**Desalination & Water Purification Research and Development Program Report No. XXX**

# Developing a Biotechnology with a Reactor to Grow Microalgae for Biodiesel Production from Reusing Waste Concentrate and Anaerobic Digested Sludge

**Prepared for Reclamation Under Agreement No.**

**R10AC80283**

*By*

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**Denver, Colorado**
**December 2014**

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# Acknowledgements

This thesis is an achievement that I accomplished with help from a group of people whom I respect and am proud to know in person. I first would like to thank my advisor, Dr. Abbas Ghassemi, for his guidance and support. I am grateful to him for making me a part of his research group and giving me the opportunity to pursue my passion for optics. I am honored to have been his student.

I would also like to thank the members of my thesis committee, Dr. Paul Andersen, Dr. Reza Foudazi, and Dr. Delia Valles-Rosales, for taking time out of their busy schedules to evaluate my thesis and provide valuable advice.

In addition, I want to take this opportunity to thank Dr. Jalal Rastegary, the program manager Jim Loya, Dr. Myint Maung, Roseann Thompson, Eahsan Shahriary, Patrick DeSimio, and Marzie Ghasempour for helping me during my thesis.

I would like to thank Nasser Khazeni for being my mentor and providing his support, especially in statistical analysis.

Certainly, I would like especially to thank the Bureau of Reclamation for their support under the contract R10AC80283.

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# Glossary

## Acronyms and Abbreviations

| | |
|---|---|
| BBM | Bold's Basal Medium |
| BGNDRF | Brackish Groundwater National Desalination Research Facility |
| CFTRI | Prescribed medium |
| CWA | Clean Water Act |
| DC | Direct current |
| dH2O | Desalinated Water |
| ED | Electrodialysis |
| EDR | Electrodialysis Reversal |
| EPA | U.S. Environmental Protection Agency |
| GHG | Greenhouse gas |
| GMO | Genetically modified organisms |
| IGD | Imperial gallon(s) per day |
| IPPC | The International Panel on Climate Change |
| J/1 | Modified Johnson medium |
| MED | Multi-effect distillation |
| MF | Microfiltration |
| Mgal | Millions of gallons |
| MSF | Multistage flash evaporation |
| MVC | Mechanical vapor compressor |
| MWCO | Molecular weight cutoff |
| NF | Nanofiltration |
| NM | New Mexico State |
| NMSU | New Mexico State University |
| NPDES | National Pollutant Discharge Elimination System |
| RO | Reverse Osmosis |
| SADS | Supernatant from Anaerobic Digested Sludge |
| TDS | Total dissolved solids |
| TVC | Thermo vapor compressor |
| UF | Ultra filtration |
| U.S | United States |
| US $ | United States Dollar(s) |
| VC | Vapor compression |
| WHO | World Health Organization |
| WWTP | Wastewater treatment plant |

# Chemical Symbols

| | |
|---|---|
| $CO_2$ | Carbon Dioxide |
| CO | Carbon Monoxide |
| $HCO_3^-$ | Bicarbonate |
| $CO_3^{2-}$ | Carbonate |
| BaCl | Barium Chloride |
| $NaH_2PO_4$ | Monosodium Phosphate |
| NaCl | Sodium Chloride |
| $NaNO_3$ | Sodium Nitrate |
| $Na_2SiO_3$ | Sodium Silicate |
| $NO_3$ | Nitrate |
| $N_2O$ | Nitrous Oxide |
| $H_2O$ | Water |
| $H_2$ | Hydrogen |
| $HPO_4^{2-}$ | Phosphoric Acid |
| $O_2$ | Oxygen |
| $NO_2$ | Nitrogen Dioxide |
| $CH_4$ | Methane |
| $NO_x$ | Nitrogen Oxides |
| $SO_x$ | Sulfur Dioxide |
| $Mg^{2+}$ | Magnesium |
| $Ca^{2+}$ | Calcium |
| N | Nitrogen |
| $SO_4^{-2}$ | Sulfate Ion |
| $NaNO_3$ | Sodium Nitrate |

# Executive Summary

Desalination technologies have significant potential to alleviate global water shortages, but they also produce a highly saline byproduct, desalination concentrate, which must be disposed of properly to avoid adverse environmental impacts. One possible solution is additional treatment of the concentrate, but this increases desalination's costs and energy use; other current options for the disposal of the concentrate, including evaporation ponds and deep well injection, are not sustainable.

To help resolve the problem of concentrate disposal and make inland desalination systems more sustainable and economically feasible, the objective of this research is to provide an alternative method for disposing of concentrate from inland desalination systems by using the concentrate as a nutrient for the production of microalgae, turning a substance that is typically a waste product into a potentially useful resource. Since this same approach could possibly be used to safely and productively dispose of certain other wastes, the use of supernatant anaerobic digested sludge (SADS) from wastewater treatment plants (WWTPs) as a nutrient for microalgae production was also investigated. The research hypothesis is that microalgae can effectively treat both the reject brine and the SADS, decreasing the financial cost and environmental effects of disposing of these substances.

In this experiment, bioreactors were constructed to grow microalgae using concentrate from the desalination of inland brackish water at the Brackish Groundwater National Desalination Research Facility (BGNDRF) in Alamogordo, New Mexico. Additional experiments were conducted to evaluate SADS as a nutrient supplement. The SADS was provided by a wastewater treatment plant in Las Cruces, NM.

The experiment included three microalgae species: *Dunaliella salina, Spirulina platensis*, and a third, unknown species of microalgae from the BGNDRF evaporation pond. These species, selected because of their ability to grow in highly saline environments, were grown in bioreactors that were filled with concentrate with different levels of salinity and directly exposed to sunlight, $CO_2$, and nutrient resources.

To evaluate the growth of the microalgae species, both optical density measurements and dry weight measurements were used.

# Chapter 1: INTRODUCTION

## 1.1 Water Scarcity

According to the U.S. Census Bureau (U.S. Census, 2011), the world population reached seven billion on March 12, 2012, and is expected to reach 8 billion in 2026. By 2042, the global population is expected to reach 9 billion (EPA, 2012). With such an extraordinary rise in population, the demand for fresh water will continue to increase over the subsequent decades: in the last century, water withdrawals increased six fold, while the global population only increased by three fold (United Nations Population Fund, 2003) (EPA, 2012). The water scarcity resulting from population growth is exacerbated by the facts that water resources are unevenly distributed on the earth's surface and only a small percentage of fresh water is readily accessible for human use as fresh surface water. Although the total volume of water on earth is approximately 1.4 billion $m^3$, only about 2.5 percent of it is fresh water (about 35 million $m^3$) (UNESCO, 1999), and most of this freshwater is either underground or locked in glaciers. Surface fresh water, easily usable by humans, constitutes only 0.01 percent of the total water on earth (Gunawansa and Bhullar, 2013). In terms of water distribution, Canada has fully a tenth of the global surface fresh water (Kalogirou, 2005), while Brazil, China, Russia, the U.S., Canada, India, Indonesia, Colombia, and the Democratic Republic of Congo collectively possess 60 percent of the available fresh water in the globe (World Business Council for Sustainable Development, 2005). In sum, fresh water is already scarce throughout much of the world, and it is expected that the global water consumption will double in the next 20 years. Therefore, finding a new source of fresh water is essential.

President John F. Kennedy, during a speech dedicated to the first seawater desalination plant in the U.S., said:

> *"No water resources program is of greater long-range importance than our efforts to convert water from the world's greatest and cheapest natural resources – our oceans – into water fit for our homes and industry. Such a break-through would end bitter struggles between neighbors, states and nations."*

Today, more than 50 years later, this statement is still true.

In 2005, the average daily water consumption in the U.S. was about 410,000 million gallons, of which 328,000 million gallons per day (80%) were withdrawn from surface water and the remaining 20 percent were from ground water (Barber, 2009). As demonstrated by Figure 1.1, the main uses of water include agriculture, industrial use, and domestic use. According to the United States Geological Survey (USGS, 2009), the total withdrawal of fresh water for

agricultural applications in the year 2005 was estimated to be 128,000 million gallons per day, while the industrial sector had a share of 228,600 million gallons per day (with thermoelectric power withdrawals of 210,000 million gallons per day) and the water withdrawal for domestic applications was estimated to be 25,600 million gallons per day (USGS, 2009). As can be seen in Figure 1.1, as the income of countries increases, the use of water for industrial purposes increases, rising from 10 percent in low- and middle-income countries to 59 percent in high-income countries (World Business Council for Sustainable Development, 2005).

Water security is a major feature of national security due to its direct impact on national independence. With the world population's current growth rate and the expansion of the global economy, it is expected that, by 2025, 60 percent of people who live in arid countries will have limited access to fresh water (Alameddine and El-Fadel, 2007). It is also anticipated that by 2025, approximately 90 percent of the fresh water now available worldwide will have been consumed and rendered unusable (Pasta et al., 2012), leading to a major portion of the world's population (about 75 percent) facing water shortages in 2050 (UNESCO, 2003). Currently, almost 1.8 million people – most of them children – die annually due to water-borne diseases (World Health Organization, 2004). Such conditions are compelling motivations to find new water resources, and desalination – a process which removes salts from salty water to produce fresh water – could provide such a resource.

Fresh water is differentiated from various forms of salty water by its levels of total dissolved solids (TDS), identified by the World Health Organization (WHO) as the measure of all organic and inorganic substances that are dissolved in water; usually, the main constituents are calcium, chloride, sodium, magnesium, and sulfates (WHO, 2008). According to the Water Quality Association (WQA), water is classified into the following categories, based on the level of TDS (WQA, 1999).

In the past several years, the western United States has suffered, and continues to suffer, from moderate to severe drought. This, coupled with fast economic growth in the Southwest which has led to increased demand for water (Brady et al., 2009), has stressed the existing water resources in the region. Throughout most of the western U.S., the water level in rivers has decreased and the water levels in reservoirs have been reduced. To help meet demand, these scarce water resources could be augmented by pumping and desalting brackish groundwater that has total dissolved solids exceeding 1000 mg per liter. However, the potential for desalination is limited in inland areas due to economic factors and the challenges associated with disposing of concentrate, a highly saline waste byproduct of desalination.

Nevertheless, desalination is a promising approach for meeting water needs, and it has a long and proven history in the United States. The first seawater desalination plant in the U.S. was built in 1961 in Freeport, Texas (Arroyo et al., 2012). Currently, there are more than 260 desalination plants in the United States, and more than 95 percent of them are considered inland, brackish groundwater facilities (Mickley, 2009) as opposed to seawater desalination facilities, which

are located in coastal areas. For seawater desalination facilities, the concentrate (also called reject brine) can affordably be returned to the ocean, where it is diluted. However, the disposal of concentrate is a major problem for inland water desalination plants.

When water is desalinated, only a certain percentage of the original water volume is turned into fresh water. This fact is reflected in the water recovery rate, which is the ratio of the volume of desalinated water to the initial water volume used in the desalination unit as feedwater. The recovery rate is an important subject in the desalination industry, which has two primary subsectors: inland brackish water desalination, and seawater desalination. There are two main differences between brackish and seawater desalination systems: recovery rates and the handling of reject brine.

Inland brackish water desalination plants face finite feedwater sources and have high recovery rates of 50 percent to 75 percent; in some cases the recovery rate can reach 94 percent. As a result of this high recovery rate, the reject brine is highly concentrated, which makes concentrate disposal problematic (the high concentration is also the main reason for fouling and scaling of the membrane). To dispose of concentrate, inland desalination plants can employ several different methods, including evaporation ponds, deep well injection, and surface water discharge – among others – but all of these methods are costly and environmentally problematic.

In contrast, seawater desalination plants generally have a lower recovery rate of 40 percent to 60 percent, which is acceptable because the ocean provides a practically unlimited supply of feed water. The lower recovery rate is also prudent because the ocean has high levels of TDS, which would cause fouling at higher recovery rates. Generally, concentrate from seawater desalination plants is returned directly back to the sea, which is a cost-effective but environmentally problematic approach.

For desalination systems themselves, there are three main classes of technology (Younos and Tulou, 2005):

- Pressure-driven (membrane) processes;
- Heat- or temperature-driven processes; and
- Chemical processes.

Pressure-driven and heat-driven processes are used mainly in industrial water purification. Pressure-driven technology is less energy-intensive than temperature driven technology, but this method also delivers a lower permeate quality. The cost of desalination is subject to a plant's location and the technologies used, but, as a result of millions of dollars of research (Yuhas and Daniles, 2006), the average cost of desalination has decreased from US$20 per thousand gallons in 1980 to under US$4 per thousand gallons in 2005. The research on this subject is ongoing, and it is predicted that in the future the cost of desalination will decrease even more.

The water which is desalinated to produce fresh water is known as feedwater. As indicated in Figure 1.2, seawater is the source for about 60 percent of the total

feedwater worldwide, and brackish water, the second largest source in the world, has a share of 21.24 percent.

## 1.2 Desalination Technologies

As mentioned earlier, the two most commonly-used desalination methods are:

1. Pressure-driven processes (membrane desalination); and
2. Heat- or temperature-driven processes.

Pressure-driven processes (membrane processes) include the technologies of reverse osmosis (RO), microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF). Electrodialysis (ED) and electrodialysis reversal (EDR) can also be classified as pressure-driven processes. Each of these technologies is described below.

Reverse Osmosis (RO): RO is an example of a pressure-driven membrane process, where high pressure is used to overcome the osmotic pressure of the membrane. The high pressure forces the solid particles present in the feedwater into the membrane, where the solid particles are retained; the feedwater, however, passes through the membranes, leaving most of its solid particles behind and becoming fresh water. Over time, the membrane will be contaminated by biological fouling, causing scaling to occur. Chemical treatments can be used to remove scaling, but if chemical clearing is not effective, then the RO membrane will require replacement (Carter, 2009). RO membranes have a total recovery rate of 70-85 percent (Greenlee et al., 2009). RO is the leading technology for treating both seawater and brackish water; there are more than 16,000 desalination plants worldwide, and about half of them are RO plants (Kurz et al., 2011). In the United States, around 70 percent of desalination plants use this technology. About 7 percent of the plants using the RO method use seawater as the feedwater source (Carter, 2013; Greenlee et al., 2009; Wetterau, 2011).

Microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) are systems distinguished by the pore size of their filters. NF membranes remove bacteria from the water using the same method as RO. The pore size of NF membranes is 0.001 μm. In UF membranes, the pore size is 10 times larger than NF (typically between 0.01-0.05 μm), and the UF membranes are capable of filtering a higher molecular weight than the NF membranes. The MF filter is used to remove larger particles, and has a comparatively large pore size of 0.1-0.2 μm. MF filters are generally used in drinking water applications (Wang et al., 2008). For MF, molecular weight cutoff (MWCO) has become the central measurement instead of the normal measurement of pore size (EPA, 2010), on account of some larger organic macromolecules that can be retained in the membranes. In addition to their use in industrial desalination, the aforementioned filters are used in a wide range of industries such as pharmaceuticals, food, and metal processes, among others.

