of very few focused on system behavior and scaling correlations in the upscaling from lab to industrial sizing for CO₂ mitigation using microalgae. As this research demonstrated there are many areas for improvements and investigations in the upscale process.

Additional studies should be done with other strains of algae, such as *Chlorella vulgaris* to see if using one of the alternative water sources will increase growth rate as well. Increasing the growth rate will increase CO₂ uptake, so any change that will result in enhanced growth should be considered.

Even the largest scale reactor used in this study (188 L) is operated in batch mode, with harvesting done once a week. At the Center for Applied Energy Research, there are PBR systems of 5,500 L that are essentially run in batch mode with harvesting done every 3 to 6 days. In the future, investigations into truly continuous operation will need to be done. One of the biggest questions to answer will be what operating algae concentration facilitates the highest CO₂ mitigation rate.

In addition a long term study of algae at the larger scale could help identify and address some of the optimization needs of the operation techniques. This could include at what time and rate the algae run out of nutrients – thus providing an optimal harvesting and nutrient addition procedure for the reactor.

Appendix A: Media Calculations

Media calculations were performed to find the amount of nutrients needed to be added to make the nutrient levels equivalent to the urea recipe. The addition levels were based on ICP results provided by the Center for Applied Energy research. Literature supports that M-8 media has been shown to have very successful results on algae growth however its cost make the media an unreasonable option. Urea media it essentially the more affordable option to M-8. Therefore the ¼ lab scale urea recipe was used as the control to begin looking at alternative water sources.

Because the results for the ICP are in ppm the urea recipe needed to be converted to the units of ppm for the essential nutrients (N, Fe, K, P and Mg).

Table A.1: Urea recipe composition (g/L).

Nutrients	Urea Recipe (g/L)
Urea(NH ₂)CO	0.1375
KH ₂ PO ₄	0.0296
MgSO ₄ 7H ₂ O	0.0273
Na EDTA Fe	0.005

Phosphorous Needed

KH₂PO₄ is 22.76% Phosphorous

$$\left(\frac{0.0296 \text{ g KH}_2\text{PO}_4}{\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \right) \times 22.76\% = 6.737 \text{ ppm P}$$

Iron Needed

NaEDTAFe is 15.22% Iron

$$\left(\frac{0.005 \text{ g NaEDTAFe}}{\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \right) \times 15.22\% = 0.761 \text{ ppm Fe}$$

Sulfate Needed

MgSO₄·7H₂O is 39% Sulfate

$$\left(\frac{0.0273 \text{ g MgSO}_4\cdot 7\text{H}_2\text{O}}{\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \right) \times 39\% = 10.647 \text{ ppm SO}_4$$

Potassium Needed

KH₂PO₄ is 28.73% Potassium

$$\left(\frac{0.0296 \text{ g KH}_2\text{PO}_4}{\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \right) \times 28.73\% = 8.504 \text{ ppm K}$$

Magnesium Needed

MgSO₄·7H₂O is 9.86% Magnesium

$$\left(\frac{0.0273 \text{ g MgSO}_4\cdot 7\text{H}_2\text{O}}{\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \right) \times 9.86\% = 2.692 \text{ ppm Mg}$$

Nitrogen Needed

Urea is 46.65% Nitrogen

$$\left(\frac{0.1375 \text{ g Urea}}{\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \right) \times 46.65\% = 64.144 \text{ ppm N}_2$$

Sodium Needed

NaEDTAFe is 8.20% Sodium

$$\left(\frac{0.005 \text{ g NaEDTAFe}}{\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} \right) \times 8.20\% = 0.41 \text{ ppm Na}$$

Using the values from the ICP analysis the amounts of the nutrients needed for each water source can be estimated.

Ag Stream Calculations

Table A.2: Agricultural stream IC and ICP results (ppm).

Na	P	Fe	K	Mg	NO3	NO2	SO4
7.17	0.4	0.07	1.3	6.02	3.2	0	27.3

Table A.3: Agricultural stream media calculations.

