RESPONSE OF *ANABAENA CYLINDRICA* TO VARIATIONS IN GROWTH CONDITIONS IN PHOTO-BIOREACTORS AND ITS USE AS A BIOFERTILIZER

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Masters of Science

in

Chemical Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 2013

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of a thesis submitted by

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November 2013

DEDICATION

This thesis is dedicated to my family for all their love and support.

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ABSTRACT

Nitrogen is a vital nutrient for plant growth. Traditional methods of manufacturing nitrogen fertilizers are energy intensive and lead to the production of greenhouse gases. Nitrogen fixing cyanobacteria have shown promise as an environmentally friendly method of producing biofertilizer containing nitrogen. A potential candidate organism, *Anabaena cylindrica,* was assessed for adaptability to changes in growth conditions. Variations in flow regime of the air in the reactor, superficial velocity of the air entering the reactor, temperature, light cycle and intensity had little statistically effect over the ranges tested on either the average growth rate or final biomass concentration. The nitrogen content, chlorophyll and final biomass concentrations increased considerably to 74.2 ± 6.9 mg N/L, 12.3 ± 0.97 µg/mL and 0.81 ± 0.16 g/L, respectively, when 0.15 mM of magnesium sulfate heptahydrate and 0.005 mM of disodium ethylenediamine tetraacetate dihydrate was present in the culture medium. Magnesium is utilized within the cyanobacterial cell for many purposes. Evaluation of the growth of wheat, camelina, carrots, tomatoes and Kentucky bluegrass, when treated with cyanobacterial biofertilizer, showed statistically similar results when compared to chemically fertilized plants.

INTRODUCTION AND BACKGROUND

Introduction

Arable land used for crops must be supplemented with fertilizers to keep up with the demand of a growing global population. Nitrogen, a key nutrient and possible limiting factor in crop yield (Topre et al., 2011), is traditionally produced for fertilizers using the Haber-Bosch method. In this process, hydrogen reacts with atmospheric nitrogen (N_2) to yield ammonia. Much of the costs associated with this energy intensive method (~90%) are derived from required natural gas (Razon, 2012). Due to ever increasing electricity and fossil fuel prices (Razon, 2012), the production of nitrogen fertilizers in now estimated to require 1.2% of global energy demands (Ahlgren et al., 2008). Along with added costs, manufacturing of nitrogen fertilizers via the Haber-Bosch method represents a large fraction of the total greenhouse gas emissions from the agriculture industry (Ahlgren et al., 2008). To adjust to the changing global climate, an alternative, ecological friendly and sustainable approach is necessary.

Biomass produced by photosynthetic organisms will capture and store carbon dioxide resulting in a net removal from the atmosphere (Sanchez Fernandez et al., 2012). Green algae and cyanobacteria are two of the most commonly known microorganisms that can perform oxygenic photosynthesis. The prokaryotic cyanobacteria can colonize all kinds of environments; not only to survive, but thrive in harsh and inhospitable conditions such as extremes of pH, salt concentration, light intensity and temperature (Seckbach and Oren, 2007). Cyanobacteria are a biochemically diverse phylum of

microorganisms. A subset of cyanobacteria is capable of N_2 fixation. This group of organisms uses carbon dioxide and N_2 for their carbon and nitrogen sources, respectively. The uniqueness of their metabolism makes them a possible solution for a more energy efficient method of producing nitrogen fertilizer.

Cyanobacteria have been found to be the predominant type of nitrogen fixing organisms in rice fields. N_2 fixing cyanobacteria play an important role in nitrogen availability in rice plants. Fertilizing with a cyanobacterial culture has shown success in improving the productivity of rice. An average of 15-20% increase in the grain yield for rice has been found using a cyanobacterial biofertilizer (Innok et al., 2009). While employing a biofertilizer, a reduction of up to 50% of chemical fertilizers could be used and still obtain similar rice grain yields and quality (Osman et al., 2010). While the benefit of using a cyanobacterial biofertilizer has been well documented for rice, perhaps the success can be translated to other agricultural crops.

Diazotrophic cyanobacteria, organisms capable of growth on only N_2 as a N source, are appealing for a commercial application since they are able to grow on only a few nutrients, water, air and solar energy (Fontes et al., 1989). Cyanobacteria do not require arable land or potable water for growth; this would eliminate competition with other industries for desirable locations. Since cyanobacteria can grow on various types of wastewater (Martins et al., 2011) a source of clean water is not necessary for a biofertilizer production plant. The reduction of the requirement for a fresh water source will increase the options for manufacturing plant sites. Contamination of large growth systems with unwanted organisms is common; but with an environment with a limited

supply of nitrogen, the risk of non N_2 fixing organisms is reduced thus allowing for the dominance of only diazotrophic microorganisms.

There are two main objectives of this thesis project. The first objective was to analyze the response of a selected strain of *Anabaena cylindrica* (*A. cylindrica*) to changes in environmental growth conditions such as flow regime of the air in the reactor and superficial velocity of the air stream into a photo-bioreactor, temperature, availability of light to the culture, and nutrient additions. The other was to assess how wheat, camelina, carrots, tomatoes and Kentucky bluegrass react to fertilization with cyanobacterial biomass.

Background

Cyanobacteria

Cyanobacteria are a group of ancient gram-negative prokaryotes that are thought to have originated 3.5 billion years ago (Adams, 2000). They are considered to be among the first organisms to evolve the cellular machinery required to perform oxygenic photosynthesis and the primary producers responsible for the oxygenation of the Earth's atmosphere (Adams, 2000; Stal, 2007). Cyanobacteria are a wide and diverse phylum capable of colonizing the most extreme of environments. They display a wide array of biochemical and morphological variations (Latysheva et al., 2012). Cyanobacteria also have the ability to differentiate vegetative cells into more specialized cells. The morphologies, extremes of habitat and the function of specialized cells are discussed below.

Morphology. Cyanobacteria are a morphological diverse phylum of microorganisms. All cyanobacteria can be divided into five subsections based on their morphology and cell division characteristics (Stal, 2007). Cyanobacteria with a unicellular cellular arrangement are classified in either subsections I or II. Microorganisms in subsection I use the process of binary fission for cell division (Stal, 2007) and have the either rods or cocci for cell shape (Willey et al., 2008). While cyanobacteria in subsection II can be held together in the form of an aggregate (Willey et al., 2008); it is the cellular reproduction process of using multiple binary fissions using baeocytes, small reproductive cells that grow into full sized cells, that differentiates between subsection I and II (Stal, 2007). Subsections III, IV, and V have a filamentous cellular arrangement. Filamentous cyanobacterial species that only form vegetative cells are categorized in subsection III (Pinevich, 2008). N_2 fixing cyanobacteria are sorted into either subsections IV or V. These cyanobacterial species can use heterocysts for N_2 fixation and can differentiate vegetative cells into akinetes under certain conditions (Stal, 2007). Cyanobacteria in subsection IV have unbranched filaments in a single plane while filaments from species in subsection V will divide and branch out to form aggregates (Willey et al., 2008). The cellular arrangements of cyanobacteria are as diverse as their possible habitats and biochemical processes.

Habitat Extremes. Cyanobacteria have evolved to colonize the most extremes of environments. They have been discovered in arctic environments and in high temperature locations such as hot springs (Seckbach and Oren, 2007). Some cyanobacterial species have been known to colonize areas where the pH is very acidic or

basic or highly saline environments (Stal, 2007). These microorganisms have adapted mechanisms to their hostile environments for survival.

Microorganisms have been seen at the extremes of temperature for life. Cyanobacteria have been located in the arctic regions, such as Antarctica and Siberia permafrost, and in high temperature environments, such as hot springs (Seckbach and Oren, 2007). Mats of cyanobacteria coating of ice covered pools, underneath and in cracks of rocks, and within the soil are areas that cyanobacteria have been found in Antarctica (Seckbach and Oren, 2007). For survival in this environment, the cells have developed methods of handling the freezing temperatures. The first method is by excreting a mucopolysaccharide that alters the flow of the water during freeze up and thaw (Vincent, 2007). The second method is to adjust the lipid content within the cyanobacterial cell to keep the cytoplasmic water from freezing (Seckbach and Oren, 2007). Psychrophilic (cold-loving) cyanobacteria have slow but steady growth rates, even at optimal growth temperatures (Vincent, 2007). While thermophiles (cells thriving at high temperatures) can withstand the damaging effects of high temperatures. A temperature maximum of 73-74^{o}C is the limit for growth of cyanobacterium with a unicellular cellular arrangement (Seckbach and Oren, 2007). Filamentous cyanobacteria have shown to be less tolerant of high temperatures with a maximum temperature of 55-62^{o}C then unicellular cyanobacteria (Seckbach and Oren, 2007). Cyanobacteria have evolved adaptive mechanisms for surviving in extreme temperatures.

Other harsh environments that cyanobacteria have colonized and developed mechanisms for are high salt concentrations and high and low pH. Both unicellular and

filamentous cyanobacteria have been discovered in salt lakes, hypersaline waters, lagoons and salterns (Seckback and Oren, 2007). To combat the osmotic pressure exhibited on the cell, organic substances, such as disaccharides, glycine betaine, and glucosylglycerol, are accumulated with the cell (Seckbach and Oren, 2007). While acidophiles (cells tolerant of low pH) use proton pumps to maintain their intracellular pH close to a neutral pH of 7, this aids in the protection chlorophyll, DNA, and ATP from degradation in acidic environments (Seckback and Oren, 2007). The majority of cyanobacteria seem to be able to grow at a pH up to 10, since photosynthetic CO_2 utilization increases the pH (Seckbach and Oren, 2007). A cyanobacterium *Plectonema nostocorum* has grown in a solution up to pH 13, the highest known to support life (Seckbach and Oren, 2007). Cyanobacteria have developed many ingenious methods to adapt to their environment.

Nitrogen Fixation. Changes in the bioavailability of nitrogen in the form of ammonia (Berman-Frank et al., 2003) or nitrogen oxides (Latysheva et al., 2012) in the early Earth's environment, created an evolutionary pressure for N_2 fixation during the genetic development of diazotrophic cyanobacteria. The widespread distribution and abundance of nitrogen-fixing cyanobacterial species makes them a vital link in the global nitrogen cycle (Latysheva et al., 2012). Diazotrophic cyanobacteria help to balance the nitrogen cycle by fixing N_2 to a more bioavailable form (Bergman et al., 2013). Many cyanobacterial species are capable of diazotrophic growth, meaning they can biologically reduce N_2 through the use of a multimeric nitrogenase enzyme complex. Biological nitrogen fixation is catalyzed by the nitrogenase enzyme complex, which reduces N_2 to ammonia, as shown in equation 1 (Willey et al., 2008). Nitrogen fixation is a very energy

expensive reaction requiring 16 ATP to convert one N_2 molecule to ammonia. The high energy requirement is due to the strength of the triple bond of N_2. The other problem is this enzyme is sensitive to oxygen and can be easily degraded (Stal, 2007). Diazotrophic cyanobacteria are the only aerobic nitrogen fixers. To overcome the challenge of both photosynthesis and nitrogen fixation occurring simultaneously, multiple mechanisms have been developed to shield nitrogenase from oxygen degradation.

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16\,ADP + 16P_i \qquad \text{Eq. 1}$$

Different species of diazotrophic cyanobacteria utilize separate mechanisms to protect the nitrogenase enzyme from oxygen degradation. Which protection method is employed often depends on the morphology of the species. Single cell cyanobacteria alternate their cellular activities between photosynthesis and N_2 fixation. The genus *Gloeothece* operates in this manner. This genus has the highest rate of N_2 fixation during the dark portions of the light cycle, though reduced activity levels have been observed under light conditions (Huang and Chow, 1988). Energy stored from photosynthesis in the light is used to power the N_2 fixation during the dark phase. By isolating photosynthesis and N_2 fixation chronologically, nitrogenase is protected from the oxygen generation photosystem II. This defense mechanism can be considered a temporal segregation method.

The other method for guarding nitrogenase from harmful oxygen degradation is to physically separate photosynthesis and N_2 fixation. Vegetative cells will differentiate into specialized cells, whose main function is N_2 fixation. Filamentous diazotrophic cyanobacteria are classified based on whether heterocysts (strictly N_2 fixing cells) are

formed. In the non-heterocystous species, vegetative cells will temporarily act like heterocysts, called diazocytes; they spatially separate photosynthesis and N_2 fixation by localizing the nitrogenase enzyme complex in diazocytes (Bergman et al., 2012). By isolating N_2 fixation from photosynthesis, both processes can occur in the light. The filamentous oceanic genus *Trichodesium,* forms diazocytes and only fixes N_2 during the light phase (Bergman et al., 2012). About 42% of the global N_2 fixation is completed by the genus of *Trichodesium* (Latysheva et al., 2012). The N_2 fixed by diazotrophic marine cyanobacteria is available to other microorganisms enhancing their growth (Bergman et al., 2012). Diazocytes and heterocysts spatially sequester nitrogenase within their cells to guard the enzyme from photosynthesis, but heterocysts strictly are N_2 fixing cells.

Vegetative cells will begin to differentiate into heterocysts when no other nitrogen sources but N_2 are available. To overcome the dilemma of oxygen degradation, the outer cell wall will start to thicken. The outer wall can be broken down into two parts; the inner layer consisting of heterocyst-specific glycolipids and the other is made up of polysaccharides (Kumar et al., 2010). The permeability of the outer wall is decreased, making it more difficult for oxygen to penetrate the heterocyst from outside the filament. With the creation of an anaerobic environment conducive for N_2 fixation, heterocysts are no longer capable of performing photosynthesis. Indeed, the heterocysts lack the photosystem II genes which encode proteins necessary for oxygenic photosynthesis electron transport (Golden and Yoon, 2003). Since heterocysts are focused on purely N_2 fixation, they become dependent on the surrounding vegetative cells for their required carbon. In exchange, vegetative cells obtain nitrogen in the form of amino acids

produced in the heterocysts (Golden and Yoon, 2003). Cyanophycin granules, comprised primarily of the amino acids of arginine and aspartic acids (Willey et al., 2008), are accumulated at the ends of the heterocysts that are abutted to the vegetative cells (Kumar et al., 2010). Cyanophycin is most likely the nitrogen storage molecule for heterocysts (Stal, 2007). The nutrients are exchanged by a continuous periplasm connecting the two cell types (Kumar et al., 2010). Figure 1-1 shows the mutually beneficial relationship between the vegetative cells and heterocyst in the filament.

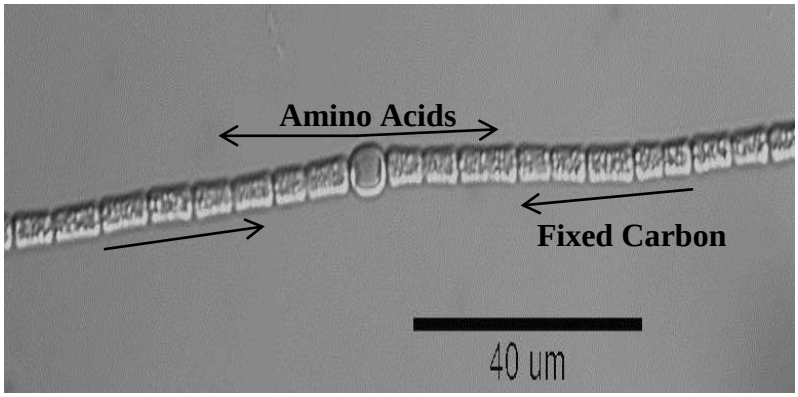

Figure 1-1. A photomicrograph of *Anabaena cylindrica* filament with a heterocyst taken on a Nikon Eclipse E800. The arrows are showing the path of nutrients between the vegetative cells and the heterocysts.

Akinetes. Cyanobacteria of the orders of *Nostocales* and *Stigonematales* (Olli et al., 2005) have a second type of specialized cell called an akinete, which is a spore-like cell that forms under unfavorable environmental conditions (Rao et al., 1987). Akinetes can withstand severe conditions and germinate once the environment is suitable for growth and ensure the ongoing survival of the microorganism. The initiation of development of akinetes and their metabolism have been under investigation for a broad range of different species.

Akinetes are substantially larger than vegetative cells and heterocysts, being upwards of 10 times the volume of a vegetative cell (Simon, 1977). In Figure 1-2, the akinetes are identified by the arrows. As akinetes develop, cyanophycin and glycogen are accumulating as nitrogen and carbon storage mechanisms (Sutherland et al., 1979). The granular appearance of the akinetes is apparent in Figure 1-2. The addition of a peptidoglycan and a multilayered extracellular envelope (Hardin and Fisher, 1995) aids in the survival of the akinete during harsh conditions. The metabolism of akinetes is considerably slower than their vegetative counterparts (Thiel and Wolk, 1983). The metabolic rates of photosynthesis and N_2 fixation are very slow while the respiration rate is increased in some akinetes (Adams and Duggan, 1999). Environmental conditions are thought to initiate the differentiation of akinetes, though the extract cause has continued be the topic of debate (Li, 1997; Rao et al., 1987; Sutherland et al., 1979; Adams and Duggan, 1999; Hori et al., 2003; Van Dok and Hart, 1996).

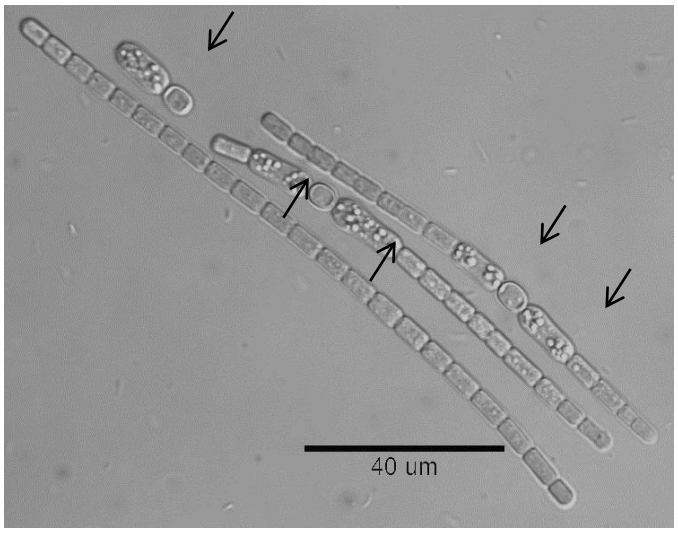

Figure 1-2. Photomicrograph of *Anabaena cylindrica* filaments containing akinetes, which are identified by the arrows, taken on a Nikon Eclipse E800 microscope.

Many possible factors or triggers have been proposed for akinete differentiation. Light limitations, depletion of nutrients, and temperature have been hypothesized to cause akinete differentiation, and the trigger for differentiation may be species, or even strain dependent (Li et al., 1997). Energy limitation has been shown to be the major factor in the initiation of akinete formation for *Nostoc* PCC 7524 (Sutherland et al., 1979). It has been discussed that the self-shading mechanism of a high cell density culture aids in the induction of akinetes (Adams and Duggan, 1999). A critical concentration of an essential nutrient that triggers the differentiation of akinetes has been studied for multiple cyanobacteria. A concentration of iron between 0.060 and 0.240 mg/L seemed to initiate akinete formation in *Anabaena cylindrica* ATCC 29414 (Hori et al., 2003). While, *Anabaena circinalis* began akinete development when the phosphate concentration was between 0.3-0.45 pg P/cell (Van Dok and Hart, 1996). The last trigger that appears to induce akinete formation is cold temperatures. Seven strains of *Anabaena* were grown at temperatures at 10 and 15^{o}C, which is cooler than their optimal temperature of 20^{o}C; five of those strains formed akinetes at higher frequencies at the cooler temperatures (Li, 1997). There does appear to be one common factor associated with the beginning of akinete differentiation; akinete development does not commence until growth of the cyanobacteria has ceased (Rao et al., 1987; Li, 1997; Sutherland et al., 1985; Hori et al., 2002; and Olli et al., 2005).

Akinetes are more resilient to environmental stresses than vegetative cells (Thiel and Wolk, 1983). Conditions such as decreased temperature and desiccation have been shown to have little effect on akinete viability (Hori et al., 2003). Studies on the genus

Anabaena of the order of *Nostocales* have shown to survive in an oven at 60^{o}C for 50 hours or direct sunlight for 10 hours (Hori et al., 2003). Even after being subjected to adverse conditions, akinetes have been able to germinate when conditions are favorable for growth.

Anabaena cylindrica

The cyanobacterium *A. cylindrica* was selected for analysis as biofertilizer for this study. It is categorized into subsection IV in the classification of cyanobacteria. It is an obligate photoautotrophic microorganism capable of forming both heterocysts and akinetes (Simon, 1977). It has an unbranching filamentous cellular arrangement. *Anabaena cylindrica* utilizes heterocysts for N_2 fixation that develop in the absence of combined N source (e.g. nitrate, nitrite, or ammonia) are evenly spaced along the filament. Akinetes of this cyanobacterium tend to differentiate adjacent to heterocysts (Simon, 1977). Akinetes of *A. cylindrica* differentiate at the highest rate approximately one week past their cell density peak (Olli et al., 2005). The diazotrophic ability with unbranched filaments makes this organism a possible candidate for a biofertilizer.

Properties of Biofertilizer

With the increasing demand for food, the need for fertilizer is also growing. Chemical fertilizers have many problems associated with their use, such as increased risk of pollution of local ecosystems and production of greenhouse gases as a result of manufacturing (Benemann, 1979). With manufacturing costs and pollution potential of chemical synthesized nitrogen fertilizers, an alternative method of producing nitrogen

fertilizer is required. The concept of farming an organism that could be used as a fertilizer while being sustainable and renewable would be a potential solution to this current problem.

N_2 fixing prokaryotes are responsible for the majority of biologically fixed nitrogen in the natural ecosystem (Benemann, 1979). Cyanobacteria that are capable of fixing N_2 have been of particular interest because they also fix inorganic carbon during photosynthesis, resulting in a removal of atmospheric carbon dioxide. Additionally, the only nitrogen source required for their growth is N_2 from the atmosphere, the most abundant form. This ability to grow and generate biomass from minimal inputs can be used to manufacture useful products, such as a biofertilizer.

Cyanobacteria are currently being used successfully as a biofertilizer in rice fields across the globe. These cyanobacterial species have been shown to fix considerable quantities of nitrogen while simultaneously preserving soil fertility (Hashem, 2001). In Indian rice fields, cyanobacteria can fix 25-30 kg nitrogen per hectare in the total omission of chemical fertilizers (Hashem, 2001). With the use of cyanobacterial biofertilizers, the average improvement on the grain yield of rice is between 15-20% (Innok et al., 2009). For rice, Pereira et al. (2009) concluded that the use of chemical fertilizers could be reduced up to 50% when used in conjunction with biofertilizers and still obtain similar yields (Pereira et al., 2009). In chemically fertilized rice fields, more than 50% of nitrogen is derived from the soil provided by N_2 fixing microorganisms (Irisarri et al., 2001).

While most of the current research has been focused on the improvement of biofertilizers in rice fields, the response of a few other agricultural crops to cyanobacterial biofertilizers has been examined. A mixture of two cyanobacteria, *Tolypothrix tenuis* and *Microchaete tenra*, has been shown to improve the growth of maize and increase the activity of the enzymes urease, phosphatase, phosphomonoesterase, protease, and dehydrogenase in the soil (Osman et al., 2010). Soil enzymes are responsible for making nutrients available for plant usage (De Cano et al., 2002). Maize was also biofertilized with *Nostoc* alone; this cyanobacterium promoted the growth of the plant (Maqubela et al., 2009). *Chlamydomonas mexicana* has been shown to boost the yields of both cotton and potatoes (Rogers and Burns, 1994). By cultivating wheat, a high nitrogen requiring plant, the dry root weight and chlorophyll were increased by using chemical and biofertilizer (Karthikeyan et al., 2007). The enhancement of plant growth in various crops as a result of biofertilizer application has shown its potential as a replacement of chemical fertilizers.

The success of some cyanobacterial biofertilizers is often dependent on its localized environment. Many physical, chemical or biological conditions can impact the growth and survival of the inoculated cyanobacteria (Irisarri et al., 2001). The nutrient levels especially nitrogen, temperature, and the soil moisture content and pH can determine the growth and nitrogen fixation rates of a cyanobacterial population (Pereira et al., 2009). Each type of cyanobacteria requires certain conditions for optimal growth. Studies have demonstrated better results were achieved when a mixed cyanobacterial inoculum containing some native strains is utilized (Pereira et al., 2009). The

effectiveness of the biofertilizer will depend on the native cyanobacterial population and soil properties.

One other benefit of using cyanobacterial biofertilizer is the improvement of soil fertility. Biofertilizers improve soil structure by excreting polysaccharide substances that bind and mesh soil particles together (Issa et al., 2007). This affects the mechanical properties of the soil, resulting in decreased erosion and an increased water holding capacity (Hashem, 2001). The improved water retention also results in a decreased need for irrigation. Another benefit of cyanobacterial biofertilizers is the potential to remediate heavy-metal laden soils by metabolizing heavy metals into more benign forms (Osman et al., 2010). Both of these benefits increase either the fertility or usability of the land. Cyanobacterial biofertilizers have numerous advantages compared to the more traditional chemically synthesized fertilizers.

CHAPTER TWO

THE RESPONSE OF *ANABAENA CYLINDRICA* TO VARIATIONS IN GROWTH CONDTIONS AND APPLICATION AS A BIOFERTILIZER

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

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Contributions: Conceived and implemented the study design. Collected and analyzed data. Wrote first draft of the manuscript.

Co-Author: Dr. Rich Macur

Contributions: Helped conceived the study design. Conducted the biofertilization study. Provided feedback on the manuscript.

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Contributions: Provided feedback on the study design. Provided comments on the manuscript.

Manuscript Information Page

Lisa Weeks, Rich Macur, Brent Peyton
Bioresource Technology
Status of Manuscript:
__x_Prepared for submission to a peer-reviewed journal
____Officially submitted to a peer-reviewed journal
____Accepted by a peer-reviewed journal
____Published in a peer-reviewed journal

Published by Elsevier

Abstract

Nitrogen is a vital nutrient for plant growth. Traditional methods of manufacturing nitrogen fertilizers are energy intensive and lead to the production of greenhouse gases. Nitrogen fixing cyanobacteria have shown promise as an environmentally friendly method of producing biofertilizer containing nitrogen. A potential candidate organism, *Anabaena cylindrica,* was assessed for adaptability to changes in growth conditions. Variations in flow regime of the air in the reactor, superficial velocity of the air within the reactor, temperature, light cycle and intensity had little statistically effect over the ranges tested on either the average growth rate or final biomass concentration. The nitrogen content, chlorophyll and final biomass concentrations increased considerably to 74.2 ± 6.9 mg N/L, 12.3 ± 0.97 µg/mL and 0.81 ± 0.16 g/L, respectively, when 0.15 mM of magnesium sulfate heptahydrate and 0.005 mM of disodium ethylenediamine tetraacetate dihydrate was present in the culture medium. Magnesium is utilized within the cyanobacterial cell for many purposes. Evaluation of the growth of wheat, camelina, carrots, tomatoes and Kentucky bluegrass, when treated with cyanobacterial biofertilizer, showed statistically similar results when compared to chemically fertilized plants.

Keywords

Anabaena cylindrica, biofertilizer, growth conditions, magnesium, temperature, superficial velocity, light intensity, light cycle, and air flow regimes

Introduction

Nitrogen (N) is an essential nutrient and is often the primary limiting factor for crop growth (Topre et al., 2011). Current methods of producing chemical nitrogen fertilizers are estimated to require up to 1.2% of the total global energy demand (Ahlgren et al., 2008) with fertilizer manufacturing representing a large fraction of the total greenhouse gas emissions from the agricultural industry (Ahlgren et al., 2008). To adjust to a changing global climate, an alternative ecologically friendly and sustainable approach is necessary for production of nitrogen fertilizers.

