LOW COST, LOW ENERGY, METHOD OF DEWATERING CULTURES OF THE GREEN MICROALGAE *NANNOCHLORIS OCULATA:* ELECTROCOAGULATION

A Thesis

by

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ABSTRACT

Microalgae have received a substantial amount of attention as an alternative fuel feedstock due to their ability to produce large quantities of lipids. The goal of this research was to determine the ideal operating parameters for electrocoagulation; a low cost, low energy method of dewatering cultures of microalgae. The objectives of this research focus on recognizing parameters that influence the overall efficiency of the process, effective electrode materials, and finally directional improvements in operating parameters contributing to a high reduction in optical density. Variables found to have a statistically significant effect on the efficiency of electrocoagulation were the electrode material, current, and duration. With no adjustment of the algae culture prior to electrocoagulation, iron and nickel were identified as the best performing electrode materials, in terms of optical density reduction. Of the materials tested, iron was found to achieve the greatest recovery of microalgae at the lowest power consumption, while staying below the threshold for animal feed tolerance. The most desirable operating parameters for electrocoagulation, within the confines of the experimental apparatus and using iron electrodes, were found to be a current of 0.3 amps and a 15 min reaction time. Increases in current and duration were found to provide the highest levels of optical density reduction. However, the average voltage, and therefore, power consumption are the highest when current and duration are maximized. Additional testing should be performed at higher currents and longer durations, in an attempt to find a peak in the optical density reduction.

DEDICATION

To my wife and son.

ACKNOWLEDGEMENTS

I would like to thank Dr. Ronald E. Lacey, for his support guidance while conducting this research, as well as his patience over the last few years.

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NOMENCLATURE

OD Optical Density

PAR Photosynthetically Active Radiation

RSM Response Surface Method

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CHAPTER I

INTRODUCTION

With a growing push for carbon emission reductions, as well as ending America's dependence on foreign oil, there is a need for an attainable alternative to petroleum based fuels. One method of meeting this demand is to produce fuels, like biodiesel, that are derived from lipids extracted from agricultural crops (Shen, 2009) . One nontraditional crop that could potentially be used as a feedstock for biodiesel production is microalgae. The growth rates of microalgae are much faster than terrestrial biomass and oil crops. Perhaps the most noteworthy aspect of microalgae is the amount of oil, or lipid, they can produce. The amount of oil that can be extracted from microalgae is greater than the quantity of oil that can be extracted from oil crops by at least one order of magnitude. Microalgae, even using a conservative estimate of 30% oil content by weight, are potentially better options when compared to traditional oil crops.

In order to replace all petroleum based diesel fuel in the United States it would require 530 billion liters (140 billion gallons) of biodiesel annually. Palm oil has the ability to produce oil, or lipids, at a rate of 5950 L ha^{-1} (Chisti, 2007a). Therefore, at 80% transesterification efficiency; 275 million acres of crop land would be required to replace petroleum diesel with palm based biodiesel. This number would equate to approximately 61% of available crop land in the United States. On the other hand, microalgae have the potential to produce 97,800 L ha-1, based on 50% lipid content. As a result, at 80% transesterification efficiency, microalgae would only require 16.7 million acres, or 3.7%

of available crop land, to meet the same demand. Additionally, microalgae do not require agricultural cropping area, but can instead be grown in places where traditional terrestrial oil producing crops cannot survive. Therefore, large scale production of microalgae for fuel would be less likely to affect food production or costs, resulting from a shift in land usage. There is currently no other potential source of lipid that can compete with the potential of utilizing microalgae as a feedstock for biodiesel productions (Shelef, 1984). Finally, the fact that algae can consume carbon dioxide makes production of algae on a large scale very attractive. Theoretically, producing 100 tons of algal biomass would fix roughly 183 tons of CO_2 (Chisti, 2007a). Mass production of microalgae, for use as an alternative fuel, has the potential to reduce an enormous amount of CO_2 that would otherwise be added to the atmosphere through combustion of traditional fossil fuels.

The cost to produce algal biomass for conversion to biofuels, on a large scale, is currently too high to be price competitive with petroleum based fuels. The costs associated with the production and harvest of microalgae must be cut significantly to make algal biofuels economically feasible (Sander, 2010). Much of the high cost of production comes from the harvesting step. Prior to harvest, microalgae are typically grown to a concentration of $1 – 2$ g l^{-1} (0.1% - 0.2% total suspended solids or TSS). Recovering algae from such a dilute solution has been a major hurdle for the industry. Regardless of the technology used to extract the oil from the algae cells, or even if it is to be converted to a bio-oil, the microalgae must be concentrated by two or more orders of magnitude prior to entering the next step in the process. Dewatering, or harvesting, can prove to be a substantial portion of the production costs, as a large amount of water must be removed to increase

concentration by one order of magnitude (Stepp, 2011). Thus, there is a need for a low cost, low energy method of dewatering microalgae solutions. Centrifugation and filtration are common methods used to separate solids from a dilute solution. However, these methods can be costly and labor intensive when utilized on the scale required to displace petroleum based fuels (Shelef, 1984).

One of the more interesting, and more promising techniques of dewatering microalgae relies on the interaction between the surface charge of the algal cells and electrolytic processes, known as electrolytic coagulation (Uduman et al., 2010), or electrocoagulation. This process requires small amounts of energy, potentially would require a minimal capital expense, and also has the ability to scale up to commercial volumes.

In this set of studies, an electrolytic coagulation method will be examined for efficacy in removing *Nannochloris oculata* from the culture medium for use in biofuels production. This electrically driven method of dewatering microalgae is attractive because it is theoretically energy efficient, safe, and cost effective. Electrolytic coagulation uses reactive, sacrificial metallic electrodes to produce positively charged ions that induce coagulation of the negatively charged microalgae cells. This results in the algae cells being removed from the solution via sedimentation. As more electricity flows through the solution, more metal is dissolved from the electrode, and therefore more ions are present. Excess ion can result in the formation of metallic hydroxides that will physically pull the algae out of solution via the mechanism of sweep flocculation.

Objectives

The goal of this research was to evaluate electrocoagulation as a method of biomass recovery from growth media for fuel production, while not limiting the use of the microalgae byproducts (items such as animal feeds that could make the utilization of microalgae for alternative fuel production more economically attractive) caused by reaching toxic levels of coagulant. The following objectives were addressed during this research:

1. Determine the significance of current, duration, electrode material, distance between electrodes, and stirring rate on both the reduction of optical density (an indicator of algae concentration), as well as the average voltage required (an indicator of electrical power consumption).
2. Determine the most practical electrode material for use in electrocoagulation separation of algae.
3. Determine directional improvements to operating conditions that maximize algae recovery during electrocoagulation.
4. Estimate scale up feasibility for an electrocoagulation algae harvesting process.

CHAPTER II

LITERATURE REVIEW

Because of the growing demand for energy, increasing energy costs, and politically unstable governments that have historically controlled the majority of crude petroleum resources, there exists a need for a domestically produced alternative. Utilizing microalgae as a feedstock for biodiesel production is one of the few technically feasible methods of displacing petroleum based fuels (Chisti, 2007a; Briggs, 2008). The prospect of algae as a source of energy was exhibited in the D.O.E.'s Aquatic Species Program (Sheehan et al., 1998). This compilation of a decade long research project sponsored by the Department of Energy demonstrated the need for algae based biofuels, not only for economic motives, but also for national security purposes.

Cost reductions are needed for production of fuel from microalgae to become a reality on a commercial scale (Uduman et al., 2010). Harvesting biomass from the growth medium can make up 20-30% to the total cost of producing biomass (Grima et al., 2003; Gudin and Therpenier, 1986).

Several different methods of removing particles from an aqueous solution have been developed for the bioseparation and wastewater treatment industries (Phoochinda et al., 2004; Tenney et al., 1969). Some of these methods have been applied to harvesting microalgae with the algal biomass being the desired product (Shelef et al., 1984). For example, centrifugation and filtration are common methods used to separate solids from a

dilute solution. However, these methods can be costly and labor intensive when utilized on the scale required to displace petroleum based fuels (Shelef et al., 1984). Furthermore, chemical coagulants have been utilized, but were not considered in this study in order to maintain the feasibility of using all by-products, like lipid extracted algae for animal feeds.

Electrocoagulation for the removal of microalgae from water as part of a water treatment process, i.e. with no goal of harvesting the biomass for fuel production, has been evaluated in several studies (Aragon et al., 1992; Alfafara et al., 2002; Azarian et al., 2007; Bukhari, 2008). Aragon et al., determined that electrocoagulation was superior to chemical flocculation because of lower cost, a shorter time needed for separation, and a lower probability that the microalgae would be contaminated with metallic hydroxides.

Electrocoagulation for the express purpose of harvesting algal biomass was examined by Poelman et al. (1997) and Gao et al. (2009), while the science and mechanisms at work are described in Mollah et al. (2004). These studies demonstrated that electrocoagulation was an effective method of harvesting algae from the culture medium. However, there are a number of operating parameters associated with the process that have not been examined. Aluminum and iron are generally assumed to be the best electrode materials for electrocoagulation of microalgae (Azarian et al., 2007; Gao et al., 2009; Mollah et al., 2004). Consequently, other potential electrode materials have not been examined that could improve the overall efficacy of the process.

Electrocoagulation of microalgae is based on surface charge and water chemistry. Algae cells carry a net negative surface charge, while positively charged ions are dispersed in the solution by an electrolytic process, resulting in charge neutralization or sweep flocculation of the cells, in cases of excess ion and metal hydroxide formation. The amount of ions dissolved into the solution through electrolysis can be determined using Faraday's Law (Fischer, 2009):

$$w = \frac{ItM}{nF}$$

where w is the quantity of electrode material dissolved (g), I is the current (A), t is the time (s), M is the relative molar mass of the electrode, n is the number of electrons in oxidation/reduction reaction, and F is the Faraday's constant (96500 C mol^{-1}). For a specified volume and concentration of algae cells, there should be a threshold to the reaction time and current intensity for the process to be optimized.

Mixing and spacing of the electrodes during electrocoagulation testing and their impact on both energy consumption and algae removal efficiency are also not examined consistently. The achievable lifetime of the electrodes are not described in the literature as well as metal contamination levels in the harvested biomass. In order to make algae production for fuel a viable alternative, excess media from the harvested microalgae needs to have the capability of being recycled, and the algal biomass must be suitable for animal feeds after lipid extraction. The metallic ion content of lipid extracted algae was not addressed in previous studies because the focus was on wastewater and not the removed algae (Azarian et al., 2007). Any technique that can help decrease the overall

cost of the feedstock increases the economic potential for success and is a necessary topic to examine. Utilizing the lipid extracted algae as a feed additive for livestock could be a major factor in the success of electrocoagulation as a harvesting method. All of these issues demonstrate a need for a significant amount of additional research to be completed in order to utilize this process to harvest microalgae for fuel production.

Electrocoagulation appears to have the potential to scale to an industrial level. Prior research has demonstrated that the process is effective when used to dewater microalgae. However, a direct comparison of the operating parameters and their efficiencies has not been made.

CHAPTER III

EXPERIMENTAL APPARATUS AND METHODOLOGY

Growth Apparatus

A system capable of maintaining a healthy culture of microalgae was required to support testing of the electrocoagulation methodology. Several liters of algae were needed on an ongoing basis for testing. To meet this need, a growth system was developed to produce and sustain over 6 liters of microalgae continuously. Several studies examined the factors that should be considered when constructing a growth system for microalgae (Chisti, 2007b; Kommareddy, 2005). The following factors were considered in establishing a robust algal growth system: species, light, mixing, temperature, carbon source and nutrients.

Nannochloris oculata was selected as the species to be utilized for these experiments. This species of microalgae is a photoautotrophic eukaryote with capabilities of reaching high lipid contents (20-50%), which makes it suitable for the purposes of the overall goal of this project, producing microalgae as an alternative fuel source. Another positive aspect of this species was the relatively rapid growth rate. The doubling rate of *Nannochloris oculata* was 3 days and the specific growth rate was 0.23 $days^{-1}$. Another contributing factor to the selection of this species was that the microalgae group at Texas A&M University had substantial experience working with this particular species.