Media Compound	Nutrient Based on	Calculation	Amount Needed (g/L)
NaEDTAFe	Na	$((0.41-7.17)/1000)/8.20\% =$	< 0.00
NAEDTAFe	Fe	$((0.761- 0.07)/1000)/15.22\% =$	0.0045
Urea	NO ₃ ⁻ + NO ₂ ⁻	$((64.144 - 3.2)/1000)/46.65\% =$	0.1306
MgSO ₄ 7H ₂ O	Mg	$((2.692- 6.02)/1000)/9.86\% =$	< 0.00
MgSO ₄ 7H ₂ O	SO ₄	$((2.692 -27.3)/1000)/9.86\% =$	< 0.00
KH ₂ PO ₄	K	$((8.504 - 1.3)/1000)/28.73\% =$	0.0250
KH ₂ PO ₄	P	$((6.737- 0.4)/1000)/ 22.76\% =$	0.0278
* Formula used for calculation: (Amount of Nutrient in the Original Recipe- ICP Amount in Water)/1000)/ % of nutrient in compound			

Pond Stream Calculations

Table A.4: Pond IC and ICP results (ppm).

Na	P	Fe	K	Mg	NO3	NO2	SO4
67.04	0.00	0.00	3.40	8.96	0.00	0.00	36.80

Table A.5: Pond media calculations.

Media Compound	Nutrient Based on	Calculation	Amount Needed (g/L)
NaEDTAFe	Na	$((0.41-67.04)/1000)/8.20\% =$	< 0.00
NAEDTAFe	Fe	$((0.761- 0.00)/1000)/15.22\% =$	0.005
Urea	$\text{NO}_3^- + \text{NO}_2^-$	$((64.144 - 0.00)/1000)/46.65\% =$	0.1375
MgSO ₄ 7H ₂ O	Mg	$((2.692- 8.96)/1000)/9.86\% =$	< 0.00
MgSO ₄ 7H ₂ O	SO ₄	$((2.692 -36.80)/1000)/9.86\% =$	< 0.00
KH ₂ PO ₄	K	$((8.504 - 3.40)/1000)/28.73\% =$	0.0178
KH ₂ PO ₄	P	$((6.737- 0.00)/1000)/ 22.76\% =$	0.0296
* Formula used for calculation: (Amount of Nutrient in the Original Recipe- ICP Amount in Water)/1000)/ % of nutrient in compound			

Urban Stream Calculations

Table A.6: Urban Stream IC and ICP results (ppm).

Na	P	Fe	K	Mg	NO3	NO2	SO4
51.7	0.3	0.06	4.2	14.5	2.7	0	83

Table A.7: Urban stream media calculations.

Urban Stream Media Calculations			
Media Compound	Nutrient Based on	Calculation	Amount Needed (g/L)
NaEDTAFe	Na	$((0.41-51.7)/1000)/8.20\% =$	< 0.00
NAEDTAFe	Fe	$((0.761- 0.06)/1000)/15.22\% =$	0.0046
Urea	NO ₃ ⁻ + NO ₂ ⁻	$((64.144 - 2.7)/1000)/46.65\% =$	0.1317
MgSO ₄ 7H ₂ O	Mg	$((2.692- 14.5)/1000)/9.86\% =$	< 0.00
MgSO ₄ 7H ₂ O	SO ₄	$((2.692 -83)/1000)/9.86\% =$	< 0.00
KH ₂ PO ₄	K	$((8.504 - 4.2)/1000)/28.73\% =$	0.01498
KH ₂ PO ₄	P	$((6.737- 0.3)/1000)/ 22.76\% =$	0.02828
* Formula used for calculation: (Amount of Nutrient in the Original Recipe- ICP Amount in Water)/1000)/ % of nutrient in compound			

Boiler Water Calculations

Table A.8: Boiler Water IC and ICP results (ppm).

Na	P	Fe	K	Mg	NO3	NO2	SO4
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Boiler water full recipe was needed due to water not containing any of the needed elements.

Process Water Calculations

Table A.9: Process water IC and ICP results (ppm).

