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Treated flue gas as an alternative CO_2 source for the open pond cultivation of the green alga, *Nannochloropsis oceanica*

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By

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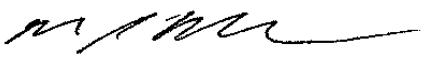
Approved by:



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Abstract

A challenge of commercial algae cultivation is maintaining a low cost of production. Addition of CO_2 is required for algae growth at this scale, and remains a significant cost. Flue gas, exhaust from fossil fuel combustion, is an attractive alternative source of CO_2 for algae cultivation because it is abundant, cheaper than conventional sources of pure CO_2, and allows for the recycling of CO_2 that would otherwise be emitted into the atmosphere. Cellana, a developer of sustainable algae-based nutraceuticals, animal feed and biodiesel, is researching and developing an on-site diesel flue gas CO_2-sourced system with commercial potential. This study aimed to evaluate the efficacy of this system in the open pond cultivation of *Nannochloropsis oceanica*. Growth performance and system cost were compared between algae grown using the flue gas system supplemented with pure CO_2 and algae grown exclusively with pure CO_2. We were able to establish novel baseline information on CO_2 usage for the open pond growth cycle unique to Cellana's cultivation procedures in addition to identifying several factors that affect CO_2 consumption. While the experimental flue gas setup resulted in an 18% reduction in productivity, it only required 4% of the pure CO_2 of the control pond to maintain algal growth, directly contributing to cost savings. No significant differences were found in daily biomass or nutrient depletion between treatments. However, residual flue gas heat did cause the experimental pond to experience temperatures exceeding 42°C, potentially reducing photosynthetic efficiency. Further investigation is required to understand what other aspects of flue gas treatment, such as particulates and toxins, may affect algal growth and biomass. The flue gas system presents a considerable cost savings with only a small reduction in productivity, which may be improved with additional

research. If modifications to the flue gas treatment and delivery system can produce quality algae with satisfactory growth performance while maintaining cost savings, expansion of the system could possess commercial viability.

Introduction

Over the last 100 years, atmospheric CO_2 concentrations have risen from 295 parts per million (ppm) to 380ppm due to combustion of fossil fuels for energy, contributing considerably to global warming and climate change (Mata et al., 2010). In addition to biological consequences of climate change such as habitat loss and increased rates of extinctions, fossil fuel dependence is unsustainable because sources are finite (Yoshihara et al., 1996). Wind, geothermal, solar, hydroelectric, and biofuel energy are being developed as sustainable alternatives to the combustion of fossil fuels (Sayre, 2010). It is clear that a host of these sources will be needed to meet current and future energy demands in addition to development of more sustainable products and practices (Mata et al., 2010). In order to confront climate change issues and ensure energy security in a pragmatic manner, it is important to develop smart technologies and practices that balance operational, economic, and environmental considerations.

The most abundant source of energy on Earth is the sun and 0.05% of this energy is harnessed in biomass through photosynthetic processes (Sayre, 2010). Unlike other renewable energy systems, biomass based energy can be converted into high-energy biofuels that are compatible with existing petroleum based infrastructure (Sayre, 2010). Additionally, the use of biofuels has the potential to reduce our carbon footprint through the sequestration of carbon through photosynthesis (Bartley et al., 2013). Thus, biofuels play an important role in our sustainable energy strategy.

Microalgae as a biofuel feedstock

Microalgae hold great promise as both a superior feedstock for biofuels and as a source of environmentally friendly bioproducts. Microalgae are unicellular, eukaryotic or prokaryotic organisms and are capable of performing photosynthesis (Alexopoulos and Bould, 1967). Over 50,000 species of microalgae exist, present in all existing aquatic ecosystems and thriving in a wide range of environmental conditions, making microalgae a versatile and resilient crop (Ma et al., 2014). Microalgae are some of the most productive organisms on earth and are the foundation of many aquatic and marine ecosystems (Sayre et al., 2010). Productivity of microalgae can be 10-150 times as high as terrestrial biofuel feedstocks such as rapeseed or soybean and can grow year round (Bartley et al., 2013). This extraordinary productivity is due in large part to higher photosynthetic efficiency than land plants and the simplicity of microalgae structure (Sayre, 2010). Microalgae do not have structural components such as stems and roots which are energetically expensive to produce and difficult to convert into biofuels (Alexopoulos and Bould, 1967; Brennan et al., 2010). Under normal growth conditions, energy is directed toward cell division, resulting in rapid growth rates (Sayre, 2010). Biomass can increase exponentially and a reproductive cycle can be as short as a few hours, meaning large amounts of biomass can be produced in a short amount of time (Brennan et al., 2010). Under stressed conditions such as low nitrogen, algae can divert reproductive energy into lipid accumulation (Dong et al., 2013). Some species are capable of producing up to 70% lipid biomass, making them the most efficient system for biofuel production (Sayre, 2010). Algae based biodiesel, produced from the transesterification of algal lipids, can directly be used as a replacement for petroleum

based diesel, having similar physical properties and comparable energy density (Guo et al., 2015). Biodiesels also burn "cleaner" than petroleum diesel, having lower carbon monoxide, particulate, hydrocarbon, sulfate, and nitrogen oxide (NOx) emissions (Negoro et al., 1991).

In addition to their high productivity, microalgae do not require arable land or fresh water to grow, unlike terrestrial crops (Brennan et al., 2010). Land usage is an important consideration for the viability of biofuel crops. Currently, 1% of arable land is used to produce biofuels, supplying 1% of global transport fuels (Brennan et al., 2010). Thus use of non-arable land is essential to producing biofuels that do not threaten food security or increase food crop cost. Cultivation of microalgae does not require arable land and uses significantly less area than other crops (Sayre, 2010). Algal cells must grow in aqueous media, however overall water usage is less than for terrestrial crops (Sayre, 2010). Because algae naturally grow in waters ranging from fresh to hypersaline, cultivated species can be selected to use brine or ocean water, and therefore do not compete with agricultural or domestic water resources (Sayre, 2010). Additionally, because microalgae require nutrient input, wastewater or agricultural runoff can be used as culture media, doubling as a method for wastewater treatment (Gantar et al., 1991).

Like all biofuel feedstocks, the cultivation of microalgae results in the uptake of the greenhouse gas CO_2 (Mata et al., 2010). However, passive diffusion of CO_2 into the culture solution is a limiting factor in algae growth, and consequently, introduction of an additional carbon source, usually solid Na_2CO_3 or $NaHCO_3$, or gaseous CO_2, is required (Brennan et al., 2010). Because a carbon source must be directly administered to the system, it is possible to use waste CO_2, such as industrial exhaust, for algae based biofuel

production. This strategy is not easily implemented with terrestrial fuel crops, which adequately grow with atmospheric CO_2. This, coupled with microalgae's superior productivity, makes microalgae especially appealing for biofuel production (Sayre, 2010).

While microalgae have many practical and economic advantages over traditional biofuel crops, current production still struggles to remain competitive with cheaper fossil fuels (Suganya et al., 2016). A universal challenge with biofuel production is the high cost of production, specifically in the high energy and expensive conversion of biomass into fuel (Brennan et al., 2019). Many algae companies that grow biofuel feedstocks are developing business models that capitalize on profits from producing other valuable bioproducts that can be manufactured from microalgae to support the more costly biofuel conversion processes (Guo et al., 2015; Brennan et al., 2010). Omega-3's, pigments such as astaxanthin, and other algal compounds used for nutraceutical supplements and pharmaceuticals hold a $5 billion market, making them some of the most valuable algae bioproducts (Cellana, 2015). Whole and defatted algal biomass can be used as a more sustainable alternative to traditional fish based feeds for aquaculture or corn or soy based diets for livestock (Cellana, 2015). Extra biomass can also be converted via fermentation into alcohol-based biofuels, allowing for additional energy to be harnessed from the biomass (Mata et al., 2010). Using all biochemical components of the algae enhances the economic viability of algae based biofuels and produces other valuable, useful, and sustainable products.