Light is essential for growth of most microalgae and is largely dependent upon the type of algae, the amount of light harvesting pigments available in the chloroplast, and the light harvesting antenna structure (Kommareddy, 2005). The photosynthetically active radiation, or PAR, spectrum is defined as 400-700nm. Different types of pigments absorb at different wavelengths. This can be used as an advantage when growing algae indoors. The light source can be chosen based on the absorption spectrum of the algae (Figure 1).

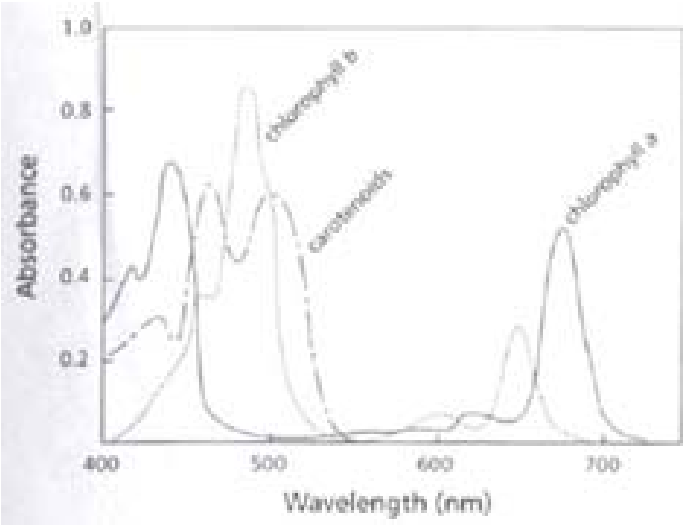

Figure 1: Absorption Spectra of Light Harvesting Pigments (Kommareddy, 2005)

Too much light, or photons, can cause the algae's antenna structure to become damaged. This is known as photoinhibition. While avoiding photoinhibition is important, it is usually more difficult to get enough PAR, to the algae. *Nannochloris oculata* is a "green algae," which means that it has chlorophyll a and b, as well as carotenoids. For this reason, these algae can absorb light effectively on the entire photosynthetically active range and the light source selection was not a critical factor to ensure the effective growth

of the algae culture. Fluorescent lights were selected for the algal growth system to minimize costs.

Good mixing in the algae solution increases light penetration by reducing the shading caused by the large number of cells in solution. Mixing also increases light-dark cycle frequency which has shown a positive relationship with cell production. Depending upon the growth method (i.e. raceway, photobioreactor, etc.), mixing can be accomplished by a variety of methods. If air or bubbles are used to create mixing, bubble size can actually influence light utilization. Smaller bubbles increase the amount and frequency of light reflected in the solution. Therefore, there are more opportunities for the light to be captured and used for photosynthesis. Mixing in the growth system was achieved through air supplied by an Aqua Culture 5-15 gallon, single-outlet aquarium pump. Supplied air was sent to a high density polyethylene manifold (detailed drawing in Appendix B1). The manifold then distributed air through throttling valves to up to six 2 liter Erlenmeyer flasks, via tubing connected to a pipette placed in the center of each flask. The air caused bubbles in the solution, creating a mixing effect in the flask. The bubbles produced by the pipette were small to increase the light-dark frequency as well as the amount of light reflected in the solution. Both have been shown to increase light utilization (Kommareddy, 2005). The airflow rate was controlled in each flask using a 3/8 inch ball valve, which served as a throttling valve. The mixing created by the air bubbles helped eliminate oxygen from the solution that was a product of photosynthesis. High levels of dissolved oxygen have adverse effects on the production of algal cultures and can cause the algae to go into photorespiration mode, stopping algae growth (Kommareddy, 2005).

The air used for mixing was filtered with an aquarium inline air filter/check valve assembly prior to entering the manifold to reduce the risk of contamination. Filtering the air was essential because airborne contaminants could be conveyed into the pure culture used for testing.

The temperature in the flasks was maintained between 20 and 30°C, in an attempt to keep the culture near the optimal temperature for the chosen species, 25°C (Cho, 2007). In order to mitigate the effects of temperature changes in the surrounding room, the flasks were placed in water baths with a thermostatically controlled electric heating element as shown in Figure 2.

Figure 2: Cultures of *Nannochloris oculata* at Various Concentrations in a Temperature Controlled Water Bath and Fluorescent Lights

Providing an abundant source of carbon helps to achieve high levels of productivity. Microalgal biomass is composed of about 50% carbon by dry weight. Bicarbonates can be put into a growth medium in the form of sodium bicarbonate for the algae to utilize. Another method of supplying carbon to the algae is by injecting carbon dioxide into the solution. Carbon dioxide dissociates in the solution to form carbonate and bicarbonate. When adding a source of carbon to the algae culture, it is important to keep the pH of the solution in mind. At low pH, the CO_2 that has been supplemented, injected, or fed into the system has not yet dissociated. In other words, inputting CO_2 decreases the pH of the solution briefly, but as the CO_2 dissociates into carbonates or bicarbonates the pH increases. It is also important to ensure that the pH of the culture remains below 10. At a pH greater than 10, photosynthesis will shut down. Maintaining a proper pH can be a constant battle for some growth systems. Nutrients required for consumption by the microalgae are provided by growth media, or mixture of different components that have been determined to be necessary for healthy cell growth. Nitrogen, phosphorous, and iron are the most essential elements for optimal growth. It is critical to have enough, or even an excess amount, of these elements in solution for use by the algae. The growth medium used was a modified f/2 formula (Appendix A). This growth medium provided a source of bicarbonates, as an additional source of inorganic carbon to the supplemented CO_2.

Culture Growth Methodology

The algae used throughout this series of testing were grown from a sample obtained from UTEX, The Culture Collection of Algae at the University of Texas in Austin, UTEX

Number LB1998, *Nannochloris oculata*. The original sample was grown through a series of transfers into fresh growth medium, allowing the culture to approximately double in concentration of algal cells. Each flask was inoculated at a 1:1 ratio with equal parts of growth medium mixed with algal culture at a concentration of 1 g/L for a resulting 0.5 g L^{-1} concentration Once six liters of algae culture was established, the normal growth regimen for testing was implemented, as described below.

During the normal growth regimen for testing, once a flask containing algae was determined to be "transfer ready" the algae solution was removed from the flask and prepared for the electrolytic coagulation procedure. The algae were determined to be "transfer ready" when the optical density was maintained at the same level for two consecutive days. In our growth apparatus, the culture was determined to be "transfer ready" at an average optical density of 0.168. By utilizing algae that were determined to be "transfer ready" for each test, the assumption was made that each of the samples used during testing were in the same growth phase.

Electrocoagulation Apparatus

A lab scale testing apparatus was developed to conduct the electrocoagulation tests. The process utilized electrolysis in order to remove the algae from solution. There are four main components necessary to perform electrolysis: a power supply, electrical leads, electrodes, and an electrolytic cell. Additional considerations for the testing apparatus were the ability to vary the current, distance between electrode materials, and stir rate, and also the ability to easily change the electrode material.

The power supply selected was a Hewlett Packard E3631A DC Power Supply 0-6V, 5A/0-25V, 1A. This power supply was able to supply a constant electrical current up to 1 amp. Standard banana clip to alligator clip electrical leads were used to construct the electrical circuit for the process as shown in Figure 3. Various electrode materials were selected for testing and included nickel, iron, zinc, tin, brass, aluminum, copper, and carbon.

One variable to be examined was the distance between the electrodes. In order to vary the distance between the electrodes as well as maintain the electrodes in an upright and parallel orientation, a clamping device, also referred to as the electrode holder, composed of 100% polytetrafluoroethylene (PTFE) was developed. Two 12” lengths of 1/4-20 threaded PTFE rod were laid parallel. Four identical rectangular pieces (1/2” x 2”x1/8”) were then cut from a sheet of PTFE. Two holes were drilled in each rectangular piece 1/4” from each end, a PTFE hex nut was threaded onto each end of the rod. Two rectangular pieces on each end of the threaded rod were placed against the hex nut spanning the two rods, essentially connecting the two threaded rods. Another set of four hex nuts secure the rectangular pieces between the hex nuts. This clamping mechanism allowed the electrodes to be slid into place and be secured at any desired distance, and also allowed the electrodes to be oriented parallel to each other. The clamping mechanism also allowed the electrodes to easily be removed when necessary. The electrode holder can be seen in Figures 3 and 4.

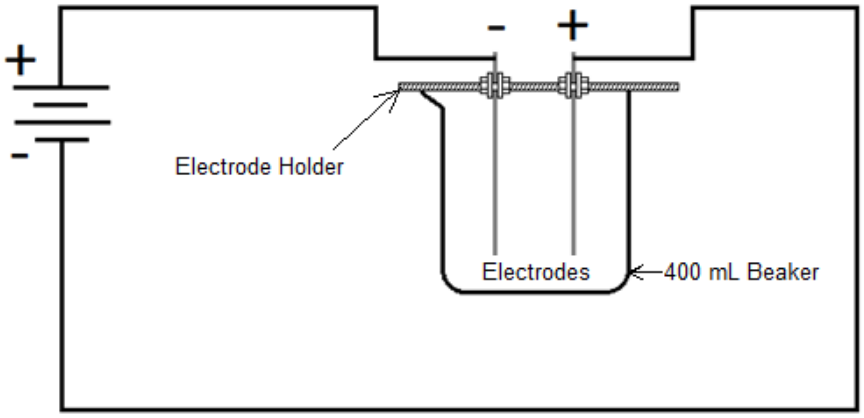

Figure 3: Electrical Circuit Used During Electrolytic Coagulation

A FisherBrand 400 mL beaker was selected to serve as the electrolytic cell, as shown in Figure 4.

Figure 4: Electrocoagulation Experimental Apparatus - Including Electrical Connections, Electrodes, Electrode Holder, and Electrolytic Cell Containing Microalgae

The size of the beaker, with its wide top, allowed for the distance between the electrodes to be varied sufficiently and the volume allowed for multiple tests to be performed for each liter of transfer ready algae. The electrolytic cell was placed on a Corning PC-410D magnetic stir plate, and a 5/16" x 1" magnetic stir bar was placed in solution to provide mixing.

Electrocoagulation Procedure

For safety purposes, the first step of the electrocoagulation procedure was to ensure that the power supply was OFF. Second, the electrodes were marked to indicate the solution level, correct orientation (up/down), and polarity of the electrodes for the duration of the experiment. The mark showing the solution level represented the limit of the active surface area on the electrode. Since the electrodes are 2.5 cm wide with a very small thickness comparatively, the thickness was considered negligible. A line was marked 6 cm from one end of each electrode. The area under this mark on the electrodes totaled 60 cm^2. An arrow indicating the correct orientation was made to ensure the active surface area was consistent in each test. The electrodes were then weighed and the pre-weight was recorded. The next step was to adjust the electrode holder to allow for the appropriate spacing between the electrodes.

The PTFE hex nuts on the PTFE threaded rod were adjusted to the correct spacing and the pre-weighed electrodes were placed into the PTFE electrode holder device. A 300 mL volume of algal solution along with a 2.5 cm magnetic stir bar were placed into a 400 mL

beaker, which served as the electrolytic cell. The electrolytic cell was placed onto a stir plate. A sample was immediately taken for measurement of optical density (See Appendix B). The electrode holder with the electrodes installed was placed into the solution. The amount of each electrode submersed was adjusted by loosening the retaining hex nuts and adjusting the electrodes. The leads from the power supply were attached according to Figure 3, making sure that the correct lead was attached to the previously labeled electrodes.

With the electrodes, electrode holder, and leads in place, the magnetic stirrer was turned on and the appropriate stir rate set according to the experimental design. The power supply was turned on, but the output remained off. The current limit was set accordingly and the voltage limit was set to the maximum value. Because of the high level of conductivity in the solution, the power supply was set using a current limited mode. A timer was set to the duration of the electrolysis experiment. The power supply output was turned on, while the timer was started simultaneously. An initial voltage reading was recorded, followed by another reading at the midpoint of the electrolysis portion of the test. After the specified amount of time, the final voltage was recorded, and the power supply output was turned off. Next, the power supply itself was turned off and the leads were unplugged from the power supply to ensure there was no risk of electric shock. The electrodes were removed from the solution. The stir rate was increased to 360 rpm for two minutes. At the conclusion of two minutes, the stir rate was decreased to 60 rpm for 20 minutes to allow the algae cells to create flocs. At the conclusion of the 20 minute period, the magnetic stir bar was removed and the stir plate turned off. The beaker was

moved to a location where it could be left undisturbed. The beaker and its contents were left undisturbed for 20 minutes. Immediately following the 20 minutes settling period, another sample was taken for optical density measurement at the same depth and location taken from the initial sample. Another sample for optical density was taken and analyzed according to the optical density measurement procedures. Finally, the electrodes were weighed and the post-weight recorded.