It is known that biomass produced by photosynthetic organisms captures and stores carbon dioxide (CO_2) resulting in a net removal from the atmosphere (Sanchez Fernandez et al., 2012). The prokaryotic cyanobacteria are photosynthetic microorganisms with a wide array of biochemical and morphological variations (Latysheva et al., 2012). A subset of cyanobacteria is capable of atmospheric nitrogen (N_2) fixation; this group of microorganisms utilizes CO_2 and N_2 for their carbon and N sources, respectively.

Diazotrophic (N_2 fixing) cyanobacteria have been shown to be the dominant source of N in rice fields. Hashem (2001) found that in Indian rice fields, cyanobacteria can fix 25-30 kg of N per hectare in the absence of chemical fertilizers. Biomass produced by diazotrophic cyanobacteria has shown promise as a biofertilizer where an average improvement in rice grain yields of 15-20% was seen with its use (Innok et al., 2009). The success of cyanobacterial fertilizers observed in rice fields has begun to translate to other agricultural crops, such as maize and wheat. Additionally, soil fertility

and structure improvement have been observed with the use of cyanobacterial biofertilizers. The physical properties of the soil were affected by extracellular polymeric substances excreted by the cyanobacteria, which increases the water holding capacity and decreases erosion (Hashem, 2001).

Diazotrophic cyanobacteria may be attractive in a commercial setting given that they can grow using solar energy with minimal nutrients (Fontes et al., 1989) and do not require arable land or often even fresh water for growth. This can significantly reduce competition with agriculture or other industries for resources and desirable locations. Contamination of large systems with unwanted organisms is also a common problem in large scale phototrophic systems, but with an environment depleted of nitrogen, the competition from non N_2 fixing microorganisms may be reduced. This potentially allows for the dominance of diazotrophic organisms.

Here we focused on two aspects important to larger scale production of cyanobacterial biofertilizers; the growth of a diazotrophic cyanobacterium under changing environmental conditions and the response of various plants to a cyanobacterial biofertilizer treatment. Changes in the growth conditions can have an impact on the productivity of the cyanobacteria. The ability of *Anabaena cylindrica* to adapt to changing growth conditions such as the flow regime and superficial velocity of the air stream in the reactor, temperature, light intensity and cycles, and nutrient additions were examined. The effect of cyanobacterial biofertilizer on wheat, camelina, carrots, tomatoes and Kentucky bluegrass was analyzed.

Methods

Organism, Growth System, and Base Medium

Anabaena cylindrica (UTEX B1611) was obtained from the University of Texas culture collection of algae. This strain was selected for its ability to fix atmospheric N_2, rapid growth in liquid N-free medium and, in early screening tests (data not shown), the lack of biofilm formation when grown in an aqueous solution.

Growth studies were carried out in closed tube photo-bioreactors with a working volume of 1.25 L. The reactors were illuminated at 42 W/m^2 using 12 T5 4 ft fluorescent lights under a 14/10 light/dark (L/D) cycle. The light intensity was measured using a Jaz Irradiance Collector, Ocean Optics, Inc. The temperature in the reactors was maintained at 25 ± 1^oC in a controlled water bath, and the photo-bioreactors were aerated with ambient air at a volumetric flow rate of 400 mL/min resulting in an average superficial gas velocity of 12.5 cm/s under slug flow conditions. BG11-N (no additional N added) (UTEX, 2005) was selected as the standard medium. All photo-bioreactor systems were autoclaved before inoculation with the cyanobacterium. The standard recipe is given in Appendix A. Samples were collected at 24 hour intervals. Cell densities were determined from direct cell counts using a Reichert hemacytometer and Leica Dm E microscope. The pH of the sample was measured using a standard pH meter (Fisher Scientific, Accument Basic AB15).

Chlorophyll and Biomass Dry Weight

The chlorophyll concentration in the cultures was measured daily. A 1 mL aliquot was centrifuged at 11,000 x g for 10 min, and the supernatant was removed. The biomass was dispersed in methanol, and the sample was vortexed and sonicated prior to incubation for 1 hour at 4^{o}C. Samples were then centrifuged again at 11,000 x g for 10 min. Absorbance of the methanol supernatant was measured at 632, 652 and 665 nm in a spectrophotometer (Genesys 10-S, Thermo Electron Corporation) and the chlorophyll a concentration was calculated using the method outlined in Ritchie (2008).

The biomass concentration as dry weight in each photo-bioreactor was measured using the following method. A known volume of the culture was centrifuged at 4,000 x g at 5 min intervals until the majority of the water was removed. The collected biomass was then lyophilized to remove the remaining water (Labconco Lyophilizer). The dried biomass was then weighed to provide a biomass concentration.

Total Nitrogen in the Biomass

The total amount of N contained in the dried biomass was measured on a carbon and nitrogen analyzer (TruSpec CN, LECO Corporation). Approximately 0.1 g of dry biomass was combusted and the composition of the resulting gas was analyzed with a thermal conductivity cell to calculate the percent of the biomass that was N.

Biofertilizer Studies

The biomass that was used as a biofertilizer for the greenhouse studies described below was grown in a 200 L open raceway pond (Separation Engineering, Inc.,

Escondido, CA) under non-sterile conditions. BG11-N medium for this study was made using non-sterile tap water. Losses of liquid volume due to evaporation during operation were replenished by directly adding tap water. The surface of the raceway was illuminated with 26 W/m^2 of light from fluorescent lights at a 14/10 L/D cycle. Air was introduced into the system at a constant rate of 2.5 L/min through a counter current diffusion gas-liquid exchange column. The counter current flow pattern and packing material in the column enhances the transfer of N_2 and CO_2 into the solution. The temperature of the raceway was maintained at 25 ± 1^oC. Biomass from the raceway pond was harvested by continuous centrifugation and stored at 4^oC prior to use as a biofertilizer.

Vananda clay soil collected from the Crow Indian Reservation in south-central Montana (USA) was used for growth experiments with wheat and camelina. The Vananda soil is common across the area and is extensively used for crop production (USDA, 2013). The soil was ground and sieved (< 2 mm) and thoroughly mixed with 50 mesh quartz sand at a 3:1 sand to soil ratio. Combining the soil with the sand provided a non-compactable soil medium and insured nutrient poor conditions. Six inch diameter pots were filled with 1800 g of the Vananda soil/sand mixture and seeded with winter wheat, var. Yellowstone and Camelina sativa, var. Suneson. The plants were housed in a temperature (22 ± 2^oC) controlled greenhouse and received both natural and artificial light to ensure at least a 14 hour light period. Automatic thermal/shade curtains were utilized to maintain temperature and decrease light intensity when necessary.

Three fertilizer treatments and a control were tested in triplicate to assess the response to the biofertilizer. The treatments included: 1) no fertilizer control, 2) commercial ammonium nitrate fertilizer, 3) half strength solution Hoagland's liquid fertilizer (Millner and Kitt, 1992) and 4) *A. cylindrica* cyanobacterial biomass. Hoagland's fertilizer contains a full suite of macro and micronutrients and represented the "best case scenario" for fertilizer composition. For the wheat experiments, 9.0 g of moist cyanobacterial biomass representing an application dose of 67 kg N per hectare (0.126 g N per pot) was added as a slurry to the pots after nine days of growth. This N application rate represents a low end rate for irrigated wheat grown in Montana (typical application rates are ~200 kg N per hectare per year) and was selected due to the short duration of these experiments (<8 weeks) where wheat was harvested prior to the boot stage. Hoagland's liquid fertilizer was applied as 12 applications over 6 weeks. After seeding, the pots were watered with 200 mL of tap water every 3 d. The experimental conditions and treatments for camelina were identical to the wheat experiments with the exception that camelina only received a dose of N equivalent to 22 kg of N per hectare per year. This N application rate represents a recommended maximum in Montana for this short season oil seed crop that is adapted to grow in nutrient poor soils (Steve Camp, personal communication).

Further experiments were conducted to evaluate the response of a wider variety of plants to biofertilizer application. For these experiments, World Vision variety carrots, Bonnie Best variety tomatoes and Scott's variety Kentucky bluegrass were evaluated. Each pot received a dose 0.126 g N per pot equating to a rate 67 kg N per hectare per

year. Using the same procedure described above with the exception that the ammonium nitrate fertilizer treatment was omitted and 100% Amsterdam silt loam soil was used. The Amsterdam soil is a relatively fertile grassland soil containing about 2% organic matter and is a common agricultural soil in the Gallatin Valley, west of Bozeman, Montana.

The plants were harvested, rinsed to remove any excess soil, and the biomass was dried at 50^{o}C for 48 h and weighed. The complete plant for wheat and camelina was harvested after 45 days of growth. The carrots were grown for 91 days before the tuber portion was harvested. The shoots of the tomato plants were collected after 84 days of growth. Kentucky bluegrass was clipped to about 2.5 cm above the soil surface after 79 days of growth. The second clipping of the bluegrass occurred 139 days after seeding. Only the clippings were used in the dried plant weight.

Statistics

All experiments were run in triplicate, with the average and standard deviation shown in the subsequent graphs and table. A statistical analysis was executed on the collected data and t-tests were employed to highlight differences in sample means between experimental conditions. The significance level was held constant at $\alpha = 0.05$.

Results and Discussion

Growth Under Standard Conditions

Anabaena cylindrica was grown in the photo-bioreactors under standard conditions to establish a baseline for growth in the system. The reactors were illuminated

with 42 W/m^2 of fluorescent light with a 14/10 light/dark cycle. The temperature of the reactors was maintained at 25 ± 1^{o}C. Ambient air was introduced into the reactor at a superficial velocity of 12.5 cm/s under a slug flow regime. Figure 2-1 shows the growth curve, as measured by direct cell counts, under baseline conditions. The average growth rate was calculated to be 0.024 ± 0.01 hr^{-1} and the final biomass concentration was 0.27 ± 0.07 g/L. This experiment established baseline for the growth rate and yield, which was used for comparison for the varied growth parameters discussed below.

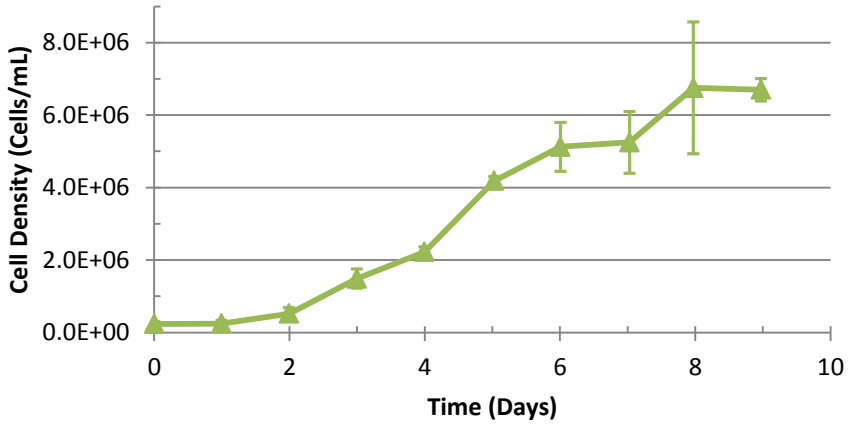

Figure 2-1. Average cell densities of a triplicate set of photo-bioreactors of *Anabaena cylindrica* grown under standard conditions, with the error bars representing standard deviations.

Response to Changes in Environmental Conditions

Variations in the environmental conditions such as the temperature and light availability to the photo-bioreactor or the addition of a gas stream and nutrients in the photo-bioreactors can affect both the rate and extent of cyanobacterial growth. Cultures that have the ability to maintain high levels of productivity under different growth conditions are desirable. Temperature, light, gas flow rate, and nutrient availability were

varied to determine the impact on average growth rate and final biomass concentration. Table 2-1 contains the average growth rates and final biomass concentrations for all photo-bioreactor experiments. The results from the statistical analysis for each of the varied parameters are included in the Appendix B.

Parameter Varied	**Average Growth Rate (1/hr)**	**Final Biomass Concentration (g/L)**
Flow Regime of the Air Stream		
Slug Flow	0.024 ± 0.01	0.27 ± 0.07
Bubble Flow	0.030 ± 0.01	0.24 ± 0.03
Air Superficial Velocity		
6.3 cm/s	0.022 ± 0.01	0.24 ± 0.02
12.5 cm/s	0.024 ± 0.01	0.27 ± 0.07
18.8 cm/s	0.017 ± 0.01	0.28 ± 0.02
Temperature		
25^{o}C	0.028 ± 0.01	0.24 ± 0.02
28^{o}C	0.035 ± 0.01	0.22 ± 0.02
30^{o}C	0.020 ± 0.01	0.21 ± 0.03
Light Cycles		
10/14 L/D	0.017 ± 0.01	0.26 ± 0.06
12/12 L/D	0.018 ± 0.02	0.26 ± 0.17
14/10 L/D	0.024 ± 0.01	0.27 ± 0.07
Light Intensity		
42 W/m^2	0.021 ± 0.01	0.27 ± 0.07
23 W/m^2	0.014 ± 0.01	0.30 ± 0.06
16 W/m^2	0.023 ± 0.01	0.25 ± 0.01

Table 2-1. A table displaying the average and standard deviation of both the growth rate and final biomass concentration for a set of triplicate photo-bioreactors for each of the varied environmental parameters.

Flow Regime of the Air Stream. The flow regime of the air in the photo-bioreactors modifies the gas exchange rate and the movement of the medium within the column. A system operating under bubble flow contains many small bubbles that rise through the column with little interaction (Yoon et al., 2012). The advantage of small bubbles is that the high surface area to volume ratio aids in the diffusion of the CO_2 and

N_2 into the solution. In contrast, the large bullet shaped bubbles of slug flow create more turbulence in the reactor (Yoon et al., 2012). Figure 2-2 shows two photo-bioreactors operating under the examined two flow regimes. Yoon et al. (2012) observed that the wake of bubbles rising under slug flow conditions appeared to increase the radial mixing when compared to bubbles rising under a bubble flow regime pattern. Yoon et al. (2012) also hypothesized that the increase of radial mixing helped with the circulation of cyanobacterial filaments from the dark to light zones of the photo-bioreactor. As shown in Table 2-1, with a constant volumetric flow rate of ambient air for each of the two flow regimes tested here, *A. cylindrica* had statistically the same average growth rates and final biomass concentrations indicating that this cyanobacterium was not sensitive to the flow regime of the air stream in this reactor system.

Air Superficial Velocity. Ambient air introduced at the bottom of the photo-bioreactor has two functions, conveying much needed CO_2 and N_2 into the system. As the bubbles rise in the column, they cause the cyanobacteria to circulate around the reactor. A high superficial velocity will increase mixing, but it can exert a greater shear stress on the cells (Yoon et al., 2012) and may be detrimental to filaments resulting in a decrease of growth rate or yield. The superficial air velocity in the reactors was calculated from the volumetric flow rate of air being pumped into the system and the cross sectional area of the photo-bioreactor. To test this, three superficial velocities (6.3, 12.5, and 18.8 cm/s) operated under a slug flow regime were investigated for their effect on cyanobacterial growth rate or final biomass concentration. *Anabaena cylindrica* grew well under all three conditions and no statistically significant differences were observed

in the final biomass concentrations. Lowering the superficial velocity to 6.3 cm/s did not appear to affect the growth rate or biomass production of this cyanobacterium. A small difference was observed in the average growth rate between 12.5 and 18.8 cm/s (p value = 0.044), where the slower growth rate at 18.8 cm/s may be attributed to a higher shear stress on the filaments, but further experiments were not run to determine if this effect was real. A moderate superficial velocity of 12.5 cm/s was used for subsequent experiments as it seemed best suited for our system and provided a lower shear stress and adequate circulation of the culture.

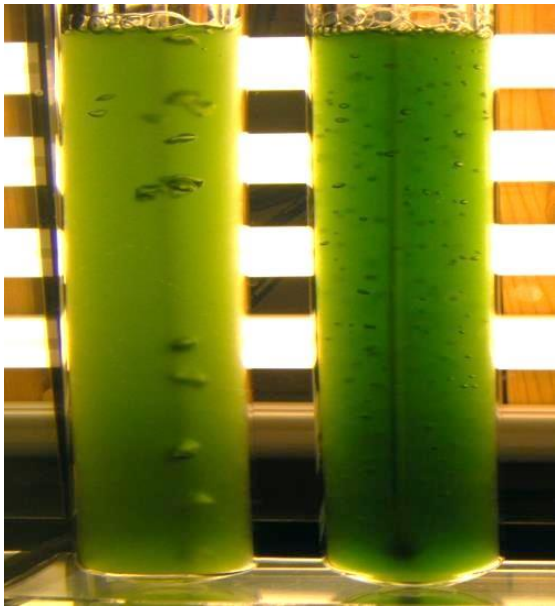

Figure 2-2. Two photo-bioreactors containing *A. cylindrica* operating under slug flow (left) and bubble flow (right) regimes.

Temperature. The effects of temperature on growth were also examined. Depending on the climate, in a commercial system, adjusting the temperature would increase the required energy inputs and operational costs. Here, *A. cylindrica* was grown

at 25, 28 and 30^{o}C. The cyanobacterium continued to grow over this temperature range and Table 2-1 shows the resulting final biomass concentrations were statistically similar *Anabaena cylindrica* (Fogg strain, IAM-M-1) has been shown to have a better specific growth rate at 24 and 27^{o}C than when the culture was grown at 35^{o}C (Yamamoto, 1976). In our tests, the growth rate at 30^{o}C was slightly slower than at lower temperatures with a p-value of 0.009.

Light Cycle and Intensity. Since cyanobacteria are photosynthetic, they require some light for growth. Under laboratory conditions, both the light cycle and intensity can be altered. A standard light cycle of 14/10 L/D was compared to light cycles of 12/12 and 10/14 L/D. Results shown in Table 2-1 show that under these conditions, changing the light cycle had no significant effect on the average growth rate or final biomass concentration. The effect of light intensity on growth was tested with a 14/10 L/D cycle. The light intensity was lowered from 42 W/m^2 to 23 and 16 W/m^2. No change in the average growth rate or final biomass concentration was observed as a function of these light intensities. Gonzalez Lopez et al. (2009) have shown that *Anabaena* can grow at irradiance levels up to 2500 µE/ (m^{2}· s), which is about six times greater than the intensity examined in this study.

Nutrient Additions

The depletion of necessary nutrients in the culture medium can limit the productivity of the cyanobacterium. The BG11-N medium contains a full suite of nutrients required for cyanobacterial growth. Through a series of additions, it was shown

that a combination of nutrients could be added to improve final biomass concentration. The combination that resulted in a large increase in final biomass concentration was 5 times the standard concentration of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) and disodium ethylenediamine tetraacetate dihydrate ($Na_2EDTA \cdot 2H_2O$). Increases of the two nutrients separately resulted in final biomass concentrations similar to the standard BG11-N medium, see Figure 2-3. The combination of the two nutrients simultaneously is required to amplify the productivity of the organism.

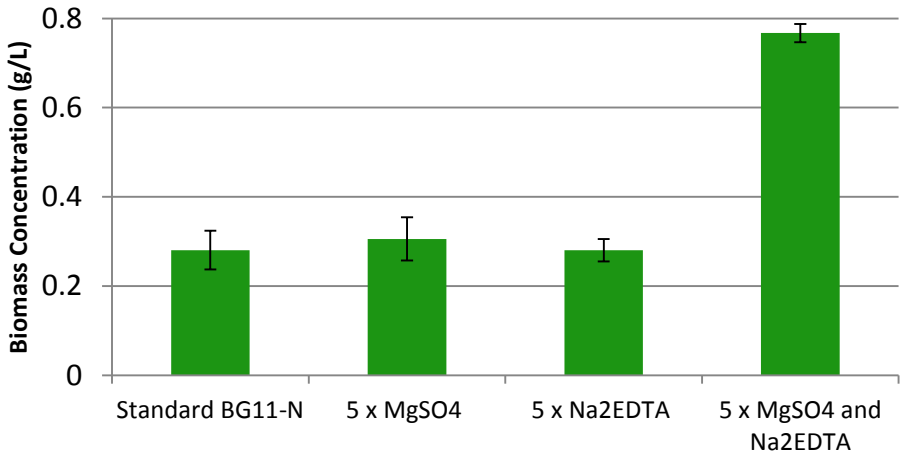

Figure 2-3. The final biomass concentration of triplicate cultures grown on four variations of the BG11-N medium: the standard concentration, 5 times the standard $MgSO_4$ concentration, 5 times the Na_2EDTA concentration and 5 times the $MgSO_4$ and Na_2EDTA concentration combined. The error bars represent the standard deviation.

Raising the initial concentration $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$ to 0.15 and 0.005 mM, respectively, resulted in a large increase in the biomass concentration, chlorophyll and N content. This enhanced medium, containing the additional $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$, and the standard BG11-N were compared in the

photo-bioreactors under the same standard growth conditions described in the previous section.

The largest improvements resulting from the additional $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$ were seen in the final amount of biomass generated and the total amount of nitrogen fixed into the biomass. As shown in Figure 2-4, the final biomass concentration of the standard BG11-N and the enhanced BG11-N were 0.27 ± 0.07 and 0.81 ± 0.16 g biomass / L, respectively. The total amount of nitrogen fixed into the biomass also increased from 18.3 ± 3.5 to 74.2 ± 6.9 mg N / L. These observed increases were significant and should drastically improve the productivity of *A. cylindrica* for use as a biofertilizer.

The culture grown in the standard BG11-N medium showed a change in the chlorophyll concentration from 2.58 ± 0.14 µg/mL compared to 12.27 ± 0.97 µg/mL in the enhanced BG11-N medium. In phototrophic organisms, chlorophyll is a light harvesting pigment that provides the energy to drive photosynthesis cycle (McKee and McKee, 2003). Magnesium plays many important roles in cell processes and in particular is an integral part of the chlorophyll molecule (Willey et al., 2008). The increased amounts of chlorophyll that were present in the culture could boost the carbon fixation rate. In addition to its importance in chlorophyll, magnesium is an important cofactor for many enzymes in the cell (Willey et al., 2008). In heterocystous cyanobacteria, ammonia is produced from N_2 fixation. The magnesium ion aids in a high activity rate of the enzyme glutamate dehydrogenase, which used in the assimilation of ammonia in many organisms (Dharmawardene et al., 1973). Magnesium has also been

shown to support a higher N_2 fixation rate than other divalent cations in a suspension of nitrogenase from *Anabaena cylindrica* (Haystead and Stewart, 1972). Magnesium ions aids in the development of an electrochemical gradient across the thylakoid membrane of chloroplasts (Scholnick and Keren, 2006). The electrochemical gradient of the ions across this membrane drives ATP synthesis (McKee and McKee, 2003).For a variety of reasons described above, one could surmise that the addition of magnesium could help cyanobacteria maintain a high rate of photosynthesis.

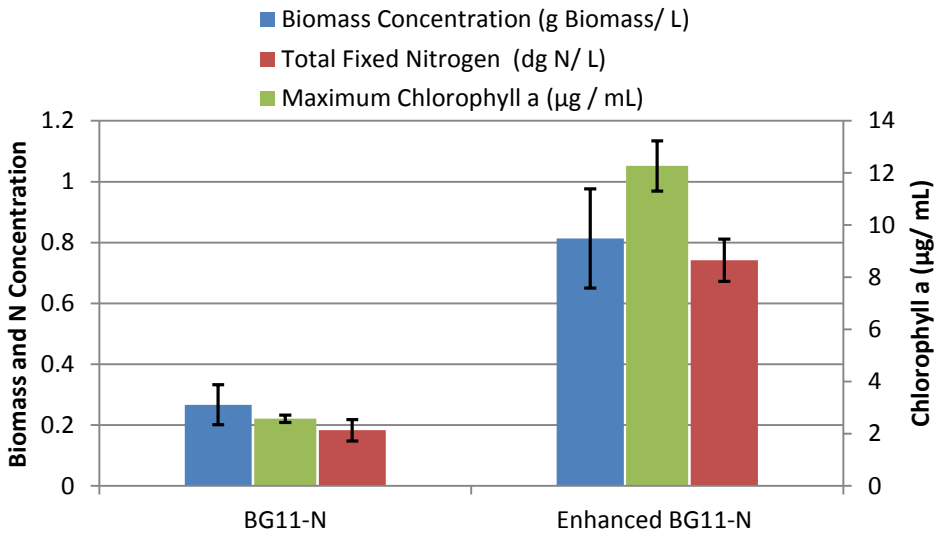

Figure 2-4. The average and standard deviation from a triplicate set of photo-bioreactors of the final biomass concentration, total fixed nitrogen and the maximum chlorophyll a concentration in the standard BG11-N medium and the enhanced BG11-N medium.

The pH of the solution increased due to the uptake of CO_2 by photosynthesis. Figure 2-5 shows the cultures grown in the enhanced BG11-N medium maintained a more alkaline pH than a culture in the standard BG11-N medium. This observation supports the idea that the additional nutrients in the enhanced medium increased the rate of photosynthesis by the cyanobacterium.

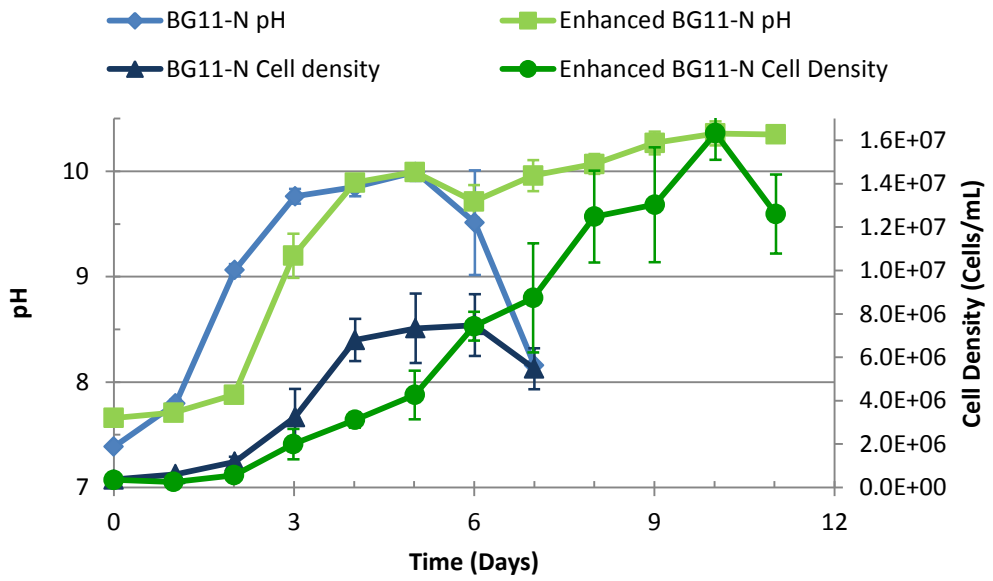

Figure 2-5. The average pH and standard deviations of a triplicate set of photo-bioreactor of the culture grown in both the standard and enhanced BG11-N media over time with the corresponding growth curve.

The additional $Na_2EDTA{\cdot}2H_2O$ has two roles in the culture medium. $Na_2EDTA{\cdot}2H_2O$ is an effective chelating agent. A chelator is often necessary to sustain a precipitate free solution in an alkaline medium (Kratz and Myers, 1955). Such a compound can also increase the bioavailability of divalent cations, such as calcium, iron and magnesium (Kratz and Myers, 1955). It has been reported that a chelating agent is important for optimal growth of cyanobacteria (Miwa and Morizan, 1988). The additional $Na_2EDTA{\cdot}2H_2O$ may have ensured that the necessary nutrients, including the extra amount of magnesium, were accessible for the organism.