Reducing cost of resources needed for growing microalgae can further save costs and increase the sustainability of production. A carbon source needs to be added to algae

cultures but can be both expensive and energy intensive to produce (Topham, 2005). In the last 25 years, there has been much research on the use of cheaper recycled carbon sources for algae growth, such as reclaimed exhaust from industrial plants as a source of CO_2 (Doucha et al., 2005; Brown et al, 1996). Using recycled resources can save money through direct decrease in operational cost but can also increase the economic value of products by enhancing marketable sustainability.

Alternative CO_2 sources for algae cultivation

Exhaust from fossil fuel combustion (flue gas) is the primary contributor to CO_2 – emissions (Mata et al., 2010). The use of flue gas as an alternative source of CO_2 for algae growth is attractive because it is cheap, sources are abundant, and results in the fixing of CO_2 that would otherwise be emitted into the atmosphere (Mata et al., 2010). However, use of flue gas possesses several challenges. At 5-20% CO_2, flue gas is more concentrated that atmospheric CO_2, but more dilute than the pure CO_2 that is typically used (Douskova et al., 2009). Consequently, equipment must be adapted to deliver larger volumes of gas. However, it has been demonstrated that the higher CO_2 content of flue gas coupled with lower O_2 concentration can affect photorespiration and photoinhibition, resulting in higher growth (Douskova et al., 2009). This is due to the reduction of the wasteful oxygenase reaction performed by the carbon-sequestering enzyme Rubisco (Douskova et al., 2009). Flue gas also contains particulate matter, SOx, NOx, heavy metals, CO, soot, and excess heat that could affect algal growth performance or biomass (Sayre, 2010). Toxins, such as SOx, NOx, heavy metals, and CO, can affect culture pH, disturb algal water balance, affect the photosynthetic apparatus, and disrupt cellular

signal cascades, potentially altering algal growth (Clemens, 2006; Lara-Gil et al., 2014). High temperatures may cause membrane instability and reduce carbon assimilation through inactivation of key photosynthetic enzymes (Heschel et al., 2014; Kobza and Edwards, 1987). However, several strains of microalgae are resilient to flue gas toxins and various simple filter systems can be used to reduce flue gas heavy metals, which can accumulate in algal biomass (Negoro et al., 1991; Douskova et al., 2009). Systems to transport or store gas as well as reduce heat are necessary, requiring additional engineering at extra cost. However, successful use of recycled CO_2 for algae cultivation not only reduces daily operational cost but also helps to mitigate CO_2 emissions and adds to the sustainability of production practices and manufactured products (Mata et al., 2010).

Study purpose and goals

Flue gas has been successfully used to grow microalgae in a number of experiments (Yoshihara et al., 1996; Zeiler et al., 1995; Doucha et al., 2005; Douskova et al., 2009). Commonly used algae varieties such as *Chorella* and *Nannochloropsis* have been shown to be well tolerant of high CO_2 concentrations and toxic NOx and SOx found in flue gas (Douskova et al., 2009, Brennan et al., 2010). However, most of these studies have been conducted at the small scale using enclosed, controlled environment photobioreactors. Few studies have tested use of flue gas for open pond cultivation, which is the most common commercial production method. This study aimed to evaluate the efficacy of using flue gas for the growth of *Nannochloropsis oceanica,* a common

microalga species for biofuel production, at the open pond scale using otherwise standard cultivation procedures. This study intends to address the following questions.

1. What is the effect of flue gas on *Nannochloropsis oceanica* daily biomass, total productivity, and nutrient usage?
2. What is the effect of the flue gas system on pure CO_2 usage and what is the cost of each setup?
3. What other natural and flue gas related factors might affect *Nannochloropsis oceanica* growth at the open pond scale?

Methods

Facility

Overview

This experiment was conducted at Cellana's Kona Demonstration Facility (KDF) located at the Natural Energy Lab of Hawai'i Authority (NEHLA) Hawai'i Ocean Science and Technology Park in Kailua-Kona, Hawai'i. The NELHA campus uses retired and renovated ocean thermal energy conversion (OTEC) infrastructure to access deep sea and surface seawater. These waters are used by NEHLA clients for aquaculture, desalination, and energy technology development (Natural Energy Lab of Hawai'i Authority, 2015).

KDF is a 6-acre, state-of-the-art production and research facility that cultivates algae for Omega-3 EPA and DHA nutritional oils, animal feed, and biofuel feedstock (Cellana, 2015). Seawater is sourced from NELHA's deep and surface seawater pipelines. Algae are cultivated using Cellana's ALDUO production technology, a hybrid system that capitalizes on the contamination protection of closed-culture photobiorectors

and the low cost and high productivity of open ponds (Cellana, 2015). This system also allows for modular expansion of units to scale-up to a commercial facility (Cellana, 2015). Cellana is located off of the local electrical grid and receives its energy from 3 diesel generators.

Ponds

The present experiment focused on large-scale algae production in open ponds. KDF facility operates 6 open ponds of the traditional paddle-wheel drive, recirculating raceway design. Paddlewheels keep cells in suspension, reducing self-shading and ensuring a homogenous culture. A single pond covers 417m^2 and has an average depth of 12cm (Huntley and Redalje, 2007). Typical pond setup results in a culture volume of 60,000L in each pond. Open ponds are not temperature controlled or sheltered from sunlight.

Organism

Cellana strain KA19

Nannochloropsis oceanica, Cellana strain KA19 was used for this experiment. *N. oceanica* KA19 is one of several strains of algae used by Cellana for the production of their line of bioproducts. Cellana's algae strains are sourced from the University of Hawaii or collected locally and have been selected for their high algae-oil yield and growth performance (Cellana, 2015).

