LIPID PRODUCTION IN ALGAE STRESSED WITH SODIUM BICARBONATE AND SODIUM CHLORIDE

by

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

of

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in

Chemical Engineering

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ABSTRACT

Microalgae may play an important role in the path to a more sustainable future by producing valuable hydrocarbons using inorganic carbon, sunlight, and non-food source competitive supplies of nitrogen and phosphorus. The prospect of growing microalgae for the production of a stable and dependable source of biofuel is plausible only if done at scale with intricate attention applied to the biochemistry, geochemistry, and environmental conditions of each system. Extreme environments with low proton activity and high salinity conditions may harbor microalgae suitable for large scale outdoor cultivation.

Several algal isolates native to Soap Lake in Washington State were screened for biofuel potential and three isolates were selected for further studies. These three isolates were characterized to assess impacts on biofuel production studying high ionic strength in the form of sodium chloride (NaCl) in excess of 18g/L, and carbon supplemented treatments through the addition of inorganic carbon in the form of sodium bicarbonate ($NaHCO_3$). Further, the ability of $NaHCO_3$ and NaCl to trigger lipid production was determined. The study was centered on understanding differences between two factors that will likely have implications in large-scale algal raceway ponds: inorganic carbon limitation, speciation, or bioavailability, and evaporative conditions resulting in high concentrations of salt.

In this study, cell concentration, cell dry weight, nitrate, pH, biofuel potential, extractable lipid potential, and DIC (dissolved inorganic carbon), were monitored over time. Isolate GK5La grown in standard medium had the highest concentration of cell dry weight at the end of the study. Cultures supplemented with sodium bicarbonate were determined to be the most efficient way to produce biofuel in the form of extractable lipids. Supplementation with sodium bicarbonate and spiking to a concentration of 18g/L sodium chloride showed to be the most productive way to make triacylglyceride (TAG). Fatty acid methyl ester (FAME) production on a concentration basis was greatest in the control treatment grown in standard medium.

INTRODUCTION

"Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs," (United Nations 1987).

This quote has a quality that resonates throughout present humanity. In the 21st century, civilization is awakening to a reality that the earth is not an endless resource capable of withstanding and tolerating abuse, but can be likened to a determined space with finite supplies. Collectively moving forward, consciousness toward future generations must guide our present society's moral compass more than growth in Gross Domestic Product (GDP) and increasing wealth for a minor fraction of the world's population.

With the global population projected to reach nine billion by 2045 (Kunzig 2011), resources used to sustain civilization may become limited; from the food consumed to the energy used to power our lights. Increased consumption and world population may combine to create a place unrecognizable by most inhabitants today if public policy does not change its current course. The United States is a fertile location for innovation and has the opportunity to lead the world in renewable energy technology, encouraging sustainable growth both domestically and abroad. Switching our energy reserves from nonrenewable organic carbon compounds (coal, natural gas, and petroleum) to cost competitive renewable sources such as solar, wind, hydropower, geothermal, and biomass (Painuly 2001) may help to preserve the earth for many more generations to come.

All of the sources listed above have strengths and weaknesses when considered as an alternative source of energy for the future. Solar energy is intermittent as the sun does not shine 24 hours a day in most locations and energy storage systems are still progressing (Shigeishi et al. 1979; Farid et al. 2004). Wind is an indirect form of solar energy with great on shore potential ($20{,}000 \times 10^9$—$50{,}000 \times 10^9$ kwh per year), however, it can be unreliable and must be stored during non-peak energy hours (Joselin Herbert et al. 2007). Geothermal resources may require a large capital investment, and those with the highest energy potential are restricted to particular geographic location, usually located near tectonic plate boundaries (Barbier 2002). Biomass requires large areas of land to grow crops and may be food source competitive depending on the type of plant produced (Field et al. 2008); however, diversity is vast among biology.

Corn ethanol has been the domestic biofuel of choice by the United States thus far due to the large swaths of land in the Midwest dedicated to its production, and an aggressive lobby in the United State's Congress. It has been successful, and in 2009, 90% of biofuel on the market was produced from corn and sugarcane ethanol (REN 21 2009), however, several drawbacks exist for this type of biofuel. Corn ethanol is food source competitive and much of the energy and carbon are used to produce the stalk and leaves of the plant. Even with corn ethanol short comings, it has helped to usher in the next generation of biofuels capable of being produced domestically and maybe even replacing fossil fuel use.

Microalgae present the potential of a reliable biofuel feedstock that can sustainably produce a domestic energy resource that reduces the United State's dependence on foreign oil, and does not appreciably contribute to global warming

through increasing atmospheric carbon dioxide levels (Cox et al. 2000). Microalgae can benefit society through several distinct venues including: energy, synthesis of high value chemical compounds, and treatment of wastewater for removal of nitrogen and phosphorus. Primarily considering energy, algae can either be harvested for their high neutral lipid content extracted as a drop-in fuel for conventional gasoline following a hydrotreating process (Ghasemi et al. 2012), or the biomass can be converted to fatty acid methyl ester (FAME) or biodiesel through transesterification with an acidic or basic catalyst (Huber et al. 2007; Chen et al. 2012). Two other energy sources that can be produced from microalgae are biohydrogen and methane by anaerobically digesting the algal biomass (Chisti 2007).

Transesterification is the reaction used to make biodiesel, a mix of different chain length fatty acid methyl ester (FAME) carbon chains, capable of being run in diesel engines (Gong and Jiang 2011). The upside to making biodiesel from algae is that both nonpolar and polar lipid fractions are transesterified to create the product. This work will consider extractable lipid produced in the form of FFA, MAG, DAG, and TAG for hydrotreating to produce an upgraded fuel, and FAME produced by transesterification to produce conventional biodiesel.

Though specific growth rates of eukaryotic organisms lag behind those of bacteria, the speed at which they grow may be fast enough to harvest algae from raceway ponds in a reasonable time to feasibly produce biofuel (Slade and Bauen 2013). Furthermore, it has been demonstrated that microalgae can be stressed in multiple ways to accumulate a wide variety of lipid molecules. Nutrient limitation stresses have been shown to increase lipid content and include nitrogen (Wang et al. 2009), phosphorus

(Valenzuela et al. 2012) (Stockenreiter et al. 2011), and silica limitation (Hildebrand et al. 2012). Nutrient and non-nutrient related stresses such as addition of sodium bicarbonate and sodium chloride respectively, have also been shown to induce microalgae to accumulate stores of energy rich compounds such as starches and oil rich lipid bodies (Gardner et al. 2012; Mutanda et al. 2011). Additionally, organic carbon sources such as sugars can increase biofuel potential (Liang et al. 2009). Identifying and characterizing alga isolates that can be stressed to accumulate lipid through a sodium chloride trigger, similar to the sodium bicarbonate trigger (Gardner et al. 2012; Valenzuela et al. 2012), would be beneficial in commercial scale open pond algal cultures, as evaporation may increase salt concentration.

The purpose of this study was to isolate and characterize algal strains obtained from Soap Lake, Washington. These strains may have the potential to be used in outside raceway ponds for biofuel production. Further, understanding the effects of sodium chloride and sodium bicarbonate on the rate and extent of lipid accumulation could improve the lipid productivity of algal cultures. Initial screens of algal isolates were performed in flasks and assessed growth, lipid accumulation (indicated by Nile Red fluorescence), and pH of the system. Three strains were chosen based on performance in the initial screen and were scaled up to 1.25-liter photo bioreactors.

BACKGROUND

Biofuel

The use of finite fossil fuel reserves as the main source of energy and chemical feedstock worldwide threatens the quality of life for future generations. Increased use of fossil fuels leads to a higher concentration of carbon dioxide in the atmosphere that has been linked to global warming (Cox et al. 2000), a serious issue that could put a large percentage of the world's population at risk. The dependency of growing economies on the production of crude oil produced in unstable regions of the world sets the stage for even more international conflict. The reliance on an energy source that is finite, becoming scarcer, difficult to extract economically, and increasing in price, may negatively impact domestic industries. Even more so, with an ever increasing population seeking a higher standard of living like that enjoyed by people in first world economies, the consumption of fossil fuels will increase (Turner 1999). The economic system of capitalism does not always operate with a moral compass (Evensky 2005), and to circumvent certain plights in the future, a viable alternative energy source must be developed. Biofuel derived from algae is a sustainable and viable alternative to fossil fuel (Chisti 2007).

Biodiesel is produced by transesterifying free fatty acids, monoacylglycerides, diacylglycerides, triacylglycerides, and polar phospholipids stemming from membranes, to produce methyl ester fuel molecules and byproduct glycerol (Vijayaraghavan and Hemanathan 2009). The term "biodiesel" refers to mono-alkyl esters derived from long

chain fatty acids that are capable of being burned in diesel engines with little or no modification.

"Drop-in" fuels can be blended with conventional fuels and refined much the same. Raw non-polar lipids are subjected to a hydrotreating process in which the glycerol backbone from the triglyceride molecule is converted to propane and the remaining lipid chains are converted to straight chained fully saturated fatty acids (Huber et al. 2007). Hydrotreating in oil refineries is traditionally used to remove impurities from inlet streams including S, N, and heavy metals. The hydrotreating process is a way to upgrade algal lipids to straight chain paraffin molecules for use in conventional fuels.

Water/Salinity

Water provides an environment essential for algal growth and reproduction, photosynthesis, and essential nutrient availability of inorganic carbon, nitrogen, and phosphorus (Murphy and Allen 2011). Irrigation for terrestrial crops in the United States is the foremost use of freshwater supplies and accounts for up to 85% of total consumptive water use (Pate et al. 2011; Resources, Studies, and Sciences 2012). Estimates place the amount of water used to produce 1 gallon of algal derived biodiesel between 500 and 3400 gallons of water (Yang et al. 2011).

Considering that only 3% of the total water on earth is freshwater (Stiassny 2011), it is imperative to utilize non-fresh water sources when culturing algae to avoid stressing an already constrained resource. The largest and most easily tapped source of non-potable water can be found in the earth's oceans. Conveniently, the United States is bordered by both the Atlantic and Pacific oceans that together offer a practically limitless supply of

ocean water with the investment of pipeline infrastructure. Another water source exists in saline aquifers residing under the continental United States (Figure 2.1).

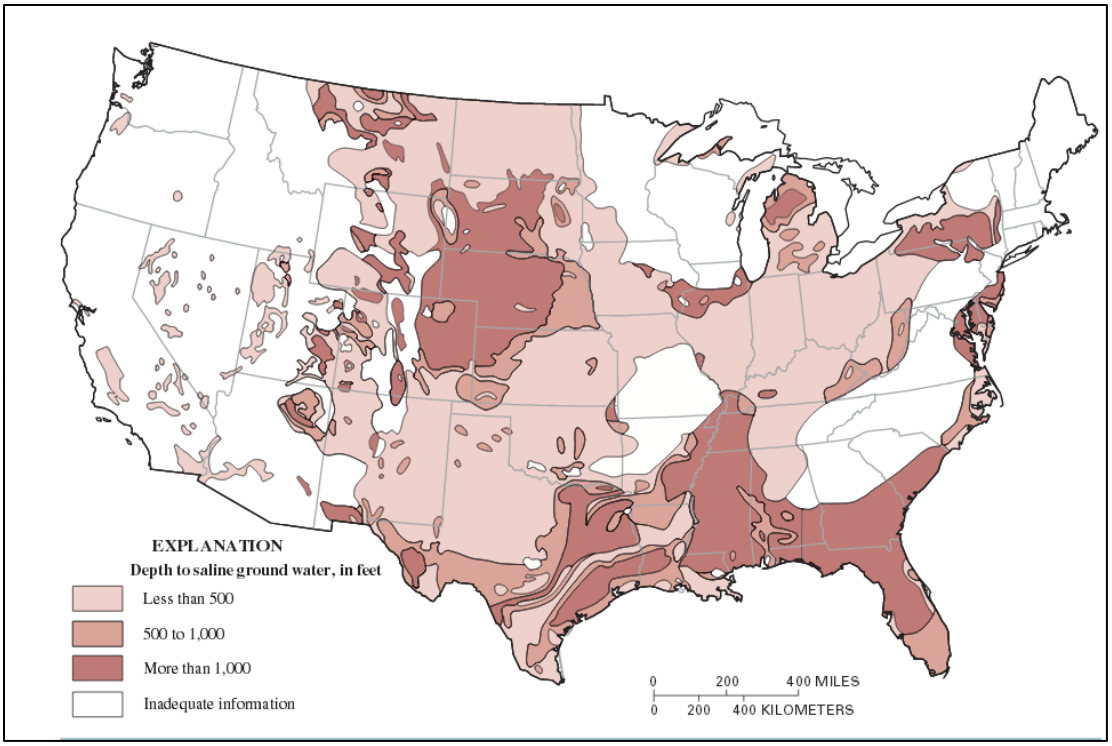


Figure 2.1 Map of the United States indicating locations of depth to saline aquifers beneath the surface.
Source: http://pubs.usgs.gov/fs/fs075-03/pdf/AlleyFS.pdf (USGS 2003)

Saline aquifers are a resource that are mined in parts of the desert southwest (Subhadra and Edwards 2010). Often times saline and freshwater aquifers are connected in the subsurface. The development and use of saline aquifers affect fresh water resources due to hydraulic connectivity within the aquifer system that includes freshwater (USGS 2003). Caution must be taken when pumping from saline aquifers to insure neighboring freshwater aquifers are not affected by drawdown or hydraulic gradients. Changes to the hydraulic gradient in the system through pumping will likely impact the freshwater

supply at some point in time, making understanding the issue imperative before any ground water resources are exploited (USGS 2003).

Hypersaline environments or brines are loosely classified as exceeding salt concentrations of 10% or 100g/L TDS (total dissolved solids) (Litchfield 1998). Hypersaline bodies of water exist on earth in such notable places as Mono Lake in California and The Great Salt Lake in Utah. These salty lakes and inland seas are formed as water flows into depressions lacking outlets, leaving the only mechanism for water to leave is through evaporation.

Microbial communities in saline systems are represented by each of the three domains of life; *Bacteria*, *Archaea*, and *Eukarya*. The salt tolerant microorganisms indigenous to these environments are referred to as halophiles. Halophiles come in two different types: obligate halophiles and those that are better described as halotolerant (Litchfield 1998). Increasing salinity affects microorganisms in three distinct ways: osmotic stress, ionic stress, and changes in cellular ionic ratios (Kirst 1989). Distinguishing between each of these stresses is difficult because they are all related through an increase in salinity. For most cells, introduction to an environment in excess of 0.2 M of salt would lead to dehydration and eventually death as water exits the cell due to osmosis. Halophiles avoid osmotic stress through the accumulation of compatible solutes and ions within their cellular bodies that counteract the effect of high salinity (Kumar and Bandhu 2005). The United States has several salt and alkaline lakes that should present ideal locations to isolate and study algal strains that have the potential for high lipid activity in saline ponds (Mutanda et al. 2011; Jones and Mayfield 2012).

Soap Lake

Located in the rain shadow of the Cascade Range and in the semi-arid environment of east-central Washington State, Soap Lake is a meromictic high alkalinity and salinity lake. Salinity has changed over time due to groundwater inputs from the Columbia Basin Irrigation project (Mundorff and Bodhaine 1954); but today it is near 17.5g/L at the top of the lake (Walker et al. 1975). The pH of the lake typically ranges from 9.8 to 10.2. The halocline layer exhibits a stark change of environments when considering chemical composition and density.

The Grand Coulee is an ancient riverbed that was formed from the cataclysmic floodwaters of Lake Missoula. The Grand Coulee is separated into the Upper Grand Coulee and Lower Grand Coulee by Dry Falls. From Dry Falls, a chain of lakes are formed that empty into one another: Deep Lake, Park Lake, Blue Lake, Alkali Lake, Lenore Lake, Little Lenore Lake, and finally Soap Lake. Salinity in the Grand Coulee increases north to south. In the upper Grand Coulee, minerals are dissolved in groundwater, and then are concentrated by evaporation as the water makes its way down the chain of lakes until the terminal Soap Lake is reached (Castenholz 1960). Soap Lake presents a promising location for isolating algal halophiles and alkalaphiles, however, the production of algal biofuel also depends on the nutrients the organism is able to utilize for growth.

Nutrient Considerations

Nitrogen

Algae consume nutrients including carbon, nitrogen, and phosphorus throughout their growth cycle (Provasoli 1958). The commonly used composition for algal biomass is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti 2007; Doran 1995). Using this composition, it can be estimated that nitrogen makes up roughly 3.3% of algal biomass produced, which may end up being a large amount of needed nitrogen if algal biofuel replaces a considerable part of the domestic transportation fuel supply. In biological systems, nitrogen is bioavailable in the form of ammonia, nitrate, nitrite, urea, or organic nitrogen. From a sustainability standpoint, the amount of nitrogen (in the form of nitrate, ammonia, or nitrogen gas) needed to sustain a large algal biofuel facility may make the prospect of using algal biofuel unfeasible. More concisely, without recycling nitrogen the amount of nitrate used to grow algae may make the entire process less sustainable and less cost effective.

Nitrogen is fixed industrially through the Haber-Bosch process, in which 1 molecule of nitrogen is reacted with 3 molecules of hydrogen over catalytic beds at high temperature and pressure (Galloway and Cowling 2002; Pate et al. 2011). The reaction was ground breaking as it provided an efficient way to fix nitrogen from the atmosphere to be used as fertilizer to grow crops, and in turn, truly shaped human society today (Mulder 2003). More than half of the food in the world eaten today is produced using nitrogen fertilizer (Galloway and Cowling 2002). If algal derived biofuels made up a significant portion of the United States energy consumption, there would certainly be

strain placed on nitrogen markets that would affect prices of agriculture due to increased demand of fertilizer (National Academy of Science 2012).

Phosphorus

Phosphorus is another essential nutrient that must be supplied to algal operations to sustain growth in ponds. It is needed to form deoxyribonucleotides (DNA), Ribonucleotides (RNA), and phospholipids in the algal cellular membrane. Phosphorus makes up less than 1% of algal biomass using the composition $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti 2007; P 1995). Phosphorus is a resource primarily recovered through open pit mining throughout the world (Cordell et al. 2009). Concern has grown over the supply of phosphorus. Peak production should be met within the next 50 to 100 years and will decrease thereafter as reserves are further removed (Cordell et al. 2009).

More concisely, supplies are limited and use is increasing, however, outlook for phosphorus is not necessarily bleak. Cellular solids left over from algae, manure, and human wastewaters all contain phosphorus. The extraction and recycling of phosphorus from these low value feedstocks may lead to more sustainable agriculture and biofuel production. Remaining cellular solids from algae after the lipids have been extracted can be broken down further within anaerobic digesters. After carrying out a thorough analysis on nitrogen and phosphorus requirements for scaled up algal raceway ponds, Pate et al. (2011) concluded that without recycling the process would be unsustainable due to the demand on natural resources (Cordell et al. 2009; Vaccari 2009). If nitrogen and phosphorus in left over harvest water are recycled back through the process, the sustainability of the operation could increase dramatically (Rösch et al. 2012).

Carbon

To increase biomass and synthesize lipid molecules, algae must have a source of carbon. Carbon comes in three different forms: inorganic, organic, and synthetic. Organic carbon sources are derived from plants and animals. Algae that can utilize these for growth are referred to as mixotrophic or heterotrophic. Inorganic carbon sources are those derived from minerals or gases such as carbon dioxide. Organic and synthetic forms are not considered and only inorganic forms are discussed and studied in this thesis.

Dissolved inorganic carbon in solution comes in the following states: aqueous carbon dioxide, carbonic acid, bicarbonate, and carbonate. Aqueous carbon dioxide, carbonic acid, and bicarbonate are the most bioavailable forms that can be used for the production of biofuel from microalgae (Giordano et al. 2005). The concentration of carbon dioxide in the atmosphere is rising due to anthropogenic emissions from the combustion of fossil fuels (Cox et al. 2000). Increasing levels of carbon dioxide in the atmosphere and carbonic acid in the oceans, are a result of growing economies and rising standards of living ever since the industrial revolution, and have recently been linked to climate change (Cox et al. 2000).

For algae, inorganic carbon is assimilated into biomass through carbon concentrating mechanisms. Carbon has been suggested to limit growth of algae due to the half saturation of RUBISCO (Urabe et al. 2003). The half saturation constant (K_m) for RUBISCO in plants ranges between 15 and 25 μM and can even exceed 200 μM in some cyanobacteria (Moroney and Somanchi 1999).

Introduction of a new technology that produces fuel primarily through the consumption of carbon dioxide may limit the extent of climate change through reduction of greenhouse gas emissions. Algal derived biofuels have the potential to greatly reduce greenhouse gas emissions and in some cases even become carbon negative after applying spent carbon to soil after extraction (Mathews 2008).

Lipid Induction Stresses

Algae are grown in raceway ponds or photobioreactors until stationary phase, and then stressed to accumulate lipid by pH, light intensity, nitrogen limitation, temperature, salt stress, sodium bicarbonate trigger, and culture age (Gardner et al. 2011; Boussiba et al. 1987; Gardner et al. 2012). The operation is conducted in this way because more lipids are produced under unfavorable or stressed conditions (Hu et al. 2008). The four most useful stresses studied in this work are nitrogen limitation, pH stress, sodium bicarbonate addition, and salt stress (sodium chloride).

Nitrogen Limitation

Nitrogen limitation may be the most practiced method to increase both extractable lipid potential and biofuel potential of microalgae. After nitrogen concentration in solution diminishes (in the form of ammonium, nitrate, or urea), the algal cell cycle changes from a pathway of rapid reproduction and division, to the pooling of carbon in the form of lipid molecules as opposed to starches or proteins (Wang et al. 2009). Nitrogen limitation also decreases photosynthetic efficiency through the degradation of chlorophyll and increase in carotenoid pigments (Berges et al. 1996). Nitrogen limitation

is a viable way to increase microalgal biofuel production; however, it comes with the constraint that lipid molecules are synthesized as secondary metabolites, after cells have reached stationary phase and have run out of nitrate. From a commercialization standpoint, waiting a relatively long time (at least 7 days in a culture of *Nannochloropsis*) (Bondioli et al. 2012) for nitrogen to run out in solution before neutral lipids are accumulated decreases the economic viability of the process. Research has and is currently being carried out to find other stress mechanisms to more efficiently produce biofuel from algal lipid.

Sodium Bicarbonate

Sodium bicarbonate is a form of inorganic carbon that previous studies have shown can increase biofuel production in industrial microalgae strains (Gardner et al. 2013). Studies have shown sodium bicarbonate can be used as a trigger to increase lipid production as indicated by Nile Red fluorescence in *Scenedesmus* sp. WC-1 and *Phaeodactylum tricornutum* (Gardner et al. 2012). At concentrations above 50mM, sodium bicarbonate stops cell replication and stresses microalgae into accumulating lipids (Gardner et al. 2012). Furthermore, the addition of sodium bicarbonate in excess of 23.8mM (2g/L) at the beginning of growth has been shown to increase FAME production along with chlorophyll and carotenoid pigments (White et al. 2012). Low dissolved inorganic carbon concentrations have been shown to inhibit growth (Gardner et al. 2013), so the addition of carbon to solution in raceway ponds may need to be monitored and occur frequently to keep carbon limited conditions from occurring. The addition of more

inorganic carbon will increase the ionic strength in solution, and may potential cause another stress to induce lipid accumulation.

Salt Stress

A likely place to invest in infrastructure to build algal production facilities lies in the deserts of the world that receive ample amounts of sunshine where land is relatively flat, cheap, and co-located near seawater. Some examples of likely places may be Southern California in the United States or the outback of Australia. In these locations, evaporation may have a large impact on raceway ponds with high surface area to water ratios. This is largely unavoidable, but increasing salinity as a result of evaporation may aid in the productivity algal biofuel facilities. This is because increased levels of sodium chloride have been shown to reduce photosynthetic activity, limit cell growth, and induce lipid production in a number of aquaculture organisms including: *Scenedesmus*, *Botryococcus*, *Dunaliella*, and *Nannochloropsis* (Pal et al. 2011; Rao et al. 2007; Kaewkannetraet al. 2012; Takagi et al. 2006). In the case of *Dunaliella*, growth was not inhibited and lipid content increased from 60% to 67% of the cell dry weight after increasing the concentration of NaCl from 0.5M to 1M (Takagi et al. 2006).

High salinity conditions are managed within higher plants through several mechanisms including: selective accumulation/exclusion of ions, control of specific ion uptake, compartmentalization of ions on the cellular level, production of compatible solutes, changes in photosynthetic pathways, induction of antioxidative enzymes, and induction of hormones (Kumar and Bandhu 2005). With this slew of evolutionary traits,

plant species are adept in controlling and mediating changing environments with respect to salinity.

Though all of the above mechanisms are important, Na^+/H^+ enzyme antiporters may be the most intuitive mechanisms relating to the regulation of ionic homeostasis and play an important role in overall cell well-being. Antiporters aid in the transport of ions (Na^+/H^+) into and out of the cell to maintain proper ion concentrations. Salt strain leads to a high ratio of potassium to sodium ions in the cell's cytoplasma in order to alleviate ionic stress. This is mediated through K^+ and Na^+ transporters and H^+ pumps that generate a driving force to establish the high ratio of potassium to sodium (Zhu 2001). Excess calcium ions in solution seemingly increase the ratio of potassium to sodium while decreasing the toxic impact on cellular tissue (Zhu et al. 1998; Liu and Zhu 1997; Rengel 1992). A common trait among organisms of varying halotolerant backgrounds is the use of vacuoles and older cell tissue to accumulate excess ions to keep ionic concentration in the cytoplasma low and regulated (Kumar and Bandhu 2005). Compatible solutes such as glycerol are synthesized as well and stored in the cytosol.

METHODS

Throughout the study several measurements were made to track key variables over time. This section provides a detailed analysis of the techniques used in each experiment to collect and record data.

Sampling and Media Types

Robert Gardner and Kelly Kirker carried out sampling for algal isolates from Soap Lake Washington. Enrichments were made and started by Rob Gardner. Several different media types were used to culture and study different isolates. The different media types used in the preliminary screen were: AM6, AM6SIS, AM6(1.8), AM6SIS(1.8), AsP_2(1.8), AM6 (5.1), AM6SIS(5.1), and AsP_2(5.1). AM6 was the control medium used throughout the study. AM6SIS was AM6 medium adjusted to allow growth of diatoms through the addition of 2mM sodium metasilicate and vitamin solutions. AM6(1.8) and AM6SIS(1.8) were the basic media with 1.8% sodium chloride. Similarly, AM6(5.1) and AM6SIS(5.1) were the basic media with 5.1% sodium chloride. The AsP_2(1.8) used in the study was a marine medium. AsP_2(5.1) was that same marine medium with 5.1% sodium chloride. Below is the detailed medium composition.

Table 3.1. AM6 medium composition

Component	Amount in medium (g/L)
Sodium Nitrate	0.33
Magnesium Sulfate-$7H_2O$	0.075
Calcium Chloride-$2H_2O$	0.025
Sodium Chloride	0.025

Table 3.1 - Continued

Ferric Ammonium Citrate	0.01
Potassium Phosphate	0.25
Sodium Carbonate	0.25
Trace Element Solution	1mL

Table 3.2. AM6SIS medium composition

Component	Amount in medium (g/L)
Sodium Nitrate	0.33
Magnesium Sulfate-7H_2O	0.075
Calcium Chloride-2H_2O	0.025
Sodium Chloride	0.025
Ferric Ammonium Citrate	0.01
Potassium Phosphate Dibasic	0.25
Sodium Carbonate	0.25
Trace Element Solution	1mL
Sodium Metasilicate-9H_2O	0.5684
Vitamin B12 Solution	1mL
S3 Vitamin Solution	1mL

Table 3.3. AsP_2(1.8) medium composition

Component	Amount in medium (g/L)	Stock Solution Concentration (g/L)
NaCl	18	-
$MgSO_4$-7H_2O	5	-
KCl	0.6	-
$CaCl_2$-2H_2O	0.735	-
Na_2SiO_3-9H_2O	0.15	-
Na_2EDTA	0.03	-
$NaNO_3$	0.05	-
H_3BO_3	1mL	34
K_2HPO_4	1mL	5
$FeCl_3$-6H_2O	1mL	3.84
$ZnCl_2$	1mL	0.313
$MnCl_2$-4H_2O	1mL	4.32
$CoCl_2$ -6H_2O	1mL	0.012
$CuCl_2$ -H_2O	1mL	0.003

Table 3.3 - Continued

Vitamin B12 Solution	1mL	0.002
S3 Vitamin	1mL	-

Table 3.4. $AsP_2(5.1)$ medium composition

Component	Amount in medium (g/L)	Stock Solution Concentration (g/L)
NaCl	18	-
$MgSO_4$-$7H_2O$	5	-
KCl	0.6	-
$CaCl_2$-$2H_2O$	0.735	-
Na_2SiO_3-$9H_2O$	0.15	-
Na_2EDTA	0.03	-
$NaNO_3$	0.05	-
H_3BO_3	1mL	34
K_2HPO_4	1mL	5
$FeCl_3$-$6H_2O$	1mL	3.84
$ZnCl_2$	1mL	0.313
$MnCl_2$-$4H_2O$	1mL	4.32
$CoCl_2$-$6H_2O$	1mL	0.012
$CuCl_2$-H_2O	1mL	0.003
Vitamin B12 Solution	1mL	0.002
S3 Vitamin Solution	1mL	-

Table 3.5. Trace element solution composition.

Component	Quantity (g/L)
Boric Acid	0.6
Manganese Chloride-$4H_2O$	0.25
Zinc Chloride Anhydrous	0.02
Copper Chloride-$2H_2O$	0.015
Sodium Molybdate-$2H_2O$	0.015
Cobalt Chloride-$6H_2O$	0.015
Nickelous Chloride-$6H_2O$	0.01
Vanadium Pentoxide Anhydrous	0.002
Potassium Bromide	0.01

Table 3.6. S3 Vitamin solution composition.

Component	Quantity (g/L)
Inositol	5
Thymine	3
Thiamine HCl (B1)	0.5
Nicotinic acid (niacin)	0.1
Ca pantothenate	0.1
p-Aminobenzoic acid	0.01
Biotin (vitamin H)	0.001
Folic Acid	0.002

Algal Isolation and Culturing

Algae were isolated from the basic media outlined above. Media were amended with 2% agar prior to autoclaving when pouring agar plates for isolation purposes. Though not all algal strains can grow on agar plates, the method was used because it is often seen as the most reliable and cost effective method for the isolation of microorganisms. A titanium loop was flame sterilized and dipped into liquid enrichment prior to streaking on an agar plate. Algal colonies were grown with indoor ambient air and under fluorescent lighting in a 14-10 light cycle until colonies appeared on the plates from each of the media types. Unialgal colonies were transferred to liquid media by the use of a sterile Pasteur pipette. This transfer technique included melting and stretching a Pasteur pipette in a Bunsen burner, allowing the pipette to cool long enough to break, in part creating a new finer tip, and then extracting colonies from the plate by picking and transferring to 1ml of sterile media. After a thick culture had grown in the test tube, the

procedure was repeated by streaking the new culture onto sterile agar medium. This process was continued for three times until a pure axenic culture was obtained.

To verify each of the isolates were free of bacteria, agar plates were made for each of the media types with the addition of 0.5 g/L yeast extract and 0.5 g/L of glucose. A 100 μL aliquot of the culture was spread onto the plate and given enough time for the liquid to dry. The plates were stored in a cardboard box away from light. If no colonies were found growing on the plates within 10 days, the culture was deemed clean of bacterial contamination. If algal colonies were found growing on the plates it added evidence that the particular strain was capable of heterotrophic growth.

Cell Counts

The most sensitive method used to monitor growth of each of the algal isolates was through counting cells after placement onto a hemacytometer (Andersen 2005). For some isolates, sonication was needed to break up aggregated clumps of cells that could not be counted. Under the microscope, cells were counted in each of the four squares until at least 400 cells were counted to provide an accurate representation of the sample based upon statistics (Andersen 2005).

Cell Dry Weight

A 15ml falcon tube was weighed prior to centrifuging 10mL of sample culture at 1380xg. The pellet was washed twice with 5mL of deionized water before being frozen at -20°C. Pellets were dried through lyophilization for 24 hours to ensure all water had

sublimated. A final mass of the tube was determined on the balance and the difference was defined as cell dry weight for that sample.

Optical Density

A BioTek PowerWave XS spectrophotometer (Bio-Tek Instruments, USA) was used to measure absorbance in each culture to measure growth over time. Absorbance was read at 750nm to minimize chlorophyll a and b interference in the measurement. The reference absorbance used was un-inoculated media measured at 750nm.

Nile Red

Nile Red (9-diethylamino-5H-benzo[alpha]phenoxazine-5-one) (Sigma Aldrich, USA) fluorescence measurements were modified and adapted from Cooksey et al. (1987). A Nile Red stock solution was prepared by adding 0.5 mg of Nile Red to 10mL of acetone. Four microliters of Nile Red stock solution was added to 1mL of dispersed culture (aggregations were sonicated until a homogenous suspension was obtained). Samples were diluted either 1:5, 1:10, or 1:20 depending on the cell concentration to ensure fluorescence values were recorded in the linear range. Cell concentrations had to be diluted to avoid 'self-quenching' leading to inaccuracies in reported Nile Red fluorescence values. The stain time prior to reading fluorescence was 15 minutes.

Specific Nile Red fluorescence was determined by dividing Nile Red fluorescence by the number of cells determined using a hemacytometer and multiplying by 1000. The factor of 1000 in the calculation yields a value of Nile Red fluorescence per 1000 cells.

pH

The pH of each solution was measured using an Accumet AP71 pH probe. The pH probe was calibrated before each use to ensure accurate measurements were recorded. The pH was checked at each sample point

Chlorophyll Determination

Hot Ethanol Extraction

Chlorophyll extraction and quantification measurements with ethanol were adapted from Harris (1989). Hot ethanol extraction was used to spectrophotometrically estimate chlorophyll a, b, and total chlorophyll concentration from algal cultures over time. In this modified method, 1mL of culture was added to a 1.5mL microcentrifuge tube, centrifuged at 16,000xg for 5 minutes, and the aqueous medium was separated from the pellet by pipetting. One milliliter of 95% ethanol was added to the pellet and vortexed for 10 seconds. The microcentrifuge tube was immersed in a 80°C hot water bath for 10 minutes, after which, it was once again vortexed. The microcentrifuge tube was then centrifuged for 3 minutes at 16,000xg and 200µL of the supernatant was removed and dispensed into a clear 96 well polystyrene plate. Absorbance was read at 665nm and 649nm to determine chlorophyll a, b, and total chlorophyll concentration.

Hot Methanol Extraction

Quantification of chlorophyll a, b, total chlorophyll, and total carotenoid concentrations in methanol was adapted from Ordog et al. (2011). Mantoura and Llewellyn (1983) suggest chlorophyll concentration is underestimated using methanol as

a solvent, however, the method worked well to extract pigment from green algal cells, and estimate total carotenoid content. Thus the method was later employed over hot ethanol extraction. Absorbance was read at 666, 653, and 470 nm, to estimate carotenoid concentration and chlorophylls a, b, and total concentrations.

IC Measurements to Determine Nitrate, Phosphate, and Sulfate

To record precise measurement of nitrate, phosphate, and sulfate concentrations over time, ion chromatography was used to quantify concentrations of these anions in the media. An IonPac AS9-HC Anion-Exchange Column (Dionex, USA) with a 9mM sodium carbonate buffer set at 1mL per minute was used to elute and separate different ions moving through the column. The conductivity detector was a CD20 (Dionex, USA) and the temperature for was set at 21 °C. Chromelion software (Thermo Fisher, Waltham, MA) was used to analyze data from the IC. For media with sodium chloride concentrations in excess of 18 g/L, the chloride peak on the anion column was so large nitrate could not be determined by IC.

Nitrate for High Salt Media

To quantify nitrate concentrations for media containing high sodium chloride (excess of 18g/L), the NAS Szechrome (Polysciences, Warrington, PA) assay was employed. The range of the NAS Szechrome reagent was between 0 and 25 mg/L nitrate, making 1:10 and 1:20 dilutions necessary for early time points in experiments. One milliliter of culture volume was transferred into a microcentrifuge tube and centrifuged at 16,000xg for 3 minutes. The supernatant was separated from the pellet through pipetting,

and transferred to a new microcentrifuge tube. Depending on the expected concentration of nitrate, dilutions were made at this step (1:5, 1:10, or 1:20). Then a 100µL volume of sample was pipetted into a microcentrifuge tube, and 1mL of the prepared NAS reagent was added. After incubation between 10 to 60 minutes, 200 µL of sample was added to a clear polystyrene well plate and read at 450nm. A standard curve of known nitrate concentrations in the media solution was analyzed on every well plate to calculate unknown culture nitrate concentrations.

18S DNA Extraction and Identification

Extraction

A 10mL culture volume was centrifuged in a 15mL conical plastic tube for 5 minutes at 1380xg to pellet the algal biomass. Biomass was resuspended in 1mL of sterile nanopure water and then transferred to a 2mL conical screw cap microcentrifuge tube and centrifuged for 1 min at 14,500xg, after which the supernatant was separated from the pellet and discarded. A volume of 200 µL of extraction buffer (1M NaCl, 70mM Tris, 30mM NaEDTA, pH 8.6) was added to the tube and centrifuged for 1 min at 14,500xg. The supernatant was discarded using a sterile pipette tip. Then 500 µL of extraction buffer was added along with enough glass beads to fill the conical bottom of the microcentrifuge tube, 200 µL of chloroform, and 125 µL of 2% CTAB (Cetyltrimethyl Ammonium Bromide) extraction solution. The contents were agitated in a Fast Prep shaker on setting 6.5 for a total of 45 seconds. After centrifuging at 12,000xg at 4°C for 15 min, 0.6 mL of the aqueous phase was removed and transferred to a new microcentrifuge tube. To the microcentrifuge tube, 40 µL of 3M-sodium acetate and 480

µL of 95% ethanol were added. The contents were mixed by vortexing and left at -20°C overnight.

The next day, the extracted DNA was pelleted at 12,000xg for 15 minutes at 4°C, and the supernatant was discarded. The pellet was washed with 20 µL of 80% ethanol and centrifuged again at 12,000xg for 15 minutes at 4°C, and the supernatant was discarded. The tubes were allowed to air dry under a sterile hood, and then the DNA was resuspended in 50 µL of Tris-HCl.

Amplification

DNA concentration was quantified using Qubit® (Grand Island, New York). The extracted DNA was amplified with the following 18S primers UNI7F (5'ACCTGGTTGATCCTGCCAG 3') and 1534R (5'TGATCCTTCYGCAGGTTCAC 3'). A total of 5µL of sample was added to 25µL of GoTAQ® (Promega, USA) Green Master Mix, 5µL of bovine serum albumin (BSA), 2.5µL of forward primer, 2.5µL of reverse primer, and 15µLDNase/RNase free water, to make a total 50µL PCR reaction. The amount of sample DNA added to the PCR reaction was adjusted based on the assayed concentration. In the thermocycler, initial denaturation was at 95 °C for 2 minutes, followed by forty 30 second cycles at 94°C, 1 minute at 52°C, 1.15 min at 72°C, and a final extension of 7min at 72°C.

Gel Verification and Sequencing

The amplicons were run on a 0.7% agarose gel to validate size. Amplification product was cleaned using a QIAquick PCR Purification Kit (Qiagen). Samples were then submitted to Functional Biosciences (Madison, WI) for DNA sequencing, aligned

using Jalview (version 2.8). Then the identity was found through the use of Megablast searches (NCBI).

Dissolved Inorganic Carbon (DIC)

Ten milliliters of culture volume was filtered into a 13x100mm borosilicate glass tube. Then dilutions were made in deionized water before quantification with a prepared standard curve on a Scalar DIC analyzer.

Concentration of DIC was computed using a standard curve made from water sparged with nitrogen and mixed with a set amount of equimolar sodium carbonate and sodium bicarbonate to make dilutions between 0 and 250 ppm dissolved inorganic carbon. At each use, phosphoric acid was changed out, and the signal was auto zeroed to carbon to record analytically correct peaks from the instrument.

Lipid Analysis

Neutral Lipid Quantification

Extraction, analysis, and quantification of neutral lipid components was adapted from (Lohman et al. 2013). Neutral lipids were recovered through a modified Bligh and Dyer method (Bligh and Dyer 1959). Bead beating was used to rupture cells in the presence of chloroform. Neutral lipids were extracted into the organic solution (chloroform) and washed with a sodium chloride solution to separate any polar components.