Biofertilization Studies

All biomass used as the biofertilizer was grown in a 200 L raceway pond. The enhance BG11-N medium formulated with tap water was used. To generate enough

biomass for all the biofertilization studies, multiple batches were required. About 90% of the total volume of the raceway was harvested; while the remainder was used for inoculate the next batch. This process continued without any observable changes in either the growth rate or yield.

To assess the value of cyanobacterial biomass as a biofertilizer, plant growth studies were conducted to examine the effects on the growth of several types of plants. Plant growth with the cyanobacterial biofertilizer was compared to plants grown with traditional chemical fertilizer or no fertilizer controls. Five types of vegetation were examined: wheat, camelina, carrots, tomatoes, and Kentucky bluegrass.

Both the wheat and camelina responded well to the cyanobacterial biofertilizer. There was a significant increase in the dried biomass of the wheat grown with the cyanobacterial biofertilizer when compared to the other three treatments. The average plant weight grown with the cyanobacterial biofertilizer is about 3.5 times greater than the average plant weight of the control. This large increase in the growth of the wheat can be seen in Figure 2-6. For camelina, the total dried biomass of the plants grown with the biofertilizer was consistent with the other treatments, but was visually difference between than the fertilizer free control as seen in Figure 2-7. The plants treated with the cyanobacterial biofertilizer produced about 3 times more plant mass grown in the absence of any fertilizer. Figure 2-8 shows the average dried plant weights for the wheat and camelina experiment.

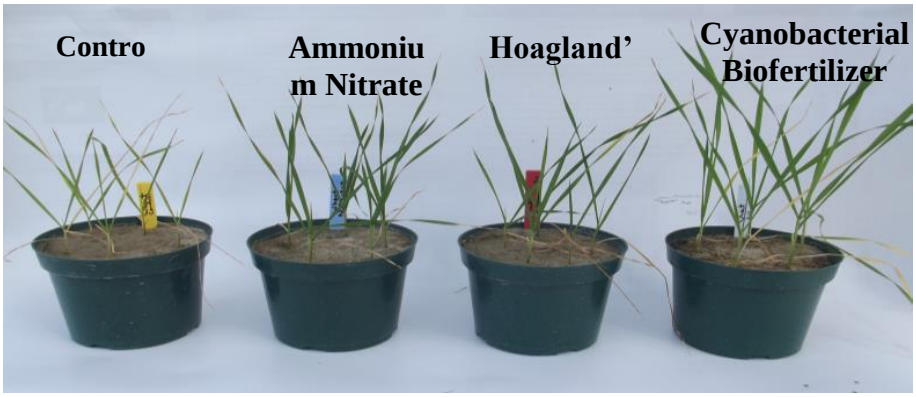

Figure 2-6. A photograph showing the differences in the plant growth of wheat using the four fertilizer treatments. For scale, the pots are 6 in diameter at the top.

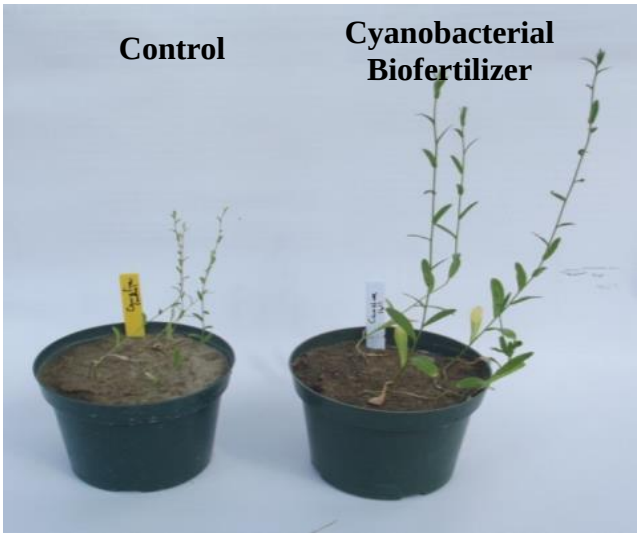

Figure 2-7. A photograph camelina grown without fertilizer addition (control) and another grown with the cyanobacterial biofertilizer. For scale, the pots are 6 in diameter at the top.

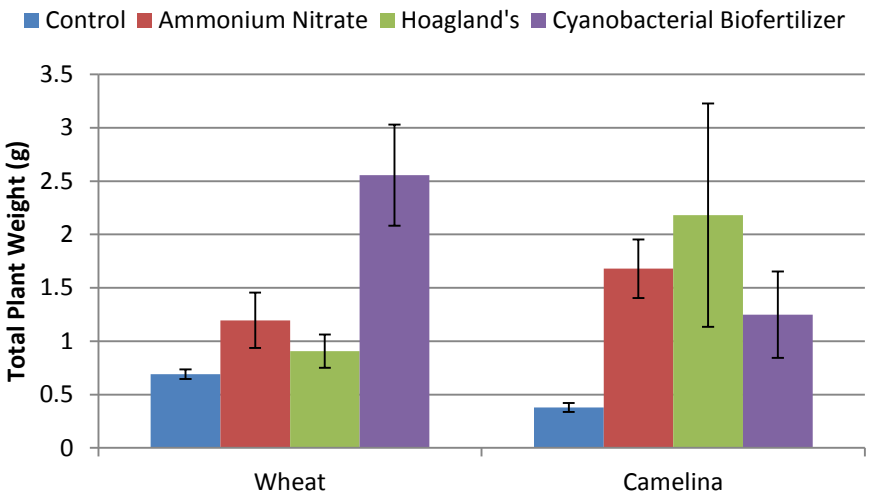

Figure 2-8. Dried biomass weight (average and standard deviation) of wheat and camelina grown under each of the four fertilizer treatments.

To evaluate of the effectiveness of the cyanobacterial biofertilizer on other plants, we also tested on three more types of flora: carrots, tomatoes, and Kentucky bluegrass. All three types of plants showed the same trend. Both the chemical fertilizer of Hoagland's and the cyanobacterial biofertilizer grew appreciably better than the control receiving no fertilizer additions. Figure 2-9 shows visually that for the carrots and Kentucky bluegrass, growth with the cyanobacterial biofertilizer was as good as with the conventional fertilizer method. Figure 2-10 shows the average dried plant weight. For both the cyanobacterial biofertilized and Hoagland's fertilized plants the average plant weights were very similar.

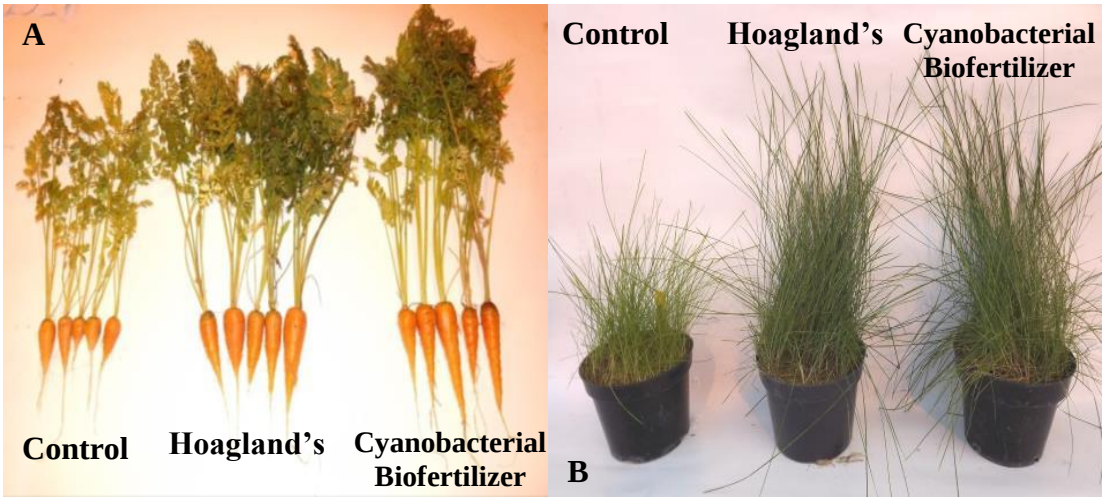

Figure 2-9. Photographs of the A) carrots and B) Kentucky bluegrass grown with the three different fertilizer treatments.

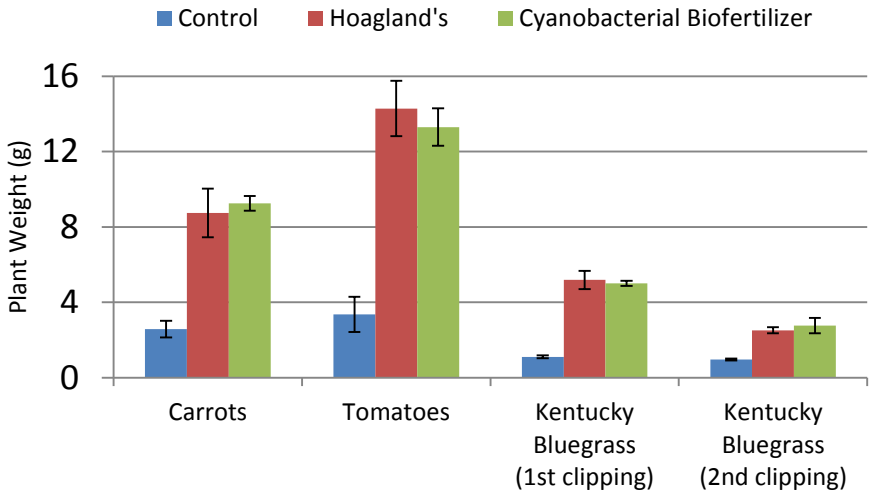

Figure 2-10. Average dry weight and standard deviation for carrots, tomatoes, and the first and second clippings for Kentucky bluegrass.

In Indian rice fields, diazotrophic cyanobacteria can fix 25-30 kg N per hectare per year in the absence of any chemical fertilizers (Hashem, 2001). In a similar study on rice fields conducted in Chile, the use of a biofertilizer a reduction of up to 50% of chemical fertilizers was possible, while still obtaining the same grain yield (Pereira et al.,

2009). Osman et al. (2012) reported that chemical fertilizer treatment could be reduced up to half when used in conjunction with a biofertilizer on pea plants. The results of our biofertilizer studies are consistent with previously reported studies. The final dried weight of all five plant types treated with only cyanobacterial biofertilizer were comparable to the Hoagland's or ammonium nitrate chemical fertilizers. This demonstrated the effectiveness of a cyanobacterial biomass for use as a biofertilizer.

Summary and Conclusions

The cyanobacterium, *A. cylindrica,* was shown to be a hardy and robust microorganism. It was able to adjust to a variety of changes in environmental growth conditions without losing productivity. Differences in flow regimes of the air stream, superficial velocities of the air, temperatures, light cycles and intensities had little statistical significant effect on the average growth rate or final biomass concentration. The addition of surplus $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$ into the standard BG11-N medium gave a significant increase in the final biomass and chlorophyll concentrations, along with the total nitrogen content of the biomass. The extra nutrients are thought to help maintain cellular activities such as photosynthesis while significant additional work is necessary. These characteristics indicate that cyanobacteria can be successfully grown at a commercial scale for possible use as a biofertilizer. The analysis on the biofertilization studies performed here indicate that biomass of *A. cylindrica* is a potent biofertilizer. Dried plant weights from the five types of plants treated with the biofertilizer were statistically much higher than the unfertilized controls and statistically

similar to the chemically fertilized plants. This study revealed that diazotrophic cyanobacteria may be a viable option for an ecological friendly and sustainable method for fixing N for fertilizers.

Acknowledgments

We would like to thank the Accelergy Corporation for their support of this project. We would like to express our gratitude to Todd Pedersen for his hard work during the response to changing environmental conditions segment and to Casey Doney for beginning the biofertilizer studies.

OVERALL CONCLUSIONS

Suggestions for Future Work

The use of N_2 fixing cyanobacteria shows merit as a capable biofertilizer. To make the concept of cyanobacterial biofertilizer successful and to replace traditional fertilizers, two areas of the entire process needed to be examined. The first area is the production and harvesting of the biomass. The second is the agricultural use of the biofertilizer, from the application to the life cycle of the cyanobacteria on the soil surface. Further work is needed in the following issues to enhance and improve the whole process.

- Many different designs are available for large scale growth of microorganisms. Determination of the best system for diazotrophic cyanobacteria would be necessary. An open pond or lagoon would provide the highest rate of N_2 and carbon dioxide diffusion, but is more susceptible to contamination. A closed system will provide a more controlled environment. The location of the facility along with the chosen cyanobacterium will aid in the determination of the growth system employed.
- This study focused on the sensitivity of *A. cylindrica* to changes in growth conditions, but further work on increasing the productivity of the cyanobacterium could be completed. Controlling the growth conditions to the optimal level for this particular microorganism would improve the efficiency of large scale production.

- This research examined the BG11-N medium for a limiting nutrient. The other aspect would be to minimize that quantity of nutrients added to the growth system while still obtaining the same rate and extent of cyanobacterial growth. Also, exploring other chemical compounds to substitute in for one of the original components. For example, $Na_2EDTA{\cdot}2H_2O$ can be costly. An investigation into other possible chelators could reduce the reactant costs at an industrial scale.
- An effective method for harvesting the biomass is needed. Continuous centrifugation was used on our laboratory scale but is not necessarily a cost effective technique on every scale. A sedimentation basin could be a successful approach for continuous harvesting of biomass. Since *A. cylindrica* is a filamentous cyanobacterium, a screen or mesh system could also be applicable. There could be clogging or plugging issues with this process that should be explored.
- Additional analysis of the effects of different application methods of the cyanobacterial biofertilizer on the plant response is necessary. The application method is tied in to the harvesting method and transportation of the cyanobacterial biofertilizer from the production plant to the field. One potential idea is to have a direct application from the large scale on-site growth system. This would eliminate any dewatering and transportation costs.
- Further work is needed on establishing the behavior of the cyanobacteria after being applied to the soil surface. The major question is if the cyanobacteria are viable or capable of growth in the soil environment. If this is possible, further

quantities of fixed nitrogen would available to the plant. The cyanobacteria may provide a continued supply of nitrogen, thus reducing the chances of nitrogen being a limiting nutrient in plant growth and yield.

- Evaluation of the long term effects of the constant application of a cyanobacterial biofertilizer is also undetermined. The introduction of any foreign microorganism can have an effect on the local ecosystem. Furthermore, the overuse of chemical fertilizers has shown to have consequences for the environment. The outcome of continuous use of a biofertilizer on the surrounding areas will also need further investigation.
- The plant response to cyanobacterial biofertilizer was conducted on a small scale in a greenhouse. Larger studies in a field setting are crucial before this technology could be implemented.

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APPENDICES

APPENDIX A

GROWTH MEDIUM

Table A-1. Final molar concentrations of components of both standard and enhances BG11-N media

Component	Standard BG11-N	Enhanced BG11-N
	Final Concentration	Final Concentration
K_2HPO_4	0.22 mM	0.22 mM
$MgSO_4 \cdot 7H_2O$	0.03 mM	0.15 mM
$CaCl_2 \cdot 2H_2O$	0.24 mM	0.24 mM
Citric Acid$\cdot H_2O$	0.012 mM	0.012 mM
Ammonium Ferric Citrate	0.02 mM	0.02 mM
$Na_2EDTA \cdot 2H_2O$	0.002 mM	0.005 mM
Na_2CO_3	0.18 mM	0.18 mM
BG11-N Trace Metals Solution	1 mL	1 mL

Table A-2. Final molar concentrations of components of the trace metal solutions used in the BG11-N medium

Component	Final Concentration (µM)
H_3BO_3	46
$MnCl_2 \cdot 4H_2O$	9
$ZnSO_4 \cdot 7H_2O$	0.77
$Na_2MoO_4 \cdot 2H_2O$	1.6
$CuSO_4 \cdot 5H_2O$	0.3
$Co(NO_3)_2 \cdot 6H_2O$	0.17

APPENDIX B

EXPERIMENTAL DATA FROM PHOTO-BIOREACTORS

All experiments were conducted with *Anabaena cylindrica* UTEX B1611 under the same standard conditions except for the studied variation of parameters. The standard conditions were 1.25 L of BG11-N medium operated under slug flow regime with 400 mL/min of ambient air entering the reactor resulting in a superficial velocity of 12.5 cm/s. The light intensity was at about 42.5 W/m^2 at a 14/10 light/dark cycle. The temperature of the reactors was kept at 25 ± 1^oC in a controlled water bath. All measurements were taken using the procedure outlined in the methods section above.

Table B-1. A table displaying the results of the t-test statistical analysis between each of the varied parameters

Comparison	Growth Rate P-Value	Final Biomass P-Value
Air Flow Regime		
Bubble vs. Slug	0.288	0.519
Superficial Velocity of the Air		
6.3 cm/s vs. 12.5 cm/s	0.764	0.616
6.3 cm/s vs. 18.8 cm/s	0.346	0.0589
12.5 cm/s vs. 18.8 cm/s	0.044	0.716
Temperature		
25^oC vs. 28^oC	0.152	0.226
25^oC vs. 30^oC	0.101	0.240
28^oC vs. 30^oC	0.007	0.429
Light Cycles		
10L/14D vs. 12L/12D	0.50	0.957
10L/14D vs. 14L/10D	0.067	0.498
12L/12D vs. 14L/10D	0.061	0.986
Light Intensities		
42 W/m^2 vs. 23 W/m^2	0.064	0.489
42 W/m^2 vs. 16 W/m^2	0.557	0.653
23 W/m^2 vs. 16 W/m^2	0.204	0.244

Table B-2. Cell densities (cells/mL) of the air flow regime variation experiment

Time (Days)	Bubble Flow					Slug Flow				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	1.71E+05	2.10E+05	2.85E+05	2.22E+05	5.78E+04	1.94E+05	2.18E+05	3.03E+05	2.38E+05	5.72E+04
1	2.98E+05	2.53E+05	1.85E+05	2.45E+05	5.66E+04	2.91E+05	2.99E+05	1.44E+05	2.45E+05	8.74E+04
2	6.58E+05	3.90E+05	3.55E+05	4.68E+05	1.65E+05	3.55E+05	5.18E+05	6.88E+05	5.20E+05	1.66E+05
3	8.20E+05	1.33E+06	1.02E+06	1.06E+06	2.57E+05	1.79E+06	1.33E+06	1.35E+06	1.49E+06	2.61E+05
4	2.47E+06	3.53E+06	3.72E+06	3.24E+06	6.74E+05	2.31E+06	2.06E+06	2.30E+06	2.22E+06	1.42E+05
5	4.46E+06	6.64E+06	6.38E+06	5.83E+06	1.19E+06	4.20E+06	4.06E+06	4.30E+06	4.19E+06	1.21E+05
6	5.18E+06	6.23E+06	6.43E+06	5.94E+06	6.71E+05	5.83E+06	5.08E+06	4.48E+06	5.13E+06	6.76E+05
7	7.50E+06	3.50E+06	5.55E+06	5.52E+06	2.00E+06	4.88E+06	6.23E+06	4.65E+06	5.25E+06	8.52E+05
	-	-	-	-	-	6.45E+06	5.10E+06	8.70E+06	6.75E+06	1.82E+06
9	-	-	-	-	-	6.83E+06	6.35E+06	6.93E+06	6.70E+06	3.07E+05
10	-	-	-	-	-	8.95E+06	9.20E+06	9.15E+06	9.10E+06	1.32E+05

Table B-3. pH measurements of the air flow regime variation experiment

Time (Days)	Bubble Flow					Slug Flow				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.57	7.55	7.42	7.51	0.08	7.25	7.25	7.23	7.24	0.01
1	7.05	7.38	7.22	7.22	0.17	7.76	7.62	7.61	7.66	0.08
2	7.2	7.55	7.4	7.38	0.18	7.42	7.4	7.41	7.41	0.01
3	7.77	7.81	7.58	7.72	0.12	8.99	9.08	8.91	8.99	0.09
4	8.82	9.18	9.49	9.16	0.34	9.71	9.7	9.77	9.73	0.04
5	9.48	9.74	9.88	9.70	0.20	9.7	9.42	9.81	9.64	0.20
6	9.28	8.59	9.17	9.01	0.37	9.9	10.02	10.16	10.03	0.13
7	9.64	8.01	7.68	8.44	1.05	9.97	10.02	10.17	10.05	0.10
8	-	-	-	-	-	9.07	9.97	10.19	9.74	0.59
9	-	-	-	-	-	9.1	9.2	9.76	9.35	0.36
10	-	-	-	-	-	8.62	8.67	8.79	8.69	0.09

Table B-4. Absorbance readings at 440 nm of the air flow regime variation experiment

Time (Days)	Bubble Flow					Slug Flow				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.055	0.052	0.062	0.056	0.005	0.056	0.055	0.061	0.057	0.003
1	0.044	0.04	0.047	0.044	0.004	0.046	0.047	0.045	0.046	0.001
2	0.061	0.052	0.053	0.055	0.005	0.057	0.063	0.06	0.060	0.003
3	0.108	0.117	0.11	0.112	0.005	0.13	0.132	0.116	0.126	0.009
4	0.221	0.261	0.245	0.242	0.020	0.211	0.212	0.201	0.208	0.006
5	0.345	0.48	0.453	0.426	0.071	0.322	0.319	0.316	0.319	0.003
6	0.437	0.512	0.513	0.487	0.044	0.396	0.408	0.395	0.400	0.007
7	0.577	0.499	0.495	0.524	0.046	0.422	0.493	0.456	0.457	0.036
8	-	-	-	-	-	0.443	0.513	0.523	0.493	0.044
9	-	-	-	-	-	0.426	0.561	0.57	0.519	0.081
10	-	-	-	-	-	0.423	0.545	0.559	0.509	0.075

Table B-5. Absorbance readings at 500 nm of the air flow regime variation experiment

Time (Days)	Bubble Flow					Slug Flow				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.045	0.041	0.041	0.042	0.002	0.045	0.046	0.048	0.046	0.002
1	0.031	0.028	0.031	0.030	0.002	0.033	0.035	0.03	0.033	0.003
2	0.043	0.034	0.034	0.037	0.005	0.039	0.044	0.041	0.041	0.003
3	0.068	0.079	0.071	0.073	0.006	0.091	0.089	0.077	0.086	0.008
4	0.133	0.172	0.161	0.155	0.020	0.132	0.126	0.123	0.127	0.005
5	0.206	0.329	0.293	0.276	0.063	0.192	0.191	0.192	0.192	0.001
6	0.243	0.348	0.344	0.312	0.060	0.234	0.24	0.218	0.231	0.011
7	0.331	0.336	0.328	0.332	0.004	0.261	0.313	0.281	0.285	0.026
8	-	-	-	-	-	0.306	0.334	0.367	0.336	0.031
9	-	-	-	-	-	0.314	0.465	0.46	0.413	0.086
10	-	-	-	-	-	0.333	0.412	0.435	0.393	0.054

Table B-6. Absorbance readings at 660 nm of the air flow regime variation experiment

Time (Days)	Bubble Flow					Slug Flow				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.031	0.03	0.034	0.032	0.002	0.032	0.033	0.034	0.033	0.001
1	0.018	0.016	0.018	0.017	0.001	0.021	0.022	0.016	0.020	0.003
2	0.026	0.019	0.017	0.021	0.005	0.023	0.027	0.025	0.025	0.002
3	0.047	0.06	0.052	0.053	0.007	0.068	0.063	0.054	0.062	0.007
4	0.106	0.145	0.128	0.126	0.020	0.107	0.099	0.096	0.101	0.006
5	0.175	0.271	0.25	0.232	0.050	0.173	0.151	0.166	0.163	0.011
6	0.22	0.276	0.285	0.260	0.035	0.202	0.205	0.187	0.198	0.010
7	0.28	0.247	0.259	0.262	0.017	0.216	0.266	0.223	0.235	0.027
8	-	-	-	-	-	0.26	0.268	0.282	0.270	0.011
9	-	-	-	-	-	0.261	0.401	0.369	0.344	0.073
10	-	-	-	-	-	0.276	0.345	0.337	0.319	0.038

Table B-7. Absorbance readings at 680 nm of the air flow regime variation experiment

Time (Days)	Bubble Flow					Slug Flow				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.055	0.052	0.062	0.056	0.005	0.035	0.034	0.036	0.035	0.001
1	0.02	0.017	0.019	0.019	0.002	0.021	0.022	0.017	0.020	0.003
2	0.029	0.022	0.02	0.024	0.005	0.026	0.028	0.027	0.027	0.001
3	0.057	0.069	0.061	0.062	0.006	0.076	0.074	0.064	0.071	0.006
4	0.133	0.175	0.158	0.155	0.021	0.134	0.129	0.124	0.129	0.005
5	0.229	0.329	0.314	0.291	0.054	0.217	0.206	0.213	0.212	0.006
6	0.287	0.331	0.345	0.321	0.030	0.261	0.267	0.255	0.261	0.006
7	0.379	0.298	0.312	0.330	0.043	0.273	0.332	0.293	0.299	0.030
8	-	-	-	-	-	0.303	0.337	0.351	0.330	0.025
9	-	-	-	-	-	0.299	0.445	0.42	0.388	0.078
10	-	-	-	-	-	0.309	0.39	0.389	0.363	0.046

Table B-8. Final biomass concentration (g/L) of the air flow regime variation experiment

Parameter Varied	A	B	C	Average	Standard Deviation
Bubble Flow	0.2604	0.2090	0.2419	0.2371	0.0260
Slug Flow	0.2043	0.2612	0.3347	0.2667	0.0654

Table B-9. Percent nitrogen of the dried biomass from the air flow regime variation experiment

Parameter Varied	A	B	C	Average	Standard Deviation
Bubble Flow	9.53	8.9	7.52	8.65	1.03
Slug Flow	7.57	6.62	6.65	6.95	0.54

Table B-10. Cell densities (cells/mL) of the superficial velocities experiment

Time (Days)	6.3 cm/s					12.5 cm/s				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	3.70E+05	2.18E+05	3.85E+05	3.24E+05	9.27E+04	1.94E+05	2.18E+05	3.03E+05	2.38E+05	5.72E+04
1	3.55E+05	7.23E+05	4.85E+05	5.21E+05	1.86E+05	2.91E+05	2.99E+05	1.44E+05	2.45E+05	8.74E+04
2	7.05E+05	1.08E+06	1.19E+06	9.88E+05	2.51E+05	3.55E+05	5.18E+05	6.88E+05	5.20E+05	1.66E+05
3	1.86E+06	2.03E+06	2.62E+06	2.17E+06	3.99E+05	1.79E+06	1.33E+06	1.35E+06	1.49E+06	2.61E+05
4	3.45E+06	4.90E+06	5.25E+06	4.53E+06	9.54E+05	2.31E+06	2.06E+06	2.30E+06	2.22E+06	1.42E+05
5	2.71E+06	4.24E+06	5.39E+06	4.11E+06	1.34E+06	4.20E+06	4.06E+06	4.30E+06	4.19E+06	1.21E+05
6	5.30E+06	5.85E+06	5.33E+06	5.49E+06	3.11E+05	5.83E+06	5.08E+06	4.48E+06	5.13E+06	6.76E+05
7	5.35E+06	6.35E+06	4.55E+06	5.42E+06	9.02E+05	4.88E+06	6.23E+06	4.65E+06	5.25E+06	8.52E+05
8	5.50E+06	3.33E+06	4.73E+06	4.52E+06	1.10E+06	6.45E+06	5.10E+06	8.70E+06	6.75E+06	1.82E+06
9	5.30E+06	4.95E+06	8.50E+06	6.25E+06	1.96E+06	6.83E+06	6.35E+06	6.93E+06	6.70E+06	3.07E+05
10	7.25E+06	5.10E+06	6.25E+06	6.20E+06	1.08E+06	8.95E+06	9.20E+06	9.15E+06	9.10E+06	1.32E+05
11	4.45E+06	7.05E+06	4.75E+06	5.42E+06	1.42E+06	-	-	-	-	-