Electrodialysis (ED) and electrodialysis reversal (EDR): ED/EDR technologies are electrochemical methods that function on the principle of the movement of an electrolyte that is subjected to an electrical field. The ED/EDR

base unit consists of a number of cells, and each cell pair contains ion- and cation-permeable membranes and a spacer. The spacer helps direct the flow of the water as the feed water passes simultaneously through all the cells. Under the influence of direct current (DC) electricity, the electrodes will split the feed into positive and negative ions. The positive ions will leave the feedwater by travelling toward the cathode through a cation exchange membrane, while the negative ions move in the opposite direction, leaving the feed stream by travelling toward the anode through an anion exchange membrane. After leaving the feed water stream (which, at this point, is desalinated), the positive and negative ions from the feedwater are trapped by oppositely charged membranes, producing the concentrate stream that contains the salts and other dissolved minerals (U.S. Department of the Interior Bureau of Reclamation, 2010; Greenlee et al., 2009). To achieve a high recovery rate and provide a self-cleaning process with less fouling and scaling of the membrane, the polarities of the electrodes are periodically reversed in EDR. With the EDR method, the recovery rate can go up to 94 percent.

Temperature-driven processes include multistage flash evaporation (MSF), multiple-effect distillation (MED), and vapor compression (VC). Each of these processes is described below.

Multiple-effect distillation: MED is the oldest desalination process used, and it consists of multiple stages or "effects." Each of these stages makes use of a series of tubes heated by internal steam. As the first step of the MED process, the salty feed water is dispersed over the tubes, and as the water reaches its boiling point, the vapor generated from the heat in the first tube transfers heat to the second tube. At this point, the process repeats itself. Distillate – the desalinated water – and brine are collected in each stage. MED is energy-efficient since it uses latent heat to boil the feedwater without any additional supply of heat after the first tube. However, MED has its problems – notably, scaling – and therefore, after MSF technology was released, the use of MED decreased significantly.

Multistage Flash Evaporation: In 1957, the first large MSF units were installed and built in the Middle East by Westinghouse Company. This system consisted of four flash stages with two units, which produced a total of 1 million imperial gallons per day (IGD) of fresh water from seawater (Al-Modaf and Al-Wazzan, 2001). MSF units, in general, are composed of three sections: heat rejection, heat recovery, and heat input (brine heater). The first two sections comprise a plant. These stages are connected to each other, and each stage consists of a heat exchanger and a condensate collector. The input feed will start boiling and evaporating by keeping the pressure in the first flash chamber lower than the corresponding saturation pressure. This will cause the water vapors to cool down and condense to form the distillate, and the latent heat generated from the condensation is used to heat the new seawater in the tubes. Finally, the distillate produced in each stage is collected and then pumped into a storage tank (Khawaji et al., 2008).

Vapor Compression: In this type of desalination technology, mechanical energy replaces thermal energy. VC operates by reducing the vapor pressure in

order to reduce the boiling point temperature (EPA, 2005). Two methods are used to compress vapor pressure: an ejector system, thermal vapor compression (TVC), which is driven by an external source of pressure, and mechanical vapor compression (MVC). TVC units are larger than the MVC units, yet both of these units have small capacities compared to MEDs or MSDs.

A problem with all desalination processes is the difficulty disposing of reject brine. This is one of the key factors that must be considered before a desalination plant is installed. This issue is particularly important for inland desalination plants. When a method for disposing concentrate has been selected, the two main concerns are the economic costs and the environmental effects (Mickley, 2009) that result from the highly saline nature of the concentrate; some studies indicate that the salinity of reject brine (concentrate) can reach 85,000 mg/L (Abdul-Wahab and Al-Weshahi, 2009), which is double the salinity of seawater.

## 1.3 Comparison

As shown in Table 1.2, the cost of electrodialysis desalination techniques is lower than the cost of the other technologies. Multiple effect distillation (MED) and multi-stage flash desalination (MSF) have a higher cost than RO processes and produce the same efficiency.

## 1.4 EDR and RO Comparison

As shown in Table 1.3, in which RO and EDR are compared, EDR has a higher efficiency and lower cost, while RO has the upper hand when the feed has a higher conductivity.

## 1.5 Wastewater Treatment Plant

The purpose of treating wastewater is to avoid pollution problems in receiving waters. In particular, the main water quality concern in the wastewater treatment plants is nitrogen (Richard et al., 2009). To minimize pollution problems, the first objective of wastewater treatment is to reduce the volume of the waste by removing its liquid portion, producing a sludge. The second objective is to decompose the highly putrescible organic matter into relatively stable or inert organic and inorganic compounds. When these two objectives are achieved, an anaerobic digested sludge is produced. The characteristics of such sludge are shown in Appendix B.

Within wastewater treatment plants, there are three fundamental levels of treatment:

1. Primary treatment, in which water is piped into large tanks and allowed to settle to remove particulate solids. This level is sometimes referred to as mechanical treatment;
2. Secondary (biological) treatment, in which microorganisms are used to remove more contaminating solids. In the absence of oxygen, the microorganisms consume the organic matter as food and convert it to carbon dioxide, methane, water, and energy for their own growth and reproduction. This step removes the dissolved organic matter that escapes primary treatment. The resulting product is called anaerobic digested sludge. The process itself is sometimes called biological treatment; and
3. Tertiary treatment, which is simply additional treatment beyond secondary treatment. This step can remove more than 99 percent of all the impurities from sewage, producing an effluent of almost drinking-water quality through disinfection, typically with chlorine.

## 1.6 Research Issue and Solution

The main difference between seawater and brackish water is the amount of TDS each contains, as can be seen in Table 1.1. Desalination technologies have significantly increased worldwide access to large quantities of drinkable water by converting non-potable saltwater into fresh water. Most of the seawater desalination units dispose of the reject brine back into the ocean, as this approach is less expensive; however, this procedure is very harmful to the environment because the high salt concentration, high temperature, and other chemical elements such as anti-scaling additives affect the aquatic environment.

The disposal methods for concentrate from inland water desalination plants are not very efficient due to their high cost and the harm they cause to the environment. After the dissolved salt is removed from saltwater to make freshwater, the salt is left in a concentrate stream (also called a reject brine) which has a very high level of total dissolved salt. Despite the problem mention above, approximately half of the concentrate streams produced from desalination plants in the United States are disposed of by the following standard disposal methods: discharge to surface water or sewers, containment in deep wells, or disposal in evaporation ponds and land applications (Mickley, 2009).

From an economic standpoint, disposing of the concentrate can be very expensive, and can vary from 5 percent to 30 percent of the total cost of desalination (Hordagui, 1997; Mohamed et al., 2005). The desalination industry and its customers are affected by the significant cost associated with disposing of the concentrate; therefore, reducing the costs is one of the main concerns in the industrial desalination sector.

An affordable and sustainable method for disposing of the concentrate could preserve the environment and reduce the cost of potable water by reducing the financial burden on the industrial desalination plants. This present study showed that microalgae can be used to treat the concentrate from desalination

plants by using the dissolved carbon and nutrients in the waste stream as media for growth, eliminating the salts by metabolizing them. The microalgae species can also produce biofuels and other useful products while they treat the waste concentrate.

## 1.7 Hypothesis

The hypothesis of this research is that concentrate can be used as a growth medium for algae because it contains nutrients and minerals that can be used by algae; Table 1.4 shows some of the results from an analysis of the four groundwater wells from the Brackish Groundwater National Desalination Research Facility, highlighting the elements that algae can use as nutrients.

In addition to these elements, the groundwater wells at BGNDRF also contained other elements such as dissolved oxygen chloride, bicarbonate silica, bromide, barium, iron, silica, organic carbon, selenium, copper, chloride, and fluoride.

The elements listed in Table 1.5 and Appendix B can be consumed by algae, providing a possible path for using the concentrate as a growth medium for algae and reducing the environmental impacts of the reject brine, potentially making this an affordable and sustainable method. This approach could also be combined with another waste stream: supernatant anaerobic digested sludge (SADS) from wastewater treatment plants (WWTP). This stream contains phosphorus and nitrogen, critical elements for algae growth (Pankaj and Awasthi, 2013). By utilizing these two waste streams (SADS and concentrate) it should be possible to produce a useful microalgae.

The bicarbonate identified in the groundwater wells at BGNDRF is an inorganic carbon source, and can improve the growth of algae cultures in carbon storage compared to $CO_2$ (Gardner et al., 2013). Concentrate from brackish groundwater desalination dissolves more $HCO_3^-$ than that from seawater desalination. *Spirulina* grows in high $CO_3^{2-}$ and $HCO_3^-$ water (Richmond, 1986). $CO_3^{2-}$, $HCO_3^-$ and alkaline-rich microalgae consume dissolved inorganic carbon as a primary carbon source and sulfate as a macronutrient. Desalination concentrate from brackish groundwater can be treated by microalgae which consume bicarbonate and sulfate. *Dunaliella* species are native to salt water (Borowitzka, 2009) and can tolerate a wide pH range (Gimmler et al., 1989), making them one of the most environmentally tolerant eukaryotic organisms recognized, capable of surviving in salinities ranging from seawater (3% NaCl) to NaCl saturation (31% NaCl) (Ginzburg, 1989).

## 1.8 Research Objective

As part of ongoing water research on treatment of desalination concentrate at New Mexico State University, this study was conducted to:

1. Determine the feasibility of integrated algae cultivation (*Dunaliella salina*, *Spirulina platensis*, and the strain from the BGNDRF evaporation pond) by using desalination concentrate as a growth medium and SADS as nutrients; and
2. Determine the feasibility of reducing the salinity level of concentrate by using it as a medium for microalgae production.

## 1.9 Approach

The experimental evaluation of the research objective was performed by varying the level of conductivity in the bioreactors, using the native non-GMO microalgae *Dunaliella salina*, *Spirulina platensis*, an unknown species of microalgae strain acquired from the BGNDRF evaporation pond, SADS from the wastewater treatment plant, and natural concentrate from the Brackish Groundwater National Desalination Research Facility in Alamogordo, NM. During each experiment, measurements were taken for dry weight, optical density, temperature, conductivity, and pH, and algae samples were collected and analyzed.

# Conclusions

Based on the experiments conducted in this study, the following conclusions can be made:

- Due to microorganisms growing with microalgae, the maximum dry weights of *D. salina* and *S. platensis* grown in desalination concentrate and supplied with SADS (1.36–1.49 g/L) are more than the dry weights of these same species when supplied with BBM and F/2, due to the manner in which the microorganism promotes microalgae growth. The maximum dry weight concentrations of *D. salina* and *S. platensis* grown in desalination concentrate and supplied with SADS are comparable to those in the literature.
- This study demonstrates the feasibility of using concentrate as a growth medium using SADS as a nutrient to grow algae culture.
- A combination of lower conductivity in the medium (25,442 and 25,100 µS/cm) and the use of SADS enhanced the growth of *D. salina* and *S. platensis*.
- The amount of the conductivity reduction was significant in BGNDRF species strains in 110 days.

These results suggest that using microalgae for reducing the conductivity of desalination concentrate and SADS by using the concentrate as a growth medium and SADS as an additional source of nutrients is better than using traditional methods for disposing of the concentrate from the desalination units, which have high costs and adverse environmental effects. High TDS levels, however, limited the ability of specific algae species to grow in the concentrate and reduce its conductivity.

The results also suggested that the BGNDRF strain can be used for concentrate management at salinity levels below 35,000 µS/cm. The BGNDRF species grew well at these levels, and since SADS is known to contain elements and ions that algae consume in their growth process – namely, ammonia nitrogen, sodium, calcium, magnesium, and potassium – it can be deduced that the BGNDRF species consumed some of these elements and ions in order to grow, reducing the overall salinity of the concentrate. The extent of this reduction could be explored in future research.

# Recommendations

Recommendations for future research are listed below:

- Experiments can be conducted at different TDS levels to establish the optimal growth rate and can be performed on a large scale.
- Different species of microalgae can be cultured with the reject concentrate to study their growths and the conductivity reductions.
- The ion and element content of the growth media could be determined before and after algae growth to identify the specific ions and elements that the algae species remove.

# Chapter 2: LITERATURE REVIEW

This chapter offers a description of the seven typical disposal methods for concentrate and provides details on these methods. This topic is followed by a discussion of algae species selection, and then algae-based concentrate treatment, energy security, and the potential for biofuel production from microalgae. The chapter concludes with a broad outline on carbon dioxide emissions.

## 2.1 Concentrate Disposal Methods

The list below shows the different methods used by desalination plants for the disposal of reject brine, starting with the most common. The information is based on a survey conducted by Michael C. Mickley that explored the concentrate disposal methods used by desalination plants that have more than 300 membranes (Mickley, 2009) and which treat at least 25,000 gallons of water per day (GPD). As reported in Mickley's research, the leading methods for concentrate disposal are:

- Surface water discharge,
- Discharge to sewer,
- Land application,
- Deep well injection,
- Evaporation ponds,
- Spray irrigation, and
- Zero liquid discharge.

Each of these methods is discussed below.

Surface Water Discharge: In this method, concentrate is discharged into surface water such as oceans or lakes. Since 1977, as a result of the Clean Water Act (CWA) passed in 1972, desalination plants have had to obtain a permit from the National Pollutant Discharge Elimination System (NPDES) to dispose of the concentrate in any surface water. The administrator of the EPA may also issue a permit to discharge. The concentrate is permitted to contain a medium or high level of TDS, depending on the technology used by the plants (Doremus and Tarlock, 2013).

Discharge to Sewer: In this method, concentrate is discharged into sewer systems. To make sure the disposed concentrate meets wastewater regulations designed to prevent adverse effects to the sewer system, this method also requires a permit issued under the NPDES (Mickley, 2006).

Land Application: This method is the most efficient option in locations where the climate is dry and sunny and where large plots of land are available at low cost. This method is usually used for small desalination plants (Mickley, 2009).

Deep Well Injection: This method consists of injecting wastewater 1000 to 8000 feet into the earth through a deep well. Generally, only large plants use this disposal method. This method may be considered storage instead of disposal, since the wastewater stays in the wells and does not disperse. Due to increase

concerns over the contamination of 300,000 injection wells (Mickley, 2006), the United States Congress added regulations for underground injection control to the CWA in 1979.

Evaporation Ponds: This method follows the same basic approach used to produce salt from seawater, and it works by pumping concentrate into shallow, artificial ponds, where the water evaporates and leaves the solids behind. These residual solids can then be dumped into landfills or sold if they are considered a valuable substance. This disposal method is usually used by small-sized plants (< 1 million gallons) in the southwestern United States, where evaporation ponds are the most suitable method for disposing of concentrate as evaporation rates are high in the dry, sunny climate, and large plots of land are available at low cost. The NPDSE currently does not require a permit for disposal of concentrate using evaporation ponds (Mickley, 2006).

Spray Irrigation: This method is similar to the sprinklers commonly used to water lawns, gardens, and golf courses. As with evaporation ponds, this process requires a relatively dry, sunny climate and available land, and is usually used for small desalination plants with low concentrate flow rates. For spray irrigation, the concentrate must be pre-treated or diluted to reduce the salinity of the wastewater; this method requires a permit from NPDSE (Mickley, 2006).

Zero Liquid Discharge: This method works by recycling the concentrate for different purposes within the desalination plant and reducing the amount of waste water. At the end of this process, the concentrate is reduced to a sludge-like material or dry salt (zero liquid), which can be disposed of as a solid. This is the most expensive method for disposing of the concentrate – because of its high energy demands, it can encompass more than 60 percent of a plant's capital cost. Consequently, this method is usually followed only when no other disposal options are feasible (Mickley, 2006).

