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Optimization of Algae Production Using Concentrate

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by

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Executive Summary

Desalination can alleviate global water scarcity by removing salt from saline water, but all desalination technologies also produce undesirable concentrate, which contains all of the substances removed from feedwater during desalination. Concentrate is saline to a degree that can threaten the health of many living organisms, and current disposal methods for concentrate are costly, environmentally harmful, and wasteful. Water is precious, and the world's volume of concentrate is a large and mostly unused water source.

One of the promising approaches for feasibly and sustainably using desalination concentrate is using it as a growth medium for halotolerant algae. If enough microalgae growth is achieved in the concentrate, the microalgae could be harvested to produce biomass for biofuel, turning a former waste into a valuable product. Furthermore, microalgae consume nutrients from the concentrate, reducing the levels of total dissolved solids, heavy toxic metals in concentrate and facilitating its safe disposal.

To improve the feasibility of using concentrate to grow microalgae, this study investigates how modified desalination concentrate – with different levels of phosphorus, nitrogen, and CO_2 – affects the growth of microalgae species *Chlorella sorokiniana* and *Nannochloropsis oculata*. Based on biomass production measures, an optimal growth medium composition was determined, and a predictive model for biomass production was developed.

The present report consists of two parts:

I. Lab-scale cultivation of microalgae in desalination concentrate and modification of desalination concentrate to optimize its suitability as a growth medium for microalgae, and

II. Pilot-scale cultivation of microalgae in desalination concentrate.

Both reports include data on ion removal during microalgae growth.

Contents

PART II

Part I: Lab-Scale Cultivation of Microalgae in Desalination Concentrate

CHAPTER 1 - INTRODUCTION

The energy and water crises are among the highest-priority challenges to be tackled in the century in which we live. These crises are interrelated: producing energy typically takes large amounts of water (e.g., dammed or running water for hydroelectric plants, steam in nuclear or thermal plants, and water in fracking fluid), and producing clean water takes large amounts of energy (e.g., in thermal and membrane desalination processes). Therefore, solutions that can address both crises simultaneously have a special appeal. In the following sections, the energy and water crises are described, and the use of impaired water to grow algae and produce biofuels is introduced as a potential solution.

1.1 The Energy Crisis

Energy crises mainly result from escalated demands for energy, energy pricing policies, oil import quotas, and depletions of domestic oil and gas reserves (Altin et al., 2001). In 2013, global energy consumption increased by 2.3%, a 0.5% acceleration over 2012. Oil remains the leading fuel, representing 32.9% of global energy consumption (BP, 2014), but the share of oil in global energy consumption is declining. Renewable energy sources are becoming more prominent, and recent conditions where pricing policies brought about overproduction have led to the projected depletion of accessible fossil fuels. The recent growth in U.S. fossil fuel production, as shown in Figure 1, is unlikely to be sustainable.

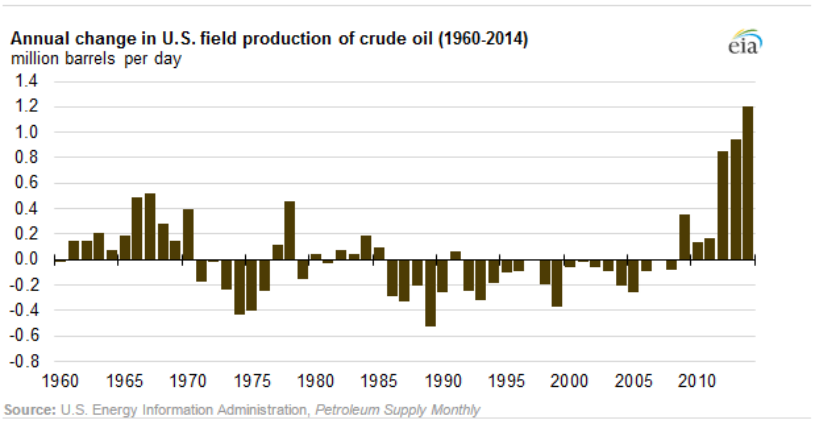

Figure 1. Changes in U.S. fossil fuel production, 1960-2014 (EIA, 2012).

Record growth in fossil fuel consumption also indicates that global CO_2 emissions have grown correspondingly, accelerating climate change. While the use of fossil fuels may continue for years, their finite and non-renewable nature along with their climatic impacts require an immediate effort to reduce growth rates for energy consumption and explore sustainable alternatives for the future.

Biofuels, which are made of living organisms and their byproducts, are seen as a large part of the solution to the energy crisis due to their less-polluting properties and their ability to be produced domestically. The first known biofuels were solid fuels such as wood, sawdust, grass cuttings, domestic refuse, charcoal, agricultural waste, non-food energy crops, and dried manure – i.e., biomass (Ankita, 2013). However, producing this kind of biomass is labor intensive and significant energy inputs are required for harvesting, processing, delivering, and burning the biomass, and then disposing of the residue. Another drawback is that solid biofuels have proven to have limited ability to reduce greenhouse gas emissions (Altin, 2001). Liquid biofuels produced from food crops are also problematic. These fuels can be produced from substances such as converted sugar, starch, vegetable oil, and animal fat, but their use – which has increased at a rate of 7% - competes with food production and can contribute to famine (IEA, 2006). The best biofuels so far are liquid fuels from aquatic organisms such as algae. These organisms are low-input, high-yield feedstocks, which contain a viable source of lipids, from which biodiesel, bioethanol, and bio-hydrogen can be produced through thermochemical or biochemical conversion processes (Ankita, 2013). Algae offer several other benefits including year-round production, no competition with food crops, low land space requirements, and little need for clean water (Chisti, 2008). Contemporary research on microalgae growth is mainly focused on utilizing wastewater as a growth medium, since the availability of water is the only limiting factor for large-scale algae cultivation.

1.2 The Water Crisis

The World Health Organization estimates that more than 750 million people lack access to safe water: that is, 1 in 9 people (Organization, 2012). These numbers are predicted to worsen with industrialization, urbanization, and a growing global population that is expected to reach 9 billion by 2050.

Although the population of the world is increasing, the amount of available freshwater is relatively constant at approximately 200,000 km^3, or about 2.5% of the total water available on earth. The relationship between population and water availability is shown in Figure 2.

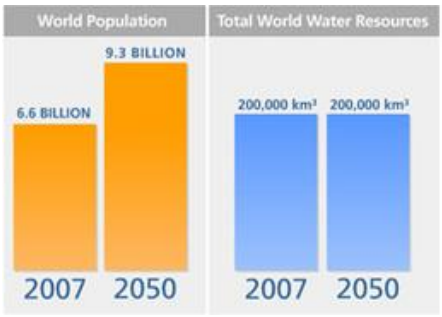

Figure 2. World population in relation to freshwater resources (UNICEF, 2014).

Furthermore, only about 1.3% of freshwater is available on surface of the earth in lakes, rivers, and streams (Figure 3). The remaining freshwater is held in glaciers and groundwater, sources which can be accessible, but which are more difficult to utilize (Altan et al., 2012).

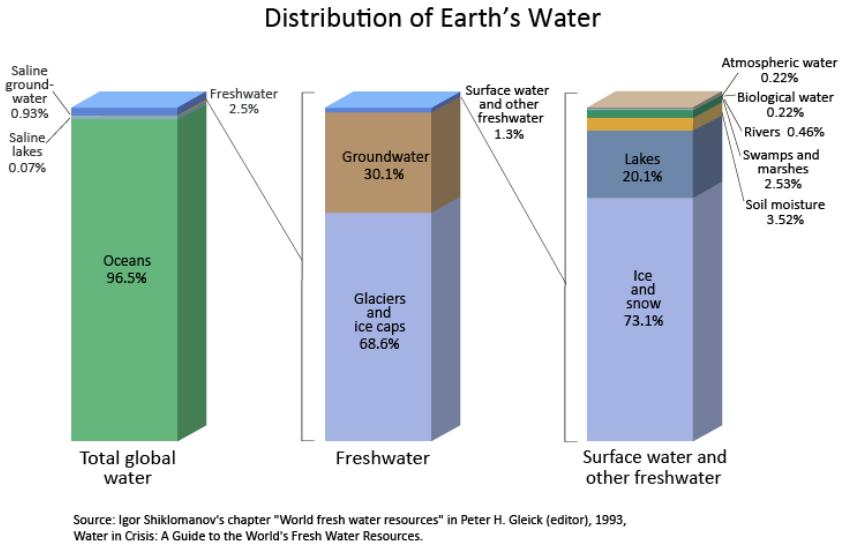

Figure 3. Distribution of water on earth (Gleick, 1993).

As information on population growth and the availability of freshwater suggests, the current scenario is ominous. Freshwater is finite, and much of its limited quantity is difficult to access. Furthermore, different sectors often compete for the use of freshwater: increases in one sector deprive another, usually household

consumption (Gleick, 1993). Currently, the agricultural sector is a dominant user of freshwater (Chisti, 2008). Under present approaches, the growth of algae would drive agricultural water consumption still higher. Current technologies require a considerable amount of water to grow algae in aqueous suspension: it takes 6000 gallons of water to cultivate 1 gallon of algae oil (Altan et al., 2012). This water not only provides a growth environment, but it also delivers nutrients, removes waste products, and acts as a thermal regulator (Cynthia, 2011). While the water for algae cultivation can be reused in theory, in practice water is lost to evaporation in open ponds at a rate that varies with the climate, temperature, humidity, precipitation, and wind velocity of the location.

1.3 Desalination

Just as energy stress has driven a search for alternative energy sources, water stress has driven a search for alternative water sources. Among alternatives, the reclamation of saline water through desalination processes has been extensively researched and has the potential to provide plentiful drinking water. Desalination can be applied to waters with varying levels of salinity, such as brackish groundwater, estuarine water, or seawater; in some regions, it provides the primary source of drinking water. All desalination technologies produce a product stream of fresh water (called permeate) and a highly concentrated stream of salts and other rejected materials. The latter stream is called desalination concentrate or reject brine, and its safe disposal has been a costly impediment to the installation of desalination plants.

Desalination technology started primarily with thermal process (e.g., flash distillation), but as a result of technological advances, membranes have become a more cost-effective alternative, so membrane technologies comprise an increasing percentage of new desalination systems. Other advances include emerging technologies such as forward osmosis, low temperature distillation, pressure retarded osmosis, and graphene membranes. Hybrid plants and reverse osmosis are gaining wider use in the Middle East, which has traditionally been home to facilities using more energy-intensive thermal technologies (Mike, 2014).

1.3.1 Membrane Desalination

In membrane desalination, reverse osmosis has been the most widely used technology, but other prominent technologies include electrodialysis (ED) and nanofiltration (NF) (Hafez and El-Manharawy, 2002). The differences among the primary desalination technologies are summarized in Table 1 and Figure 4. ED membranes, which typically are used only for brackish water desalination, operate under an electrical current that causes ions to move through parallel membranes (Greenlee et al., 2009). NF, in which pressure is applied to drive water through small pores, has been used to reduce the passage of particles between 1 and 5 nm in size.

Table 1. Differences among filtration processes (Greenlee et al., 2009).

Particular	MF	UF	NF	RO
Membrane	Porous isotropic	Porous asymmetric	Finely porous asymmetric/composite	Nonporous asymmetric/composite
Pore size	50 nm–1 μm	5–20 nm	1–5 nm	–
Transfer mechanism	Sieving and adsorptive mechanisms (the solutes migrate by convection)	Sieving and preferential adsorption	Sieving/electrostatic hydration/diffusive	Diffusive (solutes migrate by diffusion mechanism)
Law governing transfer	Darcy's law	Darcy's law	Fick's law	Fick's law
Typical solution treatment	Solution with solid particles	Solution with colloids and/or macromolecules	Ions, small molecules	Ions, small molecules
Typical pure water flux (L m^{-2} h)	500–10 000	100–2000	20–200	10–100
Pressure requirement (atoms)	0.5–5	1–10	7–30	20–100

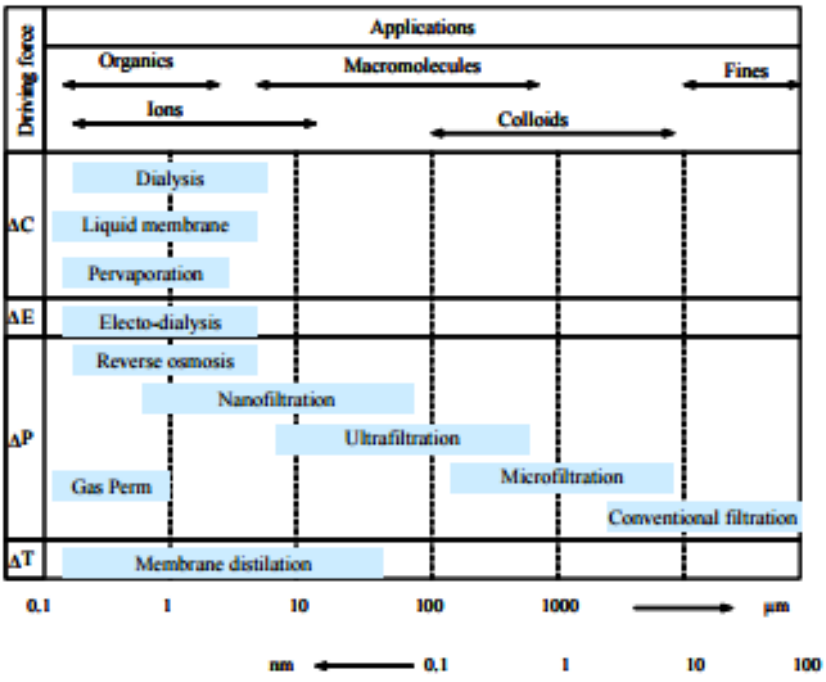

Figure 4. Characteristics of different membrane processes (Shon, 2013).

RO, the most commonly used membrane process, is characterized by an operating pressure that usually is fairly high (20 to 100 bar) and the capacity to remove very fine particles. NF has pore sizes of 1–5 nm and a higher water permeability than RO membranes, so it operates at lower pressure (7 to 30 bar). UF membranes, which have still larger pore sizes of 5 to 20 nm, retain fine colloids, macromolecules, and microorganisms, and operate with a pressure range of 1 to 10 bar. The other membrane technologies used in liquid separation processes are microfiltration (MF), electrodialysis reversal (EDR), and liquid membrane (LM).

1.3.2 Desalination Cost

Over the last decade, the cost for membrane desalination has decreased drastically due to technological advances. All indicators are that the costs associated with the technology will continue to decrease as technologies and efficiencies improve. However, cost comparisons depend on more than the characteristics of the technology. Operating conditions – including feed water characteristics, finished water quality goals, intake type, and disposal method – all play a large role in the overall cost of water (Hafez and El-Manharawy, 2002). Typical cost breakdown for a seawater reverse osmosis (SWRO) desalination plant (Figure 5).

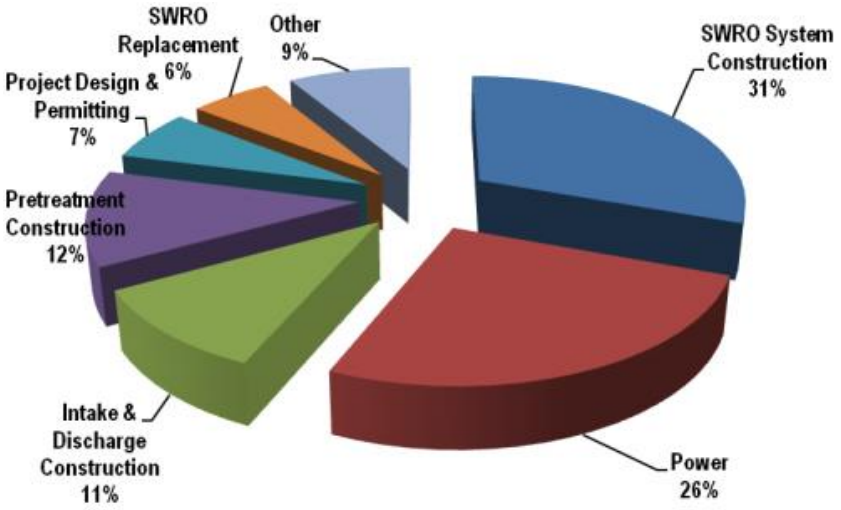

Figure 5. Typical cost breakdown of a sea water reverse osmosis desalination plant (WateRuse, 2009).

The feed water quality affects the pretreatment system required, and typically the pretreatment cost ranges from US$0.5M/MGD to US$1.5M/MGD. The lower cost represents a single-stage filtration system, and any additional stages increase this cost. The intake and discharge costs associated with plants are approximately 11 to 12% of the total plant cost (Figure 5). An open intake system typically would cost US$0.5-1.5M/MGD, but prices up to US$3M/MGD are possible for complex tunnel and offshore intake systems (Table 2). For discharge, there are several methods to dispose of concentrate, and their cost differs with the varying complexities of the discharge systems.

Table 2. Concentrate disposal methods (WateReuse, 2012)

Disposal Method	Construction Cost	
	(US$ MM / MGD)	(US$ MM /acre-foot/day)
New Outfall w/Diffusers	2.0 – 5.5	0.7 - 1.8
Power Plant Outfall	0.2 – 0.6	0.07 - 0.20
Sanitary Sewer	0.1 – 0.4	0.03 - 0.13
WWTP Outfall	0.3 – 2.0	0.1 - 0.7
Deep Well Injection	2.5 – 6.0	0.8 - 2.0
Evaporation Ponds	3.0 – 9.5	1.0 - 3.1
Zero-Liquid Discharge	5.5 – 15.0	1.8 - 4.9

The disposal techniques vary from low- to high-end pricing, and disposal approaches have a considerable impact on the budgeting for a desalination plant. Much of the cost is attributable to environmental regulations put in place by federal agencies, which require permits and formal evaluations of disposal methods in order to protect the environment (Hafez and El-Manharawy, 2002).

In the effort to reduce the cost of concentrate disposal and thereby increase the economic feasibility of desalination plants, microalgae are an auspicious tool. Some researchers have demonstrated that microalgae can be cultivated in desalination concentrate (Hussein et al., 2015; Khaled, 2012), and the microalgae use materials in the concentrate as nutrients, reducing the levels of total dissolved solids (TDS) in the concentrate and facilitating its safe disposal (Shirazi, 2014), all while producing raw materials for biofuels that can alleviate the energy crisis.