Nannochloropsis

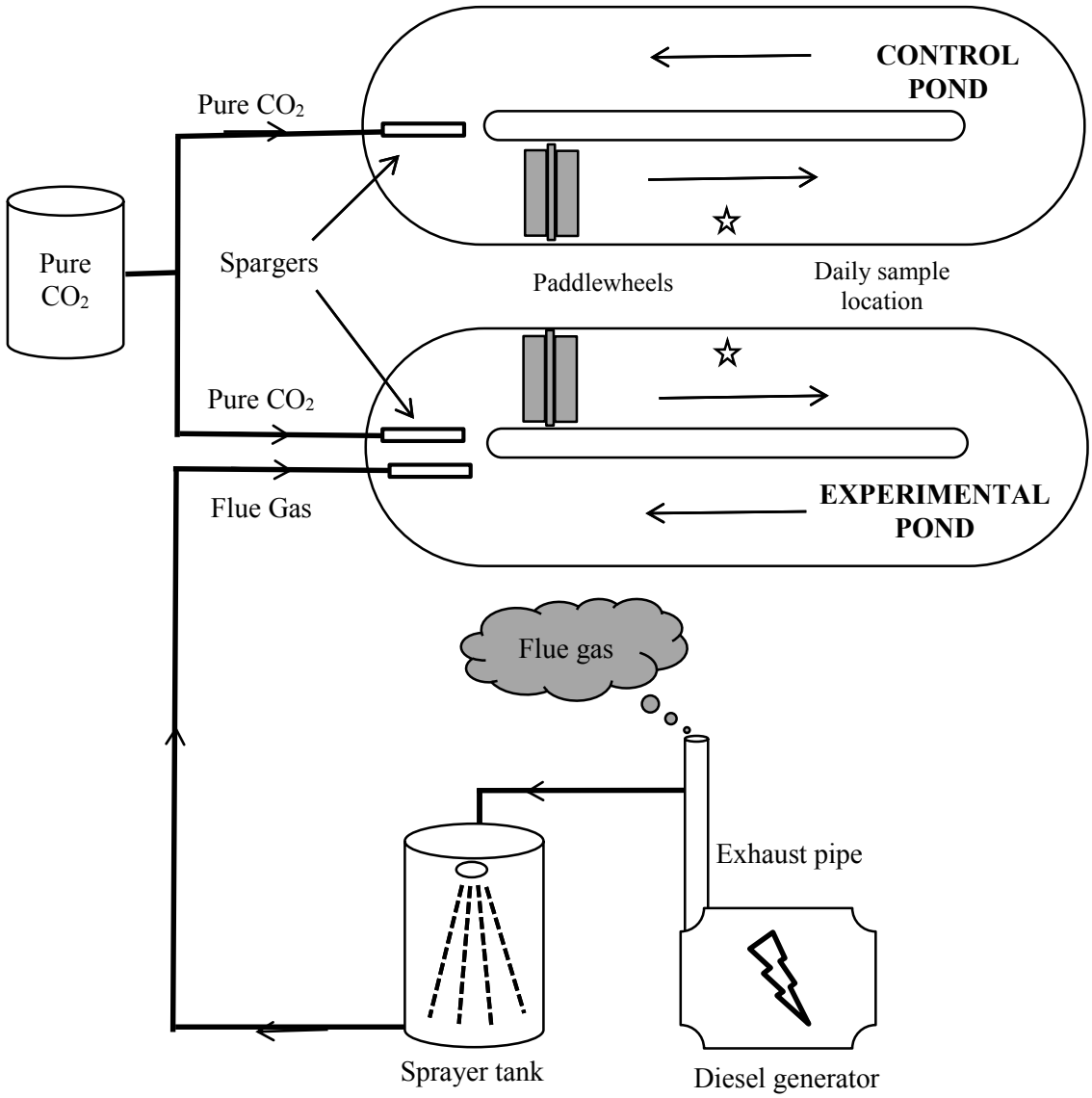
Microalgae of the genus *Nannochloropsis* are widely used in aquaculture because of their high EPA content and exceptional growth under extreme environmental conditions (Pal et al., 2013). Marine species of *Nannochloropsis* occupy coastal areas and estuaries where high freshwater and tidal influx results in rapid and dramatic salinity change (Pal et al., 2013). Because of this, *Nannochloropsis* can tolerate both hypersaline and hyposaline environments with little effect on growth, an attractive quality for outdoor cultivation where ponds are exposed to rainfall and evaporation (Sukenik et al., 2009). Salinity tolerance also allows for flexibility in water sourcing for cultivation. *Nannochloropsis* also grows well in nutrient depleted conditions and will overproduce lipid triacylglycerols (TAGs), a fat key for biofuel feedstock production, when nitrogen-starved (Pal et al., 2013). Lastly, *Nannochloropsis* is able to acclimate to a range of irradiances. Together, these qualities make microalgae strains of *Nannochloropsis* robust and versatile and thus attractive for general algae cultivation of bioproducts.

Carbon dioxide sources

Flue gas was diverted from the exhaust pipe of one of three diesel generators located on Cellana's site. Gas was diverted from muffler flue and directed to a tank where seawater spray cooled the flue gas to below 40°C and reduced soot. Downstream air filters further removed particulate matter. After treatment, flue gas was introduced to the pond culture via a submerged sparger that increases gas to culture surface area by production of small bubbles (Diagram 1).

Pure CO_2 was sourced from storage tanks filled by a local gas supplier. Pure CO_2 was also delivered to pond culture via a submerged sparger (Diagram 1).

Diagram 1: Schematic of pond setup.



Experimental design

Pond setup

To test the efficacy of flue gas as an alternative CO_2 source, three rounds of binary ponds consisting of an experimental and control treatment were conducted consecutively between July 17th and August 25th, 2015 at Cellana's Kona Demonstration Facility. Pond setup was intended to mimic Cellana's standard algae cultivation techniques. Adjacent raceway open ponds were filled seawater and inoculated with a strain of *Nannochloropsis* (KA19) sourced from a smaller scale closed-culture photobioreactor (PBR). Nutrients were added to ponds based on inoculation density. The growth period of the experiment lasted for ten days or until nutrients were depleted from the system. Algae biomass was then harvested after the last day of the growth cycle.

CO_2 administration

An automated process control system used pH triggers (Table 1) to regulate injection of carbon dioxide sources to the ponds to maintain pH between 7.7-8.3. Experimental ponds received flue gas as the primary CO_2 source. If flue gas was insufficient to keep pH below the upper threshold, supplementary backup pure CO_2 was turned on. The control pond received only pure CO_2.

Table 1: Experimental and control pond pH triggers for CO_2 sources.

Control pond pH triggers

	On	Off
Pure CO_2	7.9	7.7

Experimental pond pH triggers

	On	Off
Flue gas (primary)	7.9	7.7
Pure CO_2 (backup)	8.3	7.7

Data collection

Process control system data

Via the process control system, pH and temperature were recorded respectively every minute and every five minutes from an Omega temperature-pH probe submerged in the pond culture. Flow rate data was sourced from GF Signet flow meters on the pure CO_2 injection pipes. Equipment limitations did not allow for a flow meter to be installed on the flue gas system, however timing of flue gas injections could be determined from flue gas blower on/off data.

Daily sample collection

Daily samples were collected each morning at 9:00am from the same collection point on each pond, just downstream of the paddlewheel to ensure samples were well mixed (Diagram 1). A 50mL sample was collected for OD and nutrient analysis. One to two liters of sample was collected for AFDW measurements depending on culture density to ensure sufficient biomass for analysis. Samples were analyzed for optical density (OD), ash-free dry weight (AFDW), and nutrient concentration.

Optical density procedure

Two mL of culture sample was prepared in a 3mL cuvette. OD was determined by measuring absorbance at 440nm using a Beckman Coulter UV-VIS spectrophotometer. If absorbance was measured at greater than 0.70, the sample was diluted to bring reading below 0.70 and dilution factor was multiplied into final OD value. Flocculated samples were sonicated for 1 minute using an ultrasonic homogonizer to separate clumped masses. Low settings were used to prevent cells from rupturing.

AFDW procedure

AFDW is a measure of organic algal biomass. A volume of algae culture sample was vacuum filtered through a pre-combusted, pre-weighed glass microfiber filter to separate algae cells from culture liquid. Filtered volume was calculated from OD measurements to ensure at least 5mg of dry algae biomass would be collected on the filter. Sample filters were dried in a 100°C oven for 4 hours to dehydrate biomass. Dry biomass was determined by weighing (with an electronic balance) the filter with sample and subtracting filter weight. Sample filters were then combusted in a combustion oven at 500°C for 4.5 hours to remove organic material. Remaining ash was weighed, deducted from dry biomass values, and divided by filtered culture volume to produce the used AFDW (mg dry ash-free biomass/L culture) values.

