INVESTIGATION OF MICROALGAE GROWTH KINETICS USING

COAL-FIRED FLUE GAS AS A CARBON SOURCE

A Thesis

Presented to

The Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Engineering,

Specialization in Bioengineering

by

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June 2011

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COMMITTEE MEMBERSHIP

TITLE: INVESTIGATION OF MICROALGAE GROWTH KINETICS
                                 USING COAL-FIRED FLUE GAS AS A CARBON SOURCE

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DATE SUMBITTED: June 2011

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ABSTRACT

Investigation of Microalgae Growth Kinetics using Coal-Fired Flue Gas as a Carbon Source

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Energy related carbon dioxide (CO_2) emissions make up the majority of the United States' greenhouse gas emissions. Emissions must be alleviated to reduce the effects of global climate change. Microalgae cultivation sequesters CO_2 while producing biomass. Algal biomass can provide a renewable feedstock for biofuel and electricity production, and ingredients for pharmaceuticals, nutraceuticals, pigments and cosmetics. Utilizing microalgae to mitigate CO_2 emissions encourages energy independence by providing a feedstock for biofuels and offers other potentially profitable avenues for the uses of biomass. This study focused on investigating the algal growth kinetics of microalgae cultivated with artificial coal-fired flue gas.

Two algal strains, *Chlorella vulgaris* and *Tetraselmis sp.* were cultivated in lab scale photobioreactors to assess the feasibility of using flue gas as a carbon source for microalgae growth. The microalgae growth kinetics were compared between flue gas and pure CO_2 treatments for each algal strain. Both microalgae species were able to grow under flue gas dosing. The differences in growth characteristics for *Chlorella* were statistically insignificant between the two gas dosing treatments. *Tetraselmis* yielded identical maximum specific growth rates among the two gas treatments, while the biomass production was greater using CO_2. At a 95% confidence interval, the difference in biomass production between the gas treatments ranged from 45 to 225 mg/L. The decrease in biomass production for *Tetraselmis* was the only sign of growth inhibition from flue gas. Overall, *Chlorella vulgaris* and *Tetraselmis sp.* are capable of fixating CO_2 from coal-fired flue gas.

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

1.1 Background Information

In 2009 the United States anthropogenic greenhouse gas (GHG) emissions totaled to 6,600 million metric tons of carbon dioxide equivalent (EIA, 2009). Energy related carbon dioxide (CO_2) emissions make up the majority of total GHG emissions at approximately 82% of the total emissions. Within the energy sector, coal utilization contributes 35% of CO_2 emissions as seen in Figure 1. Electricity production from coal fired power plants make up 93% of the total coal derived emissions, resulting to approximately 1,750 million metric tons of CO_2 emitted in 2009 from the combustion of coal (EIA, 2009).

Figure 1: Carbon dioxide emissions by coal combustion, 2009 (Adapted from EIA, 2009)

Global GHG emissions from human activities are the driving force for climate change and the evidence of global climate change is indisputable (IPCC, 2007). Carbon dioxide is the dominate GHG and CO_2 emissions have steadily risen since the industrial revolution, and are projected to increase globally by 1.3% per year (International Energy Outlook, 2010). Therefore, emission mitigation strategies must be implemented to reduce CO_2 emissions and slow the effects of global climate change.

1.2 Coal Fired Power Plants

Currently, coal provides about one half of all electricity generated in the United States (DOE, 2011). To sustain the United States electric energy demand, fossil fuel will continue to be used as an energy resource. One quarter of the global coal reserves are located in the United States ensuring the prolonged usage of coal as an energy resource. The combustion of coal releases an assortment of toxic gases into the atmosphere. Such gases are commonly known as flue gases, and include carbon dioxide (CO_2), nitrogen oxides (NO_x), sulfur dioxide (SO_2) and particulate matter (PM; DOE, 2011).

1.2.1 Flue Gas Characteristics

The typical constituents of coal fired flue gas are 80% nitrogen, 10-15% carbon dioxide, 5-10% oxygen, 100-150 ppm nitrogen oxides, 300-500 ppm sulfur dioxide and approximately 50 mg/m^3 particulate matter (Oilgae, 2011). The precise flue gas composition depends on the type of coal being burned and the combustion characteristics.

Carbon dioxide is the primary GHG responsible for global warming. CO_2 has become the basis for determining the global warming potential of other GHGs. The sheer quantity of CO_2 emissions has made CO_2 the most important GHG in need of a sequestration mechanism.

Nitric oxide (NO) and nitrogen dioxide (NO_2) are known as NO_x because the two gases cycle between each other in the atmosphere. NO can oxidize in the presence of ozone (O_3) forming NO_2. NO_2 in turn can be reduced back to NO by photolysis (Sawyer et al., 2003). Due to the constant transformations between NO to NO_2, a steady state concentration is reached with NO as the dominant species. At high temperature conditions, those similar to coal combustion, the thermodynamics favor the formation of NO. Therefore, typical combustion exhaust streams contain 90% NO (Ozkan et al., 1995). NO_x has been linked to the formation of acid rain and photochemical smog (DOE, 2011).

The consequences of emitting GHGs include global climate change, acid rain, smog and ozone depletion. Seeing that coal will continue to be used for electricity generation and GHG emissions must be alleviated to reduce the effects of global climate change, methods need to be developed and implemented for GHG abatement.

1.3 Greenhouse Gas Regulation

The U.S. Environmental Protection Agency (EPA) requires annual reporting of GHGs by specified sources, usually power plants. Known as the Mandatory Reporting of Greenhouse Gases Rule 74 FR 5620, this mandate requires industries to report their emissions in an attempt to accurately monitor the United States' GHG emissions (EPA, 2011). Although this mandate records GHG emissions, it does not regulate the quantity of GHGs emitted.

Carbon trading has been proposed, and would place a "cap" or upper limit on the amount of pollutants emitted (EPA, 2009). The cap is set lower than the historical emissions in an effort to decrease GHG emissions. Carbon trading would encourage emission abatement strategies because emissions exceeding the pollutant allowance would be fined. With carbon trading likely to become a reality, industries will be forced to buy additional allowances for their GHG emissions or invest in mitigation mechanisms.

In 2010 the United Nations Framework Convention on Climate Change announced their global atmospheric CO_2 concentration cap at 450 parts per million (ppm), although this goal is a non-legally binding agreement (Global CCS Institute, 2010). To achieve a stable atmospheric CO_2 concentration of 450 ppm, GHG emissions must be reduced by 80% (Stern, 2007; Global CCS Institute, 2010). Recalling that energy-related emissions accounted for 82% of total emissions in 2009, such a reduction would require energy sector emissions to be eliminated (Global CCS Institute, 2010).

1.4 Present Carbon Capture and Storage Methodology

Currently, the method of carbon capture and storage (CCS) is to inject CO_2 into geologic formations. CCS entails three distinct steps: carbon isolation, transportation and storage (DOE, 2011). Carbon isolation is achieved through various separation techniques isolating the CO_2 from the flue gas. Then the CO_2 is compressed and transported to the storage site. Long term carbon storage sites include geologic formations such as oil and gas reservoirs, methane coal beds, and saline formations as illustrated in Figure 2 (Global CCS Institute, 2010). The main goals in pumping CO_2 into geologic formations are to 1) store CO_2 while maintaining the environmental integrity of the geologic formation and 2) enhance the recovery of hydrocarbons yielding value-added byproducts. Pumping CO_2 into oil and gas reservoirs can improve oil and gas recovery and is known as enhanced oil recovery (EOR; Global CCS Institute, 2010). Methane coal beds are used in a similar fashion to oil and gas reservoirs, and by pressurizing a coal bed with CO_2 the methane is displaced for more efficient methane recovery. Saline formations are believed to have large carbon loading capacities making them a viable long term solution for carbon sequestration. However, saline formations lack the aspect of value-added byproducts found in EOR and enhanced methane recovery. The biggest hurdle remaining for saline formation sequestration is proving that this method is environmentally acceptable. Containing the carbon dioxide within the saline formation is of highest priority to guarantee that it does not permeate through the earths subsurface and ultimately contaminate groundwater. To fully illustrate the early stages of development for CCS, there are 234 globally recognized CCS projects, and 77 provide both capture and storage while only 8 of the 77 are in operation (Global CCS Institute, 2010).