Although studies have investigated the use of other impaired waters for algae cultivation (Woertz and Feffer, 2009), few studies have focused on desalination concentrate as a growth medium for microalgae. In the few studies that have been conducted, the main focus has been reducing the salinity of the concentrate solution during algae growth (Hussein et al., 2015; Shirazi, 2014). None of the researchers have investigated the growth of two algae strains, *C. sorokiniana* (fresh water strain) and *N. oculata* (marine strain) using the concentrate stream, and no study has yet investigated the effects of N, P, and CO_2 (three main parameters for algae growth (Grobbelaar, 2004) on the biomass production of the two target microalgae strains.

CHAPTER 2: LITERATURE REVIEW

The first sections of this chapter describe the strains of microalgae used in lipid quantification and characterization studies. The latter section provides information associated with nutrients required for the microalgae and phycoremediation capability of algae species. This section comprehensively provides information on the effect of nutrient and CO_2 capacity used in growth process.

2.1 Microalgae strains

Microalgal products have potential as food supplements, fertilizer, and biofuel feedstocks. Microalgae can also be used for CO_2 sequestration since photoautotrophic algal cultures have the potential to remove CO_2 from the atmosphere, helping alleviate the trend toward global warming.

To reach this goal, it is especially important to achieve two objectives: 1) identifying the microalgae species that performs best in fixing CO_2 (Takagi, 2000), and 2) improving the economic feasibility of microalgae production. For achieving the latter objective, one promising approach is seeking additional value for the system through development of multifunctional, integrated systems, such as combined waste treatment and aquaculture farms (Pedroni et al., 2003). In such systems, algae can sequester CO_2 and produce materials for valuable products while performing other valuable services (e.g., waste water treatment).

In selecting the appropriate microalgae from the more than 72,500 microalgae species (Guiry, 2012), multiple criteria should be considered. As reported by Mata et al. (2010), these criteria include:

- High growth rate
- High performance in competitive mass nature and tolerance to predators
- Appropriate lipid content and energy yield based on type of fuel desired from biomass
- Tolerance to changes in environmental conditions, including resistance to variations in temperature, nutrient inputs (salinity), and light levels
- Availability of nutrients, especially CO_2 when carbon fixation is the goal
- Possibility of obtaining other valuable chemicals
- Degree of easiness of biomass isolation

- Less complex structure and, as a result, easier oil extraction

Some microalgae species, such as *Chlorella*, *Spirulina*, and *Dunaliella*, already have commercial value. *Chlorella* and *Spirulina* are used as food supplements, and *Dunaliella* is a source of beta-carotene (Graham, 2000). *Chlorella* has also been studied for use in CO_2 sequestration and has been shown to grow in conditions of up to 20% CO_2 (Guiry, 2012). For all of these species, commercial profit from biomass production will potentially reduce operational and capital costs for CO_2 sequestration.

The use of marine microalgae for biological CO_2 sequestration has also been considered. Marine algae offer several advantages, including the ability to use plentiful and cheap seawater and brackish water directly as growing media, thereby reducing the costs of microalgae cultivation. CO_2 sources, such as power plants, are also located along coastal areas (Barsanti, 2006), where seawater is available in practically unlimited quantities. Therefore, in addition to algae species that already have demonstrated commercial potential (e.g., *Chlorella*, *Spirulina*, and *Dunaliella*), marine microalgae merit investigation.

One particularly interesting marine microalgae is *Nannochloropsis oculata*, which has a high lipid content of 30% (Malakootian, 2014). Many microalgae can accumulate lipids due to excess photosynthate, and some species can accumulate lipids under heterotrophic or environmental stresses such as nutrient deficiency. The *N. oculata* cultured in 2%, 5%, and 10% CO_2 in a semi-continuous system with a high-cell density of inoculum can be grown optimally in 2% CO_2 (Sheng-Yi, 2009).

From among freshwater algae strains, *Chlorella sorokiniana* has a high growth rate and is tolerant to high irradiance, high temperature, and high CO_2 concentrations (Matsukawa, 2000). Therefore, this strain has clear benefits for use in outdoor cultures and is a good candidate for a CO_2 fixation/conversion system.

2.2 Effect of CO_2 on microalgae growth

Biological CO_2 mitigation is an attractive process since, while achieving CO_2 fixation through photosynthesis, it produces biomass energy as a byproduct (Chang, 2011). Biological mitigation of CO_2 by microalgae mainly focuses on two CO_2 sources: flue gas (with 10–20% CO_2) and air in a closed space (generally around 1.0% CO_2) (Jajesniak, 2014; Matsukawa, 2000). However, to maximize the efficiency of CO_2 removal through bio-regenerative systems, two important factors must be addressed: the need for free CO_2, and the need for superior mechanisms for concentrating carbon.

Presently, nearly all pilot-scale algae cultures depend on purchased CO_2 that contributes substantially (~50%) to the cost of producing biomass (Giordano, 2005). Unless CO_2 is available free, cultivation of algae for fuels is not feasible (Chisti, 2007). Also, even once CO_2 is available, it must be concentrated to levels that are usable by algae. Many algae and cyanobacteria are known to possess mechanisms for concentrating carbon dioxide from the culture mediums into cells (Giordano, 2005), but carbon dioxide absorption from the standard atmosphere into the culture medium is never sufficiently fast to rapidly grow a large concentration of algae. Due to inadequacies in natural carbon concentrating mechanisms, supplementing an algae culture with CO_2 nearly always enhances the biomass growth rate compared to what is possible under a normal atmosphere. Therefore, to enhance algae growth using high concentrations of CO_2, most large-scale cultivation units are situated near coal-fired power stations, which produce plentiful CO_2 as a waste gas.

Algae require 45 pounds of CO_2 to produce a gallon of biodiesel (Pienkos, 2007), and an average power station produces 400 tons of CO_2 in an hour (EIA, 2014). The amount of CO_2-rich flue gas produced from these coal burning power stations can therefore be used productively in algae cultivation. The expected level of CO_2 in a typical flue gas from a power plant is in the range of 10-20% (Mijeong, 2003), which meets or exceeds the amount required for most strains of microalgae.

2.3 Effect of nutrients on microalgae growth

In addition to CO_2, about 30 elements are important to ensure autotrophic growth. According to the amount required by the microalgae, these essential nutrients are grouped into two categories: 1) macronutrients, which are required in the culture medium in relatively large concentrations of g/L; and 2) micronutrients (trace elements), which are required in the culture medium in mg/L or less (Procházková et al., 2013). Most algae nutrient solutions contain both macronutrients (N, P and K) and micronutrients (Raghawan et al., 2008). Key nutrients essential for autotrophic microalgae are shown in Table 3 (Grobbelaar, 2004). If any of these nutrients exist naturally in the water used to grow algae, such waters will provide an economic advantage.

Table 3. Nutrients essential for autotrophic microalgae and elemental composition of algal cells (Grobbelaar, 2004)

Element	Component added to culture medium	Concentration in culture medium	Cell composition (mg g^{-1} dry weight)
C	CO_2, HCO_3^-, CO_3^{2-}	g L^{-1}	175–650
O	O_2, H_2O	g L^{-1}	205–330
H	H_2O	g L^{-1}	29–100
N	NH_4^+, NO_3^-, NO_2^-, urea etc.	g L^{-1}	10–140
Na	Inorganic salts, i.e. NaCl, Na_2SO_4, Na_3PO_4	g L^{-1}	0.4–47
K	Inorganic salts, i.e. KCl, K_2SO_4, K_3PO_4	g L^{-1}	1–75
Ca	Inorganic salts, i.e. $CaCl_2$, $CaCO_3$	g L^{-1}	0.0–80
P	Inorganic salts, i.e. Na or K phosphates	g L^{-1}	0.5–33
S	Inorganic salts, i.e. $MgSO_4 \cdot 7H_2O$, or amino acids	g L^{-1}	1.5–16
Mg	Inorganic salts, i.e. Mg sulphates or chlorides	g L^{-1}	0.5–75
Cl	As Na^+, K^+, Ca^{2+} or NH_4^+ salts	g L^{-1}	*
Fe	In complex with metal ion buffer (e.g. EDTA)	mg L^{-1}	0.2–34
Zn	Inorganic salts, i.e. $ZnSO_4$, $ZnCl_2$	mg L^{-1}	0.005–1.0
Mn	Inorganic salts, i.e. $MnSO_4$, $MnCl_2$	mg L^{-1}	0.02–0.24
Br	As Na^+, K^+, Ca^{2+} or NH_4^+ salts	mg L^{-1}	*
Si	$Na_3SiO_3 \cdot 9H_2O$	mg L^{-1}	0–230
B	H_3BO_3	mg L^{-1}	0.001–0.25
Mo	Na^+ or NH_4^+ molybdate salts	µg L^{-1}	0.0002–0.001
V	$Na_3VO_4 \cdot 16H_2O$	µg L^{-1}	*
Sr	As sulphates or chlorides	µg L^{-1}	*
Al	As sulphates or chlorides	µg L^{-1}	*
Rb	As sulphates or chlorides	µg L^{-1}	*
Li	As sulphates or chlorides	µg L^{-1}	*
Cu	As sulphates or chlorides	µg L^{-1}	0.006–0.3
Co	Vitamin B_{12}, sulphates or chlorides	µg L^{-1}	0.0001–0.2
I	As Na^+, K^+, Ca^{2+} or NH_4^+ salts	µg L^{-1}	*
Se	SeO_3^{2-}, SeO_4^{2-}	ng L^{-1}	0–0.9[a]

* Data not available

When considering the composition of growth waters, it is important to distinguish between freshwater, marine, and halotolerant/halophilic algae species. Seawater has a relatively constant pH and composition of major ions (Na^+, K^+, Mg_2^+, Ca_2^+, Cl^-, SO_4^2, HCO_3^-, CO_3^{2-}), whereas freshwaters have highly variable compositions. Microalgae species that grow in particular waters are appropriately adapted to the chemistries of those waters. For example, when growing in freshwater containing high concentrations of particular metals (e.g., copper) that are toxic to the majority of other phytoplankton species, cells possess particular detoxification or tolerance mechanisms (Sunda, 2005). Another key adaptation possessed by certain species is that, if the concentration of an essential nutrient falls below a required level, the cells interpret the limitation and produce a specific set of genes in order to alter their physiology and adapt to the deficiency (Cade-Menun and Paytan, 2010).

2.3.1 Key elements for microalgae growth

The absolutely essential elements for microalgae growth are nitrogen, phosphorus, and carbon. The roles played by each of these elements are discussed in the following sections.

2.3.1.1 Nitrogen

Nitrogen is an important macronutrient for microalgae: it is the most abundant cellular macromolecule in the form of proteins and nucleic acids. Nitrogen usually is supplied in forms such as NO_3^-, NO_2^-, NH_4^+, and $(NH_2)_2CO$ (urea). The preferred N form for many algae is NH_4^+ since it can be incorporated directly into organic compounds. NH_4^+ concentrations greater than 25 μM are often reported to be toxic for some algal species, so NO_3- is used more often in synthetic culture media (Barsanti, 2006).

2.3.1.2 Phosphorus

Like nitrogen, phosphorus is an essential macronutrient for the growth of algae. Algal biomass usually contains less than 1% of P, but P levels can exceed 3% by dry weight under certain conditions. The particular importance of phosphorus is in the biosynthesis of nucleic acid and phospholipids, protein function modification, and energy transfer (Powell, 2009). Algae primarily acquire P as inorganic phosphate in the form of either $H_2PO_4^-$ or HPO_4^{2-}.

Larger amounts of P, orthophosphate monoesters (including sugar phosphates, inositol phosphates, and orthophosphate diester degradation products), are found in seawater phytoplankton grown under high light conditions as compared to the same algae grown under low light conditions (Chang, 2011). In freshwater phytoplankton, P is often the main growth-limiting nutrient and it is stored as intracellular polyphosphate. Polyphosphate bodies in eukaryotic algae represent another form of cell protection from metal toxicity, as they can bind incoming metals in a detoxified complex (Cade-Menun and Paytan, 2010).

2.3.1.3 Carbon

Carbon is an essential component of all algae cultures and represents 50% of cell dry weight. Consequently, a limitation in this macronutrient stops biosynthesis (Chisti, 2007). Depending on the source from which carbon is drawn, microalgae species can be divided into autotrophs and heterotrophs. Autotrophic organisms use solar energy to convert and utilize inorganic forms of carbon such as CO_2, carbonate, or bicarbonate. Heterotrophs, in contrast, use the chemical energy of organic forms of carbon (e.g., acetate or glucose) for their metabolic activities (Pires, 2015). Prolonged deprivation of carbon affects photosynthetic energy acquisition and photosynthetic efficiency.

Equation (1) describes the reaction that takes place when gaseous CO_2 is dissolved into water, forming H_2CO_3*, carbonic acid. This reaction occurs between pH 6.5 and 7.5; the other alkaline species, HCO_3− and CO_3^{2-}, are not present However, dissolved carbonic acid, H_2CO_3*, is maximized when the pH is 6.5, and its presence decreases to zero when the pH is 8.5 (Sawyer and McCarty), as H_2CO_3* dissociates to H^+ and HCO_{3-} (shown in Eq. (2)) as pH reaches 7.5. The released H^+ will react with the available calcium carbonate alkalines to form HCO_{3-}, as shown in Eq. (3). More CO_2 dissolved reacts to form HCO_{3-}, Equation (4) (Maung-Thein, 2014).

$$H_2O + CO_{2\,(gas)} \leftrightarrow H_2CO_3^* \quad \text{Eq. (1)}$$

$$H_2CO_3^* \leftrightarrow H^+ + HCO_3^- \quad \text{Eq. (2)}$$

$$CaCO_3 + H^+ \leftrightarrow Ca^{2+} + HCO_3^- \quad \text{Eq. (3)}$$

$$H_2O + CO_2 + CaCO_3 \leftrightarrow Ca^{2+} + 2HCO_3^-$$

Autotrophic cultures respond to low levels of CO_2 by increasing the assimilation of the limiting nutrient and at the same time adjusting its capacity, flux rates, and intermediate storage options for the non-limiting nutrients (Giordano, 2005).

2.4 Algae metal reduction

Organic pollutants and heavy metals are considered to be a serious environmental problem during disposal of desalination concentrate. Accumulation of toxic metals e.g. Hg, Cu, Cd, Cr and Zn in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and wide range of other illnesses (Dwivedi, 2012).

Microalgae have the ability of reducing heavy metals and its toxicity effects in its habitat; therefore would be effective in reducing the heavy metal toxicity in desalination concentrate. These metals exist in form of free ions, complex ions or in particulate forms (Shaw, 1989). Toxicity of copper, zinc, cadmium, mercury is reduced by calcium and magnesium salts as a result of co-precipitation in some algae, other types of algae synthesize phytochelatins and metallothioneins that can form complexes with heavy metals and translocate them into vacuoles (Sakaguchi et al., 1981).

In order to control heavy metal levels before they are released into the environment, the treatment of the contaminated wastewaters is of great importance since heavy metal ions accumulate in living species with a permanent toxic and carcinogenic effect (Worku and Sahu, 2014).

CHAPTER 3: MATERIAL AND METHODS

3.1 Experimental Set-Up

A lab-scale bioreactor design containing thirty-six 500 mL glass photobioreactors (as shown in Figure 6) was used to conduct the experiments in this study. The photobioreactors were partially filled with 200 mL of growth medium and 50 mL of algae inoculum according to the experimental design, discussed in Section 3.3. The bioreactors were then conditioned with parameters (Table 4) and equipped with an air and CO_2 supply system.

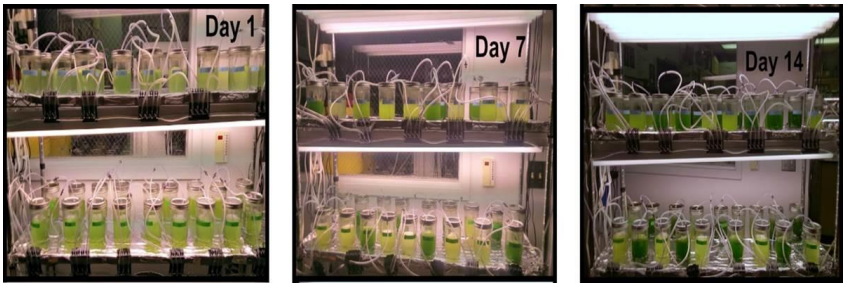

Figure 6. Bioreactor setup.

Table 4. Generalized conditions for culturing microalgae in photobioreactor.

Parameters	Value	Literature
Temp (^{0}C)	24-26	(Crowe et al., 2012; Rocha et al., 2003; Tamburic et al., 2014)
Light intensity (µmol/m².s)	2000	(Aburezq et al., 1999; Sharma et al., 2012)
Photoperiod (light:dark)	16:8	(Sforza and Simionato, 2012; Sharma et al., 2012)
pH	6.7-8.2	(Franco et al., 2012; Rocha et al., 2003)

During the cultivation period, 1 µL of algal broth was sampled at the 3rd, 6th, 10th and 14th days, and then cell count was conducted using an improved

Neubauer haemocytometer to determine the growth rate of the algae species. Deionized water was added during each sampling day to replenish the water lost through evaporation in the bioreactor.

3.2 Algae strains and culture

In this study, two strains of algae were used – *C. sorokiniana* (UTEX 1230) and *N. oculata* (UTEX 2640). These species are fresh water microalgae and marine microalgae, respectively; however, *C. sorokiniana* is halotolerant, and therefore it can grow in desalination concentrate (Ramikrishan, 2014). Growth media suitable for the strains were prepared based of recipe in Table 2: BBM was used for *C. sorokiniana*, and F/2 was used for *N. oculata*. The starting algae used for the experiment were obtained from the University of Texas Algae Collection in Austin, Texas. The algae were obtained in a volume of 500 mL, then cultured and retrieved at exponential growth stage at the concentration 3.7×107 cells/mL.