Table 2.1 indicates the percentages at which the five conventional concentrate disposal methods are used for municipal membrane desalination plants in the U.S. Such plants account for 98 percent of disposal cases in the U.S.

## 2.2 Algae Species Selection

The microalgae strain from the desalination evaporation pond at BNGDRF and two other species of halophytic microalgae, *Spirulina platensis* and *Dunaliella salina,* were selected for this study.

*Spirulina platensis* is a photosynthesizing cyanophyte (blue-green algae) that has the shape of a spiral coil and the ability to grow energetically in sturdy sunlight under hot temperatures and highly alkaline conditions (Richmond, 1986). *Spirulina* prospers in alkaline lakes where it is difficult or impossible for other organisms to live (Habib and Parvin, 2008; Kebede and Ahlgren, 1996), and it also can grow in brackish water and in high bicarbonate concentrations (Mallick, 2002). *Spirulina* can consume dissolved carbon dioxide in a water medium as a primary substrate for its growth (Habib and Parvin, 2008). For many decades, *Spirulina* has been used as a food source worldwide because it contains several

nutrients such as proteins, carbohydrates, minerals, different vitamins, and γ-linoleic acid (Ramadan et al., 1996; Teimouri, 2013). The oil content in *Spirulina platensis* ranges from 6-13 percent of algae dry weight (Chaiklahan et al., 2008).

*Dunaliella salina* is a unicellular green microalgae that is capable of prospering in high-salinity water (Fisher et al., 1997) and in strong shifts in salinity due to its intracellular osmotic metabolites (Ben-Amotz and Avron, 1973; Mishra et al., 2008; Chen and Jiang, 2009). The size of *Dunaliella salina* ranges between 5-25 μm in length and 3-13 μm in width. This species contains β-carotene in a range of 10-14 percent of algae dry weight, and is therefore often used in natural foods. The total lipid content in *Dunaliella salina* is in the range from 3.8 to 4.4 percent in terms of dry weight (Abd El-Baky et al., 2004; Weldy et al., 2007). In open lakes, microalgae growth cycles are normally limited by the availability of nutrients in the water medium. In commercial cultivation, the growth and carotenogenesis media comprise one-third of the total production cost of *Dunaliella salina* (Santos et al., 2001). If *D. salina* can be cultured from a cost-free growth medium and a cost-free nutrient, this would avoid not only about one-third of the total cost but also the $CO_2$ emissions from the fossil fuel-based manufacturing of conventional growth media and nutrients.

*Spirulina* and *Dunaliella* have been successfully cultured in a scale larger than 0.1 ha (Sheehan et al., 1998). Most of the commercial production of microalgae is from open ponds.

## 2.3 Algae-based Concentrate Treatment

Microalgae require water, light, $CO_2$, appropriate pH, suitable salinity, macronutrients (nitrates and phosphates), vitamins, and trace elements for their growth (Chisti, 2007; Brennan and Owende, 2010). By using photosynthesis, microalgae convert light into new algae biomass as the following equation indicates:

$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H + \text{sunlight} \leftrightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2$ (Stumm and Morgan, 1988).

By rearranging the above equation, the microalgae component can be expressed as:

$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)$.

The ratio of carbon-to-nitrogen-to-phosphorus is the main requirement for the growth of algae. Microalgae will grow well if all these components are available in an appropriate ratio; if fresh water is used, it takes 3726 kg water, 0.33 kg nitrogen, and 0.71 kg of phosphate to produce 1 kg of biodiesel from algae. Recycling harvested water reduces the water and nutrient usage by 84 percent and 55 percent, respectively. Using seawater or wastewater decreases the water requirement by 90% and reduces the need to supply all of the nutrients except phosphate (Yang et al., 2011). To grow 1 kg of dry microalgae, 20.3 L of water, 134 g of salt, 147 g of nitrogen, and 20 g of phosphorus are required (Batan et al., 2010). Chisti's 2008 analysis shows that algae biomass with a lipid content of 42

percent and production costs of US$217.22 per ton becomes competitive with petroleum at the price of US$60.00 per barrel. Using a value of US$0.22 per kWh for energy consumption, the production cost of 1 ton of algae biomass from desalination concentrate that had already been used to produce a crop of microalgae was estimated to be US$808.79. One ton of algal biomass can be produced during the treatment of 1443 $m^3$ of wastewater. If the credit for wastewater treatment at US$0.4 per $m^3$ is considered, the cost of 1 ton of biomass would be reduced to US$231.59. This calculation concludes that if the price of petroleum is US$63.97 per barrel, algae biomass can be a viable energy alternative.

The financial costs of both desalinization and microalgae production can be reduced by reusing the concentrate from desalination to grow the microalgae. This same practice could also reduce the environmental costs of desalinization. As mentioned before, the financial costs associated with disposing of concentrate are currently very high: in the case of inland sites, concentrate disposal adds a minimum of 15 percent to the cost of desalination (Glueckstern and Priel, 1997; Oren et al., 2010). For disposal by evaporation pond, the cost is US$1.18-10.04 per $m^3$ (Samimi and Zarinabadi, 2012). At present, the top available disposal methods – surface water disposal or discharge to wastewater treatment plants – are also highly debatable due to environmental concerns. In sum, the literature supports the contention that desalination could be made more sustainable and be done with a dramatically lower cost by using the waste concentrate produced by desalinization to grow microalgae.

Microalgae can also reduce the presence of many heavy materials in wastewater through the phycoremediation process (Pankaj and Awasthi, 2013).

## 2.4 Carbon Dioxide Emissions (Global Warming)

As sunlight reaches the earth's atmosphere and strikes the planet, the surface of the earth is heated and a portion of the sunlight is reflected back to space as infrared radiation. The main greenhouse gases - carbon dioxide ($CO_2$), nitrogen dioxide ($NO_2$), methane ($CH_4$) and steam ($H_2O$) (EPA, 2012) – absorb the infrared radiation and trap the heat, causing the earth's temperature to rise about 32 $^oC$ (59 $^oF$) to an average of 14-15 $^oC$. This natural phenomenon makes the environment warmer and more suitable for the development of human civilization; without it, the surface of the planet would be covered with a thick layer of ice (Chen et al., 2001; Samimi and Zarinabadi, 2012; Loriuset et al., 1990).

However, since the Industrial Revolution (mid-1700s) (Hettiarachchi, 2012), the use of fossil fuels and other energy sources that produce greenhouse gases (GHG) such as carbon dioxide and methane has increased dramatically, and the concentrations of these gases in the earth's atmosphere have increased by 36 percent and 148 percent, respectively; other studies indicate that the concentrations of the aforementioned gases in the atmosphere have increased by 40 percent and 160 percent, respectively (EPA, 2013).

The Intergovernmental Panel on Climate Change (IPCC) has identified carbon dioxide as the most significant anthropogenic greenhouse gas, with an 80 percent annual emission growth between 1970 and 2004 (Greenwell et al., 2010). The largest share of the total greenhouse gas emissions is also carbon dioxide (about 70 percent) (Stewart and Hessami, 2005). Increasing the presence of $CO_2$ and other greenhouse gases in the atmosphere has kept a large portion of infrared radiation from exiting the earth's atmosphere, causing the weather to become warmer (Wang et al., 2008).

Carbon dioxide alone is responsible for about 25 percent of the effect from all greenhouse gases, due to its absorption of half of the infrared radiation wavelength reflected back to space from the earth. According to the National Energy Technology Laboratory, the total $CO_2$ emissions from industrial sources is about 100 trillion cubic feet (5,090 million metric tons) per year (Nakamura, 2006), and according to the U.S. Department of Energy's Carbon Dioxide Information Analysis Center, in 2013 the global carbon dioxide emission was approximately 36 billion tons from the combustion of fossil fuels only; that same year, the cumulative emission of $CO_2$ due to all human activity since the mid-1800s reached 2015 billion tons of $CO_2$ (Carbon Dioxide Information Analysis Center, 2013). Currently, the level of carbon dioxide concentration in the atmosphere is between 300 and 400 ppm (Rosenberg et al., 2011). According to Hettiarachchi (2012), "the IPCC Special Report on Emissions Scenarios gives a wide range of future $CO_2$ scenarios, ranging from 541 to 970 ppm by the year 2100" (IPCC, 2012).

Microalgae have the capability of capturing $CO_2$ from various sources such as the atmosphere, industrial exhaust gases, and fixed $CO_2$ sources. Generally, microalgae are cultivated in two methods: open ponds (raceways), and closed systems, which are exposed to air or aerated in order for air-tolerating microalgae to capture $CO_2$ from the atmosphere for cell growth. Microalgae are considered to be the most productive carbon user, and can fix a larger amount of $CO_2$ per land area than can higher plants such as trees and sugar cane. In addition, microalgae are not subject to the loss of plant leaves due to weather and environmental conditions, a problem in higher plants which adversely affects the process of photosynthesis and therefore reduces $CO_2$ uptake (Brown and Sprague, 1992). Some studies show that $CO_2$ is captured by microalgae with an efficiency up to 50 times greater than that of higher plants (Nakamura, 2006; Demirbas, 2006). Such efficient $CO_2$ capture could help alleviate climate change effects from elevated $CO_2$ levels.

In the southwestern desert of the United States, there are favorable conditions for algae growth, such as expansive lands, warm temperatures, brackish water, and large sources of carbon dioxide (in the form of fossil fuel power plants). The feasibility of using such $CO_2$ sources to grow microalgae has already been demonstrated: in Kona, Hawaii, a commercial production plant for biofuel already supplies $CO_2$ for *Spirulina* growth using flue gases from a power plant. About 75 percent of the flue gas, which provides 67 tons of $CO_2$ per month, is efficiently absorbed into the system, supporting 36 tons/month of *Spirulina* (Pedroni et al., 2001). Globally, approximately 7 percent of $CO_2$ emissions is due

to power plant flue gases (Fisher et al., 1997). In 2010, EIA estimated that the total emission of $CO_2$ from coal power plants in New Mexico and Arizona has been 68.5 million metric tons (EIA, 2013), which can be absorbed by algae farms covering only 0.3425 percent of the area of those states (Demirbas, 2006).

Emissions from the use of fossil fuels will add more carbon dioxide to the atmosphere, and will therefore increase the climatic effects of greenhouse gases. Using biofuel from microalgae as an alternative to fossil fuels reduces the emission of greenhouse gases such as carbon dioxide, methane, and nitrous oxide. During the process of microalgae cultivation, the algae consume the carbon dioxide necessary for their growth and then release the same amount of this greenhouse gas (carbon dioxide) when they are used as biofuels. The advantage of this method is that the balance of the gas in the atmosphere will not be affected during the combustion of the biodiesel.

The application of microalgae to reduce greenhouse gas emissions can come through the development of a wastewater treatment and aquatic farming process that combines algae's waste treatment features with their ability to reduce GHG emissions and produce biofuel (Havlík et al., 2011).

As compared with petroleum diesel, the percentage decrease in greenhouse gas components and the reduction in net emissions for the production of biodiesel from microalgae and soybean feedstocks are evident in Table 2.2.

# Chapter 3: MATERIALS AND METHODS

## 3.1 Analytical Method and Sampling (Experiment Design)

Two experiments of the three experiments (for *D. salina* and *S. platensis*) are designed based on a two-level factorial design ($2^2$) with two replications for each level of conductivity in the medium. The nutrient types were considered as treatments, and dry weight and optical density were measured as responses. The third experiment (for the BGNDRF species) is designed with five different levels of conductivity as treatments, and dry weight and optical density as responses.

In all three experiments, temperature, pH, conductivity, and flow rate were monitored and measured.

The lengths of the experiments were determined by the growth behavior of the microalgae, which, in fed batch reactors, is characterized by five phases: 1) the lag phase, in which the microalgae are acclimating to the new environment and only a small increase in cell density happens; 2) the exponential phase, in which cell density increases as a function of time; 3) the phase of declining growth rate, in which the increases in cell density slow; 4) the stationary phase, in which cell density stabilizes; and 5) the death or crash phase, when cell densities drop precipitously as a result of algae die-off. This may happen due to an obstruction of light caused by the high cell density, an increase in the toxicity of the growth medium due to the buildup of algae's natural wastes, the competitive effects of indigenous bacteria and protozoa, and/or a depletion of nutrients. The experiments were run until the algae reached their death or crash phase, so the lengths of the experiments varied with the lengths of the algae's different growth phases. The BGNDRF strain, since its characteristics were previously unknown, was allowed to grow for a longer period to ensure that it had reached the crash phase.

The *D. salina* microalgae species was cultivated at the New Mexico State University Laboratory. *Arthrospira (Spirulina) platensis* was cultivated at the University of Texas at Austin. A previously unknown species from a desalination concentrate pond in Alamogordo, NM was also grown in a single reactor. The three species were grown in cleaned used bottles (3.785 L volume) with desalination concentrate as the growth medium and SADS from a wastewater treatment plant as a nutrient (Table 3.1). Desalination concentrate samples were collected from the desalination concentrate ponds of the Brackish Groundwater National Desalination Research Facility located in Alamogordo, New Mexico. Anaerobic digested sludge was collected from the wastewater treatment plant in

Las Cruces, NM. Desalination concentrate and anaerobic digested sludge were separately centrifuged for 3 minutes at 10,000 rpm to separate the heavy particles and collect the supernatants. These supernatants were used in the studies as a nutrient. Dry weight concentration and the optical density of growth culture were used to identify the microalgae growth. About 10 mL of cell suspension samples were withdrawn from the reactor and centrifuged for 3 minutes at 10,000 rpm; then, the supernatant was decanted, and the remaining wet microalgae (slurries) were dried at 103-105 °C in an oven to measure the dry weight concentrations of the microalgae. These measurements were taken in accordance with the SM 2540D procedures (American Public Health Association, 2005; Valigore et al., 2012). The same volume of supernatant of each sample was also dried in the same oven to obtain the correct TDS concentration from the wet microalgae (slurries) to get the TDS-free dry weight concentration of the microalgae. The optical density of the growth culture was measured with a spectrophotometer (Hach DR/2010) at a 560 nm wavelength, the same wavelength recommended by Concaset et al., 2013. The 560 nm wavelength was chosen to correspond to the peak absorption rate for chlorophyll. Scans were performed in cuvette tubes. The pH was measured with a Cole Parmer pH meter AB15 Accumet Basic. The conductivity was measured with the Hach sensION5 conductivity meter. Information on the growth of each of the three algae species is presented below.

- *D. salina*: This experiment was performed during November-December 2011, for a total of 41 days. The parameter measurements were taken on an average of every two days. Dry weight concentrations were measured at 18 points, the optical density of the growth culture was measured at 17 points, the conductivity of growth culture was measured at 18 points, and the pH was measured at 17 points of treatment.
- *S. platensis*: This experiment was performed during January-February 2012, for a total of 34 days. The parameter measurements were taken on an average of every three days. Dry weight concentrations were measured at 10 points, optical density of growth culture was measured at 12 points, the conductivity of growth culture was measured at 13 points, and the pH was measured at 13 point of treatment.
- BGNDRF species: This experiment was performed during February-September 2012, for a total of 110 days. The parameter measurements were taken on an average of every five days. Dry weight concentrations were measured at 20 points, the optical density of the growth culture was measured at 22 points, the conductivity of growth culture was measured at 22 points, and the pH was measured at 24 points of treatment.