Response Variables

In order to examine the electrocoagulation process, three response variables were chosen: reduction in optical density, average voltage, and mass loss in the electrodes. Reduction in optical density compared the final optical density measurement to the initial measurement and was used as an estimate of the effectiveness of electrolytic coagulation occurring in the algal culture. The average voltage response was determined by taking an average of three readings of the voltage output by the power supply. One reading was taken at the beginning of each test, one reading at the midpoint of each test, and one final reading at the conclusion of the test. The average voltage was chosen as a response. The power supply used during testing supplied a constant current for each test. The current and the average voltage can easily be used to calculate estimated power consumption. Mass consumption of the electrodes was found by weighing the electrodes before and after completion of the tests. The mass consumption allowed us estimate the number of ions released into the solution. With this information, we could see if there is a correlation between the amount of ions in solution and coagulation of biomass. Additionally, the amount of the electrode consumed could provide some insight into the

potential for exceeding toxicity thresholds for animal feeds or the recyclability of the growth medium.

CHAPTER IV

FACTORS INFLUENCING ELECTROCOAGULATION OF MICROALGAE

Introduction

Electrocoagulation for the express purpose of harvesting algal biomass was examined by Poelman et al. (1997) and Gao et al. (2009), while the science and mechanisms at work are described in Mollah et al. (2004). These studies demonstrated that electrocoagulation was an effective method of harvesting algae from the culture medium. However, there are a number of operating parameters associated with the process that have not been examined. The experiments in this chapter were designed to examine a variety of factors and determine if any have a significant impact on the efficiency of the electrocoagulation process.

Objective

The objective of this study was to statistically determine if current, duration, electrode material, distance between electrodes, and stirring rate influence the efficiency of electrocoagulation.

Experimental Design

The first round of testing was essentially a factor eliminating test. A 2-level factorial design was implemented to allow the parameters with a significant effect on reduction in optical density, power consumption (average voltage), and electrode consumption to be

identified. A 2-level factorial design allowed us to test these factors simultaneously rather than examining each factor individually. The factors examined are described in Table 1.

Table 1: Factors and Corresponding High and Low Level Parameters Examined in the Factor Elimination Testing

Factor	Low Level	High Level
Electrode Material	Aluminum	Iron
Current (A)	0.1	0.5
Duration (min)	5	30
Distance (cm)	2	5
Stir Rate (rpm)	0	260

Because there were five factors being examined, the 2 level factorial design consisted of 32 runs, or tests, where algae was subjected to the electrocoagulation process. This is a significant reduction in the number of runs that would be required to achieve the same power, or probability of committing a Type II error, if conducting the tests one factor at a time. This is accomplished by reducing the number of levels for each factor to two.

Results and Discussion

The results from the series of tests conducted to determine significant factors were analyzed using Design Expert statistical software.

When examining the response variable of reduction in optical density and applying a square root transform to the data, we were able to determine that the electrode material, current, and duration all had a significant effect (p-value less than 0.05) on the reduction of optical density. Current and duration also had an interaction effect. The model had an F-value of 20.17 which implies the model is significant. Also, there is only a 0.01% chance that a model with an F-value this large could occur due to noise. From the data represented in Figures 5 and 6, we can see that given the experiment conditions an increase in current produced an increase in OD reduction. Additionally, an increase in duration yielded a larger reduction in OD.

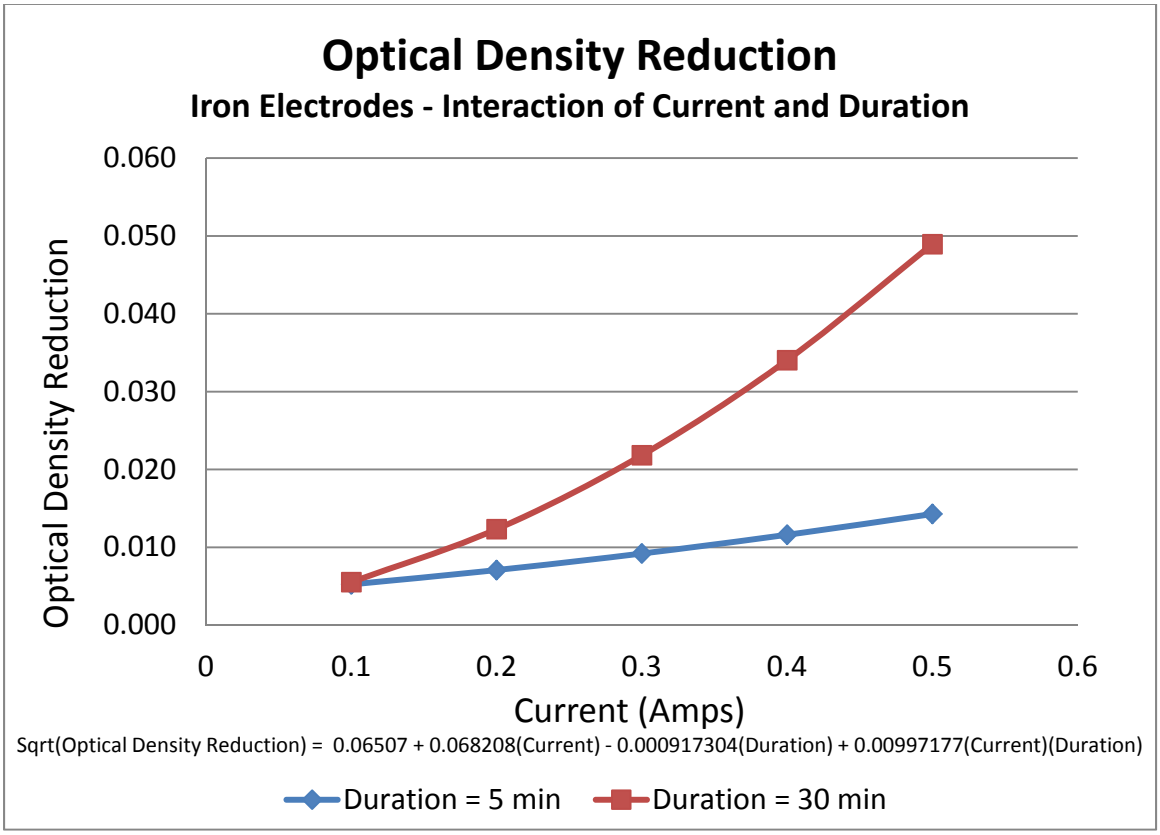

Figure 5: The Effect of Current and Duration on the Reduction of Optical Density When Using Iron Electrodes for Electrocoagulation

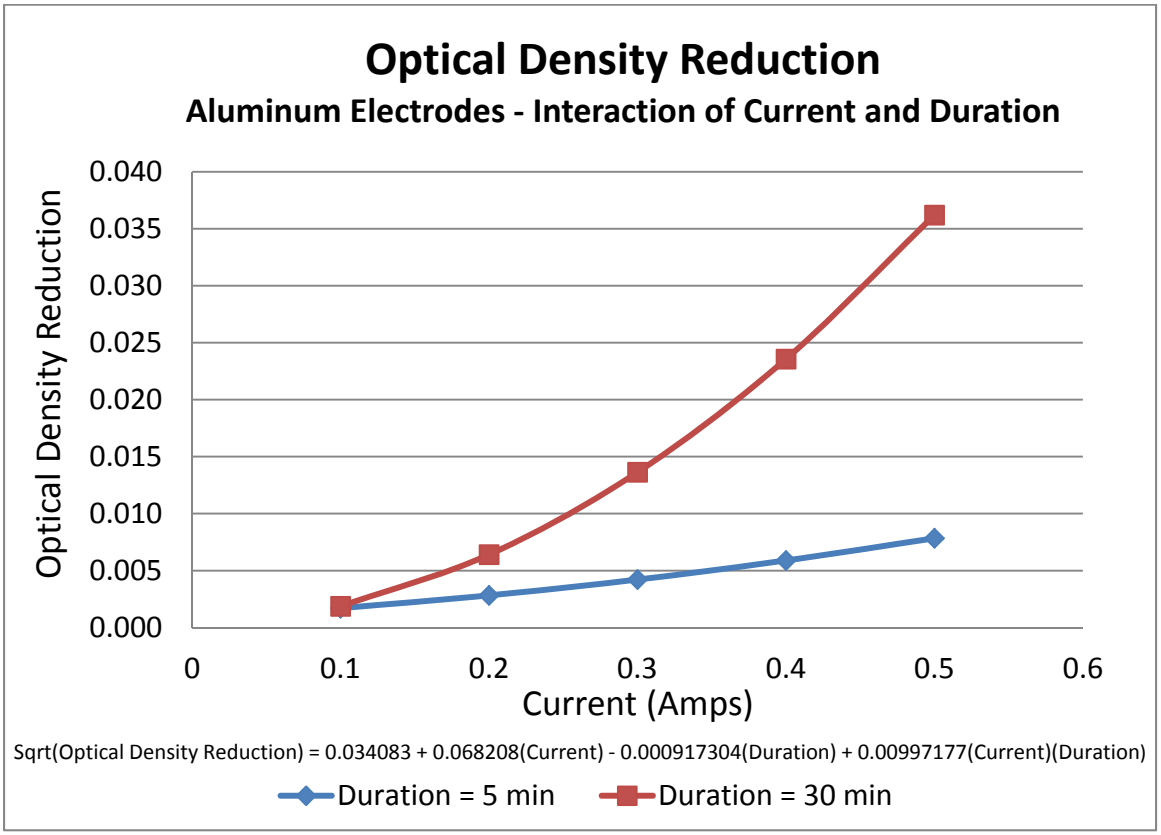

Figure 6: The Effect of Current and Duration on the Reduction of Optical Density When Using Aluminum Electrodes for Electrocoagulation

When examining the response variable of average voltage and applying a Base 10 Log transform to the data, we were able to determine that the electrode material, current, and distance all had a significant effect (p-value less than 0.05) on the average voltage. Current and distance also had an interaction effect. The model has an F-value of 140.23 which would imply the model is significant. Also, there would only be a 0.01% chance that a model with an F-value this large could occur due to noise. From the data represented in Figures 7 and 8, we can see that under the experimental conditions an increase in distance produced an increase in average voltage.