A total of 10-30 mg of dry biomass was homogenized and added to a 2mL stainless steel bead beating tube. To the tube, 0.6 g of 0.1mm zirconium beads, 0.4 g of

1mm zirconium glass beads, and two 2.5mm zirconium glass beads were added. Additionally 1mL of chloroform was added after which the tube was capped and shaken on an MP FastPrep 24 (Solon, OH). The biomass was disrupted for six 20-second cycles at 6.5m/s to break cell membranes. The contents of the 2mL stainless steel tube were emptied into a disposable glass test tube. The stainless steel glass tube was washed with 1mL of chloroform twice, emptied into the same glass test tube, and followed by 1mL of 15% NaCl. The test tube was then vortexed for 10 seconds and centrifuged (1380xg) for 2 minutes, after which 1mL of the bottom solvent layer was collected and saved in a GC vial for analysis via gas chromatography flame ionization detection (GC FID; Agilent 6890N, Santa Clara, CA). A 15m (fused silica) RTX biodiesel column (Restek, Bellefonte, PA) was used for 1μL injections under a column temperature ramp from 100 to 370 °C. The carrier gas for this technique was helium and the flow rate varied throughout the process from 1.3 mL/min (0—22min), to 1.5 mL/min (22—24min) to 1.7mL/min (24—36min). Calibration curves were constructed using the following standards: C10:0, C12:0, C14:0, C16:0, C18:0, C20:0 free fatty acid (FFA); C12:0, C14:0, C16:0, C18:0 monoacylglyerol (MAG); C12:0, C14:0, C16:0, C18:0 diacylglycerol (DAG); and C11:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0 triacylglycerol (TAG) (Sigma—Aldrich, St. Louis, MO) for quantification.

FAME Quantification

Extraction analysis, and quantification methods were adapted from (Lohman et al. 2013). In situ transesterification was used to quantify the total amount and speciation of FAME extracted from the sample of dried biomass. A total of 5-15mg was measured into

a 16x100mm screw cap glass test tube where one mL of toluene and 2mL of sodium methoxide were then added. The contents were heated to 95°C for 30 minutes, with intermittent vortexing every 10 minutes during the interval. Then 2 mL of 14% boron trifluoride in methanol were added and the process was repeated for a second time. The test tubes were removed, and allowed to cool to room temperature. Then 0.8mL of sodium chloride saturated water, 0.8ml of hexanes, and 10µL of a C23 FAME was added to each test tube and the tube was vortexed for 10 seconds. The contents were heated for an additional 10 minutes before the phases were separated by centrifugation (1380xg) for 2 minutes. One mL of the organic top layer was collected, transferred into a GC vial, and saved for GC-MS analysis (Agilent 6890N GC and Agilent 5973 Networked MS). GC-MS analysis was carried out according to a published protocol (Bigelow et al. 2011). One-microliter samples were injected onto a 30m x 0.25mm Agilent HP-5MS column (0.25µm film thickness). The column temperature started at 80 °C and ramped at 14 °C/min to a final temperature of 310 °C where it was held for 3 minutes. The injector temperature was set at 250 °C and the detector temperature was set at 280 °C. Helium was the carrier gas and the flow through the column was set at 0.5mL/min. Calibration curves were constructed using a 28-component fatty acid methyl ester standard prepared in methylene chloride ("NLEA FAME mix": Restek, Bellefonte, PA). Quantifications of peaks were made with the nearest calibration standard based on retention time and were performed in Agilent MSD Chem Station software (Version D.02.00.275).

Experimental Setup

Preliminary Isolate Screen

All preliminary studies were conducted in 250ml baffled shaker flasks. The starting volume in each flask was limited to 150ml to provide proper mixing and aeration that facilitated growth, however this small volume restricted the amount of sample that could be extracted throughout the study. An example of the experimental setup can be found in Figure 4.2

Biological triplicates were not used in this screen, and instead each treatment was represented by one biological replicate. Each isolate was grown in the basic sample media [AM6, AM6SIS, $AsP_2(1.8)$, and $AsP_2(5.1)$] to evaluate growth characteristics. The growth characteristics measured were pH, cell dry weight, and the amount of lipid accumulated as indicated by Nile Red fluorescence at a gain of 100, when exposed to varying environmental conditions. The gain on the instrument amplifies the signal. Later on in the scaled up experimental set up, the gain was set at 75 to minimize the signal to noise ratio.

The length of each study was approximately four weeks, but differed based upon a number of factors including growth rate, lipid accumulation, final volume, and time available. As this was a qualitative microalgae isolate screen for biofuel potential, evaporation was not accounted for. Sampling included withdrawing an aliquot of 3ml from each shaker flask to monitor: pH, Nile Red fluorescence, cell counts, and optical density at 750nm.

In Depth Scaled Up Studies

To retrieve more samples from experiments to monitor a wide range of other parameters, reduce evaporation, and have a more controlled aeration environment, triplicate studies in 1.25 L photobioreactors were conducted (Figure 3.1).



Figure 3.1. Example of scaled up experimental environment including photobioreactor tubes sparged with air and temperature controlled in an aquarium.

PRELIMINARY ISOLATE SCREEN

Algae grown in both AsP_2 media types experienced limited growth throughout the entirety of the study. The AsP_2 media types were modeled to resemble seawater and never enhanced growth of any of the isolates compared to AM6 and AM6SIS. This could be due to the elevated levels of sodium chloride or limited concentration of nitrate as compared to the AM6 medium. Though all of the isolates were isolated from high pH and high saline environments, osmotic and ionic stress imparted on cells in this extreme environment is the most probable reason for why the growth was limited.

Isolate GK5La

Isolate GK5La is a green microalga that grew dense cultures quickly relative to the other isolates studied. Media AM6 and AM6SIS yielded the fastest growth rate and cell concentrations over time. The maximum specific growth rate in AM6 medium was 0.76 d^{-1} and the maximum cell concentrations in AM6 medium was $5.03x10^7$ cells/mL (Figure 4.1). Growth rate of isolate GK5La slowed in AsP_2(1.8) (specific growth rate = 0.49 d^{-1}) likely due to the high concentration of sodium chloride present, imparting a higher ionic/osmotic stress upon the cells. This stress led to a low maximum cell concentration ($6.4x10^6$ cells/mL) (Figure 4.1). AsP_2 (5.1) medium inhibited isolate GK5La, and no growth was observed. Absorbance read at 750nm showed trends over time that agreed with data collected from cell concentration data.

Figure 4.1 shows that the pH in solution increased along with growth in the culture. Isolate GK5La growth in AM6 medium resulted in maximum pH values near

11.7 twenty days into the experiment. Maximum pH values of 8.7 in AsP_2(1.8) and 8.15 in AsP_2(5.1) were observed. A lower pH in solution indicates decreased photosynthetic activity. Since isolate GK5La was native to an alkaline lake (pH near 10), the starting pH near 7.8 may have inhibited growth of the organism.

Analysis of growth in the varying media types led to interesting observations regarding cell morphology as seen in Figure 4.3. In AM6 and AM6SIS, the cells routinely configured themselves in groupings of 2, 4, and 8 cocci, linked together in chains. In AsP_2(1.8) medium, the cells arranged themselves in a cross like arrangement consisting of 4 compartments. These cells appeared rough and grouped together more so than those in AM6 and AM6SIS and looked as if completing the cell cycle was inhibited.

Figure 4.3 shows cells grown in AM6 and AM6SIS contained no visible lipid bodies after a period of 30 days. However, cells grown in AsP2(1.8) appeared to have several small lipid bodies visualized by fluorescence microscopy. To assess the stress causing accumulation of lipid, as indicated by Nile Red staining, further sets of experiments were planned regarding isolate GK5La.

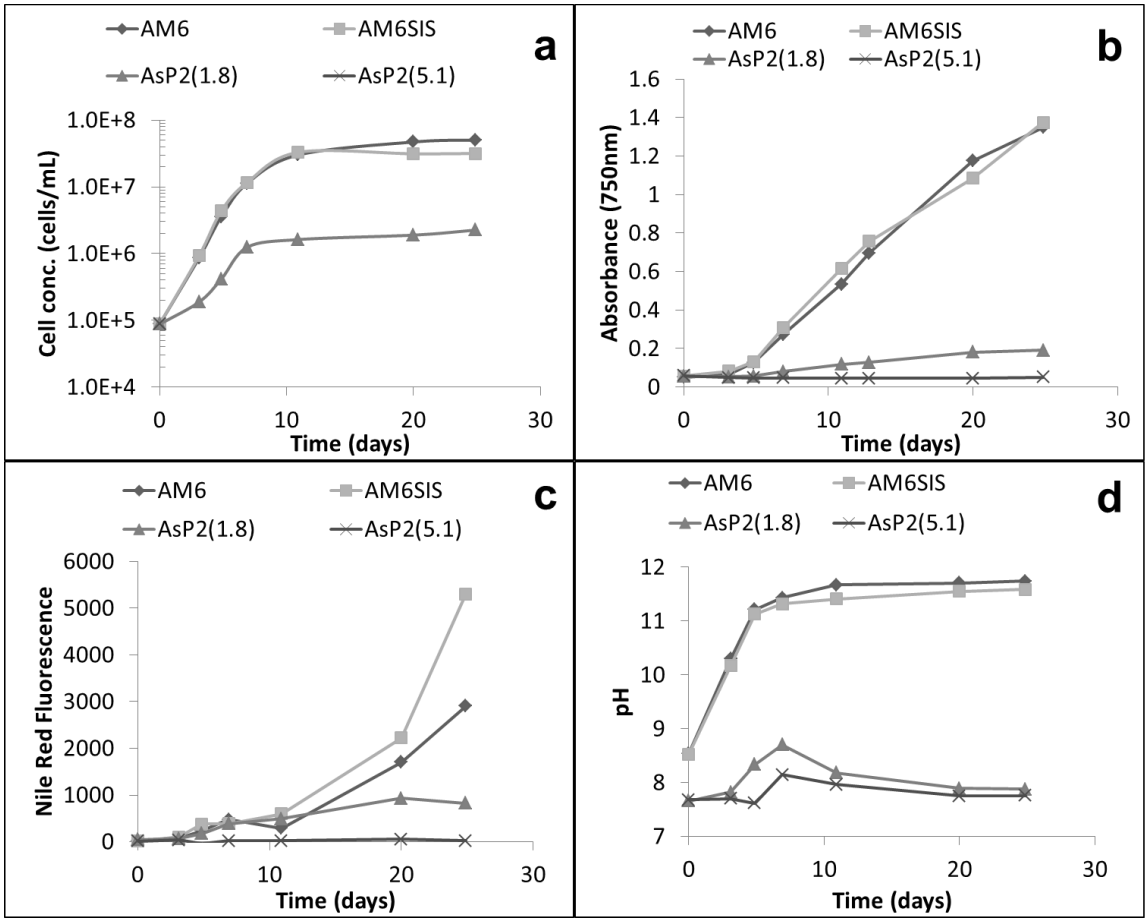


Figure 4.1. (a) Cell density,(b) absorbance at 750nm, (c) Nile Red fluorescence, and (d) pH, for cultures of isolate GK5La grown in AM6 (♦), AM6SIS (■), AsP_2(1.8) (▲), and AsP_2(5.1) (✖). The isolate was unable to grown in AsP_2(5.1).

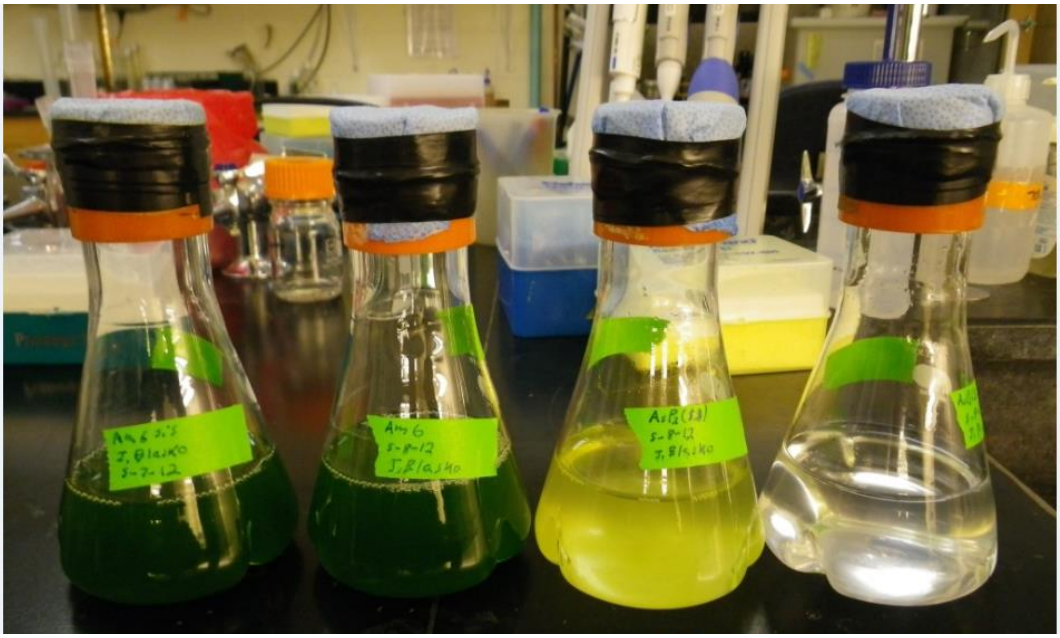


Figure 4.2. Growth of isolate GK5La in the preliminary experimental environment. From the left, isolate GK5La is grown in AM6SIS, AM6, AsP_2(1.8), and AsP_2(5.1).

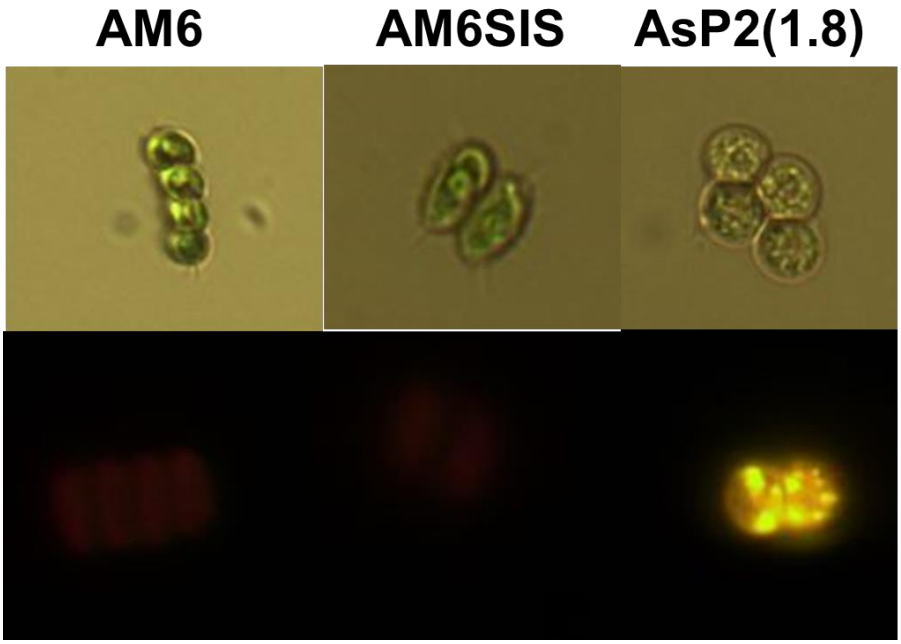


Figure 4.3. Micrograph of isolate GK5La grown in different media with corresponding Nile Red flourescent imaging of neutral lipid bodies below.

Isolate GK5La Sodium Chloride Experiments (Flasks)

Isolate GK5La was cultured in AM6 prior to being pelleted, washed, and re-suspended in two different media types, AM6(1.8) and AsP_2(1.8). Cell concentration and Nile Red fluorescence were monitored over time throughout the experiment. The 'sodium chloride stress' resulted in increased Nile Red fluorescence for both treatments as seen in Figure 4.4. The far higher Nile Red fluorescence value in AM6(1.8) on day 26 (11,740 compared to 1,720 fluorescence units) is hard to diagnose, however regarding cell counts it is most likely due to AM6(1.8) imparting more stress on the organism and causing it to accumulate long term energy storage in the form of lipid. Cell counts over time indicated growth was not completely arrested at this concentration of sodium chloride, however growth was significantly inhibited for both media types. The lower cell concentration of AM6(1.8) compared to AsP_2(1.8) as seen in Figure 4.4a may not statistically be significant however biological replicates were not used in this experiment.

Another thick culture of isolate GK5La was treated under the same method in the preceding paragraph, however, one part was resuspended in AM6(1.8) and the other was resuspended in AM6. Figure 4.5 shows that cells resuspended in AM6 did not accumulate lipid as indicated by Nile Red fluorescence over the 24 day period of study. In contrast to AM6, cells that were resuspended in AM6(1.8), accumulated lipid bodies as indicated by Nile Red imaging and fluorescence measurements (maximum = 46,748 fluorescence units) shown in Figure 4.5b. Figure 4.5c shows that the pH of both AM6 and AM6(1.8) increased after the experiment began. The maximum pH reached in AM6

was 12.0 at day 8 and the maximum pH reached in AM6(1.8) was 11.3 at day 20, shifting inorganic carbon speciation in solution predominantly to carbonate.

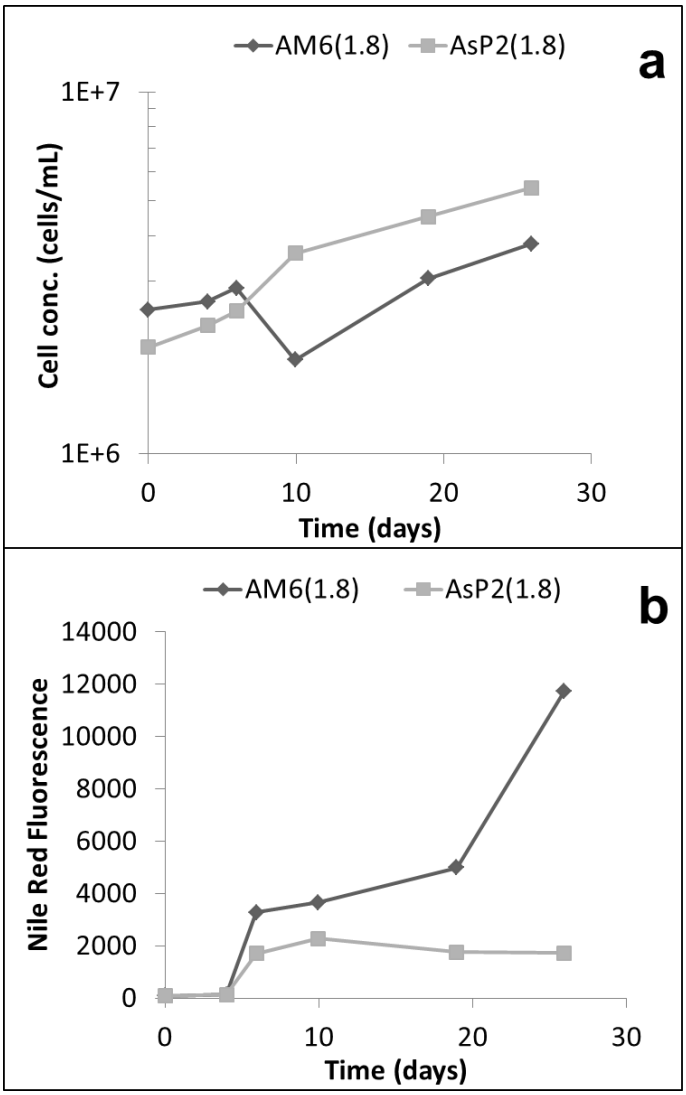


Figure 4.4. (a) Cell density and (b) Nile Red fluorescence, for cultures of isolate GK5La grown in AM6(1.8) (♦) and AsP2(1.8) (■).

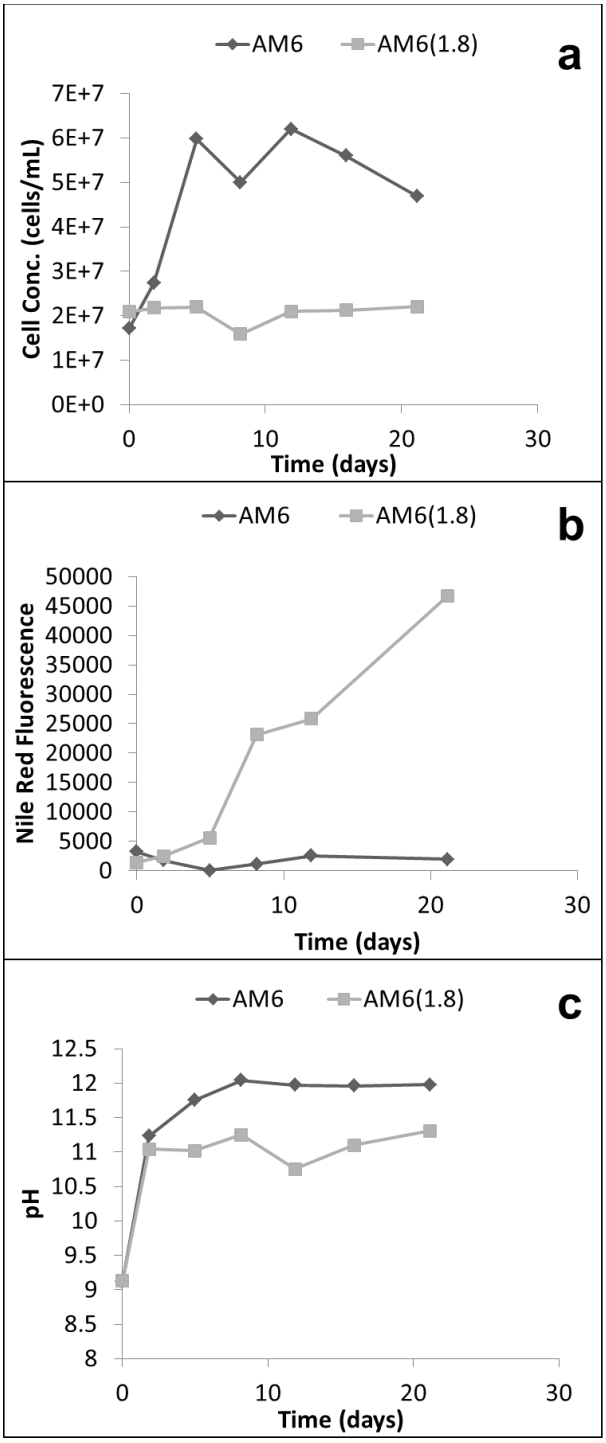


Figure 4.5. (a) Cell density, (b) Nile Red fluorescence, and (c) pH, for isolate GK5La grown in AM6 (♦) and AM6(1.8) (■).

The next round of experimentation consisted of growth in two different culture conditions with biological triplicates. The two treatments studied were AM6 and AM6(1.8) in flasks containing 150mL of medium on a shaker table with cloth caps. Prior to inoculation in each flask, isolate GK5La was grown under the inoculating condition it would be subjected, (AM6 for AM6 and AM6(1.8) for AM6(1.8)), washed and resuspended in fresh medium before inoculation in the study. Isolate GK5La in the presence of 1.8% sodium chloride was inhibited compared to isolate GK5La grown in sodium chloride deplete AM6 as shown in Figure 4.6. This resulted in a smaller specific growth rate and final biomass concentration as compared to AM6. The specific growth rate in AM6 was 0.96 d^{-1} and the specific growth rate in AM6(1.8) was 0.64 d^{-1}. The maximum cell concentration in AM6 was $6.28x10^7$ (day 26) and the maximum cell concentration in AM6(1.8) was $1.15x10^7$ cells/mL (day 26). Sodium chloride limited growth and productivity of isolate GK5La in AM6 medium.

Maximum pH in AM6 was 11.9 on day 8 and the maximum pH in AM6(1.8) was 11.1 reached on day 13 in the study (Figure 4.6). The difference observed indicates that AM6 provided a better environment for growth since a rise in pH by the organism is attributed to the export of OH^- ions to achieve charge neutrality after importing nitrogen in the form of nitrate (NO_3^-) (Eustance et al. 2013), or bicarbonate (HCO_3^-). More bicarbonate is imported relative to nitrate, so the rise in pH is mainly to due to inorganic carbon fixation.

While growth rate and final biomass concentration were low in the presence of sodium chloride, lipid content as indicated by Nile Red fluorescence was increased. Figure 4.6 demonstrates cells exposed to the sodium chloride stress recorded significantly

higher fluorescence values than cells grown in sodium chloride deplete media. Given that cells grown in the media containing 18 g/L sodium chloride have lower final biomass concentrations, culturing the organism in this medium may not be an advantageous strategy for harvesting the highest attainable lipid content per cell.

Time is an important variable for algal biofuel operations. A major drawback to isolate GK5La cultured in 18 g/L sodium chloride containing medium is the long length of time it takes algal cells to reach their most stressed out and productive state as indicated by Nile Red fluorescence measurements. A more productive algal culturing condition with respect to isolate GK5La would include growing the organism quickly in AM6 and then spiking to a concentration of 18 g/L sodium chloride after stationary-phase was reached. This and more culturing conditions with respect to isolate GK5La are studied in scaled up photobioreactors in the next chapter.

Isolate GK2Lg

Isolate GK2Lg is a diatom that aggregated to form flocks in solution, and biofilm on the sides of the beveled flasks over time. Divalent cations have been studied and shown to act as bridges between biofilms in solution (Huang and Pinder 1995). To prevent the formation of biofilm, and to obtain more accurate and reliable cell concentration data, the dependence on Ca^{2+} was investigated. Without the presence of Ca^{2+}, and at low levels of Ca^{2+}, growth was significantly inhibited although aggregation decreased as well. Thus, throughout the screen sonication was used to disperse flocks in

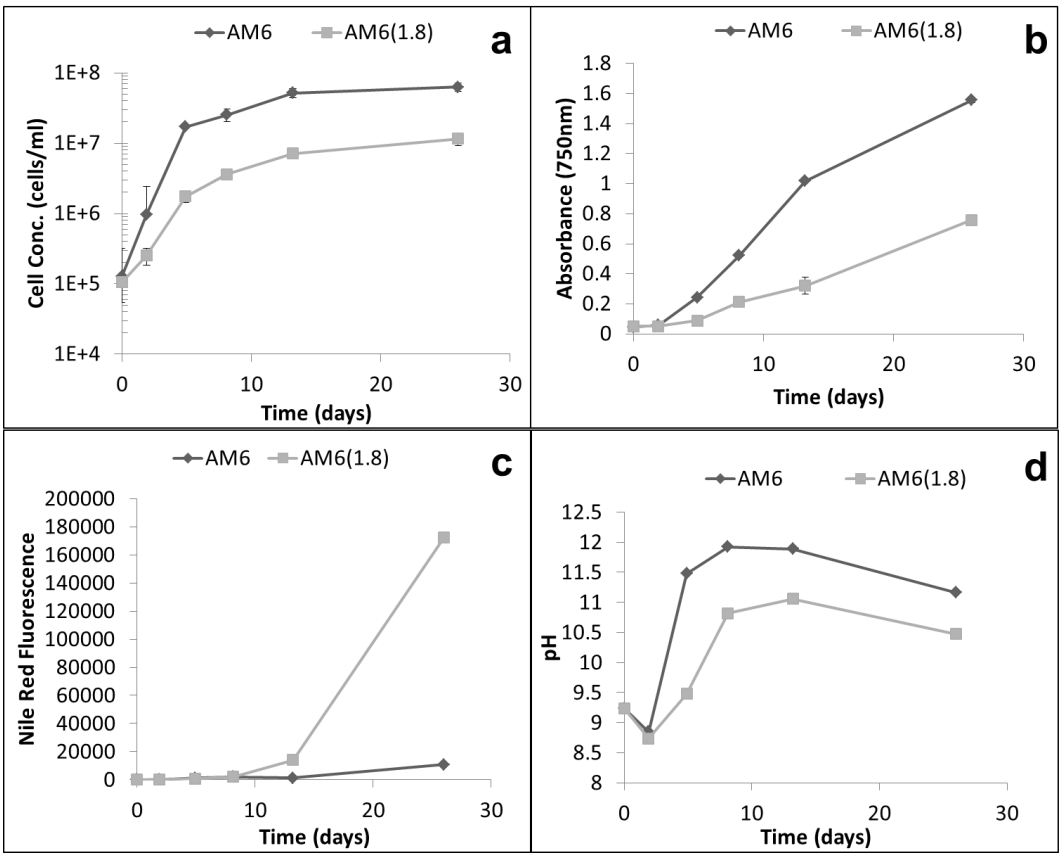


Figure 4.6. (a) Cell density, (b) absorbance at 750nm, (c) Nile Red fluorescence, and (d) pH, for triplicate cultures of isolate GK5La grown in AM6 (♦) and AM6(1.8) (■). Error bars represent standard deviations of triplicate treatments. Some error bars are not visible since they are smaller than the markers.

samples taken from the experiment to record accurate cell concentrations while the calcium chloride concentration of the medium was left unchanged.

Isolate GK2Lg performed best in AM6SIS, turning the solution turbid, and forming thick brown biofilm on the sides of the flasks (Figure 4.8). The specific growth rate of isolate GK2Lg grown in AM6SIS was 0.5 d^{-1}. The isolate survived in each of the media types even though growth was limited in AM6 and AsP_2(5.1). The maximum cell concentrations in AM6 and AsP_2(5.1) were $4.4x10^5$ cells/mL and $6.78x10^5$ cells/mL, respectively.

The pH in solution stayed low compared to the other green algal isolates studied. The maximum pH in the study was 10.0 recorded in AM6SIS on day 18. The next highest pH reached in solution was 8.4 in AM6, followed by 8.1 in AsP_2(1.8), and finally 8.0 in AsP_2(5.1). The pH recorded for these media was low due to the limited growth of isolate GK2Lg. The pH may have also been lower in the AsP_2 media types due to the limited nitrate in solution as compared to AM6 (0.05g/L compared to 0.33g/L, respectively).

Lipid increased in solution over time, as indicated by Nile Red fluorescence shown in Figure 4.7. AM6 medium does not have silicon in its composition; therefore, after introduction to the AM6 environment, isolate GK2Lg was immediately limited of an essential nutrient, and stressed to produce neutral lipids, once again shown in Figure 4.7. After cell growth was arrested, lipid began to accumulate in the cells as indicated by Nile Red fluorescence (Figure 4.7). Since growth of isolate GK2Lg in AsP_2(1.8) and AsP_2(5.1) was not substantial, little lipid accumulated in these cultures as indicated by Nile Red fluorescence.

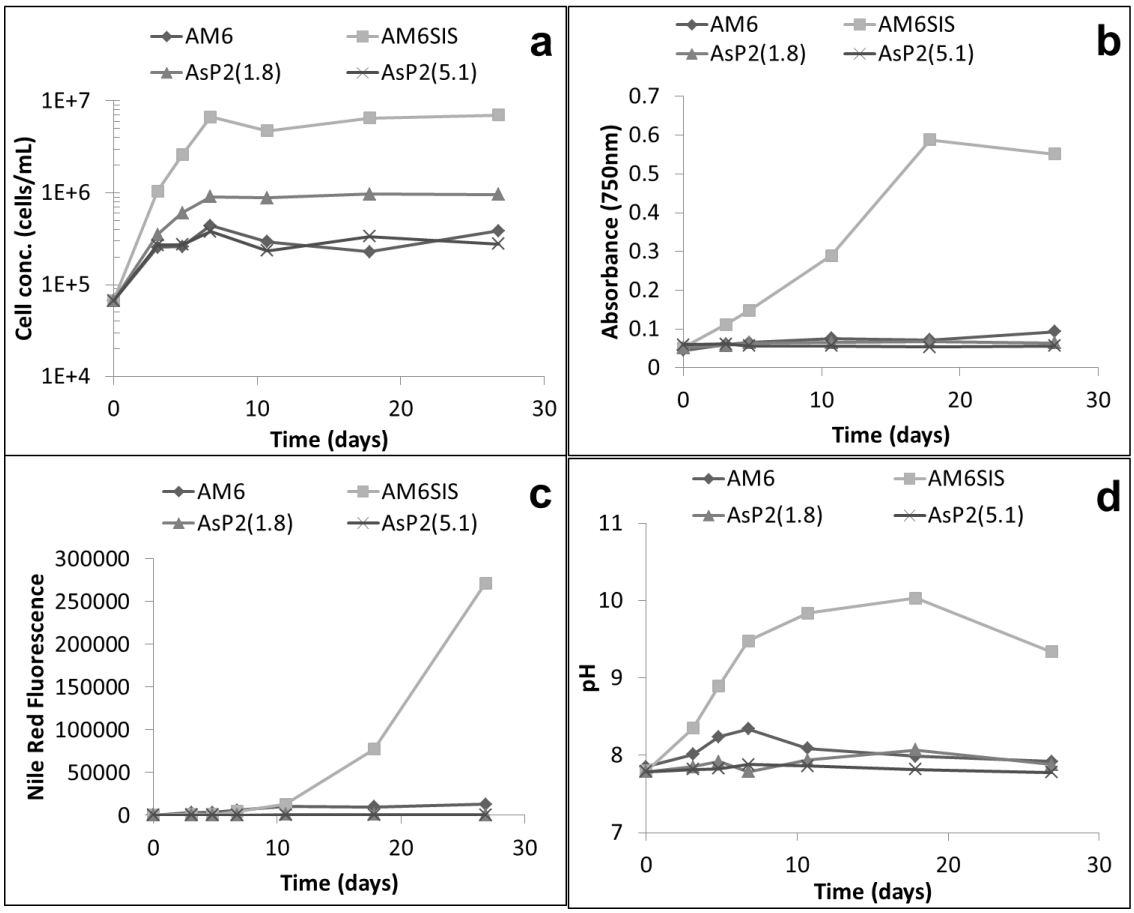


Figure 4.7. (a) Cell density, (b) absorbance at 750nm, (c) Nile Red fluorescence, and (d) pH for cultures of isolate GK2Lg grown in AM6 (♦), AM6SIS (■), AsP2(1.8) (▲), and AsP2(5.1) (✖).

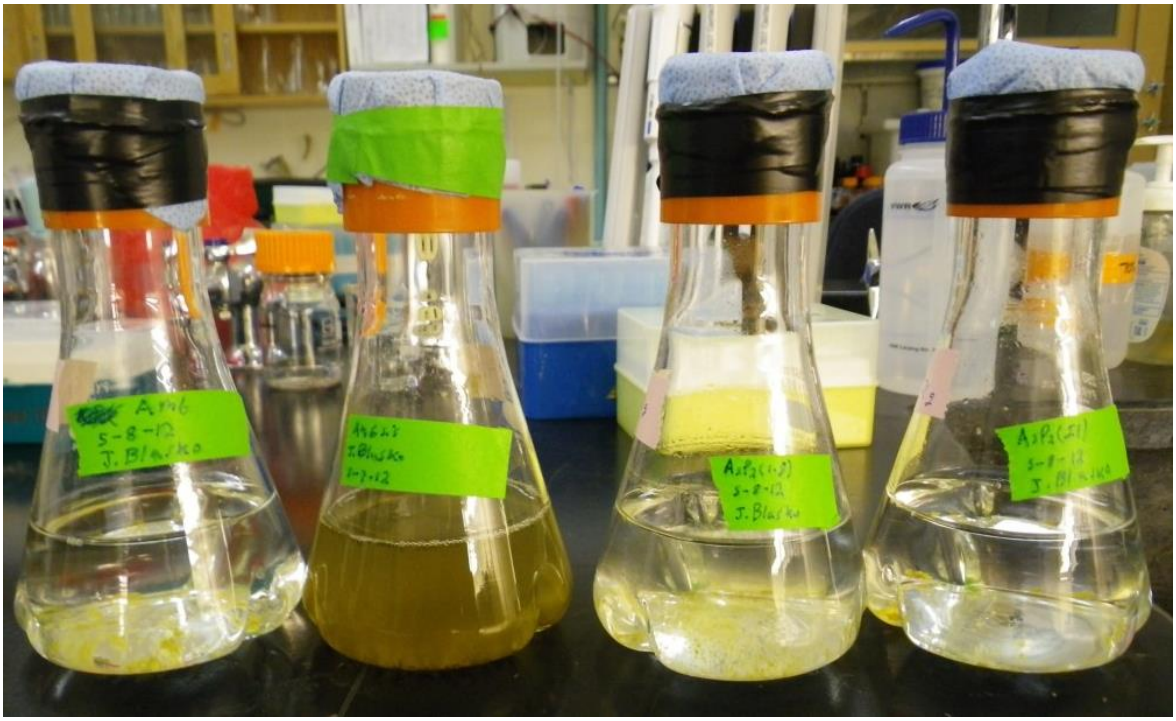


Figure 4.8. Image showing growth of isolate GK2Lg in the preliminary experimental environment. Isolate GK2Lg grown in (from left to right) AM6, AM6SIS, $AsP_2(1.8)$, and $AsP_2(5.1)$.

Isolate GK6-G2

Isolate GK6-G2 is a small cyanobacterium that appears microscopically similar to *Microcystis aeruginosa*. It is capable of growing dense blue-green cultures in a relatively short time as seen in Figure 4.10. Like all of the isolates tested, GK6-G2 grew best in AM6 and AM6SIS, although increases in cell concentration were also recorded in $AsP_2(1.8)$ and $AsP_2(5.1)$. The maximum specific growth rate in the experiment was 0.96 d^{-1} recorded in AM6SIS. The maximum specific growth rate in $AsP_2(1.8)$ media was 0.43 d^{-1}. The media composition of $AsP_2(1.8)$ and $AsP_2(5.1)$ led to a lower maximum specific growth rate when compared to AM6 and AM6SIS.

Isolate GK6-G2 recorded its maximum cell concentration in the experiment in AM6SIS, growing to $1.73x10^7$ cells/mL on day 22 (Figure 4.9). In the high salinity environment of $AsP_2(1.8)$ the highest cell concentration was $3.42x10^6$ cells/mL on day 8 in the study. Isolate GK6-G2 did not increase in cell concentration throughout the study in $AsP_2(5.1)$.

The pH in AM6 reached its maximum at 11.2 on day 8 and the pH in AM6SIS reached its maximum value on day 8 at 11.3 (Figure 4.9). After day 8, both AM6SIS and AM6 media decreased sharply, and both cultures turned from blue-green to yellow indicating chlorophyll degradation. The pH in both $AsP_2(1.8)$ and $AsP_2(5.1)$ did not increase drastically throughout the study period due to limited growth.

Figure 4.9 shows Nile Red fluorescence did not increase in the study relatively to the other isolates in AM6 and AM6SIS media indicating that isolate GK6-G2 may not be a good candidate for biofuel production. AM6 and AM6SIS accumulated similar amounts of lipid in the culture based on Nile Red fluorescence. AsP2(1.8) and AsP2(5.1) had very little measured Nile Red fluorescence when compared to both of the AM6 based media. The reason for the limited fluorescence signal in the AsP2 media could be due to the difference in salt concentration or media composition; however, more experimentation is needed to determine this. The highest Nile Red fluorescence was recorded in AM6 on day 19 at a value of 7540 fluorescence units.

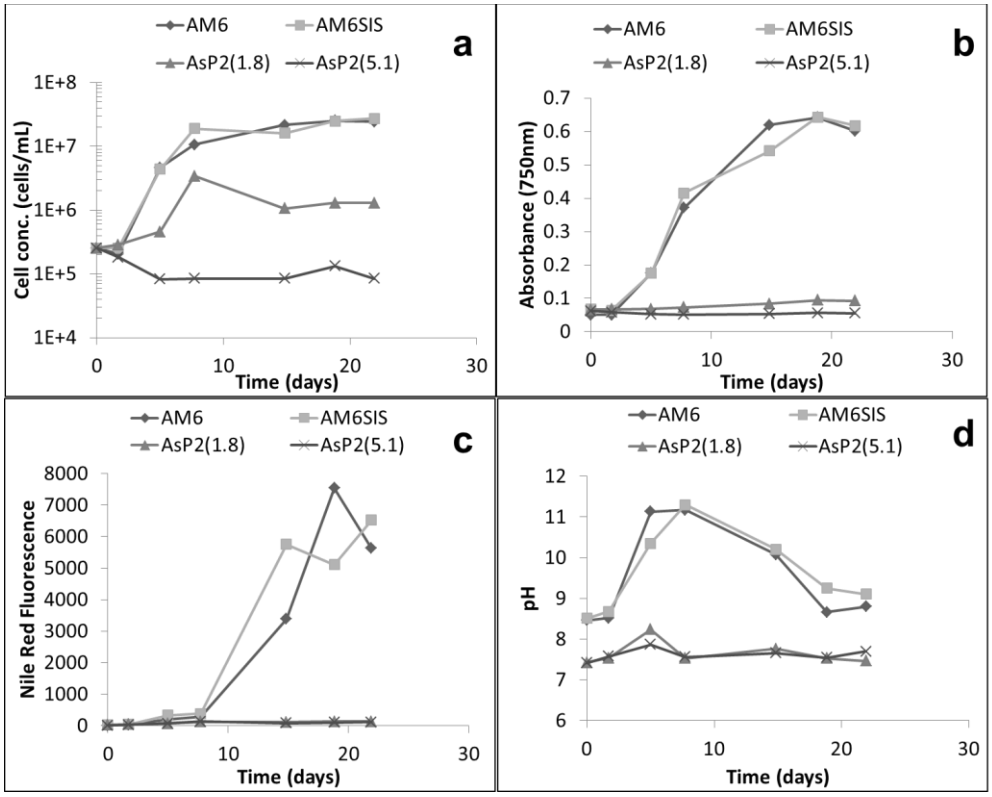


Figure 4.9. (a) Cell density, (b) absorbance at 750nm, (c) Nile Red flourescence, and (d) pH, for cultures of isolate GK6-G2 grown in AM6 (♦), AM6SIS (■), AsP2(1.8) (▲), and AsP2(5.1) (✖).