Na	P	Fe	K	Mg	NO3	NO2	SO4
8.0	0.196	0.00	1.8	35.0	2.3	0.00	60.4

Table A.10: Process water media calculations.

Media Compound	Nutrient Based on	Calculation	Amount Needed (g/L)
NaEDTAFe	Na	$((0.41-8.0)/1000)/8.20\% =$	< 0.00
NAEDTAFe	Fe	$((0.761- 0.00)/1000)/15.22\% =$	0.0046
Urea	NO ₃ ⁻ + NO ₂ ⁻	$((64.144 - 2.3)/1000)/46.65\% =$	0.1317
MgSO ₄ 7H ₂ O	Mg	$((2.692- 35.0)/1000)/9.86\% =$	< 0.00
MgSO ₄ 7H ₂ O	SO ₄	$((2.692 -60.4)/1000)/9.86\% =$	< 0.00
KH ₂ PO ₄	K	$((8.504 - 1.8)/1000)/28.73\% =$	0.01498
KH ₂ PO ₄	P	$((6.737- 0.196)/1000)/ 22.76\% =$	0.02828
* Formula used for calculation: (Amount of Nutrient in the Original Recipe- ICP Amount in Water)/1000)/ % of nutrient in compound			

Well Water Calculations

Table A.11: Well water IC and ICP results (ppm).

Na	P	Fe	K	Mg	NO3	NO2	SO4
6.8	0.00	0.00	1.8	35.0	2.3	0.00	61.0

Table A.12: Well water media calculations.

Media Compound	Nutrient Based on	Calculation	Amount Needed (g/L)
NaEDTAFe	Na	$((0.41-6.8)/1000)/8.20\% =$	< 0.00
NAEDTAFe	Fe	$((0.761- 0.00)/1000)/15.22\% =$	0.005
Urea	NO ₃ ⁻ + NO ₂ ⁻	$((64.144 - 2.3)/1000)/46.65\% =$	0.1326
MgSO ₄ 7H ₂ O	Mg	$((2.692- 35.0)/1000)/9.86\% =$	< 0.00
MgSO ₄ 7H ₂ O	SO ₄	$((2.692 -61.0)/1000)/9.86\% =$	< 0.00
KH ₂ PO ₄	K	$((8.504 - 1.8)/1000)/28.73\% =$	0.0233
KH ₂ PO ₄	P	$((6.737- 0.00)/1000)/ 22.76\% =$	0.0296
* Formula used for calculation: (Amount of Nutrient in the Original Recipe- ICP Amount in Water)/1000)/ % of nutrient in compound			

Tap Water Calculations

Table A.13: Tap water IC and ICP results (ppm).

Na	P	Fe	K	Mg	NO3	NO2	SO4
0.00	0.228	0.00	2.85	11.9	0.4	0.2	55.5

Table A.14: Tap water media calculations.

Media Compound	Nutrient Based on	Calculation	Amount Needed (g/L)
NaEDTAFe	Na	$((0.41-0.00)/1000)/8.20\% =$	0.005
NAEDTAFe	Fe	$((0.761- 0.00)/1000)/15.22\% =$	0.005
Urea	$\text{NO}_3^- + \text{NO}_2^-$	$((64.144 - 0.6)/1000)/46.65\% =$	0.1362
MgSO ₄ 7H ₂ O	Mg	$((2.692- 11.9)/1000)/9.86\% =$	< 0.00
MgSO ₄ 7H ₂ O	SO ₄	$((2.692 -55.5)/1000)/9.86\% =$	< 0.00
KH ₂ PO ₄	K	$((8.504 - 2.85)/1000)/28.73\% =$	0.0197
KH ₂ PO ₄	P	$((6.737- 0.228)/1000)/ 22.76\% =$	0.0286
* Formula used for calculation: (Amount of Nutrient in the Original Recipe- ICP Amount in Water)/1000)/ % of nutrient in compound			

Appendix B: Upscale Calculations

Table B.1: Lab-scale nutrient amounts used for tap water.