Figure 2: Geological carbon storage (Global CCS Institute, 2010)

1.5 Carbon Capture and Storage Alternative: Microalgae Cultivation

A viable alternative emission sequestration methodology is the cultivation of microalgae. CO_2 fixation by microalgae grown outdoors is considered the best way to sequester CO_2 because the solar energy utilization is much higher than that of terrestrial plants (Tapie and Bernard, 1988). As microalgae require CO_2 to undergo photosynthesis, cultivating microalgae provides a living carbon sink that continually produces algal biomass. The biomass can be used for the production of biofuels, pharmaceuticals, nutraceuticals, pigments and cosmetics (Oilgae, 2011). Algal biomass can even be used as a fuel source to generate electricity. Microalgae cultivation has a large potential for successful GHG mitigation due to their rapid reproduction, versatile living conditions, and variety of applications to utilize the biomass. Similar to EOR, algal biomass provides the ingredients to produce value added byproducts. With the multitude of uses for biomass, potentially profitable markets exist to help offset the capital cost of implementing microalgae cultivation as a CCS method.

1.6 Microalgae Introduction

Algae are a diverse group of aquatic organisms. In the past blue-green algae and cyanobacteria were included in the classification of "algae". However cyanobacteria are

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prokaryotic organisms and lack a defined nucleus. Therefore, cyanobacteria/blue-green algae are now classified within the *Bacteria* domain. Algae are in the *Eukarya* domain due to a membrane enclosed nucleus, making them eukaryotic organisms. Algae exist as autotrophic and heterotrophic organisms. Autotrophs require CO_2 as their exclusive carbon supply, while heterotrophs utilize organic carbon for energy, metabolism and growth (Sigee, 2005). Algae are subdivided into two classes- macroalgae and microalgae.

The largest and most complex forms of macroalgae are commonly known as kelp. Microalgae can exist as individual cells, in cell colonies, or as long filamentous chains (Sheeler and Bianchi, 1987). Microalgae cells range in size from a couple micrometers (μm) to a few hundred micrometers. Microalgae lack features of higher order plants such as roots, stems, and leaves (Lee, 1999). Able to perform photosynthesis, microalgae produce oxygen while consuming atmospheric CO_2. Photoautotrophic microalgae obtain sunlight for energy and CO_2 provides the carbon supply, both of which are necessary for reproduction. Due to the abundant microalgae population, there is large domain of environmental conditions acceptable for cultivation. Microalgae growth is governed by light and nutrient supply, as well as the environmental parameters influencing growth for the specified algal strain.

1.6.1 Growth Requirements

Microalgae have a specific set of requirements for growth, similar to that of other photosynthetic plants. Basic inputs for microalgae growth include water, sunlight, nutrients and an acceptable range of environmental conditions specific to the algal species. Environmental conditions like temperature, pH, salinity, and dissolved gases all affect the growth characteristics of microalgae. Growth inputs have an optimum range of supply, and providing the optimum growth conditions yields the largest algal population. Generally speaking, larger algal populations result to greater quantities of algal biomass. Figure 3 illustrates how each growth variable (gradient) has a range of influence which can be either too little, too great, or within an optimum

range. Managing the algal growth variables to remain within an optimum range of tolerance produces the greatest amount of biomass, yielding the largest carbon consumption.

Figure 3: Optimizing growth inputs to maximize population

1.6.2 Light "Photo" Requirements

Microalgae require a light period as well as a dark period to grow, known as a photoperiod (South and Whittick, 1987). The light period allows photosynthesis to occur while the dark phase allows the algae to respire. The photosynthesis reaction is shown below as Equation 1. Chloroplasts absorb light energy, and in the presence of CO_2 and water, convert the captured energy into potential chemical energy (Sheeler and Bianchi, 1987). In this way photosynthesis transforms light energy along with CO_2 and water into chemical energy in the form of carbohydrates and releases oxygen in the process. During the dark phase, respiration follows the same equation proceeding in the opposite direction.

$$6CO_2 + 6H_2O + light \leftrightarrow C_6H_{12}O_6 + 6O_2 \qquad \text{\textit{Equation 1}}$$

1.6.3 Nutrient Requirements

Carbon is an indispensable nutrient for the growth of microalgae. CO_2 is a key ingredient driving photosynthesis and is the primary GHG to be sequestered. When CO_2 is injected into

water it becomes carbonic acid (H_2CO_3), thus lowering the water pH (Sawyer et al., 2003). The following set of equilibrium equations illustrates how H_2CO_3 behaves in an aqueous solution and identifies the carbonic species that are consumed by microalgae according to the pH.

$$CO_2(gas) + H_2O\ (aq)\ \rightarrow H_2CO_3\ (aq) \qquad\qquad \textit{Equation 2}$$

$$H_2CO_3\ \leftrightarrow H^+ + HCO_3^- \qquad\qquad \textit{Equation 3}$$

$$HCO_3^-\ \leftrightarrow\ H^+ + CO_3^{2-} \qquad\qquad \textit{Equation 4}$$

Besides carbon, the next most important nutrients are nitrogen, phosphorous, and potassium. Nitrogen (N), phosphorous (P), and potassium (K) are the fundamental macro nutrients required by all plants, and usually plant fertilizers are categorized by their N-P-K ratios. Various micro nutrients such as calcium, iron, magnesium, sulfur, zinc, and various trace elements are also necessary to fully satisfy the nutrient requirements of microalgae. Because microalgae are suspended in water, the nutrient availability is great, and therefore maintaining sufficient nutrient levels is essential for optimum algal growth.

1.6.4 Environmental Requirements

Environmental conditions play an important role governing the growth of microalgae. Environmental parameters such as temperature, pH, salinity, and dissolved oxygen ultimately affect the success of algal cultivation. The microalgae population is composed of around 100,000 identified species, and currently 2,800 different strains are available for purchase (Sheehan et al., 1998; UTEX, 2011). Therefore, the optimum environmental conditions are specific to the individual algal strain.