3.3 Design of experiment

The first set of experiments was designed to investigate how ROC and blended ROC affect the growth of the two studied algae strains. Six different growth media were used in the experimental design. For *C. sorokiniana*, these growth media were BBM, ROC, B-ROC, and deionized (DI) water. For *N. oculata*, the growth media were F/2, ROC, F-ROC, and DI water. The DI water was used as a control. The F/2 and BBM media were prepared by adding growth medium components, trace elements, and vitamin solutions to 950 mL of DI water, based on the components shown in Table 5.

ROC was obtained from the RO pilot plant desalination system at the Brackish Groundwater National Desalination Research Facility (BGNDRF) in Alamogordo, New Mexico. BGNDRF has access to four brackish groundwater wells with a wide range of salinities.

Table 5. Components of the F/2 (Guillard, 1978) and BBM (Stein, 1973) growth media.

Component	F/2	BBM
$NaNO_3$ (g/L)	75.00	25.00
$NaH_2PO_4 \cdot H_2O$ (g/L)	5.00	17.50
$Na_2SiO_3 \cdot 9H_2O$ (g/L)	30.00	30.00
F2 Trace Metal Solution (mL)	1.00	
F2 Vitamin Solution (mL)	0.50	
NaCl (g/L)		2.50
H_3BO_3 (g/L)		5.75
Trace Elements		
$FeCl_3 \cdot 6H_2O$ (g/L)	3.15	
$Na_2EDTA \cdot 2H_2O$ (g/L)	4.36	

$CuSO_4 \cdot 5H_2O$ (g/L)	9.80		
$Co(NO_3)_2.6H_2O$ (g/L)		0.05	
$Na_2EDTA \cdot 2H_2O$ (g/L)		4.36	

The details of the utilized RO system and the conditions and availabilities of the brackish water in BGNDRF are provided by Karimi et al. (2015). The electrical conductivities (ECs) of the different growth media are given in Table 6. Additionally, the ionic concentration of the ROC, which was obtained from ICP-OES analysis, is provided in Table 7.

Table 6. Electrical conductivities of media.

Medium	BBM	F/2	ROC	B-ROC	F-ROC
EC (mS/cm)	13.2	15.4	9.78-10.44	11.32-11.6	12.4-13.7

Table 7. Ion content in the desalination concentrate.

Ion	K^+	Na^+	Mg^{2+}	Ca^{2+}	TN*	S	P
Concentration (mg/L)	40.5	880	461.2	905.2	193	1491	17.8

* Total N

A second set of experiments was conducted to determine how different levels of P, N, and CO_2 affect microalgae growth in ROC. The resulting data was used to develop a predictive model that can be used to optimize microalgae growth in concentrate. In this set of experiments, two levels of P (15 and 75 parts per thousand [ppt]) and two levels of N (15 and 75 ppt) were added to ROC at three different CO_2 concentrations based on the volume percentage (0.03% [ambient air], 2%, and 5%).

The fractional factorial design was developed for experiments at different combinations of different factors, as shown in Table 8. For the P and N columns in this table, H indicates 75 ppt and L indicates 25 ppt. In the CO_2 column, L indicates 0.03% CO_2 by volume, M indicates 2% CO_2 by volume, and H indicates 5% CO_2 by volume. Since preliminary experiments identified N as an essential nutrient in growth media for microalgae, most combinations in this set of experiments fixed N at the highest investigated level of 75 ppt. The growths of the two algae strains were measured using the cell counting methods described in

Section 2.3, and the maximum growth of algae was observed during the 10th day, shown in the growth charts for C. sorokiniana (Figure 7) and *N. oculata* (Figure 8). Therefore, the response for data analysis was considered the amount of algae growth by day 10.

Table 8. Combinations of different factors.

P (Level)	N (Level)	CO_2 (Level)
H	L	L
H	L	M
H	L	H
L	H	L
L	H	M
L	H	H
H	H	L
H	H	M
H	H	H

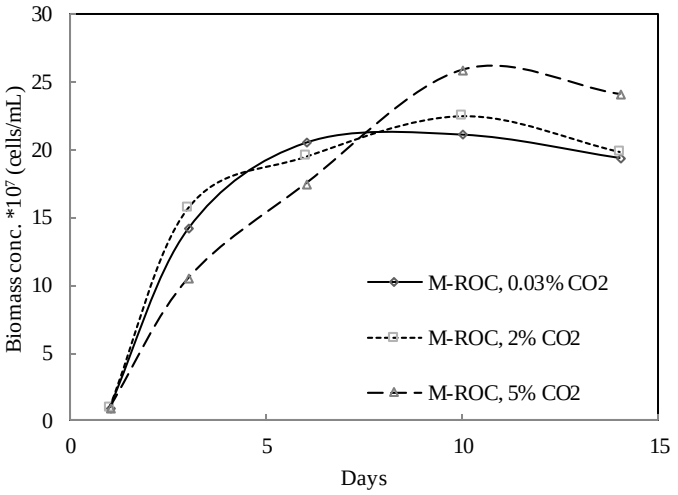

Figure 7. Growth pattern for *C. sorokiniana* species in M-ROC.

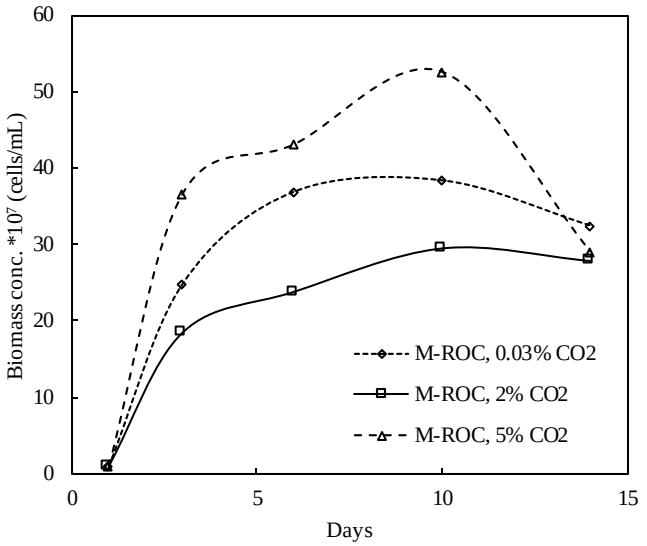

Figure 8. Growth pattern for *N. oculata* species in M-ROC.

At the end of the analysis, the best combination of nutrient were studied for ion content removal. Supernatant from each strain were analyzed on ICP-OES spectroscopy, and the heavy metal concentrations were evaluated.

3.4 Calculations and statistical analysis

Algae growth was reported in concentrations of cells per milliliter of culture, using the equation for Neubauer chamber calculation (Oscar, Technical note-Neubauer chamber cell counting, 2009), shown in Eq. (5):

$$Concentration\ (cells/mL) = \frac{(No.of\ cells \times 10{,}000)}{(No.of\ square \times dilution)} \qquad \text{Eq. (5)}$$

In the first set of experiments, the visual differences between the growths of algae in different growth media were investigated, and statistical analysis was conducted to discern how the studied parameters affect the growth of algae. Equation 1 was also used for the second set of experiments, and from the results, statistical analysis using Minitab 16 was conducted to develop a predictive model for algae growth.

Algae metal reductions were analyzed using difference in metal concentrations before and after culture. The concentrations were from ICP-OES analysis of the

desalination water before the experiment, and supernatant of algae culture after the experiment.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Effect of Media on Growth of Microalgae

As shown in Figures 7 and 8, the exponential growth of algae cells ended at approximately the tenth day of the experiment for both of the algae strains. Experimental results indicated that the growths for these algal strains varied between the species and among the different media under ambient air CO_2 levels, with the cell concentrations for the strains ranging from 5×10^7 cells/mL to 2.3×10^8 cells/mL for C. *sorokiniana* and 5×10^7 cells/mL to 3.7×10^8 cells/mL for *N. oculata*. The effect of media on the growth of C. sorokiniana is shown in Figure 9, which depicts that, although the concentration of main nutrients such as N and P in B-ROC was half the concentration in the BBM media, the maximum growth of C. sorokiniana was obtained when B-ROC was used as the media. The main reason for the better growth of C. sorokiniana in the medium of B-ROC can be attributed to its lower salinity compared to BBM, because C. sorokiniana is a fresh water strain and can survive in fresh water media. However, C. sorokiniana could not grow well in the DI water and ROC media because of the lack of nutrients. Additionally, the growth of C. sorokiniana was lower in ROC compared to two other conditions, because although ROC had less salinity compared to B-ROC, it had little N, a main nutrient required for algae growth (Raghawan, 2008).

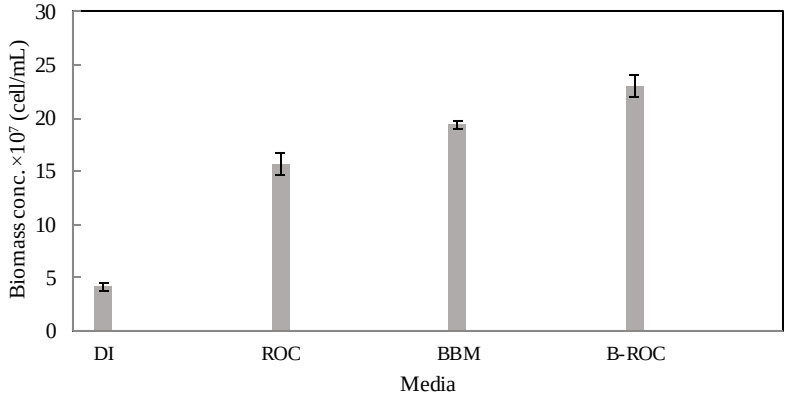

Figure 9. *C. sorokiniana* growth in different media at ambient air CO_2 level.

The influence of media on the growth of *N. oculata* was also investigated, and the results are shown in Figure 10, which shows that the maximum growth of *N. oculata* was obtained when F-ROC was used as the growth media. Although there was no significant difference between *N. oculata* growth in F-ROC and *N. oculata* growth in F/2, the absence of a significant difference means that F-ROC can replace F/2, thereby offering dual benefits for managing brine in inland desalination plants and cultivating *N. oculata* as biomass for biofuel production at lower cost. However, similar to the results observed for the growth of *C. sorokiniana*, ROC resulted in lower growth due to the lack of main nutrients such as N and P. The DI water, used as a control medium for both algae strains, resulted in a small amount of growth for both algae strains.

In a comparison of the growth results for both algae strains, it was found that *N. oculata* grew faster than *C. sorokiniana* in the investigated media, likely because *N. oculata* is a marine algae species and the concentrate media are highly saline. Additionally, *N. oculata* grown in F-ROC media under ambient air conditions produced the highest growth at the 10^{th} day, with a cell concentration of 3.7×10^8 cells/mL.

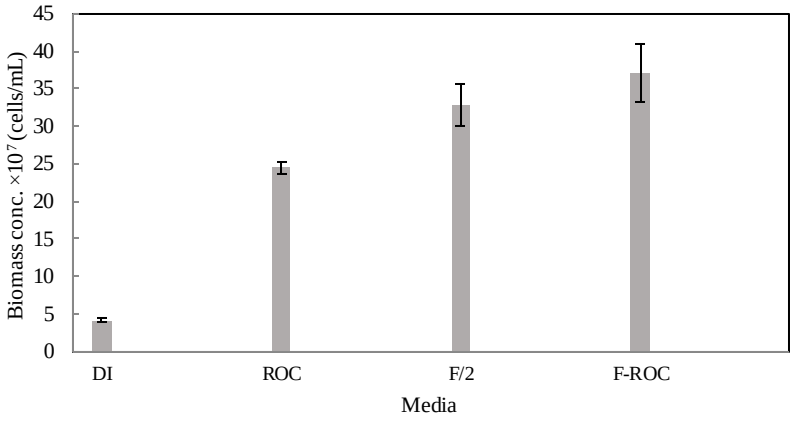

Figure 10. *N. oculata* growth in different media under ambient air CO_2 level.

As compared to ROC and DI water, the conventional media exhibited higher levels of algae growth, which is attributable to the N and P levels in conventional media. This finding led to further study of the concentrate water to produce even better results, which are presented in the following section.

4.2 Predictive Model

In the literature, both wastewater and desalination concentrate have been considered as potential sources of water and media for the production of algal biomass (Hussein, 2013). However, it has been a challenge to select proper conditions under which algae can be grown in concentrate, as there are no established criteria as of yet. According to our experience in algae-based biofuel research and as shown in Section 3.1, a promising medium is expected to satisfy the following requirements: 1) have a high nutrient content, 2) produce a high growth rate, and 3) produce a high cell density at the end of stationary growth stage. According to our recent findings on the effects of media on algae growth, M-ROC was chosen for further investigation on the influence of P, N, and CO_2 levels.

A fractional factorial design of experiments was developed, and the effects of the target parameters were modeled. Linear regression analysis using Minitab software was employed for this purpose. The model was derived using algae concentration as the dependent variable, with N, P, and CO_2 concentrations as the independent variables. In the regression analysis, not only were the three main independent variables taken into account, but all possible interactions among the parameters were incorporated into the model to investigate the possibility that different parameters may have different effects at different levels of other parameters.

In pre-analysis before the regression model was developed, the partial least squares method was used to screen the significant parameters. The selection plot from the procedure determined that the optimal model should have 4 terms for *C. sorokiniana* and 5 terms for *N. oculata*. Loading plots from the partial least squares procedure further clarified the interaction terms that should be eliminated to yield the optimal model: for *C. sorokiniana*, P × N, CO_2, and P × N × CO_2 were eliminated; for *N. oculata*, P × N and P × N × CO_2 were eliminated.

Regression analysis was conducted for the *C. sorokiniana* strain, and the effects of the studied parameters were investigated. As shown in Table 5, the levels of individual parameters, such as P and N, had significant effects on the growth of *C. sorokiniana*, while the effect of CO_2 depended on the level of N and P, as shown by p-values less than 0.05. Based on the effective variables and their interactions, a linear regression model was developed (Equation 6).

Table 5. Factors significance in regression model for *C. sorokiniana*

Source	Coef.	SE Coef.	*T*-value	*p*-value
Constant	13.094	0.926	14.15	0

P	-0.0395	0.0117	-3.36	0.004
N	0.0756	0.0102	7.39	0
P*CO_2	-0.000615	0.000227	-2.71	0.015
N*CO_2	0.001099	0.000239	4.6	0
R^2	94.88%		R^2 (adj)	93.67%
R^2 (pred)	91.99%		No. of obs.	21

$$C.\ sorokiniana\ concentration = 13.094 - 0.0395 \times P + 0.0756 \times N - 0.000615\ P \times CO_2 + 0.001099\ N \times CO_2 \qquad Eq.\ (6)$$

As shown in the regression equation, increases in N promote the growth of *C. sorokiniana*. The growth of this algae strain also proved more sensitive than *N. oculata* to variations in the concentration of N. Notably, P had a negative coefficient for microalgae growth in ROC, a result very different from findings in conventional media, where P serves as an essential nutrient. This result merits further research. The effects of P and CO_2 and the effects of N and CO_2 were not independent of each other, as shown by their combined variables. Therefore, an optimum condition should be chosen in consideration of the interactions between these variables. The interaction graphs for the above-mentioned variables are plotted in Figures 11 and 12, which show the interactions between P and CO_2 and N and CO_2, respectively. In the experiments behind Figure 11, N was held constant at 75 ppt, and in the experiments behind Figure 12, P was held constant at 75 ppt. As can be seen in Figure 11, higher levels of CO_2 yielded higher *C. sorokiniana* growth. From Figure 12, it is determined that, although a higher concentration of CO_2 resulted in a higher growth of *C. sorokiniana* at the studied concentration for P when N levels were above 44 ppt, a lower concentration of CO_2 yielded better results at 75 ppt P when the concentration of N was kept at levels lower than 44 ppt.

Ultimately, considering the data reported in Figures 11 and 12, the maximum growth of the *C. sorokiniana* strain (2.68×10^8 cells/mL) in the studied range of parameters can be obtained at higher concentrations of CO_2 and N but at lower concentrations of P.

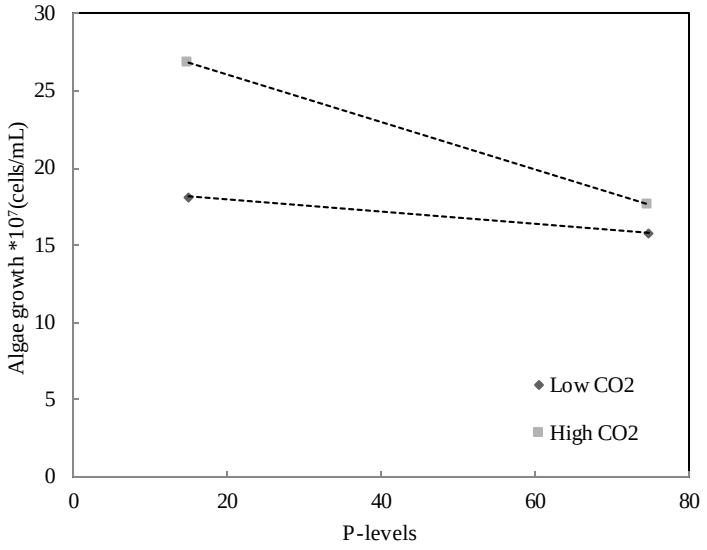

Figure 11. The effect of P-CO_2 interaction on the growth of *C. sorokiniana* at 75 ppt of N.

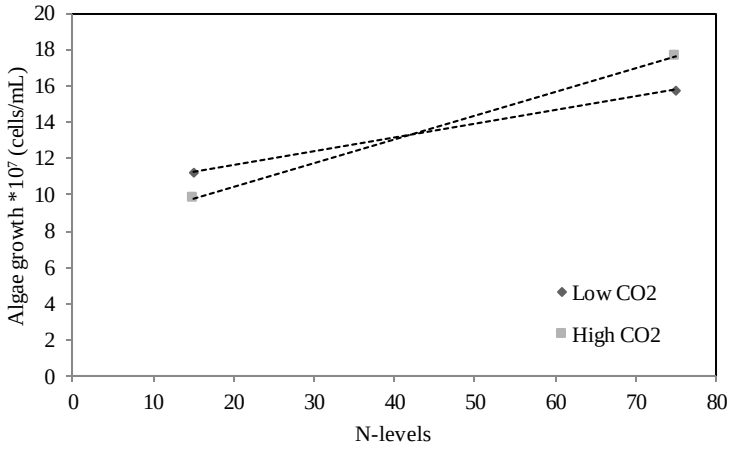

Figure 12. N-CO_2 interaction effect for *C. sorokiniana* at 75 ppt of P.