KH ₂ PO ₄	0.031 g/L
NaEDTAFe	0.0053 g/L
Urea	0.148 g/L
*Note: Urea is approximately a 1 to 1 conversion from lab to industrial grade.	

Converted g/L to moles of K, P and Fe:

$$\frac{0.031 \text{ g KH}_2\text{PO}_4}{\text{L}} \times \frac{1 \text{ mol KH}_2\text{PO}_4}{136 \text{ g KH}_2\text{PO}_4} \times \frac{1 \text{ mol K}}{1 \text{ mol KH}_2\text{PO}_4} = 0.00023 \frac{\text{mol}}{\text{L}} \text{ of K}$$

$$\frac{0.031 \text{ g KH}_2\text{PO}_4}{\text{L}} \times \frac{1 \text{ mol KH}_2\text{PO}_4}{136 \text{ g KH}_2\text{PO}_4} \times \frac{1 \text{ mol P}}{1 \text{ mol KH}_2\text{PO}_4} = 0.00023 \frac{\text{mol}}{\text{L}} \text{ of P}$$

$$\frac{0.0054 \text{ g NaEDTAFe}}{\text{L}} \times \frac{1 \text{ mol NaEDTAFe}}{367.08 \text{ g NaEDTAFe}} \times \frac{1 \text{ mol Fe}}{1 \text{ mol NaEDTAFe}} = 0.0000147 \frac{\text{mol}}{\text{L}} \text{ of Fe}$$

To supplement for K, Potash (KCL) fertilizer was used. However, fertilizer is measure with “available” Potassium given by K₂O. To find the amount of Potash needed the mol/L of K were converted to g/L of Potash:

$$\frac{0.00023 \text{ mol of K}}{\text{L}} \times \frac{1 \text{ mol K}_2\text{O}}{2 \text{ mol K}} \times \frac{94.195 \text{ g K}_2\text{O}}{1 \text{ mol K}_2\text{O}} = 0.0108 \frac{\text{g}}{\text{L}} \text{K}_2\text{O}$$

From the bag of fertilizer only 60% of the theoretical amount can dissolve or used.

$$\frac{0.0108 \frac{\text{g}}{\text{L}} \text{K}_2\text{O}}{60\%} = 0.018 \frac{\text{g}}{\text{L}} \text{ of Potash}$$

To supplement for P, Triple Super Phosphate fertilizer (CaH₄P₂O₈) was used. However, fertilizer is measure with “available” Phosphorous given by P₂O₅. To find the amount of Triple Super Phosphate (TSP) needed the mol/L of P were converted to g/L of TSP:

$$\frac{0.00023 \text{ mol of P}}{\text{L}} \times \frac{1 \text{ mol P}_2\text{O}_5}{2 \text{ mol P}} \times \frac{141.94 \text{ g P}_2\text{O}_5}{1 \text{ mol P}_2\text{O}_5} = 0.0163 \frac{\text{g}}{\text{L}} \text{P}_2\text{O}_5$$

From the bag of fertilizer only 44% of the theoretical amount can dissolve or used.

$$\frac{0.0163 \frac{\text{g}}{\text{L}} \text{P}_2\text{O}_5}{44\%} = 0.037 \frac{\text{g}}{\text{L}} \text{ of TSP}$$

To supplement for Fe, Sprint 330 fertilizer ((FeDTPA)NaH) was used. To find the amount of Sprint 330 needed the mol/L of Fe were converted to g/L of Sprint 330:

$$\frac{0.0000147 \text{ mol of Fe}}{\text{L}} \times \frac{1 \text{ mol (FeDTPA)NaH}}{1 \text{ mol P}} \times \frac{79.85 \text{ g (FeDTPA)NaH}}{1 \text{ mol (FeDTPA)NaH}} = 0.00117 \frac{\text{g}}{\text{L}} \text{(FeDTPA)NaH} = 0.00117 \frac{\text{g}}{\text{L}} \text{ Sprint 330}$$

Table B.2: Amount of fertilizer (commercial) nutrients needed for the trailer reactor (188L).