## 3.2 Contents in Reactors

All reactors were filled with desalination concentrate and seed microalgae as shown in Table 3.1. Reactors D1, D2, S1, and S2 were fed with SADS as

nutrients. Reactors D3 and D4 were fed with Bold's Basal Medium (BBM) (Nichols and Bold, 1965), and reactors S3 and S4 were fed with F/2 (Guillard and Ryther, 1962). Reactors R1, R2, R3, R4, and R5 were fed with SADS. The characteristics of SADS, Bold's Basal Medium, and F/2 are shown in Appendices B, C, and D, respectively. The reactors were bubbled 8 hours a day with air from the environment, which contained $CO_2$ at 0.0387 percent by volume. All reactors were directly exposed to sunlight from 9:00 a.m. to 5:00 p.m. in New Mexico State University, Las Cruces, NM. Sunlight radiation data were not collected since sunlight radiation varies with time (from 9:00 a.m. to 5:00 p.m.) during the day and also with the location of the reactor surface. The reactors were illuminated with light bulbs on holidays when the reactors were in the lab. The radiation from the light bulbs to the reactors was not collected since the exposure time to the light bulbs was negligible compared to the exposure time to sunlight. SADS, F/2, and BBM were fed periodically as fed-batch culture.

The conductivities of the concentrate varied somewhat due to the process used to increase the salinity to needed levels. The concentrate taken from the desalination systems at BGNDRF had relatively low levels of conductivity, which varied slightly around 6280 μS/cm. Since the goal of this research was to investigate the growths of different algae species at elevated and significantly different salinities (differences of about 7000 μS/cm), the concentrate salinity had to be increased. This was done by boiling the concentrate to reach the desired salinity levels, and since this process is not completely controllable, the final salinity levels for different experiments were slightly different.

## 3.3 Statistical Analysis

After data collection (biomass measurement), based on the hypothesis and research questions, two way analysis of variance and regression techniques were used to find the differences and relationships between predictors (nutrients and conductivities) and response (biomass).

# Chapter 4: RESULT AND DISCUSSION

## 4.1 Introduction

A $2^2$ factorial experiment was conducted for two experiments (*D. salina* and *S. platensis*) and the third experiment (BGNDRF species) was designed using five different levels of conductivity. These experiments were carried out for two reasons: (1) to evaluate microalgae's ability to reduce conductivity in desalination concentrate; and (2) to investigate the feasibility of integrated algae cultivation using desalination concentrate and supernatant from anaerobic digested sludge (SADS). This chapter presents the results obtained from these experiments.

## 4.2 Batch Reactors (*Dunaliella salina* and *Spirulina platensis*)

Table 4.1 shows the highest dry weight achieved from the study, along with optical density, pH, culturing day, and temperature at which the highest dry weight was attained.

The growths of *D. salina* and *S. platensis* in two different nutrients with time are shown in Figures 4.1(a) and 4.2(a). Reactors D1, D2, S1, and S2 were supplied with SADS, while D3, D4, S3, and S4 were supplied with BBM and F/2, respectively. *D. salina* required 37-39 days to reach maximal growth. With SADS as a nutrient, *D. salina* needed 37 days to reach maximal growth, which was 1.40 g/L of dry weight when grown in 31,800 μS/cm conductivity and 1.56 g/L dry weight when grown in a conductivity of 25,442 μS/cm. With BBM as a nutrient, *D. salina* required 37-39 days to reach maximal growth, which was 1.04 g/L of dry weight when grown in 31,800 μS/cm conductivity and 0.84 g/L dry weight when grown in 25,442 μS/cm conductivity. *S. platensis* needed less time than *D. salina* to reach maximal growth, taking 14-20 days to reach the maximal growth as determined by the highest point in the dry weight graph. With SADS as a nutrient, *S. platensis* needed 14-24 days to reach maximal growth, which was 1.46 g/L of dry weight when grown in 35,800 μS/cm conductivity and 1.96 g/L dry weight when grown in 25,100 μS/cm conductivity. In F/2 nutrient, *S. platensis* required 20 days to reach the maximal growth of 1.28 g/L of dry weight when grown in 35,800 μS/cm conductivity and 0.66 g/L dry weight when grown in

25,100 µS/cm conductivity. SADS provided higher yields than BBM (*D. salina*) and F/2 (*S. platensis*). The lower conductivity improved the yield while SADS was fed into reactors.

Figures 4.1(b) and 4.2(b) show the optical density of the cultures with time. The highest optical densities (*D. salina* 2.25; *S. platensis* 1.62) occurred in the lower conductivities (*D. salina* 25,442 µS/cm; *S. platensis* 25,100 µS/cm) with the SADS as nutrient for both *D. salina* and *S. platensis*.

Figures 4.1(c) and 4.2(c) depict the conductivity changes in the growth media with culturing time. The conductivities in cultures of *D. salina* in reactors D1 and D2 decreased with time, as shown in Figure 4.1(c). This decrease is due to microalgae consuming the necessary ions (phosphorus, nitrate+nitrite, calcium, sulfate, magnesium, sodium, and potassium) for their growth in the concentrate stream. The conductivities of the cultures in reactors S3 and S4 increased with time, as shown in Figure 4.2(c). Although *S. platensis* consumed the ions in the medium necessary for its growth, the F/2 nutrient had a higher conductivity than the culturing desalination concentrate, so the conductivity level increase may have been due to the contribution from F/2 nutrient. The nutrients added into reactors S1, S2, S3, and S4 are shown in Figure 4.2(d). As algae grow, they require more nutrients; therefore, the amount of nutrient added was increased with time.

Figures 4.1(e) and 4.2(e) show the pH of the media with time. These data show that *D. salina* and *S. platensis* grew in pH 8-9, as shown in Figures 4.1(e) and 4.2(e). The temperature of medium for *D. salina* was 60-90 ºF, as shown in Figure 4.1(f); the temperature of medium for S. *platensis* was 65-95 ºF, as shown in Figure 4.2(f). $CO_2$ was supplied from air, which was bubbled into reactors.

As previously mentioned, this experiment was a full-factorial design ($2^2$). The Analysis of Variance for the experiment (*D. salina)* is shown in Table 4.2

After finding a statistically significant difference, it was necessary to use multiple comparisons to find the significant nutrient and the best combination of conductivity and nutrient to control Type I error and increase the power of our statistical analysis; we therefore used Tukey's test.

As can be seen in the ANOVA in Table 4.2, conductivity levels had no effect on the level of biomass production for *D. salina* (P-value >0.05). Tukey's test also showed no difference, a result that could be due to *D. salina*'s ability to grow in high levels of salinity and in strong changes in salinity. Therefore, the level of conductivity, in the range between 25,443 and 31,800 µS/cm, did not significantly affect the growth rate of *D. salina*, as shown in Figure 4.3.

Tukey's test did, however, show a significant difference between the different types of nutrient; in this system, using SADS as a nutrient provided more biomass than BBM did, as indicated in Figure 4.4.

Tukey's test showed no significant difference among means of nutrient-conductivity interaction when using the same type of nutrient at different conductivity levels, as indicted in Figure 4.5.

The four treatment combinations in the design are shown graphically in Figure 4.6, where "A" refers to the effect of Factor A (level of conductivity), "B" refers to the effect of Factor B (type of nutrient), and "AB" refers to the AB

interaction. In the $2^2$ design, the low and high levels of A and B are denoted by "-" and "+," respectively, on the A and B axes.

The four treatment combinations in the design are also represented by lowercase letters as shown in Figure 4.6.

The main effective parameter on this system is type of nutrient (factor B) and the interaction between the level of conductivity and type of nutrient (factor AB), as shown in Table 4.3.

The regression model in a ($2^2$) factorial design is:

$$Y = \beta o + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \varepsilon$$

Where $X_1$ is a coded variable that represents the level of conductivity, $X_2$ is a coded variable that represents the type of nutrient, and the βs are regression coefficients.

$$X_1 = \frac{\text{Cond} - (\frac{(\text{Cond at low} + \text{Cond at high})}{2})}{(\text{Cond at high} - \text{Cond at low})/2}$$

$$X_1 = \frac{\text{Cond} - 28{,}621}{3{,}179}$$

If the level of conductivity is at a high level (Conductivity = 31,800 µS/cm), then $X_1$=+1; if the level of conductivity is at low level (25,442 µS/cm), then $X_1$=-1.

$$X_2 = \frac{\text{TN} - ((\text{TN at low} + \text{TN at high}))/2}{(\text{TN at high} - \text{TN at low})/2}$$

$$X_2 = \frac{\text{TN} - (\text{BBM} - \text{SADS})/2}{(\text{SADS} - \text{BBM})/2}$$

If the type of nutrient is at a high level (SADS), then X1=+1, and if the level of conductivity is at a low level (BBM), then $X_1$=-1.

The fitted regression model is:

$$Y = 1.19 + \left(\frac{0.045}{2}\right) X_2 + \left(\frac{0.145}{2}\right) X_1 X_2$$

This model can be used to obtain the predicted value of Y; the residuals are the difference between the observed and the fitted values of Y.

$Y = \beta o + \beta 2\ (-1) + \beta 12\ (-1)\ (-1) = 1.24$

e1 = 1.56 - 1.24 = 0.32

e2 = 1.42 - 1.24 = 0.18

$Y = \beta o + \beta 2\ (-1) + \beta 12\ (+1)\ (-1) = 1.095$

e3 = 1.38 - 1.095 = 0.285

e4 = 1.4 - 1.095 = 0.305

Y = βo + β2 (+1) + β12 (-1) (+1) = 1.14

e5 = 0.84 - 1.14 = -0.3

e6 = 0.8 - 1.14 = -0.34

Y = βo + β1 (+1) + β12 (+1) (+1) = 1.285

e7 = 1.04-1.285 = -0.245

e8 = 0.98-1.285 = -0.305

After substituting the relationships between the natural and coded variables, the following regression model is obtained:

$$Y=1.19+\left(\frac{0.045}{2}\right)\left(\frac{TN-(BBM+SADS)/2}{(SADS-BBM)/2}\right)+\left(\frac{0.145}{2}\right)\left(\frac{Cond-28{,}261}{3{,}179}\right)\left(\frac{TN-(BBM+SADS)/2}{(SADS-BBM)/2}\right)$$

Where Y is maximum biomass (g/L), TN is type of nutrient, BBM is Bold's Basal Medium, SADS is supernatant from anaerobic digested sludge, and Cond is conductivity (µS/cm).

Figure 4.7 shows that there are no points further than + 0.4 or – 0.4, and that there is no issue of outliers; therefore, we can trust our regression analysis.

As for *S. platensis*, the analysis of variance for this full-factorial design ($2^2$) experiment is shown in Table 4.4.

Again, after finding a significant difference, we needed to use multiple comparisons to find the significant nutrient and best combination of conductivity and nutrient to control Type I error and increase the power of our statistical analysis; we therefore used Tukey's test.

As can be seen in the ANOVA in Table 4.4, conductivity had no impact on biomass production (P-value > 0.05). Tukey's test also showed no difference. Therefore, it can be concluded that the level of conductivity, in the range between 25,100 and 35,800 µS/cm, did not affect the growth rate of *S. platensis* significantly, as shown in Figure 4.8.

Tukey's test did show a significant difference between the types of nutrients (P-value < 0.05); in this system, the use of SADS as a nutrient provided more biomass in comparison to F/2, as indicated in Figure 4.9.

Tukey's test showed significant difference among means of nutrient-conductivity interaction, and the highest levels of biomass were seen in the combination of a conductivity of 25,100 µS/cm and SADS as a nutrient; also, the lowest biomass production was seen in the interaction between a conductivity of 25,100 µS/cm and F/2 (P-value < 0.05), as indicated in Figure 4.10.

Again, the four treatment combinations in the design are shown graphically in Figure 4.11, where "A" refers to the effect of Factor A (level of conductivity), "B" refers to the effect of Factor B (type of nutrient), and "AB" refers to the AB interaction. In the $2^2$ design, the low and high levels of A and B are denoted by "-" and "+," respectively, on the A and B axes. The four treatment

combinations in the design are also represented by lowercase letters, as shown in Figure 4.11.

The main effective parameter on this system is type of nutrient (Factor B) and the interaction between the level of conductivity and the type of nutrient (Factor AB).

The regression model in a $2^2$ factorial design is:

$$Y=\beta o+\beta_1 X_1+ \beta_2 X_2 + \beta_{12}X_1X_2 +\varepsilon$$

Where $X_1$ is a coded variable that represents the level of conductivity, $X_2$ is a coded variable that represents the type of nutrient, and the βs are regression coefficients.

$$X_1 = \frac{\text{Cond}-(\frac{(\text{Cond at low}+\text{Cond at high})}{2})}{(\text{Cond at high}-\text{Cond at low})/2}$$

$$X_1 = \frac{\mathbf{Cond-28{,}700}}{\mathbf{5350}}$$

If the level of conductivity is at a high level (Conductivity = 35,800 µS/cm), then $X_1$ = +1; if the level of conductivity is at low level (25,100 µS/cm), then $X_1$ = -1.

$$X_2 = \frac{\text{TN}-(\text{TN at low}+\text{NT at high})/2}{(\text{TN at high}-\text{NT at low})/2}$$

$$X_2 = \frac{\text{TN}-(\text{F2}+\text{SADS})/2}{(\text{SADS}-\text{F2})/2}$$

If the type of nutrient is at a high level (SADS), then $X_2$=+1, and if the level of conductivity is at low level (F/2), then $X_2$ = -1.

The fitted regression model for *S. platensis* is:

$$Y = 1.32 + (\frac{0.09}{2})\ X_2 + (\frac{0.52}{2})\ X_1X_2$$

Similar to the previous calculations, this model can be used to obtain the predicted value of Y and the residuals.

$Y = \beta o + \beta 2\ (-1) + \beta 12\ (-1)\ (-1) = 1.53$

e1 = 1.88 - 1.535 = 0.345

e2 = 1.96 - 1.535 = 0.425

$Y = \beta o + \beta 2\ (-1) + \beta 12\ (+1)\ (-1) = 1.32$

e3 = 1.52 - 1.32 = 0.2

e4 = 1.46 - 1.32 = 0.14

$Y = \beta o + \beta 2\ (+1) + \beta 12\ (-1)\ (+1) = 1.58$

e5 = 0.6 - 1.58 = -0.98

e6 = 0.66 - 1.58 = -0.92

Y = βo + β1 (+1) + β12 (+1) (+1) = 1.32

e7 = 1.2 - 1.32 = -0.12

e8 = 1.28 - 1.32 = -0.04

The final regression model is:

$$Y = 1.32 + \left(\frac{0.09}{2}\right)\left(\frac{TN-(F2+SADS)/2}{(SADS-F2)/2}\right) + \left(\frac{0.52}{2}\right)\left(\frac{Cond-28{,}700}{5350}\right)\left(\frac{(TN-(F2+SADS)/2}{(SADS-F2)/2}\right)$$

where Y is maximum biomass (g/L), TN is type of nutrient, F2 is F/2 Medium, SADS is supernatant from anaerobic digested sludge, and COND is conductivity (μS/cm).

Figure 4.12 shows that there are no points further than +1 or –1, and that there is no issue of outliers; therefore, we can trust our regression analysis.