1.7 Project Goals

The main purpose of this project was to investigate and evaluate the use of coal fired flue gas as a carbon source for microalgae cultivation. Lab scale photobioreactors were used to grow microalgae and demonstrate that microalgae cultivation provides a viable CCS method. Analytical methods were used to model the algal growth kinetics. Using the biomass produced

and the maximum specific growth rate a comparison was drawn between flue gas and pure CO_2 dosing for each algal strain. The purpose of this project was broken down into 4 specific project outcomes:

1) Confirm the feasibility of cultivating microalgae with flue gas as a carbon source

2) Maximize algal biomass production

3) Quantify microalgae growth kinetics

4) Analyze the growth kinetics among gas dosing treatments

1.8 Project Importance

Industrial emissions are becoming increasingly ascribed to global climate change as identified by the Intergovernmental Panel on Climate Change (IPCC). GHG emissions are beginning to be regulated and therefore methods for reducing emissions will be implemented. As said by the IPCC, all energy related emissions must be eliminated to effectively stabilize the atmospheric CO_2 concentration at 450 ppm. Further, utilizing microalgae as a CCS method gives rise to a biofuel feedstock that could help the United States become independent of foreign oil. Overall, the importance of sustainability and environmental consciousness is greater than ever, and seeing that energy related emissions will not cease, the need to mitigate GHGs is unprecedented. Cultivating microalgae provides a biological mechanism for sequestering CO_2 and provides a renewable feedstock for biofuels. This is of utmost value, the fact that one process, *cultivating microalgae* satisfies two prevalent global needs; the need to reduce CO_2 emissions while producing a renewable feedstock for biofuels. Here the old saying, to *kill two birds with one stone* has never been more appropriate.

1.9 Experimental Strategy

Two microalgae species were chosen for this experiment. One freshwater and one saltwater strain were selected for growth for the reason that power plants are located near bodies of water for cooling, whether it is fresh or salt water. The water body adjacent to the power plant would have to be used as growth medium to fulfill the large water demand for microalgae cultivation. *Chlorella vulgaris* was the chosen freshwater strain, and *Tetraselmis sp.* was the saltwater strain used. Originally, both algal strains were to be tested in 9 trials of week-long growth periods. Due to the financial constraints on the project only 3 trials were carried out. In addition, the growth period was reduced to 5 days. Using twelve lab-scale phtotobioreactors (PBRs), a spilt plot design was implemented to maintain consistency among gas treatments and growth trials. Therefore, both algal strains and both gas dosing techniques were applied for every growth trial. Figure 4 illustrates the split plot design incorporating two microalgae strains, *Chlorella* and *Tetraselmis*, and two gas dosing regimens. Pure CO_2 dosing was the control variable for algal growth, while flue gas dosing was the variable of interest. The algal growth characteristics under flue gas dosing were compared to the algal growth characteristics exhibited by the control variable per algal species. Executing 3 growth trials with this experimental design yielded 9 replicates per algal strain for each gas treatment.

	Flue Gas Dosing	CO$_2$ Dosing
Chlorella	3	3
Tetraselmis	3	3

Figure 4: Number of samples for one growth trial using a split plot experimental design

Each photobioreactor (PBR) configuration aimed to maintain optimum environmental conditions such as light influx, temperature and pH. Setting these variables at the optimum level per algal species promoted the fastest reproductive rates and resulted in the largest carbon sequestering capacities. The photoperiod was the same for each algal strain. The temperature was controlled for *Chlorella* only due to its optimum temperature at 29°C (Mehlitz, 2009). *Tetraselmis* being the saltwater strain prefers cooler water and therefore *Tetraselmis* was subject to ambient temperature conditions. The pH was monitored and maintained at relatively constant levels by gas injection. Equal nutrient supplements were provided upon inoculation. Through these methods the variables effecting microalgae growth were isolated, effectively eliminating the influence on algal growth from such variables. Maintaining consistent environmental conditions allowed the variable of interest, *flue gas dosing,* to be compared against the control variable, *CO_2 dosing*. The experimental design intended to maximize algal biomass production and confirm flue gas as a carbon substitute for algal cultivation. The hypothesis was to determine whether or not a statistically significant difference existed between microalgae grown with two different carbon sources, flue gas verse pure CO_2.

CHAPTER II

LITERATURE REVIEW

2.1 Introduction

Algal flue gas mitigation has been studied for the past couple of decades and poses as a viable biological mechanism to capture and utilize CO_2. Using flue gas from various combustion processes as a carbon source for algal growth has been proven on a lab scale. Microalgae cultivation successfully assimilates CO_2 from flue gas and can grow with minimal inhibition in the presence of NO_x and SO_x (Yoshihara et al., 1996; Doucha et al., 2005). Supplying flue gas for microalgae cultivation and CO_2 fixation can occur in two ways, by direct flue gas injection or separating the CO_2 from the exhaust stream. Isolating CO_2 from exhaust streams is an unfavorable precursor for algal cultivation in terms of the energy and cost requirements to separate CO_2. Direct flue gas injection into algal cultures brings arise to issues pertaining to the high temperatures of flue gas, and the presence of NO_x and SO_x (Madea et al., 1995). For this reason, microalgae strains tolerant to high temperatures and resilient to the presence of NO_x and SO_x have historically been sought to be cultivated as a biological mechanism to mitigate flue gas emissions. Previous studies have focused on identifying microalgae strains capable of withstanding direct flue gas injection, the effectiveness of CO_2 mitigation (flue gas decarbonization), NO_x serving as a potential nitrogen source for algal growth, and the economic analysis of implementing microalgae cultivation as a flue gas emission mitigation strategy.

2.2 Case Studies

The case studies highlighted for the investigation of this project were chosen to illustrate the progression of cultivating microalgae with flue gas. They are not the only studies pertaining to this area of research; however they represent the development of utilizing microalgae cultivation to mitigate flue gas emissions.

2.2.1 Microalgae Strain Selection

In a report published by Maeda et al. (1995) titled, *CO_2 Fixation from the Flue Gas on Coal-fired Thermal Power Plant by Microalgae*, the microalgae strain *Chlorella sp. T-1* was identified as being able to grow under direct flue gas injection conditions. A series of growth treatments were performed to determine the algal resistance to temperature, CO_2, NO_x and SO_x variations. *Chlorella sp. T-1* was grown in 600 mL batch cultures and demonstrated resilience up to 40°C. Algal CO_2 resistance favored 10-50% CO_2 concentrations delivered at 0.5L/min. The same concentration of NO_x and SO_x in flue gas, half the concentration, and double the concentration resulted in no effect on algal growth. Through this study *Chlorella sp. T-1* was found to be a successful candidate for growth in severe environmental conditions such as those experienced by using flue gas as a carbon source for microalgae cultivation.

2.2.2 Mitigation Effectiveness

Using microalgae as a carbon fixer for flue gas emissions requires a high degree of CO_2 mitigation efficiency if industrial implementation is to take place. Ultimately, microalgae must sequester a significant fraction of CO_2 from power plant exhaust streams to effectively provide a mitigation strategy. Doucha et al. (2005) performed a study to determine the degree of CO_2 mitigation or "flue gas decarbonization". In their publication titled, *Utilization of Flue Gas for Cultivation of Microalgae (Chlorella sp.) in an Outdoor Open Thin-layer Photobioreactor* they achieved 10-50% CO_2 consumption by microalgae grown in a 330 L photobioreactor. Their photobioreactor was characterized by a 55 m^2 culture surface area with an algal suspension thickness of 6 mm. They further deduced that increasing the flue gas injection rate decreased the degree of CO_2 mitigation. In a CO_2 mass balance on flue gas containing 8% CO_2, the efficiency of microalgae CO_2 biofixation was determined. Upon flue gas injection, half of the CO_2 content in the flue gas was lost due to culture medium saturation. Of the remaining 50%, approximately 10% was lost from suspension, resulting to 40% of the CO_2 supplied within the flue gas to be

utilized by the algal cells. This flue gas decarbonization efficiency was specific to the bioreactor design, algal strain used, and environmental parameters surrounding microalgae growth.