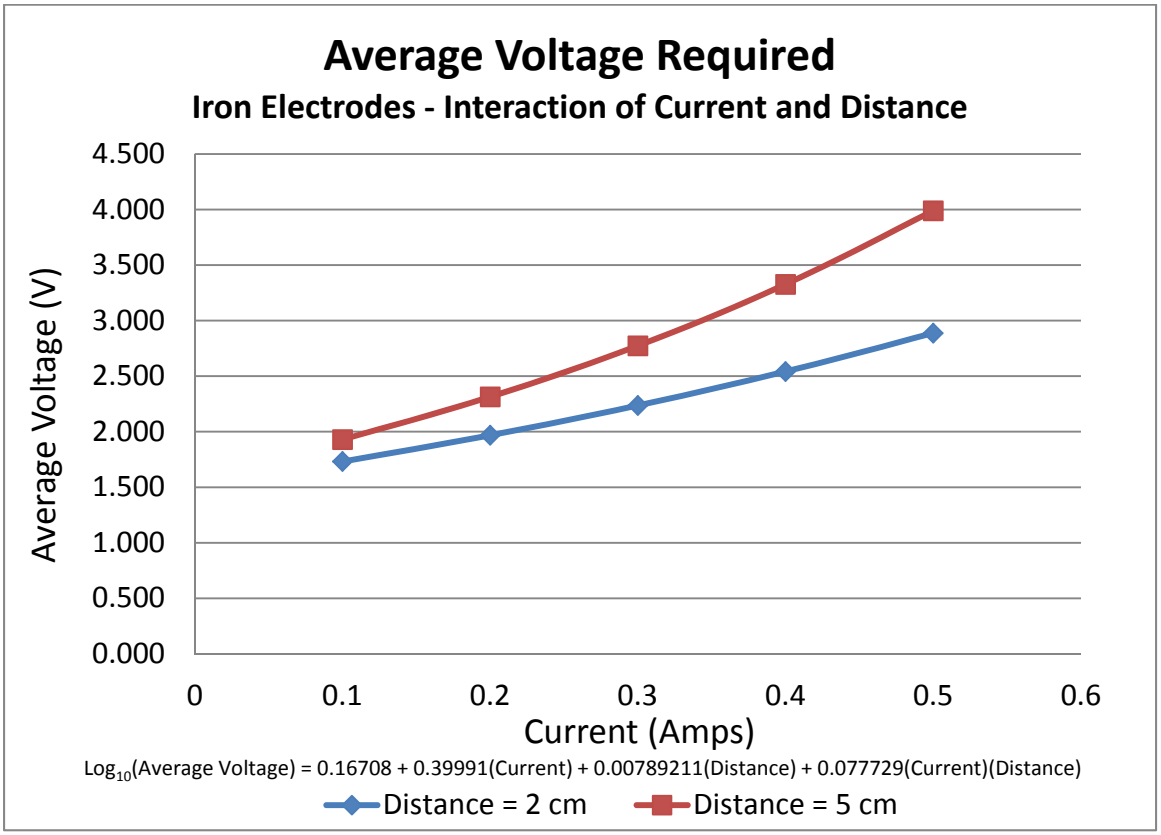

Figure 7: The Effect of Distance and Current on the Average Voltage When Using Iron Electrodes for Electrocoagulation

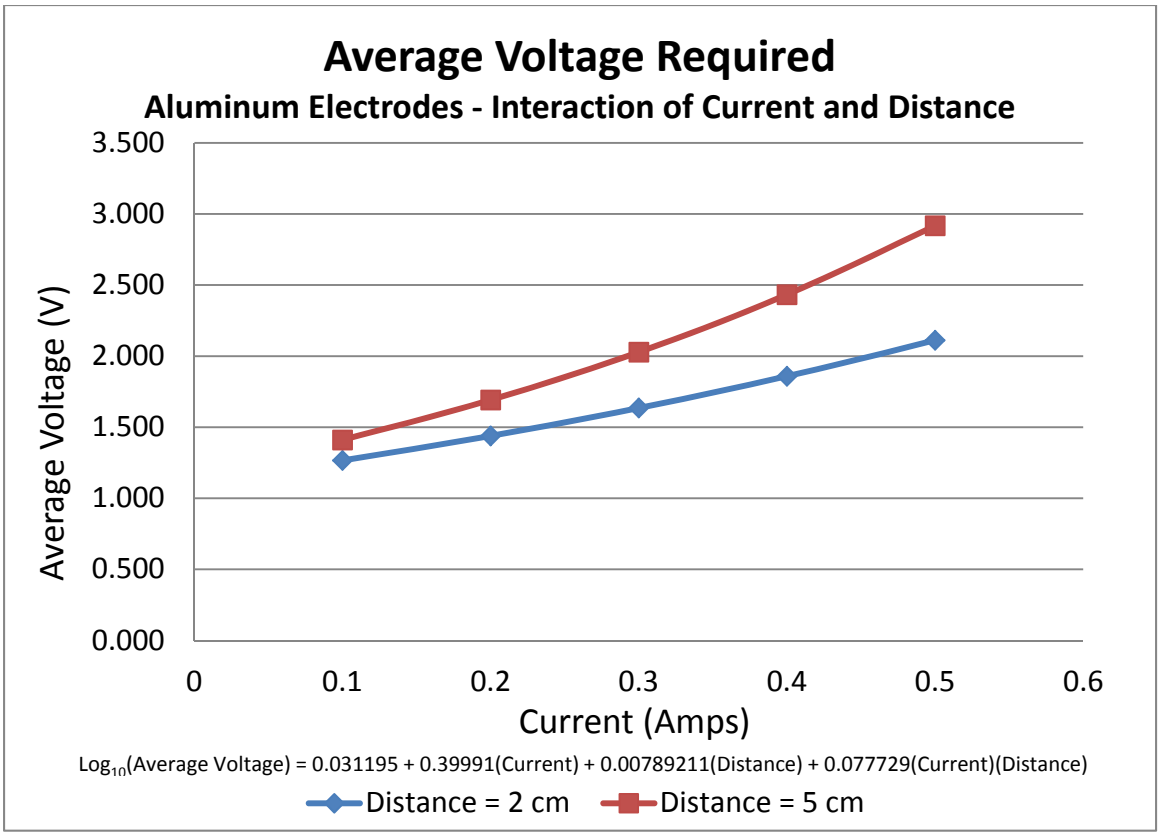

Figure 8: The Effect of Distance and Current on the Average Voltage When Using Aluminum Electrodes for Electrocoagulation

When examining the response variable of electrode consumption and applying a square root transform to the data, we were able to determine that the electrode material, current, and duration all had a significant effect (p-value less than 0.05) on the amount of electrode consumed. Current and duration also had an interaction effect. The model has an F-value of 32.29 which would imply the model is significant. Also, there would only be a 0.01% chance that a model with an F-value this large could occur due to noise. From the data represented in Figures 9 and 10, we can see that at the experimental conditions, an increase in current or duration increased the amount of electrode material that was

dissolved the solution, and also that the electrode material itself played a significant role in the amount of electrode consumed. This result aligns with Faraday's law.

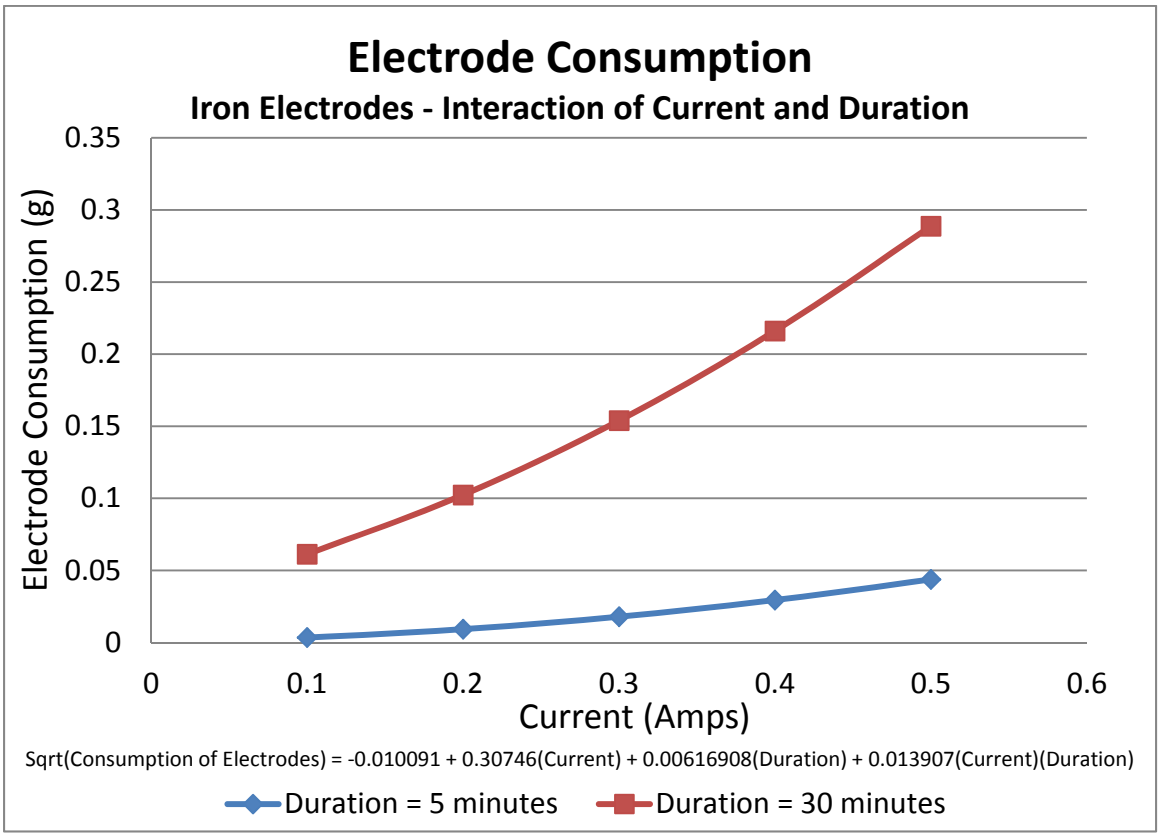

Figure 9: The Effect of Current and Duration on the Amount of Electrode Consumed When Using Iron Electrodes for Electrocoagulation

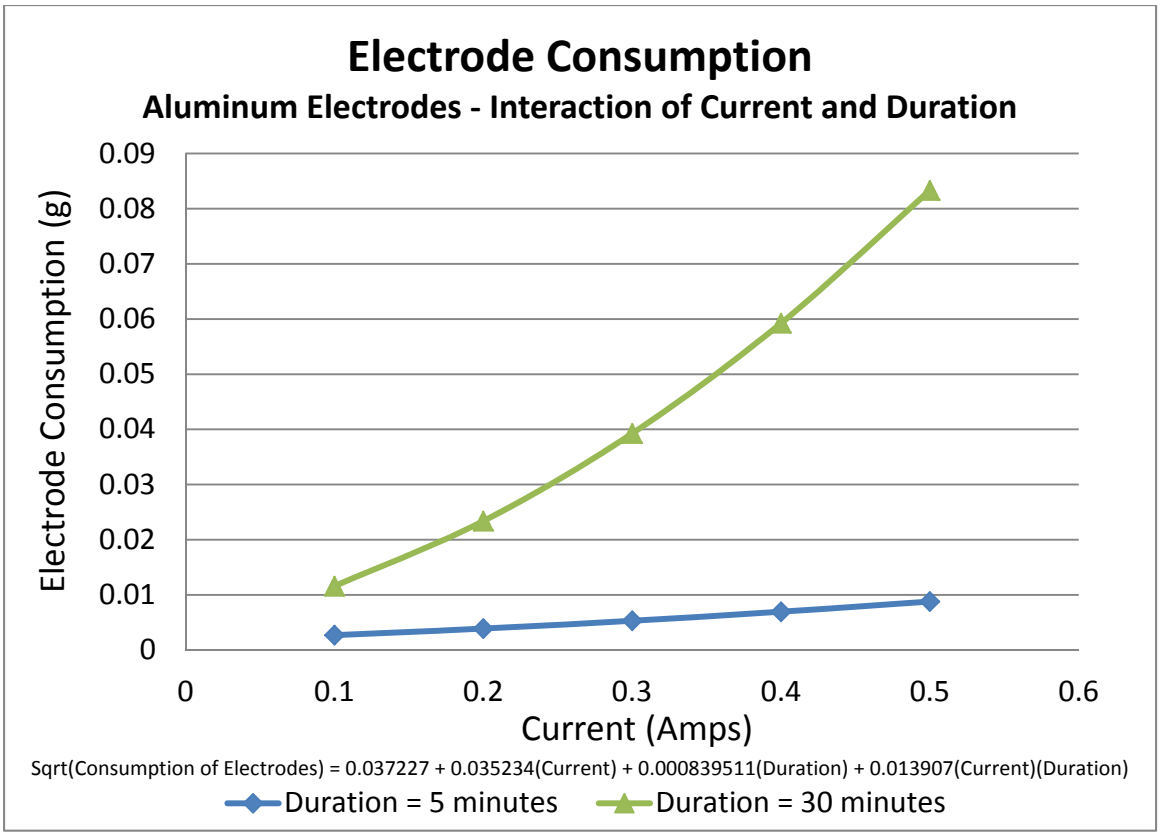

Figure 10: The Effect of Current and Duration on the Amount of Electrode Consumed When Using Aluminum Electrodes for Electrocoagulation

Conclusion

The results from the factor eliminating tests suggested that electrode material, current, and duration all had a significant effect on the amount of electrode consumed as well as the amount of reduction in optical density. The average voltage was affected by electrode material, current, and distance between electrodes. As current and distance between the electrodes were increased, the average voltage also increased. Because the data suggested that distance did not play a significant role in O.D. reduction, and had an adverse effect on the average voltage required, the distance was held constant at the low value of 2 cm

for the following experiments. Also, because the stir rate had no significant impact on the response variables, it was held constant at the low level of 0 rpm stir for the subsequent experiments. Utilizing the low levels for each of the insignificant factors reduced the energy consumption and increased the attractiveness of this process for the dewatering of microalgae. One item of note is that distance and stir rate may have a more significant impact when scaling up the process. Electrophoresis, or the movement due to electrical fields, in the electrolysis chamber at this scale, could potentially be creating a sufficient amount of mixing during the electrolysis process. Even though these factors were not found to be significant on this scale, it would be prudent to examine them during the scale up process. Further research will be need during the scale up process.