The partial least squares method was also applied to choose the appropriate variables in the regression analysis. On the basis of this method, the interactions P × N and P × N × CO_2 were eliminated for the *N. oculata* strain; the loading plots from the partial least squares procedure showed their insignificance.

Regression analysis was conducted for the *N. oculata* strain, and the effects of the studied parameters were investigated. As shown in Table 6, the levels of individual parameters, such as P, N, and CO_2, have significant effects on the growth of *N. oculata*. Additionally, the effect of CO_2 varies with the levels of N and P, as shown by *p*-values below 0.05 for these interaction terms. Based on the effective variables and their interactions, a linear regression model is presented, as shown in Equation 7.

Table 6. Factor significance in regression model for *N. oculata* growth.

Source	Coef.	SE Coef.	T-value	P-value
Constant	13.31	4.53	2.94	0
N	0.1119	0.0471	2.37	0.029
P	0.2806	0.0445	6.31	0
CO_2	0.621	0.14	4.45	0
P*CO_2	-0.01159	0.00138	-8.39	0
N*CO_2	0.00464	0.00144	3.21	0.005
R^2	93.80%		R^2 (pred)	89.78%
R^2 (adj)	92.08%		No. of obs.	23

$$N.\ oculata\ concentration = 13.31 + 0.1119 \times N + 0.2806 \times P + 0.621 \times CO_2 - 0.01159\, P \times CO_2 + 0.00464\, N \times CO_2 \qquad Eq.\ (7)$$

As shown in Equation 7, increasing the concentration of N in the medium has a purely positive effect on the growth of the *N. oculata* strain, a result similar to the findings for *C. sorokiniana*.

Figures 13 and 14 show the effects of combinations of factors on the predicted growth values of *N. oculata*. In Figure 13, N was held constant at 75 ppt; in Figure 14, P was held constant at 75 ppt. As illustrated in these figures, although a higher concentration of CO_2 is typically desired in the studied range of P, a lower concentration of CO_2 is preferred at P levels of 75 ppt and N levels below 55 ppt. From these figures, it is concluded that the maximum growth for *N. oculata* (6.5×10^8 cells/mL) occurs at the highest concentrations of N and CO_2 but at a lower concentration of P.

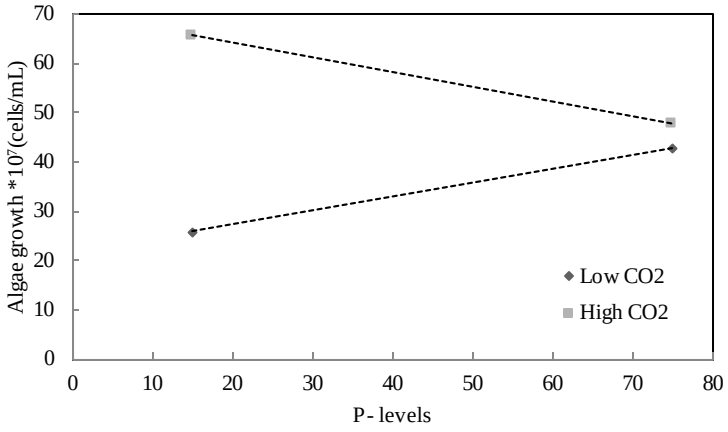

Figure 13. P-CO_2 interaction effect for *N. oculata* 75 ppt of N.

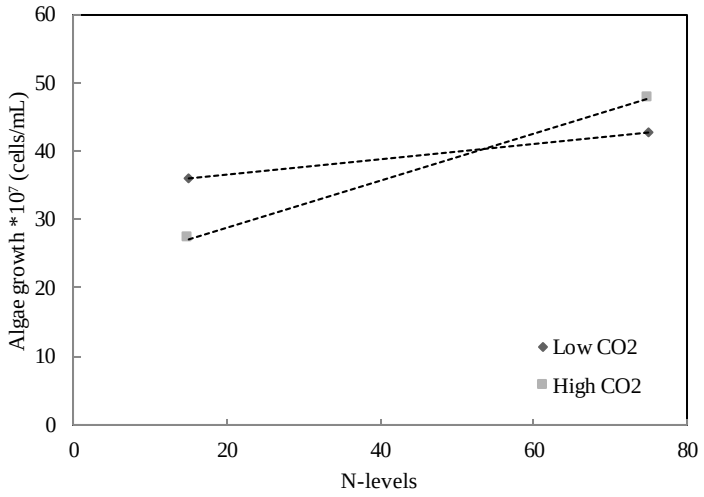

Figure 14. P-CO_2 interaction effect for *N. oculata* 75 ppt of P.

4.3 Ion removal

Figures 15 and 16 offer an analysis of the concentrations of heavy metals before culturing and after the culturing period for both algae species. This result showed

a reduction in metal concentration for *C. sorokiniana* species at all CO_2 levels. Significant reductions in metal concentration were observed at 2% and 5% CO_2 levels. Copper metal was the exception, as it showed an increase in concentration at the end of experiment.

N. oculata species increased the concentrations of the metals at the end of experiment, with the exception of bismuth. These increases in metal concentration may be attributed to bacterial or protozoan breakdown or conversion of other metals present in the desalination water, leading to the accumulation of those heavy metals.

As observed from the results, the differences in species affected the reduction of and tolerance for the heavy metals.

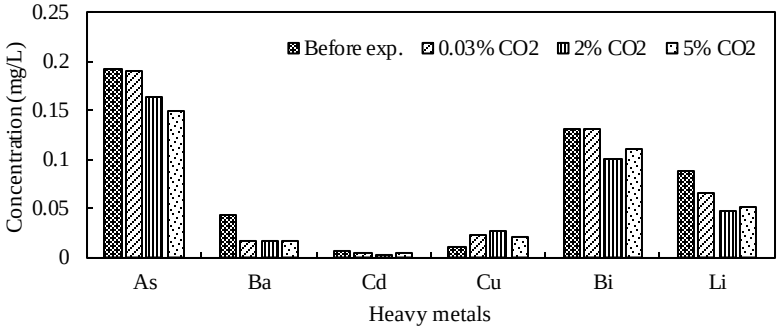

Figure 15. Ion removal in *C. sorokiniana*.

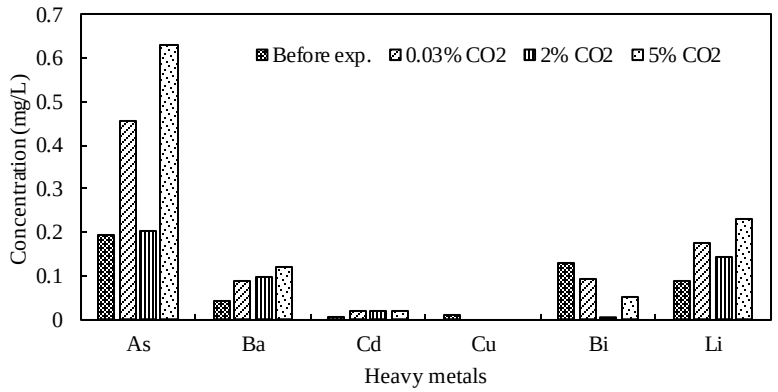

Figure 16. Ion removal in *N. oculata*.

CHAPTER 5: CONCLUSION

The results obtained in this research show that desalination concentrate can be utilized as a microalgae growth medium; however, adding nutrients, such as N, resulted in better microalgae growth. The modified desalination concentrate provided the optimum conditions for cultivating algae. This study suggests that, if N and CO_2 are maintained at a high ratio to P in concentrate water, both freshwater and marine strains of algae could thrive well. In unmodified ROC, *N. oculata* showed better performance than *C. sorokiniana*, achieving roughly 55% higher growth. The use of such species in a growth medium of concentrate or modified concentrate could help alleviate water scarcity in society while providing biomass to curb energy stress.

Differences in heavy metal reduction were observed with the selected microalgae species, as heavy metal concentrations increased in the cultivation media for *N. oculata* but decreased in the cultivation media for *C. sorokiniana*. This suggests that *Chlorella* species is among the species that can tolerate elevated heavy metal concentrations.

Although the algae strains showed good performance in this laboratory experiment, more intensive investigations regarding even lower levels of P and higher levels of N and CO_2 should be implemented in the future, particularly under field stress conditions (like light stress, salt stress, etc.). Lipid content for the strains could also be investigated for nutrient fluctuations. Additionally, future research could pursue an explanation for the negative impacts of high P, usually regarded as a nutrient, when concentrate or blended concentrate is used as a growth medium.

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Part II: Pilot Scale Cultivation of Microalgae in Desalination Concentrate

Abstract

Recent research has demonstrated that microalgae grow well in desalination concentrate. The concentrate can serve as a growth medium for the algae, while the algae remove contaminants from the concentrate. However, more investigation is necessary before this method can be implemented on a practical scale. The majority, if not all, of the published research in this field has been completed at the lab scale. Therefore, this research performs a bench-scale cultivation of microalgae in concentrate (brine) to investigate how well *Chlorella sorokiniana* (UTEX 1230) grows in brackish water desalination concentrate. UTEX 1230 was cultivated in two indoor raceway ponds with different concentrations of brine. The experiment was repeated once. Cell population growth, contaminant removal, and the evaporation rates of each pond were examined.

CHAPTER 1: INTRODUCTION

The viability of future generations depends on our commitment to live sustainably today. Five major sustainability concerns are water, food, energy, environmental health, and economic stability. Our current practices in these five areas are unsustainable, and the population growth in most of the world exacerbates the problem. Microalgae cultivated in desalination concentrate can contribute to a viable future in all of these areas.

Treating saline water sources is necessary to meet the growing demand for water resulting from population growth and industrialization. The expansion of saline water treatment is largely inhibited by financial and environmental concerns involved with concentrate disposal. Since algae are enhanced by and remove contaminants from desalination concentrate, their cultivation can alleviate the economic and environmental obstacles hindering the expansion of desalination techniques.

Algae can also contribute to a more sustainable food supply. Microalgae cultivation has the benefits of first-generation biofuels without the disadvantages of requiring arable land or competing with crops. In addition, microalgae may be used for producing nutritional supplements or as an ingredient in animal feed.

Furthermore, microalgae cultivation in concentrate can alleviate the energy crisis by providing feedstock for biodiesel, ethanol, or biogas production. Moreover, algae-based fuels can be used without net carbon dioxide emissions.

Growing microalgae in concentrate can also generate economic value by producing various products and reducing the cost of concentrate disposal. For all of these reasons and more, algae grown in concentrate medium can be a solution to many sustainability concerns.

According to a literature review, the "only two feasible methods available for large-scale production of microalgal biomass" are raceway ponds and tubular photobioreactors [1]. However, the vast majority, if not all, published research on microalgae concentrate management has been conducted at the laboratory scale (Hussein W.Z., 2014; Hussein, Myint, & Ghassemi, 2014; Matos, Morioka, & Sant'Anna, 2011; Matos et al., 2013; Maos et al., 2014; Matos et al., 2015; Matos et al., 2014; Morioka et al., 2014; Myint, 2014; Myint, Ghassemi, and Nirmalakhandan, 2010). This echoes a problem that inhibits algae-based biofuels

in general. As reported by the Food and Agriculture Organization of the United Nation, "Due to a lack of industrial scale experiments, there is insufficient knowledge to adequately judge the economic viability" of algae-based biofuels; "Productivity data is often extrapolated from small experiments and not always presented clearly and consistently" [15].

Chlorella sorokiniana (UTEX 1230) is a freshwater species of microalgae that is adaptive to highly saline environments. In previous lab-scale experiments, UTEX 1230 grew the best in desalination concentrate when compared to six other prospective species. It was therefore chosen to be cultivated in two indoor raceway ponds on a pilot scale. The experiment was run for 31 days under conditions that have been successful in previous research projects. After the first experimental run, the experiment was repeated with a length of 24 days to gain perspective on the variability of the results.

CHAPTER 2: MATERIALS AND METHODS

Desalination concentrate was taken from the Brackish Groundwater Desalination Research Facility (BGNDRF) in Alamogordo, New Mexico. This concentrate was obtained via reverse osmosis desalination of brackish water. The desalination concentrate in the first run of the experiment came from Well 2 at BGNDRF, whose water chemistry has been analyzed in the past and is somewhat consistent. Previous analyses of Well 2 water can be found at the referenced website [16]. An analysis of the ions in concentrate from Well 2 can be found in Paruchuri's referenced work, though this concentrate was produced by electrodialysis reversal (EDR) rather than the RO used in this experiment [13]. Trace element analyses of the concentrate used in both of the experimental runs are located in the appendix of this paper.

Chlorella sorokiniana (UTEX 1230) was obtained from the University of Texas and scaled up in a laboratory. It was subsequently cultivated in 10-L bioreactors at the same facility as the indoor ponds. The bioreactor algae were fed with the same nutrient source as the indoor ponds.

UTEX 1230 was cultivated in two 35-foot indoor raceway ponds at the New Mexico State University WERC A-Mountain greenhouse in Las Cruces, NM. One pond (Pond 7) contained only desalination concentrate and nutrients. The other pond (Pond 8) contained nutrients and concentrate that had been diluted with city water to approximately half of its original electrical conductivity. Both indoor ponds were in a humidified greenhouse. Ambient air was bubbled through both ponds using a 2-horsepower pump during the first run and a 1-horsepower pump during the second run. During the first run of the experiment, a paddle wheel in each pond operated at 24 rpm to ensure raceway circulation. During the second run, an air lift system was constructed to replace the paddle wheels, taking advantage of the air delivery system that was already running. The indoor raceway ponds used in the experiments are shown in Figures 1 and 2, and the bioreactors used to cultivate the inoculating algae are shown in Figure 3.

In Table 1, information is tabulated on the conditions of the ponds at the beginning of each experimental run. The listed information includes the volume of algae taken from the bioreactors for pond inoculation, the amount of nutrients initially fed to each pond, the depths of the ponds, and the ponds' electric conductivities after pond inoculation and feeding.

Table 1. Pond conditions on Day 1.

Volume of Inoculant		Amount of Miracid		Depth (in) Ponds 7/8		Conductivity (mS): Ponds 7/8	
Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
33 L each	50 L each	1 lb. each	2 lb. each	12/11.5	13/13	8.21/4.40	8.6/4.3

Figure 1. Indoor raceway ponds for algae cultivation. Experiment Run 1.

A commercial fertilizer (Miracid) was used as the nutrient for algae cultivation. This fertilizer has a 30:10:10 ratio of N:P:K and also contains trace elements. It has worked well as a nutrient source in previous experiments. The fertilizer was fed to the ponds at an amount in excess of a previously established feeding quantity based on nitrogen (0.1 g N/L/month). In experimental runs 1 and 2, both ponds were fed on day 15 after measurements had been taken (1 lb. in Experiment

1 and 2 lbs. in Experiment 2). In Experiment 1, both ponds were fed about 1/3 lb. of fertilizer on day 27 after measurements had been taken.

The first experimental run ran for 31 days, and the second run ran for 24 days. Depth, pH, and electrical conductivity typically were measured five times per week. Temperature was also measured five times per week during the first run. Photos were taken of the ponds throughout the experiment. Cell count and cell sizing were conducted twice each week. A water analysis was completed once each week.

Conductivity was measured using a Hach sensION5 Conductivity Meter. The pH was measured using an Accumet® Basic AB15 pH meter. Temperature was measured using a multimeter. Cell counts were measured using a Hausser Scientific Hemocytometer. Cell counts measured the average UTEX 1230 population in a 4-nL volume. Cell sizing was completed by approximating the average diameter of the UTEX 1230 using an Olympus BX60 microscope. Water analysis was conducted via EPA method 200.7, which uses inductively coupled plasma optical emission spectroscopy (ICP-OES), after algae were filtered out of the mediums with a 0.2 µm filter. Routine measurements began on day -1 of the experimental runs. Raw data are listed in the appendix.

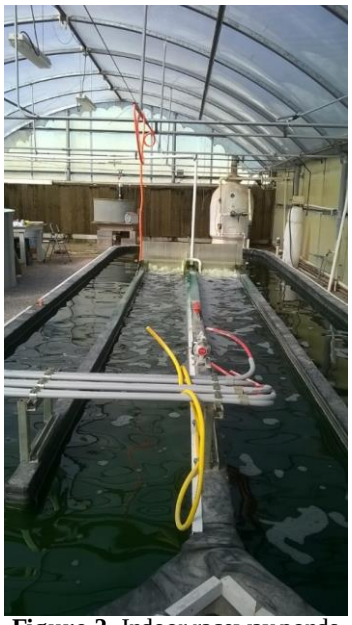


Figure 2. Indoor raceway ponds for algae cultivation. Experiment Run 2.

Figure 3. Bioreactors for Pond Inoculation.

Algae growth in each pond was contrasted to examine the effects of brackish water desalination concentrate on UTEX 1230 growth. Also, evaporation rates and contaminant removal were examined in each pond.

CHAPTER 3: RESULTS AND ANALYSIS

3.1 Population Analysis

During the first run of the experiment, both ponds exhibited healthy cultivation until after population measurements were taken on day 12 (see Figure 4). Pond 7 initially had a stronger growth curve, but it had a lower cell count than Pond 8 on day 15. After a drop in the populations of both ponds between days 15 and 19, the ponds exhibited small recoveries. Finally, the ponds exhibited large declines in cell population after Day 22.

Pond 7 showed higher cell counts than Pond 8 throughout the second run. Both ponds demonstrated population declines after days 6-8. While Pond 8 had a small recovery after its initial population drop, similar to the two ponds during the first run, Pond 7 merely exhibited an increase in population growth rate slope (a less negative slope in Figure 4 between day 12 and day 15).