Novakovic et al. (2005) published their work titled, *Air-Lift Bioreactors for Algal Growth on Flue Gas: Mathematical Modeling and Pilot-Plant Studies* and demonstrated a greater carbon uptake efficiency. Thirty triangular air lift bioreactors were used for cultivation each with a volume of 30 L. Flue gas was constantly administered into each bioreactor at a flow rate of 600-800 mL/min. They reported a CO_2 removal efficiency of $82.3 \pm 12.5\%$ on sunny days and $50.1 \pm 6.5\%$ on cloudy days. Further, the biomass production was consistent with the carbon removal efficiency. The high decarbonization efficiency may have been due to the specialized bioreactor in operation; however it reveals that a carbon uptake efficiency of 80% is achievable.

2.2.3 Nitrogen Oxides as a Nitrogen Supply

Studies have been performed to determine whether or not NO_x can provide a supplemental nitrogen source for microalgae growth. Nagase et al. (2001) explored the ways in which NO is utilized by microalgae. Their publication titled, *Uptake Pathway and Continuous Removal of Nitric Oxide from Flue Gas using Microalgae* assumed that two possible pathways existed. The first possible pathway being the oxidation of dissolved NO into nitrate or nitrite. The second pathway was direct diffusion of NO into the cells. The results concluded that little NO was oxidized in the culture medium, and therefore the majority of NO diffused into the algal cells.

The aforementioned study by Doucha et al. (2005) determined the effectiveness of *Chlorella sp.* to denitrify flue gas. About 10% of the NO_x from the entering flue gas was able to be absorbed in the culture solution.

In a study conducted by Yoshihara et al. (1996) titled, *Biological Elimination of Nitric Oxide and Carbon Dioxide from Flue Gas by Marine Microalga NOA-113 Cultivated in a Long Tubular Photobioreactor* the effects of NO concentration, flow rate, and algal uptake of NO were

investigated. A 4L vertical column bioreactor was used to cultivate a marine microalga strain NOA-113. The optimum gas flow rate was 100 mL/min causing a 51% elimination of NO. Approximately half of the NO supplied from the flue gas was consumed at NO concentrations of 100 and 300 ppm.

The denitrification of flue gas could potentially supply an additional nitrogen source for microalgae to grow. Although the works previously listed do not conclude the same reduction in NO_x by microalgae, it is evident that NO_x can be mitigated through microalgae cultivation.

CHAPTER III

MATERIALS AND METHODS

3.1 Microalgae Culturing Equipment

Bioreactors have long been used for microbial growth and fermentation. The word photobioreactor (PBR) stems from the historical use of bioreactors in the biotechnology industry with the addition of "photo" implying the reactor itself is transparent allowing an influx of light energy. Photobioreactors (PBRs) served as the holding tank allowing algal growth and carbon fixation. Twelve vertical column Plankton Reactors (Aqua Medic, Plankton Reactor, Bissendorf, Germany) were used and for the remainder of this report will be called PBRs. Each PBR was a transparent plastic cylinder with a holding volume of 2.25 L. A fluorescent light (18W, 6700K, 1300 lm; Aqua Medic, Plankton Light Reactor, Bissendorf, Germany) provided the necessary light for photosynthesis to occur. As microalgae grow the pH of the algal slurry increases due to the consumption of the carbonic species present. By setting a pH target point, CO_2 or flue gas was injected into the aqueous solution upon reaching the upper target value. The upper pH limit was set at 7.5 and 8.0 for *Chlorella* and *Tetraselmis* respectively. Injecting CO_2 into the aqueous solution forms carbonic acid, thus lowering the pH. Therefore, the pH was in constant balance between algal carbon fixation and gas injection. Twelve pH meters (Milwaukee, SMS 122, Romania) continuously monitored the pH of the algal solution. They were coupled with CO_2 control valves (Red Sea, CO_2 Magnetventil, Israel) which acted as the gas dosing solenoid, in essence maintaining a constant pH level by supplying the proper amount of carbon. Ambient air pumps (Fusion Quiet Power, 400, Taiwan) were used to continuously agitate the culture and keep the algal solution homogeneous. Digital thermometers (Coralife, ESU Reptile) monitored the aqueous solution temperature. Submersible heaters (Marine Land, Stealth Pro, China) were used for cultivating *Chorella* and were set at 30°C. Ambient temperature conditions were sufficient for Tetraselmis as its optimum temperature was around 22°C. Combining this set of cultivation

equipment allowed the major growth variables to be controlled and maintained at constant levels. Keeping the temperature, pH, and nutrient levels constant enabled the variable in question, *the effect of direct flue gas injection* to be investigated. Each PBR was accompanied by the same additional components to make twelve complete PBR sets as seen in Figure 5 and 6.

Figure 5: Laboratory PBR schematic   Figure 6: Laboratory PBR in use

3.2 Cultivation Method

Each growth trial was limited to 5 days of growth. Upon inoculation algal samples would be taken for analysis. For the remainder of the growth period samples were taken at approximately the same time of day. The algal strains were expected to follow a typical non-continuous batch culture growth curve. Batch growth is characterized by 4 distinct phases: lag, exponential, stationary and death. The 4 phases are depicted in Figure 7 and briefly explained below.

Figure 7: Typical batch culture growth curve (Adapted from Shuler and Kargi, 2002)

1. **Lag:** Immediately after inoculation the culture experiences a lag phase as it acclimates to the new environmental conditions.

2. **Exponential:** After the culture has fully adapted to the batch conditions the culture begins reproducing exponentially. This is the optimum growth seen throughout the growth cycle, and the cell population doubles at regular time intervals, known as the doubling time (t_d).

3. **Stationary Phase:** The stationary phase begins after exponential growth decelerates and the microorganism population is maintained. At this point the culture has reached its maximum population. At stationary phase the growth rate is equal to the death rate, and the population is held constant.

4. **Death Phase:** The death phase occurs once the maximum population has been supported for a period of time and the culture begins to die faster than it can reproduce. Usually nutrient depletion or toxic product accumulation causes the microorganism population to decline (Shuler and Kargi, 2002).

3.2.1 Maintaining Inoculum

In order to begin batch growth for each trial, algal inoculum was maintained throughout the study. *Chlorella* and *Tetraselmis* inoculum were grown in two 2 liter Erlenmeyer flasks. The idea in maintaining the culture inoculum was to keep each strain in a subdued yet healthy condition. The photoperiod was 1:1, at 12 hours of light and 12 hours of dark. Ambient air was constantly diffused into the media to provide agitation and minimal CO_2. In this way each strain grew slowly and after a week of inhibited growth the culture was ready for inoculation. The goal was to have each strain at a transmittance of 40% for inoculation.

3.2.2 Aseptic Techniques

All twelve PBRs were taken apart and sanitized before inoculation. All other equipment in contact with the algal solution was also cleaned including the submersible heaters, the pH probes, the thermometers, and the algal sampling ports. A phosphoric acid solution (Star San, Five-Star, Commerce City, CO) was used for all equipment sanitation. Maintaining aseptic culturing techniques was essential to avoid contamination and culture crashes.

3.2.3 Inoculation Ingredients

Each PBR had a holding volume of 2.25L, however the displacement caused by the submersible heater, pH probe and thermometer yielded an operating volume of 2.0L. In addition, two inches of head space was required to accommodate the algal uproar upon gas dosing. Beginning inoculation, 200 mL of algal solution at a transmittance of 40% was used. The remaining 1800 mL was filled with distilled water. Distilled water was used for *Chlorella*, while *Tetraselmis* required a salt water additive called *Instant Ocean*. Salt water was made with distilled water and Instant Ocean to an achieved specific gravity between 1.020 - 1.024 at 25°C. *Schultz Plant Food Plus* provided the necessary nutrients and the nutrient breakdown is seen in Table 1. Liquid plant food of was administered in doses of 1.5 mL per PBR. This was the only nutrient supply for the duration of the growth period besides the gas dosing. The photoperiod was set at 2:1 resulting to 16 hours of light and 8 hours of dark. Once all twelve PBRs were inoculated, the growth period began and the first samples were collected for analysis.