Nutrient	Amount (g)
Urea	28.02
Potash	3.40
Triple Super Phosphate	7.00
Sprint	0.22
*Numbers were based on the reactor volume of 50 gallons (189.3 L).	

Lighting regime comparison Calculations:

$$\text{Operating Percentage} = \frac{\text{Operating Capacity}}{\text{Full Capacity}} = \frac{40 \text{ Hz}}{60 \text{ Hz}} = 66.6\%$$

$$\begin{aligned} \text{Pumping Rate} &= \text{Available Pumping Rate} * \text{Operating Percentage} = 67 \text{ gpm} * 66.6\% \\ &= 44.7 \text{ gpm} \end{aligned}$$

$$\text{Reservoir Resonance} = \frac{\text{Reservoir Volume}}{\text{Pumping Rate}} = \frac{30 \text{ gallons}}{44.7 \text{ gpm}} = 0.671 \text{ min}$$

$$\text{System Resonance} = \frac{\text{System Volume}}{\text{Pumping Rate}} = \frac{54 \text{ gallons}}{44.7 \text{ gpm}} = 1.2 \text{ min}$$

$$\text{Reservoir Resonance \%} = \frac{\text{Reservoir Resonance Time}}{\text{System Resonance}} = \frac{0.671 \text{ min}}{1.2 \text{ min}} = 55\%$$

$$\text{Tubes Resonance \%} = 1 - \text{Reservoir Resonance Time} = 1 - 55\% = 45\%$$

Appendix C: Steripen Standardization

Table C.1: Initial coliform and *E. coli* in the water sample before using the SteriPEN Freedom.

Sample ID	Coliform Large	Coliform Small	Coliform MPN	Ecoli Large	Ecoli Small	Ecoli MPN
Control 1	32	2	50.4	1	0	1.0
Control 2	30	5	50.4	1	0	1.0
Control 3	32	7	59.1	2	0	2.0



Figure C.1: Initial pond water sample without sterilization.



Figure C.2: Appearance of samples after the use of the steripen.

References

- Andersen, R. A. 2005. *Algal culturing techniques*. Academic Press. Burlington, MA.
- Association, K. C. 2011. Kentucky Coal Facts K. D. o. Energy, ed. Frankfort
- Association, W. C. 2009. Coal and Electricity Available at:
<http://www.worldcoal.org/coal/uses-of-coal/coal-electricity/>. December 5, 2012.
- Bell, B. 2012. New Environmental Rules Keep Pressure on Coal-Fired Generation. *Power* 156(8):58.
- Cassidy, K. O. 2011. Evaluating algal growth at different temperatures. MS thesis. Lexington, Kentucky: University of Kentucky, Department of Biosystems and Agricultural Engineering.
- Chaumont, D. 1993. Biotechnology of algal biomass production: a review of systems for outdoor mass culture. *Journal of applied phycology* 5(6):593-604.
- Chinnasamy, S., A. Bhatnagar, R. W. Hunt and K. C. Das. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology* 101(9):3097-3105.
- Chiu, S.-Y., C.-Y. Kao, M.-T. Tsai, S.-C. Ong, C.-H. Chen and C.-S. Lin. 2009. Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration. *Bioresource Technology* 100(2):833-838.
- Corsini, M. and M. Karydis. 1990. An algal medium based on fertilizers and its evaluation in mariculture. *Journal of applied phycology* 2(4):333-339.
- Crofcheck, C. X. E, A. Shea, M. Montross, M. Crocker, R. Andrews. 2013. Influence of media composition on the growth rate of *Chlorella vulgaris* and *Scenedesmus acutus* utilized for CO₂ mitigation. *Journal of Biochemical Technology*, in press.
- Daneshmand, S., A. Mortaji and Z. Mortaji. 2012. Investigation and Design Seawater Desalination with Solar Energy. *Life Science Journal* 9(3).
- Dettore, C. 2009. Comparative Life-Cycle Assessment of Bottled vs. Tap Water Systems. University of Michigan, Natural Resources and Environment, Ann Arbor.
- Energy, U. S. D. o. July 1998. Annual Energy Review 1997. Energy Information Administration, Office of Energy Markets and End Use DOE/EIA-0384. Washington, D.C.
- EPA. 2009. Water on Tap what you need to know. 1-31. EPA.