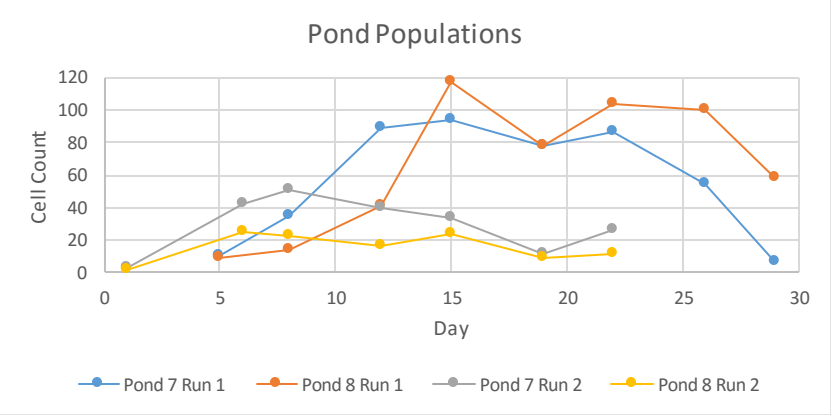
At the beginning of the second run, both ponds demonstrated higher cell counts than their counterparts from the first run (likely due to a higher volume of inoculant). Still, the ponds exhibited unhealthy cell counts by Day 12. In all four pond cultivations, an increase in cell population growth rate was followed by a decrease in growth rate, which was in turn followed by an increase, then a decrease, in population growth rate.

The first-run population drops after day 22 are likely due to foreign species growing in the ponds. Beginning on this day, foreign species were observed to be prevalent in Pond 7 during cell counts. In addition, occasional cells of a contaminating species were found in Pond 8 on both day 19 and day 22.

The contaminating species in Pond 8 had an appearance similar to *Volvox* species, but it was much smaller. The entire cell had a diameter that was only a few times as large as that of a UTEX 1230 cell; it was more prevalent in later cell counts. During the cell count on day 26, foreign species were spread throughout Pond 8. Pond 7 continued as the more highly contaminated pond for the rest of the experiment.

Similarly, the second run's early population drops are likely due to foreign organisms that were observed in the ponds on day 8. On day 12, foreign species appeared to be more dominant in Pond 7 than in Pond 8.

Figure 4. Cell counts of UTEX 1230 in cultivation ponds.



The reasons for the initial population drops in the first run are less clear than the reasons for the other population drops. Still, it is hypothesized that the initial population drops in the first run were also due to contamination, which was demonstrated by algae cells sticking together. The cells may have been stuck together by a biofilm-like substance that is described in later paragraphs. It was noted that many algae cells in Pond 7 were in small groups on day 15. Moreover, the appearance of the algae in both ponds was different on this day than on preceding days, with the algae cells appearing to have an extra coating. On day 19, both ponds had sporadic groups of algae that appeared to be stuck together. The groups of algae were sparser in Pond 8, which could explain why Pond 8 had a better recovery between day 19 and day 22.

The early deceleration of algae growth in Pond 7 during the first run may also be attributable to researchers harvesting algae in Pond 7, but not in Pond 8. This harvesting was conducted again toward the end of the experiment. The days of these harvests are listed in Table 2, along with other events that may have affected experimental measurements.

Uncertainty in interpreting the results of fed-batch experiments is not unheard of. This phenomena is referred to in another research paper: "Despite the application of [batch and fed-batch cultivations] on the evaluation of biomass composition, the microorganisms are submitted to many variables during cultivation, especially nutrient concentration, which makes it difficult to match a biomass composition variation to a certain cause" [8].

Table 2. Events that may have affected experimental measurements.

Run	Day(s)	Event that May Have Affected Measurements
1	4-5	There was a blow-off in the air pump during the weekend.
1	8	Both ponds were refilled with city water to replace evaporative losses after measurements were taken.
1	15	The ponds were fed 1 lb. of fertilizer each after measurements.
2	15	The ponds were fed 1 lb. of fertilizer each after measurements.
1	22	Both ponds were refilled with city water to replace evaporative losses after measurements were taken.
1	27	The ponds were fed about 1/3 lb. of fertilizer each after measurements.
1	13 – 18	First harvest in Pond 7: after Day 13 measurements and before Day 18 measurements.
1	20 – 24	Second harvest in Pond 7.

During the first run, the foreign species in Pond 7 were often more dominant than those in Pond 8 during cell counts. This was also observed on day 12 of Experiment 2. In addition, the ponds were contaminated earlier during the second run of the experiment than during the first run. It is proposed that these two phenomena can be explained by a factors that was specific to the facility in which the algae were cultivated: how much contaminated air was blowing over each algae cultivation pond.

The wet wall in the greenhouse had algae growing in it. This was typically combatted by bleaching the water that was fed into the wet wall. However, the wet wall was not algae-free during either experimental run. Particularly, during the second run, the wet wall was bleached much less often than during the first experimental run. Upon examining the algae from the wet wall under the microscope during the second run, it was found that the algae were fused together by what appeared to be a biofilm (Figure 5). During the second experimental run, algae bound in a similar biofilm-like substance were also observed in the foam of the ponds after their populations had dropped (Figure 6). This could explain why the ponds were contaminated more quickly during the second run.

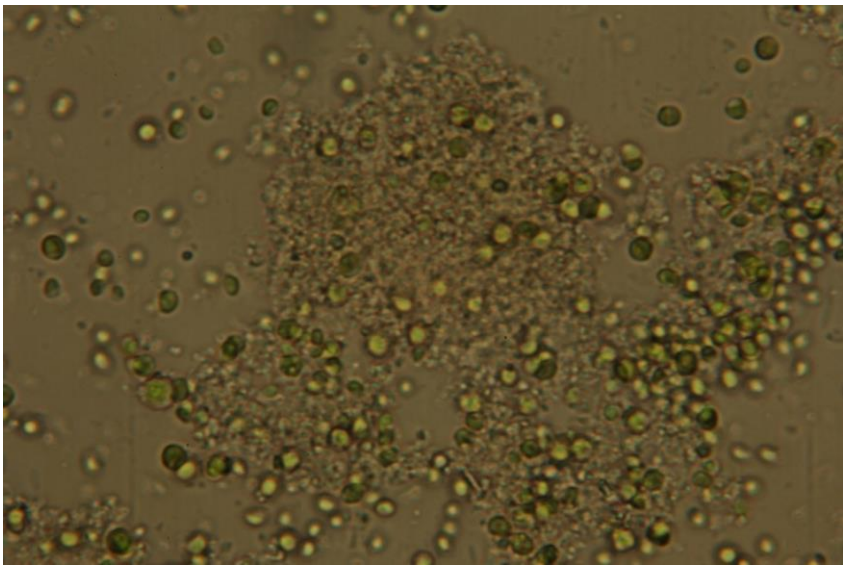


Figure 5. Algae biofilm from wet wall sample on day 20 of the second experimental run.

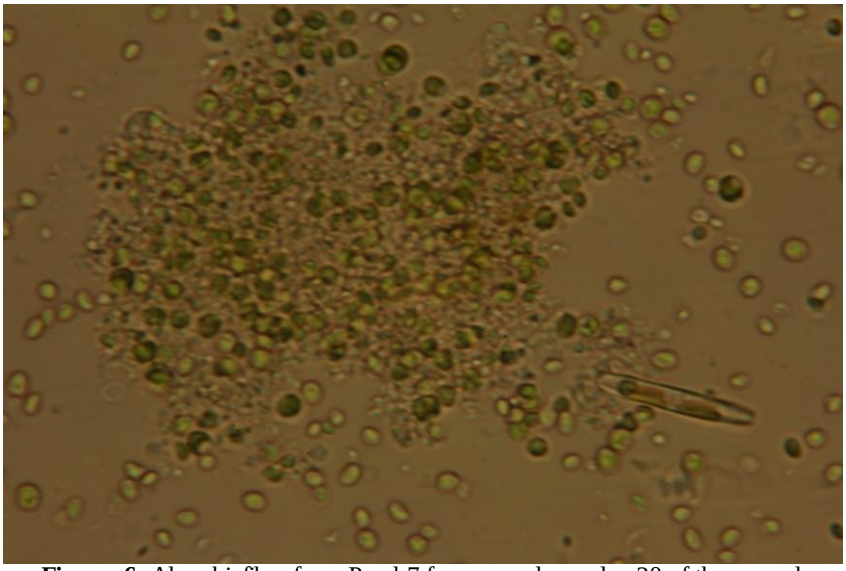


Figure 6. Algae biofilm from Pond 7 foam sample on day 20 of the second experimental run.

The tendency of Pond 7 to be more affected by contamination than Pond 8 may be explained by three factors. First, the wet wall in the greenhouse blew directly over Pond 7, while it did not directly blow over Pond 8; there was an obstruction in front of part of Pond 8. The second factor was Pond 7's proximity to a greenhouse entrance. Oftentimes, the entrance was left open for a short period, allowing outside wind to blow over the ponds. Since Pond 7 was closer to this entrance, it was more exposed to the contamination source. Pond proximity to the greenhouse entrance may have also contributed to the variety of contaminating species in the ponds that were not noted in the wet wall. A third possible reason for Pond 7's tendency to be more contaminated may be related to the water chemistry of the pond. However, it is expected that this is not the case. Further experimentation, in which contamination is better controlled, or in which the two growth media switch locations, may be worthwhile.

If pond composition did not significantly affect how readily ponds were contaminated, the cultivation medium used for Pond 7 (desalination concentrate and nutrients) appears to be a better growth medium than the medium in Pond 8 (nutrients and desalination concentrate diluted with city water to half of the concentrate's normal conductivity). Pond 7 displayed better population counts than Pond 8 during the periods when the ponds were not contaminated.

3.2 pH Analysis

Microalgae population declines were associated with unusual changes in pH. In other research, microalgae health was also linked to pH changes in a medium with high TDS [17]. Further investigation into using pH as an indicator of pond health may be warranted. The pH measurements for cultivation ponds during the first experimental run are plotted in Figure 7. The pH measurements from the second run are omitted because of a discrepancy in the validity of pH measurements.

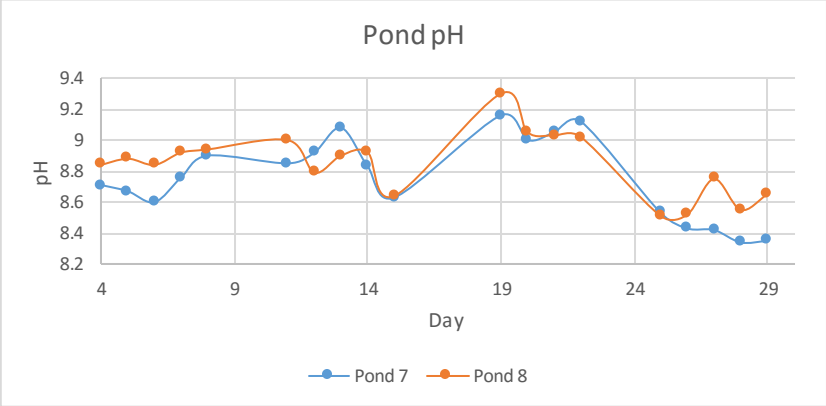


Figure 7. pH of cultivation ponds during the first experimental run.

3.3 Conductivity and Depth Analysis

Overall, the conductivities of the growth mediums were not significantly reduced. This can be seen by the correlation between conductivity and depth measurements during the first run. If conductivities are examined at points where a pond has the same depth on different days, it becomes apparent that only small differences are observed in conductivities over time. This is probably due to the composition of the fertilizer that was used.

This conclusion is supported by previous research. Spirulina platensis growth in desalination concentrate with F/2 as nutrients led to an increase in the conductivity of the growth medium (Hussein W. Z., 2014). Conversely, S. platensis growth in concentrate with supernatant anaerobic digested sludge (SADS) as nutrients led to a decrease in conductivity. This indicates that nutrient source affects conductivity reduction.

A lack of conductivity reduction, however, does not necessarily discount the current approach in the field of concentrate management. This approach could be incorporated with concentrate disposal via evaporation ponds, providing a revenue stream for owners of evaporation ponds. Still, it is likely that better concentrate management prospects would appear if a different nutrient source were used.

Figure 3. Electrical conductivity of cultivation ponds.

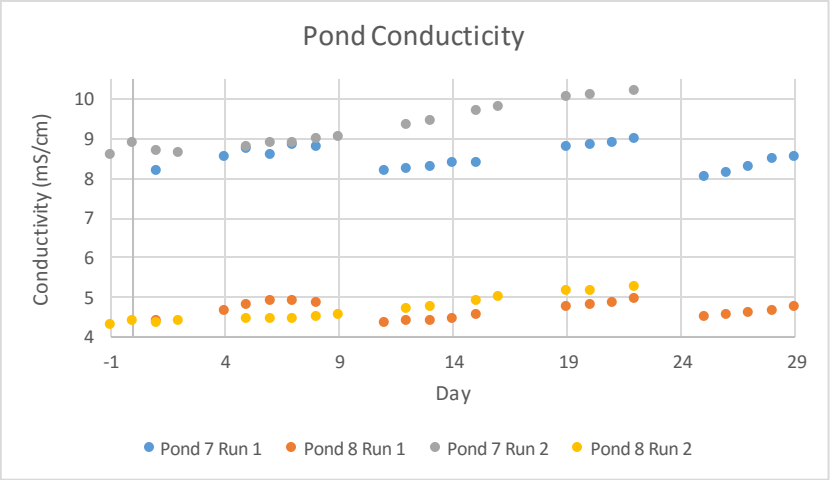
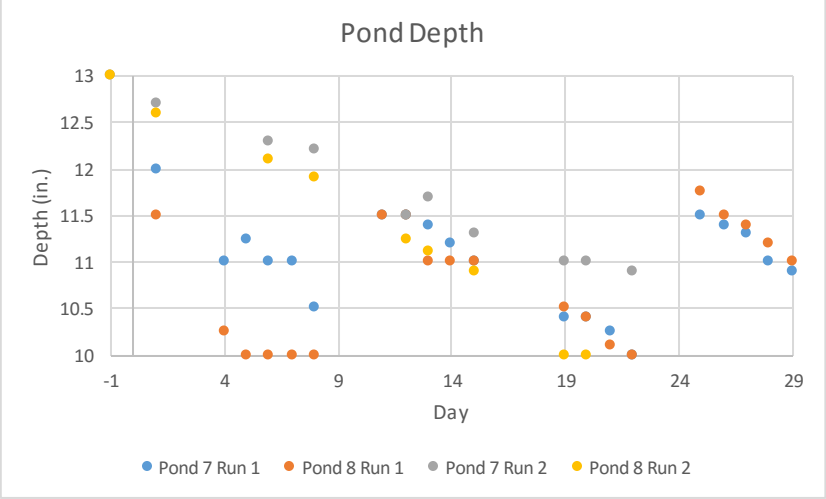


Figure 4. Depth of cultivation ponds.



The average daily evaporative loss was 0.13"/day in both ponds during the first run. Since each pond has a surface area of approximately 172 ft^2, this equates to a water loss of 14 gal/day/pond. Local weather data are available at the referenced website, and morning temperature measurements of the indoor ponds are listed at

the end of this article [18]. Day 1 of the first run was 5/6/15 and Day 29 was 6/5/15.

During the second run, the average daily water loss was 0.09”/day in Pond 7 and 0.14”/day in Pond 8. It is expected that Pond 8 had a small leak, likely caused by the abrasive cleaning methods that are necessary to clean ponds between cultivations if they become contaminated. The lower water loss in Pond 7 during the second run compared to the first run is expected since the outside weather was cooler during the second experiment. Local weather data are available at the referenced website [18]. Day -1 of the second run was 9/15/15 and day 22 was 10/8/15.

3.4 Element Removal Analysis

Though no significant decreases in the conductivities of the two ponds were observed, water analyses demonstrated that certain elements were removed from the growth medium by the microalgae. The following elements were removed in at least one of the four cultivations: Al, As, B, Ba, Cd, Fe, Mn, Ni, Tl, Zn, Bi, Ca, Li, Mg, P, Sr, K, SiO2, Na, S and Cu. Tables 3 through 6 contain data from the water analyses. The numbers in the central part of the table represent adjusted concentrations of contaminants in mg/L. Boxes highlighted in blue indicate elements whose presence increased.

Concentrations were adjusted by multiplying each concentration by h'/h. Here, h' is the height of the pond on the given day and h is the initial height of the given pond during a cultivation. For example, h = 12” for Pond 7 during the first run. Also, h was treated as a variable ($\hat{h}$) in Pond 8 during the second run because of the suspected leak. The variable $\hat{h}$ was calculated using the following formula:

$$\hat{h} = h - (h'_7 - h'_8) \times \frac{h}{0.5(h + h'_8)} = h\left(1 - \frac{h'_7 - h'_8}{0.5(h + h'_8)}\right)$$

Notably, Table 2 states that algae were fed fertilizer before the measurements on Day 22 and Day 29 of the first run. Therefore, the ***% Decrease*** columns describe the decrease in concentration of a given element between the first and third measurements.

Table 3. Analyses of water samples from Pond 7 Run 1 after microalgae were filtered out of the medium. Concentrations are represented in mg/L.

	Day						
Element	1	8	15	22	29	% Decrease	MDL
Al	0.1101	0.0896	0.088825	0.070166667	0.069033	19.3	0.0026
As	0.2018	0.136763	0.146025	0.124833333	0.118538	27.6	0.0174
B	0.8854	0.8631	0.862492	0.876666667	0.86328	2.6	0.05
Ba	0.0521	0.042263	0.045925	0.039083333	0.047597	11.9	0.001
Be	ND	ND	ND	ND	ND		0.0002
Cd	0.0028	0.002013	ND	ND	0.001726	100	0.001
Co	ND	ND	ND	ND	ND		0.002
Cr	ND	ND	ND	ND	ND		0.003
Fe	0.2235	ND	ND	0.007416667	0.019893	100	0.004
Mn	0.0602	0.017763	0.003575	0.005666667	0.008902	94.1	0.0017
Mo	0.0177	0.0203	0.020717	0.023833333	0.02398	-17.0	0.0017
Ni	0.0051	0.004113	0.003025	ND	ND	40.7	0.002
Pb	ND	ND	ND	ND	ND		0.0027
Se	ND	ND	ND	ND	ND		0.0135
Tl	0.0227	ND	ND	ND	ND	100	0.0068
V	ND	ND	ND	ND	ND		0.0017
Zn	0.195	0.122063	0.113758	0.16675	0.212096	41.7	0.0013
Bi	0.1174	0.09555	0.083783	0.062666667	0.065854	28.6	0.0052
Ca	769.2	583.1875	550.1833	547.1666667	594.2317	28.5	0.0971
Li	0.1251	0.1204	0.124117	0.120833333	0.129801	0.8	0.0014
Mg	400.3	362.5125	347.6917	347.3333333	350.8892	13.1	0.0185
P	6.09	0.607163	0.760833	0.513583333	1.928392	87.5	0.0251
Sr	13.45	12.03125	11.77917	11.825	12.0445	12.4	0.0012
K	23.98	21.04375	19.855	27.05	32.07325	17.2	0.2101
SiO2	56.86	53.2	54.90833	53.75	56.135	3.4	0.0151
Na	1276	1128.75	1085.333	1086.666667	1111.8	14.9	0.0483
S	1502	1420.125	1402.5	1505.833333	1377.033	6.6	0.5
Cu	0.0804	0.015313	0.012925	0.02175	0.034063	83.9	0.0023

* % Decrease between Day 1 and Day 15

^ Method Detection Limit [19].