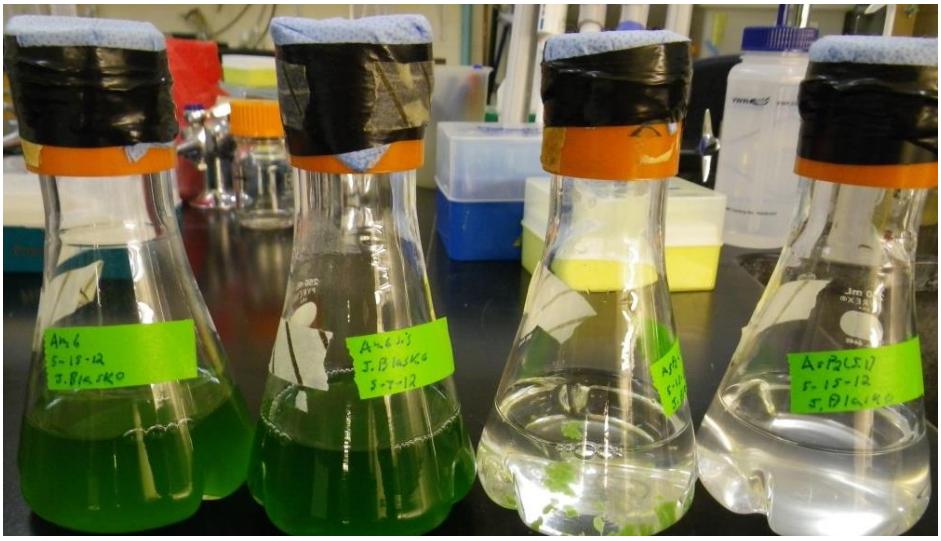


Figure 4.10. Image showing growth in the preliminary experimental environment of isolate GK6-G2. Isolate GK6-G2 grown in from left to right AM6, AM6SIS, AsP_2(1.8), and AsP_2(5.1).

Isolate GK3L

This isolate is a green alga that looks similar to *nannochloropsis gaditana,* grows slowly compared to the other isolates, and is very small. All of the media tested allowed for high final cell concentrations except for $AsP_2(5.1)$, likely due to its high concentration of sodium chloride (Figure 4.11). Though isolate GK3L did reach the highest cell concentration among the algae tested, it grew slowly reducing its potential for biofuel production. On day 22, isolate GK3L had a maximum cell concentration of $2.27x10^8$ cells/mL in AM6SIS, followed closely by AM6 with $1.46x10^8$ cells/mL. Figure 4.11 shows that growth was limited in $AsP_2(1.8)$ and $AsP_2(5.1)$ and the maximum cell concentration recorded was $7.48x10^7$ cells/mL and $2.23x10^6$ cells/mL, respectively. Again due to its small size, even though the final cell concentrations were relatively high, final cell dry weight was similar to the other isolates. Absorbance measured at 750nm agreed with cell counts measured over time (Figure 4.11).

For the AM6 medium, pH crested at 11.8 and eventually began decreasing after day 15 as shown in Figure 4.11. Isolate GK3L grown in $AsP_2(1.8)$ only increased to a maximum pH of 8.4. This was interesting since cell growth was comparable to AM6 and the nitrogen source was nitrate, thus it was expected the pH would rise higher.

As shown in Figure 4.11, isolate GK3L shows reaction to salt stress similar to isolate GK5La. The medium that experienced the largest increase in Nile Red was $AsP_2(5.1)$. This indicates that this organism may respond to a salt trigger as indicated by specific Nile Red fluorescence measurements. In fact, its specific Nile Red fluorescence was more than an order of magnitude higher than for media without additional salt, or

with a lower concentration of salt (1.8%). Later on in the growth cycle of this organism, lipid accumulated in other media types without salt as nitrogen became depleted, though still not on the same order of magnitude as the 5.1% salt media. The differences noted could be due to noise from the experimental measurement, but more experimentation is needed to determine this.

This organism is a candidate for biofuel production because it grows to a high cell concentration, reacts to salt stress to accumulate a moderate amount of lipid, and it can tolerate very saline solutions (up to 51 g/L). The only drawbacks to this organism are its small size and slow growth rate.

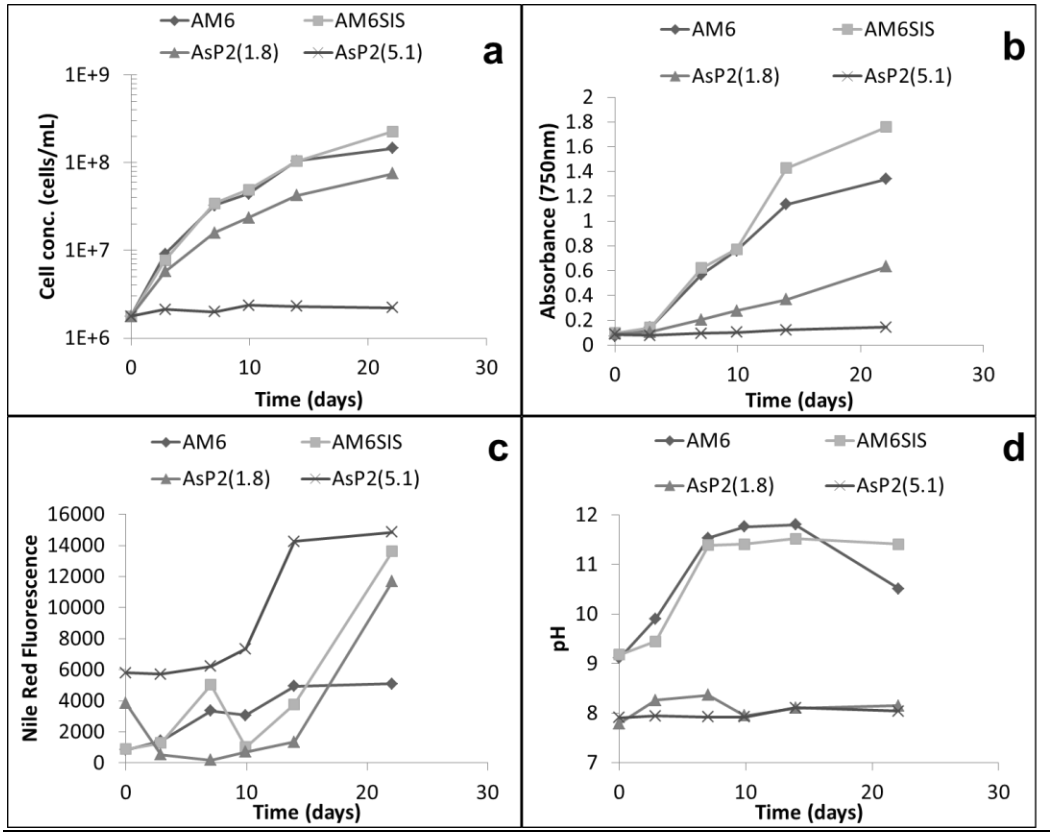


Figure 4.11. (a) Cell density, (b) absorbance at 750nm, (c) Nile Red fluorescence, and (d) pH, for cultures of isolate GK3L grown in AM6 (♦), AM6SIS (■), AsP2(1.8) (▲), and AsP2(5.1) (✖).

GK3L Salt Spike

A turbid culture of GK3L was grown in flasks before being pelleted, washed twice, and resuspended in sterile AM6 and AM6(5.1) media. These media types were used to understand the effect of 51g/L sodium chloride on the isolate with respect to Nile Red fluorescence.

As shown in Figure 4.12, the isolate grew better in AM6 compared to AM6(5.1). The final concentration of isolate GK3L in AM6 was $1.98x10^8$ cells/mL, whereas the final concentration in AM6(5.1) was only $4.48x10^7$ cells/mL. Few data points were taken over time since this was a screening experiment.

The pH of both systems agreed well with cell growth. Figure 4.12 shows AM6 increased to a maximum pH value of 11.5 while AM6(5.1) stayed stationary throughout the period of the study, only reaching a maximum pH of 9.5.

Nile Red fluorescence measured in the system indicated that isolate GK3L reacted to salt stress to produce neutral lipid stores as shown in Figure 4.12. Nile Red fluorescence was studied further over time because it was the main parameter of interest in the experiment. Both media tracked well with one another until day 20. Afterwards isolate GK3L registered far higher total Nile Red fluorescent signal over the course of the study. This is even more significant when considering there were was a lower cell concentration in AM6(5.1).

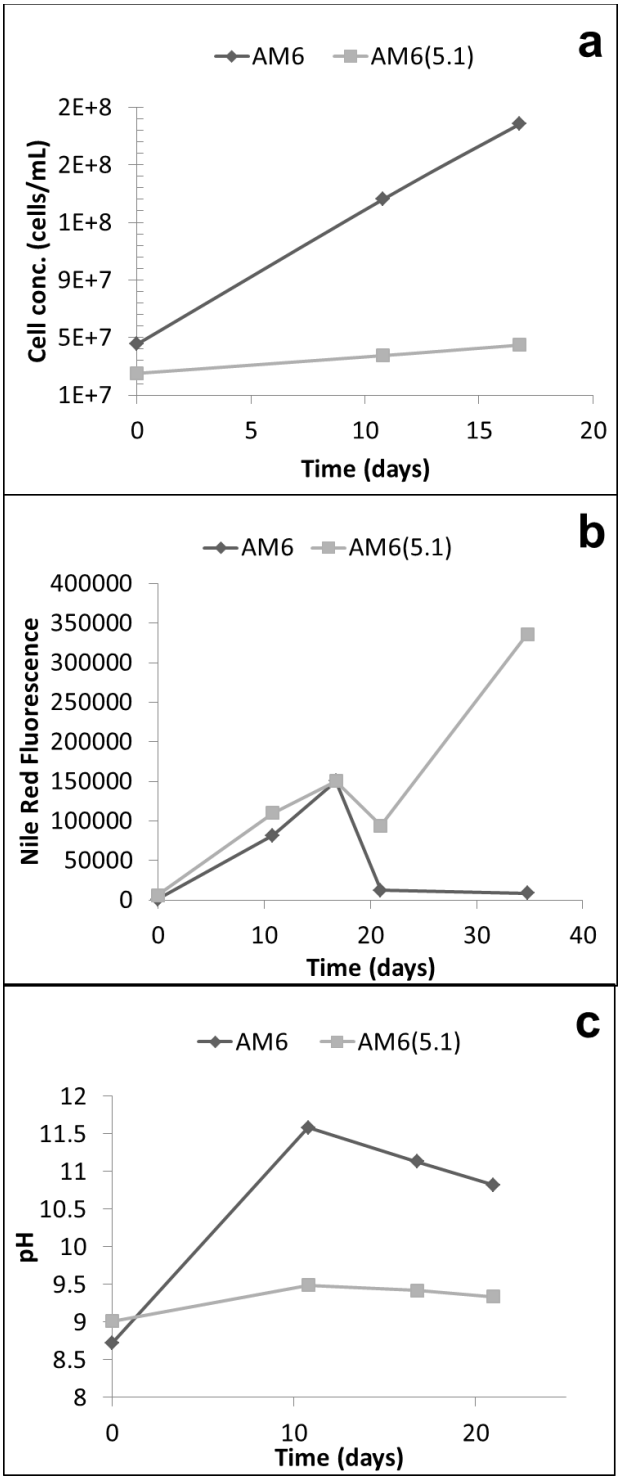


Figure 4.12. (a) Cell density, (b) Nile Red fluorescence, and (c) pH, for cultures of isolate GK3L grown in AM6 (♦) and AM6(5.1) (■).

Salt Spike 2

The next study pertaining to isolate GK3L included testing growth in two different culture conditions with biological triplicates. AM6 and AM6(5.1) were the two media tested in shaker flasks with cloth caps, each containing 150mL of medium.

In the presence of 51 g/L sodium chloride isolate GK3L was inhibited compared to the control grown in AM6. Until day 10, there was a very distinct lag in growth of isolate GK3L in AM6(5.1). Subsequently the culture assumed a similar growth rate to the culture grown in AM6. This delay in growth, due to high concentration of sodium chloride, lead to a difference in cell concentration on day 33 near $1.8x10^8$ cells/mL as seen in Figure 4.13.

Figure 4.13 shows Nile Red fluorescence was substantially higher in AM6(5.1) as compared to control AM6. Nile Red fluorescence increased substantially from day 21 to day 25, probably revealing the effects of both nitrate limitation and salt stress, to induce TAG accumulation. A more productive system would likely be spiking a culture of isolate GK3L, grown in AM6, with enough salt to register a biological stress in the organism to accumulate lipid.

As shown in Figure 4.13, the solution pH tracked well for both treatments, however, the control (AM6) with a higher concentration of cells registered a higher pH, reaching a maximum of 11.9 on day 21. AM6(5.1) registered a maximum pH of 11.2 on day 21 as well. Given that proton activity would be less for a high ionic strength solution, after taking into account thermodynamic relationships describing the un-idealities of high salt solutions through geochemical modeling, it would be expected that

the pH would be higher AM6(5.1). This was not the case as seen in Figure 4.13, and is most likely due to higher photosynthetic activity in AM6.

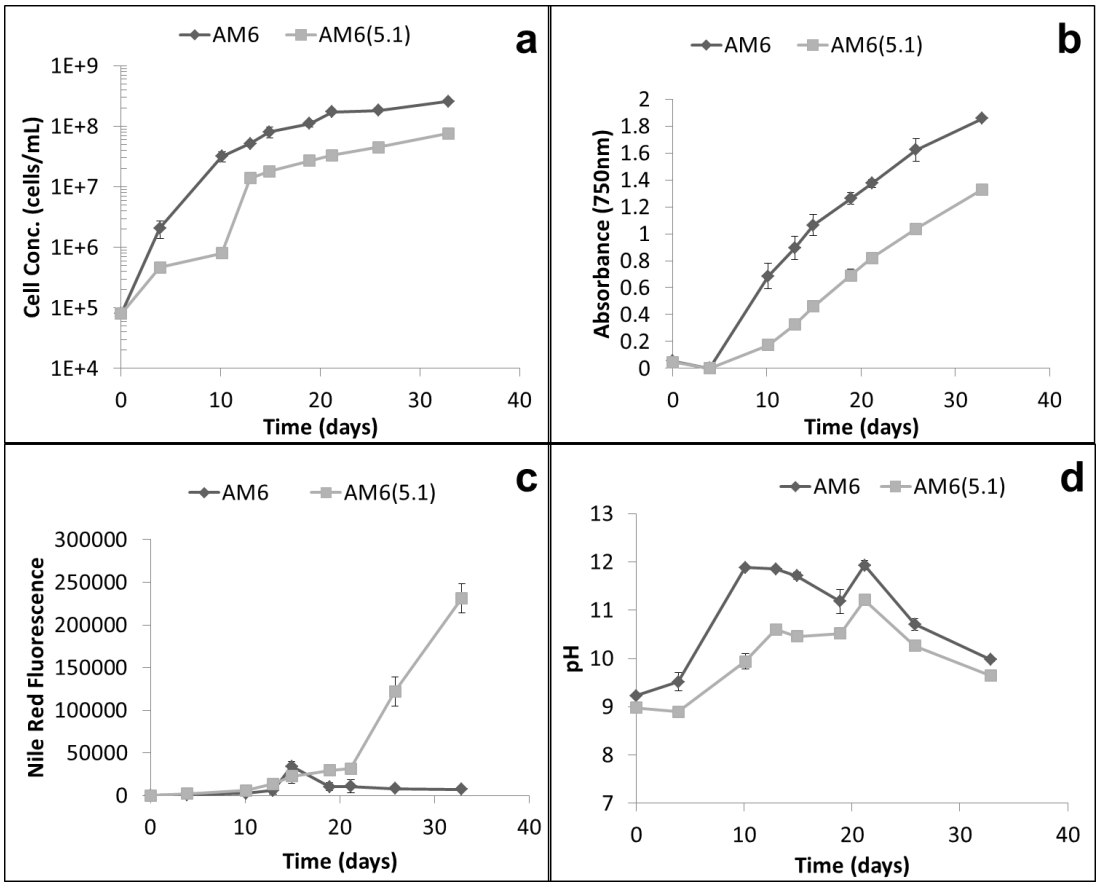


Figure 4.13. (a) Cell density, (b) absorbance at 750nm, (c) Nile Red fluorescence, and (d) pH, for triplicate cultures of isolate GK3L grown in AM6 (♦) and AM6(5.1) (■). The error bars represent standard deviation of triplicate treatments. Some error bars are not visible because they are smaller than the markers.

Isolate GK5L-G2

Isolate GK5L-G2 was a very large green alga that looked similar to typical *Botryococcus* spp. As shown in Figure 4.14, Nile Red imaging displays several small lipid bodies inside the main large membrane, highlighting this isolate's potential with respect to biofuel production. One immediate characteristic of the organism relates to

biofilm-forming properties. In flask studies, the organism stuck to the sides of the flasks making accurate cell counts a difficult task.

Cell concentration over time as shown in Figure 4.14 depicts the main drawback of the isolate's slow growth rate. The maximum cell concentration was $9.23x10^6$ in AM6SIS, a low value relative to the other isolates studied, and the maximum specific growth rate was $0.32d^{-1}$ in the same medium. Cell concentration is not a measure of biofuel productivity because it does not take into account the size of the organism. In this study cell dry weights over time would have offered more detail. The isolate grew best in AM6 and AM6SIS, but appeared inhibited in AsP_2(1.8), and appeared to die off in AsP_2(5.1). This may be due to the isolate's sensitivity to high sodium chloride concentration. Nile Red fluorescence and specific Nile Red fluorescence was high for both AM6 and AM6SIS throughout the course of the study as shown in Figure 4.14. Isolate GK5L-G2 grown in both AM6 and AM6SIS immediately reached a high maximum pH (12 and 11.7 respectively) early on in the study before eventually decreasing after day 13. The pH rose high in solution due to the limited buffering capacity of the medium and high photosynthetic activity of the isolate in AM6.

This organism may be a prospect considering biofuel because of its large size, and apparent accumulation of neutral lipid stores. In the medium of study, the growth rate was slow, but with more research and time, optimal growth conditions favoring the organism could be found.

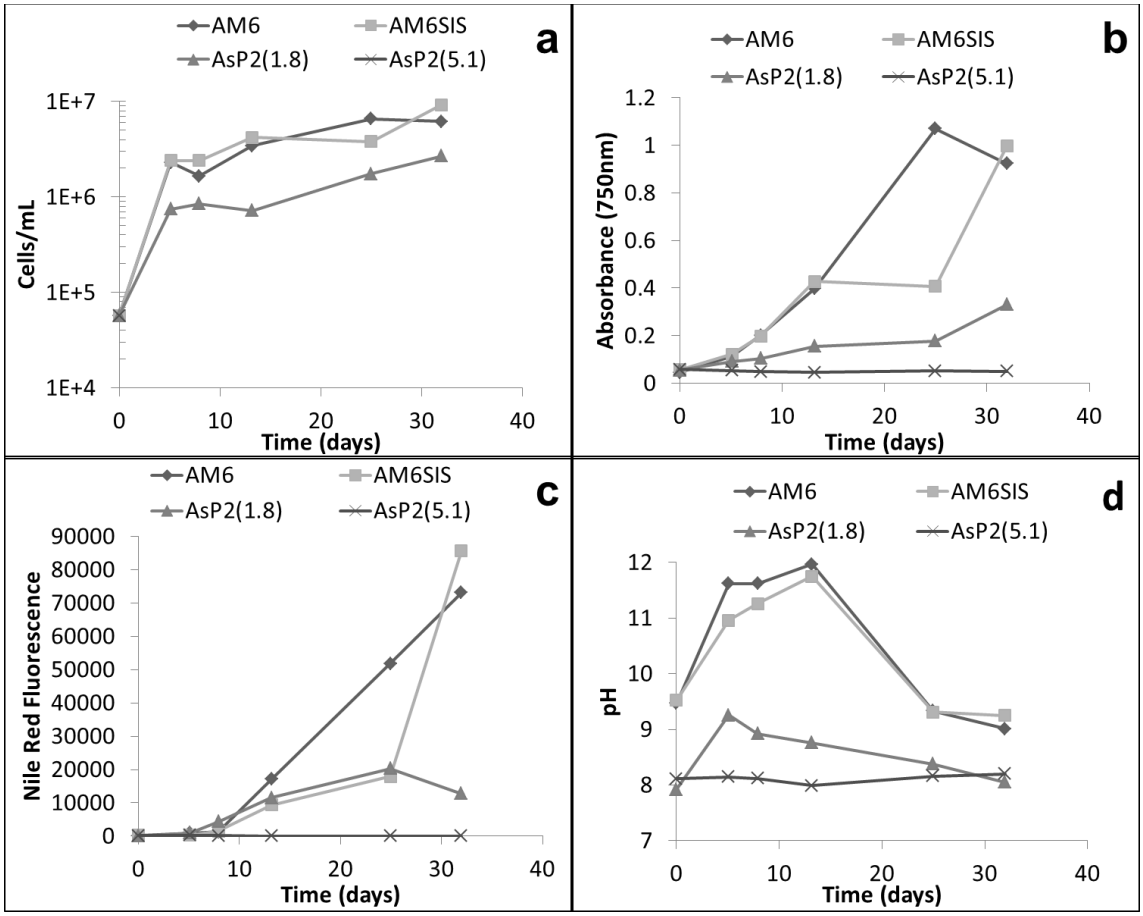


Figure 4.14. (a) Cell density, (b) absorbance at 750nm, (c) Nile Red fluorescence, and (d) pH, for cultures of GK5L-G2 grown in AM6 (♦), AM6SIS (■), AsP_2(1.8) (▲), and AsP_2(5.1) (✖).

CHAPTER 5

THE USE OF SODIUM BICARBONATE AND SODIUM CHLORIDE TO STIMULATE LIPID PRODUCTION IN AN ALGAL ISOLATE FROM SOAP LAKE, WASHINGTON

Contribution of Authors and Co-Authors

Manuscript in Chapter 5

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Contributions: Writing and Experimentation

Co-Author: Dr. Rob Gardner

Contributions: Experimental Planning

Co-Author: Dr. Egan Lohman

Contributions: Experimental Planning

Co-Author: Karen Moll

Contributions: DNA Extraction, Sequencing, and Identification

Co-Author: Luke Halverson

Contributions: Assisted in Experimentation

Co-Author: Dr. Robin Gerlach

Contributions: Experimental Planning

Co-Author: Dr. Brent Peyton

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Manuscript Information Page

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Algal Research

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_X__Prepared for submission to a peer-reviewed journal
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____ Accepted by a peer-reviewed journal
____ Published in a peer-reviewed journal

Abstract

In this paper we present data showing the effect increased levels of sodium chloride and supplementation of sodium bicarbonate have on isolate GK5La from Soap Lake, Washington (USA). Isolate GK5La was cultivated in 1.25L tubular photobioreactors under a 14:10 light cycle with nitrate as a nitrogen source. Of the conditions tested, the highest total neutral lipid content (grams of neutral lipid per culture volume) was achieved by supplementing with sodium bicarbonate and spiking to a concentration of 1.8% sodium chloride. The highest cell dry weight and biodiesel content was measured under the control condition grown in AM6 media without additional salt. Isolate GK5La stored 96-97% of FAME as C18 or C16 carbon chains, and predominantly the speciation of neutral lipid was in the form of free fatty acid. Specific Triacylglyceride (TAG) content (weight of TAG per cell dry weight) increased with increasing salinity in the medium. The inorganic carbon speciation was modeled using Visual MINTEQ over the pH range from 8-11 for AM6 media with and without 1.8% sodium chloride. Inorganic carbon speciation shifted from predominantly bicarbonate at pH 8, to carbonate at pH 12, and media containing 1.8% sodium chloride favored sodium carbonate at pH 10. Understanding the effects of carbon supplementation and increasing salinity are important for scaling microalgae for the production of biofuel in open raceway ponds.

Introduction

Microalgae may play an important role in the path to a more sustainable future for an exponentially growing human population by producing valuable hydrocarbons using inorganic carbon and sunlight. Microalgae are eukaryotic microorganisms that have the capability to efficiently synthesize lipid molecules during photosynthesis as a form of energy storage. The prospect of growing microalgae for producing a stable and dependable source of biofuel is plausible only if done at scale with consideration of biochemistry, geochemistry, and environmental conditions (Slade and Bauen 2013; Lundquist et al. 2010).

High pH conditions are favored in open raceway ponds to limit contamination (Borowitzka 1992). A pH of 10 has previously been shown to yield peak growth in cultures of *Chlorella* spp. and *Spirulina* spp. (Ramanan et al. 2010). Since pH is based on a log scale, even one pH unit difference can have a profound impact on the system of interest both biologically and chemically. Speciation of many ions in solution are thermodynamically controlled by pH. While alkaline conditions are often preferred (Borowitzka 1992), pH should be prevented from rising so high that carbonate is thermodynamically favored over bicarbonate. The carbonate ion is not a useful carbon source in microalgae cultures (Giordano et al. 2005; Raven et al. 2012; Mercado and Gordillo 2011). Bicarbonate and aqueous carbon dioxide are bioavailable forms of inorganic carbon because they can be transported into the cell via carbonic anhydrase and incorporated metabolically by ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO) (Giordano et al. 2005). The enzyme RUBISCO evolved in a high CO_2

environment, and as a result, the enzyme has a low affinity for CO_2 limiting growth of algal cultures in the relatively low atmospheric CO_2 environment of today (Moroney and Somanchi 1999). The half saturation constant (K_m) for RUBISCO in plants ranges between 15 and 25 μM and can even exceed 200 μM in some cyanobacteria (Moroney and Somanchi 1999).

Isolate GK5La was obtained from Soap Lake in Washington State (USA). Soap Lake is characterized as a high alkalinity saline lake, with pH values ranging from 9.8 to 10.2 and salinity ranging from 16.5 to 18g/L at the top of the lake to more than 100 g/L at the bottom of the lake below the halocline (Kallis et al. 2010). Isolates from this environment are likely adapted to high concentrations of Na^+, Ca^{2+}, Mg^{2+}, and K^+, along with low hydrogen ion activities (high pH values) for optimal growth.

In this paper, growth, pH, total extractable lipid content, total FAME content, and inorganic carbon consumption, are analyzed for isolate GK5La from Soap Lake. The study is centered on characterizing differences between two factors that will likely have implications in large-scale algal raceway ponds: carbon limitation, speciation, or bioavailability, and evaporative conditions resulting in high salt concentrations.

Methods

Isolation and Culturing

Algal strain GK5La was isolated from cultures collected at Soap Lake, Washington (USA). Isolate GK5La was cultured in AM6 medium adjusted to pH 9.5 prior to autoclaving. After autoclaving, the pH decreased to between 8-9. Cultures were grown in 1.25L photo bioreactors suspended in a temperature controlled aquarium and

sparged with atmospheric air (400mL/min) humidified in sterile nanopure water. The light system was set to a 14:10 light dark cycle using 12 T5 4ft fluorescent lights (350μmoles/m^2s) fixed behind it. "Salt stressed" cultures contained 18g/L sodium chloride from the beginning of the experiment. The "salt spiked" cultures received additional sodium chloride to bring the medium up to 18g/L after nitrate depletion.

Analysis of Medium Components

The pH of each solution was measured using an Accumet AP71 pH meter. The pH probe was calibrated between 7 and 10 before each use. Cell concentrations were determined using a hemacytometer, counting a minimum of 400 cells for statistical significance. Nile Red fluorescence measurements were taken 15 min after staining with 4μL/mL of culture volume as detailed in Gardner et al. (2013).

Nitrate, phosphate, and sulfate concentrations over time were determined using ion chromatography. An IonPac AS9-HC Anion-Exchange Column (Dionex) with a 9mM sodium carbonate buffer set at 1mL per minute was used as an eluent. A CD20 (Dionex) conductivity detector was used and the temperature was set at 21 °C. Thermo Fisher (Waltham, MA) software Chromelion (7.2) was used to analyze the data. For samples with sodium chloride concentrations in excess of 18 g/L, the chloride peak so large that nitrate concentrations could not be determined with this method.

To quantify nitrate concentrations at high sodium chloride concentrations, the NAS Szechrome (Polysciences Inc., USA) assay was employed. Sensitivity of the NAS Szechrome reagent was between 0 and 25 ppm nitrate, making 1:10 and 1:20 dilutions of the media necessary for time points early in the growth curve. One milliliter of culture

volume was pipetted into a microcentrifuge tube and spun down at 16000xg for 3 minutes. The supernatant was separated from the pellet through pipetting, and transferred to a new microcentrifuge tube. Then a 100µL volume of sample was pipetted into a microcentrifuge tube, and 1mL of the prepared NAS reagent was added to the same microcentrifuge tube. After incubation between 10 to 60 minutes, 200µL of sample was added to a clear polystyrene well plate and read at 450nm. A standard curve made in the medium solution was analyzed on every plate to quantify the collected absorbance data.

Cell Dry Weight

Algal cells were harvested and washed three times in deionized water through centrifugation (1380xg for 10 minutes). Cell dry weights were determined in pre-weighed 15ml Falcon tubes via lyophilization after being frozen. Pellets were dried through lyophilization for 24 hours to sublimate all moisture. The difference between the pre-weighed Falcon tube and Falcon tube containing dried algal biomass was assigned to cell dry weight.

Extractable Lipid Content Using GC-FID

Extraction, analysis, and quantification of neutral lipid components was adapted from Lohman et al. (2013). Neutral lipids were recovered through a modified Bligh and Dyer method (Bligh and Dyer 1959), bead beating dried biomass to rupture cells, chloroform to extract neutral lipids. A total of 10-30 mg of dry biomass was homogenized and added to a 2mL stainless steel bead beating tube. To the tube, 0.6 g of 0.1mm zirconium beads, 0.4 g of 1mm zirconium glass beads, and two 2.5mm zirconium glass beads were added. Additionally 1mL of chloroform was added after which the tube

was capped and shaken on an MP FastPrep 24 (MP Biomedicals, Solon, OH). The biomass was disrupted for 6 cycles of 20 seconds at 6.5m/s to rupture cell membranes. The contents of the 2mL stainless steel tube were emptied into a disposable glass test tube. The stainless steel tube was washed with 1mL of chloroform twice, emptied into the glass test tube, and followed by 1mL of 15% NaCl. The test tube was then vortexed for 10 seconds and centrifuged (1380xg) for 2 minutes, after which 1mL of the bottom solvent layer was collected and saved in a GC vial for analysis via gas chromatography flame ionization detection (GC—FID) (Agilent 6890N, Santa Clara, CA). A 15m (fused silica) RTX biodiesel column (Restek, Bellefonte, PA) was used for 1μL injections under a column temperature ramp from 100 to 370 °C using a gradient of 14°C/min. The carrier gas for this technique was helium. The flow rate varied throughout the process from 1.3 mL/min (0—22min), to 1.5 mL/min (22—24min) to 1.7mL/min (24—36min). Calibration curves were constructed using the standards: C10:0, C12:0, C14:0, C16:0, C18:0, C20:0 FFA; C12:0, C14:0, C16:0, C18:0 MAG; C12:0, C14:0, C16:0, C18:0 DAG; and C11:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0 TAG (Sigma-Aldrich, St. Louis, MO) for quantification. See Lohman et al. (2013) for details.

FAME Content Using GC-MS

Extraction analysis and quantification methods were adapted from Lohman et al. (2013). In situ transesterification was used to quantify the total amount and speciation of FAME extracted from a sample of dried biomass. A total of 5-15mg was measured into a 16x100mm screw cap glass test tube where one mL of toluene and 2mL of sodium methoxide were then added. The contents were heated at 95°C and vortexed

intermittently for 30 minutes. Then 2 mL of 14% boron trifluoride in methanol were added and the contents were heated for 30 minutes with intermittent vortexing for a second time. The test tubes were then removed, and allowed to cool to room temperature. Then 0.8mL of sodium chloride saturated water, 0.8ml of hexanes, and 10μL of C23 FAME were added to each test tube and vortexed for 10 seconds. The contents were heated for an additional 10 minutes before the phases were separated by centrifugation (1380xg) for 2 minutes. One mL of the organic top layer was collected in a GC vial and saved for GC—MS analysis (Agilent 6890N GC and Agilent 5973 Networked MS). GC—MS analysis was carried out according to the published protocol (Bigelow et al. 2011). One-microliter samples were injected onto a 30m x 0.25mmAgilent HP-5MS column (0.25μm film thickness). The column temperature started at 80 °C and ramped at 14 °C/min to a final temperature of 310 °C where it was held for 3 minutes. The injector temperature was set at 250 °C and the detector temperature was set at 280 °C. Helium was the carrier gas and the flow through the column was 0.5mL/min. Calibration curves were constructed using a 28-component fatty acid methyl ester standard prepared in methylene chloride ("NLEA FAME mix": Restek, Bellefonte, PA). Peak quantifications were made with the nearest calibration standard based on retention time and were performed in Agilent MSD Chem Station software (Version D.02.00.275).

Results and Discussion

The goal of this study was to characterize isolate GK5La from Soap Lake, Washington, and assess the impacts on biofuel production of two conditions: high ionic strength solution in the form of sodium chloride in excess of 1.8%, and carbon

supplemented treatments through the addition of inorganic carbon in the form of sodium bicarbonate.

Inorganic Carbon Supplemented Versus Carbon Limited

Isolate GK5La was grown in AM6 medium, and in the presence of excess DIC in the range of 7-10mM as seen in Figure 5.1a. Specific growth rates for both conditions were similar until day 4 when the pH value increased to 12.05 and $NaHCO_3$ (1M) was spiked into solution to a concentration of 7mM for the first time. In all sodium bicarbonate supplemented treatments, sodium bicarbonate was added after DIC measured in solution had depleted to near zero. DIC was still being consumed until day 6, after which the concentration of DIC began rising in solution as CO_2 from the atmosphere in-gassed. On day 10, DIC concentration began to decrease and the pH began to increase as seen in Figure 5.1b, prompting the second exponential growth phase before reaching stationary phase. Nitrate became limited in the control by day 9, while nitrate in the inorganic carbon supplemented culture was depleted by day 14, highlighting the large impact the inhibited growth rate after day 4 had on biofuel production as a result of nitrate depletion. The limit of detection for nitrate for both methods was between 0 and 5 ppm.

Alkaline conditions are considered a possible way to grow algae under open raceway pond conditions, however, relying only on CO_2 gas transfer as a source of carbon dioxide is inadequate and may result in inorganic carbon limited media. Sparging with a concentrated CO_2 source may be cost prohibitive, and also drop the pH in solution creating an environment potentially more conducive to bacterial growth. Organic carbon

sources such as glucose, sucrose, and lactate are relatively expensive and can be consumed by bacteria. Previous studies have shown sodium bicarbonate can be added as a form of inorganic carbon to increase cell dry weight, FAME, and pigment production (White et al. 2012; Costa et al.2003; Gardner et al. 2012).

The pH in the experimental environment is a master control variable, influencing both ion speciation in solution and cell growth. For isolate GK5La in the treatment of AM6 supplemented with inorganic carbon, on day 4, as the pH rose to 11.6 and approached 12, the carbon speciation in solution changed from bicarbonate to carbonate, the less bioavailable form of aqueous inorganic carbon (Nakajima et al. 2013; Hansen et al. 2007). This inhibited not only growth, but inorganic carbon utilization and nitrate utilization as well. Previously, the formation of carbonate ions in solution has been suggested to inhibit the bicarbonate pump (Ramanan et al. 2010). As pH decreased favoring bicarbonate once again, the culture rebounded and completely consumed the rest of the available nitrate before reaching a stationary phase for the second time.

Comparison of Salt Spiked and Salt Stressed Treatments

Isolate GK5La was inhibited after the addition of 7mM sodium bicarbonate on day 4, possibly due to increasing ionic strength in solution. To better understand growth of the isolate under ionic stress, two conditions were tested in an environment consisting of 1.8% sodium chloride. These results compare well with Kaewkannetra et al. (2012) who demonstrated that a *Scenedesmus* sp. accumulated lipid in the presence of increased salt concentration. For both treatments tested, sodium chloride was added to a

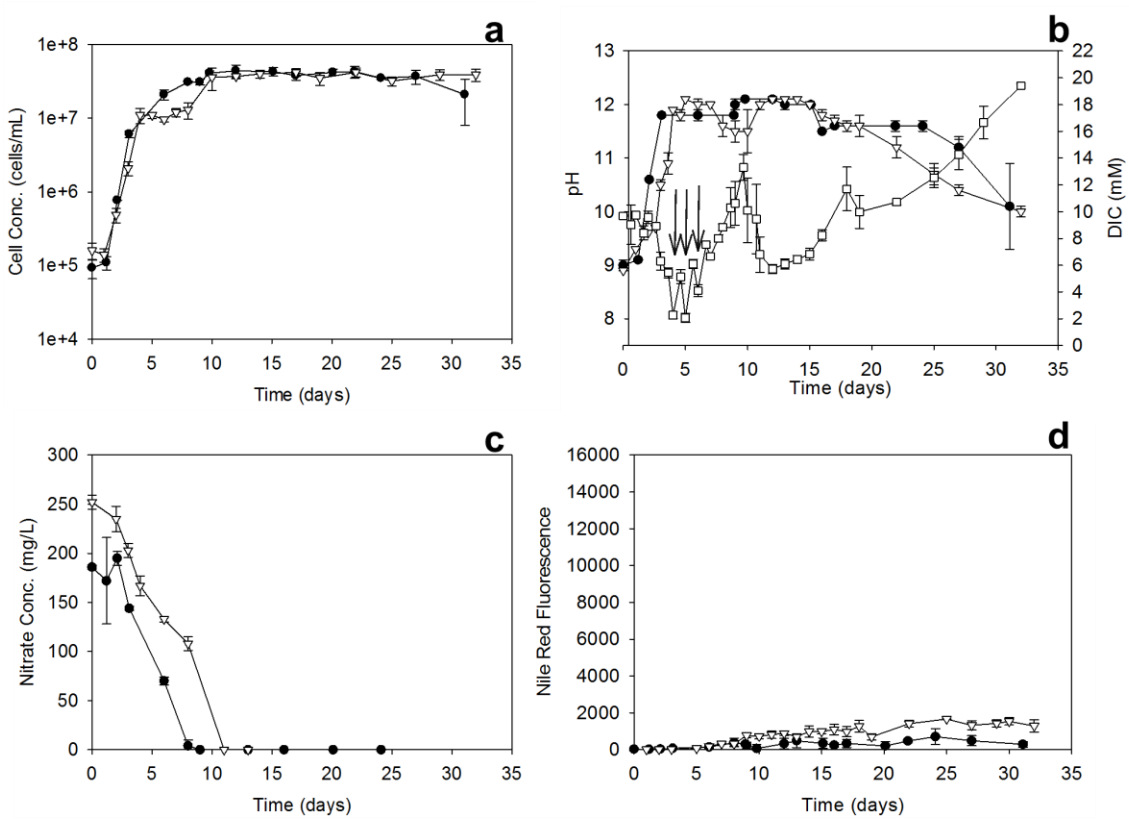


Figure 5.1. Mean and range of (a) cell density, (**b)** pH and DIC (□),(**c)** nitrate concentration, and (d) Nile Red fluorescence for triplicate cultures of isolate GK5La grown in control AM6 media (●), and AM6 media supplemented with HCO_3^-(▽). Downward arrows indicate addition of 1M filter sterilized $NaHCO_3^-$ to a concentration of 7mM.

concentration of 18g/L either at the beginning (stressed) or on day 9 (spiked) of the study. The cultures were spiked on day 9 to induce lipid accumulation after a high cell concentration was reached and the growth rate slowed. Figure 5.2a shows sodium chloride stress inhibited GK5La growth, leading to a lower max cell concentration ($1.20x10^7$cells/mL compared to $3.75x10^7$cells/mL) and lower cell dry weight (1.02 mg/mL compared to 1.34mg/mL) when compared to the salt spiked condition. The pH in the salt stressed culture was less than the salt spiked culture, indicating reduced

photosynthetic activity (Figure 5.2b). After spiking the culture to a concentration of 1.8% sodium chloride on day 9, cell division ceased (Figure 5.2) and pH began decreasing immediately. The decrease in pH was an indicator of the decline in photosynthetic activity. Sodium chloride stress inhibited growth of isolate GK5La, resulting in nitrate depleting in the culture at day 24. Nile Red fluorescence indicated that the spike culture accumulated more TAG over the course of the experiment (Figure 5.2). In the spiked culture, increase in fluorescence started on day 13, four days after receiving the addition of sodium chloride.