## 4.3 Specific Growth Rate

The specific growth rate was found from Equation 1:

$$\mu = \{Ln (Wy/Wx)\}/\{ty - tx\} \quad \text{Eq.1}$$

where Wy and Wx are the microalgae dry weight (W) at the beginning (tx) and at the end (ty) of the logarithmic growth phase (Wood et al., 2005; Huerlimann et al., 2010). The available literature found for specific growth rates of *D. salina* while culturing with NaCl solutions as a growth medium (García et al., 2007) and a manufactured chemical nutrient (Prieto et al., 2011) were used for comparison with the results from this study. The natural desalination concentrate and SADS that were used in the study and the specific growth rates (0.095-0.114) for *D. salina* in Table 4.6 and 0.019-0.034 for *S. platensis* in Table 4.7 were lower than those reported in the literature (0.12-0.47 for *D. salina* (Prieto et al., 2011) in Table 4.6 and 0.255 for *S. platensis (*Leema et al., 2010) in Table 4.7, where seawater and pretreated seawater were used as the water medium in a closed reactor. Misleading conclusions could be made when comparing the microalgae growth rate between different water mediums, different nutrients supplied, and different types and characteristics of reactors used by Pittman et al., 2011, leading to a statement that nutrient removal rates are comparable. However, microalgae growth rates are higher in artificial wastewater than in natural wastewater (Lau et al., 1995; Ruiz-Marin et al., 2010). This may be due to the increased toxicity of natural wastewaters, the competitive effects of indigenous bacteria and protozoa, or the diverse chemical composition of the natural wastewaters (Pittman et al., 2011). Natural desalination concentrate from the evaporation pond has to be used

for simulating real-world conditions (Samorı et al., 2013) and to reduce the disconnection between lab and field, noted by Sheehan et al. in 1998. The lab conditions should simulate the field situation, through approaches such as using natural concentrate in the experiments.

The lower specific growth rates of microalgae may also be due to the temperature fluctuation between daytime (open outdoor, 91.0-116.9 °F) and nighttime (in the lab, 62.5-86 °F), or the illumination problems from the color of SADS (optical density 0.58 at 560 nm wavelength). Additionally, the higher concentrations of TDS, N, $Mg^{2+}$, and $Ca^{2+}$ can be toxic to the microalgae, inhibiting their growth (Kim et al., 2013). Tredici and Zittelli, 1998 found that the biomass growth rates of outdoor cultures of *S. platensis* (1.09 and 1.26 g/L/d) were lower than those of indoor cultures (1.64-1.93 g/L/d). However, the enthalpies are similar (20.9-21.6 kJ/g). Torzillo et al., 1991 concluded that temperature and light irradiance influence the biomass composition and found that dry weight concentrations of biomass were reduced during the night due to the decrease of these two factors.

## 4.4 The Comparison of Microalgae Biomass between SADS – BBM, and SADS – F/2

Dry weight concentrations of *D. salina* supplied with SADS (1.40-1.56 g/L) were higher than the dry weight concentrations of *D. salina* supplied with BBM (0.84-1.04 g/L), as shown in Figure 4.1(a). Dry weight concentrations of *D. salina* supplied with SADS (1.46-1.96 g/L) were higher than those supplied with F/2 (0.68-1.28 g/L), as shown in Figure 4.2(a). The reason for this may be that micro-organisms grew in SADS along with microalgae, and the microorganism promoted microalgae growth. This finding agrees with the finding of Wang et al., 2010, which states that the specific growth rate of microalgae from concentrate (wastewater from sludge centrifuge) is higher than that from wastewater before and after primary settling and aeration tank. Wastewater from sludge centrifuge has more micro-organisms than the wastewater before and after primary settling and aeration tank. In cases of SADS as nutrient, low conductivity media provide higher microalgae dry weight concentrations. In the case of Bold's Basal Medium and F/2 as nutrients, higher conductivity media provided higher microalgae dry weight concentrations.

## 4.5 Comparison of Microalgae Biomass between Study and Literature

By reusing concentrate as a growth medium and SADS as a nutrient, this study achieved dry weight concentrations of 1.56 g/L for *D. Salina* (Figure 4.1(a)) and 1.96 g/L for *S. Platensis* (Figure 4.2(a)). These dry weight concentrations are comparable to the results in the literature data where seawater was used, which

are 1.06 g/L for *D. salina* and 0.8–2.9 g/L for *S. platensis*, as shown in Tables 4.4 and 4.5.

A dry weight concentration of 2.587 g/L for *S. platensis* was observed in the work of Volkmann et al., 2008, in desalinated wastewater. A dry weight concentration of 2.37 g/L of dry biomass was observed by Pandey and Tiwari, 2010, at a pH of 8.25, a temperature of 30 °C, and a light intensity of 3 Klux (Jain et al., 2011). A dry weight concentration of 2.34 g/L for *S. platensis* was found on the 27th day of culturing in a 30 percent petha waste medium supplemented with a standard medium (for example, CFTRI medium) in triplicate at 3 Klux light intensity, pH 9.5 ± 0.1, and 30 °C ± 2 under 12/12 h light/dark cycles (Jain et al., 2011). A dry weight concentration of 2.91 g/L for *S. platensis* was observed at an input $CO_2$ concentration of 10 percent on the 25th day of culturing by Ramananet et al., 2010. In the current research, a longer culturing time of 37–39 days for *D. salina* was required to reach maximal growth due to higher conductivities in the concentrate (Table 4.1) and the color from SADS, which decreased the transparency of the plastic bottles used as reactors. The growth rate of microalgae also depends on the amount of seed microalgae in the growth medium (Pittman et al., 2011; Lau et al., 1995). The growth of *D. salina* may be inhibited in desalination concentrate by brackish groundwater since this concentrate contains a high concentration of $SO_4^{-2}$ and a high concentration of $HCO_3^-$. *D. salina* prefers high pH of 11, while the pH of the growth culture was between 6.8 and 8.8, as shown in Figure 1(e). Therefore, a longer culturing time was required for *D. salina* to reach the maximum dry weight concentration compared to *S. platensis*, since *Spirulina* prospers in high $CO_3^{-2}$ and $HCO_3^-$ water (Richmond, 1986) in the pH range of 8.5–11.0 (Habib and Parvin, 2008).

The highest dry weight yields from reused concentrate as a water medium and SADS as a nutrient are comparable to the data from the literature (Tables 4.6 and 4.7). Most of the culturing time in the study is higher than the culturing time from literature, which may be due to the desalination concentrate that was used in the experiment.

## 4.6 The Microalgae Strains from the Desalination Evaporation Pond at the Brackish Groundwater National Desalination Research Facility (BGNDRF)

## 4.7 Results

The study had a duration of about 110 days in order to analyze the growths of these strains, measuring factors such as dry weight, optical density, salinity, pH, and temperature.

Table 4.8 shows the highest dry weight achieved from the study along with the optical density, pH, culturing day, and temperature at which the highest dry weight was gained.

The growth of the Brackish Ground Water National Desalination Facility (BGNDRF) species with time is shown in Figure 4.13(a) in five different conductivities; all reactors were supplied with the same amount of nutrient (SADS).

The BGNDRF species required 80-100 days to reach the maximal growth. The lower conductivity (21000 µS/cm) improved the yield of biomass (2.08 g/L); the highest conductivity (52500 µS/cm) resulted in a lower yield (1.59 g/L).

Figure 4.13(b) shows the optical density of the culture with time. The highest optical density (2.08) occurred in the lowest conductivity (21000 µS/cm); the lowest optical density (1.68) occurred in the highest conductivity (52500 µS/cm).

Figure 4.13(c) depicts the conductivity changes with culturing time. Figure 4.13(d) shows the amount of nutrient that was added during the experiment period. Unlike the experiments with *D. salina* and *S. platensis*, the nutrient addition rate was held constant for the BGNDRF strain. This was done because the BGNDRF strain was previously undiscovered and its growth characteristics and nutrient requirements were unknown.

Figure 4.13(e) displays the pH of the media with time. Data show that the BGNDRF species grew in pH 8-9.2. The temperature of medium for BGNDRF species was 75-100 ºF, as shown in Figure 4.13(f). $CO_2$ was supplied from air which was bubbled into reactors, as shown in Figure 4.13(g).

## 4.8 Mass of Conductivity Reduction

Mass of conductivity is the result of multiplying the conductivity (µS/cm) of the microalgae in the bioreactor by the actual volume of the microalgae (L) in the bioreactor; the mass of conductivity deduction from concentrate and nutrient percentage is shown in Figure 4.14. Microalgae growth rates are depicted in Figure 4.13(a) and Figure 4.15.

Overall, the mass of conductivity deductions from concentrate and nutrients (57, 54, 46, 40, and 37 percent) are inversely proportional to the original mass of conductivities (51,191; 64,502; 80,517; 104,950; 120,720 (µS/cm) (L)) in 110 days of treatment (Figure 4.14).The maximum dry weights of microalgae (2.08, 1.92, 1.85, 1.75 and 1.59 g/L) in the five different conductivities of concentrate are shown in Figure 4.15. Conductivities of culture in 110 days are significantly less than that of original concentrate from desalination. Mass of conductivity deduction is significant in 0-110 days of treatment, as shown in Figure 4.14. Desalted culture included water, green food, protein, and nutrients, which can be fed to sheep by mixing dry feed stocks to sustain cities in arid-regions (State of New South Wales, 2007; Government of Western Australia, 2007).

The ANOVA regression model in Table 4.9 shows that conductivity (predictor) explains the variation in biomass (response) (P-value <0.05).

A regression model was developed based on the maximum biomass in each reactor and the five levels of conductivity in each one.

The regression equation is:

Y= 2.353 - 0.000015 Conductivity

Y: Maximum Dry Weight (g/L)

Conductivity (µS/cm)

The regression fit for the maximum biomass and conductivity obtained has an R-squared value of 97.4% (P-value < 0.05).

Figure 4.15 shows that low conductivity of the concentrate results in a high dry weight.

The results in Figure 4.16 indicate that we can trust the regression analysis because all residuals are around the best fit line (there is no problem of normality), there are no points further than -0.04 or +0.04, there is no issue of outliers, and all the data have the same frequency.

# Chapter 5 – CONCLUSIONS

Based on the experiments conducted in this study, the following conclusions can be made:

- Due to microorganisms growing with microalgae, the maximum dry weights of *D. salina* and *S. platensis* grown in desalination concentrate and supplied with SADS (1.36–1.49 g/L) are more than the dry weights of these same species when supplied with BBM and F/2, due to the manner in which the microorganism promotes microalgae growth. The maximum dry weight concentrations of *D. salina* and *S. platensis* grown in desalination concentrate and supplied with SADS are comparable to those in the literature.
- This study demonstrates the feasibility of using concentrate as a growth medium using SADS as a nutrient to grow algae culture.
- A combination of lower conductivity in the medium (25,442 and 25,100 µS/cm) and the use of SADS enhanced the growth of *D. salina* and *S. platensis*.
- The amount of the conductivity reduction was significant in BGNDRF species strains in 110 days.

These results suggest that using microalgae for reducing the conductivity of desalination concentrate and SADS by using the concentrate as a growth medium and SADS as an additional source of nutrients is better than using traditional methods for disposing of the concentrate from the desalination units, which have high costs and adverse environmental effects. High TDS levels, however, limited the ability of specific algae species to grow in the concentrate and reduce its conductivity.

The results also suggested that the BGNDRF strain can be used for concentrate management at salinity levels below 35,000 µS/cm. The BGNDRF species grew well at these levels, and since SADS is known to contain elements and ions that algae consume in their growth process – namely, ammonia nitrogen, sodium, calcium, magnesium, and potassium – it can be deduced that the BGNDRF species consumed some of these elements and ions in order to grow, reducing the overall salinity of the concentrate. The extent of this reduction could be explored in future research.

# Chapter 6 - FUTURE RECOMMENDATIONS

Recommendations for future research are listed below:

- Experiments can be conducted at different TDS levels to establish the optimal growth rate and can be performed on a large scale.
- Different species of microalgae can be cultured with the reject concentrate to study their growths and the conductivity reductions.
- The ion and element content of the growth media could be determined before and after algae growth to identify the specific ions and elements that the algae species remove.

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# Tables

# Chapter 1

**Table 1.1. Classification of Water Based on TDS Levels (WQA, 1999)**

| Water type | Range mg/L TDS |
|---|---|
| Fresh water | < 1,000 |
| Brackish water | 1,000 - 5,000 |
| Highly brackish water | 5,000 - 15,000 |
| Saline water | 15,000 - 30,000 |
| Saline water | 15,000 - 30,000 |
| Seawater | 30,000-40,000 |
| Brines | >40,000 |

**Table 1.2. Desalination cost for various desalination technologies ($/m$^3$ freshwater - multiply by 3.8 for $/1000 gal) (Younos, 2005)**

| Process | |
|---|---|
| Multistage flash (Seawater) | 1.32-5.36 |
| Multiple-effect distillation (Seawater) | 0.46-8.5 |
| Reverse osmosis (Seawater) | 0.45-0.92 |
| Reverse osmosis (Brackish Water) | 0.37-0.7 |
| Electrodialysis (Brackish water) | 0.58 |

**Table 1.3. Compassion between RO and EDR (Eltawi et al., 2009)**

| Process | Recovery and Total dissolved solids | Pros | Cons |
|---|---|---|---|
| RO | • 30–60% recovery<br>• Possible for single pass (higher recoveries are possible for multiple passes<br>• Product water has less than 200 mg/L TDS when brackish water is the feed water source | • Lower energy requirements<br>• Relatively lower investment cost<br>• No cooling water flow<br>• Has a modular design, so maintenance does not require entire plant to shut down | • Higher costs for chemical and membrane replacement<br>• Membranes susceptible to biofouling<br>• Minimum membrane life expectancy around 5–7 years<br>• Mechanical failures possible due to high pressure operation |
| ED/EDR | • 85–94% recovery possible<br>• Product water has 140–600 mg/L TDS | • Energy usage is proportional to salts removed<br>• Operational at low to moderate pressures<br>• Higher membrane life of 7–10 years | • Only suitable for feed water up to 12,000 mg/L TDS<br>• Periodic cleaning of membranes required<br>• Leaks may occur in membrane stacks |

**Table 1.4. Analysis of the four groundwater walls from (BGNDRF) (Tetra Tech, 2010).**

| Elements/ions | Range (mg/L) |
|---|---|
| Phosphorus Total (as P) | 0.015-0.03 |
| Nitrate+Nitrite (as N) | 2.8-8.3 |
| Bicarbonate Alkalinity (as $CaCO_3$) | 150-250 |
| Calcium | 49-550 |
| Sulfate | 580-3200 |
| Magnesium | 13-340 |
| Sodium | 310-720 |
| Potassium | 2.6-5.0 |

# Chapter 2

**Table 2.1. Methods of inland concentrate disposal in the U.S. (Mickley, 2009)**

| Disposal Method | (%) |
|---|---|
| Discharged to surface water | 45 |
| Discharged to wastewater treatment plants | 27 |
| Land application | 8 |
| Deep wall injection | 13 |
| Evaporation ponds | 4 |

**Table 2.2. Net greenhouse gas emissions of conventional diesel, soybean biodiesel, and microalgae biodiesel (Batan et al., 2010)**

| Contribution | Conventional diesel | Soybean biodiesel | Microalgae biodiesel |
|---|---|---|---|
| $CO_2$ (g. $MJ^{-1}$) | 14.69 | -72.73 | -59.49 |
| $CH_4$ (g. $MJ^{-1}$) | 2.48 | 2.48 | 0.74 |
| $N_2O$ (g. $MJ^{-1}$) | 0.07 | 0.58 | -16.54 |
| Net “strain to pump” GHG ($gCO_2$-eq/MJ) | 17.24 | -71.73 | -75.29 |

# Chapter 3

### Table 3.1.Composition in reactors

| | Reactor Conductivity (μS/cm) | Desalination Volume (L) | Concentrate Seed Microalgae | Seed Microalgae | Nutrient |
|---|---|---|---|---|---|
| D11,D12 | 31,800 | 2.0 | 0.1 | *D. Salina* | SADS |
| D21,D22 | 25,442 | 2.0 | 0.1 | *D. Salina* | SADS |
| D31,D32 | 31,800 | 2.0 | 0.08 | *D. Salina* | BBM |
| D41,D42 | 25,442 | 2.0 | 0.08 | *D. Salina* | BBM |
| S11,S12 | 35,800 | 1.9 | 0.1 | *S. platensis* | SADS |
| S21,S22 | 25,100 | 1.9 | 0.1 | *S. platensis* | SADS |
| S31,S32 | 35,800 | 1.9 | 0.1 | *S. platensis* | F/2 |
| S41,S42 | 25,100 | 1.9 | 0.1 | *S. platensis* | F/2 |
| R11,R12 | 21,000 | 2.1 | 0.1 | BGNDRF species | SADS |
| R21,R22 | 27,100 | 2.1 | 0.1 | BGNDRF species | SADS |
| R31,R32 | 35,500 | 2.1 | 0.1 | BGNDRF species | SADS |
| R41,R42 | 48,500 | 2.1 | 0.1 | BGNDRF species | SADS |
| R51,R52 | 52,600 | 2.1 | 0.1 | BGNDRF species | SADS |

Note: SADS is Supernatant from Anaerobic Digested Sludge after centrifuging at 10,000 rpm for 3 min twice. BBM is Bold's Basal Medium.