Nutrient analysis procedure

Nutrient analysis was performed to determine the nutrient content of the culture medium. 10mL of culture sample was filtered through a glass microfiber filter to remove algae

cells and solids. Filtered solution was then analyzed for nitrite + nitrate and orthophosphate concentrations using a Lachat QuikChem 8500 Flow Injection Analysis System. Reagents were prepared according to Lachat Instruments instruction.

Photosynthetically active radiation

Local global photosynthetically active radiation (PAR) data was sourced from the National Renewable Energy Laboratory (Olson and Andreas, 2012). PAR was measured by a Kipp & Zonen Model CMP10 or CMP11 Pyranometer.

Data analysis

Carbon dioxide usage

To compare CO_2 usage between experiments and for culture density differences, volume pure CO_2 values were adjusted by AFDW.

Productivity

Productivity was calculated using the following equation:

$$\frac{(AFDW\ T_F - AFDW\ T_0) * V_p}{SA_P * D * 1000} = productivity$$

where AFDW T_f is the final AFDW, AFDW T_0 is the inoculation AFDW, V_p is the pond volume in liters, SA_p is the pond surface area in m^2, and D is the amount of days (including fraction of days) between the inoculation and final AFDW sample collection times. Productivity for the third experimental round was excluded from analysis due to abnormally low final biomass.

Statistical analyses

Statistical analyses were performed using JMP Version 4.00, SAS Institute 2000. ANOVAs were used to test for differences between treatments for total CO_2 usage, productivity, temperature, and nutrients. Repeated measures MANOVAs were used on daily CO_2 usage, AFDW, and OD data to account for the effect of experimental rounds occurring sequentially in time. Linear regressions were used to examine associations between orthophosphate versus nitrite + nitrate, AFDW versus OD, and CO_2 injections versus PAR. For all analyses, distributions of residuals were examined for normality.

Results

Table 2: Linear regression analyses results.

Column1	R square	Slope	F Ratio
OD vs AFDW	0.820	283.6	274.073***
Nitrate + Nitrate vs. Orthophosphate	0.384	0.0405	31.692***
Inj. length vs. hourly ave. PAR	0.022	-0.0091	6.221*
Time betw. Inj. vs. hourly ave PAR	0.067	0.0032	15.886***

***p<0.001, **p<0.01, *p<0.05, +p>0.05

Table 3: One-way ANOVA analyses results.

	Treatment
Productivity	45.691*
Nitrate D00	0.431+
Orthophosphate D00	0.227+
Nitrate D10	0.760+
Orthophosphate D10	0.516+
Nitrate slope	0.461+
Orthophosphate slope	0.516+
Temperature	563.273***

***p<0.001, **p<0.01, *p<0.05, +p>0.05

Table 4: Repeated measures MANOVAs results.

	Time	Treatment	Time*treatment
AFDW	10.931***	0.020+	1.562+
Adjusted volume pure CO_2	758.596*	16.153**	861.206*
OD	75.734**	0.704+	1.285+

***p<0.001, **p<0.01, *p<0.05, +p>0.05

Biomass and productivity

Two standard measures of biomass exist in the algae production industries, OD and AFDW. While OD and AFDW were strongly positively correlated (Figure 1, Table 2), AFDW was chosen as the standard measure of biomass for this study due to its consistency, smaller variability, and likely more accurate measure of biomass. Thus, AFDW was used for all biomass analyses.

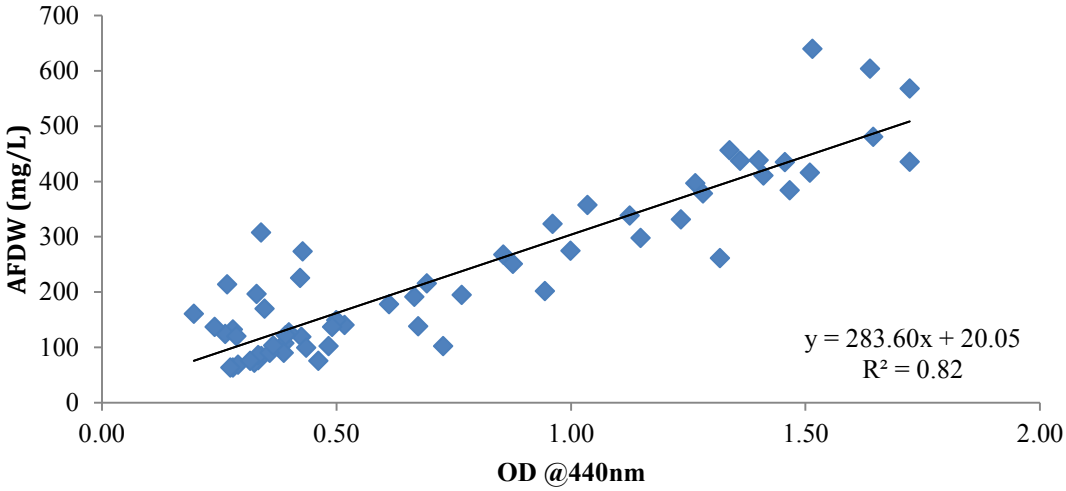


Figure 1: Regression analysis of OD by AFDW

Biomass-adjusted volume of CO_2 consumed differed significantly between growth cycle for the control ponds (Table 3). There was no significant difference in daily AFDW between flue gas treated ponds and control ponds (Figure 2, Table 4). However, average productivity for flue gas ponds was 3.95g/L/day compared to 4.81g/L/day for the control ponds, an 18% decrease (Figure 3, Table 3).

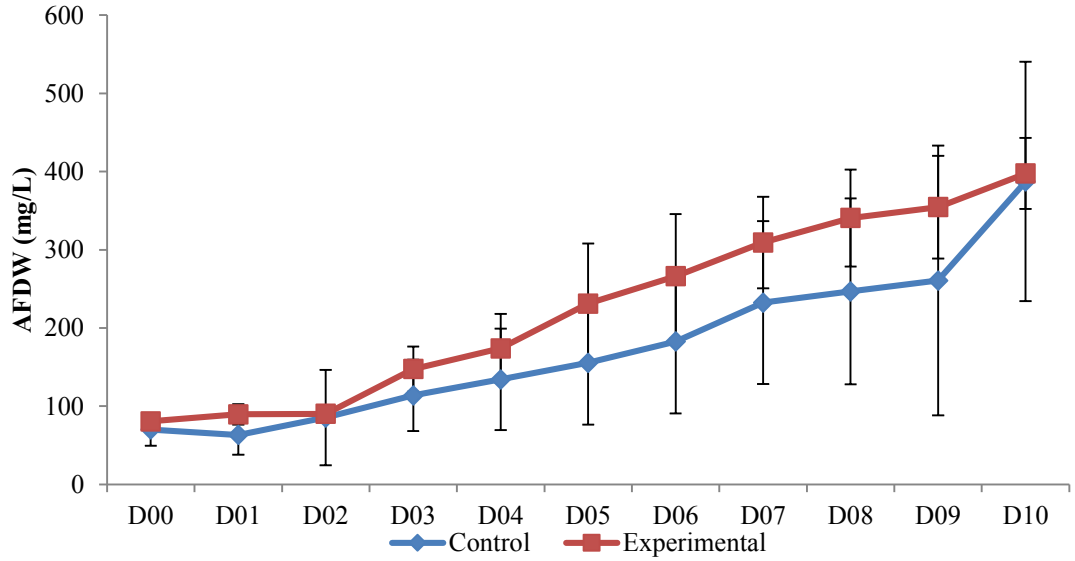


Figure 2: Mean (±1 SE) AFDW by growth cycle day between flue gas treated (experimental) and pure CO_2 treated (control) ponds