CHAPTER V

IDENTIFYING ELECTRODE MATERIALS ABLE TO ACHIEVE A HIGH LEVEL OF SEDIMENTATION OF MICROALGAE

Introduction

The results of the previous study, examining the factors that have a significant impact on the electrocoagulation of microalgae, suggest there is a statistically significant impact on the efficacy of the electrocoagulation process that is derived from the type of electrode material selected. Several studies consider electrocoagulation (Azarian et al., 2007; Gao et al., 2009; Mollah et al., 2004), but none of the studies describe the reasoning for the selection of the electrode material, other than the fact that electrode material provides positively charged ions into the solution once electricity is applied.

As discussed in the literature review, positively charged ions from sacrificial metallic electrodes can be dispersed in the solution by the electrolytic process, and because algae cells carry a net negative surface charge, the interaction between the algae and the metallic ions can result in charge neutralization or, in cases of excess ion and metal hydroxide formation, sweep flocculation of the cells.

Because certain factors were statistically determined to have an influence on the results of electrocoagulation, the only factor that was varied during this set of testing was the

electrode material. The materials examined in this testing were aluminum, copper, iron, zinc, carbon, brass, nickel, and tin.

Objective

The objective of this study was to determine the most practical materials for use as an electrode when using electrocoagulation to aid in the harvest of microalgae.

Experimental Plan

A general factorial design (blocked by replicate) was used to determine the performance of eight electrode materials: zinc, carbon, brass, tin, iron, nickel, copper, and aluminum. Carbon electrodes were included in this set of testing to establish a baseline in optical density reduction. Since no ions are added to the solution when using carbon, any reductions in optical density seen when using carbon electrodes, is either due to gravity settling or another mechanism. Holding current, duration, distance, and stir rate constant allowed a direct comparison of the various electrode materials. The response variables remained unchanged for this set of testing: reduction in optical density, power consumption (average voltage), and electrode consumption.

Results and Discussion

When comparing the results of this set of testing, it was clear that two electrodes outperformed the other electrodes when considering the reduction of optical density in the solution. Those electrode materials were nickel and iron. The results were rather surprising because some previous studies of electrocoagulation of microalgae have

utilized aluminum electrodes (Aragon et al., 1992; Azarian et al., 2007). The reductions represented here were calculated by subtracting the final OD reading from the initial OD reading. Nickel and iron electrodes were able to achieve the highest levels of optical density reduction. Each saw a a reduction of approximately 0.16. This was much greater than the next highest achieving electrode, zinc, which gave slightly more than 0.08 reduction in optical density. Tin and brass followed these electrodes with reductions of 0.053 and 0.32, respectively. Aluminum achieved an OD reduction of 0.026, which outperformed only copper and carbon. Results for each material can be seen graphically in Figure 11 and listed in Table 2.

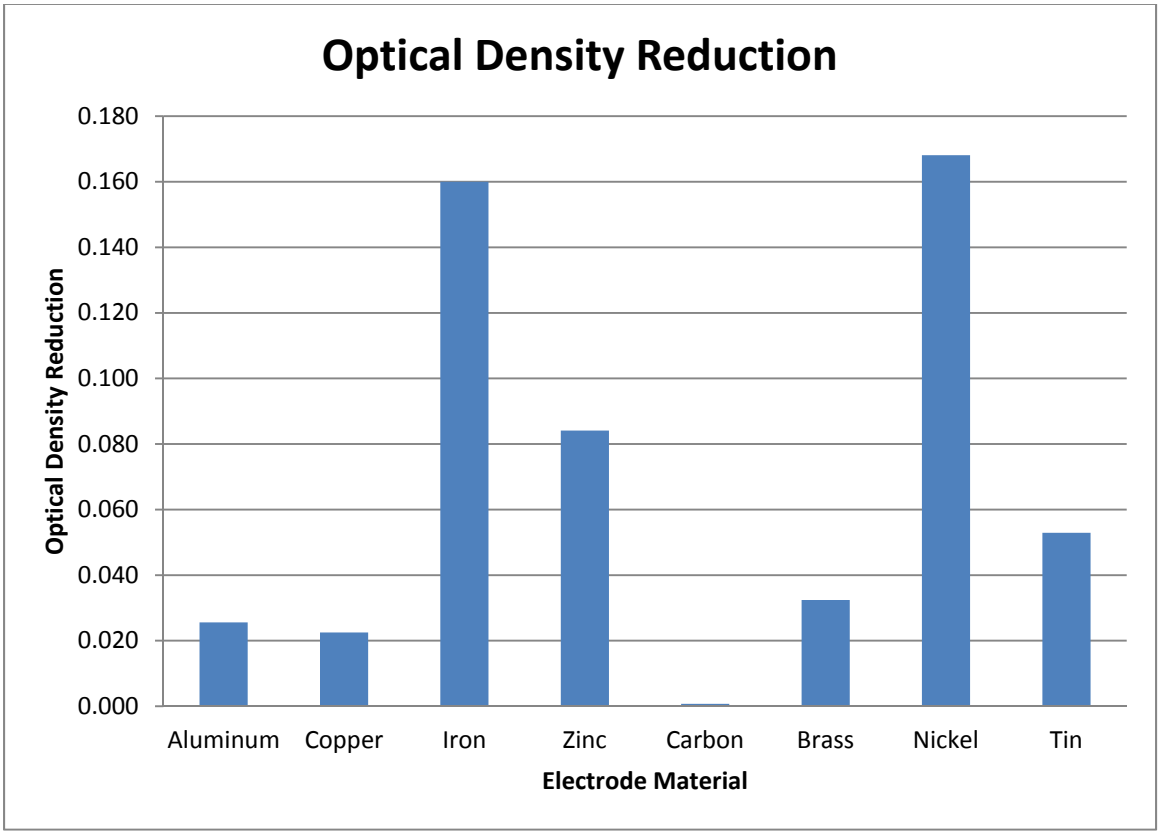

Figure 11: Material Performance Comparison of the Reduction in Optical Density During Electrocoagulation

Table 2: Material Performance Comparison of the Reduction in Optical Density During Electrocoagulation

Material	OD Reduction
Nickel	0.168
Iron	0.160
Zinc	0.084
Tin	0.053
Brass	0.032
Aluminum	0.026
Copper	0.023
Carbon	0.001

When examining the average voltage as an indication of power consumption a low average voltage is preferred. The lower the voltage required, the lower the electricity required. In our testing, we determined that zinc, aluminum, iron, and nickel were the four electrodes that required the lowest amount of energy. The average voltage of the above listed electrodes varied from 2.11 Volts (zinc) to 2.88 Volts (nickel). The remaining electrodes had an average voltage varying from 3.16 Volts (copper) to 4.55 Volts (tin). Therefore, using iron or nickel electrodes, rather than tin electrodes, would increase the energy efficiency approximately 40% when using iron electrodes and 37% when using nickel electrodes. If aluminum electrodes were determined to be a desirable option, this would increase the energy efficiency by 51% over using tin electrodes. Additionally, the cost of tin is approximately 150 times more expensive than iron, 12 times more expensive than aluminum and 1.5 times more expensive than nickel, according to 2013 Commodity Exchanges. The average voltage required for each material is shown in Figure 12, as well as Table 3.

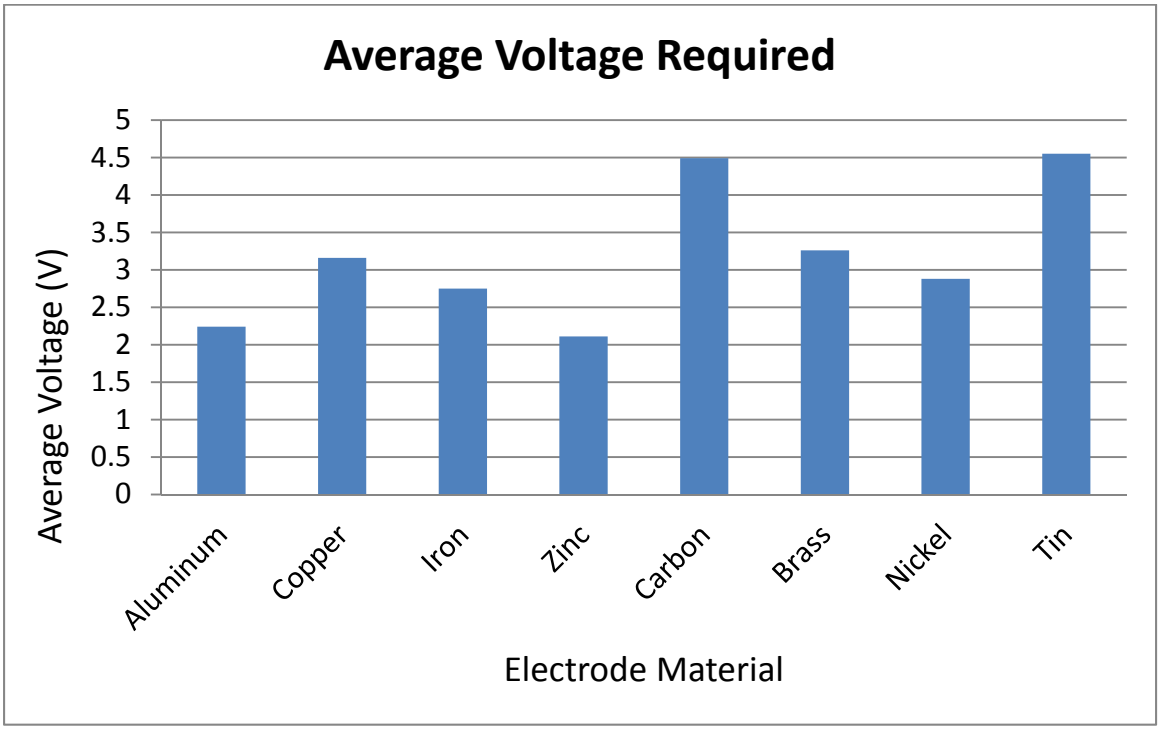

Figure 12: Material Performance Comparison of the Average Voltage Required During Electrocoagulation

Table 3: Material Performance Comparison of the Average Voltage During Electrocoagulation

Material	Avg. Voltage Required (v)
Zinc	2.11
Aluminum	2.24
Iron	2.75
Nickel	2.88
Copper	3.16
Brass	3.26
Carbon	4.49
Tin	4.55

When considering electrode consumption, the preferred response is a low amount of material consumed. The reasoning is simply that the faster the electrode is consumed, the more often the electrode will have to be replaced. Additionally, with a smaller amount of electrode consumed, the risk of introducing toxic levels of minerals into the by-products or recycled media is decreased. The electrodes that saw the smallest reduction in mass were the carbon, brass, nickel, and aluminum electrodes, shown below in Figures 13, as well as Table 4