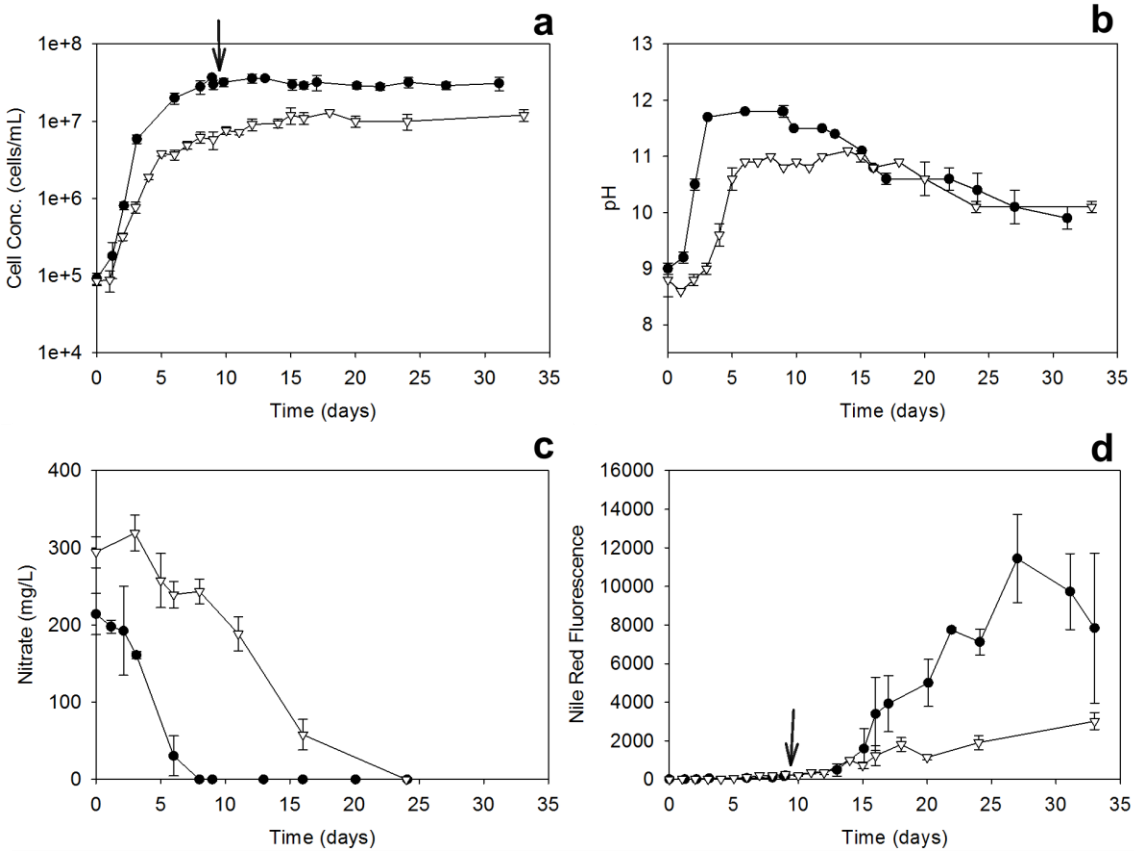


Figure 5.2. Mean and range of (a) cell density, (b) pH, (c) nitrate, and (d) Nile Red fluorescence for triplicate cultures of isolate GK5La grown in AM6 spiked to 1.8% sodium chloride (●), and AM6(1.8) (▽). Downward arrow indicates NaCl spike to a concentration of 18g/L.

Comparison of Inorganic Carbon
Supplemented Salt Spiked/Stressed

As indicated by Nile Red fluorescence, the presence of sodium chloride in excess of 1.8% increases lipid accumulation in the cultures. To find an optimized condition relating to both growth and lipid accumulation, inorganic carbon supplementation in the presence of 1.8% sodium chloride was studied. Inorganic carbon supplementation carried out similar to the treatment in Figure 5.1, where just as DIC ran out in solution the culture was supplemented with sodium bicarbonate to a concentration of 7mM. Figure 5.3a shows growth of isolate GK5La was better when spiked with sodium chloride rather than stressed with sodium chloride. This is similar to the earlier case (Figure 5.2) without additional inorganic carbon supplementation. Growth was inhibited with the first addition of inorganic carbon on day 4 and eventually rebounded by day 10. Sodium chloride was not added until nitrate had been depleted on day 12 and the cells had just reached their second stationary phase. Figure 5.3b shows the pH of the carbon supplemented salt spiked system decreased after the addition of sodium chloride, indicating decreased photosynthetic output, and even fell lower than the inorganic carbon supplemented salt stressed system toward the end of the study. The pH of the salt stressed system was lower and held near 10.5 at stationary phase. Figure 5.3 shows nitrate did not deplete in the inorganic carbon supplemented sodium chloride stressed condition until day 31. This is longer than the sodium chloride stressed condition shown in Figure 5.2, and may be attributed to the higher ionic strength of the system from the additional sodium bicarbonate added. Nile Red fluorescence for each of the treatments trended close together throughout the entirety of the study. After addition of sodium

chloride in Figure 5.2, Nile Red fluorescence began increasing four days thereafter. In Figure 5.3, Nile Red fluorescence began increasing four days after the addition of sodium chloride, but then dropped lower after day 18 and did not appreciably increase throughout the rest of the study.

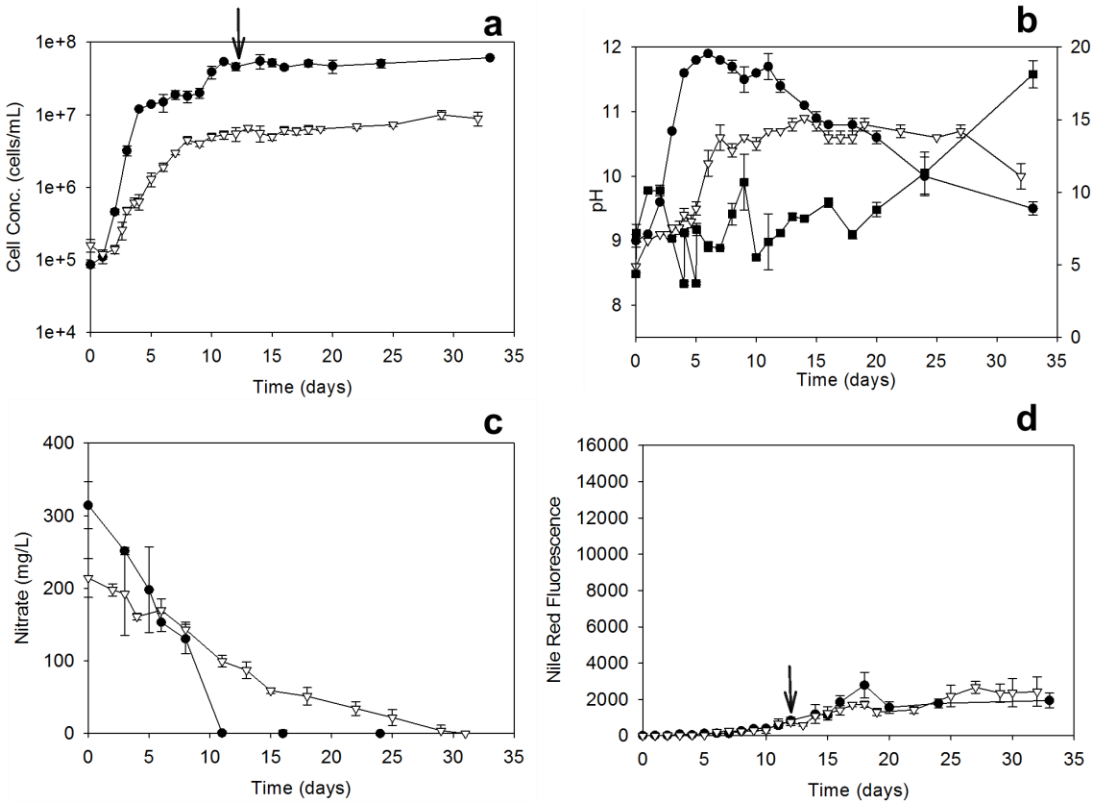


Figure 5.3. Mean and range of (a) cell density, (b) pH, and DIC (□), (c) nitrate, and (d) Nile Red fluorescence for triplicate cultures of isolate GK5La grown in AM6 supplemented with HCO_3^- and spiked to 1.8% sodium chloride (●), and AM6(1.8) supplemented with HCO_3^- (▽). Downward arrow indicates NaCl spike to concentration of 18g/L.

Comparisons of 50mM CHES Buffered
Inorganic Carbon Supplemented AM6
Media and 50mM CHES Buffered AM6 Media

It was hypothesized that the speciation of inorganic carbon in solution played a larger role than previously expected, leading to the use of CHES buffer to regulate pH in

the range favoring bicarbonate. The next two conditions tested with isolate GK5La were in AM6 media buffered with 50mM CHES, with and without added sodium bicarbonate (Figure 5.4). Figure 5.4b shows the pH without additional sodium bicarbonate stayed buffered steady in the range of 8.6-8.9. For the case that was buffered with CHES and fed with additional sodium bicarbonate, the buffer exceeded its workable range by day 7, and the pH rose to a maximum value of 11.6. With additional DIC, the maximum cell concentration ($6.3x10^7$ cells/mL) was higher relative to the CHES buffered inorganic carbon-limited medium ($3.1x10^7$ cells/mL).

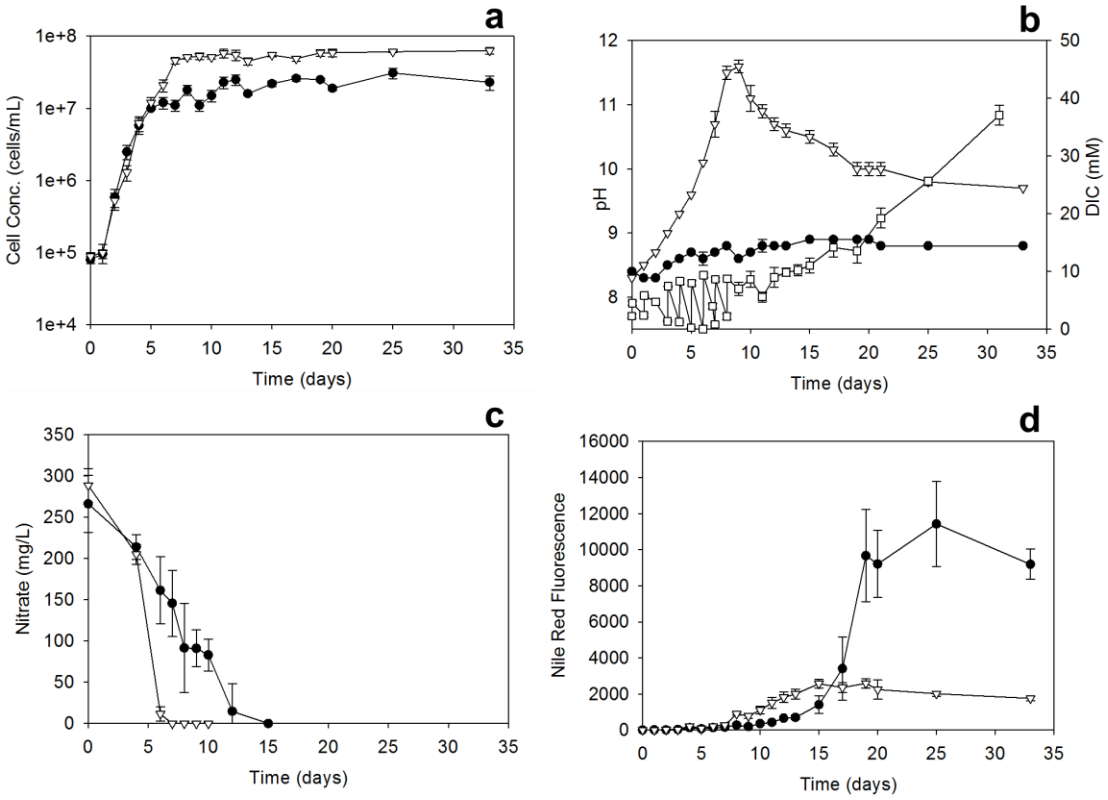


Figure 5.4. Mean and range of (a) cell density, (b) pH and DIC (□), (c) nitrate, and (d) Nile Red fluorescence for triplicate cultures of isolate GK5La grown in AM6 buffered with 50mM CHES and supplemented with HCO_3^- (▽), and AM6 buffered with 50mM CHES (●).

MINTEQ Modeling/Activity

Though the concentration of each ion in solution was added in by measured amounts, this was not truly an accurate representation of the experimental environment as concentration is only a proxy for chemical potential. Chemical potential of each species in solution is controlled by thermodynamic relationships that take into account the non-idealities that can occur from interactions among ions. Activity takes into account this non-ideality, and is a measure of effective concentration. Visual MINTEQ 3.0 (KTH Department of Land and Water Resources, 2010) is a chemical equilibrium program that models activity of ions in solution, and is a common approach taken to understand speciation of important ions in solution. AM6 medium is a simple solution with limited salts, low ionic strength, and activity coefficients near 1 for all components. In the treatments containing 18g/L of sodium chloride, however, activity coefficients may be important to understand the activity of various components such as protons or inorganic carbon species. Activity was modeled using Visual MINTEQ 3.0.

Table 5.1. MINTEQ carbon speciation modeling over the pH range 8-11 for AM6

pH	8	9	10	11
Component	%	%	%	%
CO_3^{2-}	0.609	6.091	52.682	91.9
$NaHCO_3$ (aq)	0.25	0.228	0.05	
HCO_3^-	96.748	92.154	45.794	7.931
H_2CO_3* (aq)	1.968	0.184	-	-
$MgCO_3$ (aq)	0.052	0.404	0.148	
$MgHCO_3^+$	0.151	0.12	-	-
$CaHCO_3^+$	0.106	0.056	-	-
$CaCO_3$ (aq)	0.058	0.307	0.079	-
$NaCO_3^-$	0.048	0.447	1.231	0.16

The pH sweeps pertaining to basic AM6 media (Table 5.1), and AM6 media with 1.8% sodium chloride (Table 5.2) detail the complexity involved in different speciation between both of the media just pertaining to inorganic carbon. For both cases, as pH rises from 8 to 11 the inorganic carbon equilibrium shifts from predominantly bicarbonate to increasing overall abundance of carbonate. This had a significant impact in the experimental setting considering that bicarbonate is more bioavailable than carbonate (Nakajima et al. 2013; Hansen et al. 2007). The condition in which bicarbonate was supplemented to the medium over the course of the experiment (Figure 5.1a) illustrates this conclusion most clearly. The first addition of inorganic carbon in the form of bicarbonate occurred at day 4 when the pH was near 11.7. The sodium bicarbonate added to the medium changed to sodium and carbonate ions in fractions of a second (Langmuir 1997), and potentially inhibited growth, not increasing growth as previously expected. The second boost in growth occurred once pH dropped just below 11.5.

Table 5.2. MINTEQ carbon speciation modeling over the pH range 8-11 for AM6(1.8) medium.

pH	8	9	10	11
Component	%	%	%	%
CO_3^{2-}	1.059	8.597	33.866	83.403
$NaHCO_3$ (aq)	6.561	5.272	1.347	0.03
HCO_3^-	89.191	72.276	28.584	8.304
H_2CO_3* (aq)	1.375	0.111	-	-
$MgCO_3$ (aq)	0.015	0.117	0.086	
$MgHCO_3^+$	0.059	0.045	-	-
$CaHCO_3^+$	0.045	0.031	-	-
$CaCO_3$ (aq)	0.019	0.128	0.05	-
$NaCO_3^-$	1.667	13.418	36.056	8.253

Media used in the study containing 18g/L sodium chloride had an impact on the carbon speciation in solution and the activity of certain ions including pH. The high salt medium favored sodium carbonate and carbonate at pH above 11 as shown in Table 5.2. Excess sodium cations lead to favorable conditions to form aqueous sodium carbonate in solution, creating another sink for inorganic carbon. Another affect that high salinity had the experimental environment was in activity coefficients describing the non-ideality of solution by not being near a value of 1 as predicted by Davies approximations. High salinity affected hydrogen ion behavior in solution by decreasing the activity. The pH decreased when salt was added to solution. This decrease in pH after spiking the solution to a concentration of 1.8% sodium chloride can only be attributed to lower cell productivity, also indicated by a lag in cell concentration, optical density, total chlorophyll, and final cell dry weight.

Lipid Analysis

Carbon is an essential element required for growth of all biological organisms. Photoautotrophic algae are evolved to utilize carbon dioxide, carbonic acid, and bicarbonate to increase biomass and construct macromolecules important to the health and multiplication of a population. Biofuel production (FAMEs or extractable lipids) is contingent upon the growth of algae, and the ability to accumulate a large portion of its cell mass as lipid. Inorganic carbon was supplemented to the media to increase growth rate, final cell dry weight (day 33-34), extractable lipid content, and biodiesel content.

Inorganic carbon supplementation in the form of sodium bicarbonate led to seemingly contradictory results. Medium AM6 supplemented with excess inorganic

carbon (treatment [5], Figure 5.5) compared to the control grown in AM6 [treatment 1], contained higher total neutral lipids as seen in Figure 5.5b, but similar FAME content as seen in Figure 5.5c. A similar amount of FAME content from both AM6 [treatment 1] and AM6 supplemented with sodium bicarbonate[treatment 5] suggests that on a weight per weight basis, both treatments accumulated similar amounts of lipid, but the carbon supplemented culture [treatment 5] had more nonpolar lipid stores, while the control stored the majority of its lipid in polar membranes. On a gram per liter basis there was more total neutral lipid produced in the AM6 supplemented with sodium bicarbonate[treatment 5] than the control grown in AM6 [treatment 1], but there was similar FAME quantified between both treatments as determined through a 95% confidence interval. The data suggest it would be more productive to supplement with inorganic carbon [treatment 5] if neutral lipid productivity was desired, however, if conventional biodiesel as FAME was desired, the control case, grown only in AM6 medium [treatment 1], would lead to a more productive system.

Sodium chloride proved to be an effective stressor to increase biofuel productivity in the isolate. Spiking isolate GK5La with 1.8% sodium chloride [treatment 4] after nitrate depletion was more productive than culturing in the presence of 1.8% sodium chloride [treatment 7] throughout the entire study. The treatment spiked with salt [treatment 4] had a higher cell dry weight than the salt stressed case [treatment 7], affecting both extractable lipid content and biodiesel content. Figure 5.5b shows extractable lipid content was higher in the salt spiked case [treatment 4] compared to the salt stressed case [treatment 7]. The disparity between the salt spiked [treatment 4] and the salt stressed culture [treatment 7] was minimal due to the higher cell dry weight in the

salt spiked condition [treatment 4]. FAME content between the salt spiked treatment and the salt stressed treatment were similar as determined by a 95% confidence interval. The solution pH near 12.0 just prior to spiking with salt indicated that isolate GK5La may have been carbon limited, and subsequently restricted in lipid accumulation.

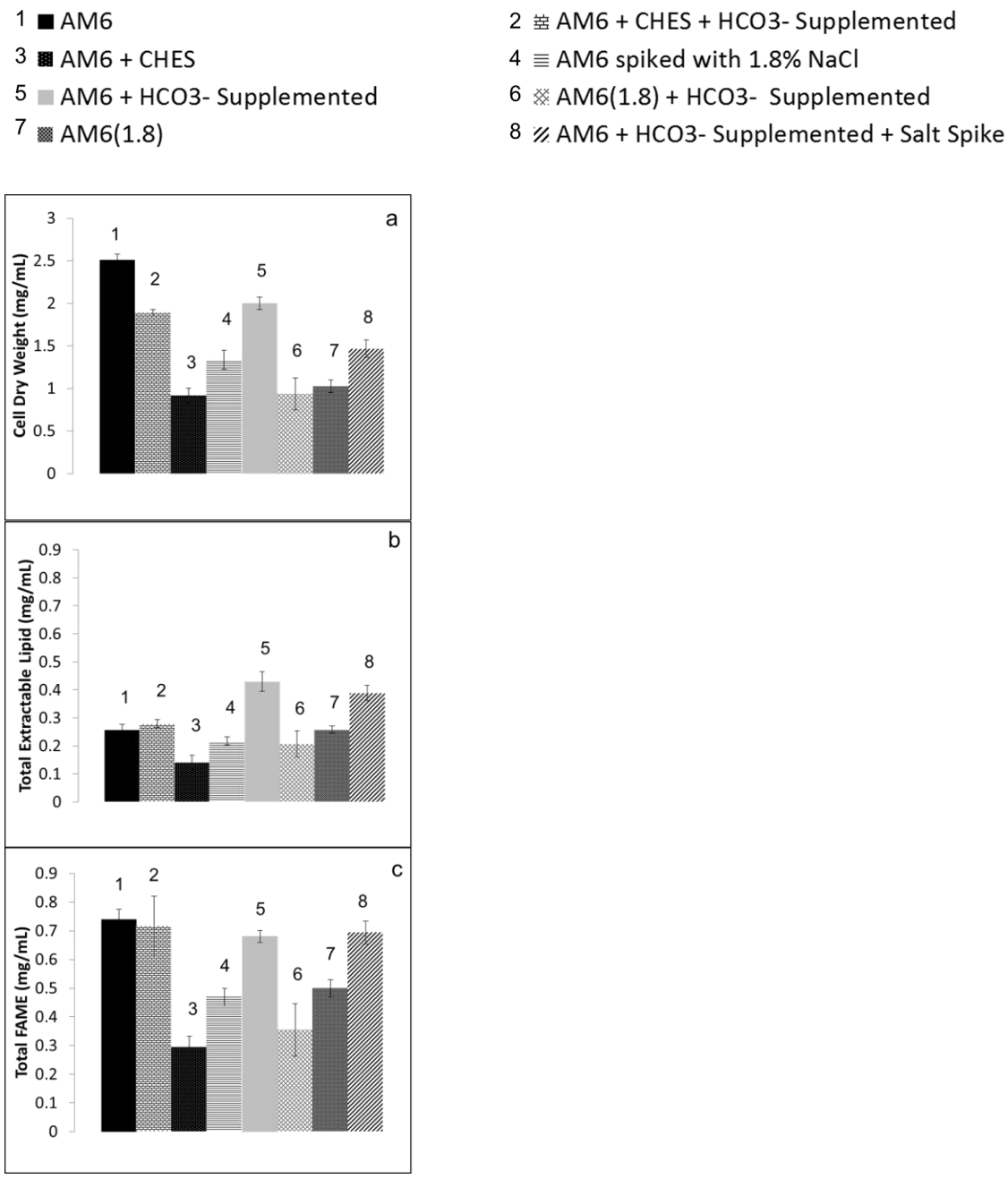


Figure 5.5. Mean and range of end point (day 33-34) (**a)** cell dry weight, (**b)** total extractable lipid, and (**c)** total FAME for each of the eight conditions tested.

Even with inorganic carbon supplementation, spiking with sodium chloride [treatment 8] after nitrate depletion showed to be a more productive culturing system. Figure 5.5 suggests that the carbon supplemented salt spiked condition [treatment 8] was more productive than the inorganic carbon supplemented salt stressed condition [treatment 6] when considering the production of both extractable lipid and total FAME on a gram per liter basis. Spiking with sodium chloride and supplementing with sodium bicarbonate [treatment 8] produced enough FAME from the culture that it was not different than the treatment cultured in AM6[treatment 1] as determined by a 95% confidence interval.

Buffering the solution pH with CHES and supplementing with inorganic carbon [treatment 2] proved more productive than only buffering with CHES [treatment 3] when considering cell dry weight, total extractable lipid content, and total FAME content. Figure 5.5a shows the 50mM CHES buffered inorganic carbon supplemented [treatment 2] condition contained roughly twice as much final cell dry weight when compared to the 50mM CHES buffered inorganic carbon limited condition [treatment 3]. This resulted in a more productive system as measured by cell dry weight, total extractable lipid content, and biodiesel content, for CHES buffered inorganic carbon supplemented treatment [treatment 2] compared to the CHES buffered treatment [treatment 3]. Conditions buffered with CHES salt controlled pH in a range predominately favoring bicarbonate for at least part of the study. With respect to cell dry weight, differences were noted between the AM6 buffered with CHES [treatment 3] and AM6 buffered with CHES and supplemented with sodium bicarbonate [treatment 2] (Figure 5.5a), highlighting the importance of pH and inorganic carbon supplementation for the production of biofuel.

Though the buffer capacity was eventually exceeded for the inorganic carbon supplemented case [treatment 2], the pH was low enough during the exponential growth phase to keep the culture from being bicarbonate limited, leading to a far higher final cell dry weight when compared AM6 buffered with CHES [treatment 3].

Comparing all the conditions in Figure 5.5 together, it can be seen that there was not an optimal culturing condition that favored cell dry weight, total lipid content, and total FAME content. Final cell dry weight was the highest for the control treatment containing only AM6 [treatment 1] media, followed by AM6 supplemented with inorganic carbon [treatment 5] and then AM6 buffered with CHES and supplemented with inorganic carbon [treatment 2]. The lowest final cell dry weight yields were AM6 buffered with CHES [treatment 3], AM6(1.8) supplemented with inorganic carbon [treatment 6], and AM6(1.8) [treatment 7]. Figure 5.5b shows the treatment containing highest extractable lipid content was AM6 supplemented with inorganic carbon [treatment 5], followed by AM6 supplemented with inorganic carbon and spiked with sodium chloride [treatment 8], and AM6 buffered with CHES and supplemented with inorganic carbon [treatment 2]. The lowest extractable lipid content was found in the treatment of AM6 buffered with CHES [treatment 1] (workable pH range 8-10). The treatment containing the highest total FAME content was the control AM6 medium [treatment 1] as shown in Figure 5.5c. Following the control were AM6 buffered with CHES and supplemented with inorganic carbon [treatment 2], AM6 supplemented with inorganic carbon and spiked with sodium chloride [treatment 8], and AM6 supplemented with inorganic carbon [treatment 5]. Once again, the treatment containing the lowest biodiesel content was AM6 buffered with CHES [treatment 3].

Specific Lipid Content

Figure 5.6 shows inorganic carbon supplementation [treatment 5] increased specific lipid composition on a weight percentage basis as compared to the control grown in AM6 [treatment 1]. AM6 [treatment 1] and AM6 supplemented with sodium bicarbonate treatments [treatment 5] stored the greatest proportion of their specific neutral lipid content in free fatty acids, and the condition supplemented with inorganic carbon [treatment 5] had higher free fatty acid content than the control [treatment 1]. Under stressed conditions, intracellular lipid is often stored in the form of TAG (Wang et al. 2009), conversely though, isolate GK5La accrued most of its stored neutral lipid in the form of free fatty acids (Figure 5.6). This led to challenges in monitoring intracellular lipid accumulation with the qualitative fluorescent lipid stain Nile Red due to its strong correlation only with TAG, but not free fatty acid (Gardner et al. 2013; Lohman et al. 2013). The results indicate the isolate from Soap Lake Washington may have been deficient in enzymes tasked with the formation of the glycerol backbone of TAG, were inhibited, or stored neutral lipid in the form of free fatty acid.

The different ways isolate GK5La was cultured in high salinity medium (spiking or stressing) affected fatty acid speciation, total specific neutral lipid content, and total specific FAME content on a weight percentage basis. Considering fatty acid speciation, Figure 5.6 shows higher amounts of FFA, MAG, and DAG in the AM6 (1.8) [treatment 7] compared to the AM6 1.8% spiked with sodium chloride [treatment 4]. Both salt stressed [treatment 7] and salt spiked treatments [treatment 4] had higher specific TAG content when compared to the control grown only in AM6 [treatment 1] as shown in Figure 5.6. As a result of the increase in free fatty acid, MAG, and DAG, the salt

stressed condition [treatment 7] had more total specific neutral lipid and higher total specific FAME than the AM6 spiked with 1.8% sodium chloride treatment [treatment 4].

Under increased sodium chloride concentration, specific TAG content increased compared to the control condition [treatment 1], while other fatty acid classes were similar. Total specific extractable lipid content on a weight per weight basis was higher in both saline conditions tested [treatment 4 and treatment 7]. This showed for isolate GK5La, saline waste water streams could be an effective means to increase not only specific TAG content, but overall extractable nonpolar lipid content as well, in part, creating another use for saline waste streams stemming from the evaporation of water and the concentration of salts in outdoor raceway ponds.

Carbon supplementation combined with sodium chloride stress through spiking after nitrate depletion [treatment 8], or stressing over the entire length of the study [treatment 6] increased specific total neutral lipid content and specific total FAME content on a weight percentage basis, while also impacting fatty acid speciation (Figure 5.6). Both of the treatments [treatment 6 and treatment 8] were similar with regard to lipid composition on a weight per weight basis. The main exception is that the carbon supplemented salt spiked condition [treatment 8] had about twice as much MAG and as a result more total specific neutral lipid content as shown in Figure 5.6. The carbon supplemented salt spike condition [treatment 8] also had more total specific FAME content on a weight per weight basis indicating that it had more non-polar and polar lipids than the inorganic carbon supplemented salt stressed treatment [treatment 6].

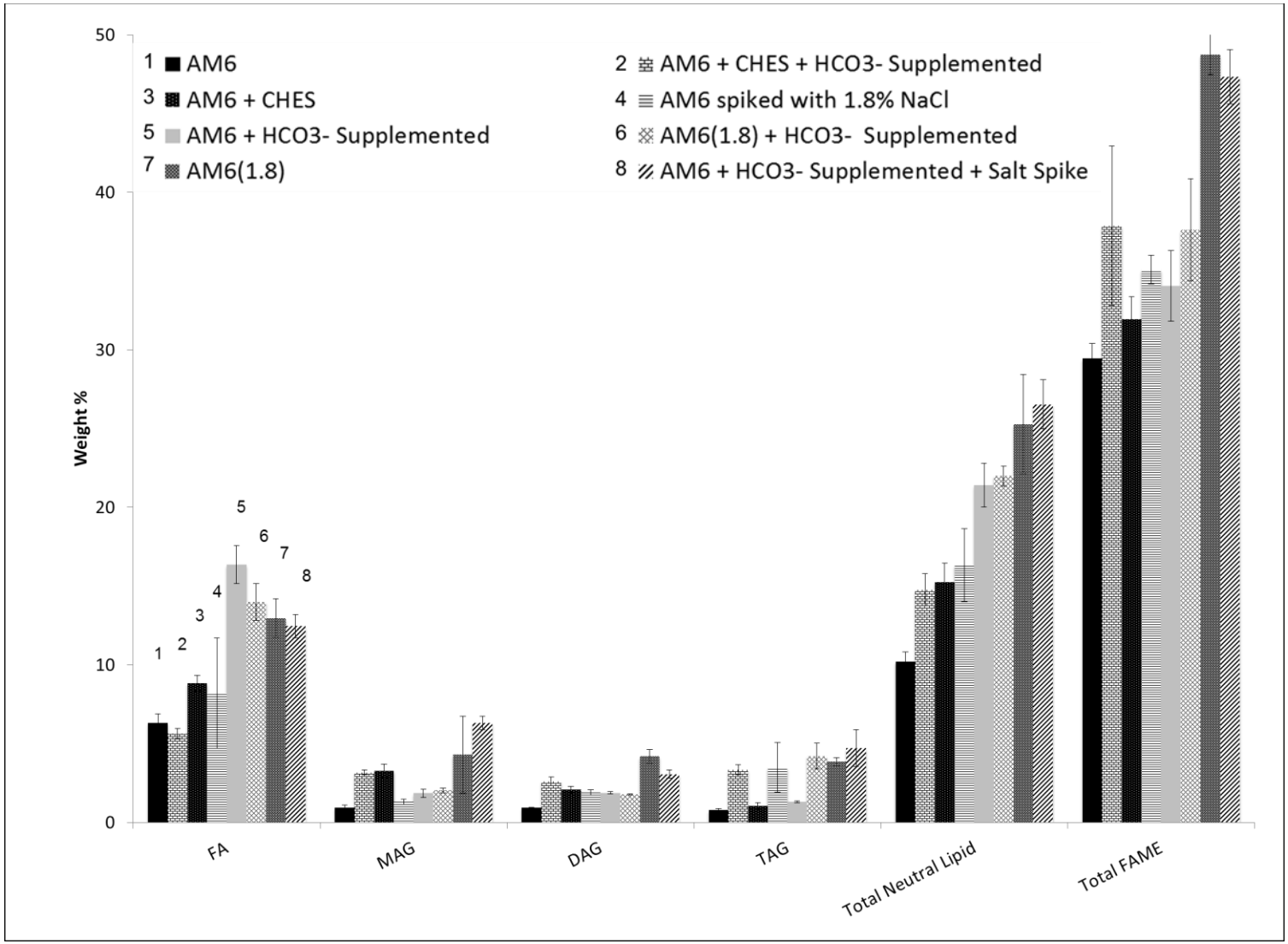


Figure 5.6. Mean and range of end point (day 33-34) weight % FA, MAG, DAG, TAG, total neutral lipid, and total FAME, for each of the eight conditions tested.

The culture conditions subjected to 50mM CHES with [treatment 2] and without inorganic carbon supplementation [treatment 3] drastically differed from one another with respect to lipid composition. Figure 5.6 shows on a weight per weight basis, more specific TAG content was present in the CHES buffered carbon supplemented condition [treatment 2], but more free fatty acid was present in the CHES buffered inorganic carbon limited condition [treatment 3], however, the outcome of these results led to similar amounts of total neutral lipids between both conditions as determined by a 95% confidence interval. More total specific FAME content on a weight per weight basis was measured in the CHES buffered inorganic carbon supplemented case [treatment 2].

Trends in speciation of neutral lipids on a weight per weight basis could be identified based on the amount of salt in solution and the degree of inorganic carbon supplementation (Figure 5.6). Inorganic carbon supplementation led to higher levels of either free fatty acid or MAG or both (the only exception was AM6 + CHES + HCO_3^- supplemented [treatment 2]). The addition of sodium chloride in excess of 18g/L (AM6(1.8) [treatment 7] and AM6 1.8% spike [treatment 4]) increased specific TAG and/or DAG content on a weight per weight basis. Conditions with high ionic stress, high pH, and the presence of excess inorganic carbon resulted in the most total specific neutral lipid content. Total specific FAME content followed the same trend except for the condition buffered with 50mM CHES and supplemented with inorganic carbon [treatment 2].

High free fatty acid content in isolate GK5La was unexpected since most microalgae store high-energy lipid molecules in the form of TAG when stressed. Under desiccating conditions, membranes in isolate GK5La may have been affected by free

radicals causing the de-esterification of lipid molecules to form free fatty acids. Bearing in mind the isolate came from a saline lake, regulation of de-esterified free fatty acids may come as a common occurrence to mediate ionic stress.

Endpoint FAME speciation for isolate GK5La under each of the culturing conditions showed several similarities with respect to carbon chain length and degree of saturation (Table 5.3). Greater than 97% of the FAME derived under each condition tested belonged to C16 and C18 chains. When isolate GK5La was supplemented with sodium bicarbonate and stressed with 1.8% sodium chloride [treatment 6] (Table 5.3), the percentage of unsaturated C18:1-3 FAME quantified was 69.2% ± 0.7, the highest recorded in the study. Growth in AM6 buffered with CHES [treatment 3] led to the lowest percentage of unsaturated C18:1-3 FAME (63.9% ± 0.5) out of all the culture conditions. The culture buffered with CHES [treatment 3] had the highest saturated C16:0 FAME content at 22.8% ± 1.5, and the condition with the lowest amount was AM6 supplemented with sodium bicarbonate and grown the presence of 1.8% sodium chloride [treatment 6] (Table 5.3). Generally, the different treatments contained similar amounts of C16:0 saturated and unsaturated FAME excluding the treatment grown in AM6 media buffered with CHES [treatment 3].

The FAME speciation data does not entirely agree with previous studies on the impact of salt stress on microalgae for the purposes of producing lipid, in that, increasing sodium chloride concentration in the medium should lead to more unsaturated C16 and C18 FAME. Studies have previously demonstrated that increases in sodium chloride can impact the degree of saturation of fatty acids produced. Zhou et al. (2013) showed

Chlorella sp. cultured in 5L triangular flasks under outdoor condition increased C18:3 FAME from 16.2% to 21.6% when exposed to 10g/L sodium chloride.

Summary and Conclusions

The studies presented here highlight the importance of dissolved inorganic carbon and sodium chloride in algal cultures for the purpose of producing biofuel. Of the 8 treatments tested, buffered conditions supplemented with sodium bicarbonate and spiked with sodium chloride to a concentration of 1.8% were shown to be the most efficient way to produce biofuel in both the forms of extractable lipid or FAME. The control condition subject to only AM6 media was the most productive with respect to FAME content (grams of FAME per liter of culture volume).

Synthetic buffers were used to control the pH in solution, but the cost of employing these at large scales is unfeasible. Sodium bicarbonate helped buffer pH throughout the experiment, but eventually its capacity was exceeded. Coupling an inexpensive buffer like sodium bicarbonate with a pH control system may be the most feasible option for facilities culturing algae for biofuel or other high value products.

Sodium bicarbonate is recovered through mining operations throughout the world and is a relatively inexpensive chemical given the potential beneficial use in the industry. Using a mined source of inorganic carbon may detract from the sustainable nature of producing fuel sources from organic plant matter, and decreases the carbon neutrality of the process. Inorganic carbon supplementation can also be in the form of carbon dioxide gas, however, this is an expensive remedy that drops the pH in solution while most of the CO_2 bubbled in solution flows to the atmosphere without being dissolved.

Table 5.3. Mean and range (standard deviation) of end point (day 33-34) weight % FAME for each of the eight conditions tested.

	AM6	AM6 spiked with 1.8% NaCl	AM6 + HCO_3^- Supplemented	AM6(1.8) + HCO_3^- Supplemented	AM6 + CHES	AM6 + CHES + HCO_3^- Supplemented	AM6 + HCO_3^- Supplemented + Salt Spike	AM6(1.8)
C12:0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
C14:0	0.3 ± 0.01	0.4 ± 0.02	0.4 ±0.01	0.4 ± 0.03	0.2 ± 0.07	0.1 ± 0.01	0.2 ± 0.04	0.2 ± 0.02
C16:3	3.7 ± 0.08	3.0 ± 0.49	2.9 ± 0.06	3.7 ± 0.15	2.6 ± 0.28	2.6 ± 0.10	2.1 ± 0.18	3.0 ± .17
C16:2	4.8 ± 0.22	5.8 ± 0.07	6.1 ± 0.16	4.2 ± 0.16	1.4 ± 0.08	3.9 ± 0.02	5.4 ± 0.35	5.3 ± 0.45
C16:1	5.6 ± 0.14	8.4 ± 0.61	6.5 ± 0.2	8.4 ± 0.3	6.1 ± 0.61	7.5 ± 0.28	10.7 ± 0.51	10.0 ± 0.68
C16:0	14.1± 0.18	14.1 ± 0.71	14.1 ± 0.24	11.2 ± 0.71	22.8 ± 1.51	18.9 ± 0.59	14.3 ± 0.58	12.9 ± 0.33
C18:1-3	67.6 ± 0.2	66.2 ± 0.80	66.4 ± 0.4	69.2 ± 0.71	63.9 ± 0.51	64.3 ± 0.48	64.1 ± 1.62	65.9 ± 1.20
C18:0	1.7 ± 0.36	0.7 ± 0.85	1.5 ± 0.06	1.4 ± 0.03	1.0 ± 0.07	1.3 ± 0.02	1.4 ± 0.05	1.1 ± 0.07
C20:5	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
C20:1	0.6 ± 0.01	0.4 ± 0.01	0.5 ± 0.09	0.2 ± 0	0.2 ± 0.01	0.3 ± 0.01	0.4 ± 0.02	0.4 ± 0.01
C20:0	0.4 ± 0.02	0.2 ± 0.03	0.3 ± 0.12	0.3 ± 0.03	0.1 ± 0.02	0.1 ± 0	0.1 ± 0.01	0.1 ± 0.01
C22:1	0.2 ± 0.12	0.0 ± 0.07	0.2 ± 0.03	0.1 ± 0.09	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
C22:0	0.2 ± 0.2	0.3 ± 0.04	0.3 ± 0.02	0.3 ± 0.07	0.3 ± 0.15	0.3 ± 0.03	0.2 ± 0.01	0.2 ± 0.02
C24:1	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
C24:0	0.2 ± 0.01	0.1 ± 0.03	0.2 ± 0.01	0.3 ± 0.12	0.3 ± 0.03	0.2 ± 0.03	0.2 ± 0.04	0.1 ± 0.01
C26:0	0.3 ± 0.01	0.3 ± 0.04	0.3 ± 0.02	0.3 ± 0.06	0.3 ± 0.04	0.3 ± 0.05	0.3 ± 0	0.2 ± 0.02
Other	0.3 ± 0.26	0.2 ± 0.1	0.2 ± 0.02	0.2 ± 0.03	0.5 ± 0.17	0.2 ± 0.21	0.5 ± 0.31	0.6 ± 0.16

Pilot scale production facilities are now being built that can produce sodium bicarbonate and hydrochloric acid by adsorbing waste carbon dioxide in a sodium hydroxide bath (Knaggs et al. 2012). The use of sodium bicarbonate derived from waste carbon dioxide may improve the sustainability of the process through carbon cycling.