Time (Days)	18.8 cm/s				
	A	B	C	Average	Standard Deviation
0	4.60E+05	2.75E+05	3.63E+05	3.66E+05	9.25E+04
1	3.25E+05	7.10E+05	7.30E+05	5.88E+05	2.28E+05
2	7.05E+05	8.75E+05	1.14E+06	9.07E+05	2.19E+05
3	2.79E+06	1.48E+06	1.91E+06	2.06E+06	6.68E+05
4	4.40E+06	3.85E+06	4.75E+06	4.33E+06	4.54E+05
5	4.73E+06	4.68E+06	4.90E+06	4.77E+06	1.18E+05
6	5.28E+06	5.60E+06	6.18E+06	5.68E+06	4.56E+05
7	8.75E+06	6.25E+06	6.30E+06	7.10E+06	1.43E+06
8	7.50E+06	8.20E+06	5.85E+06	7.18E+06	1.21E+06
9	6.90E+06	6.85E+06	9.70E+06	7.82E+06	1.63E+06

Table B-11. pH measurements of the superficial gas velocities experiment

Time (Days)	6.3 cm/s					12.5 cm/s				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.18	7.31	7.39	7.29	0.11	7.25	7.25	7.23	7.24	0.01
1	8.19	8.39	8.47	8.35	0.14	7.76	7.62	7.61	7.66	0.08
2	8.99	9.32	9.34	9.22	0.20	7.42	7.4	7.41	7.41	0.01
3	9.32	9.65	9.66	9.54	0.19	8.99	9.08	8.91	8.99	0.09
4	9.7	9.89	9.92	9.84	0.12	9.71	9.7	9.77	9.73	0.04
5	9.67	9.91	9.87	9.82	0.13	9.7	9.42	9.81	9.64	0.20
6	9.76	9.91	9.97	9.88	0.11	9.9	10.02	10.16	10.03	0.13
7	9.85	9.99	10.04	9.96	0.10	9.97	10.02	10.17	10.05	0.10
8	9.92	10.03	10.02	9.99	0.06	9.07	9.97	10.19	9.74	0.59
9	9.57	10	9.97	9.85	0.24	9.1	9.2	9.76	9.35	0.36
10	9.57	9.98	10.02	9.86	0.25	8.62	8.67	8.79	8.69	0.09
11	9.55	9.77	9.86	9.73	0.16	-	-	-	-	-

Time (Days)	18.8 cm/s				
	A	B	C	Average	Standard Deviation
0	7.35	7.23	7.17	7.25	0.09
1	7.78	7.95	7.9	7.88	0.09
2	8.39	8.51	8.48	8.46	0.06
3	9.63	9.64	9.66	9.64	0.02
4	9.91	10.07	9.99	9.99	0.08
5	10.04	10.03	10.04	10.04	0.01
6	9.97	10.09	9.99	10.02	0.06
7	9.96	10.05	10.03	10.01	0.05
8	9.69	9.74	9.66	9.70	0.04
9	7.9	8.05	8.12	8.02	0.11

Table B-12. Absorbance readings at 440 nm of the superficial gas velocities experiments

Time (Days)	6.3 cm/s					12.5 cm/s				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.053	0.056	0.053	0.054	0.002	0.056	0.055	0.061	0.057	0.003
1	0.07	0.08	0.071	0.074	0.006	0.046	0.047	0.045	0.046	0.001
2	0.109	0.113	0.114	0.112	0.003	0.057	0.063	0.06	0.060	0.003
3	0.179	0.181	0.177	0.179	0.002	0.13	0.132	0.116	0.126	0.009
4	0.261	0.239	0.245	0.248	0.011	0.211	0.212	0.201	0.208	0.006
5	0.304	0.276	0.303	0.294	0.016	0.322	0.319	0.316	0.319	0.003
6	0.368	0.319	0.349	0.345	0.025	0.396	0.408	0.395	0.400	0.007
7	0.435	0.38	0.404	0.406	0.028	0.422	0.493	0.456	0.457	0.036
8	0.46	0.417	0.467	0.448	0.027	0.443	0.513	0.523	0.493	0.044
9	0.528	0.478	0.534	0.513	0.031	0.426	0.561	0.57	0.519	0.081
10	0.574	0.512	0.559	0.548	0.032	0.423	0.545	0.559	0.509	0.075
11	0.535	0.537	0.541	0.538	0.003	-	-	-	-	-

Time (Days)	18.8 cm/s				
	A	B	C	Average	Standard Deviation
0	0.05	0.05	0.055	0.052	0.003
1	0.064	0.07	0.077	0.070	0.007
2	0.103	0.098	0.11	0.104	0.006
3	0.218	0.193	0.213	0.208	0.013
4	0.309	0.312	0.318	0.313	0.005
5	0.403	0.404	0.412	0.406	0.005
6	0.492	0.476	0.505	0.491	0.015
7	0.541	0.531	0.538	0.537	0.005
8	0.576	0.601	0.576	0.584	0.014
9	0.564	0.614	0.634	0.604	0.036

Table B-13. Absorbance readings at 500 nm of the superficial gas velocities experiment

Time (Days)	6.3 cm/s					12.5 cm/s				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.035	0.038	0.038	0.037	0.002	0.045	0.046	0.048	0.046	0.002
1	0.05	0.06	0.05	0.053	0.006	0.033	0.035	0.03	0.033	0.003
2	0.072	0.076	0.076	0.075	0.002	0.039	0.044	0.041	0.041	0.003
3	0.111	0.113	0.11	0.111	0.002	0.091	0.089	0.077	0.086	0.008
4	0.158	0.155	0.157	0.157	0.002	0.132	0.126	0.123	0.127	0.005
5	0.173	0.176	0.191	0.180	0.010	0.192	0.191	0.192	0.192	0.001
6	0.226	0.198	0.221	0.215	0.015	0.234	0.24	0.218	0.231	0.011
7	0.25	0.218	0.231	0.233	0.016	0.261	0.313	0.281	0.285	0.026
8	0.273	0.227	0.262	0.254	0.024	0.306	0.334	0.367	0.336	0.031
9	0.332	0.294	0.357	0.328	0.032	0.314	0.465	0.46	0.413	0.086
10	0.403	0.33	0.378	0.370	0.037	0.333	0.412	0.435	0.393	0.054
11	0.353	0.37	0.353	0.359	0.010	-	-	-	-	-

Time (Days)	18.8 cm/s				
	A	B	C	Average	Standard Deviation
0	0.035	0.035	0.041	0.037	0.003
1	0.044	0.051	0.056	0.050	0.006
2	0.075	0.072	0.081	0.076	0.005
3	0.146	0.121	0.135	0.134	0.013
4	0.192	0.199	0.197	0.196	0.004
5	0.242	0.247	0.239	0.243	0.004
6	0.314	0.286	0.325	0.308	0.020
7	0.365	0.341	0.344	0.350	0.013
8	0.411	0.431	0.387	0.410	0.022
9	0.406	0.442	0.473	0.440	0.034

Table B-14. Absorbance measurements at 660 nm of the superficial gas velocities experiment

Time (Days)	6.3 cm/s					12.5 cm/s				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.022	0.025	0.023	0.023	0.002	0.032	0.033	0.034	0.033	0.001
1	0.033	0.04	0.034	0.036	0.004	0.021	0.022	0.016	0.020	0.003
2	0.048	0.051	0.051	0.050	0.002	0.023	0.027	0.025	0.025	0.002
3	0.084	0.086	0.082	0.084	0.002	0.068	0.063	0.054	0.062	0.007
4	0.119	0.119	0.117	0.118	0.001	0.107	0.099	0.096	0.101	0.006
5	0.129	0.14	0.149	0.139	0.010	0.173	0.151	0.166	0.163	0.011
6	0.198	0.172	0.188	0.186	0.013	0.202	0.205	0.187	0.198	0.010
7	0.196	0.17	0.18	0.182	0.013	0.216	0.266	0.223	0.235	0.027
8	0.204	0.173	0.198	0.192	0.016	0.26	0.268	0.282	0.270	0.011
9	0.236	0.222	0.284	0.247	0.033	0.261	0.401	0.369	0.344	0.073
10	0.296	0.235	0.26	0.264	0.031	0.276	0.345	0.337	0.319	0.038
11	0.237	0.274	0.238	0.250	0.021	-	-	-	-	-

Time (Days)	18.8 cm/s				
	A	B	C	Average	Standard Deviation
0	0.023	0.022	0.026	0.024	0.002
1	0.029	0.037	0.04	0.035	0.006
2	0.052	0.05	0.059	0.054	0.005
3	0.11	0.09	0.103	0.101	0.010
4	0.149	0.155	0.151	0.152	0.003
5	0.194	0.198	0.19	0.194	0.004
6	0.264	0.244	0.279	0.262	0.018
7	0.274	0.248	0.25	0.257	0.014
8	0.307	0.326	0.275	0.303	0.026
9	0.297	0.32	0.353	0.323	0.028

Table B-15. Absorbance readings at 680 nm of the superficial gas velocities experiment

Time (Days)	6.3 cm/s					12.5 cm/s				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.025	0.026	0.025	0.025	0.001	0.035	0.034	0.036	0.035	0.001
1	0.035	0.042	0.035	0.037	0.004	0.021	0.022	0.017	0.020	0.003
2	0.057	0.062	0.061	0.060	0.003	0.026	0.028	0.027	0.027	0.001
3	0.108	0.108	0.104	0.107	0.002	0.076	0.074	0.064	0.071	0.006
4	0.161	0.152	0.151	0.155	0.006	0.134	0.129	0.124	0.129	0.005
5	0.186	0.178	0.191	0.185	0.007	0.217	0.206	0.213	0.212	0.006
6	0.243	0.21	0.227	0.227	0.017	0.261	0.267	0.255	0.261	0.006
7	0.273	0.238	0.249	0.253	0.018	0.273	0.332	0.293	0.299	0.030
8	0.286	0.254	0.28	0.273	0.017	0.303	0.337	0.351	0.330	0.025
9	0.312	0.293	0.336	0.314	0.022	0.299	0.445	0.42	0.388	0.078
10	0.352	0.304	0.33	0.329	0.024	0.309	0.39	0.389	0.363	0.046
11	0.31	0.342	0.314	0.322	0.017	-	-	-	-	-

Time (Days)	18.8 cm/s				
	A	B	C	Average	Standard Deviation
0	0.024	0.023	0.026	0.024	0.002
1	0.03	0.036	0.041	0.036	0.006
2	0.059	0.055	0.064	0.059	0.005
3	0.132	0.113	0.128	0.124	0.010
4	0.194	0.199	0.199	0.197	0.003
5	0.258	0.26	0.26	0.259	0.001
6	0.318	0.305	0.329	0.317	0.012
7	0.342	0.325	0.327	0.331	0.009
8	0.368	0.387	0.345	0.367	0.021
9	0.357	0.383	0.413	0.384	0.028

Table B-16. Final biomass concentrations (g/L) of the superficial gas velocities experiment

Parameter Varied	A	B	C	Average	Standard Deviation
6.3 cm/s	0.2562	0.2247	0.2499	0.2436	0.0167
12.5 cm/s	0.2043	0.2612	0.3347	0.2667	0.0654
18.8 cm/s	0.2595	0.2919	0.2976	0.2839	0.0205

Table B-17. Percent nitrogen content of the dried biomass from the superficial gas velocities experiment

Parameter Varied	A	B	C	Average	Standard Deviation
6.3 cm/s	7.15	7.88	7.48	7.53	0.37
12.5 cm/s	7.57	6.62	6.65	6.95	0.54
18.8 cm/s	6.8	6.62	6.58	6.67	0.12

TableB-18. Cell densities (cells/mL) of the temperature variation experiment

Time (Days)	25°C					28°C				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	3.19E+05	3.06E+05	3.39E+05	3.21E+05	1.64E+04	3.64E+05	4.04E+05	3.31E+05	3.66E+05	3.63E+04
1	4.45E+05	4.70E+05	6.55E+05	5.23E+05	1.15E+05	6.65E+05	6.03E+05	5.58E+05	6.08E+05	5.40E+04
2	1.32E+06	8.00E+05	1.93E+06	1.35E+06	5.66E+05	1.31E+06	1.33E+06	9.10E+05	1.18E+06	2.35E+05
3	1.47E+06	1.66E+06	2.57E+06	1.90E+06	5.88E+05	3.80E+06	4.18E+06	1.75E+06	3.24E+06	1.31E+06
4	5.23E+06	5.33E+06	5.98E+06	5.51E+06	4.07E+05	7.68E+06	6.98E+06	5.75E+06	6.80E+06	9.74E+05
5	3.70E+06	8.03E+06	6.05E+06	5.93E+06	2.17E+06	5.93E+06	7.00E+06	9.08E+06	7.33E+06	1.60E+06
6	9.50E+06	8.35E+06	5.30E+06	7.72E+06	2.17E+06	5.95E+06	7.75E+06	8.75E+06	7.48E+06	1.42E+06
7	7.80E+06	7.65E+06	5.30E+06	6.92E+06	1.40E+06	4.45E+06	5.68E+06	6.30E+06	5.48E+06	9.41E+05

Time (Days)	30°C				
	A	B	C	Average	Standard Deviation
0	3.61E+05	2.86E+05	3.58E+05	3.35E+05	4.23E+04
1	7.03E+05	7.45E+05	7.45E+05	7.31E+05	2.45E+04
2	1.35E+06	1.48E+06	1.24E+06	1.36E+06	1.23E+05
3	1.58E+06	1.92E+06	2.36E+06	1.95E+06	3.91E+05
4	4.40E+06	3.40E+06	3.03E+06	3.61E+06	7.11E+05
5	4.48E+06	4.48E+06	5.28E+06	4.74E+06	4.62E+05
6	3.60E+06	4.33E+06	3.85E+06	3.93E+06	3.68E+05
7	2.98E+06	3.03E+06	2.93E+06	2.98E+06	5.00E+04

Table B-19. pH measurements of the temperature variation experiment

Time (Days)	25°C					28°C				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.2	7.5	7.3	7.33	0.15	7.4	7.37	7.39	7.39	0.02
1	7.23	7.32	7.3	7.28	0.05	7.84	7.77	7.78	7.80	0.04
2	7.39	7.44	7.36	7.40	0.04	9.03	9.03	9.13	9.06	0.06
3	9.36	9.67	9.7	9.58	0.19	9.7	9.75	9.84	9.76	0.07
4	9.51	9.94	10.02	9.82	0.27	9.76	9.86	9.93	9.85	0.09
5	9.7	10.12	10.15	9.99	0.25	9.95	9.94	10.09	9.99	0.08
6	9.86	10.21	10.23	10.10	0.21	9.66	8.96	9.92	9.51	0.50
7	10	10.25	10.28	10.18	0.15	8.14	8.16	8.18	8.16	0.02

Time (Days)	30°C				
	A	B	C	Average	Standard Deviation
0	7.66	7.58	7.59	7.61	0.04
1	8.65	8.72	8.62	8.66	0.05
2	9.54	9.53	9.68	9.58	0.08
3	9.67	9.71	9.9	9.76	0.12
4	9.71	9.71	9.92	9.78	0.12
5	9.9	9.98	10.06	9.98	0.08
6	9.21	9.32	10.13	9.55	0.50
7	8.86	8.12	9.83	8.94	0.86

Table B-20. Absorbance readings at 440 nm of the temperature variation experiment

Time (Days)	25°C					28°C				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.039	0.039	0.040	0.039	0.001	0.047	0.048	0.052	0.049	0.003
1	0.051	0.047	0.059	0.052	0.006	0.071	0.070	0.069	0.070	0.001
2	0.090	0.090	0.100	0.093	0.006	0.140	0.143	0.134	0.139	0.005
3	0.174	0.168	0.193	0.178	0.013	0.279	0.301	0.243	0.274	0.029
4	0.283	0.279	0.289	0.284	0.005	0.381	0.410	0.375	0.389	0.019
5	0.367	0.384	0.377	0.376	0.009	0.510	0.540	0.513	0.521	0.017
6	0.495	0.460	0.474	0.476	0.018	0.518	0.525	0.569	0.537	0.028
7	0.499	0.502	0.484	0.495	0.010	0.468	0.472	0.511	0.484	0.024

Time (Days)	30°C				
	A	B	C	Average	Standard Deviation
0	0.046	0.046	0.050	0.047	0.002
1	0.075	0.076	0.077	0.076	0.001
2	0.146	0.141	0.151	0.146	0.005
3	0.195	0.219	0.224	0.213	0.016
4	0.287	0.328	0.315	0.310	0.021
5	0.346	0.374	0.399	0.373	0.027
6	0.377	0.405	0.437	0.406	0.030
7	0.340	0.370	0.434	0.381	0.048

Table B-21. Absorbance readings at 500 nm of the temperature variation experiment

Time (Days)	25°C					28°C				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.026	0.028	0.027	0.027	0.001	0.033	0.033	0.037	0.034	0.002
1	0.036	0.032	0.044	0.037	0.006	0.050	0.048	0.048	0.049	0.001
2	0.065	0.063	0.073	0.067	0.005	0.088	0.095	0.084	0.089	0.006
3	0.112	0.111	0.137	0.120	0.015	0.181	0.196	0.132	0.170	0.033
4	0.200	0.189	0.192	0.194	0.006	0.240	0.250	0.243	0.244	0.005
5	0.213	0.264	0.230	0.236	0.026	0.334	0.376	0.362	0.357	0.021
6	0.343	0.297	0.305	0.315	0.025	0.370	0.392	0.421	0.394	0.026
7	0.349	0.344	0.312	0.335	0.020	0.320	0.324	0.353	0.332	0.018

Time (Days)	30°C				
	A	B	C	Average	Standard Deviation
0	0.032	0.033	0.036	0.034	0.002
1	0.052	0.057	0.055	0.055	0.003
2	0.096	0.096	0.103	0.098	0.004
3	0.116	0.141	0.138	0.132	0.014
4	0.170	0.224	0.205	0.200	0.027
5	0.234	0.244	0.259	0.246	0.013
6	0.275	0.289	0.271	0.278	0.009
7	0.228	0.249	0.264	0.247	0.018

Table B-22. Absorbance readings at 660 nm of the temperature variation experiment

Time (Days)	25°C					28°C				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.016	0.018	0.018	0.017	0.001	0.021	0.021	0.023	0.022	0.001
1	0.023	0.021	0.030	0.025	0.005	0.034	0.035	0.034	0.034	0.001
2	0.046	0.045	0.053	0.048	0.004	0.069	0.077	0.066	0.071	0.006
3	0.080	0.081	0.101	0.087	0.012	0.154	0.166	0.107	0.142	0.031
4	0.155	0.144	0.150	0.150	0.006	0.209	0.218	0.205	0.211	0.007
5	0.158	0.206	0.176	0.180	0.024	0.283	0.326	0.307	0.305	0.022
6	0.263	0.225	0.226	0.238	0.022	0.310	0.343	0.349	0.334	0.021
7	0.259	0.257	0.216	0.244	0.024	0.274	0.273	0.300	0.282	0.015

Time (Days)	30°C				
	A	B	C	Average	Standard Deviation
0	0.022	0.022	0.023	0.022	0.001
1	0.038	0.039	0.037	0.038	0.001
2	0.072	0.071	0.077	0.073	0.003
3	0.090	0.111	0.107	0.103	0.011
4	0.138	0.184	0.166	0.163	0.023
5	0.189	0.195	0.210	0.198	0.011
6	0.206	0.224	0.204	0.211	0.011
7	0.163	0.192	0.189	0.181	0.016

Table B-23. Absorbance readings at 680 nm of the temperature variation experiment

Time (Days)	25°C					28°C				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.018	0.020	0.019	0.019	0.001	0.023	0.023	0.023	0.023	0.000
1	0.027	0.025	0.033	0.028	0.004	0.039	0.039	0.037	0.038	0.001
2	0.052	0.051	0.059	0.054	0.004	0.083	0.090	0.079	0.084	0.006
3	0.101	0.102	0.119	0.107	0.010	0.186	0.202	0.149	0.179	0.027
4	0.186	0.178	0.186	0.183	0.005	0.263	0.280	0.255	0.266	0.013
5	0.221	0.253	0.236	0.237	0.016	0.353	0.397	0.369	0.373	0.022
6	0.328	0.291	0.296	0.305	0.020	0.363	0.389	0.403	0.385	0.020
7	0.322	0.322	0.288	0.311	0.020	0.320	0.322	0.348	0.330	0.016

Time (Days)	30°C				
	A	B	C	Average	Standard Deviation
0	0.022	0.022	0.023	0.022	0.001
1	0.043	0.044	0.042	0.043	0.001
2	0.087	0.086	0.091	0.088	0.003
3	0.120	0.141	0.139	0.133	0.012
4	0.181	0.221	0.211	0.204	0.021
5	0.233	0.249	0.267	0.250	0.017
6	0.245	0.264	0.270	0.260	0.013
7	0.205	0.232	0.259	0.232	0.027

Table B-24. Final biomass concentration (g/L) of the temperature variation experiment

Parameter Varied	A	B	C	Average	Standard Deviation
25°C	0.2542	0.2204	0.2366	0.2371	0.0169
28°C	0.2163	0.1956	0.2354	0.2158	0.0199
30°C	0.1754	0.2400	0.2033	0.2062	0.0324

Table B-25. Percent nitrogen content of the dried biomass from the temperature variation experiment

Parameter Varied	A	B	C	Average	Standard Deviation
25°C	7.50	8.09	7.70	7.76	0.30
28°C	7.41	7.63	7.46	7.50	0.12
30°C	6.6	6.87	6.96	6.81	0.19

Table B-26. Cell densities (cells/mL) of the light cycle experiment

Time (Days)	10 Light / 14 Dark					12 Light / 12 Dark				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	4.61E+05	5.50E+05	5.00E+05	5.04E+05	4.45E+04	4.16E+05	4.94E+05	5.16E+05	4.75E+05	5.25E+04
1	6.93E+05	4.95E+05	3.23E+05	5.03E+05	1.85E+05	4.98E+05	3.53E+05	5.73E+05	4.74E+05	1.12E+05
2	8.80E+05	1.04E+06	8.00E+05	9.07E+05	1.22E+05	6.70E+05	7.25E+05	7.70E+05	7.22E+05	5.01E+04
3	1.97E+06	1.80E+06	1.57E+06	1.78E+06	2.01E+05	1.77E+06	2.71E+06	1.50E+06	1.99E+06	6.35E+05
4	4.13E+06	3.43E+06	5.43E+06	4.33E+06	1.01E+06	4.63E+06	4.80E+06	4.38E+06	4.60E+06	2.14E+05
5	5.18E+06	4.13E+06	4.58E+06	4.63E+06	5.27E+05	4.90E+06	5.80E+06	6.45E+06	5.72E+06	7.78E+05
6	5.40E+06	6.50E+06	6.25E+06	6.05E+06	5.77E+05	6.50E+06	7.15E+06	6.85E+06	6.83E+06	3.25E+05
7	8.85E+06	7.55E+06	8.55E+06	8.32E+06	6.81E+05	1.08E+07	7.30E+06	9.00E+06	9.03E+06	1.75E+06
8	6.45E+06	1.24E+07	1.04E+07	9.73E+06	3.02E+06	1.20E+07	1.11E+07	1.32E+07	1.21E+07	1.08E+06
9	6.25E+06	5.95E+06	8.90E+06	7.03E+06	1.62E+06	1.01E+07	1.17E+07	1.22E+07	1.13E+07	1.10E+06
10	8.80E+06	1.00E+07	9.20E+06	9.33E+06	6.11E+05	7.85E+06	6.20E+06	5.60E+06	6.55E+06	1.17E+06
11	5.23E+06	6.23E+06	6.60E+06	6.02E+06	7.11E+05	-	-	-	-	-

Time (Days)	14 Light / 10 Dark				
	A	B	C	Average	Standard Deviation
0	1.94E+05	2.18E+05	3.03E+05	2.38E+05	5.72E+04
1	2.91E+05	2.99E+05	1.44E+05	2.45E+05	8.74E+04
2	3.55E+05	5.18E+05	6.88E+05	5.20E+05	1.66E+05
3	1.79E+06	1.33E+06	1.35E+06	1.49E+06	2.61E+05
4	2.31E+06	2.06E+06	2.30E+06	2.22E+06	1.42E+05
5	4.20E+06	4.06E+06	4.30E+06	4.19E+06	1.21E+05
6	5.83E+06	5.08E+06	4.48E+06	5.13E+06	6.76E+05
7	4.88E+06	6.23E+06	4.65E+06	5.25E+06	8.52E+05
8	6.45E+06	5.10E+06	8.70E+06	6.75E+06	1.82E+06
9	6.83E+06	6.35E+06	6.93E+06	6.70E+06	3.07E+05
10	8.95E+06	9.20E+06	9.15E+06	9.10E+06	1.32E+05

Table B-27. pH measurements of the light cycle experiment

Time (Days)	10 Light / 14 Dark					12 Light / 12 Dark				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.67	7.63	7.67	7.66	0.02	7.38	7.28	7.39	7.35	0.06
1	7.68	7.59	7.65	7.64	0.05	7.20	7.22	7.19	7.20	0.02
2	7.30	7.57	7.46	7.44	0.14	7.56	7.39	7.50	7.48	0.09
3	7.86	8.05	8.13	8.01	0.14	8.40	8.52	8.45	8.46	0.06
4	8.94	9.56	9.55	9.35	0.36	9.86	9.83	9.90	9.86	0.04
5	9.68	9.74	9.91	9.78	0.12	10.03	9.98	10.01	10.01	0.03
6	9.62	9.96	9.96	9.85	0.20	9.94	10.09	9.94	9.99	0.09
7	9.68	10.02	10.05	9.92	0.21	10.13	10.10	10.07	10.10	0.03
8	9.68	9.91	9.91	9.83	0.13	10.09	10.05	10.06	10.07	0.02
9	9.69	10.02	9.92	9.88	0.17	9.96	10.07	10.08	10.04	0.07
10	9.91	9.93	9.98	9.94	0.04	9.05	9.10	9.23	9.13	0.09
11	9.73	10.01	8.97	9.57	0.54	-	-	-	-	-

Time (Days)	14 Light / 10 Dark				
	A	B	C	Average	Standard Deviation
0	7.25	7.25	7.23	7.24	0.01
1	7.76	7.62	7.61	7.66	0.08
2	7.42	7.40	7.41	7.41	0.01
3	8.99	9.08	8.91	8.99	0.09
4	9.71	9.70	9.77	9.73	0.04
5	9.70	9.42	9.81	9.64	0.20
6	9.90	10.02	10.16	10.03	0.13
7	9.97	10.02	10.17	10.05	0.10
8	9.07	9.97	10.19	9.74	0.59
9	9.10	9.20	9.76	9.35	0.36
10	8.62	8.67	8.79	8.69	0.09