- Foy, R. H., C. E. Gibson and R. V. Smith. 1976. The influence of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. *British Phycological Journal* 11(2):151-163.
- Freeman, S., J. C. Sharp and M. Harrington. 2008. *Biological science*. 3 ed. Prentice Hall. San Francisco, CA.
- Goswami, R. and Kalita, M.C. 2011. *Scenedesmus dimorphus* and *Scenedesmus quadricauda* : two potent indigenous microalgae strains for biomass production and CO₂ mitigation - A study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source. *Journal of Algal Biomass Utilization* 2(4):42-49.
- Graneli, E. and M. O. Moreira. 1990. Effects of river water of different origin on the growth of marine dinoflagellates and diatoms in laboratory cultures. *Journal of Experimental Marine Biology and Ecology* 136(2):89-106.
- Hanagata, N., Takeuchi, T., Fukuji, Y., Barnes, D.J. and Karube, I. 1992. Tolerance of microalgae to high CO₂ and high temperature. *Phytochemistry* 31(10):3345-3348.
- Hughes, E. and J. R. Benemann. 1997. Biological fossil CO₂ mitigation. *Energy Conversion and Management* 38, Supplement(0):S467-S473.
- Jeong, M. L., J. M. Gillis and J. Y. Hwang. 2003. Carbon dioxide mitigation by microalgal photosynthesis. *Bulletin of the Korean Chemical Society* 24(12):1763-1766.
- Keffer, J. E. and G. T. Kleinheinz. 2002. Use of *Chlorella vulgaris* for CO₂ mitigation in a photobioreactor. *Journal of Industrial Microbiology & Biotechnology* 29(5):275-280.
- Kim, M., J. Park, C. Park, S. Kim, K. Jeune, M. Chang and J. Acreman. 2007. Enhanced production of *Scenedesmus* spp. (green microalgae) using a new medium containing fermented swine wastewater. *Bioresource Technology* 98(11):2220-2228.
- Lal, R. 2008. Carbon sequestration. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363(1492):815-830.
- Laur, P. a. S., E. 2012. Producing algae-based biofuels from wastewater. *Water Resources IMPACT* 14(1):15-16.
- Lehr, F. and C. Posten. 2009. Closed photo-bioreactors as tools for biofuel production. *Current opinion in biotechnology* 20(3):280-285.
- Makareviciene, V., V. Andruleviciute, V. Skorupskaite and J. Kasperoviciene. 2011. Cultivation of Microalgae *Chlorella* sp. and *Scenedesmus* sp. as a Potential Biofuel Feedstock. *Environmental Research, Engineering and Management* 57(3):21-27.