Extractable lipid content is a measure of lipid stores in the cell that can be extracted with non-polar solvent. Treatments stressed/supplemented with sodium bicarbonate and stressed with sodium chloride resulted in the highest extractable lipid content on a weight per weight basis. Stressing cultures only with sodium bicarbonate led to high free fatty acid content while stressing with sodium chloride shifted non-polar lipid stores to TAG. Considering the pathway to vehicular fuel from this point, more FFA or TAG may be desired and the stresses above illustrate a potential control point for this isolate.

FAME content is a measure of both the non-polar and polar lipid molecules making up the cell. Although the treatment in AM6 had the least amount of intracellular non-polar lipid on a weight per weight basis, this growth condition was still the most productive system with respect to FAME content on a gram per liter of culture basis. This may suggest that some polar lipids are reconstituted into non-polar lipids when stresses are imparted on isolate GK5La.

The fatty acid profile for isolate GK5La contained low levels of TAG when not cultured in the presence of high ionic strength solution. Further isolation of algae that store the majority of their lipid in the form of free fatty acid and phospholipid may be another alternative to the seemingly unavoidable over supply of glycerol byproduct.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

The studies presented were all conducted in beveled flasks or 1.25L photobioreactor tubes, in controlled environments dissimilar from actual conditions expected in scaled up algal raceway ponds. Experimental conditions conducted in 200L raceway ponds would offer insight into a more realistic system that better represents industrial conditions and would be the next step forward to take in this area of research.

Considering the culture conditions tested, the focus of this thesis was broad enough that there were several unanswered questions pertaining to culturing Soap Lake algal isolates with the purpose of biofuel production. Speciation of inorganic carbon in solution was shown to have a profound effect on growth rate and intracellular lipid productivity. More experimentation under pH control would help answer questions regarding the effects observed among ionic stress, pH stress, and carbon speciation stress. Varying sodium chloride concentration in solution for salt stressed and spiked studies would offer insight to efficient ways to use high salt waste streams in algal biofuel operations.

The studies presented indicate inorganic carbon supplementation is important when growing algae for biofuel production purposes. Sodium bicarbonate is recovered from mining operations throughout the world and is a relatively inexpensive chemical given the potential beneficial use in the industry. Unfortunately, using a mined source of inorganic carbon cuts into the sustainable nature of producing fuel sources from organic plant sources, and would take away from the added benefit of being a near carbon neutral process. Inorganic carbon supplementation can also be in the form of carbon dioxide gas;

however, this is an expensive remedy that drops the pH in solution while most of the CO_2 bubbled in solution flows to the atmosphere without being dissolved.

In recent years, much attention has been given to the prospect of sequestering CO_2 geologically or using waste CO_2 beneficially in another industry such as algal biofuel production. Using waste CO_2 from power plants to grow algae presents four main drawbacks:

- This would require algal biodiesel ponds to be located at or very near existing power plants
- Much of the CO_2 gas bubbled into the raceway pond would be lost to the atmosphere anyway.
- The flue gas will contain impurities that could actually inhibit growth.
- The addition of CO_2 gas would cause the pH in solution to drop, creating an environment more open to contamination from other microorganisms.

Transforming waste CO_2 from power plants and other industrial operations to sodium bicarbonate by reacting it with sodium hydroxide is realistic way to sequester CO_2 into a very usable form for the algal biofuels industry. Utilizing sequestered sodium bicarbonate to grow algae for biofuel production may form a sustainable loop and an efficient way to cycle waste carbon.

Skyonics is a company currently constructing a commercial scale carbon capture to sodium bicarbonate plant in San Antonio. With an estimated selling cost of $45/ ton of sodium bicarbonate, this technology presents a way to grow algae using a cheap substrate

that also acts to buffer the medium when used in high enough concentration as shown in Chapter 5.

With further research focused upon the biology and geochemistry in scaled up raceway ponds, algal biofuel production may eventually become a reality.

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APPENDICES

APPENDIX A

EXPERIMENTAL DATA FOR CHAPTER 5

Isolate GK5La

Table A.1. Absorbance (750nm) for isolate GK5La grown on 4 different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	0.05	0.056	0.054	0.059
3	0.067	0.083	0.052	0.049
5	0.127	0.132	0.056	0.046
7	0.274	0.31	0.081	0.046
11	0.534	0.616	0.118	0.045
13	0.696	0.757	0.128	0.044
20	1.175	1.084	0.182	0.045
25	1.348	1.375	0.191	0.05

Table A.2. Cell concentration (cells/mL) for isolate GK5La grown on 4 different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	8.8E+04	8.8E+04	8.8E+04	8.8E+04
3	8.7E+05	9.4E+05	1.9E+05	0.0E+00
5	3.6E+06	4.4E+06	4.2E+05	0.0E+00
7	1.1E+07	1.2E+07	1.3E+06	0.0E+00
11	3.0E+07	3.3E+07	1.6E+06	0.0E+00
20	4.7E+07	3.1E+07	1.9E+06	0.0E+00
25	5.0E+07	3.2E+07	2.3E+06	0.0E+00

Table A.3. Nile Red fluorescence (a.u.) for isolate GK5La grown on 4 different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	17	16	49	14
3	71	101	71	33
5	237	376	174	-44
7	475	390	388	19
11	290	600	490	20
20	1700	2220	928	51
25	2910	5300	822	17

Table A.4. pH for isolate GK5La grown on 4 different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	8.54	8.53	7.66	7.68
3	10.3	10.17	7.82	7.7
5	11.21	11.13	8.34	7.62
7	11.43	11.32	8.7	8.15
11	11.67	11.41	8.18	7.96
20	11.71	11.55	7.89	7.75
25	11.74	11.59	7.88	7.76

Isolate GK5La Sodium Chloride Experiments (Flasks)

Table A.5. Cell concentration (cells/mL) for isolate GK5La grown on two different media.

Time (d)	AM6(1.8)	AsP_2(1.8)
0	2.5E+06	2.0E+06
4	2.6E+06	2.3E+06
6	2.9E+06	2.5E+06
10	1.8E+06	3.6E+06
19	3.1E+06	4.5E+06
26	3.8E+06	5.4E+06

Table A.6. Nile Red fluorescence (a.u.) for isolate GK5La grown on two different media.

Time (d)	AM6(1.8)	AsP_2(1.8)
0	75	84
4	133	129
6	3270	1695
10	3660	2270
19	4980	1760
26	11740	1720

Table A.7. Cell concentration (cells/mL) for isolate GK5La grown on two different media.

Time (d)	AM6	AM6(1.8)
0	1.7E+07	2.1E+07
2	2.7E+07	2.2E+07
5	6.0E+07	2.2E+07
8	5.0E+07	1.6E+07
12	6.2E+07	2.1E+07
16	5.6E+07	2.1E+07
21	4.7E+07	2.2E+07

Table A.8. Nile Red fluorescence (a.u.) for isolate GK5La grown on two different media.

Time (d)	AM6	AM6(1.8)
0	3257	1423
2	1740	2503
5	23	5613
8	1103	23133
12	2540	25840
21	1960	46780

Table A.9. pH for isolate GK5La grown on two different media.

Time (d)	AM6	AM6(1.8)
0	9.12	9.13
2	11.23	11.04
5	11.75	11.02
8	12.04	11.25
12	11.97	10.75
16	11.96	11.1
21	11.98	11.31

Table A.10. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	1.3E+05	1.3E+05	1.2E+05	1.3E+05	9.5E+03
2	8.6E+05	8.6E+05	1.2E+06	9.7E+05	1.9E+05
5	1.9E+07	1.7E+07	1.6E+07	1.7E+07	1.5E+06
8	2.6E+07	2.6E+07	2.5E+07	2.5E+07	5.0E+05
13	5.5E+07	5.5E+07	4.6E+07	5.2E+07	5.4E+06
26	5.8E+07	5.9E+07	7.1E+07	6.3E+07	7.4E+06

Table A.11. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	53	73	67	64	10
2	98	74	54	75	22
5	990	1210	1280	1160	151
8	1520	2660	2060	2080	570
13	1560	1280	1360	1400	144
26	9610	11540	11060	10737	1005

Table A.12. Absorbance (750nm) for isolate GK5La grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	0.05	0.05	0.05	0.05	0.00
2	0.06	0.06	0.06	0.06	0.00
5	0.24	0.25	0.24	0.24	0.01
8	0.51	0.52	0.53	0.52	0.01
13	1.02	1.02	1.02	1.02	0.00
26	1.56	1.54	1.57	1.56	0.02

Table A.13. pH for isolate GK5La grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	9.24	9.23	9.24	9.24	0.01
2	8.66	8.90	8.98	8.85	0.17
5	11.52	11.47	11.44	11.48	0.04
8	11.99	11.90	11.88	11.92	0.06
13	11.96	11.86	11.84	11.89	0.06
26	11.31	11.37	10.81	11.16	0.31

Table A.14. Cell concentration (cells/mL) for isolate GK5La grown on AM6(1.8) medium.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	1.1E+05	1.1E+05	1.0E+05	1.1E+05	3.8E+03
2	2.0E+05	3.0E+05	2.7E+05	2.5E+05	5.2E+04
5	1.8E+06	1.8E+06	1.7E+06	1.7E+06	6.9E+04
8	3.4E+06	4.0E+06	3.6E+06	3.6E+06	3.1E+05
13	6.5E+06	7.5E+06	7.5E+06	7.2E+06	5.7E+05
26	1.1E+07	1.3E+07	1.1E+07	1.2E+07	1.2E+06

Table A.15. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6(1.8) medium.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	87	71	82	80	8
2	175	198	208	194	17
5	705	820	660	728	83
8	2225	1980	1630	1945	299
13	13740	14410	14150	14100	338
26	183940	175440	157930	172437	13263

Table A.16. Absorbance (750nm) for isolate GK5La grown on AM6(1.8) medium.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	0.05	0.05	0.05	0.05	0.00
2	0.05	0.05	0.05	0.05	0.00
5	0.09	0.08	0.09	0.09	0.00
8	0.21	0.21	0.21	0.21	0.00
13	0.30	0.39	0.28	0.32	0.06
26	0.78	0.77	0.73	0.76	0.02

Table A.17. pH for isolate GK5La grown on AM6(1.8) medium.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	9.21	9.26	9.24	9.24	0.03
2	8.77	8.75	8.69	8.74	0.04
5	9.57	9.30	9.57	9.48	0.16
8	10.80	10.75	10.91	10.82	0.08
13	11.06	11.04	11.08	11.06	0.02
26	10.34	10.53	10.55	10.47	0.12

Isolate GK2Lg

Table A.18. Cell concentration (cells/mL) for isolate GK2Lg grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	6.6E+04	6.6E+04	6.6E+04	6.6E+04
3	2.6E+05	1.0E+06	3.5E+05	2.7E+05
5	2.6E+05	2.6E+06	6.1E+05	2.7E+05
7	4.4E+05	6.7E+06	9.0E+05	3.8E+05
11	2.9E+05	4.7E+06	8.8E+05	2.4E+05
18	2.3E+05	6.5E+06	9.7E+05	3.4E+05
27	3.9E+05	7.0E+06	9.6E+05	2.8E+05

Table A.19. Absorbance (750nm) for isolate GK2Lg grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	0.045	0.052	0.052	0.059
3	0.06	0.112	0.057	0.061
5	0.065	0.148	0.064	0.056
11	0.076	0.289	0.065	0.056
18	0.072	0.588	0.067	0.054
27	0.093	0.551	0.064	0.056

Table A.20. Nile Red fluorescence (a.u.) for isolate GK2Lg grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	361	222	166	125
3	3306	1396	267	142
5	3185	1285	201	88
7	6078	4480	314	187
11	10378	13065	960	215
18	9702	77230	1213	361
27	13129	271263	451	35

Table A.21. pH for isolate GK2Lg grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	7.85	7.8	7.79	7.79
3	8.01	8.35	7.85	7.82
5	8.24	8.9	7.92	7.83
7	8.34	9.48	7.79	7.88
11	8.09	9.84	7.94	7.86
18	7.99	10.03	8.07	7.82
27	7.92	9.34	7.88	7.78

Isolate GK6-G2

Table A.22. Cell concentration (cells/mL) for isolate GK6-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	2.6E+05	2.6E+05	2.6E+05	2.6E+05
2	2.1E+05	2.6E+05	2.9E+05	1.8E+05
5	4.7E+06	4.4E+06	4.6E+05	8.3E+04
8	1.1E+07	1.9E+07	3.4E+06	8.5E+04
15	2.2E+07	1.6E+07	1.1E+06	8.5E+04
19	2.5E+07	2.5E+07	1.3E+06	1.3E+05
22	2.4E+07	2.7E+07	1.3E+06	8.5E+04
27	2.9E+07	3.5E+07	1.5E+06	1.2E+05
34	3.4E+07	5.4E+07	2.5E+06	1.1E+05

Table A.23. Absorbance (750nm) for isolate GK6-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	0.05	0.067	0.066	0.062
2	0.05	0.064	0.067	0.058
5	0.176	0.175	0.068	0.052
8	0.371	0.415	0.073	0.051
15	0.619	0.542	0.084	0.053
19	0.642	0.643	0.095	0.056
22	0.602	0.617	0.093	0.055

Table A.24. Nile Red fluorescence (a.u.) for isolate GK6-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	5	18	18	14
2	34	45	43	26
5	195	330	67	69
8	290	380	123	139
15	3390	5760	112	80
19	7540	5110	130	100
22	5627	6523	137	118
27	8343	9190	126	72
34	7700	8200	240	131

Table A.25. pH for isolate GK6-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	8.46	8.51	7.43	7.42
2	8.52	8.68	7.54	7.58
5	11.13	10.35	8.24	7.87
8	11.17	11.3	7.53	7.56
15	10.08	10.21	7.76	7.66
19	8.66	9.25	7.53	7.55
22	8.8	9.11	7.47	7.7
27	8.71	9.23	7.57	7.65
34	9.03	9.24	7.84	7.82

Isolate GK3L

Table A.26. Cell concentration (cells/mL) for isolate GK3L grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	1.775E+06	1.775E+06	1.775E+06	1.775E+06
3	9.080E+06	7.750E+06	5.730E+06	2.120E+06
7	3.240E+07	3.430E+07	1.590E+07	1.985E+06
10	4.410E+07	4.910E+07	2.355E+07	2.360E+06
14	1.046E+08	1.038E+08	4.210E+07	2.290E+06
22	1.460E+08	2.268E+08	7.480E+07	2.225E+06

Table A.27. Absorbance (750nm) for isolate GK3L grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	0.073	0.096	0.091	0.085
3	0.141	0.14	0.104	0.078
7	0.569	0.622	0.205	0.094
10	0.761	0.773	0.275	0.101
14	1.134	1.428	0.368	0.121
22	1.339	1.759	0.631	0.143

Table A.28. Nile Red fluorescence (a.u.) for isolate GK3L grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	838	894	3848	5802
3	1410	1277	527	5717
7	3360	5027	179	6219
10	3060	1020	710	7332
14	4940	3780	1360	14250
22	5100	13620	11684	14856

Table A.29. pH for isolate GK3L grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	9.11	9.18	7.79	7.91
3	9.9	9.45	8.26	7.94
7	11.53	11.39	8.36	7.92
10	11.76	11.41	7.95	7.92
14	11.8	11.52	8.1	8.11
22	10.51	11.41	8.15	8.04

Isolate GK3L Salt Spike

Table A.30. Cell concentration (cells/mL) for isolate GK3L grown on two different media.

Time (d)	AM6	AM6(5.1)
0	4.5E+07	2.5E+07
11	1.5E+08	3.8E+07
17	2.0E+08	4.5E+07
21	2.0E+08	3.5E+07

Table A.31. Nile Red fluorescence (a.u.) for isolate GK3L grown on two different media.

Time (d)	AM6	AM6(5.1)
0	900	6460
11	81960	110440
17	150300	150770
21	12220	94040
35	9060	336360

Table A.32. pH for isolate GK3L grown on two different media.

Time (d)	AM6	AM6(5.1)
0	8.72	9.01
11	11.58	9.49
17	11.13	9.42
21	10.82	9.34

Table A.33. Cell concentration (cells/mL) for isolate GK3L grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	8.0E+04	8.0E+04	8.0E+04	8.0E+04	0.0E+00
4	2.8E+06	1.5E+06	2.0E+06	2.1E+06	6.6E+05
10	3.2E+07	2.5E+07	3.8E+07	3.2E+07	6.4E+06
13	5.1E+07	4.8E+07	5.6E+07	5.2E+07	3.9E+06
15	6.3E+07	8.2E+07	9.8E+07	8.1E+07	1.7E+07
19	1.0E+08	1.0E+08	1.3E+08	1.1E+08	1.5E+07
21	1.8E+08	1.6E+08	1.7E+08	1.7E+08	1.0E+07
26	1.8E+08	1.7E+08	2.0E+08	1.8E+08	1.3E+07
33	2.1E+08	2.6E+08	3.0E+08	2.6E+08	4.4E+07

Table A.34. Nile Red fluorescence (a.u.) for isolate GK3L grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	22	36	49	36	14
4	548	1149	465	721	373
10	1760	2120	3440	2440	885
13	2140	4740	11640	6173	4910
15	41100	30680	30940	34240	5942
19	6280	9240	15400	10307	4653
21	6840	19460	5460	10587	7715
26	7600	10950	5750	8100	2636
33	7600	8950	5750	7433	1606

Table A.35. Absorbance (750nm) for isolate GK3L grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	0.05	0.07	0.05	0.06	0.01
10	0.65	0.61	0.79	0.68	0.09
13	0.83	0.87	0.99	0.89	0.09
15	1.01	1.03	1.15	1.07	0.08
19	1.26	1.22	1.31	1.26	0.04
21	1.34	1.38	1.40	1.37	0.03
26	1.53	1.68	1.67	1.62	0.08
33	1.72	1.93	1.92	1.86	0.12

Table A.36. pH for isolate GK3L grown on AM6 medium.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	9.28	9.22	9.18	9.23	0.05
4	9.74	9.42	9.39	9.52	0.19
10	11.91	11.9	11.85	11.89	0.03
13	11.84	11.89	11.83	11.85	0.03
15	11.77	11.74	11.63	11.71	0.07
19	11.47	11.06	11.02	11.18	0.25
21	12.01	11.96	11.82	11.93	0.10
26	10.8	10.74	10.57	10.70	0.12
33	10	9.92	10.01	9.98	0.05

Table A.37. Cell concentration (cells/mL) for isolate GK3L grown on AM6(5.1) medium.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	8.0E+04	8.0E+04	8.0E+04	8.0E+04	0.0E+00
4	4.2E+05	5.0E+05	4.8E+05	4.7E+05	4.1E+04
10	8.8E+05	7.8E+05	7.6E+05	8.1E+05	6.1E+04
13	1.3E+07	1.5E+07	1.3E+07	1.4E+07	1.0E+06
15	1.7E+07	1.8E+07	2.0E+07	1.8E+07	1.7E+06
19	2.6E+07	2.3E+07	3.2E+07	2.7E+07	4.7E+06
21	2.9E+07	3.8E+07	3.3E+07	3.3E+07	4.1E+06
26	4.6E+07	4.4E+07	4.6E+07	4.5E+07	1.4E+06
33	7.5E+07	7.3E+07	8.2E+07	7.6E+07	5.1E+06

Table A.38. Nile Red fluorescence (a.u.) for isolate GK3L grown on AM6(5.1) medium.

Time (d)	2a	2b	2c		
0	44	61	34	46	14
4	1828	2332	2727	2296	451
10	6910	6060	5310	6093	801
13	17340	13780	9600	13573	3874
15	14600	22860	30700	22720	8051
19	32400	24480	31680	29520	4380
21	27000	36500		31750	6718
26	106800	118560	140660	122007	17191
33	243100	211700	239250	231350	17126

Table A.39. Absorbance (750nm) for isolate GK3L grown on AM6(5.1) medium.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	0.05	0.05	0.05	0.05	0.00
10	0.21	0.16	0.15	0.17	0.03
13	0.35	0.30	0.32	0.32	0.02
15	0.49	0.43	0.46	0.46	0.03
19	0.74	0.65	0.69	0.69	0.05
21	0.85	0.77	0.84	0.82	0.04
26	1.09	1.02	1.00	1.04	0.04
33	1.37	1.32	1.30	1.33	0.04

Table A.40. pH for isolate GK3L grown on AM6(5.1) medium.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	9.01	8.96	8.97	8.98	0.03
4	8.93	8.87	8.88	8.89	0.03
10	10.12	9.83	9.86	9.94	0.16
13	10.63	10.59	10.58	10.60	0.03
15	10.46	10.45	10.47	10.46	0.01
19	10.54	10.55	10.46	10.52	0.05
21	11.22	11.21	11.21	11.21	0.01
26	10.18	10.33	10.27	10.26	0.08
33	9.56	9.73	9.64	9.64	0.09

Isolate GK5L-G2

Table A.41. Cell concentration (cells/mL) for isolate GK5L-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	5.8E+04	5.8E+04	5.8E+04	5.8E+04
5	2.3E+06	2.4E+06	7.5E+05	0.0E+00
8	1.7E+06	2.4E+06	8.5E+05	0.0E+00
13	3.4E+06	4.2E+06	7.2E+05	0.0E+00
25	6.6E+06	3.8E+06	1.8E+06	0.0E+00
32	6.2E+06	9.2E+06	2.7E+06	0.0E+00
40	7.4E+06	8.8E+06	2.4E+06	0.0E+00

Table A.42. Absorbance (750nm) for isolate GK5L-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	0.045	0.056	0.055	0.058
5	0.113	0.121	0.092	0.053
8	0.201	0.198	0.104	0.048
13	0.398	0.427	0.155	0.045
25	1.07	0.407	0.177	0.051
32	0.923	0.997	0.331	0.05

Table A.43. Nile Red fluorescence (a.u.) for isolate GK5L-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	189	273	78	106
5	900	270	909	218
8	1260	1520	4326	131
13	17130	9390	11498	18
25	51840	17900	20330	66
32	73160	85640	12827	38

Table A.44. pH for isolate GK5L-G2 grown on four different media.

Time (d)	AM6	AM6SIS	AsP_2(1.8)	AsP_2(5.1)
0	9.47	9.52	7.92	8.11
5	11.62	10.96	9.26	8.15
8	11.62	11.26	8.92	8.12
13	11.97	11.75	8.76	7.99
25	9.33	9.31	8.38	8.16
32	9.01	9.25	8.05	8.2
40	9.1	9.28	7.79	7.97

APPENDIX B

EXPERIMENTAL DATA CHAPTER 6

Inorganic Carbon Supplemented vs. Carbon Limited

Table B.1. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0.0	6.3E+04	1.1E+05	1.1E+05	9.3E+04	2.7E+04
1.2	1.0E+05	1.4E+05	9.5E+04	1.1E+05	2.3E+04
2.1	7.7E+05	7.7E+05	7.7E+05	7.7E+05	2.5E+03
3.1	6.7E+06	5.4E+06	6.2E+06	6.1E+06	6.4E+05
6.0	2.5E+07	2.1E+07	1.8E+07	2.1E+07	3.4E+06
8.0	3.3E+07	3.0E+07	3.1E+07	3.1E+07	1.1E+06
8.9	3.3E+07	3.5E+07	3.4E+07	3.4E+07	1.2E+06
9.0	3.4E+07	3.0E+07	2.9E+07	3.1E+07	2.7E+06
9.8	4.0E+07	4.4E+07	3.9E+07	4.1E+07	3.0E+06
12.0	5.1E+07	3.4E+07	4.7E+07	4.4E+07	8.6E+06
13.0	5.2E+07	4.7E+07	4.6E+07	4.8E+07	3.4E+06
15.1	4.9E+07	4.2E+07	3.8E+07	4.3E+07	5.5E+06
16.0	3.8E+07	4.8E+07	5.1E+07	4.5E+07	6.9E+06
17.0	3.2E+07	4.1E+07	4.2E+07	3.8E+07	5.2E+06
20.1	4.6E+07	4.0E+07	3.9E+07	4.2E+07	4.1E+06
21.9	3.4E+07	4.8E+07	4.7E+07	4.3E+07	7.9E+06
24.1	3.6E+07	3.4E+07	3.4E+07	3.5E+07	1.3E+06
27.0	4.7E+07	3.3E+07	3.3E+07	3.7E+07	7.9E+06
31.1	2.4E+07	7.0E+06	3.3E+07	2.1E+07	1.3E+07

Table B.2. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0.0	2.1E+05	1.3E+05	1.5E+05	1.6E+05	4.2E+04
1.0	1.1E+05	1.7E+05	1.4E+05	1.4E+05	2.9E+04
1.6	4.1E+05	4.4E+05	5.4E+05	4.6E+05	6.9E+04
2.0	5.4E+05	3.7E+05	5.6E+05	4.9E+05	1.1E+05
2.6	2.2E+06	2.0E+06	2.1E+06	2.1E+06	7.5E+04
3.0	1.9E+06	2.6E+06	1.7E+06	2.1E+06	4.5E+05
3.6	6.3E+06	1.0E+07	1.2E+07	9.3E+06	2.7E+06
4.0	7.8E+06	1.1E+07	1.3E+07	1.1E+07	2.6E+06
5.0	1.0E+07	1.0E+07	1.2E+07	1.1E+07	1.1E+06
6.0	9.1E+06	9.4E+06	1.0E+07	9.6E+06	6.0E+05
7.0	1.1E+07	1.0E+07	1.3E+07	1.2E+07	1.5E+06
8.0	1.6E+07	1.4E+07	9.2E+06	1.3E+07	3.3E+06
9.0	1.7E+07	2.0E+07	3.2E+07	2.3E+07	8.0E+06
10.0	2.7E+07	3.2E+07	5.0E+07	3.6E+07	1.2E+07
11.0	3.7E+07	3.2E+07	3.5E+07	3.5E+07	2.2E+06
12.0	3.8E+07	3.9E+07	3.3E+07	3.7E+07	2.9E+06
13.0	4.3E+07	3.3E+07	4.2E+07	3.9E+07	5.4E+06
14.0	4.3E+07	3.4E+07	4.2E+07	4.0E+07	5.0E+06
15.0	3.4E+07	4.8E+07	3.3E+07	3.8E+07	8.3E+06
16.0	5.2E+07	3.9E+07	4.1E+07	4.4E+07	7.0E+06
17.0	4.8E+07	3.9E+07	3.9E+07	4.2E+07	5.0E+06
18.0	4.2E+07	2.8E+07	4.5E+07	3.8E+07	8.8E+06
19.0	3.3E+07	2.9E+07	4.3E+07	3.5E+07	6.9E+06
22.0	3.6E+07	4.7E+07	4.4E+07	4.2E+07	5.7E+06
25.0	3.5E+07	2.7E+07	3.3E+07	3.2E+07	4.4E+06
29.0	4.6E+07	3.3E+07	3.8E+07	3.9E+07	6.6E+06
32.0	4.8E+07	3.7E+07	3.3E+07	3.9E+07	7.7E+06

Table B.3. pH for isolate GK5La grown on AM6 medium in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0.0	9.0	8.9	9.1	9.0	0.1
1.2	9.1	9.2	9.2	9.1	0.0
2.1	10.6	10.6	10.6	10.6	0.0
3.1	11.8	11.8	11.7	11.8	0.0
6.0	11.9	11.8	11.8	11.8	0.1
8.9	11.8	11.8	11.9	11.8	0.0
9.0	12.1	12.0	12.0	12.0	0.1
9.8	12.1	12.1	12.0	12.1	0.0
12.0	12.1	12.0	12.0	12.1	0.0
13.0	12.0	12.0	11.9	12.0	0.1
15.1	12.0	12.0	12.0	12.0	0.0
16.0	11.5	11.5	11.5	11.5	0.0
17.0	11.6	11.6	11.6	11.6	0.0
21.9	11.7	11.6	11.7	11.6	0.1
24.1	11.6	11.5	11.7	11.6	0.1
27.0	11.2	10.9	11.4	11.2	0.2
31.1	10.1	9.4	10.9	10.1	0.8

Table B.4. pH for isolate GK5La grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time	2a	2b	2c	Average	Standard
0	8.9	8.9	8.9	8.9	0
1	9.3	9.3	9.3	9.3	0
2	9.6	9.6	9.6	9.6	0
2.6	9.7	9.7	9.8	9.7	0
3	10.5	10.5	10.7	10.5	0.1
3.6	10.8	10.8	11.1	10.9	0.2
4	11.8	11.8	11.9	11.9	0
4.6	11.8	11.8	11.9	11.8	0.1
5	12.1	12.1	12.1	12.1	0
6	12	12	12.1	12	0.1
7	12	12	12	12	0
8	11.8	11.7	11.4	11.6	0.2
9	11.7	11.5	11.2	11.5	0.2
10	11.1	11.5	12	11.5	0.4
11	11.9	12	12.1	12	0.1
12	12.1	12.1	12.2	12.1	0
13	12.1	12.1	12.1	12.1	0
14	12.1	12.1	12.1	12.1	0
15	12	11.9	12	12	0
16	11.9	11.8	11.8	11.8	0.1
17	11.8	11.6	11.6	11.7	0.1
18	11.7	11.5	11.6	11.6	0.1
19	11.7	11.4	11.6	11.6	0.2
22	11.2	11	11.5	11.2	0.2
25	10.6	10.6	10.9	10.7	0.2
27	10.3	10.3	10.6	10.4	0.1
32	9.9	9.9	10	10	0.1

Table B.5. Nitrate concentration (mg/L) for isolate GK5La grown on AM6 medium in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0.0	185	188	185	186	2
1.2	188	122	206	172	44
2.1	189	204	194	195	7
3.1	143	144	146	144	2
6.0	72	66	73	70	4
8.0	0	0	11	4	6
9.0	0	0	0	0	0
13.0	0	0	0	0	0
16.0	0	0	0	0	0
20.1	0	0	0	0	0
24.1	0	0	0	0	0

Table B.6. Nitrate concentration (mg/L) for isolate GK5La grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0.0	260	248	248	252	7
2.0	237	247	221	235	13
3.0	195	208	206	203	7
4.0	174	171	156	167	10
6.0	129	135	134	133	3
8.0	104	103	117	108	7
11.0	0	0	0	0	0
13.0	0	0	0	0	0

Table B.7. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0.0	25	23	38	29	8
1.2	31	29	22	27	5
2.1	37	45	11	41	6
3.1	110	80	50	80	30
6.0	260	120	60	147	103
8.0	600	140	440	393	234
8.9	300	340	280	307	31
9.0	60	420	240	240	180
9.8	120	-160	240	67	205
12.0	280	80	640	333	284
13.0	140	880	440	487	372
15.1	540	20	480	347	284
16.0	140	360	260	253	110
17.0	600	220	200	340	225
20.1	80	460	80	207	219
21.9	580	400	440	473	95
24.1	620	340	1180	713	428
27.0	780	440	240	487	273
31.1	460	160	260	293	153

Table B.8. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0.0	9	-5	-9	-2	9
1.0	14	4	18	12	7
2.0	18	24	19	20	3
3.0	23	22	25	23	2
4.0	60	-20	-70	-10	66
5.0	120	30	60	70	46
6.0	130	220	220	190	52
7.0	360	310	300	323	32
8.0	170	420	360	317	131
9.0	620	820	870	770	132
10.0	740	850	580	723	136
11.0	600	840	1020	820	211
12.0	800	1020	840	887	117
13.0	820	720	580	707	121
14.0	1140	1180	640	987	301
15.0	1100	1020	840	987	133
16.0	1360	1160	780	1100	295
17.0	1180	1100	660	980	280
18.0	1200	1620	1000	1273	316
19.0	600	600	900	700	173
22.0	1200	1480	1560	1413	189
25.0	1680	1660	1660	1667	12
27.0	1040	1500	1440	1327	250
29.0	1240	1420	1640	1433	200
30.0	1680	1320	1620	1540	193
32.0	1420	1540	920	1293	329

Comparison between Salt Spiked and Salt Stressed

Table B.9. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium spiked to 1.8% sodium chloride at day 9 in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0.0	7.5E+04	1.1E+05	9.5E+04	9.2E+04	1.5E+04
1.2	1.3E+05	2.9E+05	1.3E+05	1.8E+05	8.9E+04
2.1	7.0E+05	8.5E+05	8.9E+05	8.1E+05	9.9E+04
3.1	5.4E+06	5.6E+06	6.8E+06	5.9E+06	7.5E+05
6.0	2.4E+07	1.8E+07	1.9E+07	2.0E+07	3.3E+06
8.0	3.5E+07	2.7E+07	2.4E+07	2.8E+07	5.5E+06
8.9	3.6E+07	3.9E+07	3.8E+07	3.7E+07	1.6E+06
9.0	3.1E+07	3.3E+07	2.5E+07	3.0E+07	4.1E+06
9.8	3.5E+07	2.8E+07	3.4E+07	3.2E+07	3.9E+06
12.0	3.6E+07	3.0E+07	4.0E+07	3.6E+07	4.8E+06
13.0	3.6E+07	3.4E+07	3.7E+07	3.6E+07	1.5E+06
15.1	3.4E+07	3.1E+07	2.5E+07	3.0E+07	4.8E+06
16.0	3.2E+07	2.6E+07	2.9E+07	2.9E+07	3.0E+06
17.0	2.4E+07	3.3E+07	3.8E+07	3.2E+07	7.2E+06
20.1	3.1E+07	2.6E+07	3.0E+07	2.9E+07	2.7E+06
21.9	2.9E+07	2.5E+07	2.9E+07	2.8E+07	2.6E+06
24.1	3.6E+07	3.4E+07	2.7E+07	3.2E+07	4.8E+06
27.0	3.3E+07	2.7E+07	2.8E+07	2.9E+07	3.1E+06
31.1	3.8E+07	2.7E+07	2.7E+07	3.1E+07	6.1E+06

Table B.10. Cell concentration (cells/mL) for isolate GK5La grown on AM6(1.8) medium in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	9.3E+04	9.0E+04	7.3E+04	8.5E+04	1.1E+04
1	1.2E+05	6.5E+04	8.3E+04	8.8E+04	2.7E+04
2	3.3E+05	3.5E+05	2.8E+05	3.2E+05	3.6E+04
3	8.4E+05	8.4E+05	6.2E+05	7.7E+05	1.3E+05
4	2.1E+06	1.8E+06	1.8E+06	1.9E+06	1.6E+05
5	3.9E+06	3.9E+06	3.5E+06	3.8E+06	2.3E+05
6	3.1E+06	4.2E+06	3.7E+06	3.7E+06	5.5E+05
7	4.8E+06	5.5E+06	4.4E+06	4.9E+06	5.6E+05
8	6.7E+06	6.8E+06	5.0E+06	6.2E+06	1.0E+06
9	4.8E+06	7.5E+06	5.1E+06	5.8E+06	1.5E+06
10	7.0E+06	8.6E+06	7.2E+06	7.6E+06	8.7E+05
11	7.2E+06	7.7E+06	6.7E+06	7.2E+06	5.0E+05
12	1.1E+07	7.8E+06	8.7E+06	9.1E+06	1.6E+06
14	1.1E+07	9.2E+06	8.3E+06	9.4E+06	1.2E+06
15	1.4E+07	1.2E+07	8.6E+06	1.2E+07	2.9E+06
16	1.2E+07	9.2E+06	1.3E+07	1.1E+07	1.9E+06
18	1.3E+07	1.4E+07	1.3E+07	1.3E+07	5.3E+05
20	1.1E+07	1.1E+07	8.1E+06	1.0E+07	1.6E+06
24	7.9E+06	1.0E+07	1.2E+07	1.0E+07	2.3E+06
33	1.9E+07	2.9E+07	2.2E+07	1.2E+07	2.1E+06

Table B.11. pH for isolate GK5La grown on AM6 medium spiked to 1.8% sodium chloride at day 9 in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0.0	8.9	8.9	9.1	9.0	0.1
1.2	9.1	9.3	9.2	9.2	0.1
2.1	10.4	10.6	10.7	10.5	0.1
3.1	11.7	11.7	11.7	11.7	0.0
6.0	11.8	11.8	11.8	11.8	0.0
8.9	11.8	11.8	11.8	11.8	0.0
9.0	11.8	11.9	11.8	11.8	0.1
9.8	11.5	11.5	11.5	11.5	0.0
12.0	11.5	11.5	11.5	11.5	0.0
13.0	11.4	11.5	11.4	11.4	0.0
15.1	11.1	11.1	11.1	11.1	0.0
16.0	10.8	10.8	10.8	10.8	0.0
17.0	10.6	10.7	10.6	10.6	0.1
21.9	10.6	10.8	10.4	10.6	0.2
24.1	10.4	10.7	10.2	10.4	0.3
27.0	9.9	10.4	9.8	10.1	0.3
31.1	9.7	10.1	9.9	9.9	0.2

Table B.12. pH for isolate GK5La grown on AM6(1.8) medium in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	9.1	8.6	8.7	8.8	0.3
1	8.6	8.6	8.6	8.6	0.0
2	8.9	8.8	8.8	8.8	0.1
3	9.2	9.0	8.9	9.0	0.1
4	9.8	9.6	9.5	9.6	0.2
5	10.7	10.6	10.4	10.6	0.2
6	10.9	10.9	10.9	10.9	0.0
7	10.9	10.9	10.9	10.9	0.0
8	11.0	11.0	10.9	11.0	0.0
9	10.8	10.9	10.8	10.8	0.0
10	10.9	10.9	10.9	10.9	0.0
11	10.8	10.9	10.8	10.8	0.0
12	11.0	11.0	11.0	11.0	0.0
14	11.1	11.1	11.1	11.1	0.0
15	11.1	10.9	10.9	11.0	0.1
16	10.8	10.8	10.8	10.8	0.0
18	10.9	10.9	10.9	10.9	0.0
20	10.5	10.9	10.4	10.6	0.3
24	10.2	10.2	9.9	10.1	0.1
33	10.0	10.2	10.1	10.1	0.1

Table B.13. Nitrate concentration (mg/L) for isolate GK5La grown on AM6 medium spiked to 1.8% sodium chloride at day 9 in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	222	184	236	214	27
1	189	201	205	198	8
2	147	257	174	193	58
3	163	156	165	161	5
6	51	39	1	30	26
8	0	0	0	0	0
9	0	0	0	0	0
13	0	0	0	0	0
16	0	0	0	0	0
20	0	0	0	0	0
24	0	0	0	0	0

Table B.14. Nitrate concentration (mg/L) for isolate GK5La grown on AM6(1.8) medium in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	281	318	284	294	20
3	315	344	298	319	23
5	224	294	255	258	35
6	259	230	230	239	17
8	256	249	225	244	16
11	183	170	213	189	22
16	50	81	43	58	20
24	0	-1	-1	-1	1

Table B.15. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium spiked to 1.8% sodium chloride at day 9 in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0.0	9	11	12	10	1
1.2	10	12	10	10	1
2.1	16	11	8	12	4
3.1	87	50	29	55	30
6.0	71	87	58	72	15
8.0	75	42	166	94	65
8.9	150	83	258	164	88
9.0	299	141	150	197	89
13.0	208	441	840	496	320
15.1	582	1522	2670	1592	1046
16.0	1547	3286	5349	3394	1903
17.0	2995	3178	5598	3924	1453
20.1	3618	5473	5914	5002	1218
21.9	7853	7786	7611	7750	125
24.1	6713	7894	6763	7123	668
27.0	9059	13617	11662	11446	2287
31.1	8352	11987	8859	9733	1969
33.0	3643	11313	8568	7841	3886

Table B.16. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6(1.8) medium in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	-7	-3	18	3	13
1	12	60	15	29	27
2	22	20	24	22	2
3	6	29	11	15	12
4	14	23	28	22	7
5	78	61	57	65	11
6	160	70	90	107	47
7	90	290	240	207	104
8	280	160	160	200	69
9	160	290	330	260	89
10	180	170	230	193	32
11	440	390	280	370	82
12	290	460	290	347	98
14	1060	920	1020	1000	72
15	700	920	560	727	181
16	720	1740	1230	1230	510
18	1640	2230	1570	1813	363
20	1030	1290	1100	1140	135
24	2290	1880	1550	1907	371
33	2530	3390	3130	3017	441