Table 4. Analyses of water samples from Pond 8 Run 1 after microalgae were filtered out of the medium. Concentrations are represented in mg/L.

	Day						
Element	1	8	15	22	29	% Decrease	MDL
Al	0.0511	0.038435	0.039217	0.027130435	0.029078	23.3	0.0026
As	0.0925	0.065304	0.078339	0.053565217	0.068583	15.3	0.0174
B	0.4436	0.439478	0.452052	0.474086957	0.49567	-1.9	0.05
Ba	0.0539	0.040522	0.047539	0.040608696	0.042948	11.8	0.001
Be	ND	ND	ND	ND	ND		0.0002
Cd	0.0012	0.001043	ND	0.001304348	0.001052	100	0.001
Co	ND	ND	ND	ND	ND		0.002
Cr	ND	ND	ND	ND	ND		0.003
Fe	0.3022	ND	ND	0.003826087	0.010139	100	0.004
Mn	0.1247	ND	ND	ND	0.003061	100	0.0017
Mo	0.0097	0.010609	0.012435	0.014	0.014061	-28.2	0.0017
Ni	0.0024	ND	ND	ND	ND	100	0.002
Pb	ND	ND	ND	ND	ND		0.0027
Se	ND	ND	ND	ND	ND		0.0135
Tl	0.0087	ND	ND	ND	ND	100	0.0068
V	ND	ND	ND	0.002434783	ND		0.0017
Zn	0.1579	0.103217	0.099478	0.162782609	0.201252	37.0	0.0013
Bi	0.0517	0.044609	0.045339	0.03373913	0.034243	12.3	0.0052
Ca	353.3	296.4348	300.5391	316.0869565	337.1739	14.9	0.0971
Li	0.0913	0.086435	0.090774	0.09373913	0.102061	0.6	0.0014
Mg	181.7	169.6522	169.4	175.3913043	177.8174	6.8	0.0185
P	4.939	0.233739	0.312304	0.717304348	1.220522	93.7	0.0251
Sr	6.351	5.929565	5.984957	6	6.160957	5.8	0.0012
K	18.02	15.16522	14.40522	23.47826087	26.88783	20.1	0.2101
SiO_2	39.29	37.05217	41.03478	40.69565217	44.1913	-4.4	0.0151
Na	582.7	538.2609	541.6783	564.2608696	582.1391	7.0	0.0483
S	722.5	683.1304	685.1565	702.4347826	708.5913	5.2	0.5
Cu	0.0759	0.012957	0.01253	0.031565217	0.038548	83.5	0.0023

* % Decrease between Day -1 and Day 15

^ Method Detection Limit [19].

Table 5. Analyses of water samples from Pond 7 Run 2 after microalgae were filtered out of the medium. Concentrations are represented in mg/L.

	Day				
Element	-1	8	15	% Decrease	MDL ^
Al	0.0756	0.109425	0.23052	-204.9206349	0.0026
As	0.1235	0.448772	0.396369	-220.9467456	0.0174
B	1.1	1.296015	1.362085	-23.82587413	0.05
Ba	0.0194	0.01952	0.016168	16.66137986	0.001
Be	ND	0.003097	ND		0.0002
Cd	ND	ND	0.009301		0.001
Co	ND	ND	ND		0.002
Cr	ND	ND	ND		0.003
Fe	0.6729	ND	ND	100	0.004
Mn	0.0944	ND	0.016863	82.13657106	0.0017
Mo	ND	ND	ND		0.0017
Ni	0.0059	0.013514	ND	100	0.002
Pb	ND	ND	ND		0.0027
Se	ND	ND	ND		0.0135
Tl	0.0061	0.074795	0.039202	-542.6607818	0.0068
V	ND	ND	ND		0.0017
Zn	0.1242	0.057809	0.049807	59.89780751	0.0013
Bi	0.1112	0.237149	0.223827	-101.2832042	0.0052
Ca	704.6	619.0092	641.5792	8.944190921	0.0971
Li	0.0865	0.072543	0.05876	32.06936416	0.0014
Mg	471.4	429.44	442.7862	6.069971607	0.0185
P	8.258	ND	1.904485	76.93770144	0.0251
Sr	10.56	11.32723	11.39562	-7.913024476	0.0012
K	15.45	16.43246	13.98592	9.476226039	0.2101
SiO_2	31.99	35.20169	35.45592	-10.83439536	0.0151
Na	973.3	924.1031	943.1154	3.101265323	0.0483
S	1606	1434.908	1410.762	12.15681579	0.5
Cu	0.1219	0.018582	0.011822	90.30226541	0.0023

^ Method Detection Limit [19].

Table 6. Analyses of water samples from Pond 8 Run 2 after microalgae were filtered out of the medium. Concentrations are represented in mg/L.

	Day				
Element	-1	8	15	% Decrease	MDL
Al	0.0559	0.048106	0.099367	-77.75856568	0.0026
As	0.2725	0.113092	0.09659	64.55414013	0.0174
B	0.8278	0.546612	0.592644	28.40733169	0.05
Ba	0.0444	0.006095	0.027771	37.45337694	0.001
Be	ND	0.000938	0.000174		0.0002
Cd	0.0065	0.001875	0.003298	49.26506614	0.001
Co	ND	ND	ND		0.002
Cr	ND	ND	ND		0.003
Fe	0.7599	ND	ND	100	0.004
Mn	0.1577	0.003095	0.003558	97.74374063	0.0017
Mo	ND	ND	ND		0.0017
Ni	0.0107	0.002157	ND	100	0.002
Pb	ND	ND	ND		0.0027
Se	ND	ND	ND		0.0135
Tl	0.059	0.010221	ND	100	0.0068
V	ND	0.002063	ND		0.0017
Zn	0.1309	0.071362	0.051983	60.28779201	0.0013
Bi	0.1281	0.060203	0.080188	37.40210922	0.0052
Ca	313.6	274.6659	295.4976	5.772445324	0.0971
Li	0.0851	0.06733	0.067431	20.76294655	0.0014
Mg	192.5	177.5154	183.3734	4.741086939	0.0185
P	8.532	0.628571	2.58875	69.65834505	0.0251
Sr	5.244	4.706548	5.038646	3.915970694	0.0012
K	19.92	17.66714	17.50422	12.12741092	0.2101
SiO2	30.35	27.39157	28.95096	4.609702096	0.0151
Na	420.6	394.5099	405.1051	3.684000109	0.0483
S	619	625.0079	584.0525	5.645791959	0.5
Cu	0.1466	0.042574	0.018919	87.0949592	0.0023

^ Method Detection Limit [19].

During the cultivations, there were a few elements that increased in concentration. These elements have been contrasted by a highlighted *% Decrease*. Molybdenum is the most prominent of the highlighted elements. Also, some elements in Pond 7 Run 2 increased in concentration during the first interval, then decreased during the second interval. There is no clear explanation for these phenomena. It is also notable that the elements in Table 7 were removed to below their detection limits.

Table 7. Elements that were removed to below their detection limits during cultivations.

Element	Pond 7, Run 1	Pond 8, Run 1	Pond 7, Run 2	Pond 8, Run 2
Cd	✓	✓		
Fe	✓	✓	✓	✓
Mn		✓		
Ni		✓	✓	✓
Tl	✓	✓		✓

In many cases, a contaminant was removed more during the first interval of cultivation than during the second interval. This has implications when using algae to remove specific elements from concentrate with specific water chemistries. More algae or a longer residence time may not be better.

In the following figures, the contaminant removal data is represented in graphical form.

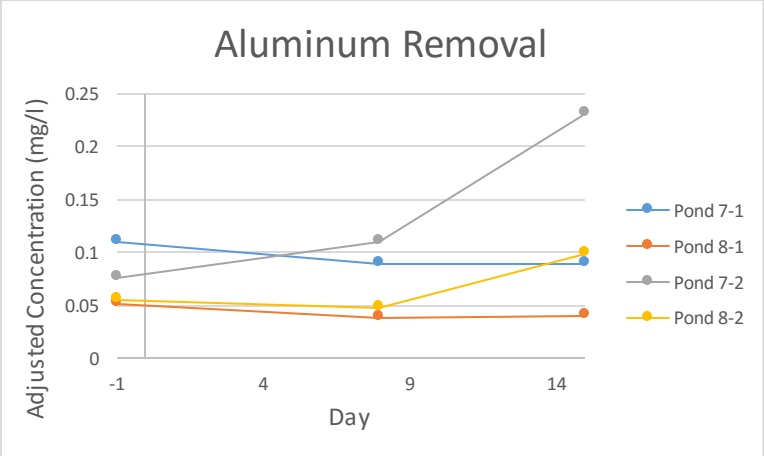


Figure 5. Aluminum removal during pond cultivations.

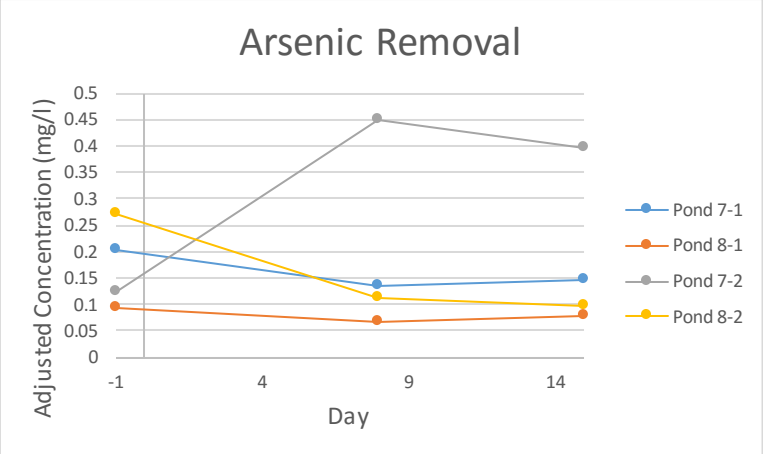


Figure 6. Aluminum removal during pond cultivations.

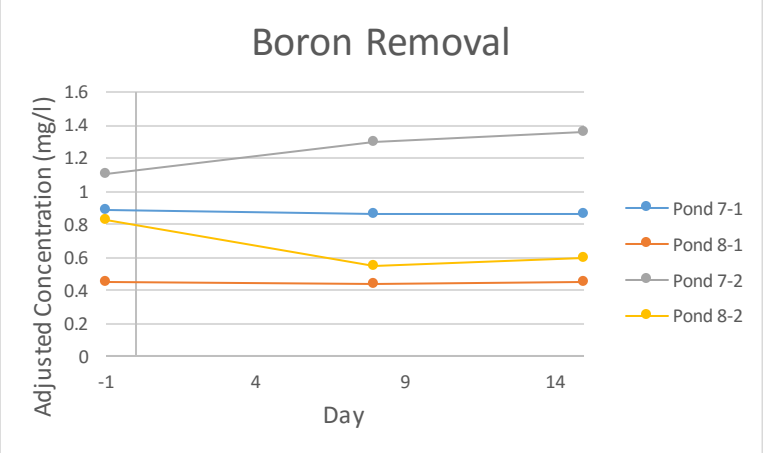


Figure 7. Boron removal during pond cultivations.

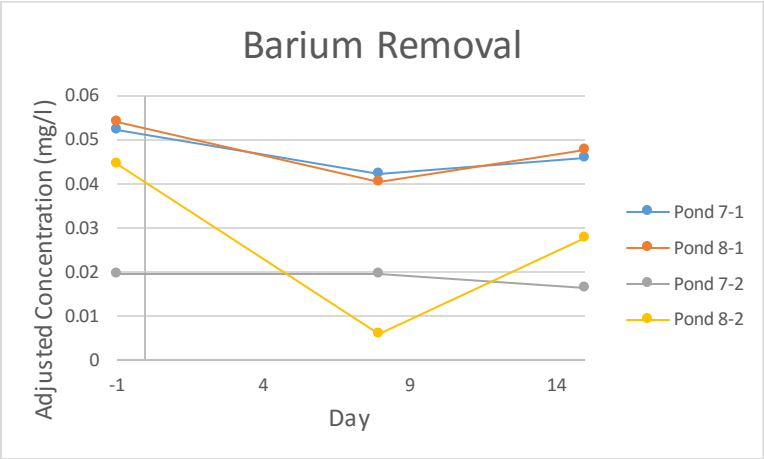


Figure 8. Barium removal during pond cultivations.

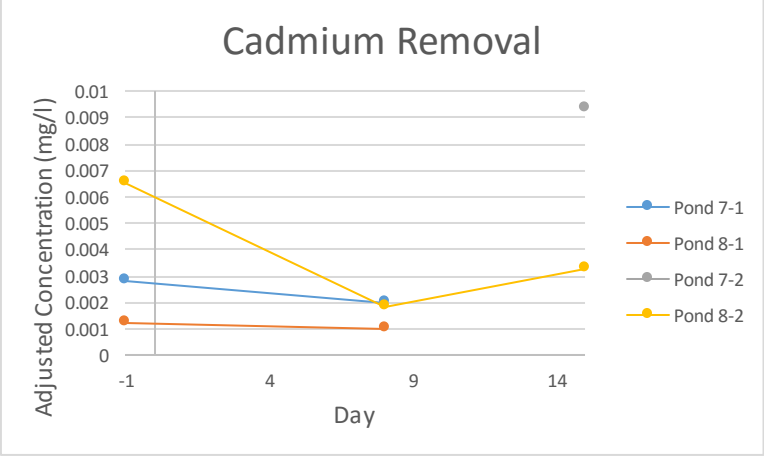


Figure 9. Cadmium removal during pond cultivations.

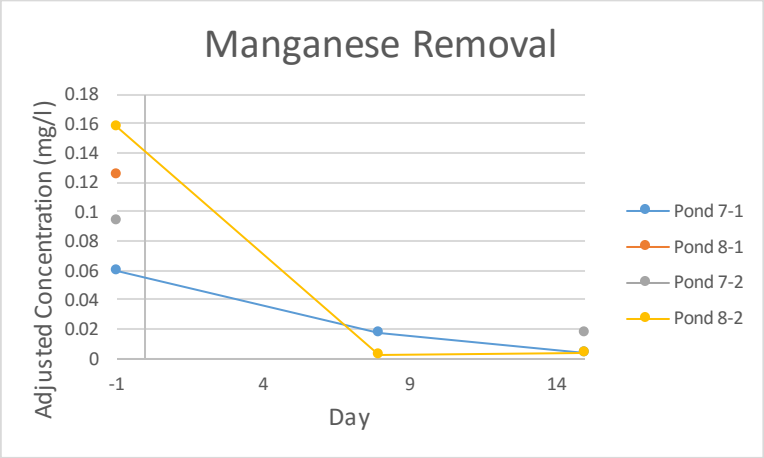


Figure 10. Manganese removal during pond cultivations.

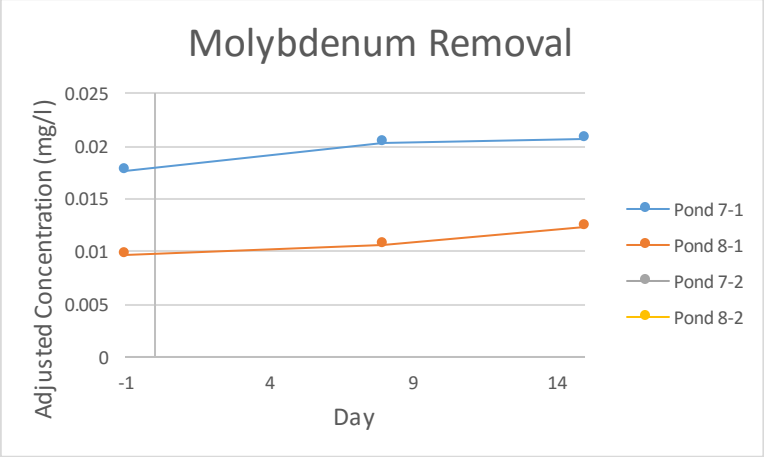


Figure 11. Molybdenum removal during pond cultivations.

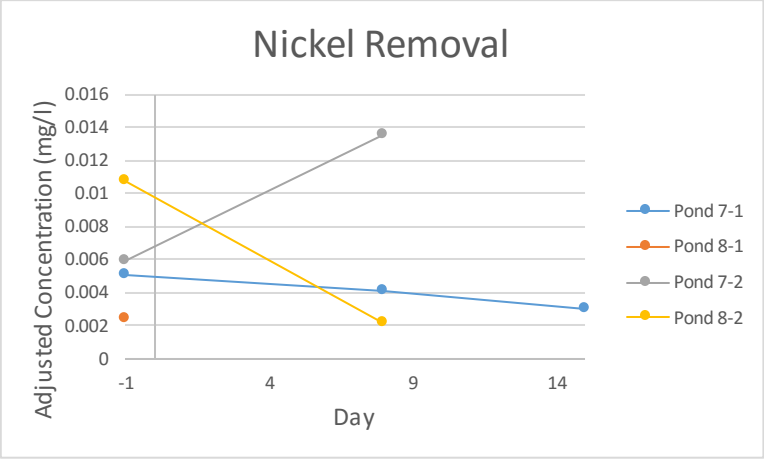


Figure 12. Nickel removal during pond cultivations.