Table B-28. Absorbance readings at 440 nm for the light cycle experiment

Time (Days)	10 Light / 14 Dark					12 Light / 12 Dark				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.051	0.047	0.045	0.048	0.003	0.057	0.052	0.059	0.056	0.004
1	0.055	0.053	0.051	0.053	0.002	0.061	0.058	0.065	0.061	0.004
2	0.070	0.067	0.064	0.067	0.003	0.082	0.074	0.081	0.079	0.004
3	0.097	0.105	0.106	0.103	0.005	0.129	0.124	0.123	0.125	0.003
4	0.148	0.175	0.190	0.171	0.021	0.249	0.240	0.248	0.246	0.005
5	0.244	0.217	0.263	0.241	0.023	0.360	0.316	0.331	0.336	0.022
6	0.311	0.280	0.330	0.307	0.025	0.465	0.409	0.456	0.443	0.030
7	0.371	0.331	0.410	0.371	0.040	0.551	0.474	0.486	0.504	0.041
8	0.411	0.417	0.488	0.439	0.043	0.603	0.587	0.570	0.587	0.017
9	0.489	0.443	0.546	0.493	0.052	0.612	0.576	0.655	0.614	0.040
10	0.584	0.527	0.595	0.569	0.037	0.634	0.525	0.597	0.585	0.055
11	0.601	0.538	0.580	0.573	0.032	-	-	-	-	-

Time (Days)	14 Light / 10 Dark				
	A	B	C	Average	Standard Deviation
0	0.056	0.055	0.061	0.057	0.003
1	0.046	0.047	0.045	0.046	0.001
2	0.057	0.063	0.060	0.060	0.003
3	0.130	0.132	0.116	0.126	0.009
4	0.211	0.212	0.201	0.208	0.006
5	0.322	0.319	0.316	0.319	0.003
6	0.396	0.408	0.395	0.400	0.007
7	0.422	0.493	0.456	0.457	0.036
8	0.443	0.513	0.523	0.493	0.044
9	0.426	0.561	0.570	0.519	0.081
10	0.423	0.545	0.559	0.509	0.075

Table B-29. Absorbance readings at 500 nm for the light cycle experiment

Time (Days)	10 Light / 14 Dark					12 Light / 12 Dark				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.038	0.035	0.032	0.035	0.003	0.043	0.039	0.045	0.042	0.003
1	0.040	0.038	0.036	0.038	0.002	0.044	0.040	0.048	0.044	0.004
2	0.050	0.047	0.045	0.047	0.003	0.063	0.054	0.060	0.059	0.005
3	0.072	0.073	0.075	0.073	0.002	0.094	0.090	0.087	0.090	0.004
4	0.099	0.124	0.139	0.121	0.020	0.193	0.184	0.187	0.188	0.005
5	0.166	0.139	0.170	0.158	0.017	0.253	0.207	0.232	0.231	0.023
6	0.211	0.178	0.208	0.199	0.018	0.317	0.268	0.320	0.302	0.029
7	0.245	0.213	0.275	0.244	0.031	0.370	0.302	0.325	0.332	0.035
8	0.252	0.290	0.319	0.287	0.034	0.431	0.430	0.382	0.414	0.028
9	0.294	0.266	0.367	0.309	0.052	0.427	0.408	0.476	0.437	0.035
10	0.394	0.346	0.398	0.379	0.029	0.443	0.357	0.393	0.398	0.043
11	0.400	0.335	0.375	0.370	0.033	-	-	-	-	-

Time (Days)	14 Light / 10 Dark				
	A	B	C	Average	Standard Deviation
0	0.045	0.046	0.048	0.046	0.002
1	0.033	0.035	0.030	0.033	0.003
2	0.039	0.044	0.041	0.041	0.003
3	0.091	0.089	0.077	0.086	0.008
4	0.132	0.126	0.123	0.127	0.005
5	0.192	0.191	0.192	0.192	0.001
6	0.234	0.240	0.218	0.231	0.011
7	0.261	0.313	0.281	0.285	0.026
8	0.306	0.334	0.367	0.336	0.031
9	0.314	0.465	0.460	0.413	0.086
10	0.333	0.412	0.435	0.393	0.054

Table B-30. Absorbance readings at 660 nm for the light cycle experiment

Time (Days)	10 Light / 14 Dark					12 Light / 12 Dark				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.024	0.022	0.020	0.022	0.002	0.026	0.024	0.029	0.026	0.003
1	0.023	0.023	0.022	0.023	0.001	0.028	0.025	0.030	0.028	0.003
2	0.033	0.032	0.032	0.032	0.001	0.041	0.036	0.040	0.039	0.003
3	0.051	0.053	0.055	0.053	0.002	0.067	0.062	0.064	0.064	0.003
4	0.075	0.096	0.110	0.094	0.018	0.152	0.140	0.144	0.145	0.006
5	0.137	0.111	0.144	0.131	0.017	0.214	0.172	0.194	0.193	0.021
6	0.171	0.149	0.172	0.164	0.013	0.260	0.222	0.265	0.249	0.024
7	0.202	0.183	0.231	0.205	0.024	0.300	0.248	0.266	0.271	0.026
8	0.209	0.254	0.275	0.246	0.034	0.350	0.360	0.323	0.344	0.019
9	0.234	0.229	0.308	0.257	0.044	0.318	0.324	0.386	0.343	0.038
10	0.333	0.295	0.325	0.318	0.020	0.324	0.279	0.297	0.300	0.023
11	0.307	0.248	0.296	0.284	0.031	-	-	-	-	-

Time (Days)	14 Light / 10 Dark				
	A	B	C	Average	Standard Deviation
0	0.032	0.033	0.034	0.033	0.001
1	0.021	0.022	0.016	0.020	0.003
2	0.023	0.027	0.025	0.025	0.002
3	0.068	0.063	0.054	0.062	0.007
4	0.107	0.099	0.096	0.101	0.006
5	0.173	0.151	0.166	0.163	0.011
6	0.202	0.205	0.187	0.198	0.010
7	0.216	0.266	0.223	0.235	0.027
8	0.260	0.268	0.282	0.270	0.011
9	0.261	0.401	0.369	0.344	0.073
10	0.276	0.345	0.337	0.319	0.038

Table B-31. Absorbance readings at 680 nm for the light cycle experiment

Time (Days)	10 Light / 14 Dark					12 Light / 12 Dark				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.024	0.022	0.022	0.023	0.001	0.028	0.026	0.030	0.028	0.002
1	0.024	0.024	0.023	0.024	0.001	0.028	0.026	0.030	0.028	0.002
2	0.036	0.036	0.035	0.036	0.001	0.042	0.039	0.043	0.041	0.002
3	0.056	0.062	0.065	0.061	0.005	0.074	0.071	0.074	0.073	0.002
4	0.091	0.115	0.126	0.111	0.018	0.166	0.160	0.168	0.165	0.004
5	0.164	0.142	0.177	0.161	0.018	0.251	0.211	0.228	0.230	0.020
6	0.208	0.192	0.222	0.207	0.015	0.307	0.277	0.308	0.297	0.018
7	0.250	0.229	0.284	0.254	0.028	0.373	0.315	0.331	0.340	0.030
8	0.275	0.306	0.337	0.306	0.031	0.394	0.397	0.381	0.391	0.009
9	0.323	0.300	0.377	0.333	0.040	0.389	0.387	0.449	0.408	0.035
10	0.388	0.351	0.386	0.375	0.021	0.377	0.330	0.359	0.355	0.024
11	0.385	0.330	0.357	0.357	0.028	-	-	-	-	-

Time (Days)	14 Light / 10 Dark				
	A	B	C	Average	Standard Deviation
0	0.035	0.034	0.036	0.035	0.001
1	0.021	0.022	0.017	0.020	0.003
2	0.026	0.028	0.027	0.027	0.001
3	0.076	0.074	0.064	0.071	0.006
4	0.134	0.129	0.124	0.129	0.005
5	0.217	0.206	0.213	0.212	0.006
6	0.261	0.267	0.255	0.261	0.006
7	0.273	0.332	0.293	0.299	0.030
8	0.303	0.337	0.351	0.330	0.025
9	0.299	0.445	0.420	0.388	0.078
10	0.309	0.390	0.389	0.363	0.046

Table B-32. Final biomass concentration (g/L) of the light cycle experiment

Parameter Varied	A	B	C	Average	Standard Deviation
10 Light / 14 Dark	0.2161	0.2357	0.3233	0.2584	0.0571
12 Light / 12 Dark	0.0916	0.2665	0.4349	0.2643	0.1717
14 Light / 10 Dark	0.2043	0.2612	0.3347	0.2667	0.0654

Table B-33. Percent nitrogen content of the dried biomass from the light cycle experiment

Parameter Varied	A	B	C	Average	Standard Deviation
10 Light / 14 Dark	7.80	7.95	7.75	7.83	0.10
12 Light / 12 Dark	7.42	7.12	7.56	7.37	0.22
14 Light / 10 Dark	7.57	6.62	6.65	6.95	0.54

Table B-34. Cell densities (cells/mL) of the light intensity experiment

Time (Days)	42.45 W/m^2					23.95 W/m^2				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	1.94E+05	2.18E+05	3.03E+05	2.38E+05	5.72E+04	2.95E+05	3.70E+05	2.68E+05	3.11E+05	5.31E+04
1	2.91E+05	2.99E+05	1.44E+05	2.45E+05	8.74E+04	5.60E+05	3.25E+05	7.10E+05	5.32E+05	1.94E+05
2	3.55E+05	5.18E+05	6.88E+05	5.20E+05	1.66E+05	6.95E+05	7.05E+05	8.75E+05	7.58E+05	1.01E+05
3	1.79E+06	1.33E+06	1.35E+06	1.49E+06	2.61E+05	2.00E+06	2.19E+06	2.06E+06	2.08E+06	9.71E+04
4	2.31E+06	2.06E+06	2.30E+06	2.22E+06	1.42E+05	2.93E+06	3.85E+06	2.93E+06	3.23E+06	5.34E+05
5	4.20E+06	4.06E+06	4.30E+06	4.19E+06	1.21E+05	2.68E+06	2.35E+06	4.40E+06	3.14E+06	1.10E+06
6	5.83E+06	5.08E+06	4.48E+06	5.13E+06	6.76E+05	4.90E+06	2.93E+06	3.15E+06	3.66E+06	1.08E+06
7	4.88E+06	6.23E+06	4.65E+06	5.25E+06	8.52E+05	5.35E+06	3.65E+06	5.60E+06	4.87E+06	1.06E+06
8	6.45E+06	5.10E+06	8.70E+06	6.75E+06	1.82E+06	5.95E+06	6.65E+06	5.10E+06	5.90E+06	7.76E+05
9	6.83E+06	6.35E+06	6.93E+06	6.70E+06	3.07E+05	7.35E+06	6.35E+06	3.65E+06	5.78E+06	1.91E+06
10	8.95E+06	9.20E+06	9.15E+06	9.10E+06	1.32E+05	3.60E+06	6.20E+06	5.55E+06	5.12E+06	1.35E+06

Time (Days)	16 W/m^2				
	A	B	C	Average	Standard Deviation
0	2.49E+05	2.21E+05	2.30E+05	2.33E+05	1.40E+04
1	3.10E+05	3.40E+05	3.15E+05	3.22E+05	1.61E+04
2	3.45E+05	5.28E+05	6.15E+05	4.96E+05	1.38E+05
3	9.40E+05	1.02E+06	8.15E+05	9.25E+05	1.03E+05
4	1.85E+06	1.93E+06	2.29E+06	2.02E+06	2.34E+05
5	2.43E+06	2.95E+06	2.89E+06	2.75E+06	2.87E+05
6	3.73E+06	4.45E+06	5.55E+06	4.58E+06	9.19E+05
7	2.98E+06	3.91E+06	3.96E+06	3.62E+06	5.56E+05

Table B-35. pH measurements of the light intensity experiment

Time (Days)	42.45 W/m^2					23.95 W/m^2				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.25	7.25	7.23	7.24	0.01	7.08	7.5	7.22	7.27	0.21
1	7.76	7.62	7.61	7.66	0.08	8.47	8.64	8.52	8.54	0.09
2	7.42	7.4	7.41	7.41	0.01	8.99	9.12	8.97	9.03	0.08
3	8.99	9.08	8.91	8.99	0.09	9.76	9.91	9.89	9.85	0.08
4	9.71	9.7	9.77	9.73	0.04	9.96	10.03	10.06	10.02	0.05
5	9.7	9.42	9.81	9.64	0.20	10	10.1	10.17	10.09	0.09
6	9.9	10.02	10.16	10.03	0.13	10.05	10.15	10.17	10.12	0.06
7	9.97	10.02	10.17	10.05	0.10	10.1	10.2	10.2	10.17	0.06
8	9.07	9.97	10.19	9.74	0.59	10.04	10.14	10.19	10.12	0.08
9	9.1	9.2	9.76	9.35	0.36	10.09	10.2	10.06	10.12	0.07
10	8.62	8.67	8.79	8.69	0.09	9.92	9.94	9.9	9.92	0.02

Time (Days)	16 W/m^2				
	A	B	C	Average	Standard Deviation
0	7.79	8.06	8.02	7.96	0.15
1	7.46	7.71	7.66	7.61	0.13
2	7.49	7.71	7.65	7.62	0.11
3	8.93	9.31	9.32	9.19	0.22
4	9.64	9.72	9.79	9.72	0.08
5	9.81	9.91	9.93	9.88	0.06
6	9.54	9.87	9.9	9.77	0.20
7	9.95	10.12	10.06	10.04	0.09

Table B-36. Absorbance readings at 440 nm for the light intensity experiment

Time (Days)	42.45 W/m^2					23.95 W/m^2				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.056	0.055	0.061	0.057	0.003	0.047	0.045	0.048	0.047	0.002
1	0.046	0.047	0.045	0.046	0.001	0.065	0.058	0.065	0.063	0.004
2	0.057	0.063	0.060	0.060	0.003	0.097	0.094	0.098	0.096	0.002
3	0.130	0.132	0.116	0.126	0.009	0.18	0.19	0.194	0.188	0.007
4	0.211	0.212	0.201	0.208	0.006	0.25	0.235	0.278	0.254	0.022
5	0.322	0.319	0.316	0.319	0.003	0.328	0.309	0.353	0.330	0.022
6	0.396	0.408	0.395	0.400	0.007	0.391	0.369	0.408	0.389	0.020
7	0.422	0.493	0.456	0.457	0.036	0.465	0.549	0.524	0.513	0.043
8	0.443	0.513	0.523	0.493	0.044	0.486	0.497	0.529	0.504	0.022
9	0.426	0.561	0.570	0.519	0.081	0.548	0.559	0.567	0.558	0.010
10	0.423	0.545	0.559	0.509	0.075	0.542	0.568	0.619	0.576	0.039

Time (Days)	16 W/m^2				
	A	B	C	Average	Standard Deviation
0	0.051	0.052	0.049	0.051	0.002
1	0.061	0.065	0.060	0.062	0.003
2	0.083	0.086	0.087	0.085	0.002
3	0.145	0.159	0.156	0.153	0.007
4	0.238	0.234	0.249	0.240	0.008
5	0.305	0.314	0.315	0.311	0.006
6	0.400	0.443	0.433	0.425	0.023
7	0.454	0.489	0.486	0.476	0.019

Table B-37. Absorbance readings at 500 nm for the light intensity experiment

Time (Days)	42.45 W/m^2					23.95 W/m^2				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.045	0.046	0.048	0.046	0.002	0.032	0.029	0.032	0.031	0.002
1	0.033	0.035	0.03	0.033	0.003	0.047	0.04	0.046	0.044	0.004
2	0.039	0.044	0.041	0.041	0.003	0.065	0.061	0.067	0.064	0.003
3	0.091	0.089	0.077	0.086	0.008	0.121	0.125	0.128	0.125	0.004
4	0.132	0.126	0.123	0.127	0.005	0.149	0.128	0.164	0.147	0.018
5	0.192	0.191	0.192	0.192	0.001	0.188	0.169	0.205	0.187	0.018
6	0.234	0.24	0.218	0.231	0.011	0.227	0.191	0.208	0.209	0.018
7	0.261	0.313	0.281	0.285	0.026	0.269	0.258	0.311	0.279	0.028
8	0.306	0.334	0.367	0.336	0.031	0.3	0.308	0.319	0.309	0.010
9	0.314	0.465	0.46	0.413	0.086	0.371	0.369	0.355	0.365	0.009
10	0.333	0.412	0.435	0.393	0.054	0.342	0.371	0.41	0.374	0.034

Time (Days)	16 W/m^2				
	A	B	C	Average	Standard Deviation
0	0.038	0.038	0.036	0.037	0.001
1	0.044	0.048	0.044	0.045	0.002
2	0.057	0.061	0.061	0.060	0.002
3	0.095	0.105	0.099	0.100	0.005
4	0.144	0.144	0.150	0.146	0.003
5	0.179	0.182	0.176	0.179	0.003
6	0.240	0.299	0.295	0.278	0.033
7	0.253	0.273	0.266	0.264	0.010

Table B-38. Absorbance readings at 660 nm for the light intensity experiment

Time (Days)	42.45 W/m^2					23.95 W/m^2				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.032	0.033	0.034	0.033	0.001	0.022	0.019	0.022	0.021	0.002
1	0.021	0.022	0.016	0.020	0.003	0.032	0.027	0.033	0.031	0.003
2	0.023	0.027	0.025	0.025	0.002	0.046	0.044	0.049	0.046	0.003
3	0.068	0.063	0.054	0.062	0.007	0.097	0.1	0.103	0.100	0.003
4	0.107	0.099	0.096	0.101	0.006	0.116	0.095	0.13	0.114	0.018
5	0.173	0.151	0.166	0.163	0.011	0.145	0.126	0.164	0.145	0.019
6	0.202	0.205	0.187	0.198	0.010	0.183	0.149	0.164	0.165	0.017
7	0.216	0.266	0.223	0.235	0.027	0.204	0.189	0.235	0.209	0.023
8	0.26	0.268	0.282	0.270	0.011	0.224	0.233	0.229	0.229	0.005
9	0.261	0.401	0.369	0.344	0.073	0.271	0.262	0.241	0.258	0.015
10	0.276	0.345	0.337	0.319	0.038	0.23	0.258	0.282	0.257	0.026

Time (Days)	16 W/m^2				
	A	B	C	Average	Standard Deviation
0	0.024	0.024	0.022	0.023	0.001
1	0.032	0.034	0.031	0.032	0.002
2	0.044	0.047	0.047	0.046	0.002
3	0.076	0.085	0.082	0.081	0.005
4	0.126	0.125	0.131	0.127	0.003
5	0.156	0.162	0.158	0.159	0.003
6	0.215	0.272	0.256	0.248	0.029
7	0.213	0.246	0.236	0.232	0.017

Table B-39. Absorbance readings at 680 nm for the light intensity experiment

Time (Days)	42.45 W/m^2					23.95 W/m^2				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.035	0.034	0.036	0.035	0.001	0.022	0.02	0.022	0.021	0.001
1	0.021	0.022	0.017	0.020	0.003	0.034	0.029	0.034	0.032	0.003
2	0.026	0.028	0.027	0.027	0.001	0.05	0.05	0.053	0.051	0.002
3	0.076	0.074	0.064	0.071	0.006	0.115	0.121	0.123	0.120	0.004
4	0.134	0.129	0.124	0.129	0.005	0.155	0.139	0.175	0.156	0.018
5	0.217	0.206	0.213	0.212	0.006	0.2	0.185	0.224	0.203	0.020
6	0.261	0.267	0.255	0.261	0.006	0.249	0.223	0.251	0.241	0.016
7	0.273	0.332	0.293	0.299	0.030	0.285	0.279	0.323	0.296	0.024
8	0.303	0.337	0.351	0.330	0.025	0.301	0.31	0.32	0.310	0.010
9	0.299	0.445	0.42	0.388	0.078	0.343	0.345	0.326	0.338	0.010
10	0.309	0.39	0.389	0.363	0.046	0.306	0.332	0.361	0.333	0.028

Time (Days)	16 W/m^2				
	A	B	C	Average	Standard Deviation
0	0.024	0.024	0.022	0.023	0.001
1	0.034	0.036	0.033	0.034	0.002
2	0.049	0.051	0.052	0.051	0.002
3	0.091	0.101	0.099	0.097	0.005
4	0.157	0.156	0.165	0.159	0.005
5	0.203	0.208	0.208	0.206	0.003
6	0.268	0.306	0.31	0.295	0.023
7	0.303	0.33	0.323	0.319	0.014

Table B-40. Final biomass concentration (g/L) of the light intensity experiment

Parameter Varied	A	B	C	Average	Standard Deviation
42.45 W/m^2	0.2043	0.2612	0.3347	0.2667	0.0654
23.95 W/m^2	0.3385	0.3473	0.2340	0.3066	0.0631
16 W/m^2	0.2551	0.2378	0.2469	0.2466	0.0087

Table B-41. Cell densities (cells/mL) of the standard and enhanced BG11-N media

Time (Days)	BG11-N					Enhanced BG11-N				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	1.08E+05	1.24E+05	1.05E+05	1.12E+05	1.02E+04	2.38E+05	3.50E+05	4.68E+05	3.52E+05	1.15E+05
1	9.88E+04	1.18E+05	1.34E+05	1.17E+05	1.75E+04	3.15E+05	2.58E+05	2.05E+05	2.59E+05	5.50E+04
2	2.93E+05	2.61E+05	2.89E+05	2.81E+05	1.71E+04	4.38E+05	4.78E+05	7.75E+05	5.63E+05	1.84E+05
3	6.25E+05	6.73E+05	6.28E+05	6.42E+05	2.67E+04	2.01E+06	2.70E+06	1.30E+06	2.00E+06	7.00E+05
4	9.80E+05	1.06E+06	1.09E+06	1.04E+06	5.41E+04	3.48E+06	2.78E+06	3.10E+06	3.12E+06	3.50E+05
5	1.78E+06	1.68E+06	1.39E+06	1.62E+06	2.03E+05	3.00E+06	5.13E+06	4.68E+06	4.27E+06	1.12E+06
6	2.55E+06	2.81E+06	1.98E+06	2.45E+06	4.28E+05	7.05E+06	7.05E+06	8.20E+06	7.43E+06	6.64E+05
7	7.45E+06	3.38E+06	2.98E+06	4.60E+06	2.48E+06	1.10E+07	6.00E+06	9.25E+06	8.73E+06	2.52E+06
8	2.63E+06	2.53E+06	2.58E+06	2.58E+06	5.00E+04	1.36E+07	1.01E+07	1.39E+07	1.25E+07	2.11E+06
9	-	-	-	-	-	1.57E+07	1.04E+07	1.30E+07	1.30E+07	2.65E+06
10	-	-	-	-	-	1.77E+07	1.53E+07	1.60E+07	1.63E+07	1.23E+06
11	-	-	-	-	-	1.47E+07	1.15E+07	1.16E+07	1.26E+07	1.82E+06

Table B-42. pH measurements of the standard and enhanced BG11-N media

Time (Days)	BG11-N					Enhanced BG11-N				
	A	**B**	**C**	**Average**	**Standard Deviation**	**A**	**B**	**C**	**Average**	**Standard Deviation**
0	7.25	7.3	7.18	7.24	0.06	7.70	7.62	7.66	7.66	0.04
1	7.12	7.21	7.12	7.15	0.05	7.78	7.64	7.71	7.71	0.07
2	7.12	7.3	7.21	7.21	0.09	7.93	7.83	7.88	7.88	0.05
3	7.46	7.82	7.9	7.73	0.23	9.15	9.43	9.02	9.20	0.21
4	9.47	9.85	9.96	9.76	0.26	9.89	9.88	9.91	9.89	0.02
5	9.64	10.01	10	9.88	0.21	9.93	9.99	10.06	9.99	0.07
6	9.51	9.94	10.06	9.84	0.29	9.89	9.59	9.66	9.71	0.16
7	9.56	9.86	9.95	9.79	0.20	10.03	9.79	10.06	9.96	0.15
8	7.97	8.59	9.83	8.80	0.95	10.06	9.98	10.17	10.07	0.10
9	-	-	-	-	-	10.30	10.15	10.36	10.27	0.11
10	-	-	-	-	-	10.43	10.23	10.42	10.36	0.11
11	-	-	-	-	-	10.31	10.32	10.42	10.35	0.06

Table B-43. Absorbance readings at 440 nm for the standard and enhanced BG11-N media

Time (Days)	BG11-N					Enhanced BG11-N				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.043	0.038	0.043	0.041	0.003	0.017	0.016	0.045	0.026	0.016
1	0.048	0.046	0.044	0.046	0.002	0.044	0.044	0.050	0.046	0.003
2	0.065	0.061	0.060	0.062	0.003	0.063	0.067	0.083	0.071	0.011
3	0.115	0.111	0.104	0.110	0.006	0.157	0.162	0.167	0.162	0.005
4	0.217	0.200	0.188	0.202	0.015	0.275	0.254	0.290	0.273	0.018
5	0.374	0.342	0.283	0.333	0.046	0.366	0.349	0.430	0.382	0.043
6	0.503	0.451	0.357	0.437	0.074	0.538	0.449	0.605	0.531	0.078
7	0.563	0.516	0.466	0.515	0.049	0.633	0.511	0.742	0.629	0.116
8	0.479	0.494	0.490	0.488	0.008	0.760	0.634	0.908	0.767	0.137
9	-	-	-	-	-	0.858	0.763	1.041	0.887	0.141
10	-	-	-	-	-	0.972	0.874	1.199	1.015	0.167
11	-	-	-	-	-	1.147	0.999	1.329	1.158	0.165

Table B-44. Absorbance readings at 500 nm for the standard and enhanced BG11-N media

Time (Days)	BG11-N					Enhanced BG11-N				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.030	0.028	0.027	0.028	0.002	0.012	0.012	0.032	0.019	0.012
1	0.035	0.033	0.031	0.033	0.002	0.031	0.030	0.035	0.032	0.003
2	0.046	0.042	0.042	0.043	0.002	0.042	0.046	0.062	0.050	0.011
3	0.077	0.074	0.069	0.073	0.004	0.110	0.117	0.118	0.115	0.004
4	0.130	0.116	0.113	0.120	0.009	0.179	0.162	0.178	0.173	0.010
5	0.211	0.189	0.157	0.186	0.027	0.201	0.201	0.244	0.215	0.025
6	0.277	0.243	0.190	0.237	0.044	0.339	0.286	0.374	0.333	0.044
7	0.411	0.325	0.262	0.333	0.075	0.418	0.315	0.459	0.397	0.074
8	0.297	0.315	0.280	0.297	0.018	0.512	0.411	0.576	0.500	0.083
9	-	-	-	-	-	0.548	0.503	0.651	0.567	0.076
10	-	-	-	-	-	0.616	0.575	0.763	0.651	0.099
11	-	-	-	-	-	0.773	0.726	0.810	0.770	0.042

Table B-45. Absorbance readings at 660 nm for the standard and enhanced BG11-N media

Time (Days)	BG11-N					Enhanced BG11-N				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.018	0.016	0.015	0.016	0.002	0.007	0.006	0.023	0.012	0.010
1	0.020	0.021	0.018	0.020	0.002	0.020	0.019	0.022	0.020	0.002
2	0.029	0.028	0.027	0.028	0.001	0.028	0.031	0.044	0.034	0.009
3	0.056	0.055	0.050	0.054	0.003	0.083	0.088	0.094	0.088	0.006
4	0.098	0.089	0.085	0.091	0.007	0.138	0.126	0.135	0.133	0.006
5	0.173	0.160	0.129	0.154	0.023	0.156	0.170	0.194	0.173	0.019
6	0.226	0.204	0.152	0.194	0.038	0.276	0.235	0.311	0.274	0.038
7	0.336	0.255	0.212	0.268	0.063	0.367	0.267	0.399	0.344	0.069
8	0.225	0.267	0.197	0.230	0.035	0.439	0.339	0.485	0.421	0.075
9	-	-	-	-	-	0.460	0.416	0.546	0.474	0.066
10	-	-	-	-	-	0.517	0.479	0.659	0.552	0.095
11	-	-	-	-	-	0.670	0.637	0.679	0.662	0.022