# Chapter 4

## Table 4.1. Maximum dry weight concentration in reactors

| Reactors | Seed microalgae | Nutrient | Where highest dry weight occurs (Average) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Dry weight (g/L) | Optical density | pH | Temp (°F) | Culturing day |
| D1 | *D. salina* | SADS[1] | 1.36 | 2.00 | 8.5 | 75 | 37 |
| D2 | *D. salina* | SADS[1] | 1.49 | 2.25 | 8.2 | 76 | 37 |
| D3 | *D. salina* | BBM[2] | 1.04 | 1.35 | 8.4 | 76 | 37 |
| D4 | *D. salina* | BBM[2] | 0.84 | 1.36 | 8.2 | 74 | 39 |
| S1 | *S. platensis* | SADS[1] | 1.41 | 0.12 | 8.6 | 74 | 14 |
| S2 | *S. platensis* | SADS[1] | 1.98 | 1.62 | 8.9 | 78 | 24 |
| S3 | *S. platensis* | F/2 | 1.24 | 0.43 | 8.5 | 73 | 20 |
| S4 | *S. platensis* | F/2 | 0.68 | 0.23 | 8.4 | 74 | 20 |

[1]SADS is supernatant from anaerobic digested Sludge after centrifuging at 10,000 rpm for 3 min twice.
[2]BBM is Bold's Basal Medium.

## Table 4.2. Analysis of variance for *D. salina.*

| SOV | df | ss | adj ss | Ms | F | P-value |
|---|---|---|---|---|---|---|
| Conductivity | 1 | 0.00405 | 0.00405 | 0.00405 | 1.29 | 0.32 |
| Nutrient | 1 | 0.55125 | 0.55125 | 0.55125 | 175.00 | 0.000 |
| Conductivity*Nutrient | 1 | 0.04205 | 0.04205 | 0.04205 | 13.35 | 0.000 |
| Error | 4 | 0.01260 | 0.01260 | 0.00315 | | |
| Total | 7 | 0.60995 | | | | |

**Table 4.3. Analysis of variance for *D. salina***

| Factors | P-value | Effect |
|---|---|---|
| A (level of conductivity) | >0.05 | Non-Significant |
| B (type of nutrient) | <0.05 | Significant |
| AB | <0.05 | Significant |

**Table 4.4. Analysis of variance for *S. platensis***

| SOV | df | ss | adj ss | Ms | F | P-value |
|---|---|---|---|---|---|---|
| Conductivity | 1 | 0.01620 | 0.01620 | 0.01620 | 6.48 | 0.064 |
| Nutrient | 1 | 1.18580 | 1.18580 | 1.18580 | 474.32 | 0.000 |
| Conductivity*Nutrient | 1 | 0.54080 | 0.54080 | 0.54080 | 474.32 | 0.000 |
| Error | 4 | 0.01000 | 0.01000 | 0.00250 | | |
| Total | 7 | 1.75280 | | | | |

**Table 4.5. Analysis of variance for *S. platensis***

| Factors | P-value | Effect |
|---|---|---|
| A (level of conductivity) | >0.05 | Non-Significant |
| B (type of nutrient) | <0.05 | Significant |

| | | |
|---|---|---|
| AB | <0.05 | Significant |

**Table 4.6. Comparison of dry weight and specific growth rate between the study data and literature values for *D. salina***

| *D. salina* data from the study | | | | | *D. salina* data from literature | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Water medium | Nutrient | Dry weight, g/L | Culturing time, day | Specific growth rate,$d^{-1}$ | Water medium | Nutrient | Dry weight, g/L | Culturing time, day | Specific growth rate, $d^{-1}$ | Type of reactor | Ref. |
| Conc.[a] | SADS | 1.56 | 37 | 0.095 | Sea Water | $NaNO_3$ | 1.06 | 17 | N/A | N/A | Huang et al., 2011 |
| Conc.[b] | SADS | 1.4 | 37 | 0.097 | Sea Water | F/2 | 0.33 | Semi-continuous | 0.12-0.33 | Open | Prieto et al., 2011 |
| Conc.[a] | BBM | 1.04 | 37 | 0.114 | Sea Water | F/2 | 0.53 | 25,bench | 0.33 | Open | Prieto et al., 2011 |
| Conc.[b] | BBM | 0.84 | 39 | 0.106 | Sea Water | F/2 | 1.65 | Semi-continuous | 0.22-0.46 | Closed | Prieto et al., 2011 |
| | | | | | Sea Water | F/2 | 2 | 25,bench | 0.47 | Closed | Prieto et al., 2011 |
| | | | | | 10% NaCl | J/1 | N/A | N/A | 0.28 | N/A | García et al.,2007 |

Note: BBM is Bold's Basal Medium; Conc.[a] is desalination concentrate which has conductivity 31,800 µS/cm; Conc.[b] is desalination concentrate which has conductivity 25,442 µS/cm; and SADS is supernatant from anaerobic digested sludge after centrifugation at 10,000 rpm for 3 minutes twice.

**Table 4.7. Comparison of dry weight and specific growth rate between study data and literature values for *S. platensis***

| *S. platensis* data from the study | | | | | *S. platensis* data from literature | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Water medium | Nutrient | Dry weight, g/L | Culturing time, day | Specific growth Rate, $d^{-1}$ | Water medium | Nutrient | Dry weight, g/L | Culturing time, day | Specific growth rate, $d^{-1}$ | Ref. |
| Conc.[a] | SADS | 1.52 | 14 | 0.03 | PS | N/A | 2.26-2.99 | 25 | 0.225 | Leema et al., 2010 |
| Conc.[b] | SADS | 1.96 | 24 | 0.032 | OP | Zarrouk's | 1.1 | 8 | N/A | Gitelson et al., 1995 |
| Conc.[a] | F/2 | 1.28 | 31 | 0.034 | DW | 50%ADE | 1.23 | 14 | N/A | Kaushik et al., 2006 |
| Conc.[b] | F/2 | 0.66 | 24 | 0.019 | 10% OM | Zarrouk's | 0.8-1.0 | 21-25 | N/A | Reichert et al., 2006 |
| | | | | | CP | $NO_3^-$ , $HCO_3^{2-}$ | 1.7 | N/A | N/A | Markou et al., 2012 |
| | | | | | Desalination WW | N/A | 2.59 | N/A | N/A | Pandey et al., 2010; Jain et al., 2011 |
| | | | | | 30% petha | CFTRI | 2.34 | 27 | N/A | Ramanan et al., 2010 |

Note: Conc.[a] is desalination concentrate which has conductivity 35,800 µS/cm; Conc.[b] is desalination concentrate which has conductivity 25,100 µS/cm; ADE is anaerobically digested distillery effluent; OM is olive oil mill wastewater; NAOCI was used to decrease the phenol concentration and turbidity; PSS is pretreated sea water; OP is open pond, 2.5 $m^2$; DW is distilled water; CP is closed photo bioreactor; CFTRI is prescribed medium (Jain et al., 2011); and SADS is supernatant from anaerobic digested sludge after centrifugation at 10,000 rpm for 3 minutes twice.

## Table 4.8. Maximum dry weight concentration in reactors

| Reactors | Seed microalgae | Desalination concentrate conductivity (µS/cm) | Nutrient | Where highest dry weight occurs (Average) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Dry weight (g/L) | Optical density | pH | Temp (°F) | Culturing time (days) |
| R1 | BGNDRF | 21,000 | SADS | 2.08 | 4.7 | 8.2 | 77 | 90 |
| R2 | BGNDRF | 27,000 | SADS | 1.92 | 3.85 | 8.4 | 77 | 80 |
| R3 | BGNDRF | 35,000 | SADS | 1.85 | 3.96 | 8.4 | 77 | 80 |
| R4 | BGNDRF | 42,500 | SADS | 1.75 | 1.86 | 8.7 | 82 | 100 |
| R5 | BGNDRF | 52,500 | SADS | 1.59 | 1.68 | 8.6 | 84 | 100 |

Note: BGNDRF is the Brackish Groundwater National Desalination Research Facility Microalgae species; and SADS is supernatant from anaerobic digested sludge after centrifugation at 10,000 rpm for 3 min twice.

## Table 4.9.Analysis of variance for BGNDF species

| SOV | df | ss | F | P-value |
|---|---|---|---|---|
| Regression | 1 | 0.140206 | 150.54 | 0.001 |
| Error | 3 | 0.002794 | 0.000931 | |
| Total | 4 | 0.143000 | | |

# Figures

## Chapter 1

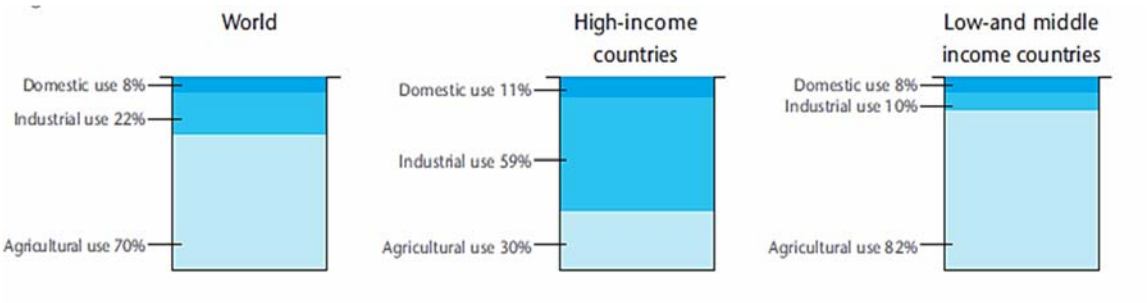



**Figure 1.1. Main uses of water (WBCSD, 2005)**

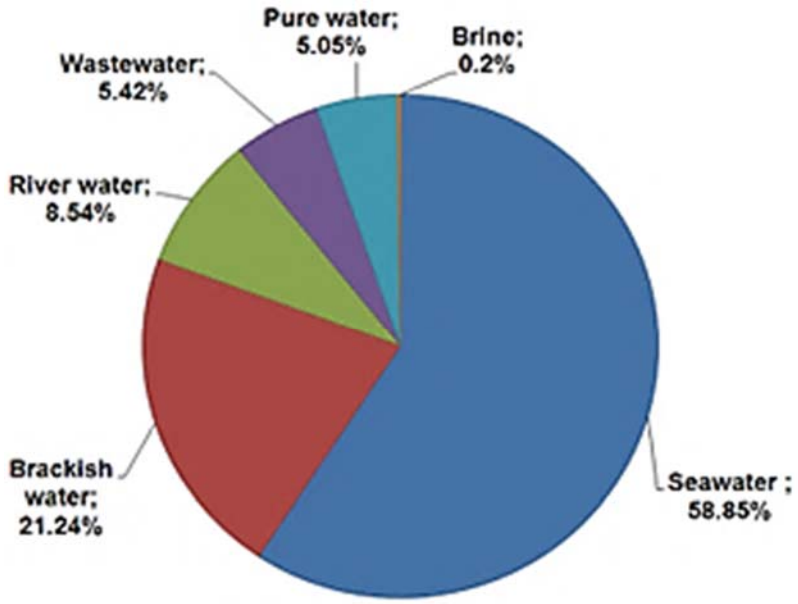
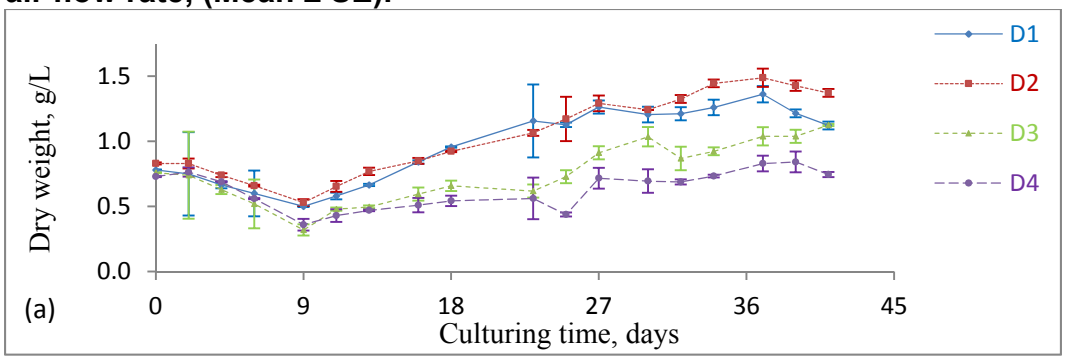
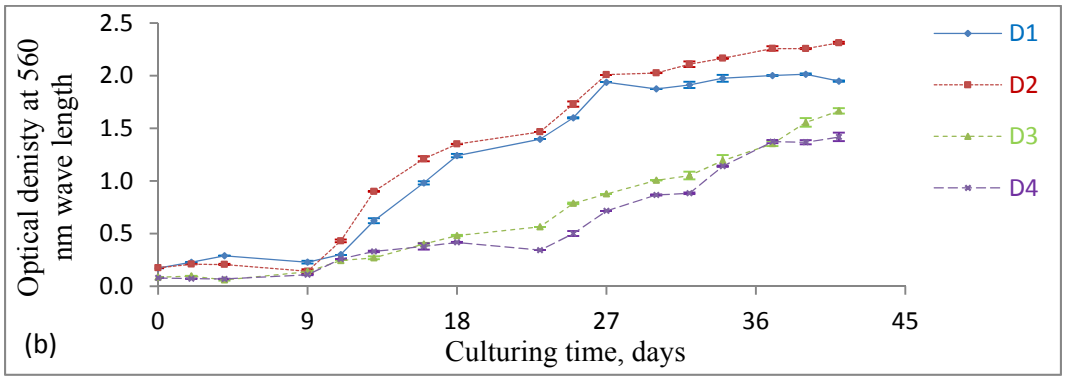