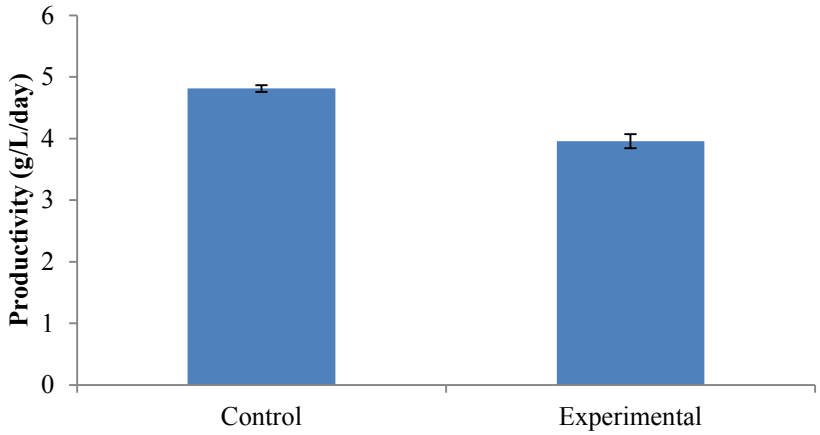


Figure 3: Mean (±1 SE) productivity for flue gas treated ponds (experimental) compared to ponds grown with pure CO_2.

Nutrient depletion

There was a strong correlation between nitrate + nitrite and orthophosphate concentration, suggesting nitrate + nitrite and orthophosphate are used at a similar rate (Figure 4, Table 2). There was no significant difference in nitrate + nitrite concentration by day between treatments (Figure 5). There was no significant difference in Day 0 and Day 10 orthophosphate or nitrate + nitrite concentration and there was no significant difference in slope of depletion for either nutrients (Table 3).

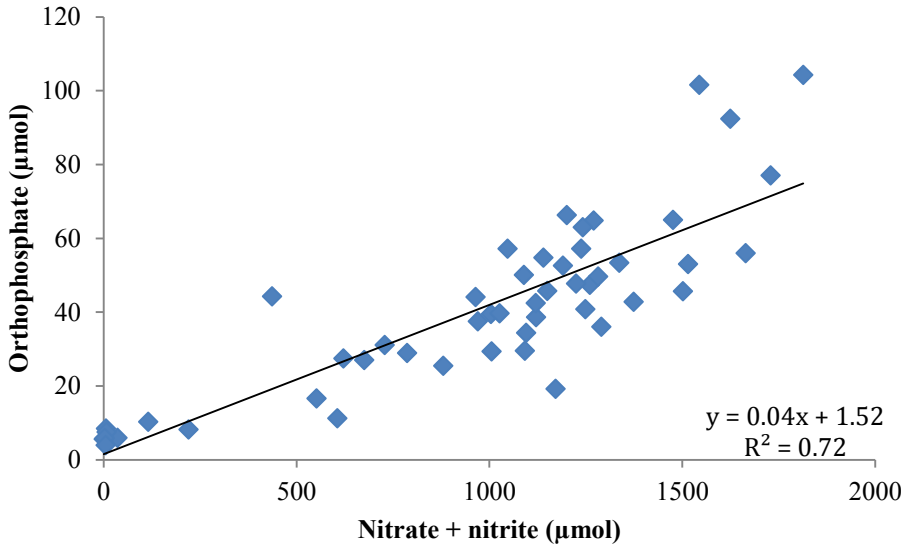


Figure 4: Nitrate + nitrite concentration versus orthophosphate concentration.

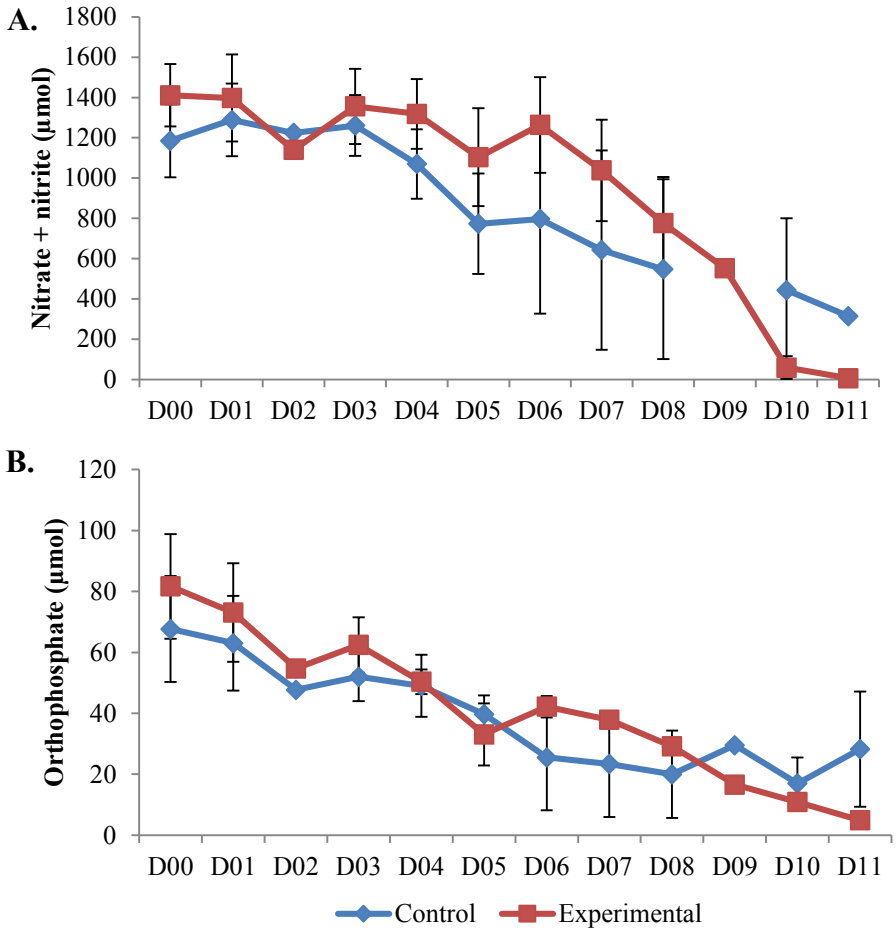


Figure 5: **A**. Mean (±1 SE) depletion of nitrate + nitrite for ponds treated with flue gas (experimental) and ponds receiving pure CO_2 (control) by day. **B**. Mean (±1 SE) depletion of orthophosphate for experimental and control ponds by day.

CO_2 usage

Control pond pure CO_2 usage per unit algae varied greatly between experimental rounds. Usage generally decreased from day 1 to day 10, however there was a relative spike in CO_2 usage on day 5 (Figure 6). Pure CO_2 usage was significantly greater by day for the control ponds than the experimental ponds, with the experimental ponds only requiring additional CO_2 on day 5 (Figure 7, Table 4). Pure CO_2 usage, overall total and adjusted for biomass, was significantly greater for the control system than for the

experimental pond (Figure 8). For the control system, total pure CO_2 usage averaged 61,045L, while the experimental system averaged 726L (Figure 8).