Table B-46. Absorbance readings at 680 nm for the standard and enhanced BG11-N media

Time (Days)	BG11-N					Enhanced BG11-N				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.018	0.017	0.015	0.017	0.002	0.007	0.006	0.026	0.013	0.011
1	0.020	0.021	0.018	0.020	0.002	0.024	0.023	0.025	0.024	0.001
2	0.032	0.030	0.029	0.030	0.002	0.033	0.036	0.048	0.039	0.008
3	0.064	0.063	0.057	0.061	0.004	0.099	0.104	0.108	0.104	0.005
4	0.125	0.118	0.111	0.118	0.007	0.178	0.162	0.179	0.173	0.010
5	0.229	0.212	0.172	0.204	0.029	0.224	0.222	0.266	0.237	0.025
6	0.302	0.279	0.217	0.266	0.044	0.273	0.285	0.266	0.275	0.010
7	0.389	0.324	0.291	0.335	0.050	0.436	0.332	0.499	0.422	0.084
8	0.280	0.302	0.279	0.287	0.013	0.523	0.419	0.620	0.521	0.101
9	-	-	-	-	-	0.584	0.513	0.710	0.602	0.100
10	-	-	-	-	-	0.665	0.598	0.843	0.702	0.127
11	-	-	-	-	-	0.832	0.742	0.913	0.829	0.086

Table B-47. Final biomass concentrations (g/L) of the standard and enhanced BG11-N media

Parameter Varied	A	B	C	Average	Standard Deviation
BG11-N	0.2205	0.2214	0.2094	0.2171	0.0067
Enhanced BG11-N	0.8283	0.9689	0.6431	0.8134	0.1634

Table B-48. Chlorophyll a concentration (μg/mL) of both the standard and enhanced BG11-N media

Time (Days)	BG11-N					Enhanced BG11-N				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.029	0.034	0.034	0.032	0.002	0.066	0.074	0.066	0.069	0.004
1	0.056	0.050	0.050	0.052	0.004	0.059	0.048	0.066	0.058	0.009
2	0.116	0.116	0.116	0.116	0.000	0.042	0.034	0.050	0.042	0.008
3	0.387	0.411	0.337	0.379	0.038	0.050	0.050	0.050	0.050	0.000
4	1.064	0.939	0.889	0.964	0.090	0.066	0.064	0.074	0.068	0.005
5	2.109	1.795	1.474	1.793	0.317	0.140	0.158	0.165	0.154	0.013
6	2.570	2.362	2.085	2.339	0.243	0.443	0.413	0.403	0.420	0.021
7	2.678	2.414	2.646	2.579	0.144	1.022	1.121	0.899	1.014	0.111
8	2.432	2.324	2.588	2.448	0.133	1.623	1.901	1.402	1.642	0.250
9	-	-	-	-	-	2.744	3.228	2.183	2.718	0.523
10	-	-	-	-	-	3.179	3.846	2.544	3.190	0.651
11	-	-	-	-	-	3.912	4.686	2.973	3.857	0.857
12	-	-	-	-	-	4.920	5.743	3.321	4.661	1.232
13	-	-	-	-	-	5.850	6.446	4.161	5.486	1.186
14	-	-	-	-	-	6.085	7.784	4.371	6.080	1.707
15	-	-	-	-	-	7.828	9.230	4.679	7.246	2.331
17	-	-	-	-	-	8.875	10.654	5.810	8.446	2.450
19	-	-	-	-	-	9.812	12.142	7.252	9.736	2.446
21	-	-	-	-	-	11.161	12.932	6.764	10.286	3.176
23	-	-	-	-	-	12.894	12.341	8.810	11.348	2.216
25	-	-	-	-	-	12.850	12.715	10.094	11.886	1.554
27	-	-	-	-	-	13.379	12.159	11.114	12.217	1.134
29	-	-	-	-	-	13.197	12.340	11.270	12.269	0.965

Table B-49. Percent nitrogen content of the dried biomass of standard and enhanced BG11-N media

Parameter Varied	A	B	C	Average	Standard Deviation
BG11-N	7.57	6.62	6.65	6.95	0.54
Enhanced BG11-N	9.09	8.31	10.38	9.26	1.05

APPENDIX C

BIOFERTILIZATION STUDIES EXPERIMENTAL STUDIES

Table C-1. Dried plant weight (g) of the wheat grown under the four fertilizer treatments

Wheat	Control			Ammonia Nitrate Fertilizer			Hoagland's Liquid Fertilizer			Cyanobacterial Biofertilizer		
	All	Root	Stalk	All	Root	Stalk	All	Root	Stalk	All	Root	Stalk
A	0.729	0.362	0.367	0.955	0.476	0.479	0.933	0.397	0.536	2.415	1.006	1.409
B	0.642	0.319	0.323	1.47	0.82	0.65	0.74	0.363	0.377	2.169	1.016	1.153
C	0.705	0.404	0.301	1.16	0.548	0.612	1.05	0.511	0.539	3.083	1.673	1.41
Average	0.692	0.362	0.330	1.195	0.615	0.580	0.908	0.424	0.484	2.556	1.232	1.324
Standard Deviation	0.045	0.043	0.034	0.259	0.181	0.090	0.157	0.078	0.093	0.473	0.382	0.148

Table C-2. Dried plant weight (g) of the camelina grown under the four fertilizer treatments

Camelina	Control			Ammonia Nitrate Fertilizer			Hoagland's Liquid Fertilizer			Cyanobacterial Biofertilizer		
	All	Root	Stalk	All	Root	Stalk	All	Root	Stalk	All	Root	Stalk
A	0.347	0.149	0.198	1.562	0.962	0.6	2.272	1.512	0.76	1.697	0.896	0.801
B	0.364	0.127	0.237	1.992	1.334	0.658	3.181	2.069	1.112	1.135	0.449	0.686
C	0.427	0.188	0.239	1.483	1.064	0.419	1.093	0.65	0.443	0.914	0.357	0.57
Average	0.379	0.155	0.225	1.679	1.120	0.559	2.182	1.410	0.772	1.249	0.567	0.686
Standard Deviation	0.042	0.031	0.023	0.274	0.192	0.125	1.047	0.715	0.335	0.404	0.288	0.116

Table C-3. Dried plant weight (g) of carrots and tomatoes grown under three fertilizer treatments

Plant Type	Carrots			Tomatoes		
Fertilizer Treatment	**Control**	**Hoagland's**	**Cyanobacterial Biofertilizer**	**Control**	**Hoagland's**	**Cyanobacterial Biofertilizer**
A	2.073	7.484	9.008	3.02	12.7	12.71
B	2.8	10.059	9.037	2.65	14.53	12.73
C	2.858	8.676	9.708	4.41	15.61	14.44
Average	2.577	8.740	9.251	3.360	14.280	13.293
Standard Deviation	0.437	1.289	0.396	0.928	1.471	0.993

Table C-4. Dried plant weight (g) of the first and second clipping of the Kentucky Bluegrass grown under three fertilizer treatments

Plant Type	Kentucky Bluegrass (1st clipping)			Kentucky Bluegrass (2nd clipping)		
Fertilizer Treatment	**Control**	**Hoagland's**	**Cyanobacterial Biofertilizer**	**Control**	**Hoagland's**	**Cyanobacterial Biofertilizer**
A	1.04	5.64	4.9	1.018	2.341	2.346
B	1.2	5.25	5.16	0.951	2.582	2.775
C	1.08	4.67	4.97	0.929	2.629	3.159
Average	1.107	5.187	5.010	0.966	2.517	2.760
Standard Deviation	0.083	0.488	0.135	0.046	0.155	0.407

APPENDIX D

NUTRIENT VARIATIONS IN FLASK EXPERIMENTAL DATA

Nutrient variation experiments were carried out in 250 mL baffled shaker flasks. The cultures were continuously shaken at 120 rpm. The flasks were illuminated with 21 W/m^2 of fluorescent light at a 14/10 light/dark cycle. All cultures were grown in the standard BG11-N medium with the notated changes in the nutrient concentrations for that given modification. All sample measurements were taken following the procedure outlined in the methods section.

Table D-1. Cell densities (cells/mL) of four initial phosphate concentrations

Time (Days)	0.022 mM					0.055 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.50E+05	6.80E+05	1.05E+06	8.27E+05	1.97E+05	8.23E+05	6.23E+05	8.95E+05	7.80E+05	1.41E+05
1	6.53E+05	8.05E+05	8.78E+05	7.78E+05	1.15E+05	8.75E+05	5.40E+05	4.83E+05	6.33E+05	2.12E+05
2	1.98E+06	1.89E+06	2.67E+06	2.18E+06	4.27E+05	2.33E+06	1.34E+06	1.51E+06	1.73E+06	5.29E+05
3	3.72E+06	4.98E+06	4.84E+06	4.51E+06	6.91E+05	4.14E+06	4.22E+06	4.74E+06	4.37E+06	3.26E+05
5	7.44E+06	1.33E+07	1.08E+07	1.05E+07	2.95E+06	8.44E+06	1.04E+07	9.64E+06	9.51E+06	1.01E+06
6	7.04E+06	1.02E+07	9.72E+06	8.97E+06	1.69E+06	1.04E+07	7.60E+06	1.07E+07	9.59E+06	1.73E+06
7	-	-	-	-	-	9.20E+06	9.76E+06	1.03E+07	9.75E+06	5.40E+05

Time (Days)	0.22 mM					0.44 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	9.93E+05	6.88E+05	8.95E+05	8.58E+05	1.56E+05	9.35E+05	5.60E+05	4.08E+05	6.34E+05	2.71E+05
1	5.88E+05	7.88E+05	7.30E+05	7.02E+05	1.03E+05	6.28E+05	8.08E+05	9.30E+05	7.88E+05	1.52E+05
2	1.32E+06	1.81E+06	1.26E+06	1.46E+06	3.02E+05	1.31E+06	1.47E+06	1.75E+06	1.51E+06	2.23E+05
3	4.30E+06	4.00E+06	5.30E+06	4.53E+06	6.81E+05	3.60E+06	3.90E+06	3.38E+06	3.63E+06	2.61E+05
5	1.48E+07	1.02E+07	7.00E+06	1.07E+07	3.92E+06	1.97E+07	1.77E+07	1.00E+07	1.58E+07	5.09E+06
6	9.16E+06	8.80E+06	8.60E+06	8.85E+06	2.84E+05	9.68E+06	1.09E+07	1.26E+07	1.11E+07	1.47E+06
7	8.96E+06	9.08E+06	1.13E+07	9.79E+06	1.33E+06	1.83E+07	1.26E+07	1.71E+07	1.60E+07	2.99E+06
8	1.23E+07	8.04E+06	1.28E+07	1.10E+07	2.61E+06	1.32E+07	1.03E+07	1.56E+07	1.30E+07	2.68E+06
9	1.09E+07	1.22E+07	7.08E+06	1.01E+07	2.65E+06	1.33E+07	1.24E+07	1.23E+07	1.26E+07	5.56E+05

Table D-2. pH measurements of four initial phosphate concentrations

Time (Days)	0.022 mM					0.055 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	6.98	6.71	6.73	6.81	0.15	7.66	7.25	7.38	7.43	0.21
1	8.22	7.66	7.7	7.86	0.31	8.69	8.41	8.6	8.57	0.14
2	8.49	8.51	8.54	8.51	0.03	9.26	8.77	9.19	9.07	0.27
3	9.6	9.72	9.7	9.67	0.06	9.8	9.77	9.83	9.80	0.03
5	9.42	9.58	9.59	9.53	0.10	9.91	9.91	9.89	9.90	0.01
6	9.42	9.51	9.49	9.47	0.05	9.84	9.83	9.84	9.84	0.01
7	-	-	-	-	-	8.62	8.56	8.7	8.63	0.07

Time (Days)	0.22 mM					0.44 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.91	7.87	7.86	7.88	0.03	7.92	7.82	7.84	7.86	0.05
1	8.74	8.9	8.95	8.86	0.11	8.67	8.6	8.62	8.63	0.04
2	9.26	9.41	9.23	9.30	0.10	9.16	9.07	9.18	9.14	0.05
3	9.64	9.76	9.71	9.70	0.06	9.65	9.71	9.72	9.69	0.04
5	9.75	9.77	9.81	9.78	0.03	9.84	9.94	9.95	9.91	0.06
6	9.72	9.81	9.83	9.79	0.06	9.97	10.04	10.01	10.01	0.04
7	9.27	9.41	9.29	9.32	0.08	10.03	10.08	10.03	10.05	0.03
8	7.74	7.73	7.69	7.72	0.03	9.19	8.48	9.88	9.18	0.70
9	7.62	7.78	7.69	7.70	0.08	7.31	7.34	7.33	7.33	0.02

Table D-3. Absorbance readings at 500 nm for four initial phosphate concentrations

Time (Days)	0.022 mM					0.055 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.023	0.023	0.022	0.023	0.001	0.025	0.023	0.022	0.023	0.002
1	0.04	0.036	0.042	0.039	0.003	0.036	0.038	0.04	0.038	0.002
2	0.067	0.072	0.072	0.070	0.003	0.058	0.058	0.07	0.062	0.007
3	0.095	0.127	0.14	0.121	0.023	0.121	0.117	0.123	0.120	0.003
5	0.276	0.351	0.348	0.325	0.042	0.272	0.29	0.323	0.295	0.026
6	0.26	0.329	0.342	0.310	0.044	0.319	0.359	0.33	0.336	0.021
7	-	-	-	-	-	0.35	0.405	0.329	0.361	0.039

Time (Days)	0.22 mM					0.44 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.035	0.032	0.038	0.035	0.003	0.022	0.023	0.023	0.023	0.001
1	0.043	0.044	0.041	0.043	0.002	0.039	0.037	0.04	0.039	0.002
2	0.064	0.075	0.076	0.072	0.007	0.07	0.069	0.065	0.068	0.003
3	0.129	0.148	0.141	0.139	0.010	0.143	0.155	0.126	0.141	0.015
5	0.327	0.305	0.307	0.313	0.012	0.35	0.438	0.398	0.395	0.044
6	0.336	0.336	0.334	0.335	0.001	0.408	0.407	0.45	0.422	0.025
7	0.401	0.362	0.379	0.381	0.020	0.487	0.512	0.519	0.506	0.017
8	0.403	0.384	0.403	0.397	0.011	0.579	0.58	0.573	0.577	0.004
9	0.396	0.374	0.399	0.390	0.014	0.551	0.533	0.64	0.575	0.057

Table D-4. Final biomass concentration (g/L) of the four initial phosphate concentrations

	A	B	C	Average	Standard Deviation
0.022 mM	0.1950	0.1709	0.2026	0.1895	0.0166
0.055 mM	0.1973	0.2408	0.2627	0.2336	0.033
0.22 mM	0.3029	0.1972	0.31124	0.2705	0.0636
0.44 mM	0.3045	0.3817	0.3484	0.3449	0.0387

Table D-5. Cell densities (cells/mL) of four initial nitrate concentrations

Time (Days)	0 mM					6.33 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	4.55E+05	2.78E+05	4.25E+05	3.86E+05	9.50E+04	3.60E+05	3.43E+05	3.63E+05	3.55E+05	1.09E+04
1	1.17E+06	6.30E+05	7.00E+05	8.33E+05	2.94E+05	7.55E+05	8.40E+05	8.40E+05	8.12E+05	4.91E+04
2	1.04E+06	1.11E+06	1.10E+06	1.08E+06	3.79E+04	8.40E+05	1.07E+06	1.01E+06	9.73E+05	1.19E+05
3	2.14E+06	3.64E+06	2.04E+06	2.61E+06	8.96E+05	3.44E+06	2.70E+06	2.30E+06	2.81E+06	5.78E+05
4	5.92E+06	7.12E+06	7.60E+06	6.88E+06	8.65E+05	8.08E+06	6.72E+06	4.80E+06	6.53E+06	1.65E+06
5	1.26E+07	8.88E+06	8.24E+06	9.89E+06	2.33E+06	9.68E+06	1.08E+07	8.08E+06	9.52E+06	1.37E+06
6	9.76E+06	2.10E+07	7.12E+06	1.26E+07	7.35E+06	1.55E+07	1.71E+07	1.06E+07	1.44E+07	3.42E+06
7	7.76E+06	1.01E+07	1.81E+07	1.20E+07	5.41E+06	1.28E+07	1.30E+07	1.02E+07	1.20E+07	1.57E+06
8	1.46E+07	9.84E+06	1.19E+07	1.21E+07	2.37E+06	5.12E+06	5.20E+06	1.70E+07	9.12E+06	6.86E+06
Time (Days)	12.2 mM					24.1 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	3.85E+05	2.30E+05	2.18E+05	2.78E+05	9.33E+04	2.00E+05	4.95E+05	3.95E+05	3.63E+05	1.50E+05
1	4.65E+05	1.35E+06	3.15E+05	7.10E+05	5.59E+05	9.00E+05	4.90E+05	6.80E+05	6.90E+05	2.05E+05
2	1.18E+06	1.22E+06	2.35E+06	1.58E+06	6.64E+05	1.57E+06	9.50E+05	1.14E+06	1.22E+06	3.18E+05
3	4.52E+06	2.22E+06	2.04E+06	2.93E+06	1.38E+06	3.88E+06	2.22E+06	2.52E+06	2.87E+06	8.85E+05
4	5.20E+06	5.92E+06	3.80E+06	4.97E+06	1.08E+06	5.04E+06	3.76E+06	6.80E+06	5.20E+06	1.53E+06
5	8.08E+06	5.56E+06	6.32E+06	6.65E+06	1.29E+06	6.00E+06	5.92E+06	5.92E+06	5.95E+06	4.62E+04
6	9.28E+06	8.40E+06	7.28E+06	8.32E+06	1.00E+06	6.32E+06	1.04E+07	3.52E+06	6.75E+06	3.46E+06
7	1.30E+07	1.30E+07	8.48E+06	1.15E+07	2.59E+06	7.36E+06	8.72E+06	7.68E+06	7.92E+06	7.11E+05
8	9.12E+06	6.52E+06	6.40E+06	7.35E+06	1.54E+06	9.32E+06	7.40E+06	6.08E+06	7.60E+06	1.63E+06

Table D-6. pH measurements of four initial nitrate concentrations

Time (Days)	0 mM					6.33 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.32	7.60	7.49	7.47	0.14	7.68	7.57	7.13	7.46	0.29
1	6.78	6.94	6.85	6.86	0.08	7.32	7.05	7.18	7.18	0.14
2	7.15	7.39	7.29	7.28	0.12	8.09	7.78	7.93	7.93	0.16
3	8.70	8.62	8.78	8.70	0.08	9.82	9.63	9.64	9.70	0.11
4	9.48	9.50	9.6	9.53	0.06	10.24	10.29	10.29	10.27	0.03
5	9.67	9.65	9.74	9.69	0.05	10.28	10.32	10.33	10.31	0.03
6	9.71	9.66	9.79	9.72	0.07	8.45	8.73	8.81	8.66	0.19
7	9.09	9.24	9.20	9.18	0.08	7.52	7.59	7.53	7.55	0.04

Time (Days)	12.2 mM					24.1 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.78	7.69	7.73	7.73	0.05	7.90	7.78	7.83	7.84	0.06
1	7.53	7.43	7.49	7.48	0.05	7.66	7.59	7.62	7.62	0.04
2	8.37	8.27	8.27	8.30	0.06	8.80	8.60	8.63	8.68	0.11
3	9.78	9.70	9.60	9.69	0.09	9.85	9.87	9.88	9.87	0.02
4	10.29	10.29	10.22	10.27	0.04	10.31	10.37	10.38	10.35	0.04
5	10.12	10.2	10.31	10.21	0.10	9.74	9.74	9.74	9.74	0.00
6	8.96	9.02	8.96	8.98	0.04	9.17	9.51	9.40	9.36	0.17
7	7.94	7.65	7.80	7.80	0.15	8.08	7.89	8.01	7.99	0.10

Table D-7. Absorbance readings at 500 nm of four different initial nitrogen concentrations

Time (Days)	0 mM					6.3 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.022	0.020	0.021	0.021	0.001	0.015	0.015	0.012	0.014	0.002
1	0.027	0.027	0.027	0.027	0.000	0.027	0.028	0.026	0.027	0.001
2	0.054	0.048	0.049	0.050	0.003	0.052	0.042	0.049	0.048	0.005
3	0.073	0.080	0.076	0.076	0.004	0.125	0.090	0.086	0.100	0.021
4	0.136	0.147	0.148	0.144	0.007	0.172	0.163	0.171	0.169	0.005
5	0.262	0.253	0.232	0.249	0.015	0.361	0.298	0.304	0.321	0.035
6	0.328	0.302	0.321	0.317	0.013	0.382	0.360	0.379	0.374	0.012
7	0.396	0.368	0.378	0.381	0.014	0.380	0.393	0.403	0.392	0.012

Time (Days)	12.2 mM					24.1 mM				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.014	0.014	0.011	0.013	0.002	0.014	0.019	0.011	0.015	0.004
1	0.031	0.025	0.024	0.027	0.004	0.036	0.025	0.025	0.029	0.006
2	0.053	0.042	0.044	0.046	0.006	0.067	0.048	0.044	0.053	0.012
3	0.100	0.104	0.079	0.094	0.013	0.120	0.097	0.099	0.105	0.013
4	0.171	0.154	0.148	0.158	0.012	0.194	0.156	0.181	0.177	0.019
5	0.272	0.239	0.248	0.253	0.017	0.250	0.226	0.251	0.242	0.014
6	0.340	0.362	0.371	0.358	0.016	0.338	0.279	0.295	0.304	0.031
7	0.335	0.391	0.392	0.373	0.033	0.330	0.299	0.361	0.330	0.031

Table D-8. Final biomass concentrations (g/L) of four different initial nitrate concentrations

	A	B	C	Average	Standard Deviation
0 mM	0.3491	0.3259	0.3133	0.3294	0.0182
6.3 mM	0.2991	0.3414	0.3351	0.3252	0.0228
12.2 mM	0.3509	0.3227	0.3807	0.3477	0.0346
24.1 mM	0.3632	0.3267	0.3362	0.3420	0.0189

Table D-9. Cell densities (cells/mL) of four combinations of the macro nutrients and trace metals

Time (Days)	1 x Macro : 1 x Trace Metals					1 x Macro : 5 x Trace Metals				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	8.95E+05	8.80E+05	5.98E+05	7.91E+05	1.68E+05	8.15E+05	7.23E+05	6.20E+05	7.19E+05	9.75E+04
2	1.14E+06	1.22E+06	1.06E+06	1.14E+06	8.01E+04	1.36E+06	8.50E+05	1.18E+06	1.13E+06	2.56E+05
3	1.67E+06	1.44E+06	1.41E+06	1.50E+06	1.42E+05	1.51E+06	1.43E+06	1.67E+06	1.54E+06	1.22E+05
4	2.98E+06	3.35E+06	2.30E+06	2.88E+06	5.33E+05	2.79E+06	2.79E+06	2.66E+06	2.75E+06	7.51E+04
5	4.14E+06	5.32E+06	4.02E+06	4.49E+06	7.18E+05	5.92E+06	5.86E+06	4.82E+06	5.53E+06	6.18E+05
6	6.92E+06	7.76E+06	8.28E+06	7.65E+06	6.86E+05	7.92E+06	7.40E+06	7.48E+06	7.60E+06	2.80E+05
7	2.28E+07	1.14E+07	9.70E+06	1.46E+07	7.13E+06	1.59E+07	1.90E+07	2.25E+07	1.91E+07	3.30E+06
8	2.81E+07	1.91E+07	2.01E+07	2.24E+07	4.93E+06	1.70E+07	1.07E+07	1.02E+07	1.26E+07	3.79E+06
9	1.74E+07	1.61E+07	1.76E+07	1.70E+07	8.14E+05	1.88E+07	1.31E+07	1.95E+07	1.71E+07	3.51E+06
Time (Days)	**5 x Macro : 1 x Trace Metals**					**5 x Macros : 5 x Trace Metals**				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	6.60E+05	6.90E+05	7.28E+05	6.93E+05	3.38E+04	8.30E+05	8.53E+05	8.95E+05	8.59E+05	3.30E+04
2	1.58E+06	1.26E+06	1.26E+06	1.37E+06	1.86E+05	1.02E+06	1.24E+06	8.60E+05	1.04E+06	1.88E+05
3	1.96E+06	1.78E+06	1.49E+06	1.74E+06	2.37E+05	2.05E+06	1.81E+06	1.67E+06	1.84E+06	1.89E+05
4	2.92E+06	2.18E+06	3.81E+06	2.97E+06	8.16E+05	3.92E+06	3.01E+06	3.55E+06	3.49E+06	4.58E+05
5	5.92E+06	7.04E+06	7.92E+06	6.96E+06	1.00E+06	6.36E+06	5.28E+06	7.96E+06	6.53E+06	1.35E+06
6	1.66E+07	9.60E+06	1.26E+07	1.29E+07	3.53E+06	8.16E+06	1.46E+07	1.10E+07	1.12E+07	3.21E+06
7	2.32E+07	1.62E+07	2.44E+07	2.13E+07	4.43E+06	1.79E+07	1.21E+07	9.50E+06	1.32E+07	4.30E+06
8	2.77E+07	1.84E+07	1.91E+07	2.17E+07	5.18E+06	1.47E+07	2.91E+07	2.19E+07	2.19E+07	7.20E+06
9	3.56E+07	2.14E+07	4.04E+07	3.25E+07	9.88E+06	3.90E+07	3.10E+07	3.58E+07	3.53E+07	4.03E+06
10	3.54E+07	3.78E+07	3.14E+07	3.49E+07	3.23E+06	2.36E+07	2.38E+07	1.94E+07	2.23E+07	2.48E+06
11	3.00E+07	3.10E+07	2.36E+07	2.82E+07	4.01E+06	-	-	-	-	-

Table D-10. pH measurements of four combinations of the macro nutrients and trace metals

Time (Days)	1 x Macro : 1 x Trace Metals					1 x Macro : 5 x Trace Metals				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.51	7.44	7.50	7.48	0.04	7.95	7.86	7.91	7.91	0.05
2	7.76	7.79	7.93	7.83	0.09	8.03	7.93	8.03	8.00	0.06
3	8.07	8.00	7.89	7.99	0.09	8.22	8.12	8.16	8.17	0.05
4	8.65	8.86	9.04	8.85	0.20	9.15	9.21	9.22	9.19	0.034
5	9.37	9.68	9.70	9.58	0.19	9.4	9.43	9.44	9.42	0.02
6	9.45	9.71	9.73	9.63	0.16	9.3	9.25	9.29	9.28	0.03
7	9.51	9.69	9.77	9.66	0.13	9.33	9.25	9.32	9.30	0.04
8	9.80	9.92	9.92	9.88	0.07	9.39	9.39	9.39	9.39	0.00
9	9.93	10.06	10.06	10.02	0.08	9.62	9.63	9.69	9.65	0.04
Time (Days)	**5 x Macro : 1 x Trace Metals**					**5 x Macros : 5 x Trace Metals**				
	A	**B**	**C**	**Average**	**Standard Deviation**	**A**	**B**	**C**	**Average**	**Standard Deviation**
0	8.68	8.55	8.67	8.63	0.07	8.75	8.75	8.74	8.75	0.02
2	8.48	8.34	8.46	8.43	0.08	8.62	8.56	8.55	8.58	0.04
3	8.86	8.73	8.90	8.83	0.09	9.00	9.09	9.00	9.03	0.05
4	10.06	10.21	10.27	10.18	0.11	10.2	10.33	10.32	10.28	0.07
5	10.36	10.54	10.53	10.48	0.10	10.35	10.50	10.46	10.44	0.08
6	10.46	10.45	10.56	10.49	0.06	10.46	10.50	10.53	10.50	0.04
7	10.46	10.51	10.54	10.50	0.04	10.40	10.49	10.54	10.48	0.07
8	10.62	10.62	10.66	10.63	0.02	10.56	10.62	10.63	10.60	0.04
9	10.73	10.77	10.75	10.75	0.02	10.68	10.73	10.70	10.70	0.03
10	10.66	10.7	10.71	10.69	0.03	10.56	10.61	10.56	10.58	0.03
11	10.72	10.76	10.74	10.74	0.02	-	-	-	-	-