Comparison Between Inorganic Carbon Supplemented Salt Spiked and Salt Stressed

Table B.17. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	7.8E+04	9.3E+04	8.8E+04	8.6E+04	7.6E+03
1	1.1E+05	9.5E+04	1.4E+05	1.1E+05	2.1E+04
2	4.6E+05	5.0E+05	4.2E+05	4.6E+05	4.0E+04
3	3.8E+06	3.0E+06	2.9E+06	3.2E+06	5.0E+05
4	1.2E+07	1.2E+07	1.1E+07	1.2E+07	5.1E+05
5	1.4E+07	1.4E+07	1.5E+07	1.4E+07	4.9E+05
6	1.1E+07	1.9E+07	1.4E+07	1.5E+07	4.2E+06
7	1.6E+07	2.2E+07	1.8E+07	1.9E+07	2.7E+06
8	2.1E+07	1.8E+07	1.4E+07	1.8E+07	3.2E+06
9	2.0E+07	2.3E+07	1.7E+07	2.0E+07	3.0E+06
10	3.0E+07	4.6E+07	4.0E+07	3.9E+07	7.7E+06
11	5.4E+07	5.6E+07	5.2E+07	5.4E+07	1.7E+06
12	5.2E+07	4.5E+07	4.1E+07	4.6E+07	5.6E+06
14	4.3E+07	5.6E+07	6.7E+07	5.5E+07	1.2E+07
15	4.7E+07	4.9E+07	5.9E+07	5.2E+07	6.4E+06
16	4.7E+07	4.7E+07	4.2E+07	4.5E+07	3.0E+06
18	5.8E+07	4.7E+07	4.9E+07	5.1E+07	5.5E+06
20	4.4E+07	3.8E+07	5.9E+07	4.7E+07	1.0E+07
24	4.7E+07	5.9E+07	4.6E+07	5.1E+07	7.1E+06
33	5.9E+07	6.2E+07	6.1E+07	6.1E+07	1.4E+06

Table B.18. Cell concentration (cells/mL) for isolate GK5La grown on AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time	2a	2b	2c	Average	Standard
0.0	1.9E+05	1.3E+05	1.7E+05	1.6E+05	3.2E+04
1.0	1.4E+05	1.0E+05	1.1E+05	1.2E+05	1.8E+04
1.6	2.7E+05	2.0E+05	2.0E+05	2.2E+05	4.5E+04
2.0	1.6E+05	1.3E+05	1.4E+05	1.4E+05	1.9E+04
2.6	3.5E+05	2.1E+05	2.4E+05	2.6E+05	7.1E+04
3.0	5.2E+05	5.0E+05	4.1E+05	4.8E+05	5.6E+04
3.6	6.7E+05	5.1E+05	6.9E+05	6.2E+05	1.0E+05
4.0	8.1E+05	5.5E+05	5.5E+05	6.4E+05	1.5E+05
5.0	1.6E+06	1.2E+06	1.1E+06	1.3E+06	2.8E+05
6.0	1.7E+06	1.9E+06	2.2E+06	1.9E+06	2.5E+05
7.0	2.9E+06	3.2E+06	3.0E+06	3.0E+06	1.7E+05
8.0	4.0E+06	4.9E+06	4.8E+06	4.5E+06	5.0E+05
9.0	4.3E+06	4.2E+06	3.6E+06	4.0E+06	3.6E+05
10.0	4.5E+06	5.6E+06	4.8E+06	5.0E+06	5.4E+05
11.0	4.6E+06	5.3E+06	6.0E+06	5.3E+06	6.9E+05
12.0	4.1E+06	6.5E+06	5.8E+06	5.5E+06	1.2E+06
13.0	7.1E+06	6.7E+06	5.9E+06	6.6E+06	5.8E+05
14.0	6.8E+06	5.9E+06	4.1E+06	5.6E+06	1.4E+06
15.0	5.6E+06	4.9E+06	4.6E+06	5.0E+06	5.2E+05
16.0	6.3E+06	5.3E+06	6.7E+06	6.1E+06	7.5E+05
17.0	6.6E+06	5.3E+06	6.0E+06	6.0E+06	6.8E+05
18.0	6.5E+06	5.4E+06	7.1E+06	6.3E+06	8.6E+05
19.0	6.3E+06	6.1E+06	6.8E+06	6.4E+06	3.5E+05
22.0	7.4E+06	6.4E+06	7.1E+06	6.9E+06	5.2E+05
25.0	7.7E+06	6.8E+06	7.5E+06	7.3E+06	4.7E+05
29.0	1.0E+07	1.1E+07	8.5E+06	1.0E+07	1.4E+06
32.0	9.0E+06	9.0E+06	5.7E+06	8.9E+06	1.9E+06

Table B.19. pH for isolate GK5La grown on AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	9.0	9.1	9.1	9.0	0.1
1	9.1	9.1	9.2	9.1	0.0
2	9.6	9.6	9.6	9.6	0.0
3	10.6	10.7	10.7	10.7	0.0
4	11.6	11.6	11.6	11.6	0.0
5	11.8	11.8	11.8	11.8	0.0
6	11.9	11.9	11.9	11.9	0.0
7	11.8	11.8	11.8	11.8	0.0
8	11.8	11.7	11.6	11.7	0.1
9	11.7	11.2	11.4	11.5	0.2
10	11.6	11.7	11.6	11.6	0.0
11	11.5	11.8	11.8	11.7	0.2
12	11.4	11.5	11.5	11.4	0.1
14	11.1	11.1	11.1	11.1	0.0
15	10.8	10.9	10.9	10.9	0.1
16	10.7	10.8	10.8	10.8	0.0
18	10.7	10.9	10.9	10.8	0.1
20	10.4	10.7	10.6	10.6	0.1
24	9.7	10.3	10.1	10.0	0.3
33	9.4	9.6	9.5	9.5	0.1

Table B.20. pH for isolate GK5La grown on AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time	2a	2b	2c	Average	Standard
0.0	8.6	8.6	8.6	8.6	0.0
1.0	9.0	9.0	9.0	9.0	0.0
2.0	9.1	9.1	9.1	9.1	0.0
2.6	9.1	9.1	9.1	9.1	0.0
3.0	9.3	9.2	9.2	9.2	0.0
3.6	9.3	9.2	9.2	9.2	0.1
4.0	9.5	9.4	9.4	9.4	0.1
4.6	9.4	9.3	9.3	9.3	0.1
5.0	9.7	9.5	9.5	9.5	0.1
6.0	10.4	10.0	10.0	10.2	0.2
7.0	10.8	10.6	10.4	10.6	0.2
8.0	10.5	10.4	10.3	10.4	0.1
9.0	10.7	10.6	10.6	10.6	0.0
10.0	10.5	10.4	10.4	10.5	0.1
11.0	10.7	10.7	10.7	10.7	0.0
12.0	10.7	10.7	10.7	10.7	0.0
13.0	10.9	10.8	10.8	10.8	0.1
14.0	10.9	10.9	10.9	10.9	0.0
15.0	10.9	10.7	10.7	10.8	0.1
16.0	10.7	10.6	10.6	10.6	0.1
17.0	10.7	10.6	10.6	10.6	0.1
18.0	10.7	10.6	10.6	10.6	0.1
19.0	10.8	10.6	10.8	10.8	0.1
22.0	10.8	10.7	10.8	10.7	0.1
25.0	10.6	10.5	10.6	10.6	0.0
27.0	10.8	10.8	10.6	10.7	0.1
32.0	10.2	9.9	9.8	10.0	0.2

Table B.21. Nitrate concentration (mg/L) for isolate GK5La grown on AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	344	280	319	314	32
3	252	246	256	252	5
5	164	164	266	198	59
6	139	163	158	153	13
8	150	130	110	130	20
11	-3	3	2	1	3
16	0	0	0	0	0
24	0	0	0	0	0

Table B.22. Nitrate concentration (mg/L) for isolate GK5La grown on AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	222	184	236	214	27
2	189	201	205	198	8
3	147	257	174	193	58
4	163	156	165	161	5
6	188	160	162	170	16
8	132	148	150	143	10
11	97	108	93	99	8
13	95	93	74	87	12
15	60	62	55	59	4
18	57	60	38	51	12
22	42	38	24	34	9
25	32	24	10	22	11
29	13	0	0	4	8
31	-1	-1	-1	-1	0

Table B.23. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	5	14	6	8	5
1	22	16	22	20	3
2	16	23	9	16	7
3	170	80	30	93	71
4	110	-30	70	50	72
5	50	110	250	137	103
6	150	130	130	137	12
7	70	90	200	120	70
8	180	270	360	270	90
9	310	400	450	387	71
10	310	460	440	403	81
11	710	500	470	560	131
12	970	640	920	843	178
14	1770	770	1030	1190	519
15	1280	1140	1000	1140	140
16	2180	1450	1910	1847	369
18	3570	2200	2590	2787	706
20	1860	1230	1590	1560	316
24	1750	1560	2040	1783	242
33	1500	2320	2000	1940	413

Table B.24. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time	2a	2b	2c	Average	Standard
0	0	3	-4	0	4
1	-2	3	4	2	3
2	8	10	11	10	1
3	12	12	12	12	0
4	17	20	19	19	1
5	36	35	38	36	2
6	208	193	223	208	15
7	216	245	362	275	77
8	187	262	291	247	54
9	291	329	245	288	42
10	453	337	125	305	167
11	919	682	420	674	250
12	957	724	570	750	195
13	653	553	549	585	59
14	1248	1123	932	1101	159
15	1622	1214	894	1244	365
16	1256	1701	1260	1406	256
17	1576	1751	1813	1714	123
18	1639	1635	1917	1730	162
19	1356	1468	1098	1307	190
22	1589	1456	1223	1422	185
25	2837	2042	1697	2192	584
27	2920	2778	2304	2667	322
29	2516	2749	1788	2351	501
30	3003	2591	1497	2364	778
32	3340	2146	1797	2428	809

Table B.25. Free fatty acid composition over time for isolate GK5La grown in AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
15	5.59	5.96	5.98	5.84	0.22
20	9.16	9.69	14.51	11.12	2.95
25	5.67	12.82	9.93	9.47	3.59
33	11.89	13.31	12.15	12.45	0.76

Table B.26. Free fatty acid composition over time for isolate GK5La grown in AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
15	5.54	5.82	6.42	5.93	0.45
20	8.50	10.88	9.80	9.73	1.19
25	7.15	7.02	8.31	7.49	0.71
33	11.85	12.75	14.27	12.96	1.22

Table B.27. Monoacylglyceride composition over time for isolate GK5La grown in AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
15	2.88	2.63	3.03	2.85	0.20
20	3.41	3.35	4.74	3.83	0.79
25	2.80	2.61	3.95	3.12	0.73
33	6.33	6.72	5.89	6.31	0.41

Table B.28. Monoacylglyceride composition over time for isolate GK5La grown in AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
15	3.23	3.47	3.40	3.37	0.13
20	3.23	3.98	3.89	3.70	0.41
25	3.23	3.18	4.06	3.49	0.49
33	2.85	2.92	7.10	4.29	2.44

Table B.29. Diacylglyceride composition over time for isolate GK5La grown in AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
15	2.58	2.20	2.43	2.41	0.19
20	1.97	2.68	2.13	2.26	0.37
25	2.20	1.07	2.73	2.00	0.85
33	3.34	2.96	2.87	3.06	0.25

Table B.30. Diacylglyceride composition over time for isolate GK5La grown in AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
15	1.52	1.73	2.10	1.78	0.29
20	1.72	1.34	1.91	1.66	0.29
25	2.51	2.55	2.78	2.62	0.15
33	3.98	4.71	3.85	4.18	0.46

Table B.31. Triacylglyceride composition over time for isolate GK5La grown in AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
15	7.74	4.78	5.04	5.85	1.64
20	3.07	4.03	1.89	3.00	1.07
25	6.13	0.87	5.67	4.22	2.91
33	6.04	4.25	3.85	4.72	1.16

Table B.32. Triacylglyceride composition over time for isolate GK5La grown in AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
15	4.49	5.80	3.45	4.58	1.18
20	2.67	1.58	3.90	2.72	1.16
25	6.38	7.35	5.40	6.38	0.98
33	3.96	4.05	3.54	3.85	0.27

Table B.33. Total neutral lipid composition over time for isolate GK5La grown in AM6 medium supplemented with sodium bicarbonate and spiked to 1.8% sodium chloride in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
15	18.79	15.58	16.48	16.95	1.66
20	17.62	19.74	23.28	20.21	2.86
25	16.81	17.38	22.28	18.82	3.01
33	27.60	27.25	24.76	26.54	1.55

Table B.34. Total neutral lipid composition over time for isolate GK5La grown in AM6(1.8) medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
15	14.77	16.83	15.37	15.66	1.06
20	16.12	17.78	19.50	17.80	1.69
25	19.27	20.10	20.54	19.97	0.65
33	22.64	24.42	28.76	25.27	3.15

Comparison Between 50mM CHES Buffered Inorganic Carbon
Supplemented AM6 Media and 50mM CHES Buffered AM6 Media

Table B.35. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium buffered with CHES in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	7.8E+04	9.0E+04	7.3E+04	8.0E+04	9.0E+03
1	8.5E+04	9.3E+04	1.1E+05	9.6E+04	1.3E+04
2	4.0E+05	6.4E+05	7.3E+05	5.9E+05	1.7E+05
3	2.5E+06	3.1E+06	2.0E+06	2.5E+06	5.6E+05
4	7.4E+06	5.0E+06	5.1E+06	5.8E+06	1.4E+06
5	9.9E+06	1.0E+07	1.0E+07	1.0E+07	1.5E+05
6	1.0E+07	1.4E+07	1.1E+07	1.2E+07	2.2E+06
7	1.3E+07	1.0E+07	1.0E+07	1.1E+07	1.9E+06
8	1.5E+07	2.0E+07	1.8E+07	1.8E+07	2.8E+06
9	1.4E+07	1.1E+07	1.0E+07	1.1E+07	1.9E+06
10	1.7E+07	1.2E+07	1.7E+07	1.5E+07	2.7E+06
11	2.8E+07	2.2E+07	2.0E+07	2.3E+07	4.3E+06
12	2.9E+07	2.4E+07	2.0E+07	2.5E+07	4.2E+06
13	1.6E+07	1.5E+07	1.7E+07	1.6E+07	8.3E+05
15	2.0E+07	2.3E+07	2.4E+07	2.2E+07	2.0E+06
17	2.8E+07	2.4E+07	2.6E+07	2.6E+07	2.2E+06
19	2.6E+07	2.5E+07	2.4E+07	2.5E+07	1.2E+06
20	1.8E+07	1.9E+07	2.0E+07	1.9E+07	1.2E+06
25	2.8E+07	3.7E+07	2.8E+07	3.1E+07	5.1E+06
33	2.8E+07	1.8E+07	2.3E+07	2.3E+07	5.2E+06

Table B.36. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	8.5E+04	8.0E+04	1.0E+05	8.9E+04	1.2E+04
1	1.0E+05	1.3E+05	7.0E+04	1.0E+05	3.0E+04
2	6.4E+05	3.7E+05	5.7E+05	5.3E+05	1.4E+05
3	1.6E+06	1.0E+06	1.2E+06	1.3E+06	3.1E+05
4	4.6E+06	7.4E+06	6.5E+06	6.2E+06	1.4E+06
5	1.4E+07	1.0E+07	1.3E+07	1.2E+07	2.2E+06
6	2.6E+07	2.0E+07	1.8E+07	2.1E+07	4.0E+06
7	5.0E+07	4.1E+07	4.7E+07	4.6E+07	4.5E+06
8	5.2E+07	4.8E+07	5.4E+07	5.1E+07	2.7E+06
9	5.9E+07	5.0E+07	4.9E+07	5.3E+07	5.5E+06
10	5.3E+07	5.0E+07	4.8E+07	5.1E+07	2.4E+06
11	6.6E+07	5.1E+07	5.6E+07	5.8E+07	7.7E+06
12	4.6E+07	5.6E+07	6.3E+07	5.5E+07	8.9E+06
13	4.1E+07	5.1E+07	4.4E+07	4.5E+07	5.2E+06
15	5.1E+07	5.7E+07	5.5E+07	5.5E+07	3.1E+06
17	4.6E+07	5.4E+07	4.7E+07	4.9E+07	4.1E+06
19	6.2E+07	5.2E+07	6.3E+07	5.9E+07	6.0E+06
20	5.2E+07	6.6E+07	6.0E+07	5.9E+07	7.2E+06
25	6.2E+07	6.2E+07	5.9E+07	6.1E+07	1.5E+06
33	6.0E+07	5.8E+07	7.1E+07	6.3E+07	6.7E+06

Table B.37. pH for isolate GK5La grown on AM6 medium buffered with CHES in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	8.5	8.4	8.4	8.4	0.0
1	8.3	8.3	8.3	8.3	0.0
2	8.3	8.3	8.3	8.3	0.0
3	8.5	8.5	8.5	8.5	0.0
4	8.7	8.6	8.6	8.6	0.0
5	8.7	8.7	8.7	8.7	0.0
6	8.5	8.7	8.7	8.6	0.1
7	8.7	8.7	8.7	8.7	0.0
8	8.8	8.8	8.8	8.8	0.0
9	8.6	8.7	8.7	8.6	0.0
10	8.7	8.8	8.7	8.7	0.0
11	8.7	8.8	8.7	8.8	0.1
12	8.8	8.8	8.8	8.8	0.0
13	8.9	8.8	8.8	8.8	0.0
15	9.0	8.9	9.0	8.9	0.0
17	8.9	8.9	8.9	8.9	0.0
19	8.9	8.9	8.9	8.9	0.0
20	8.9	8.9	8.9	8.9	0.0
21	8.8	8.8	8.8	8.8	0.0
25	8.8	8.7	8.7	8.8	0.0
33	8.8	8.8	8.8	8.8	0.0

Table B.38. pH for isolate GK5La grown on AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	8.4	8.3	8.3	8.3	0.0
1	8.5	8.5	8.6	8.5	0.0
2	8.8	8.7	8.8	8.7	0.0
3	9.0	9.0	9.0	9.0	0.0
4	9.3	9.3	9.3	9.3	0.0
5	9.7	9.6	9.6	9.6	0.0
6	10.1	10.0	10.0	10.1	0.0
7	10.9	10.7	10.5	10.7	0.2
8	11.6	11.5	11.4	11.5	0.1
9	11.7	11.7	11.5	11.6	0.1
10	11.1	11.3	11.0	11.1	0.2
11	10.8	11.0	10.8	10.9	0.1
12	10.6	10.8	10.6	10.7	0.1
13	10.6	10.8	10.5	10.6	0.1
15	10.5	10.7	10.4	10.5	0.1
17	10.3	10.4	10.2	10.3	0.1
19	10.1	10.1	9.9	10.0	0.1
20	10.0	10.1	10.0	10.0	0.1
21	10.0	10.1	9.9	10.0	0.1
25	9.8	9.8	9.8	9.8	0.0
33	9.7	9.7	9.7	9.7	0.0

Table B.39. Nitrate concentration (mg/L) for isolate GK5La grown on AM6 medium buffered with CHES in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	305	237	257	266	35
4	201	230	211	214	15
6	204	158	123	161	41
7	183	151	103	146	40
8	154	65	56	92	54
9	117	76	80	91	22
10	105	77	68	83	19
12	54	-4	-5	15	34
15	0	0	0	0	0

Table B.40. Nitrate concentration (mg/L) for isolate GK5La grown on AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	270	286	310	289	20
4	193	207	217	205	12
6	5	22	8	12	9
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0

Table B.41. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium buffered with CHES in tube reactors.

Time (d)	1a	1b	1c	Average	Standard Deviation
0	1	-2	8	2	5
1	4	11	15	10	6
2	-4	9	21	9	13
3	21	43	31	32	11
4	210	40	140	130	85
5	200	-60	80	73	130
6	210	90	100	133	67
7	300	130	50	160	128
8	230	380	220	277	90
9	310	150	120	193	102
10	330	460	290	360	89
11	520	360	400	427	83
12	660	650	670	660	10
13	650	620	840	703	119
15	840	1670	1710	1407	491
17	1430	4070	4730	3410	1746
19	6900	10190	11930	9673	2554
20	7200	10880	9550	9210	1863
25	8950	11700	13640	11430	2357
33	10160	8640	8780	9193	840

Table B.42. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (d)	2a	2b	2c	Average	Standard Deviation
0	-2	6	3	2	4
1	9	5	14	9	5
2	32	16	36	28	11
3	37	38	26	34	7
4	200	310	140	217	86
5	-10	100	200	97	105
6	210	170	260	213	45
7	300	230	320	283	47
8	940	950	810	900	78
9	630	800	910	780	141
10	960	1030	1340	1110	202
11	1250	1450	1830	1510	295
12	1590	1710	2140	1813	289
13	1700	2060	2240	2000	275
15	2400	2480	2840	2573	234
17	2050	2430	2590	2357	277
19	2320	2850	2610	2593	265
20	1690	2320	2730	2247	524
25	2010	1880	2160	2017	140
33	1620	1760	1890	1757	135

Table B.43. Free fatty acid composition over time for isolate GK5La grown in AM6 medium buffered with CHES in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
8	4.80	4.36	5.00	4.72	0.33
15	7.41	8.20	5.82	7.14	1.21
20	8.51	6.61	4.63	6.58	1.94
25	9.42	8.42	6.56	8.13	1.45
33	8.78	8.35	9.34	8.82	0.49

Table B.44. Free fatty acid composition over time for isolate GK5La grown in AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
8	5.07	4.79	3.98	4.61	0.56
15	7.45		7.19	7.32	0.18
20	4.38	4.91	8.63	5.97	2.31
25	4.49	4.92	5.35	4.92	0.43
33	5.91	5.72	5.31	5.65	0.30

Table B.45. Monoacylglyceride composition over time for isolate GK5La grown in AM6 medium buffered with CHES in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
8	1.78	1.93	1.87	1.86	0.07
15	3.62	3.45	2.60	3.22	0.54
20	3.32	2.64	2.13	2.70	0.59
25	3.07	3.67	2.89	3.21	0.41
33	2.99	3.07	3.77	3.28	0.43

Table B.46. Monoacylglyceride composition over time for isolate GK5La grown in AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
8	1.58	1.29	1.40	1.42	0.15
15	2.44		2.63	2.53	0.14
20	1.96	2.51	2.41	2.29	0.29
25	2.37	2.45	2.50	2.44	0.07
33	3.32	3.16	3.03	3.17	0.15

Table B.47.Diacylglyceride composition over time for isolate GK5La grown in AM6 medium buffered with CHES in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
8	0.96	0.86	0.95	0.92	0.05
15	1.78	1.51	1.08	1.46	0.35
20	1.82	1.89	1.76	1.82	0.06
25	2.12	2.24	1.88	2.08	0.18
33	1.95	1.96	2.31	2.07	0.21

Table B.48.Diacylglyceride composition over time for isolate GK5La grown in AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
8	0.54	0.62	0.55	0.57	0.04
15	0.90		0.86	0.88	0.03
20	2.09	1.99	1.68	1.92	0.21
25	1.99	2.13	2.10	2.07	0.07
33	2.80	2.71	2.30	2.60	0.27

Table B.49.Triacylglyceride composition over time for isolate GK5La grown in AM6 medium buffered with CHES in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
8	0.03	0.02	0.02	0.02	0.00
15	0.03	0.03	0.03	0.03	0.00
20	0.37	0.42	1.24	0.67	0.49
25	3.08	1.08	1.12	1.76	1.14
33	1.18	0.83	1.18	1.06	0.20

Table B.50. Triacylglyceride composition over time for isolate GK5La grown in AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
8	0.01	0.01	0.02	0.01	0.00
15	0.05		0.06	0.06	0.00
20	3.77	2.82	1.71	2.77	1.03
25	3.32	3.40	3.33	3.35	0.04
33	3.64	3.41	2.99	3.34	0.33

Table B.51.Total neutral lipid composition over time for isolate GK5La grown in AM6 medium buffered with CHES in tube reactors.

Time (days)	1a	1b	1c	Standard Deviation	
8	7.57	7.18	7.84	7.53	0.33
15	12.84	13.18	9.52	11.85	2.02
20	14.01	11.56	9.76	11.78	2.13
25	17.70	15.42	12.45	15.19	2.63
33	14.90	14.20	16.60	15.24	1.23

Table B.52. Total neutral lipid composition over time for isolate GK5La grown in AM6 medium buffered with CHES and supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Standard Deviation	
8	7.20	6.70	5.94	6.61	0.63
15	10.84		10.74	10.79	0.08
20	12.20	12.24	14.43	12.96	1.27
25	12.17	12.90	13.28	12.78	0.56
33	15.66	15.00	13.63	14.77	1.03

Isolate GK5La Overall Lipid Analysis

Table B.53. End point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK5La grown under 8 different treatments in tube reactors. Units are shown in (%).

Culture Condition		FA	MAG	DAG	TAG	Total Neutral Lipid	Total FAME
AM6	Tube 1	6.51	1.11	0.90	0.69	10.28	29.26
	Tube 2	5.69	0.82	0.95	0.89	9.54	28.60
	Tube 3	6.78	0.89	0.94	0.74	10.75	30.46
AM6 spiked with 1.8% NaCl	Tube 1	7.40	1.37	2.00	3.89	16.45	34.36
	Tube 2	12.04	1.42	1.71	1.73	18.57	36.11
	Tube 3	5.18	1.13	2.01	4.81	13.95	34.77
AM6 + HCO_3^- Supplemented	Tube 1	17.24	1.82	1.86	1.31	22.23	36.19
	Tube 2	15.01	1.60	1.82	1.37	19.80	34.22
	Tube 3	16.84	2.13	1.95	1.26	22.19	31.73
AM6(1.8) + HCO_3^- Supplemented	Tube 1	14.76	2.19	1.80	3.95	22.70	41.11
	Tube 2	12.64	1.97	1.75	5.15	21.52	34.68
	Tube 3	14.55	1.92	1.75	3.55	21.76	37.03
AM6 + CHES	Tube 1	8.78	2.99	1.95	1.18	14.90	32.34
	Tube 2	8.35	3.07	1.96	0.83	14.20	30.30
	Tube 3	9.34	3.77	2.31	1.18	16.60	33.13
AM6 + CHES + HCO_3^- Supplemented	Tube 1	5.91	3.32	2.80	3.64	15.66	32.08
	Tube 2	5.72	3.16	2.71	3.41	15.00	41.33
	Tube 3	5.31	3.03	2.30	2.99	13.63	40.20
AM6 + HCO_3^- Supplemented + Salt Spike	Tube 1	11.89	6.33	3.34	6.04	27.60	48.81
	Tube 2	13.31	6.72	2.96	4.25	27.25	47.78
	Tube 3	12.15	5.89	2.87	3.85	24.76	45.45
AM6(1.8)	Tube 1	11.85	2.85	3.98	3.96	22.64	47.34
	Tube 2	12.75	2.92	4.71	4.05	24.42	49.85
	Tube 3	14.27	7.10	3.85	3.54	28.76	48.99

Table B.54. Average end point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK5La grown under 8 different treatments in tube reactors. Units are shown in (%).

Culture Condition	FA	MAG	DAG	TAG	Total Neutral Lipid	Total FAME
AM6	6.33	0.94	0.93	0.77	10.19	29.44
AM6 spiked with 1.8% NaCl	8.20	1.31	1.91	3.48	16.32	35.08
AM6 + HCO_3^- Supplemented	16.36	1.85	1.88	1.32	21.40	34.05
AM6(1.8) + HCO_3^- Supplemented	13.98	2.03	1.77	4.21	21.99	37.61
AM6 + CHES	8.82	3.28	2.07	1.06	15.24	31.92
AM6 + CHES + HCO_3^- Supplemented	5.65	3.17	2.60	3.34	14.77	37.87
AM6 + HCO_3^- Supplemented + Salt Spike	12.45	6.31	3.06	4.72	26.54	47.35
AM6(1.8)	12.96	4.29	4.18	3.85	25.27	48.73

Table B.55. Standard deviation end point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK5La grown under 8 different treatments in tube reactors. Units are shown in (%).

Culture Condition	FA	MAG	DAG	TAG	Total Neutral Lipid	Total FAME
AM6	0.57	0.15	0.02	0.10	0.61	0.94
AM6 spiked with 1.8% NaCl	3.50	0.16	0.17	1.58	2.31	0.91
AM6 + HCO_3^- Supplemented	1.19	0.27	0.07	0.05	1.39	2.24
AM6(1.8) + HCO_3^- Supplemented	1.16	0.14	0.03	0.83	0.62	3.25
AM6 + CHES	0.49	0.43	0.21	0.20	1.23	1.46
AM6 + CHES + HCO_3^- Supplemented	0.30	0.15	0.27	0.33	1.03	5.05
AM6 + HCO_3^- Supplemented + Salt Spike	0.76	0.41	0.25	1.16	1.55	1.72
AM6(1.8)	1.22	2.44	0.46	0.27	3.15	1.28

Table B.56. 95% confidence interval for mean specific free fatty acid content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	FA (weight%)	CL	CU
AM6	1.41	6.33	4.92	7.74
AM6 spiked with 1.8% NaCl	8.70	8.21	-0.49	16.90
AM6 + HCO3- Supplemented	2.95	16.36	13.41	19.32
AM6(1.8) + HCO3- Supplemented	2.90	13.98	11.08	16.89
AM6 + CHES	1.23	8.82	7.59	10.06
AM6 + CHES + HCO3- Supplemented	0.76	5.65	4.88	6.41
AM6 + HCO3- Supplemented + Salt Spike	1.88	12.45	10.57	14.33
AM6(1.8)	3.04	12.96	9.92	16.00

Table B.57. 95% confidence interval for mean specific monoacylglyceride content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	MAG (weight %)	CL	CU
AM6	0.38	0.94	0.56	1.32
AM6 spiked with 1.8% NaCl	0.39	1.31	0.92	1.69
AM6 + HCO3- Supplemented	0.66	1.85	1.19	2.51
AM6(1.8) + HCO3- Supplemented	0.36	2.03	1.67	2.38
AM6 + CHES	1.07	3.28	2.21	4.34
AM6 + CHES + HCO3- Supplemented	0.36	3.17	2.81	3.53
AM6 + HCO3- Supplemented + Salt Spike	1.03	6.31	5.28	7.34
AM6(1.8)	6.05	4.29	-1.76	10.34

Table B.58. 95% confidence interval for mean specific diacylglyceride content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	DAG (weight%)	CL	CU
AM6	0.07	0.93	0.86	1.00
AM6 spiked with 1.8% NaCl	0.42	1.91	1.48	2.33
AM6 + HCO3- Supplemented	0.17	1.88	1.71	2.04
AM6(1.8) + HCO3- Supplemented	0.07	1.77	1.69	1.84
AM6 + CHES	0.51	2.07	1.56	2.58
AM6 + CHES + HCO3- Supplemented	0.66	2.60	1.94	3.27
AM6 + HCO3- Supplemented + Salt Spike	0.62	3.06	2.44	3.68
AM6(1.8)	1.15	4.18	3.03	5.33

Table B.59. 95% confidence interval for mean specific triacylglyceride content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	TAG (weight %)	CL	CU
AM6	0.26	0.77	0.51	1.03
AM6 spiked with 1.8% NaCl	3.93	3.48	-0.45	7.40
AM6 + HCO3- Supplemented	0.14	1.31	1.18	1.45
AM6(1.8) + HCO3- Supplemented	2.07	4.22	2.15	6.29
AM6 + CHES	0.50	1.06	0.56	1.57
AM6 + CHES + HCO3- Supplemented	0.82	3.35	2.53	4.17
AM6 + HCO3- Supplemented + Salt Spike	2.90	4.71	1.82	7.61
AM6(1.8)	0.68	3.85	3.17	4.53

Table B.60. 95% confidence interval for mean specific total neutral lipid content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	Total Neutral Lipid (weight %)	CL	CU
AM6	1.52	10.19	8.67	11.71
AM6 spiked with 1.8% NaCl	5.74	16.32	10.58	22.07
AM6 + HCO3- Supplemented	3.46	21.41	17.95	24.86
AM6(1.8) + HCO3- Supplemented	1.55	21.99	20.44	23.54
AM6 + CHES	3.07	15.23	12.17	18.30
AM6 + CHES + HCO3- Supplemented	2.57	14.76	12.19	17.34
AM6 + HCO3- Supplemented + Salt Spike	3.85	26.54	22.69	30.38
AM6(1.8)	7.82	25.27	17.45	33.09

Table B.61. 95% confidence interval for mean specific total FAME content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	Total FAME (weight %)	CL	CU
AM6	2.34	29.44	27.10	31.78
AM6 spiked with 1.8% NaCl	2.27	35.08	32.81	37.35
AM6 + HCO3- Supplemented	5.55	34.05	28.49	39.60
AM6(1.8) + HCO3- Supplemented	8.08	37.61	29.52	45.69
AM6 + CHES	3.63	31.92	28.30	35.55
AM6 + CHES + HCO3- Supplemented	12.54	37.87	25.33	50.41
AM6 + HCO3- Supplemented + Salt Spike	4.28	47.35	43.07	51.62
AM6(1.8)	3.17	48.73	45.56	51.90

Table B.62. Endpoint analysis representing productivity in each treatment expressed on a concentration basis. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

Culture Condition		Cell Dry Weight Conc. (g/L)	Total Lipid Conc. (g/L)	Total FAME Conc. (g/L)
AM6	Tube 1	2.58	0.26	0.75
	Tube 2	2.44	0.23	0.70
	Tube 3	2.52	0.27	0.77
AM6 spiked with 1.8% NaCl	Tube 1	1.36	0.22	0.47
	Tube 2	1.22	0.23	0.44
	Tube 3	1.44	0.20	0.50
AM6 + HCO_3^- Supplemented	Tube 1	1.94	0.43	0.70
	Tube 2	1.98	0.39	0.68
	Tube 3	2.08	0.46	0.66
AM6(1.8) + HCO_3^- Supplemented	Tube 1	1.10	0.25	0.45
	Tube 2	0.99	0.21	0.34
	Tube 3	0.73	0.16	0.27
AM6 + CHES	Tube 1	0.86	0.13	0.28
	Tube 2	0.88	0.12	0.27
	Tube 3	1.02	0.17	0.34
AM6 + CHES + HCO_3^- Supplemented	Tube 1	1.86	0.29	0.60
	Tube 2	1.88	0.28	0.78
	Tube 3	1.93	0.26	0.78
AM6 + HCO_3^- Supplemented + Salt Spike	Tube 1	1.35	0.37	0.66
	Tube 2	1.54	0.42	0.74
	Tube 3	1.51	0.37	0.69
AM6(1.8)	Tube 1	1.10	0.25	0.52
	Tube 2	1.03	0.25	0.51
	Tube 3	0.95	0.27	0.47

Table B.63. Average endpoint analysis representing productivity in each treatment expressed on a concentration basis. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

Culture Condition	Cell Dry Weight (g/L) Conc.	Total Lipid (g/L) Conc.	Total FAME (g/L) Conc.
AM6	2.51	0.26	0.74
AM6 spiked with 1.8% NaCl	1.34	0.22	0.47
AM6 + HCO_3^- Supplemented	2.00	0.43	0.68
AM6(1.8) + HCO_3^- Supplemented	0.94	0.21	0.35
AM6 + CHES	0.92	0.14	0.29
AM6 + CHES + HCO_3^- Supplemented	1.89	0.28	0.72
AM6 + HCO_3^- Supplemented + Salt Spike	1.47	0.39	0.69
AM6(1.8)	1.03	0.26	0.50

Table B.64. Standard deviation of endpoint analysis representing productivity in each treatment expressed on a concentration basis. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

Culture Condition	Cell Dry Weight (g/L) Conc.	Total Lipid (g/L) Conc.	Total FAME (g/L) Conc.
AM6	0.07	0.02	0.04
AM6 spiked with 1.8% NaCl	0.11	0.01	0.03
AM6 + HCO_3^- Supplemented	0.07	0.03	0.02
AM6(1.8) + HCO_3^- Supplemented	0.19	0.05	0.09
AM6 + CHES	0.09	0.02	0.04
AM6 + CHES + HCO_3^- Supplemented	0.04	0.01	0.10
AM6 + HCO_3^- Supplemented + Salt Spike	0.10	0.03	0.04
AM6(1.8)	0.08	0.01	0.03

Table B.65. 95% confidence interval for mean cell dry weight in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	Cell Dry Weight (mg/mL)	CL	CU
AM6	0.17	2.51	2.34	2.69
AM6 spiked with 1.8% NaCl	0.28	1.34	1.06	1.62
AM6 + HCO3- Supplemented	0.18	2.00	1.82	2.18
AM6(1.8) + HCO3- Supplemented	0.47	0.94	0.47	1.41
AM6 + CHES	0.22	0.92	0.70	1.14
AM6 + CHES + HCO3- Supplemented	0.09	1.89	1.80	1.98
AM6 + HCO3- Supplemented + Salt Spike	0.25	1.47	1.21	1.72
AM6(1.8)	0.19	1.03	0.84	1.21

Table B.66. 95% confidence interval for mean total lipid content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	Total Lipid (mg/mL)	CL	CU
AM6	0.05	0.25	0.20	0.31
AM6 spiked with 1.8% NaCl	0.04	0.22	0.18	0.25
AM6 + HCO3- Supplemented	0.09	0.43	0.34	0.51
AM6(1.8) + HCO3- Supplemented	0.11	0.21	0.09	0.32
AM6 + CHES	0.07	0.14	0.07	0.21
AM6 + CHES + HCO3- Supplemented	0.04	0.28	0.24	0.31
AM6 + HCO3- Supplemented + Salt Spike	0.07	0.39	0.31	0.46
AM6(1.8)	0.03	0.26	0.23	0.29

Table B.67. 95% confidence interval for mean total FAME content in each of the 8 controls for isolate GK5La.

Treatment	Confidence Level	Total FAME (mg/mL)	CL	CU
AM6	0.09	0.74	0.65	0.83
AM6 spiked with 1.8% NaCl	0.07	0.47	0.40	0.54
AM6 + HCO3- Supplemented	0.05	0.68	0.63	0.73
AM6(1.8) + HCO3- Supplemented	0.23	0.35	0.13	0.58
AM6 + CHES	0.09	0.30	0.20	0.39
AM6 + CHES + HCO3- Supplemented	0.26	0.72	0.46	0.98
AM6 + HCO3- Supplemented + Salt Spike	0.10	0.70	0.60	0.80
AM6(1.8)	0.07	0.50	0.43	0.57

Table B.68. Neutral lipid speciation of endpoint analysis represented in weight percent.

Compound	AM6	AM6 + 1.8% Salt Spike	AM6 + HCO_3^- Supplemented	AM6(1.8) + HCO_3^- Supplemented	AM6 + CHES	AM6 + CHES + HCO_3^- Supplemented	AM6 + HCO_3^- Supplemented + 1.8% Salt Spike	AM6(1.8)
C10_FFA	0%	0%	0%	0%	1%	1%	1%	1%
C12_FFA	1%	0%	0%	0%	0%	0%	0%	0%
C14_FFA	2%	2%	2%	2%	1%	1%	1%	1%
C16_FFA	20%	16%	23%	18%	18%	13%	14%	14%
C18_FFA	46%	34%	50%	43%	36%	23%	30%	32%
C12_MA	0%	0%	0%	0%	0%	0%	0%	0%
C20_FFA	2%	1%	2%	1%	1%	1%	1%	1%
C14_MA	1%	1%	0%	0%	5%	3%	3%	3%
C16_MA	3%	4%	4%	4%	5%	4%	5%	5%
C18_MA	6%	5%	4%	4%	12%	14%	16%	17%
C12_DAG	3%	3%	3%	3%	4%	3%	2%	2%
C14_DAG	4%	4%	3%	2%	3%	3%	3%	2%
C11_TAG	0%	0%	0%	0%	0%	0%	0%	0%
C16_DAG	1%	1%	1%	1%	1%	2%	1%	1%
C12_TAG	4%	7%	2%	4%	1%	2%	2%	2%
C18_DAG	3%	5%	2%	3%	5%	10%	6%	8%
C14_TAG	0%	1%	0%	1%	1%	2%	1%	1%
C16_TAG	1%	4%	1%	3%	1%	5%	4%	3%
C17_TAG	0%	0%	0%	0%	0%	0%	0%	0%
C18_TAG	3%	11%	2%	10%	2%	8%	8%	6%
C20_TAG	0%	0%	0%	0%	2%	6%	3%	3%
C22_TAG	0%	0%	0%	0%	0%	0%	0%	0%

APPENDIX C

EXPERIMENTAL DATA NOT INCLUDED IN MAIN BODY

Protein Purification / Raceway Experiment

Isolate GK6-G2 turned its growth medium brown late in the growth phase and after settling out to form a biofilm. It was thought that the dark color formed in solution was a secreted extracellular protein in solution. After filtration of the solution through a 0.2 micron filter, several steps were carried out to isolate and identify the brown protein in solution.