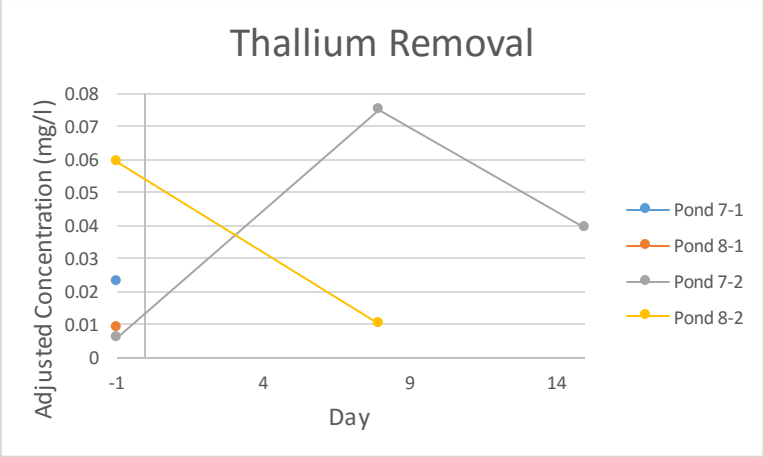


Figure 13. Thallium removal during pond cultivations.

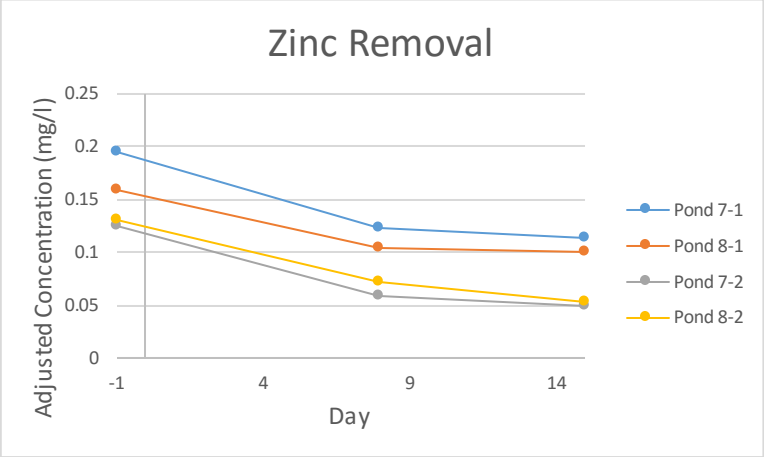


Figure 14. Zinc removal during pond cultivations.

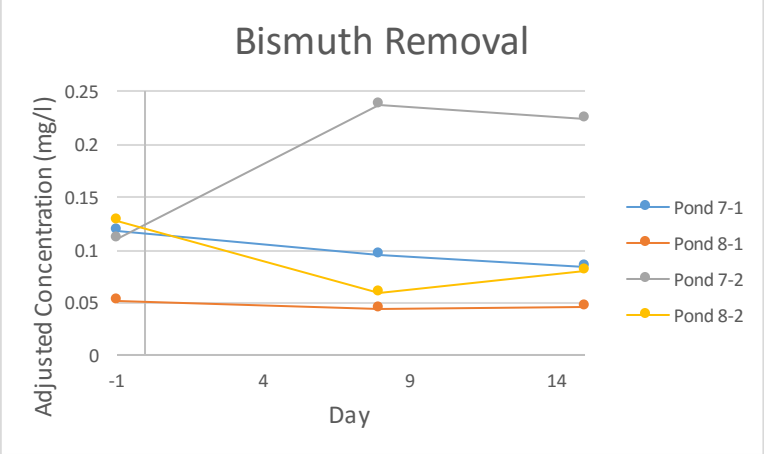


Figure 15. Bismuth removal during pond cultivations.

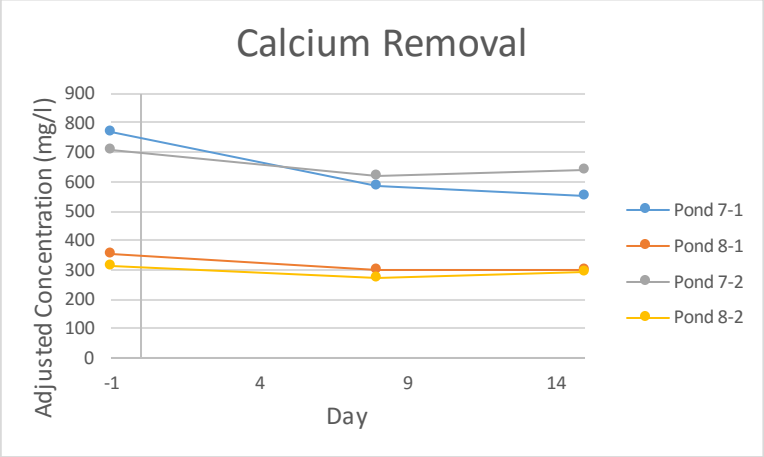


Figure 16. Calcium removal during pond cultivations.

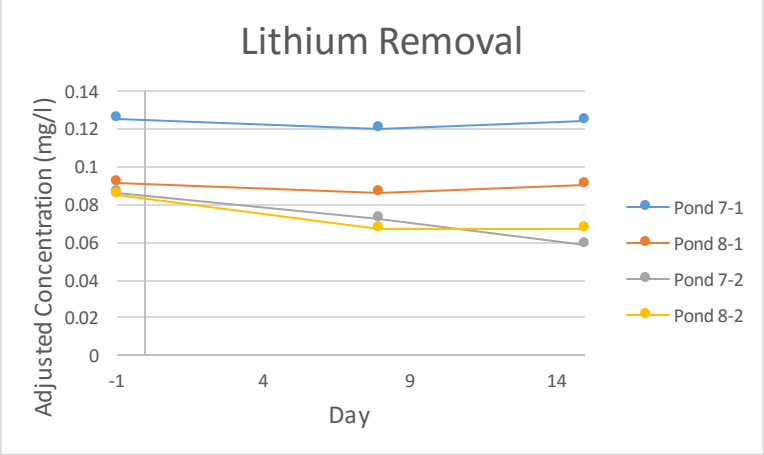


Figure 17. Lithium removal during pond cultivations.

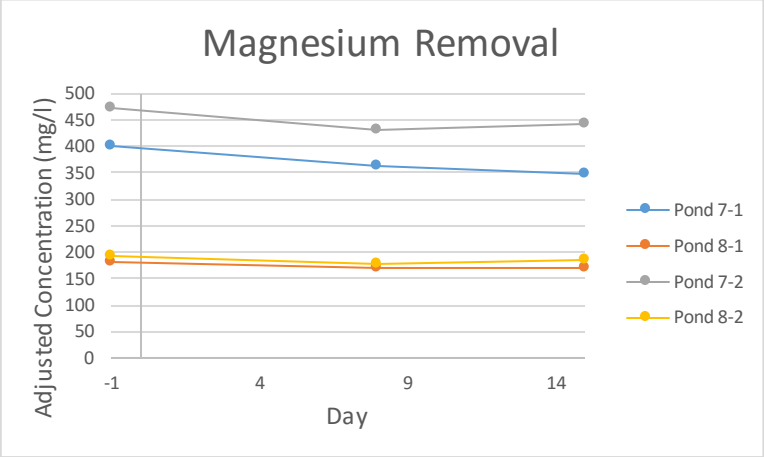


Figure 18. Magnesium removal during pond cultivations.

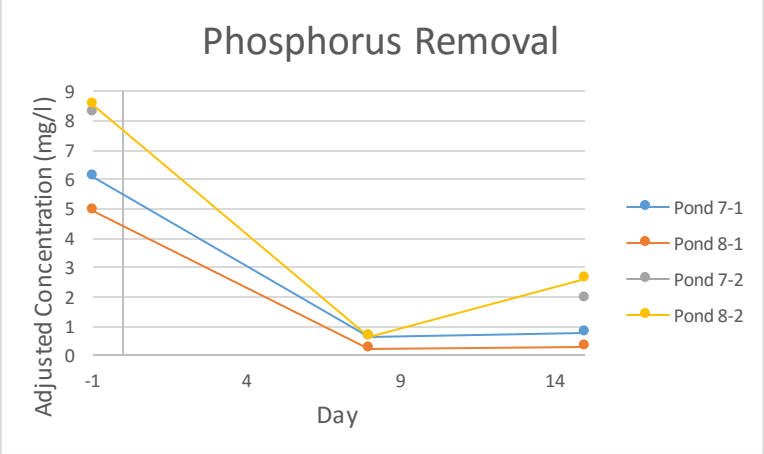


Figure 19. Phosphorus removal during pond cultivations.

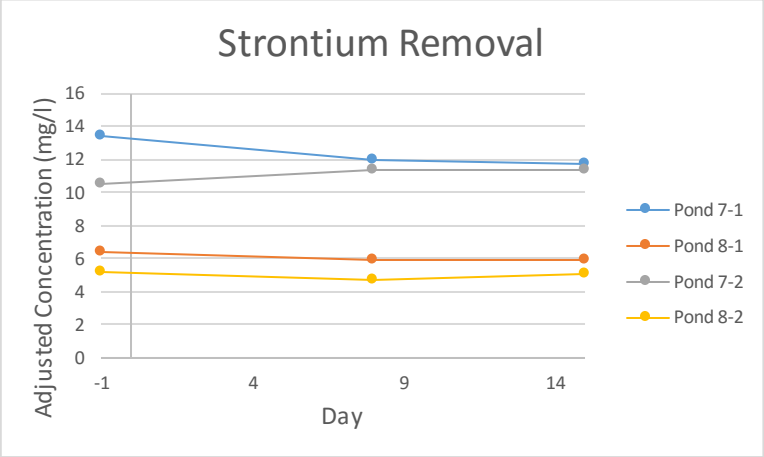


Figure 20. Strontium removal during pond cultivations.

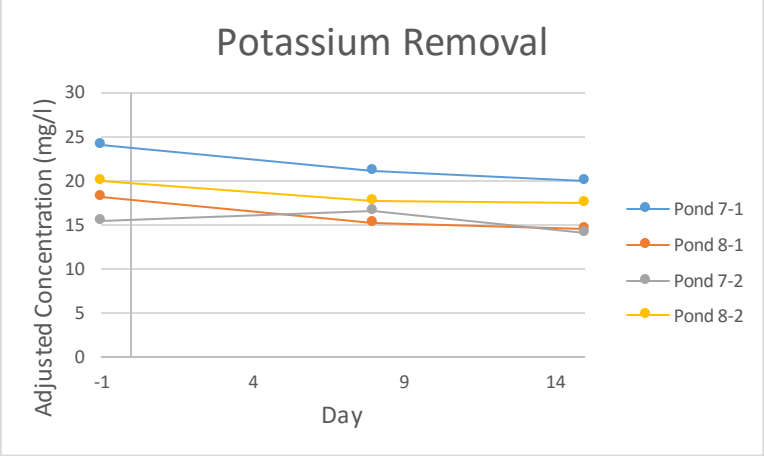


Figure 21. Potassium removal during pond cultivations.

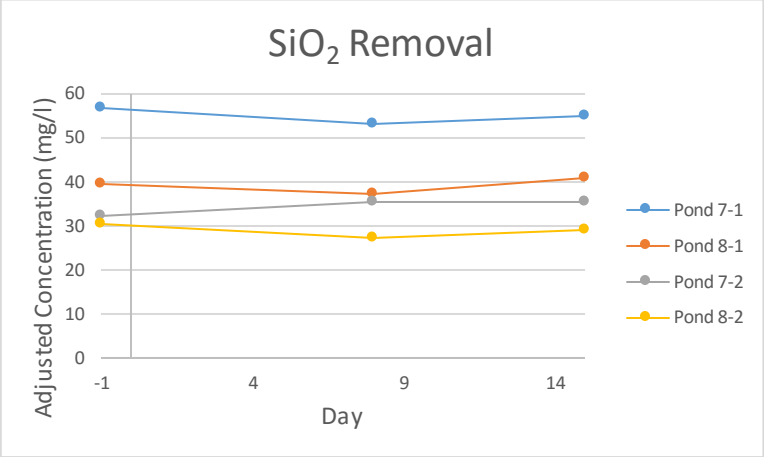


Figure 22. Silicon dioxide removal during pond cultivations.

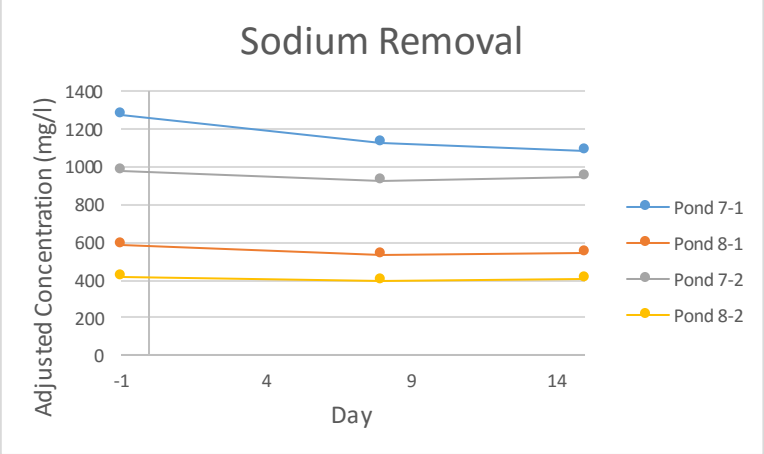


Figure 23. Sodium removal during pond cultivations.

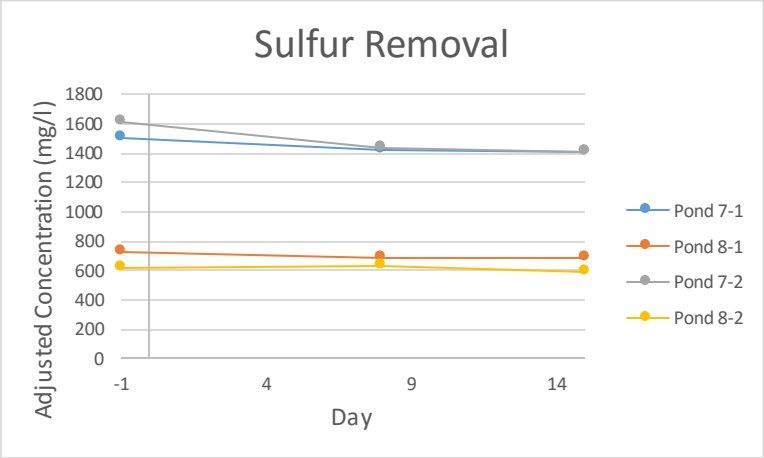


Figure 24. Sulfur removal during pond cultivations.

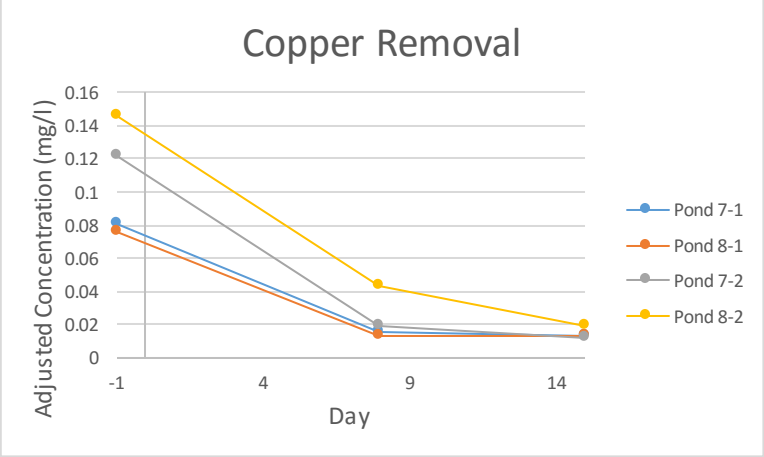


Figure 25. Copper removal during pond cultivations.

If a sum of element removals is taken over the first two intervals of the first run, there was less of an elemental increase in Pond 8 than in Pond 7. Since Pond 8 had a higher biomass-to conductivity ratio during these intervals, this appears to support a conclusion made in previous research: mass conductivity reduction is directly proportional to the mass microalgae-to-conductivity-ratio (Myint, 2014).

However, since there were unexplained increases in some elements during the first interval, and because there was an overall increase in the sum of measured concentrations in Run 1 between Day -1 and Day 15, it appears that there are factors in the experiment that were not accounted for. Therefore, further investigation is necessary to examine the claims made in previous research (Myint, 2014).

CHAPTER 4: DISCUSSION AND CONCLUSION

Chlorella sorokiniana was successfully cultivated in desalination concentrate at a pilot scale. Large amounts of cadmium, iron, manganese, nickel, thallium, zinc, phosphorus, copper and arsenic (Pond 8 Run 2) were removed from the concentrate. Aluminum, boron, barium, bismuth, calcium, lithium, magnesium, strontium, potassium, silicon dioxide, sodium, and sulfur were also removed in at least one of the four cultivations. Some of these elements had higher removals during the first period of cultivation than during the second period. This implies that higher microalgae density and longer residence times may not be better for the removal of specific contaminants.

If further investigation of microalgae growth in desalination concentrate at the pilot scale were conducted, certain changes should be made to the current experimental procedure. First, contamination factors should be controlled more closely. Second, Miracid 30:10:10 is not an appropriate nutrient source when contaminant removal is the goal. Also, cell sizing should be done by a computer. Estimation errors could have large consequences because of the cubic relation between the radius and volume of a sphere. In addition, a more complete water analysis is recommended. This would allow researchers to better understand factors involved with biomass increase and contaminant removal. Particular elemental analyses that may have been helpful in this experiment are analyses of nitrogen and chlorine.

Furthermore, it may have been helpful if a nutrient source with a known composition were used. Continuously fed processes should also be considered. In addition, minimization of variables would be ideal. For example, it would be helpful if harvesting had not taken place in Pond 7 during the first run. Moreover, there were other relevant factors in the experiment that were not analyzed, as is evidenced by the unexplained increase in many elements during the first interval.

Also, this experiment points to other investigations that may be worthwhile. Investigation into the correlation between pH and pond health may be merited. An understanding of this correlation could improve pond maintenance practices. Furthermore, two methodological developments are merited to make comparing biomass between experiments more feasible: a method for correlating algae volume with biomass and a standard method for measuring microalgal biomass.