Table D-11. Absorbance readings at 500 nm of four combinations of the macro nutrients and trace metals

Time (Days)	1 x Macro : 1 x Trace Metals					1 x Macro : 5 x Trace Metals				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.034	0.033	0.034	0.034	0.001	0.035	0.026	0.024	0.028	0.006
2	0.048	0.043	0.044	0.045	0.003	0.047	0.044	0.045	0.045	0.002
3	0.063	0.053	0.064	0.060	0.006	0.075	0.071	0.072	0.073	0.002
4	0.116	0.116	0.115	0.116	0.001	0.121	0.127	0.135	0.128	0.007
5	0.190	0.185	0.151	0.175	0.021	0.183	0.230	0.158	0.190	0.037
6	0.248	0.277	0.275	0.267	0.016	0.277	0.248	0.264	0.263	0.015
7	0.538	0.574	0.515	0.542	0.030	0.498	0.486	0.525	0.503	0.020
8	0.616	0.711	0.609	0.645	0.057	0.621	0.611	0.608	0.613	0.007
9	0.783	0.835	0.771	0.796	0.034	0.725	0.737	0.706	0.723	0.016
Time (Days)	**5 x Macro : 1 x Trace Metals**					**5 x Macros : 5 x Trace Metals**				
	A	**B**	**C**	**Average**	**Standard Deviation**	**A**	**B**	**C**	**Average**	**Standard Deviation**
0	0.029	0.040	0.021	0.030	0.010	0.063	0.034	0.061	0.053	0.016
2	0.024	0.046	0.026	0.032	0.012	0.076	0.067	0.067	0.070	0.005
3	0.068	0.073	0.076	0.072	0.004	0.147	0.114	0.096	0.119	0.026
4	0.241	0.271	0.257	0.256	0.015	0.282	0.270	0.260	0.271	0.011
5	0.343	0.446	0.381	0.390	0.052	0.379	0.316	0.342	0.346	0.032
6	0.500	0.524	0.524	0.516	0.014	0.532	0.530	0.534	0.532	0.002
7	0.874	0.980	0.943	0.932	0.054	0.869	0.957	0.930	0.919	0.045
8	1.074	1.078	1.102	1.085	0.015	1.061	1.109	1.042	1.071	0.035
9	1.200	1.239	1.178	1.206	0.031	1.220	1.191	1.161	1.191	0.030
10	1.398	1.358	1.390	1.382	0.021	1.442	1.421	1.376	1.413	0.034
11	1.571	1.671	1.570	1.604	0.058	-	-	-	-	-

Table D-12. Final biomass concentrations (g/L) of four combinations of the macronutrients and trace metals

	A	B	C	Average	Standard Deviation
1 x Macro : 1 x Trace Metals	0.2615	0.3303	0.2500	0.2806	0.0434
1 x Macro : 5 x Trace Metals	0.3043	0.2768	0.3304	0.3038	0.0268
5 x Macro : 1 x Trace Metals	0.8276	0.9119	0.7263	0.8219	0.0929
5 x Macros : 5 x Trace Metals	0.7417	0.7177	0.6934	0.7176	0.0241

Table D-13. Cell densities (cells/mL) of two sets of increased macronutrients

Time (Days)	**5 x $MgSO_4 \cdot 7H_2O$, Citric Acid$\cdot H_2O$, $Na_2EDTA \cdot 2H_2O$**					**5 x K_2HPO_4, $CaCl_2 \cdot 2H_2O$, Ammonia Ferric Citrate, Na_2CO_3**				
	A	**B**	**C**	**Average**	**Standard Deviation**	**A**	**B**	**C**	**Average**	**Standard Deviation**
0	2.54E+05	3.29E+05	1.86E+05	2.56E+05	7.13E+04	4.21E+05	4.05E+05	3.98E+05	4.08E+05	1.21E+04
1	5.10E+05	6.40E+05	4.45E+05	5.32E+05	9.93E+04	5.83E+05	4.25E+05	7.33E+05	5.80E+05	1.54E+05
2	1.50E+06	1.32E+06	1.40E+06	1.40E+06	9.02E+04	1.15E+06	1.64E+06	1.31E+06	1.36E+06	2.49E+05
4	3.60E+06	3.86E+06	4.82E+06	4.09E+06	6.43E+05	3.26E+06	1.65E+06	1.08E+06	2.00E+06	1.13E+06
5	4.92E+06	7.16E+06	5.16E+06	5.75E+06	1.23E+06	3.80E+06	2.04E+06	3.48E+06	3.11E+06	9.38E+05
6	9.15E+06	1.15E+07	9.00E+06	9.87E+06	1.37E+06	3.66E+06	2.54E+06	3.32E+06	3.17E+06	5.74E+05
7	1.06E+07	9.30E+06	7.30E+06	9.07E+06	1.66E+06	3.60E+06	2.72E+06	3.66E+06	3.33E+06	5.26E+05

Table D14. pH measurements of two sets of increased macronutrients

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$, Citric Acid$\cdot H_2O$, $Na_2EDTA \cdot 2H_2O$					5 x K_2HPO_4, $CaCl_2 \cdot 2H_2O$, Ammonia Ferric Citrate, Na_2CO_3				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.73	8.06	8.15	7.98	0.22	8.51	8.4	8.46	8.46	0.06
1	7.99	8.15	8.15	8.10	0.09	8.76	8.75	8.80	8.77	0.03
2	8.46	8.50	8.80	8.59	0.19	9.31	9.48	9.56	9.45	0.13
4	9.72	9.87	9.84	9.81	0.08	10.30	10.04	10.13	10.16	0.13
5	9.91	10.04	10.03	9.99	0.07	9.44	9.41	9.31	9.39	0.07
6	9.84	10.10	10.05	9.99	0.14	8.32	8.27	8.30	8.30	0.03
7	10.09	10.23	10.21	10.18	0.08	8.27	8.31	8.32	8.30	0.03

Table D-15. Absorbance readings at 500 nm of two set of increased macronutrients

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$, Citric Acid$\cdot H_2O$, $Na_2EDTA \cdot 2H_2O$					5 x K_2HPO_4, $CaCl_2 \cdot 2H_2O$, Ammonia Ferric Citrate, Na_2CO_3				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.020	0.018	0.016	0.018	0.002	0.001	0.00	0.00	0.00	0.001
1	0.030	0.031	0.027	0.029	0.002	0.044	0.041	0.039	0.041	0.003
2	0.072	0.079	0.080	0.077	0.004	0.074	0.090	0.082	0.082	0.008
4	0.221	0.233	0.266	0.240	0.023	0.310	0.270	0.241	0.274	0.035
5	0.301	0.371	0.299	0.324	0.041	0.280	0.333	0.317	0.310	0.027
6	0.470	0.512	0.476	0.486	0.023	0.283	0.389	0.328	0.333	0.053
7	0.593	0.599	0.510	0.567	0.050	0.310	0.373	0.337	0.340	0.032

Table D-16. Final biomass concentrations (g/L) of two sets of increased macronutrients

	A	B	C	Average	Standard Deviation
5 x $MgSO_4 \cdot 7H_2O$, Citric Acid·H_2O, $Na_2EDTA \cdot 2H_2O$	0.3805	0.4250	0.3817	0.3957	0.0253
5 x K_2HPO_4, $CaCl_2 \cdot 2H_2O$, Ammonia Ferric Citrate, Na_2CO_3	0.2115	0.3115	0.2330	0.2510	0.0532

Table D-17. Cell densities (cells/mL) of five times the standard concentration of Magnesium sulfate or EDTA salt

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$					5 x $Na_2EDTA \cdot 2H_2O$				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	3.28E+05	4.78E+05	4.46E+05	4.17E+05	7.91E+04	4.16E+05	7.83E+05	5.34E+05	5.78E+05	1.87E+05
1	1.03E+06	5.77E+05	1.03E+06	8.79E+05	2.62E+05	8.55E+05	8.45E+05	8.85E+05	8.62E+05	2.08E+04
2	1.28E+06	1.63E+06	1.30E+06	1.40E+06	1.99E+05	1.26E+06	1.55E+06	1.80E+06	1.53E+06	2.70E+05
3	3.28E+06	3.27E+06	3.35E+06	3.30E+06	4.36E+04	3.04E+06	2.42E+06	2.89E+06	2.78E+06	3.23E+05
4	8.32E+06	6.80E+06	7.20E+06	7.44E+06	7.88E+05	6.48E+06	6.52E+06	7.64E+06	6.88E+06	6.58E+05
5	8.05E+06	1.09E+07	8.20E+06	9.03E+06	1.58E+06	8.35E+06	6.55E+06	7.80E+06	7.57E+06	9.22E+05
6	1.14E+07	1.06E+07	1.18E+07	1.13E+07	6.38E+05	5.90E+06	7.45E+06	9.90E+06	7.75E+06	2.02E+06
7	9.10E+06	9.00E+06	1.05E+07	9.52E+06	8.10E+05	1.01E+07	8.80E+06	1.05E+07	9.80E+06	8.89E+05
8	-	-	-	-	-	9.90E+06	6.55E+06	7.90E+06	8.12E+06	1.69E+06

Table D-18. pH measurements of five times the standard concentration of magnesium sulfate or EDTA salt

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$					5 x $Na_2EDTA \cdot 2H_2O$				
	A	**B**	**C**	**Average**	**Standard Deviation**	**A**	**B**	**C**	**Average**	**Standard Deviation**
0	7.34	7.25	7.38	7.32	0.07	7.72	7.73	7.76	7.74	0.02
1	7.84	7.84	7.93	7.87	0.05	7.81	7.71	7.82	7.78	0.06
2	8.75	8.83	8.61	8.73	0.11	9.22	9.38	9.28	9.29	0.08
3	9.51	9.69	9.73	9.64	0.12	9.70	9.84	9.83	9.79	0.08
4	9.71	9.86	9.91	9.83	0.10	9.89	10.05	10.02	9.99	0.09
5	9.82	9.78	9.84	9.81	0.03	9.78	9.90	9.93	9.87	0.07
6	10.01	10.07	10.08	10.05	0.04	9.83	9.98	9.97	9.93	0.08
7	10.07	10.13	10.19	10.13	0.06	9.19	8.69	9.18	9.02	0.29
8	-	-	-	-	-	6.92	6.88	6.89	6.90	0.02

Table D-19. Absorbance readings at 500 nm of five times the standard concentration of magnesium sulfate or EDTA salt

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$					5 x $Na_2EDTA \cdot 2H_2O$				
	A	**B**	**C**	**Average**	**Standard Deviation**	**A**	**B**	**C**	**Average**	**Standard Deviation**
0	0.017	0.019	0.021	0.019	0.002	0.018	0.021	0.018	0.019	0.002
1	0.046	0.033	0.043	0.041	0.007	0.042	0.039	0.037	0.039	0.003
2	0.072	0.089	0.075	0.079	0.009	0.080	0.089	0.089	0.086	0.005
3	0.167	0.168	0.163	0.166	0.002	0.157	0.169	0.161	0.162	0.006
4	0.294	0.283	0.273	0.283	0.011	0.238	0.292	0.260	0.263	0.027
5	0.401	0.390	0.394	0.395	0.006	0.356	0.412	0.331	0.366	0.041
6	0.488	0.498	0.480	0.489	0.009	0.446	0.485	0.487	0.473	0.023
7	0.591	0.531	0.530	0.551	0.035	0.491	0.599	0.502	0.531	0.059
8	-	-	-	-	-	0.498	0.466	0.501	0.488	0.019

Table D-20. Final biomass concentration (g/L) of five times the standard concentration of magnesium sulfate or EDTA salt

	A	**B**	**C**	**Average**	**Standard Deviation**
5 x $MgSO_4 \cdot 7H_2O$	0.3388	0.3278	0.2500	0.3055	0.0484
5 x $Na_2EDTA \cdot 2H_2O$	0.2922	0.2974	0.2519	0.2805	0.0249

Table D-21. Cell densities (cells/mL) of five times the standard concentration of both magnesium sulfate and EDTA salt

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$				
	A	B	C	Average	Standard Deviation
0	4.88E+05	3.55E+05	4.18E+05	2.81E+05	2.52E+05
1	7.35E+05	5.18E+05	7.23E+05	6.58E+05	1.22E+05
2	1.32E+06	1.10E+06	1.77E+06	1.39E+06	3.41E+05
3	3.64E+06	4.38E+06	2.46E+06	3.49E+06	9.68E+05
4	5.40E+06	3.70E+06	6.40E+06	5.17E+06	1.37E+06
5	8.13E+06	7.93E+06	5.50E+06	7.18E+06	1.46E+06
6	8.28E+06	7.13E+06	7.03E+06	7.48E+06	6.95E+05
7	1.02E+07	1.59E+07	1.01E+07	1.21E+07	3.33E+06
8	1.48E+07	1.52E+07	1.50E+07	1.50E+07	2.00E+05
9	2.03E+07	2.21E+07	1.67E+07	1.97E+07	2.75E+06
10	2.25E+07	1.96E+07	1.92E+07	2.04E+07	1.80E+06
11	1.13E+07	2.10E+07	2.52E+07	1.92E+07	7.13E+06
12	2.97E+07	1.90E+07	2.45E+07	2.44E+07	5.35E+06

Table D-22. pH measurements of five times the standard concentration of both magnesium sulfate and EDTA salt

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$				
	A	B	C	Average	Standard Deviation
0	7.38	7.15	7.18	7.24	0.13
1	7.68	7.38	7.57	7.54	0.15
2	8.74	8.68	8.69	8.70	0.03
3	9.48	9.76	9.79	9.68	0.17
4	9.85	9.84	9.98	9.89	0.08
5	9.41	9.69	9.78	9.63	0.19
6	9.69	9.95	10.27	9.97	0.29
7	9.97	10.22	10.32	10.17	0.18
8	10.34	10.52	10.62	10.49	0.14
9	10.09	10.30	10.36	10.25	0.14
10	10.14	10.16	10.33	10.21	0.10
11	10.36	10.49	10.60	10.48	0.12
12	10.16	10.26	10.40	10.27	0.12

Table D-23. Absorbance readings at 500 nm of five times the standard concentration of both magnesium sulfate and EDTA salt

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$				
	A	**B**	**C**	**Average**	**Standard Deviation**
0	0.024	0.026	0.024	0.025	0.001
1	0.041	0.034	0.038	0.038	0.004
2	0.085	0.078	0.099	0.087	0.011
3	0.187	0.193	0.153	0.178	0.022
4	0.285	0.242	0.315	0.281	0.037
5	0.383	0.371	0.336	0.363	0.024
6	0.493	0.443	0.443	0.460	0.029
7	0.638	0.691	0.607	0.645	0.042
8	0.889	0.989	0.804	0.894	0.093
9	1.039	0.987	0.913	0.980	0.063
10	1.148	1.237	1.268	1.218	0.062
11	0.964	1.174	1.317	1.152	0.178
12	1.528	1.395	1.42	1.448	0.071

Table D-24. Final biomass concentration (g/L) of five times the standard concentration of both magnesium sulfate and EDTA salt

	A	**B**	**C**	**Average**	**Standard Deviation**
5 x $MgSO_4 \cdot 7H_2O$ and $Na_2EDTA \cdot 2H_2O$	0.7683	0.7460	0.7873	0.7672	0.0207

Table D-25. Cell densities (cells/mL) of increased concentration of divalent cations

Time (Days)	5 x Ammonia Ferric Citrate and $Na_2EDTA{\cdot}2H_2O$					5 x $CaCl_2{\cdot}2H_2O$ and $Na_2EDTA{\cdot}2H_2O$				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	8.83E+05	9.28E+05	9.28E+05	9.13E+05	2.60E+04	7.45E+05	7.18E+05	8.30E+05	7.64E+05	5.86E+04
1	4.05E+05	6.60E+05	9.10E+05	6.58E+05	2.53E+05	1.03E+06	4.53E+05	3.93E+05	6.26E+05	3.53E+05
2	2.84E+06	3.10E+06	2.52E+06	2.82E+06	2.91E+05	3.58E+06	2.62E+06	3.54E+06	3.25E+06	5.43E+05
3	5.05E+06	4.73E+06	3.35E+06	4.38E+06	9.02E+05	3.95E+06	4.60E+06	3.63E+06	4.06E+06	4.96E+05
4	8.75E+06	6.75E+06	1.06E+07	8.68E+06	1.90E+06	7.30E+06	6.15E+06	7.60E+06	7.02E+06	7.65E+05
6	9.80E+06	1.29E+07	2.09E+07	1.45E+07	5.73E+06	9.00E+06	1.22E+07	9.40E+06	1.02E+07	1.71E+06
7	9.55E+06	1.28E+07	1.13E+07	1.12E+07	1.60E+06	9.25E+06	1.29E+07	1.16E+07	1.13E+07	1.85E+06
8	1.25E+07	1.53E+07	1.15E+07	1.31E+07	1.94E+06	9.50E+06	1.15E+07	9.00E+06	1.00E+07	1.32E+06

Table D-26. pH measurements of increased concentration of divalent cations

Time (Days)	5 x Ammonia Ferric Citrate and $Na_2EDTA{\cdot}2H_2O$					5 x $CaCl_2{\cdot}2H_2O$ and $Na_2EDTA{\cdot}2H_2O$				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.18	7.47	7.32	7.32	0.15	6.69	6.55	6.60	6.61	0.07
1	8.25	8.19	8.33	8.26	0.07	7.80	7.79	7.81	7.80	0.01
2	9.18	9.49	9.61	9.46	0.22	8.99	8.76	8.93	8.89	0.12
3	9.93	10.1	10.13	10.05	0.11	9.16	9.07	9.21	9.15	0.07
4	10.01	10.06	10.18	10.08	0.09	9.28	9.28	9.36	9.31	0.05
6	7.26	7.12	7.34	7.24	0.11	9.00	9.13	9.03	9.05	0.07
7	7.39	7.34	7.45	7.39	0.06	8.72	8.57	8.69	8.66	0.08
8	7.33	7.07	7.22	7.21	0.13	7.15	7.14	7.12	7.14	0.02

Table D-27. Absorbance readings at 500 nm of increased concentration of divalent cations

Time (Days)	5 x Ammonia Ferric Citrate and $Na_2EDTA{\cdot}2H_2O$					5 x $CaCl_2{\cdot}2H_2O$ and $Na_2EDTA{\cdot}2H_2O$				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.035	0.044	0.04	0.040	0.005	0.039	0.047	0.041	0.042	0.004
1	0.053	0.050	0.062	0.055	0.006	0.078	0.053	0.066	0.066	0.013
2	0.117	0.128	0.119	0.121	0.006	0.119	0.102	0.123	0.115	0.011
3	0.187	0.185	0.182	0.185	0.003	0.151	0.176	0.154	0.160	0.014
4	0.289	0.296	0.384	0.323	0.052	0.247	0.207	0.222	0.225	0.020
6	0.454	0.490	-	0.472	0.025	0.445	0.442	0.454	0.447	0.006
7	0.457	0.471	0.445	0.458	0.013	0.488	0.572	0.544	0.535	0.043
8	0.489	0.524	0.451	0.488	0.037	0.559	0.530	0.515	0.535	0.022

Table D-28. Final biomass concentrations (g/L) of the increased concentrations of divalent cations

	A	B	C	Average	Standard Deviation
5 x Ammonia Ferric Citrate and $Na_2EDTA{\cdot}2H_2O$	0.2897	0.2679	0.2949	0.2842	0.0143
5 x $CaCl_2{\cdot}2H_2O$ and $Na_2EDTA{\cdot}2H_2O$	0.2900	0.3652	0.3756	0.3436	0.0467

Table D-29. Cell densities (cells/mL) of two combinations of magnesium sulfate and EDTA salt concentrations

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$ and 2.5 x $Na_2EDTA \cdot 2H_2O$					2.5 x $MgSO_4 \cdot 7H_2O$ and 5 x $Na_2EDTA \cdot 2H_2O$				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	9.15E+05	8.20E+05	8.20E+05	8.20E+05	8.20E+05	6.40E+05	6.40E+05	6.25E+05	6.35E+05	8.66E+03
1	1.12E+06	9.85E+05	9.85E+05	9.85E+05	9.85E+05	1.36E+06	7.20E+05	1.21E+06	1.09E+06	3.32E+05
2	1.86E+06	2.01E+06	2.01E+06	2.01E+06	2.01E+06	1.32E+06	1.62E+06	1.21E+06	1.38E+06	2.12E+05
3	2.01E+06	3.50E+06	3.50E+06	3.50E+06	3.50E+06	3.58E+06	4.31E+06	2.25E+06	3.38E+06	1.05E+06
4	4.33E+06	3.88E+06	3.88E+06	3.88E+06	3.88E+06	3.70E+06	3.83E+06	4.80E+06	4.11E+06	6.02E+05
5	4.80E+06	9.30E+06	9.30E+06	9.30E+06	9.30E+06	6.13E+06	5.08E+06	4.03E+06	5.08E+06	1.05E+06
6	6.90E+06	9.25E+06	9.25E+06	9.25E+06	9.25E+06	1.22E+07	5.70E+06	8.60E+06	8.82E+06	3.23E+06
7	8.20E+06	9.00E+06	9.00E+06	9.00E+06	9.00E+06	3.44E+07	1.51E+07	7.65E+06	1.91E+07	1.38E+07
8	6.55E+06	8.80E+06	8.80E+06	8.80E+06	8.80E+06	7.75E+06	5.80E+06	7.50E+06	7.02E+06	1.06E+06
9	5.40E+06	1.06E+07	1.06E+07	1.06E+07	1.06E+07	-	-	-	-	-
10	2.08E+07	7.60E+06	7.60E+06	7.60E+06	7.60E+06	-	-	-	-	-
11	9.60E+06	1.04E+07	1.04E+07	1.04E+07	1.04E+07	-	-	-	-	-
12	7.45E+06	2.26E+07	2.26E+07	2.26E+07	2.26E+07	-	-	-	-	-
13	5.60E+06	8.30E+06	8.30E+06	8.30E+06	8.30E+06	-	-	-	-	-
14	1.07E+07	7.10E+06	7.10E+06	7.10E+06	7.10E+06	-	-	-	-	-
15	6.05E+06	6.20E+06	6.20E+06	6.20E+06	6.20E+06	-	-	-	-	-

Table D-30. pH measurements of two combinations of magnesium sulfate and EDTA salt concentrations

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$ and 2.5 x $Na_2EDTA \cdot 2H_2O$					2.5 x $MgSO_4 \cdot 7H_2O$ and 5 x $Na_2EDTA \cdot 2H_2O$				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	7.22	7.01	7.17	7.13	0.11	7.47	7.46	7.47	7.47	0.01
1	9.01	9.00	9.16	9.06	0.09	8.96	8.94	9.19	9.03	0.14
2	9.08	8.95	9.09	9.04	0.08	9.32	9.35	9.45	9.37	0.07
3	9.22	9.43	9.49	9.38	0.14	9.43	9.57	9.64	9.55	0.11
4	9.47	9.62	9.59	9.56	0.08	9.76	9.72	9.86	9.78	0.07
5	9.81	10.00	9.94	9.92	0.10	9.95	10.01	10.04	10.00	0.05
6	9.75	9.92	9.85	9.84	0.09	9.96	10.15	10.10	10.07	0.10
7	10.00	10.25	10.27	10.17	0.15	10.19	10.32	10.34	10.28	0.08
8	10.19	10.26	10.16	10.20	0.05	10.08	10.17	10.24	10.16	0.08
9	9.75	9.82	9.92	9.83	0.09	-	-	-	-	-
10	10.35	10.48	10.44	10.42	0.07	-	-	-	-	-
11	10.10	10.34	10.24	10.23	0.12	-	-	-	-	-
12	9.57	10.31	10.3	10.06	0.42	-	-	-	-	-
13	9.85	9.86	9.92	9.88	0.04	-	-	-	-	-
14	10.19	10.38	10.27	10.28	0.10	-	-	-	-	-
15	9.57	9.94	10.18	9.90	0.31	-	-	-	-	-

Table D-31. Absorbance readings at 500 nm of two combinations of magnesium sulfate and EDTA salt concentrations

Time (Days)	5 x $MgSO_4 \cdot 7H_2O$ and 2.5 x $Na_2EDTA \cdot 2H_2O$					2.5 x $MgSO_4 \cdot 7H_2O$ and 5 x $Na_2EDTA \cdot 2H_2O$				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.030	0.033	0.028	0.030	0.003	0.029	0.037	0.029	0.032	0.005
1	0.046	0.090	0.079	0.072	0.023	0.069	0.084	0.069	0.074	0.009
2	0.108	0.135	0.118	0.120	0.014	0.100	0.119	0.099	0.106	0.011
3	0.158	0.190	0.206	0.185	0.024	-	-	-	-	-
4	0.249	0.245	0.239	0.244	0.005	0.243	0.234	0.254	0.244	0.010
5	0.340	0.431	0.332	0.368	0.055	0.361	0.317	0.313	0.330	0.027
6	0.416	0.456	0.353	0.408	0.052	0.493	0.386	0.437	0.439	0.054
7	0.575	0.570	0.580	0.575	0.005	0.929	0.938	0.545	0.804	0.224
8	0.595	0.627	0.991	0.738	0.220	0.594	0.502	0.604	0.567	0.056
9	0.681	0.767	0.641	0.696	0.064	-	-	-	-	-
10	1.090	0.808	0.803	0.900	0.164	-	-	-	-	-
11	0.959	0.975	0.924	0.953	0.026	-	-	-	-	-
12	1.056	1.563	1.519	1.379	0.281	-	-	-	-	-
13	1.160	1.243	1.540	1.314	0.199	-	-	-	-	-
14	1.534	1.464	1.559	1.519	0.049	-	-	-	-	-
15	1.675	1.615	1.629	1.640	0.031	-	-	-	-	-

APPENDIX E

AKINETE TRIGGERING EXPERIMENTAL DATA

Experiments were performed to trigger the differentiation of akinetes in an *Anabaena cylindrica* culture. All tests were completed in the 1.25 L photo-bioreactors containing a variation of the BG11-N medium. The cultures received 42.5 W/m^2 of light at a 14/10 hour light/dark cycle. The reactors were aerated at 400 mL/min resulting in a superficial velocity of 12.5 cm/s. The temperature of the reactors was maintained at 25 ± 1^oC. The cell density, pH, chlorophyll and final biomass concentration were determined by the methods outlined above. The number density of the three cell types were determined by a direct count from microscopic images. Three variations of BG11-N were examined: the removal of all phosphate, the elimination of all iron from the medium and the addition of sodium acetate into the medium.