Table 1: Liquid Macro and Micro Nutrients, 10-15-10

Schultz Plant Food Plus	
Nutrient Constituents	**Percent, %**
Total Nitrogen *Ammoniacal Nitrogen, 1.6%* *Nitrate Nitrogen, 0.2%* *Urea Nitrogen, 8.2%*	10
Available Phosphate (P_2O_5)	15
Soluble Potash (K_2O)	10
Iron (Fe)	0.10
Manganese (Mn)	0.05
Zinc (Zn)	0.05

3.2.4 Sample Collecting and Culture Monitoring

Samples for analysis were collected daily throughout the 5 day growth period. Upon analyzing the samples on the fifth and final day, the PBRs were taken apart, sanitized and put back together for the proceeding growth trial. Daily monitoring of temperature and pH were

performed to ensure no adverse effects on microalgae growth from these two variables, as well as preserving consistency among sample replicates.

3.2.5 Gas Treatments

Pure CO_2 was used as the control for cultivating both algal strains. Flue gas was purchased from Praxair and the composition was meant to imitate coal-fired flue gas. The flue gas composition can be seen in Table 2.

Table 2: Flue Gas Composition

Flue Gas Constituents	Concentration
Carbon Dioxide (CO_2)	14% (mole percent)
Nitric Oxide (NO)	100 ppm
Sulfur Dioxide (SO_2)	300 ppm
Nitrogen (N_2)	balance

3.3 Quantifying Growth Kinetics

There are multiple ways to quantify microorganism growth and each method has its advantages and disadvantages. Three methods were used to model the growth kinetics including cell counting, mass determination by volatile suspended solids (VSS) and optical density. Cell counting and optical density were performed daily throughout the growth cycle, while VSS was carried out at the beginning and end of each growth cycle.

3.3.1 Cell Counting

Microscopic inspection of microalgae is essential for cultivating monocultures. Quantifying the number of cells per unit volume is difficult, however necessary to verify culture purity. The difficulty arises in counting the microalgae cells because it is subject to human error and is labor intensive. A microscope (Motic, BA310) was used for sample inspection and cell counting. Duplicate cell counts for each sample were performed to obtain an average cell count per sample per day. A hemocytometer was used to count the number of cells. A hemocytometer

has a counting chamber defined by a known depth and a grid with known surface area. The depth is the space between the grid surface and the underside of the cover slip and the standard depth is 0.1 mm. Using the specified hemocytometer cover slip is important to maintain the intended chamber volume because the aqueous sample relies on capillary action to stay within the grid surface and therefore preserve a constant volume. The counting method was taken from *Standard Methods for the Examination of Water and Wastewater, Method 10200E and 10200F* (APHA, 1998) and called for counting the four corner squares and the center square seen in Figure 8 with circles. This method was used to quantify *Chlorella*. *Tetraselmis* is larger in diameter than *Chlorell*a and very motile, therefore the method for counting *Tetraselmis* was slightly modified. A digital picture was taken of *Tetraselmis* (Figure 9) to provide a snapshot and enable cell counting. The entire grid surface area (all 25 squares composed of 16 smaller squares were counted) was used for counting because it was more representative taking into account the larger cell size and motility of *Tetraselmis*.

Figure 8: Standard hemocytometer grid

Figure 9: Counting *Tetraselmis* at 200x

Before each sample was loaded for counting the hemocytometer and the cover slip were rinsed with distilled water and dried via *Lense Paper*. It is important to note that using *Kim Wipes* can scratch the glass due to its abrasiveness and was never used. Ensuring the hemocytometer and the cover slip were clean the sample was ready to be loaded. After mixing the sample well, a sterile *Pasteur pipet* was used to dispense the sample into the counting chamber. Caution was taken upon injecting the sample into the counting chamber because if the sample spills over the grid surface the chamber volume becomes compromised and the process must be repeated.

3.3.2 Volatile Suspended Solids

Measuring volatile suspend solids (VSS) provides a mass based method for determining organic content within an aqueous solution. As biomass is organic, VSS is an estimate for the quantity of biomass in an aqueous solution. These methods are commonly used for wastewater examination and the method was adapted from *Standard Methods for the Examination of Water and Wastewater, Method 2540* (APHA, 1998). Total suspended solids (TSS) must be found first in order to determine VSS. TSS is the total amount of solids within an aqueous sample after the sample has been filtered through a glass fiber filter. The increase in weight from the residue retained on the filter represents TSS. VSS is the difference between the weight of dried residue and the weight of residue after ignition (also known as ash weight). The result yields an estimated biomass concentration in units of mg/L. The detailed procedure is as follows:

Filter Preparation

1. Prepare G4 glass fiber filters by rinsing with distilled water under vacuum until all water is pulled through the filter.

2. Place filter in crucible and bake in furnace (550°C) for approximately 15 minutes.

3. Remove filter and crucible from furnace and place in bell jar desiccator until sample reaches room temperature.

4. Weigh filter and crucible and record A.

TSS

5. Filter uniform aqueous sample of known volume (V) through filter under vacuum.

6. Return filter to corresponding crucible.

7. Bake in oven at 103-105°C for 1 hour.

8. After baking remove from oven and allow cooling in desiccator.

9. Re-weigh dry residue, filter and crucible and record B.

10. TSS is then calculated using the equation below:

$$TSS = \frac{B - A}{V}$$

<div align="right">*Equation 5*</div>

VSS

11. Place filter with dry residue and crucible in furnace at 550°C for 5 minutes.

12. After ignition remove from furnace and allow cooling in desiccator.

13. Re-weigh ash residue, filter and crucible and record C.

14. VSS can be calculated using the equation below:

$$VSS = \frac{B - C}{V}$$

<div align="right">*Equation 6*</div>

15. Units of TSS and VSS are (mg/L) and below is the description of each recorded weight:

> A: Initial filter and crucible weight, g
>
> B: Dry weight of residue, filter and crucible, g
>
> C: Ash weight of residue, filter and crucible, g
>
> V: Volume of aqueous sample, mL

16. Conversions used:

> 1 g = 1000 mg
>
> 1 L = 1000 mL

VSS was performed at the beginning and end of each treatment. In this way the biomass produced over the duration of the treatment was found. There were no duplicates executed for this method due to the lack of resources, large number of samples and the extensive time required to obtain VSS data.

3.3.3 Optical Density

A spectrophotometer (Hach, DR3800) was used to measure the absorbance and transmittance of algal samples. The spectrophotometer seen in Figure 10 passes a light of known wavelength through an aqueous sample and measures the light entering and exiting the sample. From the measured incident and exiting light the absorbance and percent transmittance are determined. The wavelength was set at 665 nm because this value is the best estimate of chlorophyll content. Chlorophyll is not a direct measure of algal density, however it provides an estimated value. The advantages of such a method are the ease of use. It is quick, reliable and easy to replicate. The disadvantages include not being able to distinguish between dead and alive cells, and cellular conglomerates can give faulty readings. Similar to cell counting, optical density was measured daily in sample duplicates.