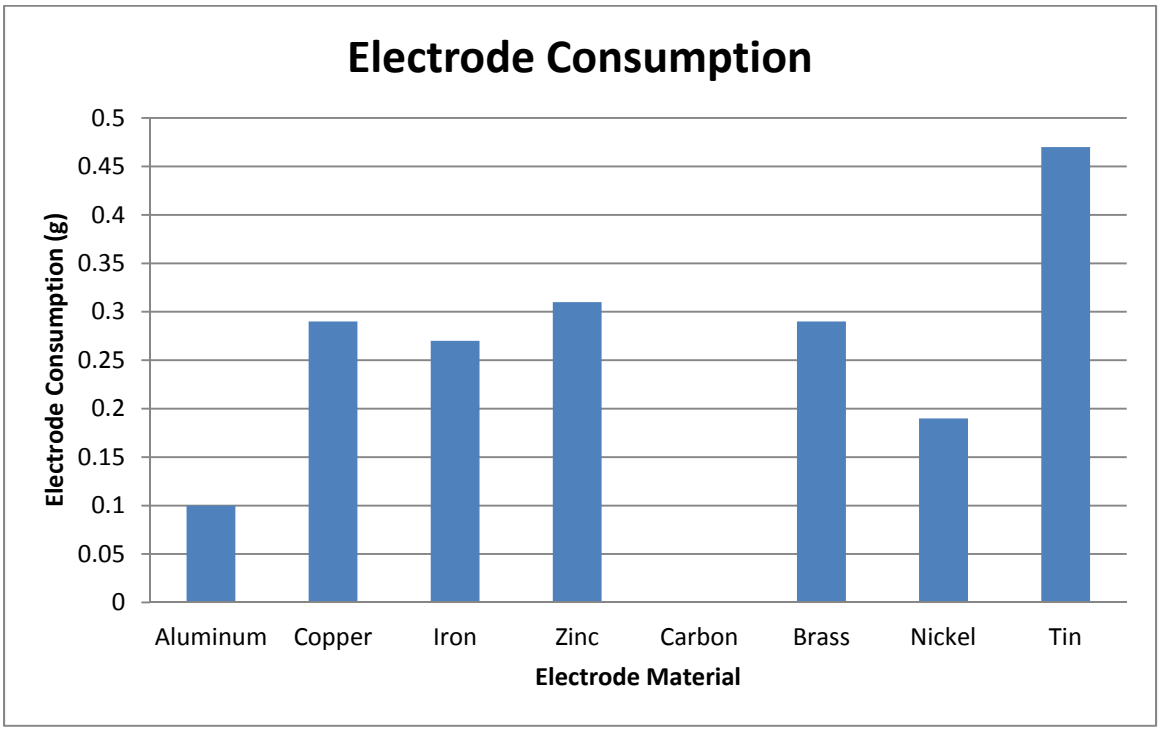

Figure 13: Material Performance Comparison of the Electrode Consumption (grams) During Electrocoagulation

Table 4: Material Performance Comparison of the Electrode Consumption During Electrocoagulation

Material	Electrode Consumption (g)
Carbon	1.1E-16
Aluminum	0.10
Nickel	0.19
Iron	0.27
Copper	0.29
Brass	0.29
Zinc	0.31
Tin	0.47

The electrode material testing showed iron and nickel electrodes to be the most efficient, with reductions in absorbance, O.D. reductions, of approximately 0.16 when compared to the initial O.D. Additionally, the settling rates of the coagulated algae using iron, when compared to nickel, was observed to be considerably different, with iron settling much faster, and should be examined further in future works. Utilizing a faster or slower settling rate could possibly be useful in the scaled up process. Based on the average voltage, the power consumed using iron and nickel was slightly greater than zinc and aluminum but less than the other tested materials.

Conclusion

The electrocoagulation process appears to be an effective method of dewatering microalgae. Utilizing iron or nickel electrodes consistently yielded the highest level of reduction in optical density. Additionally, iron and nickel are viable options, because of their above average results in voltage requirements and electrode consumption. A

response surface method analysis was later performed to help identify if a peak or saddle point in the efficiencies exists within the operating parameters.

CHAPTER VI

IDENTIFYING DIRECTIONAL IMPROVEMENTS IN OPERATING PARAMETERS OF THE ELECTROCOAGULATION PROCESS

Introduction

The results from the previous studies suggest that there are a few variables that can significantly impact the efficacy of electrocoagulation. Previous testing has also provided some insight on the performance of various electrodes in comparison to one another. This set of testing combined the results from previous testing in an effort to find directional improvements in operating parameters for electrocoagulation.

The results of the previous studies suggest there is a significant impact on the efficacy of the electrocoagulation process derived from the type of electrode material selected. Additionally, both the current used and the duration were found to play a significant role in the efficiency of the electrocoagulation process. Several studies consider electrocoagulation (Azarian et al., 2007; Gao et al., 2009; Mollah et al., 2004), but none of the studies describe the methodology used when selecting the operating parameters for the electrocoagulation process.

The only factors that were varied during this set of testing were current and duration. The electrode materials examined were the two highest performing materials, in terms of optical density reduction, from the previous set of testing: iron and nickel.

Objective

The objective of this study was to utilize the results from previous experiments and attempt to identify directional improvements in operating parameters when using electrocoagulation to aid in the harvest of microalgae.

Experimental Design

This set of testing employed an experimental design that is referred to as a Response Surface Method. The Response Surface Method (RSM) design was used to determine if the electrolytic coagulation procedure could be optimized or if directional improvements could be identified. The RSM design utilized in this set of experiments was a central composite design. There are three groups of design points for this type of experiment: two-level factorial design points, axial points, and center points. The factorial points consisted of all possible combinations of the high and low levels of current (high = 0.7 A, low = 0.3) and reaction time (high = 45 min, low = 15 min). The axial points consisted of all possible combinations of the midpoints for each factor range and a high or low level alpha. The default rotatable alpha values were used in this experiment (α = +/-1.414). Five centerpoint replicates were used randomly in the design in order to provide an accurate check for lack of fit. (Montgomery, 2009)

Results and Discussion

Based on the 2 best performing electrode materials from the earlier set of testing, RSM tests were executed in an attempt to identify directional improvements in operating conditions for the electrocoagulation process. The first material examined was iron. The OD reduction using iron electrodes can be seen in Figure 14.

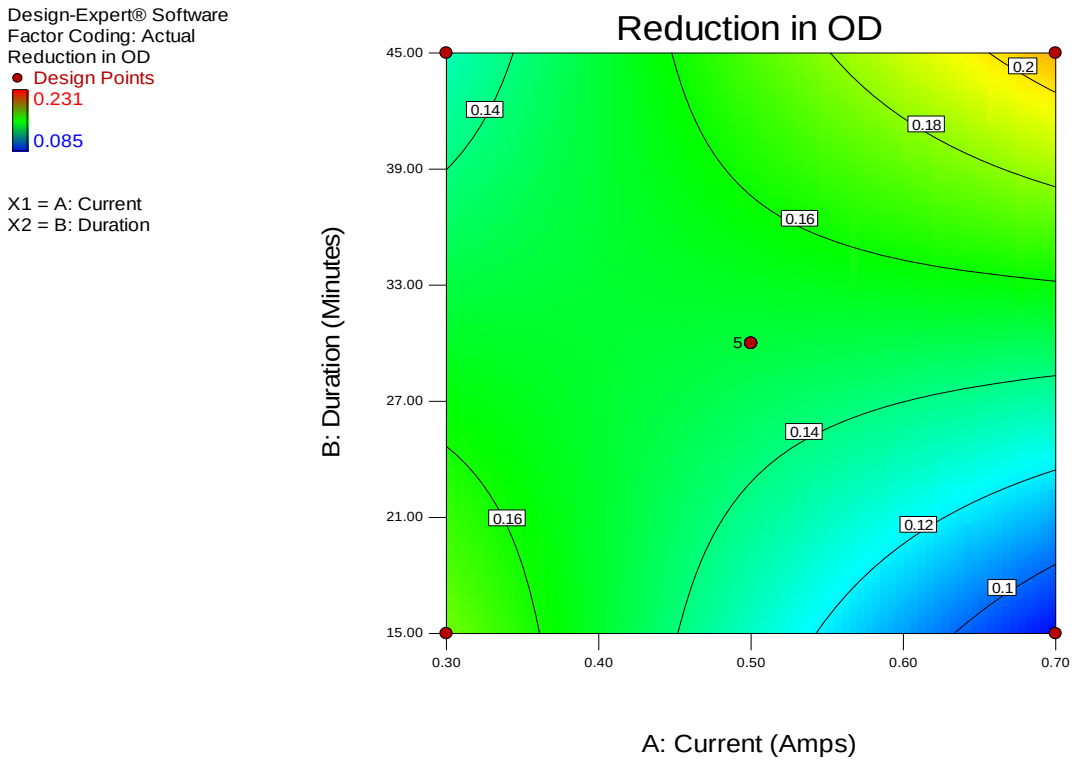

Figure 14: Reduction in Optical Density From Electrocoagulation Using Iron Electrodes Response Surface Contour

The reduction in optical density dropped significantly with low reaction times, and high current; however, when both current and duration were maximized, the greatest reduction in optical density was achieved.

As one would expect, the average voltage increased with the current, as shown in Figure 15.

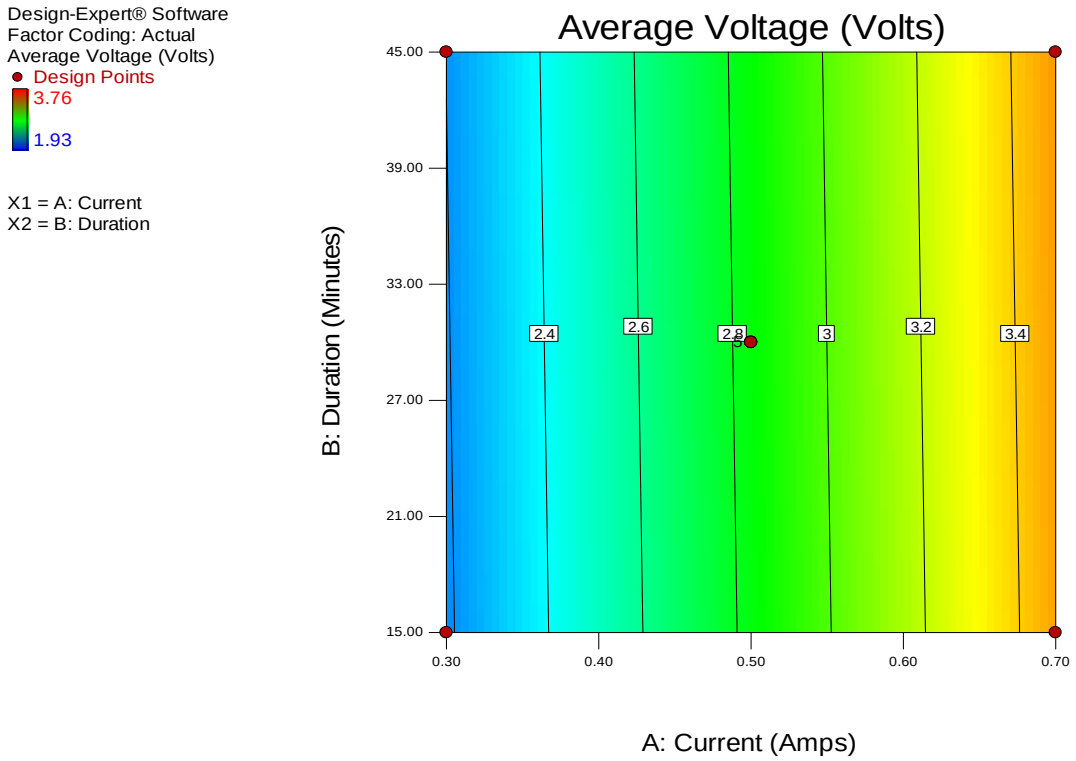

Figure 15: Average Voltage Required During Electrocoagulation Using Iron Electrodes Response Surface Contour

As expected, Figure 16 verifies that the amount of electrode consumed increases as duration and current are increased.