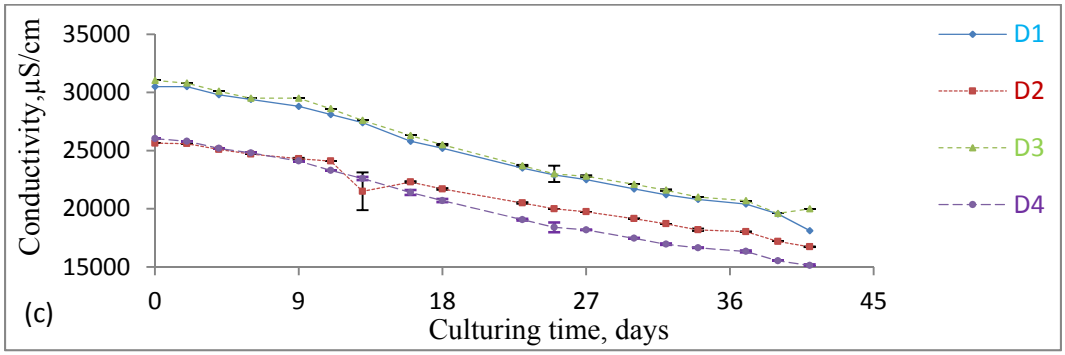

**Figure 1.2. Worldwide sources of feedwater (Pankratz, 2012)**

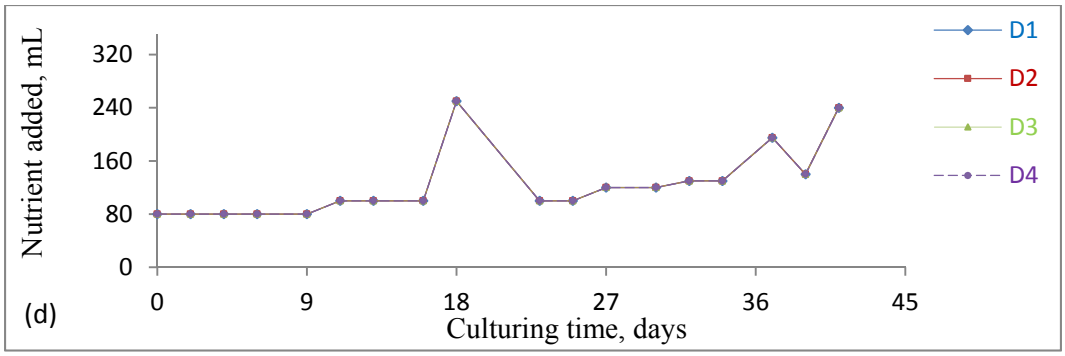
# Chapter 4

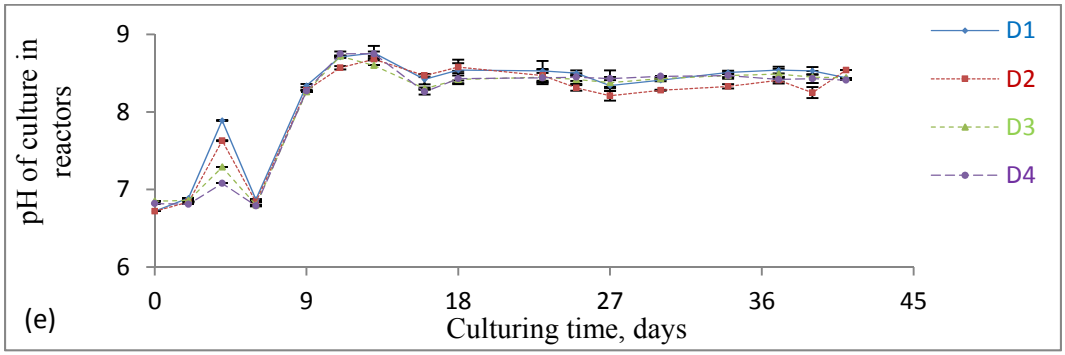
**Figure 4.1. *D. salina's* growing characteristics with culturing time: (a) dry weight; (b) optical density; (c) conductivities of medium in reactors; (d) nutrient added into reactors; (e) pH; (f) temperature; (g) air flow rate, (Mean ± SE).**

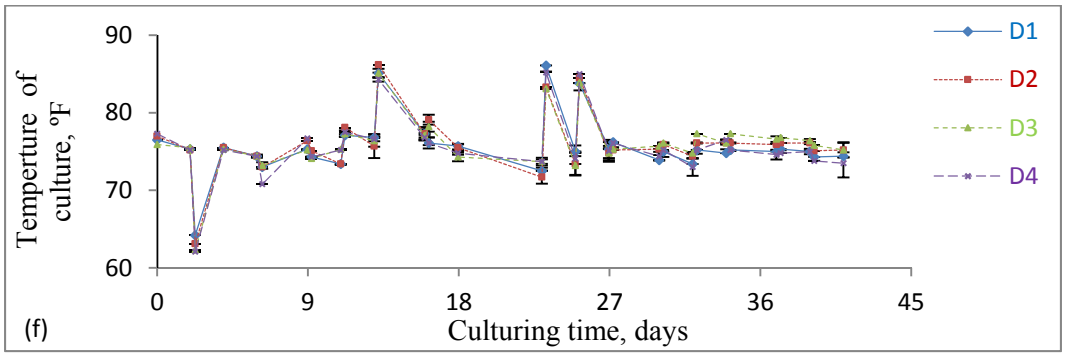


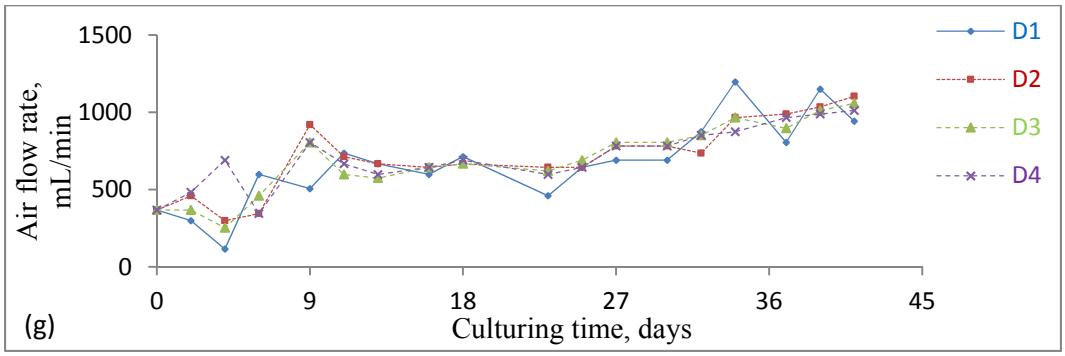





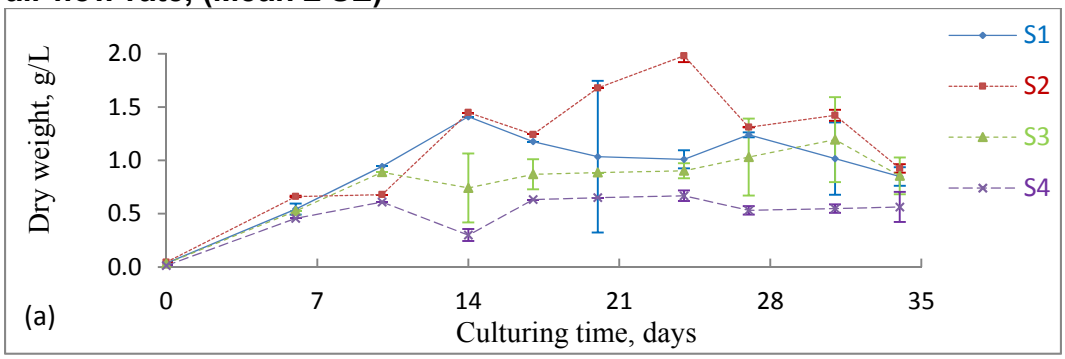



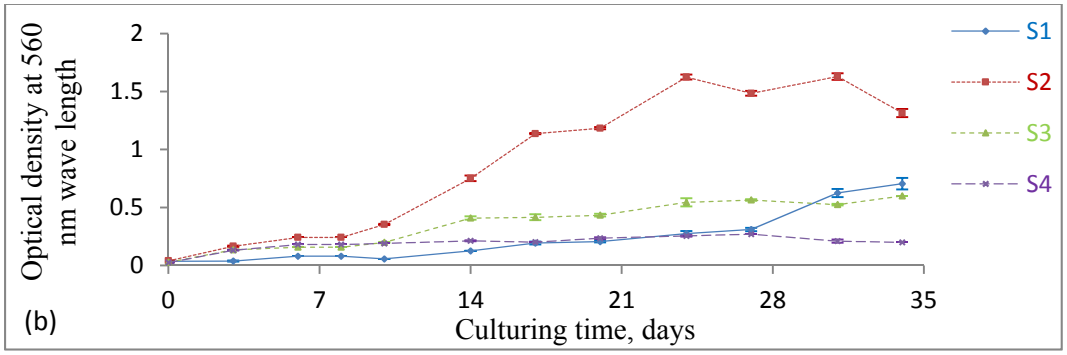



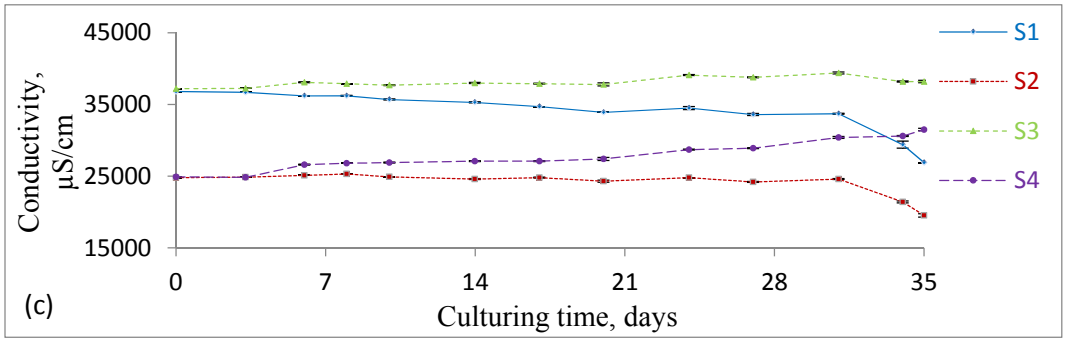



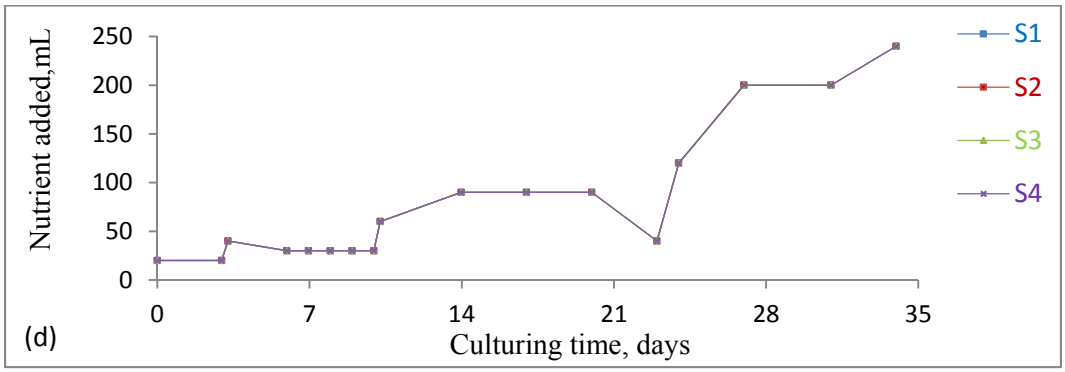

Note. SADS was added in reactors; D1 (31,800 µS/cm), D2 (25,442 µS/cm). BBM was added in Reactors; D3 (31,800 µS/cm), D4 (25,442 µS/cm).

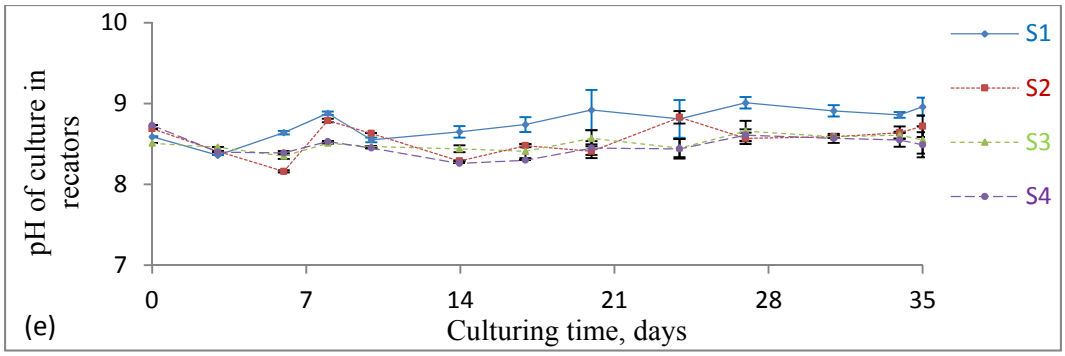
**Figure 4.2. *S. platensis's* growing characteristics with culturing time: (a) dry weight; (b) optical density; (c) conductivities of medium in reactors; (d) nutrient added into reactors; (e) pH; (f) temperature; (g) air flow rate, (Mean ± SE)**