Figure 10: Hach DR3800 spectrophotometer

3.4 Statistics for Analysis

Modeling the microalgae growth kinetics enabled a comparison between CO_2 and flue gas treatments per algal strain. The degree of difference between cultivation methods was tested to determine if flue gas inhibited algal growth. Using *Minitab 15*, an unpaired t-test was used to conclude whether there was a statistically significant difference in the growth characteristics between the gas treatments for each algal strain. The t-test assumes that the sample data is Gaussian and follows a normal distribution. Biological data can never be precisely Gaussian because the normal distribution extends infinitely in the positive and negative directions. However, many times biological data follow a near bell-shaped curve and can be approximated as Gaussian. An Anderson-Darling normality test was used to ensure that the data approximated a normal distribution. Due to the small sample size of this study, the sample data could not be determined to be decisively Gaussian, rather the sample data were concluded to not be inconsistent with a normal distribution.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Growth Parameters Optimized

The temperature and pH were maintained at optimum levels to promote the largest algal population. The average temperature and pH across all 3 growth trials are shown in Figure 11 and 12 respectively. Keeping the temperature and pH constant was important in limiting growth influences from these two variables. Persevering optimum temperature and pH levels, took away any influence on algal growth from such variables.

The use of heaters for *Chlorella* kept the temperature distribution very close to 30°C. The error bars are plus and minus one standard deviation. The standard deviation for *Chlorella* (1.1°C) was small compared to that of *Tetraselmis* (2.5°C). This was due to the temperature control provided by the submersible heaters. *Tetraselmis* was subject to ambient temperature conditions and therefore the standard deviation is much larger due to temperature fluctuations throughout the 3 growth trials.

The pH was also kept constant as seen in Figure 12. As previously mentioned, algal growth increases the pH by consuming carbonic species, which in turn activated the gas dosing solenoid and administered CO_2/flue gas into the sample. The entering CO_2 forms carbonic acid and causes the pH to decrease. This cultivation method provided the algal species with an appropriate supply of carbon, never too much and never too little. Because the flue gas contained 14% (mole percent) CO_2 the flue gas was consumed far quicker compared to pure CO_2 in order to fulfill the necessary carbon demand. The error bars are plus and minus one standard deviation. The standard deviation for *Chlorella* was 0.3 and 0.4 for *Tetraselmis*. Overall, the pH remained relatively constant. The pH values for both algal strains exceeded the pH optimums rarely.

Figure 11: *Chlorella* and *Tetraselmis* mean temperature distribution from all growth trials

Figure 12: *Chlorella* and *Tetraselmis* mean pH distribution from all growth trials

The twelve PBRs were set up in the same room as seen in Figure 13. *Tetraselmis* was subject to the temperature swings within the room. *Chlorella* was cultivated with heaters which enabled constant temperature. The pH was controlled as previously stated in section 4.1 Growth Parameters Optimized. Cultivating microalgae in this way provided optimum growth conditions per algal species. This was an essential element to quarantine growth variables, provide optimum environmental conditions to produce the greatest amount of biomass and enable the variable in question, *gas dosing treatments* to be examined.

Figure 13: The twelve PBRs in use

4.2 Analysis Strategy

Cell count data was used to model the growth kinetics of both algal species. Optical density was not used to model growth kinetics because absorbance and percent transmittance are arbitrary measures of analysis. Rather, cell count and optical density were correlated, so the cell count could be estimated by percent transmittance. The number of cells per mL yields an easy to understand growth curve, in which the maximum specific growth rate was calculated. The growth rates were then compared in a t-test to determine if there was a statistically significant difference in growth rates between gas treatments. The biomass produced over the growth period was also statistically compared by a t-test to conclude whether a significant difference existed among gas dosing treatments.

4.3 Analytical Methods Correlated

Optical density and cell counting were correlated to estimate the cell concentration from optical density. This correlation yields an easy analytical method using a spectrophotometer to estimate the number of microalgae cells per mL. As spectrophotometry is widely used throughout the biotechnology industry, optical density is usually the most used method for determining cellular concentrations. The correlations for *Chlorella* and *Tetraselmis* under both gas treatments are seen in Figures 14 to 17.

Figure 14: Correlation between analytical methods for *Chlorella* grown with CO_2

Figure 15: Correlation between analytical methods for *Chlorella* grown with flue gas

Figure 16: Correlation between analytical methods for *Tetraselmis* grown with CO_2

Figure 17: Correlation between analytical methods for *Tetraselmis* grown with flue gas

The correlation between optical density (% transmittance) and cells per mL yield a quick way to determine a sample cell density. The average transmittance and average cell count for all 3 trials were used for the correlation. The results are summarized in Table 3. The correlations are strongly tied with the coeffecient of determination (R^2) all greater than 0.96. Correlating cell density with absorbance yields a much more intuitive graph with a positive slope. However, using absorbance the slopes were identical per algal strain making the y-intercept the only differing aspect betweeen flue gas dosing and CO_2 dosing. Therefore, percent transmittance was used to correlate cell density and Equation 7 shows the governing relationship.

$$\frac{Cells}{mL} = (-slope * transmittance) + y\_intercept \qquad \textit{Equation 7}$$

As microalgae cultures grow, the cell density increases, causing a reduction in light transmitted through the sample. Therefore, the negative slope infers algal growth and increasing cell density. The gas treatment slopes for *Chlorella* and *Tetraselmis* do not differ greatly from each other, suggesting that growth between flue gas and CO_2 per algal strain is not signifcantly different. A hypothesis test will conclude if the differences in growth kinetics are significant, in the upcoming section 4.6 Inferences Based on Two Sample Populations.

Table 3: Summarized relationship between cells/mL and percent transmittance

Strain	Factor	Slope (-)	Y-Intercept	R^2
Chlorella	CO_2	111,677	1.0E+07	0.9893
Chlorella	Flue gas	106,632	1.0E+07	0.9799
Tetraselmis	CO_2	22,716	2.0E+06	0.9603
Tetraselmis	Flue gas	17,612	2.0E+06	0.9828

4.4 Modeling Algal Growth Kinetics

Algal growth kinetics were modeled with cell counting data from all 3 growth trials. Due to the strong correlation between cell counting and optical density, there was no need to include

growth curves modeled from optical density. Cell counting was used to graphically illustrate the microalgae growth kinetics. By using cell counting to model the growth kinetics, the maximum specific growth rate (μ_{max}) was deteremined. The growth rates were then analyzed to determine if there is a signficant differnce in growth between the gas dosing treatments per algal species.

Algal biomass determination by VSS was used to quantify the amount of biomass produced over the 5 day growth period. The difference between final and initial biomass concentration yielded the dry weight of ash-free biomass produced in mg/L. Similarily to the analysis of growth rates, the biomass produced was statistically analyzed to conclude whether there was a statistically signficant difference in biomass produced between flue gas and CO_2 dosing per algal strain.