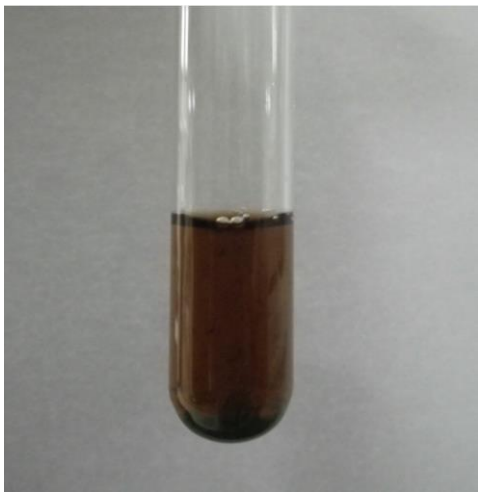


Figure C.1. Culture test tube containing isolate GK6-G2 settled on the bottom of the test tube. The brown solution above the aggregation containing isolate GK6-G2 was suspected to be an extracellular protein.

Extracellular proteins were precipitated in solution by the addition of 5 volumes of ice cold acetone and incubated at -80°C overnight followed by centrifugation. The protein pellet was washed twice with acetone, dried at room temperature for 10 minutes, suspended in loading buffer and stained with 5μL of bromophenol blue. Purified proteins were denatured by boiling for 15 minutes and then separated on a 15% SDS-PAGE polyacrylamide gel. This was followed by staining with Coomassie brilliant blue R250. The most abundant protein bands were excised, destained, with 90uL of 50% acetonitrile

in 50mM ammonium bicarbonate (pH 7.9), and vacuum dried. Gel slices were rehydrated with 1.5mg/mL DTT in 25mM ammonium bicarbonate (pH 8.5) at 56°C for 1 hour in a water bath. Gel slices were alkylated with 10mg/mL iodoacetamide (IAA) in 25mM ammonium bicarbonate (pH 8.5), and incubated at room temperature in the dark for 45 minutes. Gel slices were washed with 100mM ammonium acetate (pH 8.5) for 10 minutes, washed twice with 50% acetonitrile in 50mM ammonium bicarbonate (pH 8.5) for 10min, vacuum dried, and rehydrated with 3uL of 100µg/mL Trypsin Gold (Promega, Madison, WI) in 25mM ammonium bicarbonate (pH 8.5). Slices were covered in a solution of 10mM ammonium bicarbonate with 10% acetonitrile (pH 8.5), and digested overnight t 37°C followed by centrifugation. Peptides were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The search engine MASCOT (Matrix Science, London, UK) was used to compare masses of identified peptides to masses of sequences in the NCBInr database. Criteria for acceptable protein identification required the detection two significant peptides based on MASCOT MOWSE scores greater than 32 (p-values < 0.05). Due to keratin (skin cell) contamination, the protein was unable to be identified according to the specified criteria.

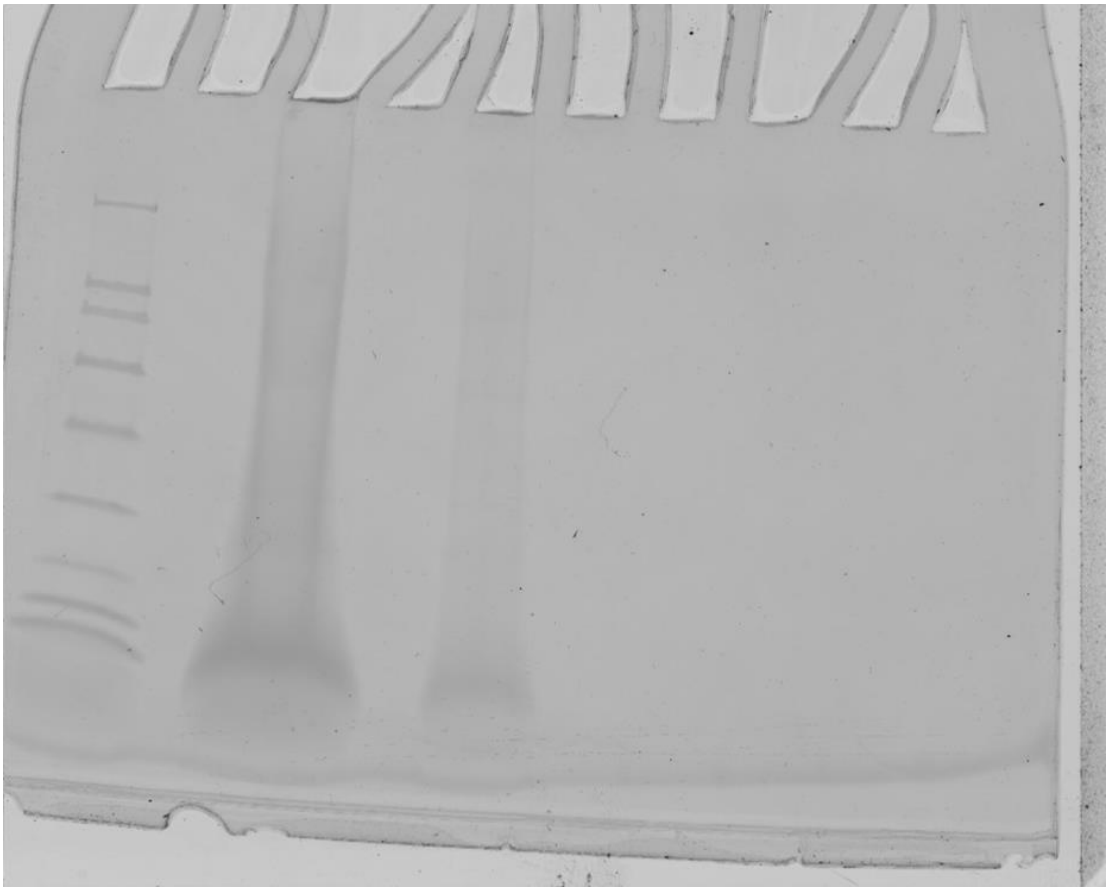


Figure C.2. Picture of the polyacrylamide gel, in which the protein was separated on, after staining with Coomassie blue. From the left is the protein ladder used, unidentified protein sample, and less concentrated unidentified protein sample.

200L Raceway Experiment

Isolate GK6-G2 was grown in a 200L raceway in AM6 medium supplemented with 18g/L sodium bicarbonate. The isolate did not produce the extracellular protein in this scaled up environment after being allowed to settle and form a biofilm on the bottom of the raceway. Several factors could have led to this including: inhibiting growth of the organism early on in the study by inoculating a low concentration of cells, not allowing the culture to photo-bleach before turning off the paddle wheel and allowing it to settle

forming a biofilm, not allowing enough time for the protein to be produced in solution, or inhibiting the production of the protein by inoculating into a non-sterile environment.



Figure C.3. The 200L raceway pond just after inoculation of isolate GK6-G2.



Figure C.4. The 200L raceway pond once isolate GK6-G2 reached stationary phase in solution.

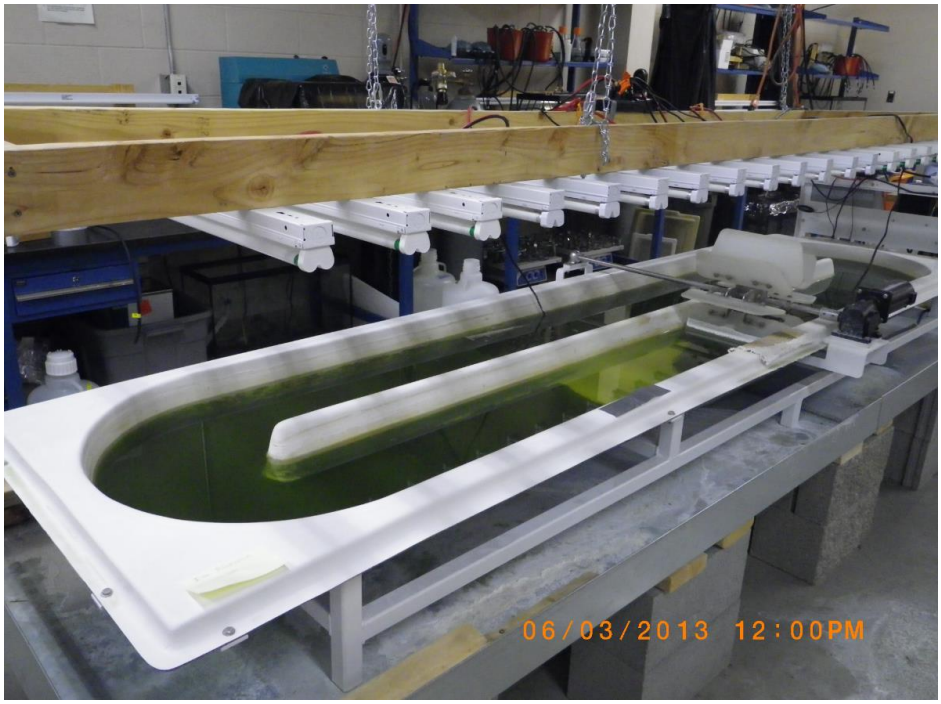


Figure C.5. The 200L raceway pond after isolate GK6-G2 was allowed to settle out.

Table C.1. Cell concentration of isolate GK6-G2 in the 200L raceway pond grown in AM6 medium buffered with 18g/L sodium bicarbonate.

Time (days)	Cell Conc.
0	5.75E+05
1	6.20E+05
2	9.50E+05
3	1.00E+06
4	9.10E+05
5	1.00E+06
6.9	1.34E+06
7	9.70E+05
17	1.35E+06
18	1.72E+06
19	2.37E+06
20	3.76E+06
21	4.90E+06
22	5.40E+06
23	6.20E+06
24	7.60E+06
25	7.20E+06
26	1.08E+07
27	9.40E+06
29	1.05E+07
31	1.37E+07
32	1.63E+07
33	1.54E+07
34	1.65E+07
35	1.66E+07
36	1.80E+07
38	1.63E+07
39	1.64E+07
41	1.70E+07

Table C.2. pH of isolate GK6-G2 in the 200L raceway pond grown in AM6 medium buffered with 18g/L sodium bicarbonate.

Time (days)	pH
0	8.71
1	9.34
2	9.47
3	9.61
4	9.76
5	9.75
6.9	9.88
7	9.73
17	10.02
18	10.1
19	10.17
20	10.22
21	10.24
22	10.32
23	10.39
24	10.38
25	10.43
26	10.56
27	10.54
29	10.43
31	10.45
32	10.46
33	10.5
34	10.49
35	10.55
36	10.53
38	10.54
39	10.62
41	10.59

Table C.3. DIC of isolate GK6-G2 in the 200L raceway pond grown in AM6 medium buffered with 18g/L sodium bicarbonate.

Time (days)	DIC (mM)
0	249
1	240
2	270
3	194
4	222
5	201
7	214
17	173
18	201
19	187
20	195
21	210
22	165
23	187
24	175
25	188
26	211
27	171
31	172
32	178
33	142
34	155
35	129
36	156
38	159
39	148
41	171

Table C.4. Absorbance (750nm) of isolate GK6-G2 in the 200L raceway pond grown in AM6 medium buffered with 18g/L sodium bicarbonate.

Time (days)	Absorbance (750nm)
0	0.01
2	0.029
3	0.021
17	0.09
18	0.118
19	0.137
20	0.193
21	0.17
22	0.23
23	0.22
24	0.32
25	0.3
26	0.35
27	0.4
32	0.47
33	0.55
34	0.59
35	0.54
36	0.59
38	0.61
41	0.66

Table C.5. Speciation of inorganic carbon in AM6 medium buffered with 18g/L sodium bicarbonate.

pH	10.009 (equilibrium)
Component (%)	
CO3-2	38.541
HCO3-	31.42
MgCO3 (aq)	0.098
CaCO3 (aq)	0.055
NaCO3-	28.784
NaHCO3 (aq)	1.091

Modified Carotenoid Extraction and Analysis (Adapted from Del Campo (2003 and 2004), Sedmak (1990), Wellburn (1994))

Isolate GK6-G2 and isolate GK4S-G2 both were visible orange or red during various parts of their growth cycle. Some strains of microalgae contain high concentrations of photosynthetic carotenoid compounds that have value in industry as natural pigments. In order to qualitatively and quantitatively analyze pigments from the two isolates, a method was developed to extract and analyze the pigments produced.

Ten milliliters of suspended sample was withdrawn into a falcon tube and the cells were subsequently pelleted through centrifugation. The cellular pellet was then washed and resuspended twice with deionized water before continuing with the extraction. Two milliliters of Methanol was added to the tube and vortexed for 20 seconds. The tube and its contents were then heated in a water bath at 55 C for 10 minutes. The tubes were removed and vortexed for an additional 20 seconds before being centrifuged again. Approximately 200uL of Methanol extract was transferred to a polystyrene 96 well plate where chlorophylls a and b, and total carotenoids were determined spectrophotometrically at wavelengths 665, 649, and 480 nm, respectively. Chlorophyll a, b, total chlorophyll, and total carotenoids were determined using the following equations.



Figure C.6. Isolate GK6-G2 pellet after chlorophyll degradation, showing high carotenoid content in its orange color.

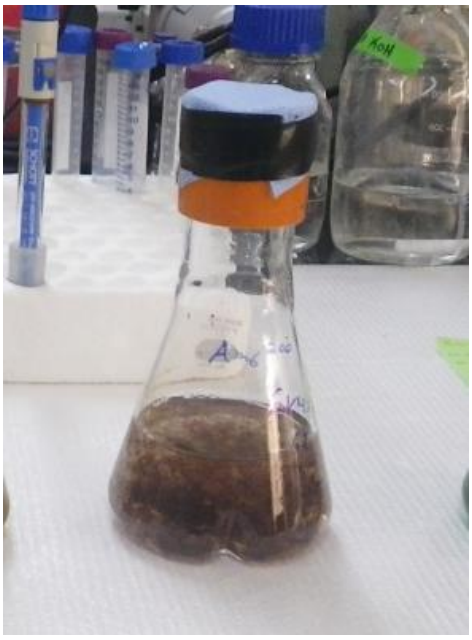


Figure C.7. Isolate GK4S-G2 grown in a 150mL beveled flask, highlighting its dark red color.

Extraction:

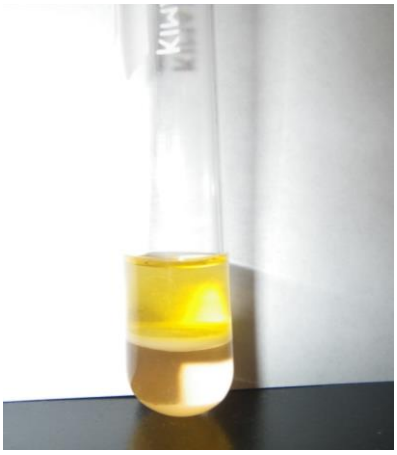


Figure C.8. Extracted pigment from isolate GK6-G2 after following the procedure outlined in Sedmak (1990).

$$\text{Chl } a\left(\text{mg L}^{-1}\right) = 17.12 \cdot A_{666} - 8.86 \cdot A_{653}$$

$$\text{Chl } b\left(\text{mg L}^{-1}\right) = 32.23 \cdot A_{653} - 14.55 \cdot A_{666}$$

$$\text{Chl } a+b\left(\text{mgL}^{-1}\right) = 2.57 \cdot A_{666} + 23.6 \cdot A_{653}$$

(Németh 1998)

$$\text{Car}\left(\text{mgL}^{-1}\right) = (1{,}000 \cdot A_{470} - 2.86 \cdot (15.65 \cdot A_{666} - 7.34 \cdot A_{653}) - 129.2 \cdot (27.05 \cdot A_{653} - 11.21 \cdot A_{666}))/221$$

(Wellburn 1994).

Figure C.9. Equations used to calculate chlorophyll a, b, total chlorophyll, and total carotenoid concentration.

HPLC analysis:

The pigment extracts were then qualitatively and quantitatively analyzed through the use of a HPLC. Approximately 500ul of methanol extract from the previous step was evaporated using compressed air, and the remaining residue was resuspended in 500uL of acetone. The pigments in acetone were then separated in a 250mm X 4mm C18 (5um) column. Eluents used were: water/ion pair reagent/methanol (1:1:18), and acetone/ methanol (1:1). The ion pair reagent was a solution of tetrabutylammonium (0.05M) and

ammonium acetate (1M) in water. The flow rate was 1 ml/min and the detection wavelengths were monitored at 470nm.

Saponification:

In order to quantify astaxanthin, astaxanthin esters were saponified to the free form molecule. This was performed by evaporating the acetone extract under nitrogen and redissolving in 1 ml of pure ethyl ether. One milliliter of 2% weight by volume KOH in methanol was added. The mixture was vortexed occasionally while being allowed to react in darkness at 0 degrees C for 15 minutes under nitrogen gas. In order to stop the reaction and remove excess alkalinity, 2ml of 10% nacl was added to the mixture and vortexed for 20 seconds. Phases were separated through centrifugation and the aqueous partition was removed and discarded. The ether phase was washed twice with 2ml of 10% NaCl and then evaporated under nitrogen gas. The residue remaining was redissolved in 1ml of acetone and centrifuged to discard particulate matter. Pigments were then analyzed using the previously mentioned HPLC method.

Enzymatic hydrolysis of carotenoid esters:

Alternatively, in order to verify that saponification did not oxidize astaxanthin and introduce artifacts, enzymatic hydrolysis was also explored. Cholesterol esterase was dissolved in 50mM tris HCL (pH 7.0) in order to make a solution having a final concentration of 4 units per ml.

In a glass test tube, 3ml of algal pigment in acetone was added to 1ml of internal standard and mixed. The glass test tube was then set in a block heater held at 37 degrees C, and 3ml of cholesterol esterase was added. The contents were mixed gently by

inversion. After 45 minutes, the tube was removed and 1 g of sodium sulfate decahydrate and 2ml of ethyl ether (petroleum ether) was added. The contents were mixed by vortexing for 30 seconds and then centrifuged at 3000rpm for 30 seconds. The ether layer was transferred to a new test tube containing 1 gram of sodium sulfate anhydrate. The ether was then evaporated and 3ml of acetone was added to the residue. The solution was then subjected to a 2 um filter and analyzed using the previously mentioned hplc method.

Internal standard:

The internal standard for the HPLC method was trans-beta-apo-8-carotenal

Other standards considered:

- Astaxanthin
- Lutein
- Beta-carotene
- Canthaxanthin
- Fucoxanthin
- Zeaxanthin
- Echinone
- Phaeophytin a and b
- Chlorophyllide a
- Chlorophyll a and b
- Phaeophorbide a and c

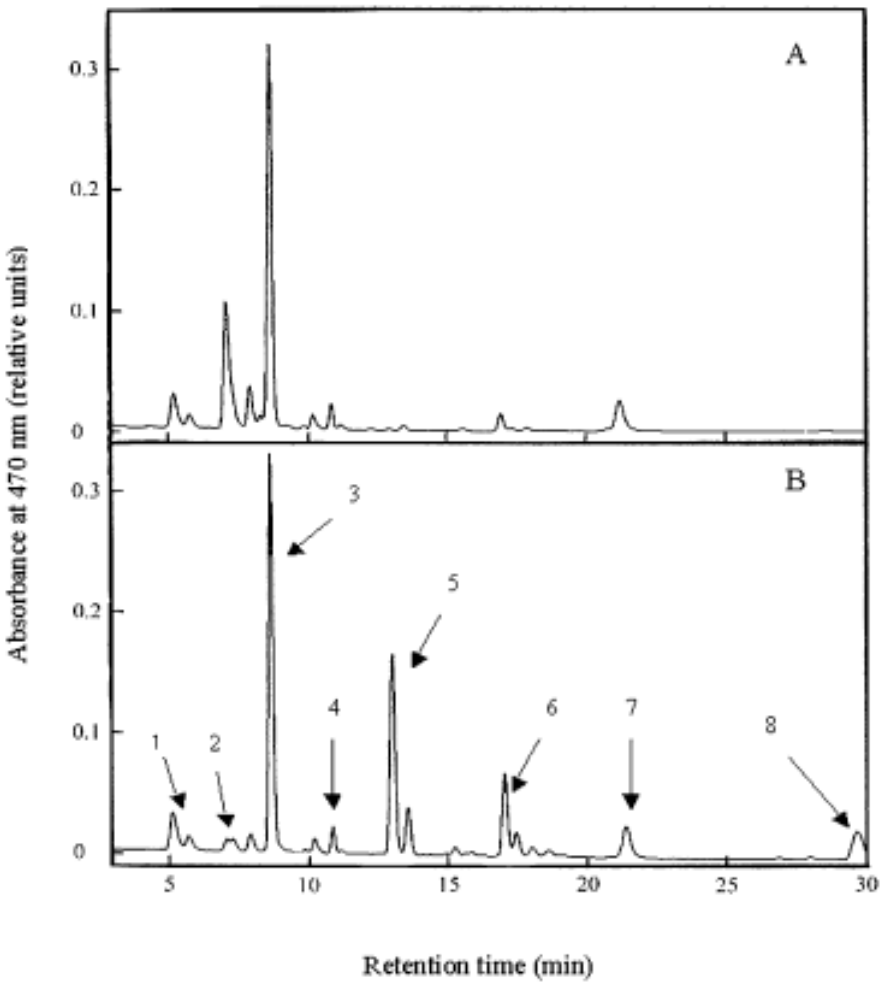


Fig. 2A, B HPLC chromatograms of pigments extracted from *C. zofingiensis* cells after 10 days growth in batch culture. **A** After saponification, **B** before saponification. *Peak 1* Violaxanthin, *peak 2* astaxanthin (free form), *peak 3* lutein, *peak 4* cantaxanthin, *peak 5* chlorophyll b, *peak 6* astaxanthin (mono)esters, *peak 7* β-carotene, *peak 8* astaxanthin (di)esters

Figure C.10. Above are examples of expected chromatograms. Source: Del Campo (2003)

Table C.6. Showing carotenoid standards available through Sigma-Aldrich and their associated cost per mass.

Description	1. mass (mg)	2. cost ($)
trans-β-Apo-8′-carotenal ≥96.0% (UV)	1000	104
Canthaxanthin (trans) analytical standard	10	75.3
β-Carotene Type II, synthetic, ≥95% (HPLC),	5	28
β-Cryptoxanthin ≥97% (TLC)	1	372
Fucoxanthin carotenoid antioxidant	10	119.5
Xanthophyll from marigold (lutein)	1	137
Zeaxanthin analytical standard	1	423.5
sum		1259.3

Isolate GK3L scaled up

Isolate GK3L was grown in AM6 media and in AM6 media supplemented with inorganic carbon in excess of 7mM. The inorganic carbon supplemented treatment grew better than the control, but most of the data points do not show deviation between the treatments. Growth of both treatments were stunted early on in the study. The control showed inhibition between day 2 and day 9 while the inorganic carbon supplemented case only showed inhibition between day 2 and day 6. After the inhibition, both treatments assumed a typical growth curve and plateaued at a high cell concentration near 2e8 cells/mL. The pH in solution increased substantially after day 9 for both treatments. The carbon supplemented treatment jumped from 9.6 to over 12 in a period of 5 days while the control made a similar jump from 8.5 to 11.7 in the same time, eventually reaching a pH of 12 near day 22. DIC measurements tell a similar story, in that, as pH is increasing, DIC is always decreasing. During the large jump in pH after day 9, DIC in

the control treatment depleted to near zero, truly representing an inorganic carbon limited culture. As photosynthetic activity of the culture decreased, DIC concentrations eventually increased to near their starting values at the beginning of the study. The inorganic carbon supplemented treatment was spiked twice with a 1M sodium bicarbonate filter sterilized solution in order to prevent DIC from running out. For both conditions tested, nitrate was depleted by day 19. Photosynthetic pigments (chlorophyll a, b, total chlorophyll, and total carotenoids were all upregulated in the inorganic carbon supplemented treatments until stationary state when the control matched. Chlorophyll concentration in both cases began decreasing after pH reached 12 at day 14, adding evidence that pH above 12 stunts photosynthetic activity in this isolate. Nitrate ran out at day 19 for both treatments as seen in, and an increase in nile red fluorescence was recorded up until harvesting the cells at day 33. Sulfate did not deplete in the study for both treatments, and phosphate did not appreciably decrease in either of the treatments.

Isolate GK3L accumulated more TAG on a weight per weight basis when supplemented with inorganic carbon compared to the carbon limited case, leading to higher total neutral potential and total FAME potential as well. Taking into account final cell dry weight, the inorganic supplemented condition was much more productive than the carbon limited condition on a g/L basis as well regarding both extractable lipid content and biodiesel content.

Table C.7. Absorbance (750nm) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0.0	0.0030	0.0030	0.0030	0.0000
1.0	0.0060	0.0060	0.0060	0.0000
2.0	0.0140	0.0130	0.0135	0.0015
3.0	0.0210	0.0190	0.0200	0.0015
4.0	0.0260	0.0190	0.0225	0.0047
5.0	0.0280	0.0210	0.0245	0.0051
6.0	0.0370	0.0230	0.0300	0.0071
7.0	0.0490	0.0290	0.0390	0.0153
8.0	0.0860	0.0470	0.0665	0.0341
9.0	0.1100	0.0500	0.0800	0.0454
10.0	0.2000	0.1000	0.1500	0.0799
11.0	0.2800	0.1800	0.2300	0.1258
12.0	0.4100	0.2600	0.3350	0.1061
13.0	0.4600	0.3400	0.4000	0.0849
14.0	0.6000	0.4600	0.5300	0.0990
15.0	0.7200	0.5200	0.6200	0.1414
16.0	0.9800	0.7400	0.8600	0.1697
18.0	1.2600	0.9800	1.1200	0.1980
19.0	1.2600	0.9600	1.1100	0.2121
19.2	1.4800	1.1400	1.3100	0.2404
19.4	1.3200	1.0200	1.1700	0.2121
19.7	1.3800	1.0200	1.2000	0.2546
20.0	1.5200	1.2200	1.3700	0.2121
22.0	1.7400	1.4000	1.5700	0.6519
23.0	1.8400	1.4800	1.6600	0.2546
25.0	2.2600	1.8200	2.0400	0.3111
27.0	2.3800	1.5800	1.9800	0.5657
29.0	2.2000	2.7200	2.4600	0.5707

Table C.8. Absorbance (750nm) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard
0	0.005	0.006	0.002	0.004	0.002
1	0.007	0.006	0.007	0.007	0.001
2	0.014	0.014	0.014	0.014	0.000
3	0.021	0.017	0.021	0.020	0.002
4	0.029	0.026	0.032	0.029	0.003
5	0.042	0.026	0.050	0.039	0.012
6	0.067	0.041	0.079	0.062	0.019
7	0.096	0.058	0.133	0.096	0.038
8	0.120	0.070	0.190	0.127	0.060
9	0.180	0.120	0.270	0.190	0.075
10	0.270	0.200	0.400	0.290	0.101
11	0.440	0.350	0.520	0.437	0.085
12	0.540	0.480	0.640	0.553	0.081
13	0.660	0.680	0.840	0.727	0.099
14	0.880	0.900	1.020	0.933	0.076
15	1.020	1.140	1.280	1.147	0.130
16	1.240	1.380	1.420	1.347	0.095
18	1.540	1.680	1.680	1.633	0.081
19	1.500	1.620	1.680	1.600	0.092
19.2	1.760	1.880	1.820	1.820	0.060
19.4	1.520	1.800	1.780	1.700	0.156
19.7	1.560	1.880	1.940	1.793	0.204
20	1.700	1.880	1.920	1.833	0.117
22	1.880	2.040	1.980	1.967	0.081
23	1.960	1.960	2.160	2.027	0.115
25	2.460	2.880	2.600	2.647	0.214
27	2.480	2.840	2.680	2.667	0.180
29	2.720	2.980	2.700	2.800	0.156

Table C.9. Cell concentration (cells/mL) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	1.3E+05	5.8E+04	9.4E+04	5.1E+04
1	4.3E+05	3.2E+05	3.8E+05	8.1E+04
2	4.5E+05	4.8E+05	4.6E+05	2.1E+04
3	5.6E+05	4.3E+05	4.9E+05	9.4E+04
4	7.1E+05	3.7E+05	5.4E+05	2.4E+05
5	7.0E+05	4.5E+05	5.8E+05	1.8E+05
6	8.5E+05	6.1E+05	7.3E+05	1.7E+05
7	1.3E+06	8.1E+05	1.0E+06	3.3E+05
8	1.0E+06	1.1E+06	1.1E+06	8.5E+04
9	6.4E+06	4.3E+06	5.4E+06	1.5E+06
10	1.4E+07	4.0E+06	8.8E+06	6.7E+06
11	1.7E+07	1.3E+07	1.5E+07	2.3E+06
12	1.8E+07	1.5E+07	1.6E+07	2.3E+06
13	2.7E+07	3.2E+07	3.0E+07	3.4E+06
14	4.2E+07	4.3E+07	4.3E+07	5.7E+05
15	5.1E+07	3.2E+07	4.2E+07	1.4E+07
16	7.5E+07	4.6E+07	6.1E+07	2.0E+07
18	9.4E+07	6.5E+07	8.0E+07	2.1E+07
19	9.3E+07	7.3E+07	8.3E+07	1.4E+07
22	1.3E+08	1.1E+08	1.2E+08	1.5E+07
23	1.5E+08	1.3E+08	1.4E+08	1.0E+07
25	1.2E+08	1.2E+08	1.2E+08	2.8E+06
27	2.1E+08	1.3E+08	1.7E+08	5.7E+07
29	2.0E+08	2.0E+08	2.0E+08	5.7E+06

Table C.10. Cell concentration (cells/mL) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	1.2E+0	7.3E+0	1.1E+0	9.8E+04	2.3E+04
1	4.4E+0	3.2E+0	4.3E+0	3.9E+05	6.7E+04
2	4.4E+0	4.2E+0	4.4E+0	4.3E+05	1.0E+04
3	6.7E+0	5.0E+0	5.4E+0	5.7E+05	8.9E+04
4	8.8E+0	5.3E+0	1.0E+0	8.0E+05	2.4E+05
5	4.9E+0	6.1E+0	1.1E+0	7.2E+05	3.1E+05
6	1.7E+0	8.6E+0	1.9E+0	1.5E+06	5.4E+05
7	1.9E+0	1.6E+0	2.8E+0	2.1E+06	5.9E+05
8	3.7E+0	2.9E+0	5.6E+0	4.1E+06	1.4E+06
9	5.5E+0	4.5E+0	1.0E+0	6.7E+06	3.0E+06
10	1.1E+0	9.1E+0	1.8E+0	1.3E+07	4.9E+06
11	2.0E+0	2.0E+0	2.7E+0	2.2E+07	4.1E+06
12	3.0E+0	2.3E+0	3.3E+0	2.9E+07	5.1E+06
13	3.8E+0	3.6E+0	5.7E+0	4.3E+07	1.2E+07
14	4.8E+0	5.1E+0	5.8E+0	5.3E+07	5.3E+06
15	6.1E+0	5.8E+0	8.5E+0	6.8E+07	1.5E+07
16	7.5E+0	9.5E+0	8.7E+0	8.6E+07	1.0E+07
18	8.4E+0	1.0E+0	1.1E+0	9.9E+07	1.3E+07
19	1.0E+0	9.3E+0	1.2E+0	1.1E+08	1.6E+07
22	1.4E+0	1.1E+0	1.6E+0	1.4E+08	2.9E+07
23	1.4E+0	1.4E+0	1.4E+0	1.4E+08	2.4E+06
25	1.0E+0	1.0E+0	1.0E+0	1.0E+08	2.3E+06
27	1.8E+0	2.0E+0	1.8E+0	1.8E+08	1.2E+07
29	2.4E+0	2.8E+0	2.1E+0	2.5E+08	3.6E+07

Table C.11. Nile Red fluorescence (a.u.) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0.0	6	3	4	2
1.0	10	17	9	5
2.0	0	-4	-2	3
3.0	23	19	16	3
4.0	161	141	126	14
5.0	107	119	95	8
6.0	373	356	295	12
7.0	215	162	157	37
8.0	318	242	239	54
9.0	310	130	211	127
10.0	180	280	222	71
11.0	130	140	197	7
12.0	120	260	190	99
13.0	120	340	230	156
14.0	260	320	290	42
15.0	280	440	360	113
16.0	300	540	420	170
18.0	1400	1500	1450	71
19.0	740	560	650	127
19.2	400	360	380	28
19.4	460	60	260	283
19.7	420	360	390	42
20.0	440	500	470	42
22.0	700	560	660	99
23.0	300	960	630	467
25.0	2320	540	1430	1259
27.0	760	400	580	255
29.0	1280	220	700	750

Table C.12. Nile Red fluorescence (a.u) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0.0	11	4	15	10	6
1.0	4	14	23	14	10
2.0	20	17	20	19	2
3.0	61	25	48	45	18
4.0	66	98	72	79	17
5.0	164	101	185	150	44
6.0	378	300	336	338	39
7.0	330	272	417	340	73
8.0	200	370	410	327	112
9.0	430	320	400	383	57
10.0	440	290	400	377	78
11.0	340	440	470	417	68
12.0	360	560	800	573	220
13.0	740	800	620	720	92
14.0	440	1360	900	900	460
15.0	1300	600	2520	1473	972
16.0	560	740	1000	767	221
18.0	4240	5140	4200	4527	532
19.0	1180	2240	2040	1820	563
19.2	1020	1100	820	980	144
19.4	760	780	1320	953	318
19.7	1140	1600	1260	1333	239
20.0	640	1520	420	860	582
22.0	840	3980	820	1880	1819
23.0	640	2460	980	1360	968
25.0	860	1580	1160	1200	362
27.0	1760	920	900	1193	491
29.0	1220	8140	1740	3700	3854

Table C.13. pH for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	8.59	8.65	8.61	0.04
1	8.35	8.54	8.41	0.13
2	8.56	8.62	8.58	0.04
3	8.62	8.59	8.61	0.02
4	8.54	8.55	8.54	0.01
5	8.61	8.59	8.57	0.01
6	8.46	8.44	8.45	0.01
7	8.78	8.63	8.62	0.11
8	8.79	8.47	8.58	0.23
9	9.92	8.9	9.22	0.72
10	11.47	10.06	10.10	1.00
11	11.33	10.89	10.25	0.31
12	11.7	11.58	11.64	0.08
13	11.54	11.53	11.54	0.01
14	11.84	11.75	11.80	0.06
15	11.79	11.7	11.75	0.06
16	11.82	11.82	11.82	0.00
18	11.78	11.83	11.81	0.04
19	11.62	11.68	11.65	0.04
19.1	11.4	11.5	11.45	0.07
19.4	10.48	10.72	10.60	0.17
19.6	11.57	11.9	11.74	0.23
20	12.01	12.1	12.06	0.06
22	11.39	11.48	11.44	0.06
23	11.35	11.41	11.38	0.04
25	11.19	11.32	11.26	0.09
27	10.92	11.34	11.13	0.30
29	11.15	11.25	11.20	0.07

Table C.14. pH for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	9.53	9.51	9.49	9.51	0.02
1	9.2	9.21	9.19	9.20	0.01
2	9.26	9.23	9.23	9.24	0.02
3	9.21	9.25	9.18	9.21	0.04
4	9.34	9.16	9.22	9.24	0.09
5	9.42	9.26	9.52	9.40	0.13
6	9.33	9.2	9.4	9.31	0.10
7	9.64	9.42	9.86	9.64	0.22
8	9.74	9.45	10	9.73	0.28
9	10.19	9.82	10.65	10.22	0.42
10	10.98	10.48	11.58	11.01	0.55
11	11.56	11.23	11.74	11.51	0.26
12	11.94	11.89	12.06	11.96	0.09
13	11.95	11.89	11.92	11.92	0.03
14	12.11	12.06	12.03	12.07	0.04
15	12.1	12.02	11.99	12.04	0.06
16	12.08	11.87	11.99	11.98	0.11
18	12.14	12.12	12.01	12.09	0.07
19	11.99	11.97	11.84	11.93	0.08
19.1	11.8	11.75	11.54	11.70	0.14
19.4	11.26	11.22	10.91	11.13	0.19
19.6	11.81	11.81	11.51	11.71	0.173205
20	12.06	12.05	11.8	11.97	0.147309
22	11.3	11.4	11.19	11.29667	0.10504
23	11.2	11.34	11.1	11.21333	0.120554
25	10.95	11.11	10.81	10.95667	0.150111
27	10.62	10.75	10.47	10.61333	0.140119
29	10.61	10.88	10.46	10.65	0.212838

Table C.15. DIC (mM) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0.0	2.8	2.8	2.8	0.0
1.0	1.9	2.0	2.0	0.1
2.0	2.6	2.7	2.6	0.1
3.0	2.7	3.1	3.0	0.3
4.0	2.7	2.6	2.6	0.1
5.0	1.9	2.1	2.0	0.1
6.0	2.0	2.1	2.1	0.0
7.0	2.9	3.0	3.0	0.0
8.0	2.2	2.4	2.4	0.1
9.0	2.0	3.4	2.8	1.0
10.0	0.0	1.9	1.6	1.3
11.0	0.6	1.1	1.7	0.4
12.0	0.2	0.2	0.2	0.0
13.0	0.3	0.3	0.2	0.0
14.0	0.1	0.1	0.1	0.0
15.0	0.5	0.4	0.5	0.0
16.0	0.4	0.2	0.3	0.1
18.0	0.6	0.4	0.5	0.2
19.0	0.3	0.2	0.3	0.1
19.2	2.2	2.0	2.1	0.1
19.4	3.9	3.8	3.9	0.1
19.7	1.4	1.3	1.4	0.1
20.0	1.1	1.0	1.1	0.1
22.0	1.8	1.5	1.6	0.2
23.0	1.6	1.3	1.5	0.2
25.0	1.4	0.9	1.1	0.4
29.0	2.5	2.2	2.0	0.3

Table C.16. DIC (mM) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0.0	7.2	6.9	7.2	7.1	0.2
1.0	7.0	7.0	6.8	7.0	0.1
2.0	8.5	7.8	7.9	8.1	0.4
3.0	9.1	9.0	8.2	8.8	0.5
4.0	8.3	8.9	8.8	8.7	0.3
5.0	5.3	5.5	5.4	5.4	0.1
6.0	6.5	7.5	5.8	6.6	0.9
7.0	8.4	8.8	8.9	8.7	0.3
8.0	6.8	7.4	5.6	6.6	0.9
9.0	7.2	8.6	6.3	7.4	1.2
10.0	5.2	6.9	3.9	5.3	1.5
11.0	3.8	5.0	3.2	4.0	0.9
12.0	2.0	2.7	2.3	2.4	0.3
12.1	4.3	5.0	4.6	4.7	0.3
13.0	2.7	3.8	4.1	3.5	0.7
13.1	6.7	7.3	7.6	7.2	0.4
14.0	5.0	5.9	6.1	5.6	0.6
15.0	5.0	6.3	6.6	6.0	0.8
16.0	5.6	7.2	7.3	6.7	0.9
18.0	6.4	6.8	8.1	7.1	0.9
19.0	6.4	7.1	8.3	7.2	1.0
19.2	8.4	10.1	10.4	9.6	1.1
19.4	10.4	11.2	12.2	11.2	0.9
19.7	9.1	9.8	11.1	10.0	1.0
20.0	9.2	9.6	10.8	9.9	0.8
22.0	10.0	9.5	10.8	10.1	0.7
23.0	10.1	9.9	11.2	10.4	0.7
25.0	10.7	10.1	11.4	10.7	0.7
29.0	10.4	10.4	11.8	10.9	0.8

Table C.17. Chlorophyll a (mg/L) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	0.08	0.08	0.08	0.00
4	0.11	0.10	0.11	0.01
7	0.24	0.27	0.25	0.02
12	5.96	3.61	4.78	1.66
15	11.17	9.35	10.26	1.29
19	12.01	12.23	12.12	0.15
23	13.75	13.28	13.52	0.33
33	12.44	13.33	12.88	0.63

Table C.18. Chlorophyll a (mg/L) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	0.12	0.08	0.07	0.09	0.03
4	0.13	0.08	0.14	0.12	0.03
7	0.48	0.32	0.76	0.52	0.22
12	6.62	6.51	8.56	7.23	1.15
15	11.93	14.89	15.65	14.16	1.97
19	11.93	15.19	14.68	13.93	1.75
23	12.78	14.05	14.34	13.72	0.83
33	11.30	13.33	12.18	12.27	1.02

Table C.19. Chlorophyll b (mg/L) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	0.06	0.06	0.06	0.00
4	0.08	0.06	0.07	0.01
7	0.10	0.27	0.18	0.12
12	0.67	0.46	0.57	0.15
15	1.38	1.07	1.22	0.22
19	1.65	1.34	1.49	0.22
23	1.53	1.55	1.54	0.02
33	1.28	1.39	1.34	0.08

Table C.20. Chlorophyll b (mg/L) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	0.15	0.06	0.04	0.08	0.06
4	0.07	0.08	0.10	0.08	0.02
7	0.11	0.07	0.14	0.11	0.04
12	0.80	0.89	0.97	0.89	0.09
15	1.47	1.92	2.01	1.80	0.29
19	1.47	1.79	1.98	1.75	0.26
23	1.49	1.40	1.52	1.47	0.06
33	1.14	1.39	1.50	1.35	0.18

Table C.21. Total chlorophyll (mg/L) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	0.14	0.14	0.14	0.00
4	0.19	0.16	0.18	0.02
7	0.33	0.54	0.44	0.14
12	6.68	4.11	5.39	1.82
15	12.65	10.50	11.57	1.52
19	13.77	13.68	13.72	0.07
23	15.40	14.96	15.18	0.31
33	13.83	14.84	14.34	0.71

Table C.22. Total chlorophyll (mg/L) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	0.27	0.14	0.11	0.17	0.08
4	0.19	0.16	0.25	0.20	0.04
7	0.60	0.40	0.91	0.64	0.26
12	7.47	7.46	9.61	8.18	1.24
15	13.51	16.94	17.81	16.09	2.27
19	13.51	17.11	16.79	15.80	1.99
23	14.38	15.57	15.99	15.31	0.83
33	12.54	14.84	13.80	13.73	1.15

Table C.23. Total carotenoids (mg/L) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1a	1b	1c	Average	Standard Deviation
0	0.00	0.00	0.00	0.00	0.00
4	0.01	0.01	0.01	0.01	0.00
7	0.01	0.04	-0.01	0.02	0.04
12	0.00	1.33	0.76	1.04	0.40
15	0.00	2.32	1.98	2.15	0.23
19	0.00	2.83	2.79	2.81	0.03
23	0.00	3.97	3.64	3.81	0.23
33	3.82	4.69	4.76	4.73	0.05

Table C.24. Total carotenoids (mg/L) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	-0.02	0.00	0.00	-0.01	0.01
4	0.02	0.01	0.01	0.01	0.01
7	0.11	0.08	0.18	0.12	0.05
12	1.34	1.24	1.70	1.43	0.24
15	2.43	2.85	3.08	2.78	0.33
19	2.86	3.64	3.62	3.38	0.45
23	3.64	4.02	4.26	3.97	0.31
33	4.21	4.94	4.50	4.55	0.37

Table C.25. Nitrate concentration (mg/L) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	248	263	255	8
4	266	282	278	11
7	291	308	282	32
12	87	143	115	40
15	33	62	47	21
19	0	0	0	0
23	0	0	0	0

Table C.26. Nitrate concentration (mg/L) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	279	311	303	298	16
4	285	287	308	293	13
7	264	313	272	283	26
12	118	180	147	148	31
15	35	22	0	19	18
19	0	0	0	0	0
23	0	0	0	0	0

Table C.27. Phosphate concentration (mg/L) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	117	122	119	3
4	124	133	132	8
7	145	147	137	17
12	95	100	97	4
15	108	120	114	9
19	106	118	112	9
23	114	115	114	0

Table C.28. Phosphate concentration (mg/L) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	128	140	136	135	6
4	137	133	146	139	7
7	140	161	149	150	11
12	104	156	172	144	36
15	106	124	104	111	11
19	110	126	126	120	9
23	111	121	111	114	6

Table C.29. Sulfate concentration (mg/L) for isolate GK3L grown on AM6 medium in tube reactors.