Table E-1. The cell densities (cells/mL) of the cultures grown with either no phosphate or iron for the akinete triggering experiment

Time (Days)	No PO_4^-					No Fe				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	1.61E+05	2.30E+05	1.79E+05	1.90E+05	3.57E+04	1.71E+05	1.60E+05	1.24E+05	1.52E+05	2.48E+04
2	5.18E+05	7.33E+05	5.05E+05	5.85E+05	1.28E+05	4.40E+05	6.43E+05	6.40E+05	5.74E+05	1.16E+05
4	1.03E+06	1.83E+06	1.54E+06	1.47E+06	4.05E+05	1.69E+06	1.46E+06	1.56E+06	1.57E+06	1.13E+05
6	1.85E+06	1.38E+06	1.98E+06	1.74E+06	3.15E+05	2.57E+06	2.81E+06	2.09E+06	2.49E+06	3.67E+05
8	2.53E+06	3.05E+06	2.23E+06	2.60E+06	4.18E+05	3.80E+06	3.88E+06	2.63E+06	3.43E+06	7.01E+05
10	1.94E+06	1.66E+06	2.33E+06	1.98E+06	3.33E+05	2.66E+06	2.55E+06	2.16E+06	2.46E+06	2.62E+05
12	2.00E+06	1.54E+06	1.81E+06	1.78E+06	2.33E+05	1.96E+06	1.85E+06	3.08E+06	2.30E+06	6.77E+05
14	4.30E+06	1.56E+06	1.05E+06	2.30E+06	1.75E+06	3.18E+06	2.63E+06	1.58E+06	2.46E+06	8.13E+05
16	1.58E+06	3.13E+06	1.53E+06	2.08E+06	9.10E+05	1.64E+06	1.51E+06	1.98E+06	1.71E+06	2.39E+05
18	1.33E+06	1.61E+06	2.60E+06	1.85E+06	6.69E+05	2.56E+06	2.55E+06	2.01E+06	2.38E+06	3.14E+05
20	1.48E+06	2.38E+06	1.78E+06	1.88E+06	4.58E+05	1.64E+06	1.15E+06	1.60E+06	1.46E+06	2.71E+05
22	1.79E+06	3.01E+06	3.38E+06	2.73E+06	8.32E+05	1.41E+06	3.01E+06	1.69E+06	2.04E+06	8.55E+05
24	1.31E+06	2.54E+06	1.55E+06	1.80E+06	6.50E+05	4.28E+06	3.23E+06	1.69E+06	3.06E+06	1.30E+06
26	1.56E+06	1.94E+06	1.73E+06	1.74E+06	1.88E+05	1.45E+06	2.88E+06	1.50E+06	1.94E+06	8.09E+05
28	1.28E+06	2.70E+06	2.79E+06	2.25E+06	8.49E+05	2.46E+06	2.19E+06	2.49E+06	2.38E+06	1.66E+05
30	1.29E+06	2.75E+06	1.91E+06	1.98E+06	7.34E+05	3.20E+06	2.60E+06	1.63E+06	2.48E+06	7.95E+05
32	-	-	-	-	-	2.33E+06	3.78E+06	3.50E+06	3.20E+06	7.70E+05

Table E-2. The pH measurements of the cultures grown with either no phosphate or iron for the akinete triggering experiment

Time (Days)	No PO_4^-					No Fe				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	6.95	7.17	7.14	7.09	0.12	7.41	7.46	7.53	7.47	0.06
2	6.81	6.45	6.49	6.58	0.20	6.95	7.14	7.23	7.11	0.14
4	8.51	8.75	9.1	8.79	0.30	9.39	9.41	9.41	9.40	0.01
6	8.6	9.07	9.3	8.99	0.36	9.72	9.81	9.83	9.79	0.06
8	8.9	9.21	9.23	9.11	0.19	9.68	9.86	9.81	9.78	0.09
10	8.69	8.87	9.02	8.86	0.17	8.6	8.37	8.25	8.41	0.18
12	6.44	6.5	7.6	6.85	0.65	7.74	7.69	7.67	7.70	0.04
14	6.58	6.4	6.45	6.48	0.09	6.78	6.88	6.99	6.88	0.11
16	6.59	6.71	6.75	6.68	0.08	7.05	7.12	7.21	7.13	0.08
18	6.46	6.54	6.55	6.52	0.05	6.85	6.96	7.05	6.95	0.10
20	7.97	7.77	7.72	7.82	0.13	7.87	7.86	7.86	7.86	0.01
22	6.56	6.44	6.52	6.51	0.06	7.11	7.15	7.26	7.17	0.08
24	6.84	6.77	6.88	6.83	0.06	7.66	8.57	8.51	8.25	0.51
26	6.83	6.95	7.01	6.93	0.09	7.55	7.63	7.73	7.64	0.09
28	7.08	6.99	7.04	7.04	0.05	7.66	7.71	7.8	7.72	0.07
30	6.32	6.19	6.37	6.29	0.09	7.21	7.28	7.39	7.29	0.09
32	-	-	-	-	-	7.16	7.82	7.69	7.56	0.35

Table E-3. Absorbance measurements at 440 nm of the cultures grown with either no phosphate or iron for the akinete triggering experiment.

Time (Days)	No PO_4^-					No Fe				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.017	0.016	0.013	0.015	0.002	0.067	0.065	0.06	0.064	0.004
2	0.05	0.052	0.032	0.045	0.011	0.106	0.098	0.096	0.100	0.005
4	0.178	0.194	0.176	0.183	0.010	0.22	0.184	0.116	0.173	0.053
6	0.339	0.296	0.272	0.302	0.034	0.337	0.303	0.317	0.319	0.017
8	0.475	0.382	0.342	0.400	0.068	0.399	0.353	0.396	0.383	0.026
10	0.569	0.391	0.399	0.453	0.101	0.418	0.345	0.386	0.383	0.037
12	0.601	0.477	0.419	0.499	0.093	0.416	0.457	0.402	0.425	0.029
14	0.604	0.474	0.429	0.502	0.091	0.43	0.344	0.374	0.383	0.044
16	0.576	0.514	0.447	0.512	0.065	0.424	0.357	0.389	0.390	0.034
18	0.532	0.503	0.467	0.501	0.033	0.45	0.357	0.39	0.399	0.047
20	0.532	0.461	0.42	0.471	0.057	0.44	0.377	0.382	0.400	0.035
22	0.555	0.503	0.46	0.506	0.048	0.443	0.403	0.393	0.413	0.026
24	0.53	0.49	0.457	0.492	0.037	0.458	0.464	0.411	0.444	0.029
26	0.54	0.494	0.458	0.497	0.041	0.472	0.513	0.484	0.490	0.021
28	0.542	0.497	0.463	0.501	0.040	0.555	0.495	0.53	0.527	0.030
30	0.535	0.495	0.461	0.497	0.037	0.625	0.489	0.549	0.554	0.068
32	-	-	-	-	-	0.537	0.394	0.364	0.432	0.092

Table E-4. Absorbance measurements at 500 nm of the cultures grown with either no phosphate or iron for the akinete triggering experiment.

Time (Days)	No PO_4^-					No Fe				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.01	0.01	0.008	0.009	0.001	0.055	0.053	0.048	0.052	0.004
2	0.032	0.033	0.032	0.032	0.001	0.08	0.074	0.071	0.075	0.005
4	0.114	0.13	0.114	0.119	0.009	0.148	0.119	0.116	0.128	0.018
6	0.242	0.205	0.182	0.210	0.030	0.225	0.214	0.207	0.215	0.009
8	0.332	0.262	0.227	0.274	0.053	0.27	0.245	0.258	0.258	0.013
10	0.391	0.303	0.266	0.320	0.064	0.291	0.245	0.254	0.263	0.024
12	0.422	0.321	0.275	0.339	0.075	0.288	0.403	0.286	0.326	0.067
14	0.443	0.317	0.283	0.348	0.084	0.31	0.249	0.251	0.270	0.035
16	0.409	0.372	0.305	0.362	0.053	0.296	0.265	0.273	0.278	0.016
18	0.389	0.36	0.334	0.361	0.028	0.332	0.262	0.276	0.290	0.037
20	0.405	0.336	0.294	0.345	0.056	0.315	0.28	0.271	0.289	0.023
22	0.438	0.388	0.42	0.415	0.025	0.325	0.299	0.285	0.303	0.020
24	0.421	0.372	0.349	0.381	0.037	0.357	0.359	0.301	0.339	0.033
26	0.436	0.378	0.339	0.384	0.049	0.349	0.402	0.355	0.369	0.029
28	0.436	0.385	0.346	0.389	0.045	0.423	0.388	0.407	0.406	0.018
30	0.434	0.388	0.347	0.390	0.044	0.488	0.386	0.422	0.432	0.052
32	-	-	-	-	-	0.537	0.394	0.364	0.432	0.092

Table E-5. Absorbance measurements at 660 nm of the cultures grown with either no phosphate or iron for the akinete triggering experiment.

Time (Days)	No PO_4^-					No Fe				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.006	0.006	0.006	0.006	0.000	0.038	0.036	0.033	0.036	0.003
2	0.036	0.028	0.029	0.031	0.004	0.059	0.056	0.054	0.056	0.003
4	0.093	0.105	0.094	0.097	0.007	0.135	0.106	0.103	0.115	0.018
6	0.176	0.143	0.13	0.150	0.024	0.196	0.194	0.183	0.191	0.007
8	0.222	0.172	0.157	0.184	0.034	0.224	0.211	0.215	0.217	0.007
10	0.251	0.198	0.184	0.211	0.035	0.245	0.221	0.213	0.226	0.017
12	0.283	0.215	0.182	0.227	0.052	0.23	0.373	0.24	0.281	0.080
14	0.302	0.203	0.185	0.230	0.063	0.247	0.21	0.195	0.217	0.027
16	0.251	0.243	0.195	0.230	0.030	0.218	0.213	0.207	0.213	0.006
18	0.24	0.221	0.215	0.225	0.013	0.246	0.195	0.2	0.214	0.028
20	0.253	0.208	0.182	0.214	0.036	0.217	0.2	0.185	0.201	0.016
22	0.28	0.246	0.317	0.281	0.036	0.221	0.204	0.193	0.206	0.014
24	0.264	0.229	0.225	0.239	0.021	0.251	0.241	0.199	0.230	0.028
26	0.279	0.227	0.203	0.236	0.039	0.224	0.268	0.225	0.239	0.025
28	0.277	0.232	0.207	0.239	0.035	0.275	0.249	0.259	0.261	0.013
30	0.275	0.234	0.203	0.237	0.036	0.306	0.242	0.282	0.277	0.032
32	-	-	-	-	-	0.338	0.25	0.216	0.268	0.063

Table E-6. Absorbance measurements at 680 nm of the cultures grown with either no phosphate or iron for the akinete triggering experiment

Time (Days)	No PO_4^-					No Fe				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.008	0.008	0.007	0.008	0.001	0.038	0.036	0.034	0.036	0.002
2	0.032	0.034	0.033	0.033	0.001	0.062	0.059	0.058	0.060	0.002
4	0.109	0.128	0.115	0.117	0.010	0.142	0.121	0.119	0.127	0.013
6	0.191	0.166	0.156	0.171	0.018	0.213	0.204	0.201	0.206	0.006
8	0.25	0.202	0.188	0.213	0.033	0.242	0.223	0.232	0.232	0.010
10	0.284	0.23	0.215	0.243	0.036	0.253	0.222	0.22	0.232	0.019
12	0.302	0.236	0.211	0.250	0.047	0.234	0.358	0.244	0.279	0.069
14	0.326	0.226	0.201	0.251	0.066	0.251	0.208	0.197	0.219	0.029
16	0.276	0.264	0.213	0.251	0.033	0.219	0.21	0.209	0.213	0.006
18	0.255	0.241	0.231	0.242	0.012	0.246	0.191	0.198	0.212	0.030
20	0.266	0.229	0.203	0.233	0.032	0.219	0.199	0.186	0.201	0.017
22	0.288	0.263	0.327	0.293	0.032	0.219	0.207	0.193	0.206	0.013
24	0.272	0.248	0.243	0.254	0.016	0.256	0.247	0.201	0.235	0.030
26	0.286	0.241	0.22	0.249	0.034	0.229	0.272	0.23	0.244	0.025
28	0.282	0.25	0.233	0.255	0.025	0.278	0.252	0.263	0.264	0.013
30	0.282	0.25	0.221	0.251	0.031	0.311	0.246	0.263	0.273	0.034
32	-	-	-	-	-	0.339	0.249	0.216	0.268	0.064

Table E-7. The chlorophyll a concentrations (µg/mL) of the cultures grown with either no phosphate or iron for the akinete triggering experiment

Time (Days)	No PO_4^-					No Fe				
	A	B	C	Average	Standard Deviation	A	B	C	Average	Standard Deviation
0	0.049757	0.057384	0.041171	0.049437	0.008111	0.06597	0.059462	0.057384	0.060938	0.004479
2	0.271506	0.303931	0.303931	0.293123	0.018721	0.287718	0.271506	0.271506	0.27691	0.00936
4	1.04597	1.04597	1.038343	1.043428	0.004403	1.0881	1.062183	1.128153	1.092812	0.033236
6	1.071888	1.11194	1.168205	1.117344	0.048385	1.935043	1.302222	1.788969	1.675411	0.331341
8	1.50568	1.375819	1.351979	1.411159	0.082721	2.019303	1.441788	1.901498	1.78753	0.30516
10	1.61378	1.581355	1.531598	1.575577	0.041395	1.812808	1.314439	1.677672	1.601639	0.257738
12	1.605194	1.555437	1.531598	1.564076	0.037551	1.557515	1.235293	1.565142	1.45265	0.188275
14	1.492664	1.426695	1.409363	1.442907	0.043954	1.449415	1.11194	1.357528	1.306294	0.174474
16	1.268838	1.277424	1.267719	1.271327	0.00531	1.327181	1.00384	1.292152	1.207724	0.177435
18	0.938989	1.127194	1.198438	1.088207	0.134046	1.176951	0.869823	1.02672	1.024498	0.153576
20	0.735531	0.981119	1.110981	0.942544	0.190674	1.068851	0.78321	0.920699	0.924253	0.142853
22	0.683696	0.905445	1.060105	0.883082	0.189198	0.921658	0.910834	1.028798	0.953763	0.065207
24	0.6101	0.848061	0.947575	0.801912	0.173406	0.863315	0.933143	0.779054	0.858504	0.077157
26	0.606903	0.788599	0.920539	0.772014	0.157474	0.803853	0.754096	0.878568	0.812172	0.062652
28	0.576556	0.772387	0.928166	0.759036	0.176185	0.870942	0.676503	0.665406	0.737617	0.115596
30	0.574478	0.76476	0.886035	0.741758	0.157047	0.813558	0.5724	0.631861	0.672606	0.125636
32	-	-	-	-	-	0.74108	0.562855	0.893798	0.732578	0.165636

Table E-8. Number fraction of akinetes (X_A) and heterocysts (X_H) in cultures grown without any phosphate in the akinete triggering experiment

Time (Days)	**A**		**B**		**C**		**X_A**		**X_H**	
	X_A	**X_H**	**X_A**	**X_H**	**X_A**	**X_H**	**Average**	**St. Dev.**	**Average**	**St. Dev.**
0	0.000	0.038	0.000	0.037	0.000	0.044	0.000	0.000	0.040	0.004
2	0.000	0.048	0.000	0.059	0.000	0.059	0.000	0.000	0.055	0.007
4	0.000	0.04	0.000	0.038	0.000	0.04	0.000	0.000	0.039	0.001
6	0.000	0.045	0.000	0.035	0.000	0.038	0.000	0.000	0.040	0.005
8	0.000	0.04	0.000	0.028	0.000	0.027	0.000	0.000	0.032	0.007
10	0.000	0.032	0.000	0.023	0.000	0.021	0.000	0.000	0.025	0.006
12	0.000	0.02	0.000	0.026	0.000	0.037	0.000	0.000	0.028	0.008
14	0.000	0.018	0.000	0.027	0.000	0.034	0.000	0.000	0.026	0.008
16	0.000	0.032	0.000	0.021	0.000	0.035	0.000	0.000	0.030	0.007
18	0.000	0.044	0.000	0.022	0.000	0.025	0.000	0.000	0.030	0.012
20	0.000	0.034	0.000	0.026	0.000	0.046	0.000	0.000	0.036	0.010
22	0.000	0.027	0.000	0.031	0.000	0.037	0.000	0.000	0.031	0.005
24	0.000	0.012	0.058	0.035	0.000	0.017	0.019	0.034	0.021	0.012
26	0.053	0.053	0.014	0.028	0.000	0.052	0.022	0.028	0.044	0.014
28	0.082	0.041	0.069	0.043	0.017	0.043	0.056	0.034	0.042	0.001
30	0.019	0.019	0.073	0.073	0.000	0.029	0.031	0.038	0.040	0.029

Table E-9. Number fraction of akinetes (X_A) and heterocysts (X_H) in cultures grown without any iron in the akinete triggering experiment

Time (Days)	**A**		**B**		**C**		**X_A**		**X_H**	
	X_A	**X_H**	**X_A**	**X_H**	**X_A**	**X_H**	**Average**	**St. Dev.**	**Average**	**St. Dev.**
0	0.000	0.051	0.000	0.043	0.000	0.048	0.000	0.000	0.048	0.004
2	0.000	0.069	0.000	0.057	0.000	0.049	0.000	0.000	0.058	0.010
4	0.000	0.041	0.000	0.050	0.000	0.048	0.000	0.000	0.046	0.005
6	0.000	0.031	0.000	0.032	0.000	0.038	0.000	0.000	0.034	0.004
8	0.000	0.042	0.000	0.032	0.000	0.036	0.000	0.000	0.037	0.005
10	0.000	0.029	0.000	0.019	0.000	0.023	0.000	0.000	0.024	0.005
12	0.000	0.030	0.000	0.017	0.000	0.021	0.000	0.000	0.023	0.007
14	0.000	0.019	0.000	0.019	0.000	0.018	0.000	0.000	0.019	0.001
16	0.000	0.000	0.000	0.010	0.000	0.009	0.000	0.000	0.006	0.005
18	0.000	0.023	0.000	0.019	0.000	0.011	0.000	0.000	0.018	0.006
20	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.005	0.008
22	0.000	0.000	0.007	0.007	0.000	0.000	0.002	0.004	0.002	0.004
24	0.000	0.006	0.022	0.032	0.000	0.009	0.007	0.012	0.016	0.014
26	0.000	0.025	0.010	0.031	0.016	0.039	0.009	0.008	0.031	0.007
28	0.004	0.031	0.062	0.018	0.027	0.027	0.031	0.029	0.025	0.007
30	0.111	0.043	0.072	0.036	0.197	0.056	0.127	0.064	0.045	0.010
32	0.069	0.042	0.034	0.079	0.057	0.034	0.054	0.018	0.052	0.024

Table E-10. Final Biomass concentration (g/L) of the cultures grown with either no phosphate or iron for the akinete triggering experiment

	A	**B**	**C**	**Average**	**Standard Deviation**
No PO_4^-	0.1687	0.1549	0.1665	0.1634	0.0074
No Fe	0.2031	0.1652	0.1772	0.1818	0.0194

Table E-11. Cell densities (cells/mL) of the culture grown in an addition of sodium acetate for the akinete triggering experiment

Time (Days)	Addition of Sodium Acetate				
	A	B	C	Average	Standard Deviation
0	1.18E+05	1.53E+05	1.18E+05	1.29E+05	2.02E+04
2	1.45E+05	2.30E+05	2.18E+05	1.98E+05	4.59E+04
4	6.70E+05	8.55E+05	6.20E+05	7.15E+05	1.24E+05
6	1.61E+06	2.33E+06	2.20E+06	2.05E+06	3.80E+05
8	1.61E+06	1.59E+06	1.34E+06	1.51E+06	1.52E+05
10	3.25E+06	2.38E+06	1.66E+06	2.43E+06	7.95E+05
12	3.00E+06	3.55E+06	1.46E+06	2.67E+06	1.08E+06
14	2.50E+06	3.15E+06	2.60E+06	2.75E+06	3.50E+05
16	3.58E+06	2.50E+06	1.61E+06	2.56E+06	9.83E+05
18	2.49E+06	2.61E+06	1.98E+06	2.36E+06	3.38E+05
20	3.53E+06	2.58E+06	1.21E+06	2.44E+06	1.16E+06
22	2.56E+06	1.84E+06	1.25E+05	1.51E+06	1.25E+06
24	3.13E+06	2.13E+06	8.63E+05	2.04E+06	1.13E+06
26	3.40E+06	1.29E+06	9.50E+05	1.88E+06	1.33E+06

Table E-12. pH measurements of the cultures grown in an addition of sodium acetate for the akinete triggering experiment

Time (Days)	Addition of Sodium Acetate				
	A	B	C	Average	Standard Deviation
0	7.4	7.52	7.6	7.51	0.10
2	7.2	7.32	7.39	7.30	0.10
4	9.37	9.35	9.39	9.37	0.02
6	10.39	10.47	10.37	10.41	0.05
8	9.74	9.23	9.14	9.37	0.32
10	8.46	8.75	8.78	8.66	0.18
12	7.76	8.67	8.82	8.42	0.57
14	8.14	8.59	8.77	8.50	0.32
16	8.83	9.16	9.24	9.08	0.22
18	9.07	9.35	9.34	9.25	0.16
20	9.42	9.57	9.55	9.51	0.08
22	9.55	9.89	9.92	9.79	0.21
24	10.17	10.15	10.1	10.14	0.04
26	9.72	10.04	10.09	9.95	0.20

Table E-13. Absorbance measurements at 440 nm of cultures grown with an addition of sodium acetate for the akinete triggering experiment

Time (Days)	Addition of Sodium Acetate				
	A	B	C	Average	Standard Deviation
0	0.034	0.038	0.045	0.039	0.006
2	0.05	0.054	0.061	0.055	0.006
4	0.134	0.145	0.143	0.141	0.006
6	0.327	0.372	0.343	0.347	0.023
8	0.541	0.48	0.565	0.529	0.044
10	0.575	0.588	0.573	0.579	0.008
12	0.642	0.553	0.6	0.598	0.045
14	0.676	0.74	0.626	0.681	0.057
16	0.674	0.636	0.585	0.632	0.045
18	0.767	0.618	0.625	0.670	0.084
20	0.671	0.584	0.601	0.619	0.046
22	0.653	0.584	0.612	0.616	0.035
24	0.65	0.653	0.6	0.634	0.030
26	0.632	0.672	0.598	0.634	0.037

Table E-14. Absorbance measurements at 500 nm of the cultures grown with an addition of sodium acetate for the akinete triggering experiment

Time (Days)	Addition of Sodium Acetate				
	A	B	C	Average	Standard Deviation
0	0.023	0.027	0.032	0.027	0.005
2	0.033	0.038	0.042	0.038	0.005
4	0.08	0.094	0.09	0.088	0.007
6	0.204	0.245	0.213	0.221	0.022
8	0.416	0.349	0.391	0.385	0.034
10	0.43	0.461	0.431	0.441	0.018
12	0.487	0.553	0.467	0.502	0.045
14	0.514	0.581	0.504	0.533	0.042
16	0.528	0.509	0.451	0.496	0.040
18	0.567	0.492	0.492	0.517	0.043
20	0.528	0.491	0.475	0.498	0.027
22	0.505	0.468	0.468	0.480	0.021
24	0.545	0.557	0.474	0.525	0.045
26	0.504	0.516	0.461	0.494	0.029

Table E-15. Absorbance measurements at 660 nm of the cultures grown with an addition of sodium acetate for the akinete triggering experiment

Time (Days)	Addition of Sodium Acetate				
	A	B	C	Average	Standard Deviation
0	0.013	0.016	0.019	0.016	0.003
2	0.021	0.024	0.025	0.023	0.002
4	0.058	0.07	0.064	0.064	0.006
6	0.158	0.194	0.168	0.173	0.019
8	0.289	0.228	0.245	0.254	0.031
10	0.43	0.461	0.431	0.441	0.018
12	0.304	0.36	0.277	0.314	0.042
14	0.315	0.357	0.301	0.324	0.029
16	0.328	0.312	0.26	0.300	0.036
18	0.333	0.295	0.287	0.305	0.025
20	0.318	0.273	0.268	0.286	0.028
22	0.29	0.276	0.267	0.278	0.012
24	0.298	0.299	0.272	0.290	0.015
26	0.311	0.3	0.264	0.292	0.025

Table E-16. Absorbance measurements at 680 nm of the cultures grown with an addition of sodium acetate for the akinete triggering experiment

Time (Days)	Addition of Sodium Acetate				
	A	B	C	Average	Standard Deviation
0	0.013	0.016	0.018	0.016	0.003
2	0.023	0.026	0.028	0.026	0.003
4	0.08	0.09	0.085	0.085	0.005
6	0.215	0.254	0.228	0.232	0.020
8	0.337	0.267	0.303	0.302	0.035
10	0.318	0.314	0.292	0.308	0.014
12	0.346	0.38	0.295	0.340	0.043
14	0.355	0.386	0.317	0.353	0.035
16	0.35	0.325	0.278	0.318	0.037
18	0.369	0.313	0.3	0.327	0.037
20	0.357	0.294	0.27	0.307	0.045
22	0.326	0.291	0.264	0.294	0.031
24	0.318	0.31	0.266	0.298	0.028
26	0.346	0.303	0.253	0.301	0.047

Table E-17. The chlorophyll a concentrations (µg/mL) of the cultures grown with an addition of sodium acetate for the akinete triggering experiment

Time (Days)	Addition of Sodium Acetate				
	A	B	C	Average	Standard Deviation
0	0.02384	0.075675	0.02384	0.041118	0.029927
2	0.091887	0.089809	0.106022	0.095906	0.008822
4	0.731216	0.710573	0.665246	0.702345	0.033746
6	1.739212	2.010717	1.878778	1.876235	0.135771
8	1.754465	1.424617	1.715532	1.631538	0.180253
10	1.489467	1.061224	1.144525	1.231739	0.227052
12	1.765311	1.037384	0.929284	1.243993	0.454698
14	1.243514	1.056587	0.877084	1.059061	0.183227
16	1.487458	0.862196	0.732334	1.027329	0.403738
18	1.259133	0.738842	0.616608	0.871528	0.341195
20	1.093649	0.832602	0.43603	0.787427	0.331129
22	1.043892	0.63282	0.277054	0.651256	0.383751
24	0.888113	0.566851	0.138607	0.53119	0.376023
26	0.748547	0.434911	0.105063	0.429507	0.321776

Table E-18. The final biomass concentration (g/L) of the cultures grown with an addition of sodium acetate for the akinete triggering experiment

	A	B	C	Average	Standard Deviation
Additional sodium acetate	0.1764	0.1812	0.1459	0.1678	0.0192

APPENDIX F

CALCULATIONS OF REQUIRED AREA FOR IMPLEMENTATION AS A BIOFERTILIZER

The area required for growth of the cyanobacterium, *Anabaena cylindrica,* to replace current usage of nitrogen on three different scales was calculated. The concentration of nitrogen per volume used in the calculation was 0.0742 g N/L, as measured in the study. A pond depth was assumed as 12 inches to allow for light penetration. According to the USDA, 12.8 million tons of nitrogen fertilizer was used in 2011. To produce that same amount of cyanobacterial biofertilizer, about 200,000 square miles of raceway ponds would be required. To replace all nitrogen fertilizer used in the state of Montana, as stated by the Montana Department of Agriculture, with cyanobacterial biofertilizer, 206,000 tons will need to be produced requiring 3,200 square miles of raceway ponds. Lastly, assuming a 5 lb N per 1,000 square feet dose of nitrogen fertilizer for the average 1/3 acre yard; a pond of 15,673 square feet is needed.

Sample Calculation:

- 12,840,000 tons of N fertilizer = 25,680 million lb N fertilizer per year
- In the study, we obtained 0.0742 g N/ L, which is equal to 0.000612 lb N/ gallon
- A total volume of raceway pond is 4.20×10^{13} gallon
- Assuming a 12 inch pond depth, the total area required is about 200,000 square miles