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APPENDIX

Trace element analyses of concentrate in both experimental runs

Element	Reported Concentration		Units	MDL
	Run 1	Run 2		
Al	0.1108	0.0797	mg/L	0.0026
As	0.1642	0.1282	mg/L	0.0174
B	0.8848	1.152	mg/L	0.05
Ba	0.052	0.019	mg/L	0.001
Be	ND	0.0005	mg/L	0.0002
Cd	0.0032	0.0046	mg/L	0.001
Co	ND	ND	mg/L	0.002
Cr	ND	ND	mg/L	0.003
Fe	0.0558	0.0097	mg/L	0.004
Mn	0.0084	0.0067	mg/L	0.0017
Mo	0.0196	ND	mg/L	0.0017
Ni	0.0041	ND	mg/L	0.002
Pb	ND	ND	mg/L	0.0027
Se	ND	ND	mg/L	0.0135
Tl	ND	ND	mg/L	0.0068
V	ND	ND	mg/L	0.0017
Zn	0.1222	0.0048	mg/L	0.0013
Bi	0.1113	0.1101	mg/L	0.0052
Ca	709.7	686.8	mg/L	0.0971
Li	0.128	0.0878	mg/L	0.0014
Mg	380.8	453.7	mg/L	0.0185
P	2.088	0.028	mg/L	0.0251
Sr	13.65	11.31	mg/L	0.0012
K	15.62	4.982	mg/L	0.2101
SiO2	57.95	34.28	mg/L	0.0151
Na	1184	958.5	mg/L	0.0483
S	1519	1504.0	mg/L	0.5
Cu	ND	ND	mg/L	0.0023

Trace element analyses of Pond 7 during Run 1 after algae were removed from the medium

	Day					
	1	8	15	22	29	MDL
Al	0.1101	0.1024	0.0969	0.0842	0.076	0.0026
As	0.2018	0.1563	0.1593	0.1498	0.1305	0.0174
B	0.8854	0.9864	0.9409	1.052	0.9504	0.05
Ba	0.0521	0.0483	0.0501	0.0469	0.0524	0.001
Be	ND	ND	ND	ND	ND	0.0002
Cd	0.0028	0.0023	ND	ND	0.0019	0.001
Co	ND	ND	ND	ND	ND	0.002
Cr	ND	ND	ND	ND	ND	0.003
Fe	0.2235	ND	ND	0.0089	0.0219	0.004
Mn	0.0602	0.0203	0.0039	0.0068	0.0098	0.0017
Mo	0.0177	0.0232	0.0226	0.0286	0.0264	0.0017
Ni	0.0051	0.0047	0.0033	ND	ND	0.002
Pb	ND	ND	ND	ND	ND	0.0027
Se	ND	ND	ND	ND	ND	0.0135
Tl	0.0227	ND	ND	ND	ND	0.0068
V	ND	ND	ND	ND	ND	0.0017
Zn	0.195	0.1395	0.1241	0.2001	0.2335	0.0013
Bi	0.1174	0.1092	0.0914	0.0752	0.0725	0.0052
Ca	769.2	666.5	600.2	656.6	654.2	0.0971
Li	0.1251	0.1376	0.1354	0.145	0.1429	0.0014
Mg	400.3	414.3	379.3	416.8	386.3	0.0185
P	6.09	0.6939	0.83	0.6163	2.123	0.0251
Sr	13.45	13.75	12.85	14.19	13.26	0.0012
K	23.98	24.05	21.66	32.46	35.31	0.2101
SiO2	56.86	60.8	59.9	64.5	61.8	0.0151
Na	1276.0	1290.0	1184.0	1304.0	1224.0	0.0483
S	1502.0	1623.0	1530	1807.0	1516.0	0.5
Cu	0.0804	0.0175	0.0141	0.0261	0.0375	0.0023

Trace element analyses of Pond 8 during Run 1 after algae were removed from the medium

	Day					
	1	8	15	22	29	MDL
Al	0.0511	0.0442	0.041	0.0312	0.0304	0.0026
As	0.0925	0.0751	0.0819	0.0616	0.0717	0.0174
B	0.4436	0.5054	0.4726	0.5452	0.5182	0.05
Ba	0.0539	0.0466	0.0497	0.0467	0.0449	0.001
Be	ND	ND	ND	ND	ND	0.0002
Cd	0.0012	0.0012	ND	0.0015	0.0011	0.001
Co	ND	ND	ND	ND	ND	0.002
Cr	ND	ND	ND	ND	ND	0.003
Fe	0.3022	ND	ND	0.0044	0.0106	0.004
Mn	0.1247	ND	ND	ND	0.0032	0.0017
Mo	0.0097	0.0122	0.013	0.0161	0.0147	0.0017
Ni	0.0024	ND	ND	ND	ND	0.002
Pb	ND	ND	ND	ND	ND	0.0027
Se	ND	ND	ND	ND	ND	0.0135
Tl	0.0087	ND	ND	ND	ND	0.0068
V	ND	ND	ND	0.0028	ND	0.0017
Zn	0.1579	0.1187	0.104	0.1872	0.2104	0.0013
Bi	0.0517	0.0513	0.0474	0.0388	0.0358	0.0052
Ca	353.3	340.9	314.2	363.5	352.5	0.0971
Li	0.0913	0.0994	0.0949	0.1078	0.1067	0.0014
Mg	181.7	195.1	177.1	201.7	185.9	0.0185
P	4.939	0.2688	0.3265	0.8249	1.276	0.0251
Sr	6.351	6.819	6.257	6.9	6.441	0.0012
K	18.02	17.44	15.06	27.0	28.11	0.2101
SiO2	39.29	42.61	42.9	46.8	46.2	0.0151
Na	582.7	619.0	566.3	648.9	608.6	0.0483
S	722.5	785.6	716.3	807.8	740.8	0.5
Cu	0.0759	0.0149	0.0131	0.0363	0.0403	0.0023

Trace element analyses of Pond 7 during Run 2 after algae were removed of the medium

	Day				
	-1	8	15	Units	MDL
Al	0.0756	0.1166	0.2652	mg/L	0.0026
As	0.1235	0.4782	0.456	mg/L	0.0174
B	1.1	1.381	1.567	mg/L	0.05
Ba	0.0194	0.0208	0.0186	mg/L	0.001
Be	ND	0.0033	ND	mg/L	0.0002
Cd	ND	ND	0.0107	mg/L	0.001
Co	ND	ND	ND	mg/L	0.002
Cr	ND	ND	ND	mg/L	0.003
Fe	0.6729	ND	ND	mg/L	0.004
Mn	0.0944	ND	0.0194	mg/L	0.0017
Mo	ND	ND	ND	mg/L	0.0017
Ni	0.0059	0.0144	ND	mg/L	0.002
Pb	ND	ND	ND	mg/L	0.0027
Se	ND	ND	ND	mg/L	0.0135
Tl	0.0061	0.0797	0.0451	mg/L	0.0068
V	ND	ND	ND	mg/L	0.0017
Zn	0.1242	0.0616	0.0573	mg/L	0.0013
Bi	0.1112	0.2527	0.2575	mg/L	0.0052
Ca	704.6	659.6	738.1	mg/L	0.0971
Li	0.0865	0.0773	0.0676	mg/L	0.0014
Mg	471.4	457.6	509.4	mg/L	0.0185
P	8.258	ND	2.191	mg/L	0.0251
Sr	10.56	12.07	13.11	mg/L	0.0012
K	15.45	17.51	16.09	mg/L	0.2101
SiO2	31.99	37.51	40.79	mg/L	0.0151
Na	973.3	984.7	1085	mg/L	0.0483
S	1606	1529	1623	mg/L	0.5
Cu	0.1219	0.0198	0.0136	mg/L	0.0023

Trace element analyses of Pond 8 during Run 2 after algae were removed from the medium

	Day				
	-1	8	15	Units	MDL
Al	0.0559	0.0513	0.1145	mg/L	0.0026
As	0.2725	0.1206	0.1113	mg/L	0.0174
B	0.8278	0.5829	0.6829	mg/L	0.05
Ba	0.0444	0.0065	0.032	mg/L	0.001
Be	ND	0.001	0.0002	mg/L	0.0002
Cd	0.0065	0.002	0.0038	mg/L	0.001
Co	ND	ND	ND	mg/L	0.002
Cr	ND	ND	ND	mg/L	0.003
Fe	0.7599	ND	ND	mg/L	0.004
Mn	0.1577	0.0033	0.0041	mg/L	0.0017
Mo	ND	ND	ND	mg/L	0.0017
Ni	0.0107	0.0023	ND	mg/L	0.002
Pb	ND	ND	ND	mg/L	0.0027
Se	ND	ND	ND	mg/L	0.0135
Tl	0.059	0.0109	ND	mg/L	0.0068
V	ND	0.0022	ND	mg/L	0.0017
Zn	0.1309	0.0761	0.0599	mg/L	0.0013
Bi	0.1281	0.0642	0.0924	mg/L	0.0052
Ca	313.6	292.9	340.5	mg/L	0.0971
Li	0.0851	0.0718	0.0777	mg/L	0.0014
Mg	192.5	189.3	211.3	mg/L	0.0185
P	8.532	0.6703	2.983	mg/L	0.0251
Sr	5.244	5.019	5.806	mg/L	0.0012
K	19.92	18.84	20.17	mg/L	0.2101
SiO2	30.35	29.21	33.36	mg/L	0.0151
Na	420.6	420.7	466.8	mg/L	0.0483
S	619	666.5	673	mg/L	0.5
Cu	0.1466	0.0454	0.0218	mg/L	0.0023

Raw data from electrical conductivity measurements

	Conductivity (mS)			
Day	Pond 7 Run 1	Pond 8 Run 1	Pond 7 Run 2	Pond 8 Run 2
-1			8.6	4.3
0			8.89	4.39
1	8.21	4.4	8.68	4.35
2			8.67	4.43
4	8.52	4.65		
5	8.73	4.83	8.80	4.44
6	8.6	4.9	8.89	4.45
7	8.86	4.89	8.89	4.46
8	8.81	4.86	8.99	4.51
9			9.06	4.56
11	8.19	4.38		
12	8.25	4.42	9.37	4.7
13	8.31	4.43	9.43	4.75
14	8.37	4.46		
15	8.38	4.55	9.71	4.89
16			9.82	5.02
19	8.79	4.76	10.04	5.15
20	8.87	4.82	10.11	5.18
21	8.9	4.85		
22	9.02	4.95	10.22	5.25
25	8.05	4.52		
26	8.15	4.55		
27	8.27	4.6		
28	8.49	4.68		
29	8.54	4.77		

Raw data from pH measurements

	pH			
Day	Pond 7 Run 1	Pond 8 Run 1	Pond 7 Run 2	Pond 8 Run 2
0			8.36	8.47
1			8.66	8.64
2			8.61	8.63
4	8.71	8.84		
5	8.67	8.88	9.56	9.39
6	8.6	8.84	8.82	8.69
7	8.76	8.92	9.33	9.15
8	8.9	8.94	8.75	8.58
9			8.89	8.68
11	8.85	9		
12	8.92	8.79	7.93	8.72
13	9.08	8.9	8.11	8.15
14	8.83	8.92		
15	8.63	8.64	8.15	8.08
16			8.42	8.39
19	9.16	9.3	8.34	8.25
20	9	9.05	8.14	6.62
21	9.05	9.03		
22	9.12	9.02	7.81	6.29
25	8.53	8.51		
26	8.43	8.52		
27	8.42	8.75		
28	8.34	8.55		
29	8.35	8.65		

Raw data from depth measurements

	Depth (in)			
Day	Pond 7 Run 1	Pond 8 Run 1	Pond 7 Run 2	Pond 8 Run 2
-1			13	13
1	12	11.5	12.7	12.6
4	11	10.25		
5	11.25	10		
6	11	10	12.3	12.1
7	11	10		
8	10.5	10	12.2	11.9
11	11.5	11.5		
12	11.5	11.5	11.5	11.25
13	11.4	11	11.7	11.1
14	11.2	11		
15	11	11	11.3	10.9
19	10.4	10.5	11.0	10.0
20	10.4	10.4	11.0	10.0
21	10.25	10.1		
22	10	10	10.9	9.8
25	11.5	11.75		
26	11.4	11.5		
27	11.3	11.4		
28	11	11.2		
29	10.9	11		

Averages of cell counts of UTEX 1230 in a 4 nanoliter volume

	Cell Count			
Day	Pond 7 Run 1	Pond 8 Run 1	Pond 7 Run 2	Pond 8 Run 2
1			2.52	2.08
5	10.4	9.4		
6			41.8	25.6
8	34.4	14.2	50.4	22
12	89.6	40.8	39.4	17
15	93.6	117.4	34	24.4
19	77.4	78.4	11.4	8.8
22	86.2	103.2	26.4	11.4
26	54.8	99.8		
29	6	57.8		

Approximations of average cell diameters.

	Average Cell Diameter (Approximate)			
	Run 1		Run 2	
Day	Pond 7 (μm)	Pond 8 (μm)	Pond 7 (μm)	Pond 8 (μm)
-1	5.0	5.0		
1			3.75	3.125
5	2.5	2.5		
6			2.5	3.0
8	2.0	2.0	2.5	2.5
12	3.0	3.0	2.5	4.0
15	3.0	3.0	2.0	3.0
19	3.0	2.5	2.5	2.5
22	2.5	3.0	2.0	2.0
26	2.5	3.0		
29	1.5-2	2.5		

Raw data from temperature measurements during the first experimental run

		Pond Temperature (°F)	
Day	Time Measured	Pond 7	Pond 8
6	10:40 AM	62	62
7	10:35 AM	61	61
8	9:20 AM	59	59
11	8:35 AM	56	57
12	9:50 AM	60	60
13	10:00 AM	61	61
14	9:25 AM	59	59
15	9:20 AM	60	60
19	10:20 AM	62	62
20	9:05 AM	57	58
21	9:20 AM	57	58
22	9:40 AM	58	58
25	9:00 AM	62	62
26	8:35 AM	63	63

27	8:55 AM	60	60
28	8:55 AM	60	60
29	10:10 AM	61	61

The following text summarizes how it was mathematically concluded that Pond 8 had a higher biomass-to-conductivity-ratio during the first run:

Day	Pond 7 Cell Count	Pond 8 Cell Count
-1		
5	10.4	9.4
8	34.4	14.2
12	89.6	40.8
15	93.6	117.4

Day	Pond 7 Avg. Radius	Pond 8 Avg. Radius	Pond 7 Avg. of Avg. Radii	Pond 8 Avg. of Avg. Radii
-1	2.5	2.5		
5	1.25	1.25	Days -1 – 8: 1.583	Days -1 – 8: 1.583
8	1	1		
12	1.5	1.5	Days 8-15:	Days 8-15:
15	1.5	1.5	1.333	1.333

Day	Pond 7 Biomass nm^3/ 4nl Water	Pond 8 Biomass nm^3/ 4nl Water
-1		
5	172.917526	156.2908408
8	571.9579707	236.0989297
12	889.6369833	405.1025549
15	929.3529201	1165.662744

Assuming biomass is proportional to volume of biomass:

Figure 11. Curve fit for relative biomass in Pond 7 (days -1-12).

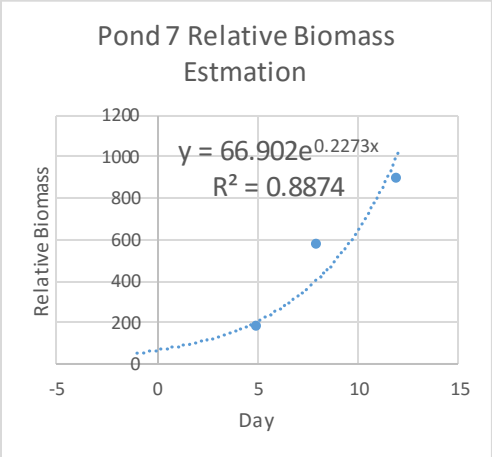


Figure 12. Curve fit for relative biomass in Pond 7 (days 12-15).

Pond 7: Days 1 - 8		
Avg. Rel. Biomass	Average Conductivity	Biomass/ Conductivity
175.47	8.56 mS	20.5

Pond 7: Days 8 - 15		
Avg. Rel. Biomass	Average Conductivity	Biomass/ Conductivity
779.94*	8.39 mS	93.0

*Used days 8-12 in *Figure 11* and days 12-15 in *Figure 12*.

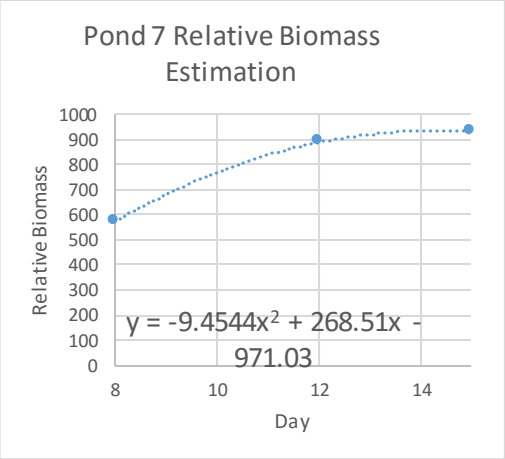
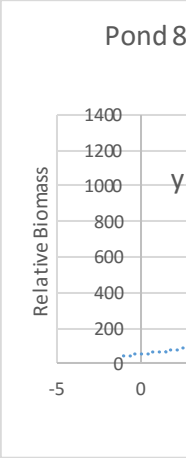


Figure 13. Curve fit for relative biomass in Pond 8.

Pond 8: Days 1 - 8		
Avg. Rel. Biomass	Average Conductivity	Biomass/ Conductivity
117.86	4.70 mS	25.1

Pond 8: Days 8 - 15		
Avg. Rel. Biomass	Average Conductivity	Biomass/ Conductivity
521.47	4.52 mS	115.4



~~Pond 8: Days 8 - 15~~		
~~Avg. Rel. Biomass~~	~~Average Conductivity~~	~~Biomass/ Conductivity~~
~~521.47~~	~~4.52 mS~~	~~115.4~~

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