Time (days)	1b	1c	Average	Standard Deviation
0	32	33	32	1
4	32	34	33	2
7	35	37	34	4
12	20	23	22	2
15	18	23	20	3
19	13	16	14	2
23	7	9	8	2

Table C.30. Sulfate concentration (mg/L) for isolate GK3L grown on AM6 medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	34	37	36	36	2
4	35	34	37	35	1
7	33	38	35	35	3
12	21	33	33	29	7
15	19	19	18	19	0
19	10	12	10	10	1
23	4	5	0	3	3

Table C.31. End point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK3L grown under 2 different treatments in tube reactors. Units are shown in (%).

Treatment		FA	MAG	DAG	TAG	Total Neutral Lipid	Total FAME
AM6	Tube 2	2.07	0.37	0.96	3.06	6.47	34.36
	Tube 3	2.55	0.37	1.09	3.49	7.51	29.08
AM6 + HCO3- Supplemented	Tube 1	3.37	0.53	1.96	11.01	16.87	41.94
	Tube 2	3.41	0.55	1.84	8.72	14.51	41.02
	Tube 3	3.08	0.46	1.92	10.10	15.56	46.65

Table C.32. Average and standard deviation of end point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK3L grown under 2 different treatments in tube reactors. Units are shown in (%).

	Treatment	FA	MAG	DAG	TAG	Total Neutral Lipid Potential	Total FAME Potential
Average	AM6	2.41	0.37	0.88	2.42	6.08	31.72
	AM6 + HCO3- Supplemented	3.28	0.51	1.91	9.94	15.65	43.20
Standard Deviation	AM6	0.30	0.00	0.27	1.50	1.65	3.73
	AM6 +HCO3-	0.18	0.05	0.06	1.15	1.18	3.02

Table C.33. Endpoint analysis representing productivity in each treatment. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

Treatment		Cell Dry Weight Conc. (mg/mL)	Total Lipid Conc. (mg/mL)	Total FAME Conc. (mg/mL)
AM6	Tube 2	2.29	0.15	0.79
	Tube 3	2.03	0.15	0.59
AM6 + HCO3- Supplemented	Tube 1	2.28	0.38	0.96
	Tube 2	2.25	0.33	0.92
	Tube 3	2.55	0.40	1.19

Table C.34. Average and standard deviation of endpoint analysis representing productivity in each treatment. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

	Cell Dry Weight Conc. (mg/mL)	Total Lipid Conc. (mg/mL)	Total FAME Conc. (mg/mL)
Average	1.94	0.12	0.55
	2.36	0.37	1.02
Standard Deviation	0.41	0.05	0.26
	0.17	0.04	0.15

Table C.35. Endpoint FAME speciation of isolate GK3L grown in AM6 medium.

% of FAME	1a	1b	1c	Average	Standard Deviation
C12:0	0.00	0.00	0.00	0.00	0.00
C14:0	0.33	0.34	0.34	0.34	0.01
C16:3	3.58	2.00	2.13	2.57	0.88
C16:2	2.34	1.45	1.51	1.77	0.50
C16:1	1.28	1.10	1.16	1.18	0.09
C16:0	20.21	21.50	20.55	20.75	0.67
C18:1-3	60.85	65.82	66.03	64.23	2.93
C18:0	2.25	1.78	1.46	1.83	0.40
C20:5	6.77	4.77	5.30	5.61	1.04
C20:1	1.18	0.67	0.91	0.92	0.26
C20:0	0.00	0.00	0.00	0.00	0.00
C22:1	0.00	0.00	0.00	0.00	0.00
C22:0	0.38	0.17	0.18	0.25	0.12
C24:1	0.00	0.00	0.00	0.00	0.00
C24:0	0.65	0.40	0.43	0.50	0.14
C26:0	0.00	0.00	0.00	0.00	0.00
Other	0.17	0.00	0.00	0.06	0.10
Total	100.00	100.00	100.00	100.00	0.00

Table C.36. Endpoint FAME speciation of isolate GK3L grown in AM6 medium supplemented with sodium bicarbonate.

% of FAME	2a	2b	2c	Average	Standard Deviation
C12:0	0.00	0.00	0.00	0.00	0.00
C14:0	0.13	0.29	0.26	0.23	0.09
C16:3	2.69	1.24	0.80	1.58	0.99
C16:2	3.83	1.34	1.12	2.10	1.50
C16:1	7.23	1.12	1.12	3.15	3.53
C16:0	18.33	20.90	21.62	20.28	1.73
C18:1-3	64.74	68.18	68.96	67.29	2.24
C18:0	1.27	1.51	1.38	1.39	0.12
C20:5	0.55	4.35	3.76	2.89	2.05
C20:1	0.29	0.62	0.51	0.47	0.17
C20:0	0.13	0.00	0.00	0.04	0.08
C22:1	0.00	0.00	0.00	0.00	0.00
C22:0	0.13	0.14	0.16	0.15	0.01
C24:1	0.00	0.00	0.00	0.00	0.00
C24:0	0.22	0.32	0.31	0.28	0.05
C26:0	0.31	0.00	0.00	0.10	0.18
Other	0.00	0.00	0.00	0.00	0.00
Total	99.85	100.00	100.00	99.95	0.08

Isolate GK2Lg

Isolate GK2Lg was grown in AM6SIS (control) and with the supplementation of additional sodium bicarbonate in the range of 7 to 10mM. The condition supplemented with inorganic carbon trended higher than the control throughout the growth curve, though specific growth rate between both conditions were similar from day 3 to day 12. This could be a result of more inorganic carbon substrate in the media, or more sodium ions in the media creating an environment the saline organism is more adapted to. Within the first 2 or 3 days the inorganic supplemented treatment outpaced the growth rate of the control allowing it to trend higher throughout the study. The observed pattern in growth

was further validated in trends found in recorded pH measurements over time. The pH in the inorganic carbon supplemented treatment trended higher throughout the experiment until day 13 when the control recorded the highest pH value of the study near 11. For both cases, pH did not increase until day 6 and the controls pH actually decreased from day 0 to day 6, a counterintuitive result since measurable growth was indicated through cell counts. DIC concentration followed very similar trends between both treatments, in that, as DIC increased in the control it also increased in the carbon-supplemented case and vice versa with decreases. One distinguishing feature between the treatments is that isolate GK2Lg used more DIC in the inorganic carbon supplemented treatment. This is established by the decreases in DIC occurring between day 4 and day 5, along with greater increases in DIC to alleviate a higher charge imbalance between day 6 and day 7. Sulfate became limiting first in the inorganic carbon-supplemented treatment at day 19 and then in the control between day 20 and 25. Nitrate was not depleted in the study.

On a weight per weight basis, carbon supplemented and carbon limited treatments of isolate GK2Lg contained similar amounts of neutral lipid stores and total FAME content, however, cell dry weight was higher in the carbon supplemented condition on day 12. Higher cell dry weight lead to both higher neutral lipid content and FAME content on a g/L basis indicating excess inorganic carbon leads to higher biofuel productivity when considering both nonpolar and polar lipid stores.

Table C.37. Absorbance (750nm) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	0.006	0.005	0.004	0.005	0.001
1	0.009	0.008	0.006	0.008	0.002
2	0.014	0.011	0.008	0.011	0.003
3	0.014	0.011	0.007	0.011	0.004
4	0.018	0.016	0.014	0.016	0.002
5	0.014	0.016	0.011	0.014	0.003
6	0.020	0.018	0.013	0.017	0.004
7	0.030	0.026	0.022	0.026	0.004
8	0.044	0.036	0.026	0.035	0.009
9	0.055	0.050	0.051	0.052	0.003
10	0.104	0.082	0.061	0.082	0.022
11	0.120	0.080	0.075	0.092	0.025
12	0.190	0.180	0.125	0.165	0.035
13	0.230	0.130	0.170	0.177	0.050
14	0.260	0.110	0.210	0.193	0.076
15	0.270	0.140	0.220	0.210	0.066
16	0.230	0.150	0.220	0.200	0.044
18	0.300	0.180	0.250	0.243	0.060
19	0.270	0.150	0.260	0.227	0.067
19.2	0.250	0.150	0.270	0.223	0.064
19.4	0.250	0.170	0.250	0.223	0.046
19.7	0.300	0.170	0.300	0.257	0.075
20	0.280	0.180	0.290	0.250	0.061
22	0.240	0.330	0.270	0.280	0.046
23	0.210	0.250	0.240	0.233	0.021
25	0.180	0.210	0.180	0.190	0.017

Table C.38. Absorbance (750nm) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	0.007	0.006	0.008	0.007	0.001
1	0.009	0.010	0.012	0.010	0.002
2	0.014	0.011	0.014	0.013	0.002
3	0.016	0.013	0.022	0.017	0.005
4	0.025	0.020	0.027	0.024	0.004
5	0.021	0.019	0.030	0.023	0.006
6	0.026	0.022	0.039	0.029	0.009
7	0.044	0.033	0.073	0.050	0.021
8	0.062	0.053	0.079	0.065	0.013
9	0.070	0.040	0.100	0.070	0.030
10	0.100	0.090	0.120	0.103	0.015
11	0.145	0.145	0.180	0.157	0.020
12	0.215	0.205	0.220	0.213	0.008
13	0.250	0.200	0.220	0.223	0.025
14	0.260	0.200	0.170	0.210	0.046
15	0.280	0.180	0.250	0.237	0.051
16	0.260	0.180	0.240	0.227	0.042
18	0.250	0.230	0.230	0.237	0.012
19	0.190	0.210	0.170	0.190	0.020
19.2	0.200	0.220	0.210	0.210	0.010
19.4	0.250	0.260	0.190	0.233	0.038
19.7	0.220	0.270	0.170	0.220	0.050
20	0.200	0.180	0.220	0.200	0.020
22	0.200	0.200	0.180	0.193	0.012
23	0.170	0.150	0.140	0.153	0.015
25	0.140	0.130	0.120	0.130	0.010

Table C.39. Cell concentration (cells/mL) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	5.5E+0	3.8E+0	3.0E+0	4.1E+04	1.3E+04
1	6.3E+0	4.0E+0	5.3E+0	5.2E+04	1.1E+04
2	5.5E+0	5.0E+0	4.3E+0	4.9E+04	6.3E+03
3	8.0E+0	6.5E+0	4.5E+0	6.3E+04	1.8E+04
4	1.6E+0	1.3E+0	1.1E+0	1.3E+05	2.4E+04
5	2.2E+0	1.4E+0	1.0E+0	1.5E+05	5.7E+04
6	2.5E+0	1.9E+0	1.9E+0	2.1E+05	3.5E+04
7	2.5E+0	3.7E+0	2.3E+0	2.8E+05	7.5E+04
8	6.4E+0	4.0E+0	3.4E+0	4.6E+05	1.6E+05
9	9.2E+0	6.1E+0	6.7E+0	7.3E+05	1.6E+05
10	1.3E+0	1.3E+0	8.6E+0	1.1E+06	2.5E+05
11	1.9E+0	1.5E+0	1.5E+0	1.6E+06	2.5E+05
12	3.1E+0	2.2E+0	1.9E+0	2.4E+06	6.3E+05
13	4.0E+0	3.5E+0	2.7E+0	3.4E+06	6.6E+05
14	6.9E+0	3.2E+0	3.5E+0	4.5E+06	2.1E+06
15	6.7E+0	2.7E+0	4.1E+0	4.5E+06	2.0E+06
16	3.4E+0	4.4E+0	6.0E+0	4.6E+06	1.3E+06
18	4.9E+0	4.7E+0	5.5E+0	5.0E+06	4.2E+05
19	5.4E+0	4.4E+0	7.0E+0	5.6E+06	1.3E+06
22	6.7E+0	7.2E+0	7.2E+0	7.0E+06	2.9E+05
23	8.2E+0	1.0E+0	5.2E+0	7.9E+06	2.5E+06
25	5.7E+0	5.0E+0	7.4E+0	6.0E+06	1.2E+06

Table C.40. Cell concentration (cells/mL) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	4.5E+0	3.8E+0	5.3E+0	4.5E+04	7.5E+03
1	8.0E+0	6.5E+0	1.2E+0	8.8E+04	2.7E+04
2	9.3E+0	5.5E+0	1.2E+0	8.9E+04	3.3E+04
3	1.3E+0	1.1E+0	1.9E+0	1.4E+05	4.1E+04
4	2.1E+0	1.6E+0	3.2E+0	2.3E+05	8.2E+04
5	2.6E+0	1.8E+0	3.3E+0	2.6E+05	7.4E+04
6	3.3E+0	2.8E+0	4.4E+0	3.5E+05	8.2E+04
7	6.3E+0	4.6E+0	8.6E+0	6.5E+05	2.0E+05
8	9.3E+0	6.7E+0	1.1E+0	9.1E+05	2.2E+05
9	1.1E+0	9.9E+0	1.7E+0	1.3E+06	4.0E+05
10	1.9E+0	1.7E+0	2.7E+0	2.1E+06	5.5E+05
11	2.9E+0	2.1E+0	3.3E+0	2.8E+06	5.9E+05
12	3.6E+0	2.6E+0	4.0E+0	3.4E+06	7.2E+05
13	4.8E+0	4.2E+0	5.1E+0	4.7E+06	4.6E+05
14	5.7E+0	3.4E+0	2.4E+0	3.8E+06	1.7E+06
15	5.2E+0	3.1E+0	5.6E+0	4.6E+06	1.3E+06
16	7.6E+0	4.3E+0	5.6E+0	5.8E+06	1.7E+06
18	5.1E+0	4.9E+0	5.7E+0	5.2E+06	4.2E+05
19	4.7E+0	5.6E+0	4.4E+0	4.9E+06	6.2E+05
22	5.6E+0	6.9E+0	6.3E+0	6.3E+06	6.5E+05
23	7.2E+0	3.3E+0	5.1E+0	5.2E+06	2.0E+06
25	5.5E+0	4.1E+0	5.8E+0	5.1E+06	9.1E+05

Table C.41. Nile Red fluorescence (a.u.) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0.0	133	160	92	128	34
1.0	159	161	111	144	28
2.0	199	167	134	167	33
3.0	279	251	150	227	68
4.0	337	331	271	313	36
5.0	488	381	262	377	113
6.0	439	500	380	440	60
7.0	814	777	683	758	68
8.0	1467	1313	913	1231	286
9.0	1340	1220	770	1110	300
10.0	3785	3124	2325	3078	731
11.0	5135	3845	3770	4250	767
12.0	5970	4545	4180	4898	946
13.0	6800	5600	7280	6560	865
14.0	7310	4680	7670	6553	1632
15.0	9200	6260	9560	8340	1810
16.0	5330	4500	6460	5430	984
18.0	10070	6590	6850	7837	1938
19.0	5010	4070	5010	4697	543
19.2	8770	5900	8150	7607	1510
19.4	7760	4150	6010	5973	1805
19.7	6230	4030	7110	5790	1586
20.0	7930	5220	10200	7783	2493
22.0	3660	6380	5220	5087	1365
23.0	2580	4910	4820	4103	1320
25.0	1870	3360	2550	2593	746

Table C.42. Nile Red fluorescence (a.u.) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0.0	174	134	202	170	34
1.0	206	168	275	216	54
2.0	272	208	375	285	84
3.0	276	274	445	332	98
4.0	598	364	762	575	200
5.0	588	501	850	646	182
6.0	807	670	1314	930	339
7.0	1411	1231	1314	1319	90
8.0	2261	1959	3388	2536	753
9.0	2120	2490	3390	2667	653
10.0	5170	3930	6330	5143	1200
11.0	6600	5810	6050	6153	405
12.0	6875	6690	7210	6925	264
13.0	9060	7770	5030	7287	2058
14.0	7900	7750	6330	7327	866
15.0	5330	6310	6680	6107	698
16.0	7980	5690	6810	6827	1145
18.0	8240	7340	4210	6597	2115
19.0	4430	5070	2430	3977	1377
19.2	5470	7390	3910	5590	1743
19.4	6160	6700	2900	5253	2056
19.7	4810	4810	3470	4363	774
20.0	4080	5010	2880	3990	1068
22.0	2160	3430	2070	2553	761
23.0	1960	2020	1730	1903	153
25.0	1170	1270	1460	1300	147

Table C.43. pH for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	9.1	9.22	9	9.11	0.11
1	8.7	8.66	8.64	8.67	0.03
2	8.8	8.75	8.73	8.76	0.04
3	8.59	8.7	8.72	8.67	0.07
4	8.5	8.6	8.59	8.56	0.06
5	8.7	8.86	8.76	8.77	0.08
6	8.63	8.72	8.6	8.65	0.06
7	8.82	9	8.81	8.88	0.11
8	8.96	9.16	8.82	8.98	0.17
9	9	9.49	8.95	9.15	0.30
10	9.48	9.9	9.35	9.58	0.29
11	9.81	10.46	9.29	9.85	0.59
12	10.35	10.78	9.86	10.33	0.46
13	11.04	10.77	10.66	10.82	0.20
14	11.31	10.77	11.21	11.10	0.29
15	10.88	10.45	11.1	10.81	0.33
16	10.62	10.54	10.85	10.67	0.16
18	10.54	10.83	10.66	10.68	0.15
19	9.59	10.48	10.68	10.25	0.58
19.16	9.1	10.05	10.08	9.74	0.56
19.41	8.86	9.5	9.76	9.37	0.46
19.66	9.14	9.98	10.03	9.72	0.50
20	9.3	10.47	10.21	9.99	0.61
22	9.08	9.94	8.96	9.33	0.53
23	9.15	8.9	8.92	8.99	0.14
25	9.12	9.02	8.93	9.02	0.10

Table C.44. pH for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	9.4	9.38	9.35	9.38	0.03
1	9.14	9.14	9.11	9.13	0.02
2	9.2	9.22	9.21	9.21	0.01
3	9.23	9.21	9.35	9.26	0.08
4	9.14	9.14	9.26	9.18	0.07
5	9.32	9.29	9.38	9.33	0.05
6	9.21	9.2	9.32	9.24	0.07
7	9.4	9.38	9.57	9.45	0.10
8	9.52	9.48	9.84	9.61	0.20
9	9.77	9.7	10.23	9.90	0.29
10	10.14	10.07	10.75	10.32	0.37
11	10.32	10.39	10.74	10.48	0.23
12	10.67	10.78	10.86	10.77	0.10
13	11.01	11.15	10.86	11.01	0.15
14	11.13	11.04	10.67	10.95	0.24
15	10.52	10.5	10.53	10.52	0.02
16	10.32	10.43	10.58	10.44	0.13
18	9.63	10.32	10.03	9.99	0.35
19	9.3	9.78	9.35	9.48	0.26
19.16	9.19	9.56	9.19	9.31	0.21
19.41	9.22	9.4	9.18	9.27	0.12
19.66	9.37	9.41	9.28	9.35	0.07
20	9.42	9.4	9.37	9.39	0.025
22	9.16	9.16	9.14	9.15	0.011
23	9.25	9.22	9.22	9.23	0.017
25	9.22	9.21	9.19	9.20	0.015

Table C.45. DIC (mM) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0.0	3.0	2.8	3.0	2.9	0.1
1.0	2.5	2.5	2.4	2.4	0.1
2.0	2.2	2.0	2.3	2.2	0.1
3.0	3.3	3.5	3.4	3.4	0.1
4.0	3.6	3.5	3.6	3.5	0.0
5.0	2.0	1.9	2.1	2.0	0.1
6.0	2.6	2.4	2.7	2.6	0.2
7.0	4.1	4.2	4.7	4.3	0.3
8.0	3.7	2.8	3.0	3.2	0.4
9.0	3.7	3.4	3.6	3.6	0.2
10.0	2.4	1.8	2.8	2.3	0.5
11.0	3.3	1.6	4.0	3.0	1.2
12.0	2.4	1.2	3.0	2.2	0.9
13.0	2.0	1.4	2.0	1.8	0.3
14.0	2.1	2.0	2.0	2.0	0.1
15.0	2.9	2.5	2.4	2.6	0.3
16.0	4.3	3.0	3.3	3.5	0.7
18.0	5.7	2.9	4.8	4.5	1.4
19.0	9.0	4.1	5.2	6.1	2.6
19.2	10.0	5.0	6.8	7.3	2.5
19.4	9.8	6.2	7.8	7.9	1.8
19.7	9.9	5.9	7.4	7.7	2.0
20.0	10.3	5.4	7.5	7.8	2.5
22.0	9.6	8.2	10.0	9.3	0.9
23.0	10.2	10.4	11.4	10.7	0.7
25.0	9.8	9.2	9.8	9.6	0.3

Table C.46. DIC (mM) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0.0	8.1	7.6	7.8	7.8	0.3
1.0	7.1	7.0	6.9	7.0	0.1
2.0	7.3	6.7	7.1	7.0	0.3
3.0	9.8	9.2	9.2	9.4	0.4
4.0	10.1	10.1	10.1	10.1	0.0
5.0	6.0	6.8	7.0	6.6	0.5
6.0	6.8	7.3	6.9	7.0	0.3
7.0	10.2	9.8	10.4	10.1	0.3
8.0	7.3	8.0	6.9	7.4	0.5
9.0	8.7	9.1	7.5	8.4	0.8
10.0	6.3	7.4	4.9	6.2	1.3
11.0	7.6	7.2	6.6	7.1	0.5
12.0	6.8	6.2	6.2	6.4	0.4
13.0	6.9	6.4	7.1	6.8	0.4
14.0	6.8	6.5	7.7	7.0	0.6
15.0	8.7	8.4	8.4	8.5	0.2
16.0	10.1	9.4	8.6	9.4	0.7
18.0	15.1	11.2	11.6	12.6	2.1
19.0	15.2	13.4	14.7	14.4	0.9
19.2	16.0	15.7	15.8	15.8	0.2
19.4	16.6	16.6	15.9	16.4	0.4
19.7	16.4	15.7	15.3	15.8	0.6
20.0	16.1	16.1	15.7	15.9	0.2
22.0	16.0	16.9	16.1	16.4	0.5
23.0	16.5	16.7	16.3	16.5	0.2
25.0	15.2	14.7	14.9	15.0	0.3

Table C.47. Chlorophyll a (mg/L) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	0.05	0.08	0.06	0.06	0.01
4	0.13	0.08	0.08	0.09	0.03
7	0.46	0.20	0.16	0.27	0.16
12	1.43	0.95	0.93	1.11	0.28
15	2.37	1.38	1.90	1.88	0.49
19	2.16	1.10	1.82	1.69	0.54
23	1.35	1.78	1.23	1.45	0.29

Table C.48. Chlorophyll a (mg/L) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	0.15	0.05	0.06	0.09	0.06
4	0.12	0.11	0.12	0.11	0.00
7	0.27	0.22	0.44	0.31	0.11
12	1.79	1.63	1.64	1.69	0.09
15	1.44	1.22	1.48	1.38	0.14
19	1.27	1.27	1.23	1.26	0.02
23	0.63	0.63	0.63	0.63	0.00

Table C.49. Total chlorophyll (mg/L) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	0.11	0.14	0.13	0.13	0.02
4	0.29	0.14	0.14	0.19	0.09
7	1.14	0.33	0.30	0.59	0.48
12	1.59	1.17	1.09	1.28	0.27
15	2.85	2.58	2.16	2.53	0.35
19	2.44	1.41	1.89	1.92	0.52
23	1.45	1.76	1.31	1.51	0.23

Table C.50. Total chlorophyll (mg/L) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	0.37	0.11	0.08	0.19	0.16
4	0.27	0.19	0.17	0.21	0.05
7	0.39	0.33	0.61	0.45	0.15
12	1.99	1.97	1.84	1.93	0.08
15	1.71	1.56	1.84	1.70	0.14
19	1.44	1.44	1.31	1.39	0.08
23	0.72	0.72	0.72	0.72	0.00

Table C.51. Total carotenoids (mg/L) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	0.00	0.00	-0.01	0.00	0.01
4	-0.02	0.02	0.01	0.00	0.02
7	-0.07	0.06	0.03	0.01	0.07
12	0.65	0.42	0.42	0.50	0.13
15	1.01	0.26	0.97	0.75	0.42
19	1.17	0.58	1.02	0.92	0.31
23	0.79	1.05	0.82	0.88	0.14

Table C.52. Total carotenoids (mg/L) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard
0	-0.03	0.00	0.01	-0.01	0.02
4	0.00	0.02	0.04	0.02	0.02
7	0.09	0.08	0.17	0.11	0.05
12	0.76	0.70	0.78	0.75	0.04
15	0.67	0.56	0.69	0.64	0.07
19	0.78	0.71	0.71	0.73	0.04
23	0.47	0.47	0.49	0.48	0.01

Table C.53. Nitrate concentration (mg/L) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	322	297	328	316	16
4	294	319	332	315	19
7	294	268	299	287	17
12	166	145	183	165	19
15	79	151	125	118	36
19	20	87	32	46	35
23	21	15	24	20	4

Table C.54. Nitrate concentration (mg/L) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	265	322	353	313	44
4	325	302	292	307	16
7	238	249	239	242	6
12	138	151	122	137	15
15	59	91	74	75	16
19	16	29	24	23	6
23	18	22	21	20	2

Table C.55. Phosphate concentration (mg/L) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	156	149	166	157	9
4	155	159	171	162	8
7	150	137	151	146	8
12	128	112	120	120	8
15	125	139	141	135	9
19	134	146	147	142	7
23	125	129	127	127	2

Table C.56. Phosphate concentration (mg/L) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	133	162	179	158	23
4	161	154	148	154	6
7	138	138	133	136	3
12	124	129	129	127	3
15	131	147	137	138	8
19	128	129	131	129	2
23	121	100	109	110	10

Table C.57. Sulfate concentration (mg/L) for isolate GK2Lg grown on AM6SIS medium in tube reactors.

Time (days)	3a	3b	3c	Average	Standard Deviation
0	38	36	39	38	2
4	35	37	39	37	2
7	34	31	35	33	2
12	23	19	23	21	2
15	6	20	10	12	7
19	0	8	0	3	5
23	0	0	0	0	0

Table C.58. Sulfate concentration (mg/L) for isolate GK2Lg grown on AM6SIS medium supplemented with sodium bicarbonate in tube reactors.

Time (days)	4a	4b	4c	Average	Standard Deviation
0	32	38	42	37	5
4	37	35	34	36	2
7	29	30	28	29	1
12	18	18	16	17	1
15	7	10	0	6	5
19	0	0	0	0	0
23	0	0	0	0	0

Table C.59. End point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK2Lg grown under 2 different treatments in tube reactors. Units are shown in (%).

Treatment		FA	MAG	DAG	TAG	Total Neutral Lipid	Total FAME
AM6SIS	Tube 1	5.95	3.14	1.12	3.98	14.19	27.19
	Tube 2	7.25	3.36	1.30	2.84	14.75	21.55
	Tube 3	6.39	3.34	1.89	3.12	14.74	23.49
AM6SIS + HCO3- Supplemented	Tube 1	5.87	3.50	1.61	3.03	14.00	26.29
	Tube 2	6.17	3.23	1.54	2.74	13.69	23.74
	Tube 3	6.01	3.72	1.66	3.28	14.67	23.75

Table C.60. Average and standard deviation of end point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK2Lg grown under 2 different treatments in tube reactors. Units are shown in (%).

	Treatment	FA	MAG	DAG	TAG	Total Neutral Lipid	Total FAME
Average	AM6SIS	6.53	3.28	1.44	3.31	14.56	24.07
	AM6SIS + HCO3- Supplemented	6.02	3.48	1.60	3.02	14.12	24.59
Standard Deviation	AM6SIS	0.66	0.12	0.40	0.59	0.32	2.87
	AM6SIS + HCO3- Supplemented	0.15	0.25	0.06	0.27	0.50	1.47

Table C.61. Endpoint analysis representing productivity in each treatment. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

Treatment	Cell Dry Weight Conc. (mg/mL)	Total Lipid Content Conc. (mg/mL)	Total FAME Content Conc. (mg/mL)
AM6SIS	0.33	0.05	0.09
	0.22	0.03	0.05
	0.26	0.04	0.06
AM6SIS + HCO3- Supplemented	0.37	0.05	0.10
	0.35	0.05	0.08
	0.38	0.06	0.09

Table C.62. Average and standard deviation of endpoint analysis representing productivity in each treatment. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

	Treatment	Cell Dry Weight Conc. (mg/mL)	Total Lipid Content Conc. (mg/mL)	Total FAME Content Conc. (mg/mL)
Average	AM6SIS	0.27	0.04	0.07
	AM6SIS + HCO3- Supplemented	0.37	0.05	0.09
Standard Deviation	AM6SIS	0.05	0.01	0.02
	AM6SIS + HCO3- Supplemented	0.01	0.00	0.01

Table C.63. Endpoint FAME speciation of isolate GK2Lg grown in AM6SIS medium.

% of FAME	3a	3b	3c	Average	Standard Deviation
C12:0	0.00	0.00	0.00	0.00	0.00
C14:0	6.07	6.35	6.35	6.26	0.16
C16:3	1.14	1.13	0.87	1.05	0.15
C16:2	0.00	0.00	0.00	0.00	0.00
C16:1	51.26	51.72	50.92	51.30	0.40
C16:0	21.17	20.85	22.45	21.49	0.85
C18:1-3	2.70	1.73	3.06	2.49	0.69
C18:0	0.40	0.48	0.52	0.47	0.07
C20:5	14.73	14.53	12.83	14.03	1.04
C20:1	0.00	0.00	0.00	0.00	0.00
C20:0	0.00	0.00	0.00	0.00	0.00
C22:6	1.60	2.02	1.80	1.81	0.21
C22:1	0.00	0.00	0.00	0.00	0.00
C22:0	0.00	0.00	0.00	0.00	0.00
C24:1	0.00	0.00	0.00	0.00	0.00
C24:0	0.94	1.19	1.19	1.11	0.15
C26:0	0.00	0.00	0.00	0.00	0.00
Other	0.00	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	0.00

Table C.64. Endpoint FAME speciation of isolate GK2Lg grown in AM6SIS medium supplemented with sodium bicarbonate.

% of FAME	4a	4b	4c	Average	Standard Deviation
C12:0	0.00	0.00	0.00	0.00	0.00
C14:0	6.48	6.31	6.50	6.43	0.10
C16:3	1.24	1.41	1.52	1.39	0.14
C16:2	0.00	0.00	0.00	0.00	0.00
C16:1	51.76	51.35	52.58	51.90	0.63
C16:0	20.18	20.26	19.20	19.88	0.59
C18:1-3	1.96	2.07	1.74	1.92	0.17
C18:0	0.54	0.43	0.39	0.45	0.08
C20:5	14.65	15.21	15.30	15.05	0.35
C20:1	0.00	0.00	0.00	0.00	0.00
C20:0	0.00	0.00	0.00	0.00	0.00
C22:6	2.07	1.89	1.81	1.92	0.13
C22:1	0.00	0.00	0.00	0.00	0.00
C22:0	0.00	0.00	0.00	0.00	0.00
C24:1	0.00	0.00	0.00	0.00	0.00
C24:0	1.14	1.07	0.96	1.06	0.09
C26:0	0.00	0.00	0.00	0.00	0.00
Other	0.00	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	0.00

Additional Isolate GK5La data

In this experiment isolate GK5La was grown in AM6 medium buffered with 18g/L sodium bicarbonate. Nitrite was mistakenly added to the medium in place of nitrate.

Table C.65. Cell concentration (cells/mL) for isolate GK5La grown on AM6 medium buffered with 18g/l sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	1.40E+05	1.15E+05	1.48E+05	1.34E+05	1.70E+04
1	1.95E+05	9.50E+04	1.43E+05	1.44E+05	5.00E+04
2	5.60E+05	5.60E+05	7.30E+05	6.17E+05	9.81E+04
3	4.20E+05	5.80E+05	4.20E+05	4.73E+05	9.24E+04
4	1.56E+06	2.72E+06	2.28E+06	2.19E+06	5.86E+05
6	7.40E+06	5.90E+06	8.10E+06	7.13E+06	1.12E+06
7	9.70E+06	9.70E+06	1.13E+07	1.02E+07	9.24E+05
8	2.13E+07	1.62E+07	1.51E+07	1.75E+07	3.31E+06
9	1.91E+07	1.31E+07	1.56E+07	1.59E+07	3.01E+06
10	2.14E+07	2.96E+07	2.60E+07	2.57E+07	4.11E+06
11	4.20E+07	3.60E+07	3.65E+07	3.82E+07	3.33E+06
13	3.00E+07	3.80E+07	3.80E+07	3.53E+07	4.62E+06
15	4.36E+07	5.70E+07	5.12E+07	5.06E+07	6.72E+06
17	4.08E+07	4.64E+07	4.20E+07	4.31E+07	2.95E+06
20	3.80E+07	4.68E+07	4.32E+07	4.27E+07	4.42E+06
25	4.72E+07	5.68E+07	5.04E+07	5.15E+07	4.89E+06
33	3.80E+07	4.24E+07	3.76E+07	3.93E+07	2.66E+06

Table C.66. pH for isolate GK5La grown on AM6 medium buffered with 18g/l sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	9.57	9.52	9.58	9.56	0.03
1	9.73	9.68	9.75	9.72	0.04
2	10.01	9.96	10.03	10.00	0.04
3	10.09	10.05	10.14	10.09	0.05
4	10.24	10.19	10.25	10.23	0.03
6	10.59	10.48	10.53	10.53	0.06
7	10.77	10.66	10.68	10.70	0.06
8	11.1	10.96	10.99	11.02	0.07
9	11.55	11.31	11.34	11.40	0.13
10	11.96	11.96	11.98	11.97	0.01
11	11.79	12.03	11.89	11.90	0.12
13	11.54	11.76	11.75	11.68	0.12
15	11.44	11.7	11.59	11.58	0.13
17	11.43	11.6	11.53	11.52	0.09
20	11.31	11.44	11.48	11.41	0.09
25	10.93	11.17	11.12	11.07	0.13
33	10.77	10.95	10.86	10.86	0.09

Table C.67. DIC (mM) for isolate GK5La grown on AM6 medium buffered with 18g/l sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	221	204	177	200	22
1	211	218	187	205	17
2	190	192	188	190	2
3	182	185	158	175	15
4	173	176	178	176	3
6	153	155	153	154	2
7	152	156	156	155	2
8	141	157	147	148	8
9	120	128	125	125	4
10	194	189	195	193	3
11	190	188	195	191	4
13	202	194	202	199	5
15	191	179	183	184	6
17	222	214	220	219	4
20	130	129	137	132	4
25	152	146	156	151	5
33	159	144	161	155	9

Table C.68. Nile Red fluorescence (a.u.) for isolate GK5La grown on AM6 medium buffered with 18g/l sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	26	25	26	26	1
1	20	20	2	14	10
2	30	24	13	22	9
3	45	31	30	35	8
6	490	430	440	453	32
8	750	610	590	650	87
9	610	430	640	560	114
11	1250	780	950	993	238
13	1500	1200	1220	1307	168
15	1900	1120	1540	1520	390
17	2420	2440	1840	2233	341
20	3300	2620	3510	3143	465
25	5080	4000	7680	5587	1892
30	3380	2840	4280	3500	727

Table C.69. Nitrite concentration (mg/L) for isolate GK5La grown on AM6 medium buffered with 18g/l sodium bicarbonate in tube reactors.

Time (days)	2a	2b	2c	Average	Standard Deviation
0	202	225	224	217	13
3	204	215	219	213	8
8	54	66	84	68	15
10	19	10	27	19	8
13	8	8	20	12	7
15	1	5	9	5	4
20	0	0	0	0	0

Table C.70. End point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK5La grown in AM6 medium buffered with 18g/L sodium bicarbonate in tube reactors. Units are shown in (%).

Treatment		FA	MAG	DAG	TAG	Total Neutral Lipid
AM6 + 18g/L NaHCO3-	Tube 1	4.62	0.49	1.85	15.75	22.72
	Tube 2	3.79	0.24	1.60	13.40	19.03
	Tube 3	3.10	0.15	1.38	14.37	19.00

Table C.71. Average and standard deviation for end point analysis of fatty acid composition, total neutral lipid, and total FAME for isolate GK5La grown in AM6 medium buffered with 18g/L sodium bicarbonate in tube reactors. Units are shown in (%).

Treatment		FA	MAG	DAG	TAG	Total Neutral Lipid
AM6 + 18g/L NaHCO3-	Average	3.84	0.29	1.61	14.51	20.25
	Standard Deviation	0.76	0.18	0.24	1.18	2.14

Table C.72. Endpoint analysis representing productivity in AM6 medium buffered with 18g/L sodium bicarbonate. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

Treatment		Cell Dry Weight	Total Lipid
AM6 + 18g/L NaHCO3-	Tube 1	1.92	0.43
	Tube 2	1.72	0.32
	Tube 3	1.76	0.33

Table C.73. Average and standard deviation for endpoint analysis representing productivity in AM6 medium buffered with 18g/L sodium bicarbonate. Cell dry weight, total lipid, and total FAME are all shown on a concentration basis.

Treatment		Cell Dry Weight	Total Lipid
AM6 + 18g/L NaHCO3-	Average	1.80	0.32
	Standard Deviation	0.105	0.061