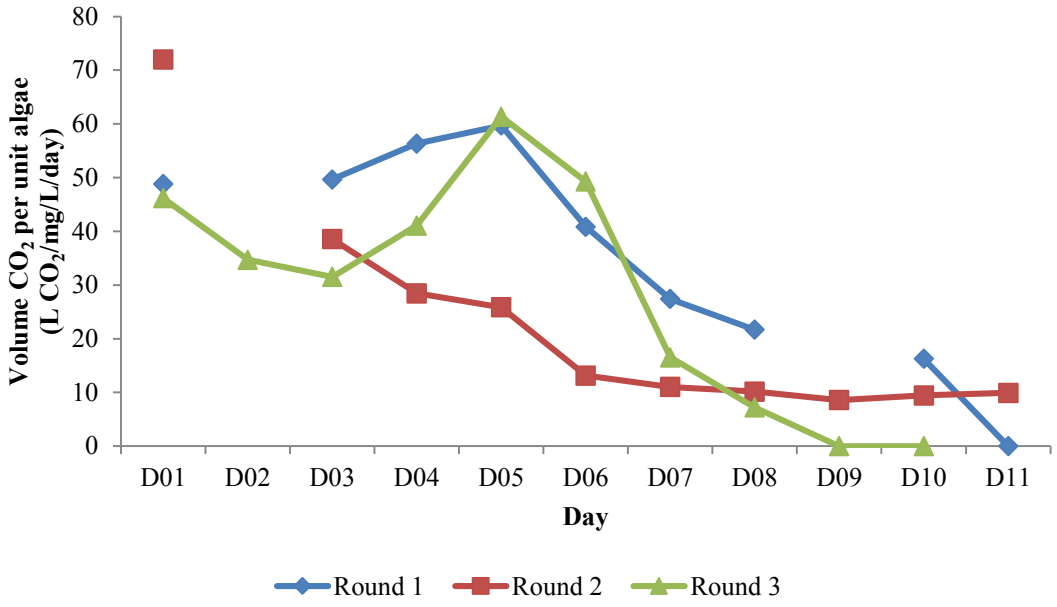


Figure 6: Volume pure CO_2 usage, adjusted by AFDW, by growth cycle day for control ponds.

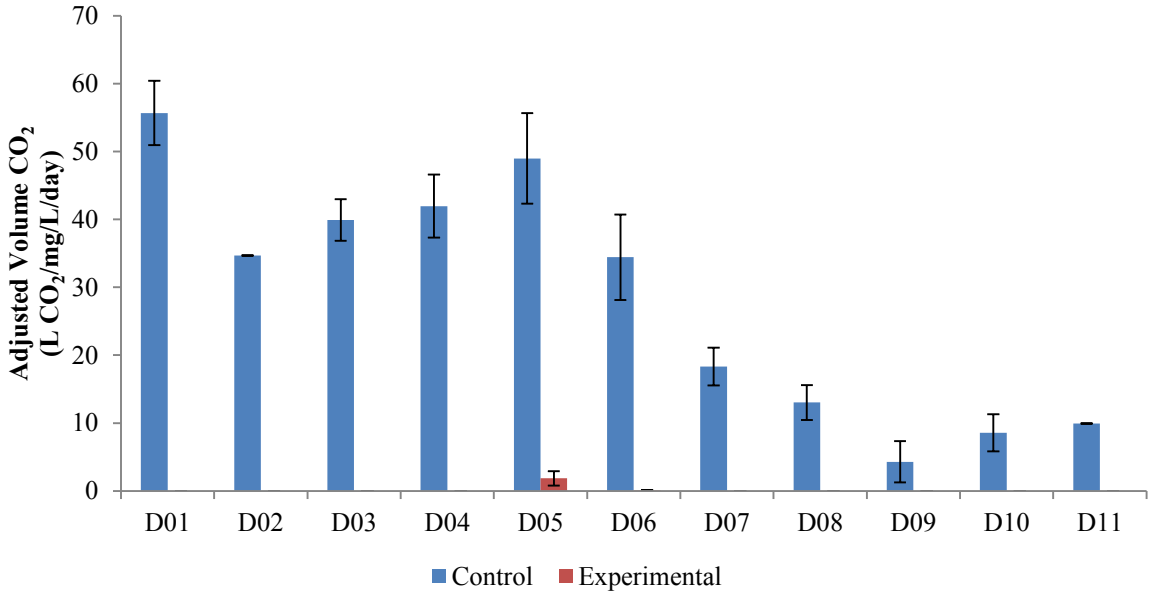


Figure 7: Mean (±1 SE) volume pure CO_2 usage, adjusted by AFDW, by growth cycle day for ponds receiving flue gas (experimental) and ponds grown with pure CO_2 (control).

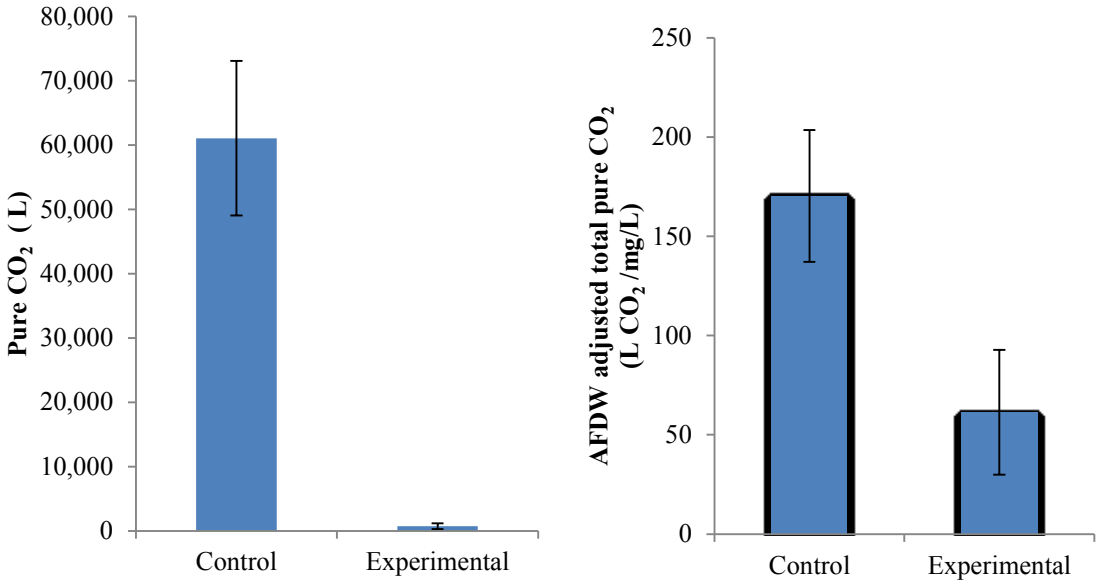


Figure 8: **A**. Mean (±1 SE) total pure CO_2 usage for ponds receiving pure CO_2 (control), and for ponds treated with flue gas, supplemented with pure CO_2 (experimental). **B**. Mean (±1 SE) pure CO_2 usage for control and experimental ponds adjusted for biomass by day 11 AFDW.

Factors affecting CO_2 usage

Photosynthetically active radiation (PAR) gradually increased throughout the day after sunrise at approximately 6:00am, peaking midday, then gradually decreasing to zero after sunset at approximately 7:00pm. PAR was associated with both length of pure CO_2 injection and the duration between injections (Figure 9, Table 2).