4.4.1 Growth Curves

The growth kinetics of *Chorella* and *Tetraselmis* resembled the expected microbial growth kinetics characterized by a lag phase, exponential growth, and stationary phase. In some cases the stationary phase was never reached due to the shortened growth period of 5 days. Seeing that the stationary phase was rarely reached the death phase was never reached. The *Chlorella* and *Tetraselmis* growth curves look strikingly similar between the two gas dosing regimens. The average maximum cell concentration for *Chlorella* was greater for flue gas at about 6.5 million cells per mL compared to 6.0 million cells per mL. However, the standard deviation for counting *Chlorella* was approximately 1 million cells per mL, making a difference of 500,000 cells irrevelent. The error bars for *Chlorella* and *Tetraselmis* are plus and minus one standard deviation. The standard deviation was strain specific due to the different methods executed to quantify cell density. Such a large standard deviation for *Chlorella* was a result to the inaccuracies in cell counting. Inaccuracies such as cellular conglomerates and not being able to distinguish between living and dead cells made enumeration difficult. The maximum cell concentration for *Tetraselmis* between the gas factors were nearly identical reaching

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approximately 1.2 million cells per mL. The standard deviation for *Tetraselmis* was about 100,000 cells per mL. The lower standard deviation for *Tetraselmis* was attributed to the larger cell size and the modified technique used for counting. The growth curves are depicted in Figures 18 to 21 for each algal strain and gas dosing treatment.

Figure 18: Growth curve for *Chlorella* grown with CO_2

Figure 19: Growth curve for *Chlorella* grown with flue gas

Figure 20: Growth curve for *Tetraselmis* grown with CO_2

Figure 21: Growth curve for *Tetraselmis* grown with flue gas

4.4.2 Maximum Specific Growth Rate

The maximun specific growth rate was calculated by taking the natural log of the cell concentration and plotting it over time. Equation 8 shows the relationship between cell concentration (x), maximum specific growth rate (μ_{max}), and time (t). Integrating Equation 8

yields a linear relationship where the maximum specific growth rate is represented by the slope of the linear portion in the plot of the natural log of cell concentration verse time.

$$\frac{dX}{dt} = \mu_{max} * X \qquad\qquad\qquad Equation\ 8$$

$$Ln(x) = (\mu_{max} * t) + Ln(x_o) \qquad\qquad Equation\ 9$$

$$t_d = \frac{Ln(2)}{\mu_{max}} \qquad\qquad\qquad Equation\ 10$$

The resulting relationship after integration can be seen in Equation 9 and is in classic *y = mx + b* form. The linear portion for determining the growth rate was comprised of cell count data from day 0 to day 3. These data points were chosen to maximum the specific growth rate. The data for day 4 and day 5 exhibited a deceleration in growth and would have decreased the growth rate if they had been included. Figures 22 to 25 were used to determine the maximum specific growth rates and the results are summarized below in Table 4. The growth rate of *Chlorella* grown with CO_2 was larger than that of flue gas. The growth rates for *Tetraselmis* were nearly identical for flue gas and CO_2 dosing. The doubling time (t_d) was also determined to give a conceptual idea of the speed at which the algal strains were growing. The doubling time was calculated by rearranging Equation 9 into the form seen in Equation 10, and represents the time required for the number of cells in the population to double during exponential growth.

Table 4: Summary of maximum specific growth rates

Strain	Factor	Growth Rate (day^{-1})	R^2	Doubling Time (days)
Chlorella	CO_2	0.8488	0.9609	0.82
Chlorella	Flue gas	0.7714	0.9175	0.90
Tetraselmis	CO_2	1.1022	0.9956	0.63
Tetraselmis	Flue gas	1.1034	0.9319	0.63

Figure 22: Maximum specific growth rate determination for *Chlorella* grown with CO_2

Figure 23: Maximum specific growth rate determination for *Chlorella* grown with flue gas

Figure 24: Maximum specific growth rate determination for *Tetraselmis* grown with CO_2

Figure 25: Maximum specific growth rate determination for *Tetraselmis* grown with flue gas

4.5 Biomass Production

Based upon VSS measurements the total algal biomass produced was determined. Biomass production was defined as the difference between final and initial biomass quantities. *Chlorella* produced more biomass being grown with flue gas while *Tetraselmis* behaved in an opposite fashion and produced more biomass being grown with CO_2. The overall biomass produced for each sample is shown in Table 5. Certain sample values were discarded due to negative biomass production values or extreme outliers.

Table 5: Mean VSS values

Estimated Biomass (Dry Weight and Ash Free)					
Strain	**Factor**	**N**	**Initial (mg/L)**	**Final (mg/L)**	**Biomass Produced (mg/L)**
Chlorella	CO_2	9	35.4	227.8	192.4
Chlorella	FLUE	8	39.0	276.9	237.9
Tetraselmis	CO_2	7	95.0	789.3	694.3
Tetraselmis	FLUE	6	110.8	670.0	559.2

4.6 Inferences Based on Two Sample Populations

An analysis on two sample populations was performed for *Chlorella* and *Tetraselmis*. A two sample t-test was carried out to determine if the average growth rates and biomass production between CO_2 and flue gas treatments differed for each algal strain. Using a confidence interval of 95% and a corresponding alpha (α) value of 0.05, a hypothesis test evaluated the difference in maximum specific growth rates and biomass production among the gas treatments. To ensure the validity of this test, the sample populations were first tested for normal distrubutions, a prerequiste to the t-test.

4.6.1 Cell Count Analysis

Using an Anderson-Darling normality test the sample populations were tested to determine if the data followed a normal distribution. For each algal strain and gas dosing treatment, the sample population data was subjected to the following hypothesis test:

Anderson-Darling Normality Test

Null Hypothesis: H_o : μ is normally distributed

Alternative Hypothesis: H_a : μ is not normally distrubuted

Seen in Figure 26, *Chlorella* grown with CO_2 dosing yielded a p-value of 0.656 which is greater than alpha of 0.05. Therefore, fail to reject the null hypothesis and conclude that the sample population of *Chlorella* grown with CO_2 is not inconsistent with a normal distribution. The Anderson-Darling normality test was performed for all sample populations and the summarized results are seen in Table 6. All the sample populations had a p-value greater than 0.05 providing that the maximum specific growth rate data per algal strain for both gas treatments could be approximated as a Gaussian distribution.

Table 6: Normality test for maximum specific growth rate data

Anderson-Darling Normality Test: Specific Growth Rate					
Strain	**Factor**	**N**	**Mean**	**StDev**	**P-Value**
Chlorella	CO_2	9	0.8488	0.1885	0.656
Chlorella	FLUE	9	0.7714	0.1556	0.428
Tetraselmis	CO_2	6	1.1022	0.1632	0.183
Tetraselmis	FLUE	6	1.1034	0.2045	0.507

Figure 26: Maximum specific growth rate data normality test for *Chlorella* CO_2

Confident that the small sample sizes were not inconsistent with a Gaussian distribution, a 2 sample t-test was performed to determine if the mean growth rates differed between the gas dosing treatments per algal strain. The 2 sample t-test was governed by the following hypothesises:

Chlorella vulgaris

Null Hypothesis: H_o : $\mu_{CO2} = \mu_{FLUE}$

Alternative Hypothesis: H_a : $\mu_{CO2} \neq \mu_{FLUE}$

Tetraselmis sp.

Null Hypothesis: H_o : $\mu_{CO2} = \mu_{FLUE}$

Alternative Hypothesis: H_a : $\mu_{CO2} \neq \mu_{FLUE}$

The resulting p-value for *Chorella* flue gas dosing compared to CO_2 dosing was 0.357. Again the p-value is greater than alpha (0.357 > 0.05) so the null hypothesis can not be rejected. The maximum specific growth rates for *Chlorella* grown with flue gas and CO_2 are not signifcantly different from one another. The p-value for *Tetraselmis* was even larger at 0.991. Such a large p-value concludes that there is little doubt that maintaining the null hypothesis is false. Table 6 summarizes the t-test results for *Chlorella* and *Tetraselmis*. Overall, there is no statistically significant difference between maximum specific growth rates for the two gas dosing treatments.