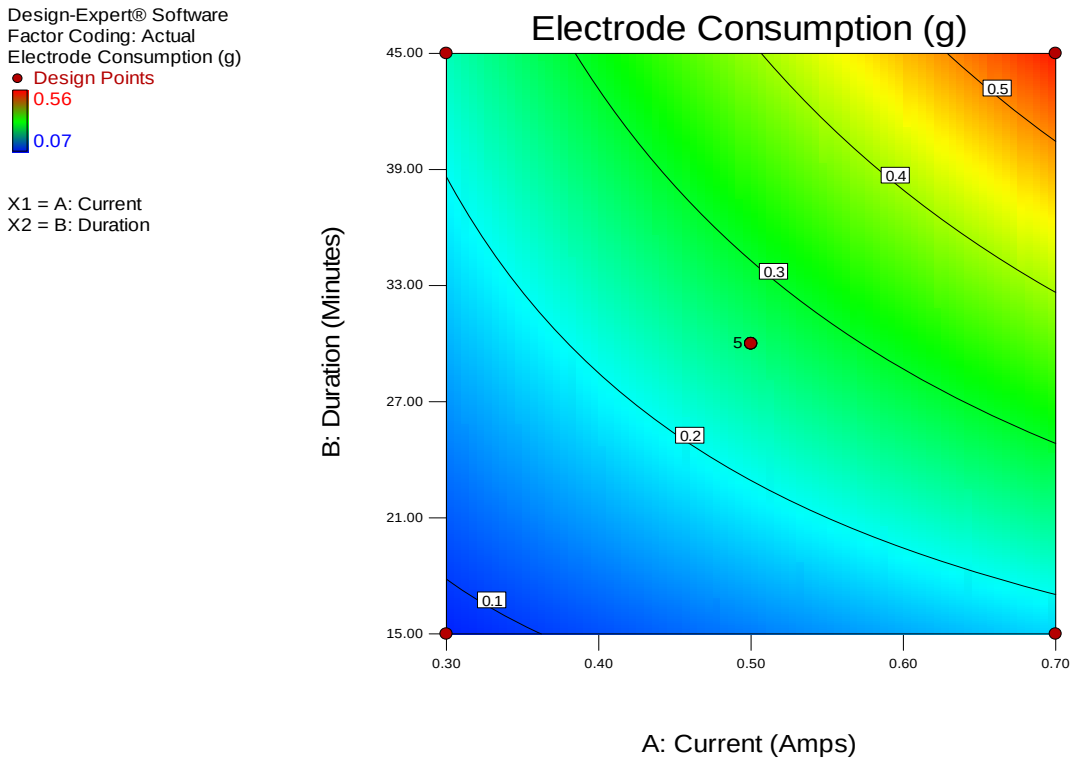

Figure 16: Electrode Consumption (in grams) During Electrocoagulation Using Iron Electrodes Response Surface Contour

With the existing experimental apparatus, the limiting factor when attempting to find a saddle point in the reduction of optical density proved to be the available current that could be supplied by the power supply. Within the operating parameters of the power supply, we were unable to achieve a peak or threshold in the reduction of optical density. Therefore, truly optimized parameters were unable to be determined. Perhaps a power supply with a capacity for higher amperage output, would allow the path of steepest ascent to be followed, in an attempt to identify a peak or saddle point in the reduction of optical density. At that point, perhaps, an optimized operating parameter could be established for the process, as a whole, using the process described below.

The Design Expert software contains an Optimization tool that allows the user to identify the level of importance for each response variable and also to assign a goal and limits for each parameter. The Optimization tool was applied using the constraints listed in Table 5 and five solutions were identified that fit the desired parameters within the confines of the experimental apparatus.

Table 5: Parameter Optimization Constraints for Electrocoagulation Utilizing Iron Electrodes

Response Variable	Goal	Lower Limit	Upper Limit	Importance
Current	minimize	0.3	0.7	2
Duration	minimize	15	45	2
Reduction in OD	maximize	0.085	0.231	5
Avg. Voltage	minimize	1.93	3.76	3
Electrode Consumption	minimize	0.07	0.56	4

The importance level for each response variable was assigned with scale up in mind. Rather than focusing solely on the reduction of optical density as the desired goal, all responses were considered in the optimization tool. A higher value assigned to the response, corresponds to a greater importance and a lower value a lower importance. The response that was assigned the highest level of importance was the maximization of reduction of optical density, because reduction in optical density is essentially the main goal of electrocoagulation for purposes of harvesting microalgae from aqueous solutions for alternative energy. Energy consumption (average velocity) and electrode consumption are irrelevant if the parameters do not provide a reduction in optical density. The

minimization of electrode consumption was chosen as the second most important constraint, in order to decrease the potential for reaching toxic mineral levels in the byproducts. It is critical to maintain usability of the majority of products and byproducts in the process of harvesting microalgae for fuel, for large scale operating to be economically feasible. In addition, the slower the electrode is consumed, the less frequent replacements will be necessary. Minimization of the average voltage was ranked next in importance level. The voltage input needed is directly correlated to the amount of energy required for the electrocoagulation process, which contributes to the overall cost of the process. Current and duration were assigned the lowest level of importance, as these are the operating conditions that were varied in order to achieve the most desirable responses for OD, electrode consumption and voltage.

Within the constraints described above, five solutions were determined by the Design Expert software. These solutions can be found in Table 6. Each solution suggests that the optimized operating conditions for electrocoagulation, within the confines of the experimental apparatus, are: a current of 0.3 amps and a 15 min reaction time. The operating conditions described above should yield a reduction in optical density of 0.17 and only consume 0.09 grams of the electrodes. The RSM parameter desirability results are shown in a a 2D contour in Figure 17.

Table 6: Response Surface Method Desirable Solutions for Electrocoagulation Using Iron Electrodes, Within the Confines of the Experimental Apparatus

	Operating Conditions		Response Variable Predicted Values			
Solution	**Current**	**Duration**	**Reduction in OD**	**Avg. Voltage**	**Electrode Consumption**	**Desirability**
1	0.300	15.00	0.173	2.183	0.086	0.825
2	0.300	15.317	0.173	2.183	0.088	0.822
3	0.306	15.000	0.172	2.201	0.088	0.817
4	0.308	15.000	0.172	2.208	0.088	0.814
5	0.300	16.254	0.172	2.184	0.092	0.812

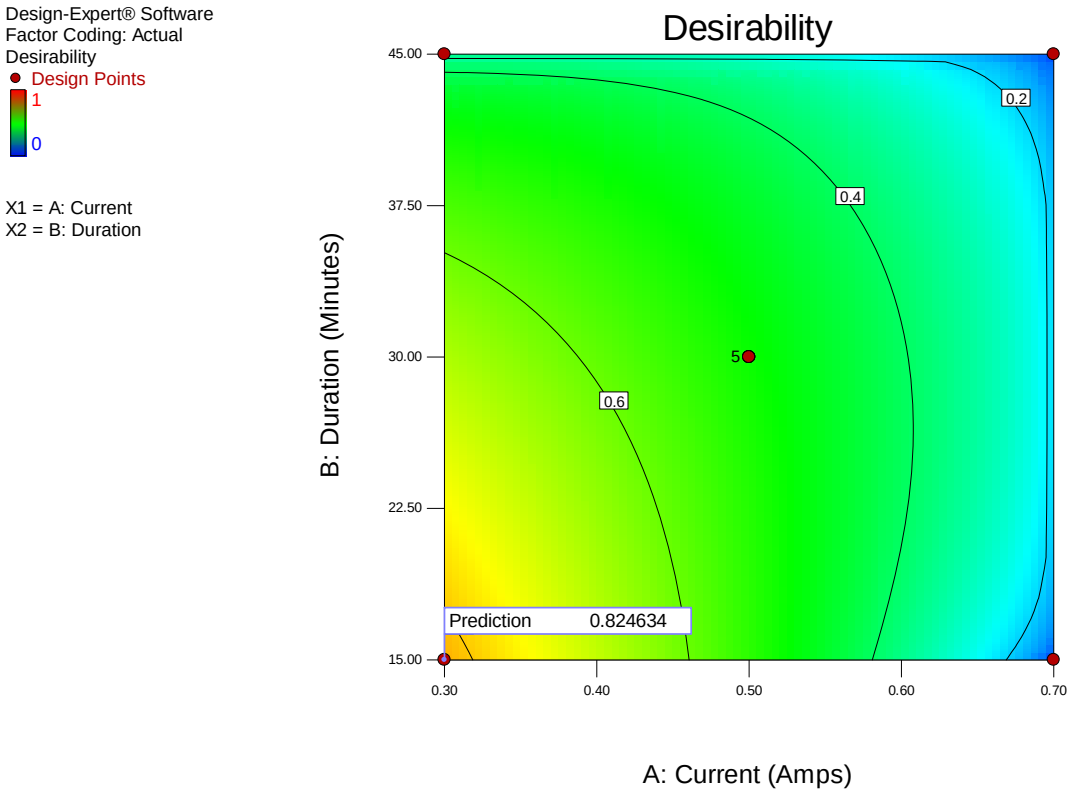

Figure 17: Electrocoagulation Using Iron Electrodes Process Parameter Desirability Response Surface Contour

The results from the testing showed that a desirable result can be achieved over a very large range of operating conditions. The wide range of operating conditions with desirable outcomes suggests that precision in operating conditions is not necessarily critical to the process. The lower amperage was likely favored due to the decrease in electricity consumption.

The second material examined was Nickel. Unfortunately, the RSM model was found to be insignificant (p-value of 0.69). Therefore, we cannot draw any statistically significant conclusions on the accumulated test data. However, it could be inferred from the results of the iron testing, in conjunction with the results from the nickel testing, that the nickel electrodes have a desirable result over a large range and that explicit ideal operating parameters may not exist.

Conclusion

The RSM test results for utilizing nickel electrodes were found to be statistically insignificant. Therefore, we were unable to determine specific desirable operating parameters, within the confines of the experimental apparatus, for this material. Conversely, the RSM testing utilizing iron electrodes allowed us to determine that, within the constraints of the experimental apparatus, the most desirable operating conditions for electrocoagulation are: a current of 0.3 amps and a 15 min reaction time. The operating conditions described above should yield a 0.17 reduction in optical density and only consume 0.09 grams of the electrodes.

During electrocoagulation, a portion of the algae will cling to the metallic ions and fall out of solution, while some ions will remain in solution. The resulting maximum, worse-case, concentration of ions in the solution or in the algae would be 0.3 g/L (300 mg/L). The maximum tolerable concentration of iron for cattle has been estimated at 500 mg Fe/kg (Donald Danforth Plant Sciences: Maximum mineral tolerances, prepared for NAABB by NMSU). Additionally, iron was considered a possible electrode with minimal downstream effects and high animal mineral tolerance (Morrison, 2012).

As current and duration were increased the amount of optical density reduction was also increased. This was identified as a potential directional improvement to the process. However, as current was increased, the average voltage was also increased. This combined with an increase in duration, yields larger power consumption. Additionally, electrode consumption was increased with current and duration. Because a maximum reduction in optical density was not achieved, additional testing following the path of steepest ascent in the RSM results for the OD reduction response should be followed until a peak or saddle point is reached. At that point, perhaps, optimal operating conditions for the electrocoagulation process could be determined.

CHAPTER VII

CONCLUSIONS

The electrocoagulation process appears to be an effective method of dewatering microalgae. Several factors were initially identified as potentially having an impact on the process of electrocoagulation: current, duration, electrode material, distance between electrodes, and stirring rate. These factors were examined to determine the statistical significance of each on the efficiency of electrocoagulation. Based on the results, it was determined that the electrode material, current, and duration all have a significant effect on the amount of electrode consumed as well as the amount of reduction in optical density. The average voltage was influenced by electrode material, current, and distance between electrodes. As current and distance between the electrodes was increased, the average voltage also increased. Because the distance between the electrodes did not play a significant role in O.D. reduction, the distance was held constant at the low value of 2 cm to minimize the energy requirements. The stir rate was also found to have no significant impact on the response variables. Therefore, to reduce the amount of energy required for the process, no stirring was used for all subsequent experiments.

Because the screening tests concluded that the electrode material did have a significant effect on the process, additional tests were conducted to examine eight electrode materials: zinc, carbon, brass, tin, iron, nickel, copper, and aluminum. Of the materials examined, iron and nickel electrodes showed the greatest reduction in optical density. No pretreating, or pH adjustment, was performed on the algae cultures prior to

electrocoagulation. Iron or nickel electrodes consistently yielded a 0.16 reduction in optical density.

A response surface method experimental design was used in an attempt to determine directional improvements in operating conditions, based on the factors found to be significant in the earlier study, for each electrode material. The response surface method allowed desirability rankings to be assigned for the response variables and operating conditions. Optical density reduction was chosen the most important response variable. It was found that higher current as well as longer duration produced the highest reduction in O.D., which was consistent with mechanisms at work in electrocoagulation (i.e. ions released into solution causing charge neutralization and sweep flocculation in cases where excess ions are present). The response surface method provided desirable operating conditions for the use of iron electrodes, within the confines of the experimental apparatus, but was unable to yield desirable operating conditions for the use of nickel electrodes. Utilizing the most desirable operating conditions (a current of 0.3 A and a 15 min reaction time), iron electrodes should yield a 0.17 reduction in optical density.