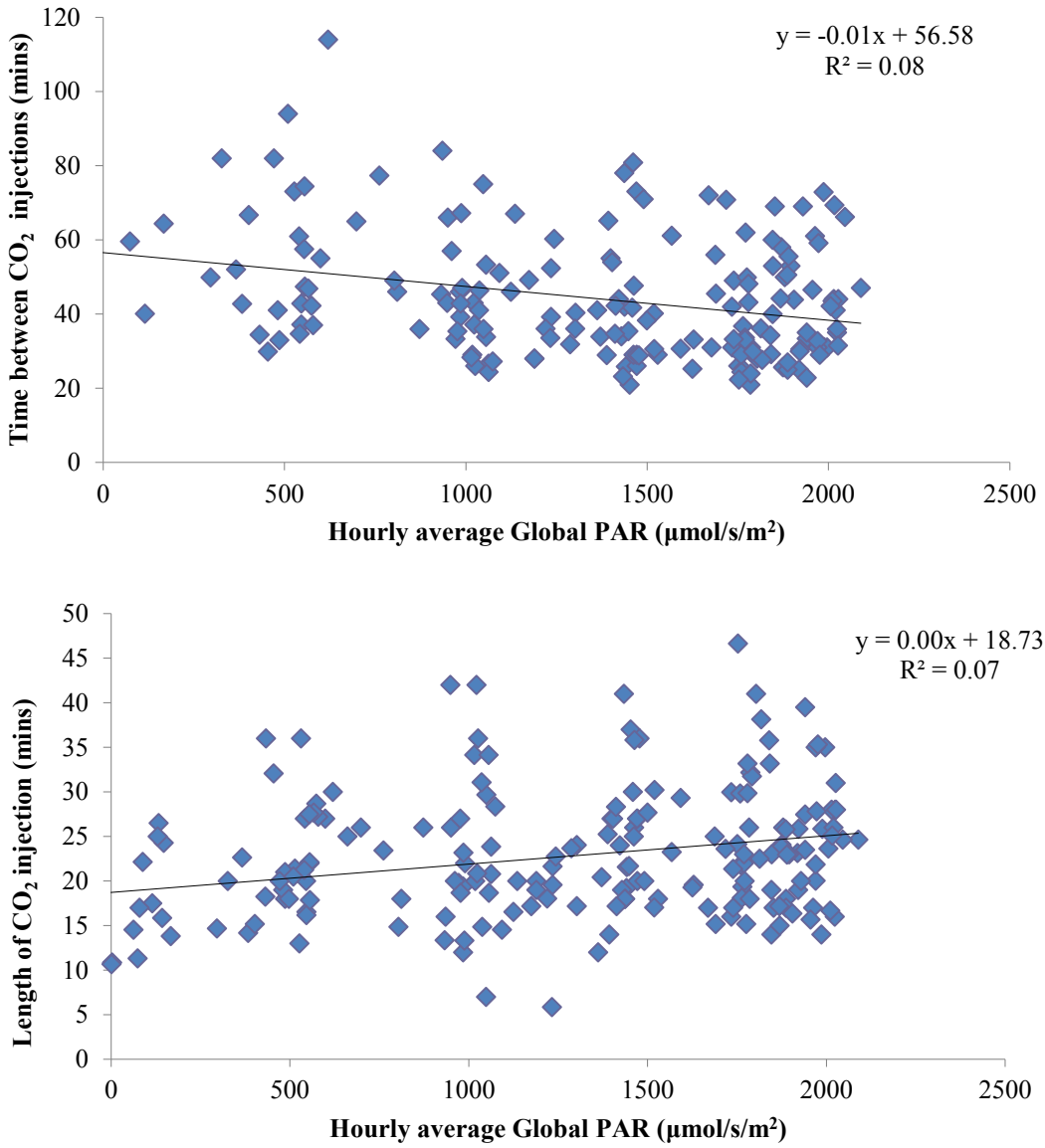


Figure 9: **A**. Linear regression of time between CO_2 injection and hourly average global PAR. **B**. Linear regression of length of CO_2 injection by hourly average global PAR

Temperature:

Temperature fluctuated between 14.0°C and 38.9°C for the control system, and 15.9°C and 41.6°C for the experimental system. Temperature increased beginning shortly after sunrise (6:30am). Temperatures were hottest midafternoon for both ponds and gradually cooled throughout the evening and nighttime hours until sunrise the next

morning. The experimental pond was on average significantly warmer than the control pond, with an average temperature of 29.15°C compared to 28.13°C for the control (Figure 10, Table 3). The experimental pond experienced peaks in temperature exceeding 3°C warmer than the control ponds for the same time. These spikes usually occurred midday to midafternoon when flue gas was engaged and sustained for several minutes.

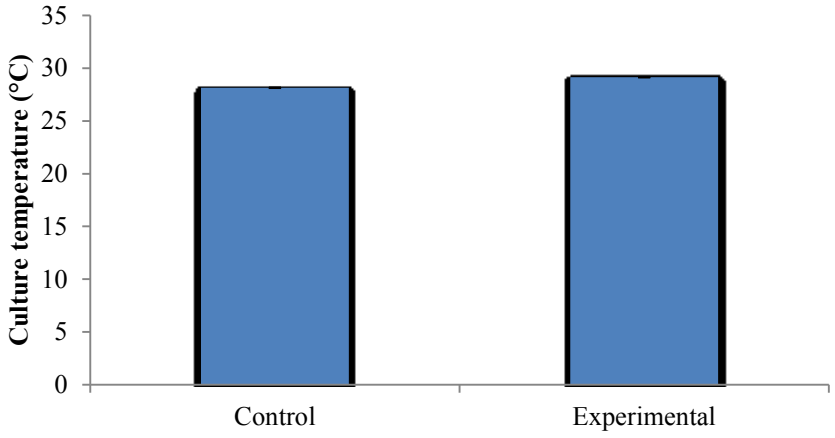


Figure 10: Mean culture temperature (±1 SE) for control and experimental ponds.

Discussion

An increase in atmospheric CO_2 due to fossil fuel combustion and consequent climate change has demonstrated our need for more sustainable energy sources and practices. Microalgae hold potential as one of the most practical, sustainable, and economically viably biofuel feedstocks because of their superior productivity and lipid content, ability to use non-arable land and non-potable water, and production of valuable co-bioproducts. Additionally, because microalgae use CO_2 for photosynthesis, they can be used to actively sequester the greenhouse gas from fossil fuel combustion. Successful use of flue gas to grow microalgae not only reduces operational cost but also increases the sustainability of algae production. Many studies have shown success in using flue gas to grow algae in small-scale systems, but commercial scale feasibility has yet to be

demonstrated. This study aimed to fill this void by testing the efficacy of flue gas to grow microalgae for biofuel and bioproduct at the open pond scale.