Table 7: Testing the difference of growth rates between gas dosing treatments

2 Sample T-Test: Specific Growth Rate						
Strain	**Factor**	**N**	**Mean**	**StDev**	**SE Mean**	**P-Value**
Chlorella	CO_2	9	0.849	0.188	0.063	0.357
Chlorella	FLUE	9	0.771	0.156	0.052	
Tetraselmis	CO_2	6	1.102	0.163	0.067	0.991
Tetraselmis	FLUE	6	1.103	0.205	0.083	

4.6.2 Algal Biomass Analysis

The same procedure was carried out to determine if there was a signifcant difference in the average algal biomass produced between gas treatmeants. First, the sample populations were tested for normality, followed by a two sample t-test.

Anderson-Darling Normality Test

Null Hypothesis: H_o : μ is normally distributed

Alternative Hypothesis: H_a : μ is not normally distrubuted

For all sample populations the p-value was greater than 0.05, concluding that despite the small sample sizes the algal biomass data can be approximated as a normal distribution. Table 8

summarizes the results from the individual normality tests. Figure 27 illustrates the normality test on *Chlorella* grown with CO_2.

Table 8: Normality test for biomass produced from VSS measurements

Anderson-Darling Normality Test: Algal Biomass					
Strain	**Factor**	**N**	**Mean**	**StDev**	**P-Value**
Chlorella	CO_2	9	192.4	51.3	0.207
Chlorella	FLUE	8	237.9	40.1	0.577
Tetraselmis	CO_2	7	694.3	84.2	0.181
Tetraselmis	FLUE	6	559.2	62.1	0.939

Figure 27: Normality test for biomass produced data on *Chlorella* grown with CO_2

The two sample t-test was performed to determine if there was a significant difference in biomass produced among the gas treatments for each algal strain. The t-test was governed by the following hypothesises:

Chlorella vulgaris

Null Hypothesis: H_o : $\mu_{CO2} = \mu_{FLUE}$

Alternative Hypothesis: H_a : $\mu_{CO2} \neq \mu_{FLUE}$

Tetraselmis sp.

Null Hypothesis: H_o : $\mu_{CO2} = \mu_{FLUE}$

Alternative Hypothesis: H_a : $\mu_{CO2} \neq \mu_{FLUE}$

Chlorella had no significant difference in the production of biomass from CO_2 and flue gas treatments. A p-value of 0.06 concluded that the null hypothesis could not be rejected. The gas treatments for *Tetraselmis* yielded a different result with a p-value of 0.008. Since the p-value was less than 0.05, the null hypothesis was rejected and concluded that there was a significant difference in the biomass produced between CO_2 and flue gas treatments. *Tetraselmis* produced more algal biomass under CO_2 dosing producing an average of aproximately 700 mg/L over the 5 day growth period compared to 560 mg/L produced under flue gas dosing. The difference between gas treatments for *Tetraselmis* biomass production ranged from 44.5 to 225.8 mg/L using a 95% confidence interval.

Table 9: Testing the difference in algal biomass production between gas dosing treatments

2 Sample T-Test: Algal Biomass						
Strain	**Factor**	**N**	**Mean**	**StDev**	**SE Mean**	**P-Value**
Chlorella	CO_2	9	192.4	51.3	17	0.060
Chlorella	FLUE	8	237.9	40.1	14	
Tetraselmis	CO_2	7	694.3	84.2	32	0.008
Tetraselmis	FLUE	6	559.2	62.1	25	

The maximum specific growth rates across gas treatments were not significantly different, and the the difference in algal biomass production was only statistically significant for

Tetraselmis. The similarities in growth characteristics using flue gas and CO_2 strongly support the feasibility of using algal cultivation as a CCS methodology. The effects of flue gas exhibited no growth inhibition for cultivating *Chlorella*. Minimal growth inhibition was seen for *Tetraselmis* and only in the form of reducing algal biomass.

CHAPTER V

CONCLUSION

5.1 Experimental Conclusion

The robust characteristics of microalgae are shown in this experiment, illustrating the ability of microalgae to adapt and survive under different carbon source treatments. Integrating microalgae cultivation alongside coal fired power plants to sequester flue gas emissions is a viable carbon capture and storage method. The additional benefit of producing biomass and providing a renewable and sustainable feedstock for biofuels further supports this CCS methodology.

5.1.1 *Chlorella vulgaris*

The differences in growth characteristics for *Chlorella* were not statistically significant between the gas dosing treatments. Maximum cell counts for both gas treatments reached about 6 million cells per mL with a standard deviation of 1 million cells per mL. The maximum specific growth rates were not significantly different at 0.849 day^{-1} and 0.771 day^{-1} for CO_2 and flue gas treatments, respectively. Biomass determination by VSS further concluded no significant difference between gas treatments; producing approximately 195 mg/L of biomass for CO_2 and 240 mg/L of biomass for flue gas dosing. The insignificant difference in growth characteristics between flue gas and CO_2 dosing for the cultivation of *Chlorella vulgaris* supports the existing literature, and proves that *Chlorella* is a viable microalgae strain to be implemented for the abatement of CO_2 emissions from coal-fired flue gas. Flue gas exhibited minimal signs of growth inhibition and the cultivation of *Chlorella vulgaris* from coal-fired flue gas was deemed successful.

5.1.2 *Tetraselmis sp.*

The quantification of growth kinetics for *Tetraselmis sp.* gave statistically significant and insignificant differences in growth, depending on the analytical method used. Generating growth curves using cell counts yielded the same maximum cell concentration of 1.2 million cells per mL for both gas treatments. The maximum specific growth rates were indistinguishable at 1.1 day^{-1} for both CO_2 and flue gas. Biomass production by VSS proved there was a statistically significant difference in the biomass produced under CO_2 and flue gas treatments. CO_2 dosing produced approximately 700 mg/L of algal biomass while flue gas only produced 560 mg/L. With a confidence interval of 95%, the difference in biomass production between the two gas treatments ranged from 45 to 225 mg/L. Although the conclusions drawn from cell counting and VSS contradict each other, *Tetraselmis* was able to grow under flue gas dosing. The lack of biomass production under flue gas treatments suggests NO and SO_2 inhibit the cell development seen in the absence of biomass. The overall result supports the fact that *Tetraselmis sp.* could be successfully cultivated with flue gas fulfilling the carbon supply.

5.2 Recommendations for Further Research

Due to the statistical limitations on small sample sizes, increasing the number of samples would strengthen the analysis performed to determine the difference between gas dosing treatments. More replicates increase statistical power and provide stronger conclusions.

Modeling algal growth kinetics with VSS would provide a growth curve with units of mass (mg/L), which is more useful than the number of cells per mL. Further, VSS should be analyzed in duplicate or triplicate measures to reduce the amount variance.

Designing the experimental setup such that gas dosing is constantly purged into the growth medium would be beneficial. Analyzing the inlet and outlet gas streams would provide essential data to complete a mass balance. This would be extremely valuable to determine the amount of gas absorbed into the aqueous solution, lost due to saturation, and the amount of

carbon utilized by the algal cells. In essence, providing a degree of mitigation effectiveness and microalgae carbon fixation efficiency is critical to implementing such a CCS strategy.

Purchasing flue gas was an ironic necessity of this experiment. Flue gas was very expensive and was depleted quickly due to the relatively small fraction of CO_2 contained within the flue gas. It would be far more sensible and environmentally friendly to work in cooperation with a power plant and have a constant supply of real flue gas emissions.

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