When considering the price difference between the top performing electrodes materials, it would appear that the use of iron would have economic advantages over the use of nickel. Iron is much less expensive than nickel. The 2013 commodity exchanges list the price of nickel at around 100 times more expensive when compared to iron. Also, the maximum tolerable concentration of iron for cattle has been estimated at 500 mg Fe/kg (Donald

Danforth Plant Sciences: Maximum mineral tolerances, prepared for NAABB by NMSU) while nickel tolerance levels have been estimated to be 100 mg/kg. This is a serious drawback for the use of nickel electrodes.

Throughout the duration of this testing, one goal was to identify potential issues when moving to an industrial scale. The studies conducted in this study were solely on electrocoagulation of an algae culture being grown for alternative fuel. The original idea was to flow cultured algae into an electrocoagulation cell and subject the culture to the process. The addition of other materials or components was not considered. For example, pH adjustment or any other additions/treatments were not made to the solution prior to electrocoagulation in this study. However, some preliminary testing has demonstrated that adjusting the pH of the algae solution can increase the efficacy of additional electrode materials (i.e. aluminum). Further studies are needed to determine if pH adjustment of the algae cultures prior to electrocoagulation would be a viable option on a large, industrial scale. Additionally, an expanded RSM test should be performed along the steepest ascent of the reduction of optical density response. To do this, a power supply with a higher current output capacity should be used. Higher current outputs and longer durations should be utilized for the future testing. Another potential issue was that after examining several samples of microalgae under a microscope, it would appear that contamination can have a negative effect on the results of the testing; more contamination tended to resulted in less reduction in O.D. This could pose serious concerns for the use of this process to dewater large open ponds and is something that certainly should be examined for scaled up processes. Perhaps the utilization of electrocoagulation as a

dewatering/harvesting method would be more attractive for cultures grown in closed photobioreactors, when compared to open ponds. As stated in previous chapters, for this process (microalgae to biodiesel) to become economically feasible, as many products and byproducts as possible should be utilized in order to maximize cost competitiveness with other fuels. Once the solution has undergone electrocoagulation, two distinct layers remain. The bottom layer contains the electrocoagulated algae cells while the top layer, or supernatant, consists of any remaining algae, unused growth media, and residual metallic ions from the sacrificial electrode. In future works, an analysis of the contents of the supernatant should likely be conducted to determine the feasibility of recycling media after the electrocoagulation process. The ability to recycle growth media from the supernatant would result in a significant cost savings and would further move this process closer to being competitive with traditional fuels. Additionally, after the lipids are extracted from the electrocoagulated algae cells for use in transesterification (for the production of biodiesel), the remaining biomass can potentially be used for animal feeds. Therefore, another area of future work would be to examine the toxicity of the electrocoagulated algae to determine if these dead algae cell bodies could be reasonably used as a feed supplement.

Another item that should likely be considered when moving towards a larger scale is the recyclability of the sacrificial metallic electrodes. As the time the electrodes are subjected to the process increases, more and more of the surface of the electrodes are dissolved into the solution. The electrodes become pitted and irregular, and the width and length of the electrodes are reduced. This makes it nearly impossible to calculate the electrode surface

area, and the amount of ion being introduced into the solution. Therefore, if the electrodes are not changed regularly, the duration or current determined to be the most desirable may change as the surface area increases with pitting, or decreases with the reduction of the length and width of the electrodes. Finally, a cost analysis will need to be completed to determine if it is economically feasible to use iron or nickel electrodes at an industrial scale.

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APPENDIX A

GROWTH MEDIUM

Chemical Name	Chemical	g/L
Potassium chloride	KCl	0.75
Calcium chloride dehydrate	CaCl2*2H2O	0.65
Magnesium Sulfate Heptahydrate	MgSO4*7H2O	7.62
Sodium nitrate	NaNO3	0.85
Boric acid	H3BO3	0.034
Sodium dihydrogen phosphate monohydrate	NaH2PO4*H2O	0.04
Commercially available water softener	Tru-Soft	21
Sodium Bicarbonate	NaHCO3	2
Iron (III) chloride hexahydrate	FeCl3*6H2O	0.00315
Manganese (II) chloride hexahydrate	MnCl2*6H2O	0.00018
Zinc sulfate heptahydrate	ZnSO4*7H2O	0.000022
Copper (II) sulfate pentahydrate	CuSO4*5H2O	0.00001
Cobalt (II) chloride hexahydrate	CoCl2*6H2O	0.00001
Deionized water	DI H2O	Balance

The above recipe is for 1 Liter of growth medium.

APPENDIX B

EQUIPMENT LIST

Thermo Fisher Scientific Genesys 20 UV-Vis Spectrophotometer Model 4001/4

Hewlett Packard E3631A DC Power Supply 0-6V, 5A/0-25V, 1A

Power supply leads with alligator clips

Corning PC-410D magnetic stir plates

VWR SympHony SB90M5

VWR micropipettes

Magnetic stir bars

400 mL beakers

2 L Erlenmeyer flasks

Aqua Culture 5-15 gallon, single outlet, aquarium pump model no. 0079285405132

Penn-Flax Aquarium Inline Air filter/Check Valve assembly CV1

Grainger Brass Ball Valve, FNPT x MNPT, 3/8 in, Model 1PZB4

Basic 10 gallon aquarium

Basic 22 inch “plug in” fluorescent light bars or cabinet lights

Thermostatically controlled electric heating element

HDPE Manifold for growth System – See Figure B1

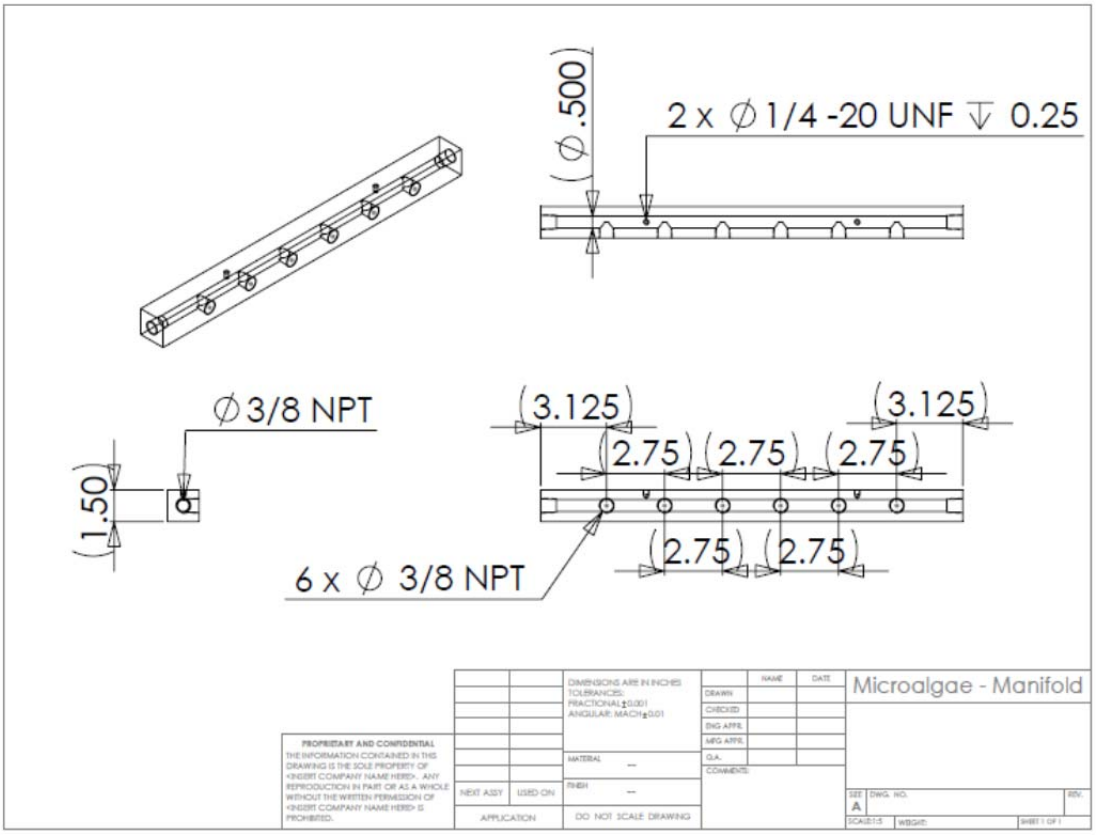


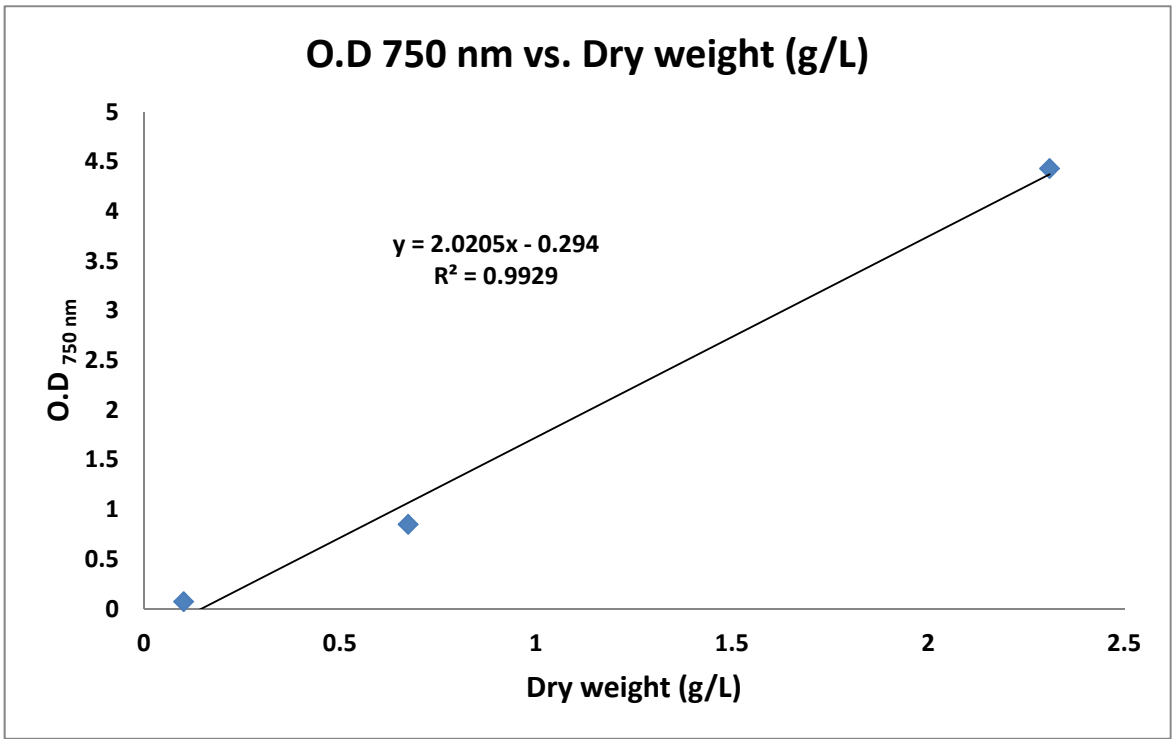
Figure B1: HDPE Manifold Specifications Used for Microalgae Growth System

APPENDIX C

OPTICAL DENSITY MEASUREMENT

The optical density of a sample was determined using a Thermo Fisher Scientific Genesys 20 UV-Vis Model 4001/4 Spectrophotometer at a wavelength of 750 nm. First, a cuvette containing 100% deionized water is used to “zero” the spectrophotometer. 1 mL of the solution is then sampled at a depth of approximately 2 inches below the liquid surface. The 1 mL sample is diluted into the linear range of the machine. A 10X dilution was determined to be adequate for the UV/Vis spectrophotometer being used. Therefore, 9 mL of deionized water is added to the 1 mL sample. Finally, the 10x diluted sample is placed into the machine and the OD determined.

In order to convert the OD to a g/L value, one can apply the equation from the growth curve show below in Figure B1. However, it is important to note that all recorded OD values were diluted 10X. Therefore, the actual OD value is 10x the indicated value.



Note: This curve was constructed using the data from dry weights taken from the growth curve. Each point was made with triplicates.

Figure C1: *Nannochloris oculata* Optical Density vs. Dry Weight (g/L) Correlation Curve (developed by Andrea Garzon)