Flue gas system summary

Use of flue gas as a CO_2 source for algae cultivation reduces cost, recycles CO_2 that would otherwise be emitted into the atmosphere, and adds to the sustainability of produced algae goods. It is clear that Cellana's flue gas treatment and deliver system can serve as a CO_2 source for growing algae at the open pond scale, as a significant amount of algal biomass was produced nearly entirely on flue gas sourced CO_2. The flue gas system also did prove a cost-saving measure, maintaining algal growth on 4% of the pure CO_2 required for the control pond. There was no significant difference in daily biomass or nutrient uptake rate between treatments. Nutrients were completely depleted by day 10 or 11, thus desired nutrient stress to induce lipid production was accomplished in the last few days. However, efficiency of production, quality of product, and cost must be balanced for commercial viability. The 18% reduction in productivity for flue gas treated algae compared to the control is significant and at 3.96mg/mL/day, productivity may not competitive with industry standards. A more in depth investigation of the direct and indirect effects of flue gas treatment on the algae and pond system will help develop improvements for this method.

Temperature

The higher average and maximum temperature in the flue gas treated ponds is the most obvious environmental difference between the ponds. Temperature, next to light

intensity, has the greatest effect on photosynthesis (Brennan et al., 2010). The flue gas treatment system employed a sea water sprayer to decrease exhaust temperature from >200°C to <40°C, however remaining heat combined with solar radiation still caused temperature spikes reaching 42°C. Other strains of *Nannochloropsis* have shown detrimental effects on photosynthetic rate at temperatures above 32°C (Sukenik et al., 2009). Moderate heat stress has been shown to inhibit net photosynthesis by the inability of Rubisco activase to maintain Rubisco's active form (Salvucci and Crafts-Brandner, 2004). Rubisco is responsible for catalyzing the fixation of CO_2 in first step of the Calvin Cycle, thus overall photosynthetic efficiency is reduced. Additionally, heat stress can promote photorespiration, in which Rubisco binds to molecular oxygen instead of CO_2, decreasing carbon assimilation. Further, thermal stress can cause thylakoid membrane instability and loss of chlorophyll, possibly reducing efficiency of photosynthesis or photon capture (Heschel et al., 2014). Cold temperatures do not seem to have the same effect on photosynthetic processes as warm temperatures, and even a small change in temperature in the supraoptimal range can have severe impacts on photosynthetic efficiencies (Salvucci and Crafts-Brandner, 2004; Sukenik et al., 2009). Thus, while both ponds experienced temperatures above optimum, the higher spikes in the flue gas treated pond may have been high enough to cause significant decrease in photosynthetic efficiency and lower productivity.

Solar radiation

Solar radiation can directly affect photosynthetic efficiency at high extremes and is intrinsically coupled with heat effects. When solar radiation is high causing excess

absorption of photons, photosystem II is downregulated to prevent permanent damage to photosynthetic components, causing photoinhibition (Franklin and Forster, 1997). If exposure to high solar radiation is extended, or coupled with other stresses such as high temperature, decreases in maximum photosynthetic rate can occur (Franklin and Forster, 1997). Additionally, UV radiation in excess or for extended periods can alter DNA, enzymes, membrane proteins, photosystem II, and cause formation of reactive oxygen species, affecting algal growth, metabolism, photosynthesis, and nitrogen fixation (Xue et al., 2005). *Nannochloropsis* has been shown to withstand high irradiance, recovering quickly from any damage at the observed irradiance levels (maximum of 2090$\mu mol^{-2}s^{-1}$) (Sukenik et al., 2009). While direct solar irradiance damage is an important consideration in outdoor cultivation, high temperatures due to high solar radiation likely have a larger effect on algae growth performance in our system since irradiance levels are within tolerance range for *Nannochloropsis*.

Other factors affecting growth performance

Our study did not explore pond salinity, toxins and heavy metals, or particulates, all of which may affect algae growth performance. Biomass composition analysis was also not included in this study, but remains a crucial element in the evaluation of the flue gas system since each biochemical component of the algal biomass is utilized. A similar, shorter term study conducted by Cellana prior to ours found comparable ash, moisture, carbohydrate, protein, lipid and fatty acid methyl ester composition of algae grown with flue gas compared to algae grown with pure CO_2 (Selinger and Bai, 2015). The same study also found negligible differences of the heavy metals arsenic, cadmium, lead, and

mercury in the flue gas grown algae compared to algae grown with pure CO_2. All levels were well below guidelines for single cell protein destined for animal feed by the International Union of Pure and Applied Chemistry (IUPAC) and Algae Biomass Organization (Hoogerheide et al., 1979; Sears et al., 2012). Cellana's prior study did involve a shorter four day growth cycle with higher inoculation density and thus had slower growth rates. Completing biomass composition and heavy metal analysis on algae grown using Cellana's standard cultivation procedures, as the present study did, would provide a more accurate understanding of how flue gas treatment affects these variables in applied cultivation practices.

Salinity and particulates could also affect algae productivity. Particulate matter could increase turbidity and as a result, increase shading of algal cells below the culture surface, decreasing photon capture and consequently photosynthetic rate. For saltwater algae species, salinity is one of the most important factors affecting growth and biochemical composition (Gu et al, 2012). Salinity can affect algae via osmotic stress, salt stress, and changes in intracellular ionic ratios (Mata et al., 2010). For marine *Nannochloropsis*, optimum salinities for maximum carbohydrate, protein, lipid production, and growth rate vary, however 28-35 PSU seems to be the best range for overall quality and performance (Bartley et al., 2013; Khatoon et al., 2014). Higher salinities, especially above 40PSU, have been shown to be of significant detriment to *Nannochloropsis* growth, despite *Nannochloropsis* being relatively versatile and resilient to changes in salinity (Khatoon et al., 2014; Fawley and Fawley, 2007). At high salinity, plasmolysis results in the accumulation of osmoprotectant solutes that stabilize metabolic enzymes and suspend algal growth (Khatoon, 2014). Additionally, increased cytoplasmic

viscosity due to water loss reduces diffusion of molecules involved in respiration, reducing overall respiration and growth (Taylor, 1958). At our study site on Keahole Point, annual precipitation is <10inches per year, so increase in salinity due to evaporation is more likely than salinity decrease from freshwater influx (Olson and Adreas, 2012). Monitoring salinity changes of the open pond cultures throughout the growth cycle, and conducting smaller scale, controlled experiments, will reveal if evaporation is great enough to negatively affect algae growth.

Lastly, our study lacked important information about flue gas composition, flow rate, and CO_2 uptake efficiency due to equipment and time limitations. Flue gas is a fairly dilute source of CO_2 compared to conventional sources, typically containing about 5-20% CO_2. When the flue gas system was activated, flow rate was fixed, but we do not know the volume or components and concentration of the gas. This information would improve our understanding of the flue gas treatment and delivery system and may expose potential improvements.

Conclusion

Because a number of physical, chemical, and biotic factors affect algae growth and composition, often in interaction, it can be difficult to discern which factors are responsible for changes in algal growth performance. We successfully sustained algal growth using a simple flue gas treatment and delivery system at the open pond scale. While productivity was reduced, we were able to decrease the cost of pure CO_2 to 4% of the control. This study is particularly valuable because it was conducted at the large scale, using standard inoculation densities and duration and thus tests real-life feasibility and

exposes practical issues not apparent in smaller scale studies. With additional research and refinement, pure CO_2 requirement will ideally be reduced to zero while producing commercially suitable algae biomass.

Using recycled flue gas not only reduces operational cost, but also mitigates Cellana's CO_2 emissions. Emissions reduction may be just as valuable to the viability of Cellana's products as cost savings because it supports their mission of producing "algae-based products for a sustainable future." Cellana's flue gas system is a demonstration of economical and sustainable green technology that meets both consumer and energy needs. Further development of smart, economic and sustainable strategies such as described in this study are required to address our modern world energy, climate, and environmental challenges.

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