- Mercado, J. M., F. Javier, L. Gordillo, F. Xavier Niell and F. L. Figueroa. 1999. Effects of different levels of CO₂ on photosynthesis and cell components of the red alga *Porphyra leucosticta*. *Journal of applied phycology* 11(5):455-461.
- Molina Grima, E., F. G. Acien Fernandez, F. Garcia Camacho and Y. Chisti. 1999. Photobioreactors: light regime, mass transfer, and scaleup. *Progress in Industrial Microbiology* 35:231-247.
- Nagase, H., Eguchi, K., Yoshihara, K., Hirata, K. and Miyamoto, K. 1998. Improvement of microalgal NO_x removal in bubble column and airlift reactors. *Journal of fermentation and bioengineering* 86(4):421-423.
- Neish, A. C. 1951. Carbohydrate Nutrition of *Chlorella Vulgaris*. *Canadian Journal of Botany* 29(1):68-78.
- Oliveira, M. A. C. L. d., M. P. C. Monteiro, P. G. Robbs and S. G. F. Leite. 1999. Growth and Chemical Composition of *Spirulina Maxima* and *Spirulina Platensis* Biomass at Different Temperatures. *Aquaculture International* 7(4):261-275.
- Ono, E. and J. L. Cuello. 2003. Selection of optimal microalgae species for CO₂ sequestration. In *Proceedings 2nd Annual Conference on Carbon Sequestration*.
- Pecegueiro do Amaral, M. F. 2012. Evaluation of Algae Concentration in Manure MANURE BASED MEDIA. Doctoral Dissertation. University of Kentucky Biosystems and Agricultural Engineering, Lexington.
- Piorreck, M., K.-H. Baasch and P. Pohl. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry* 23(2):207-216.
- Ramaraj, R., D. D. W. Tsai and P. H. Chen. 2010. Algae Growth in Natural Water Resources. *Journal of Soil and Water Conservation* 42(4):439-450.
- Raposo, M. F. d. J., S. E. Oliveira, P. M. Castro, N. M. Bandarra and R. M. Morais. 2010. On the Utilization of Microalgae for Brewery Effluent Treatment and Possible Applications of the Produced Biomass. *Journal of the Institute of Brewing* 116(3):285-292.
- Shuler, M. L. and F. Kargi. 1992. *Bioprocess Engineering: Basic Concepts*. 1st ed. Prentice Hall, Englewood Cliffs, New Jersey.
- Stuart, B. 2011. Addressing the Grand Challenge of atmospheric carbon dioxide: geologic sequestration vs. biological recycling. *Journal of Biological Engineering* 5(1).
- Subhadra, B. G. and M. Edwards. 2011. Coproduct market analysis and water footprint of simulated commercial algal biorefineries. *Applied Energy* 88(10):3515-3523.

- Sundquist, E., B. Robert, S. Faulkner, R. Gleason, J. Harden, Y. Kharaka, L. Tieszen and M. Waldrop. 2008. Carbon Sequestration to Mitigate Climate Change. U. S. G. Survey, ed.
- Tredici, M. and R. Materassi. 1992. From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. *Journal of applied phycology* 4(3):221-231.
- Van den Hoek, C., D. Mann and H. M. Jahns. 1996. *Algae: an introduction to phycology*. Cambridge University Press. Cambridge, United Kingdom.
- Waddell, A. P. a. A. 2011. 2011 Energy Profile K. D. f. E. D. a. Independence, ed. Frankfort
- Wang, B., Y. Li, N. Wu and C. Lan. 2008. CO₂ bio-mitigation using microalgae. *Applied Microbiology and Biotechnology* 79(5):707-718.
- Wang, L., M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang and R. Ruan. 2010. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied biochemistry and biotechnology* 162(4):1174-1186.
- Wang, M. 2008. Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET). A. N. Laboratory, ed. Argonne, IL.
- Wegmann, K. and H. Metzner. 1971. Synchronization of *Dunaliella* cultures. *Archives of Microbiology* 78(4):360-367.
- Wheeler, A. E., R. A. Zingaro, K. Irgolic and N. R. Bottino. 1982. The effect of selenate, selenite, and sulfate on the growth of six unicellular marine algae. *Journal of Experimental Marine Biology and Ecology* 57(2):181-194.
- Woertz, I., A. Feffer, T. Lundquist and Y. Nelson. 2009. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Engineering* 135(11):1115-1122.
- Younos, T. 2012. Water Dependency of Energy Production and Power Generation Systems. *Water Resources Impact* 14(1):9-12.
- Zhang, L., T. Happe and A. Melis. 2002. Biochemical and morphological characterization of sulfur-deprived and H₂-producing *Chlamydomonas reinhardtii* (green alga). *Planta* 214(4):552-561.

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T. Graham, C. Crofcheck, A. Shea, M. Montross, R. Andrews and M. Crocker. 2013. Investigation of Media Ingredients and Water Sources for Algae CO₂ Capture at Different Scales to Demonstrate the Correlations Between Lab-scale and Large-scale Growth. Annual Institute of Biological Engineering Meeting in Raleigh, NC. March 2013.