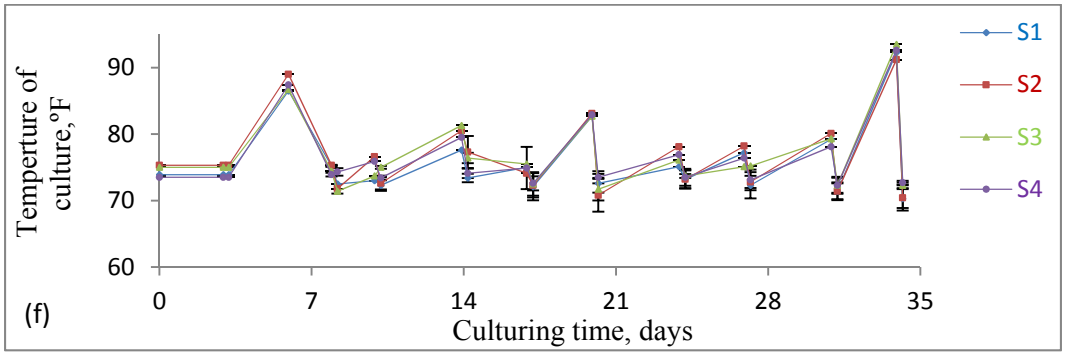


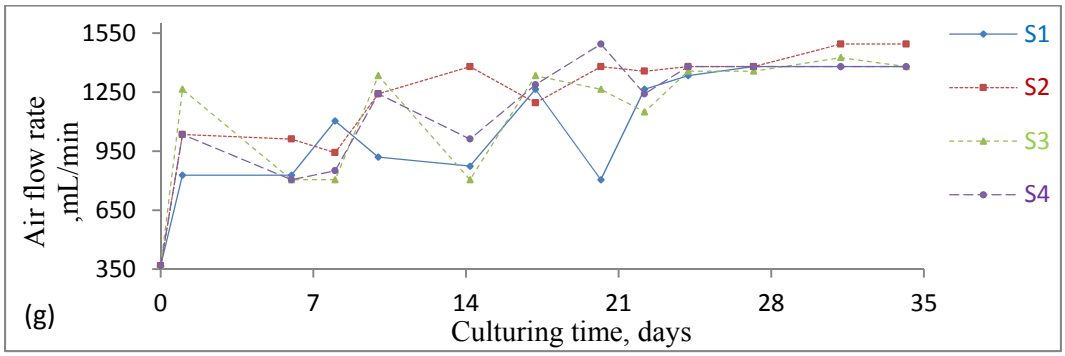






Note: SADS was added in reactors S1 (35,900 µS/cm), S2 (25500 µS/cm). F/2 was added in Reactors S3 (35,900 µS/cm), S4 (25500µS/cm)

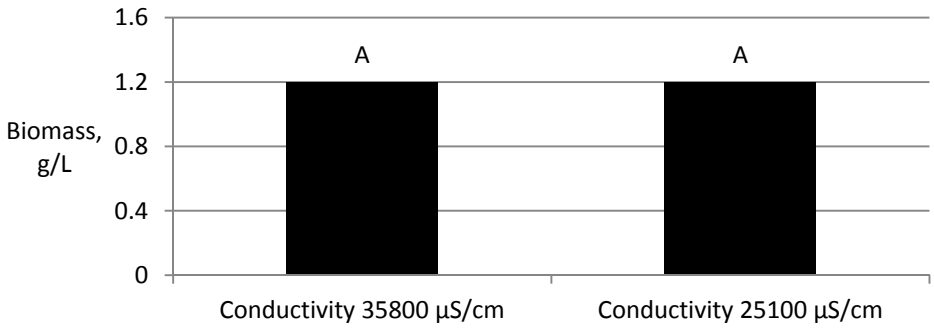



**Figure 4.3. Effect of conductivity on biomass**

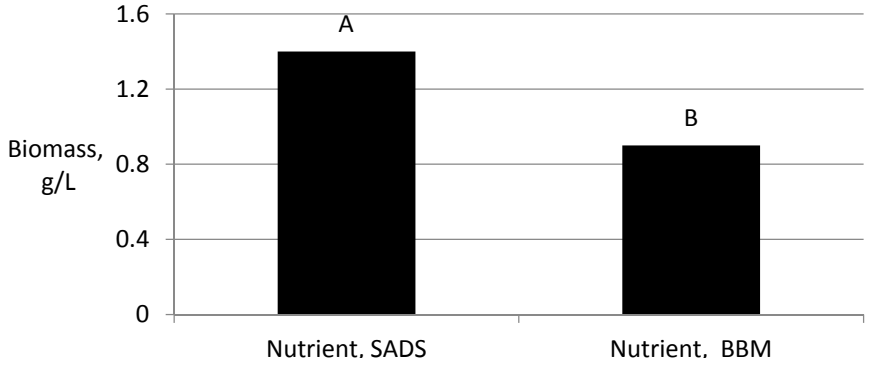



**Figure 4.4. Effect of nutrient type on biomass**

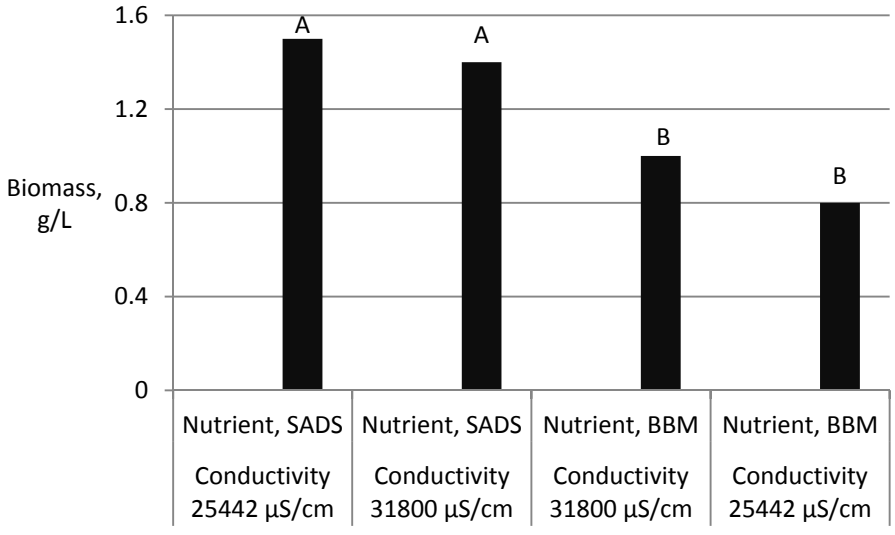



**Figure 4.5. Effect of conductivity and nutrient type on biomass**

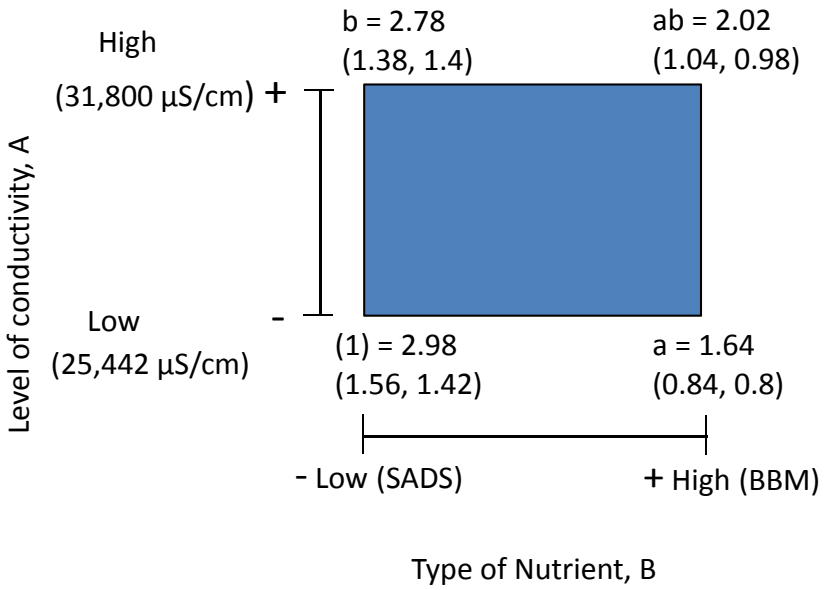



**Figure 4.6. Combination in the $2^2$ design**

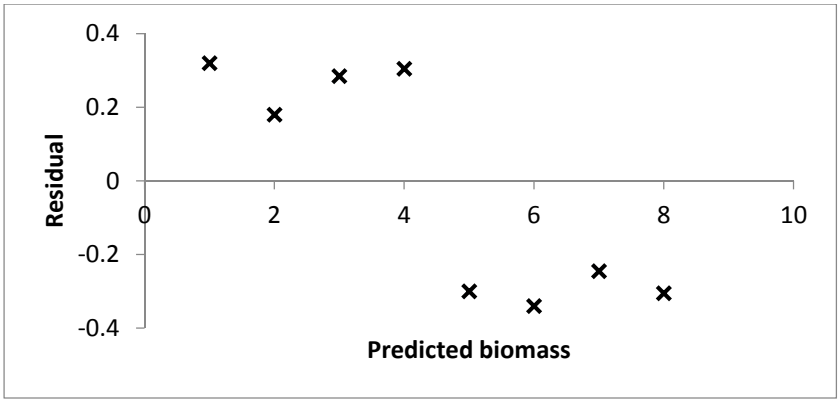



**Figure 4.7. Residuals vs. predicted maximum biomass**

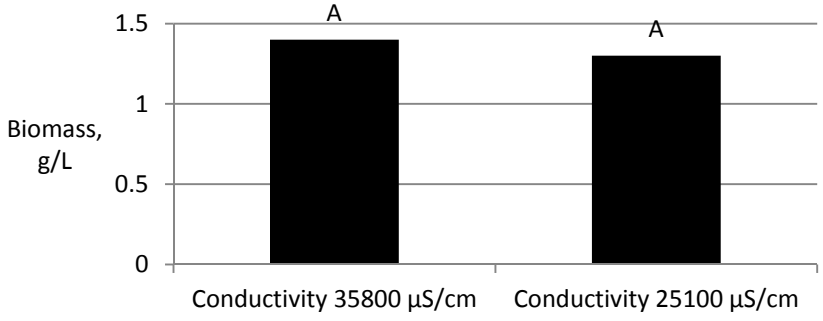



Figure 4.8. Effect of conductivity on biomass

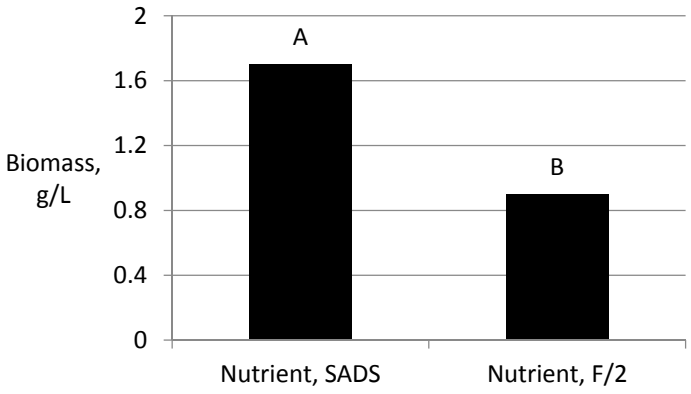



**Figure 4.9. Effect of conductivity and nutrient type on biomass**

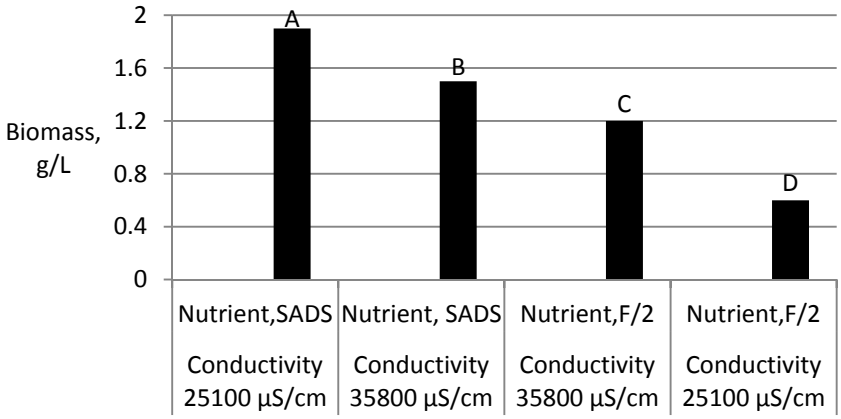



**Figure 4.10. Effect of conductivity and nutrient type on biomass**

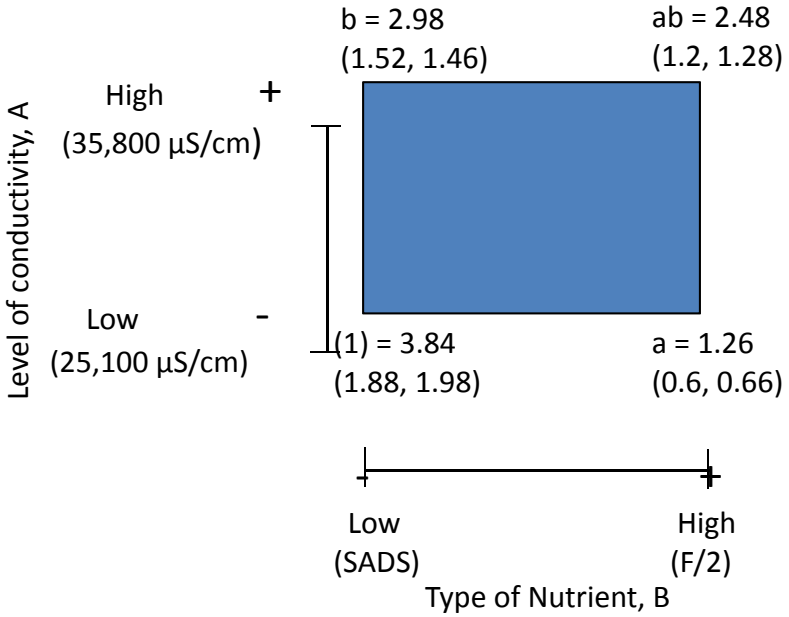



**Figure 4.11. Combination in the $2^2$ design**

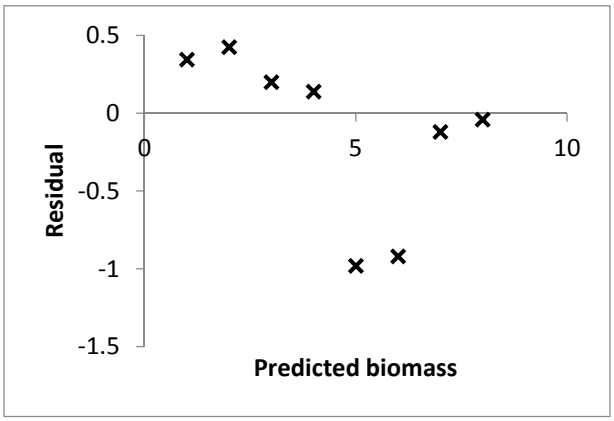
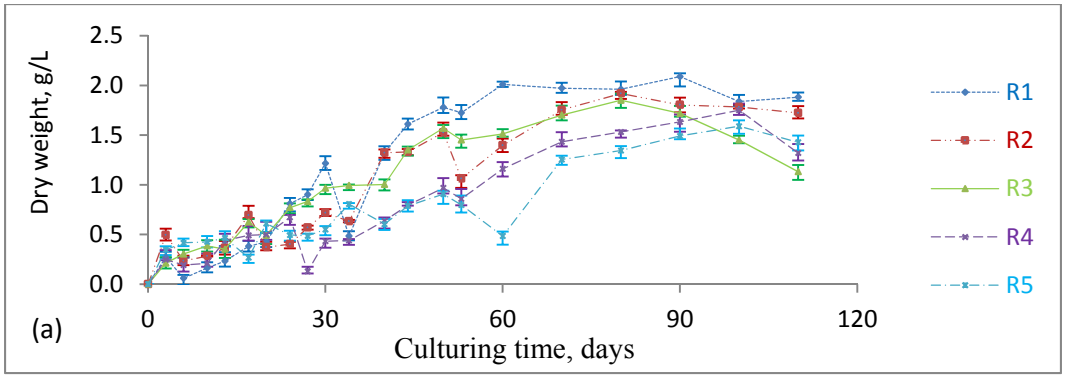
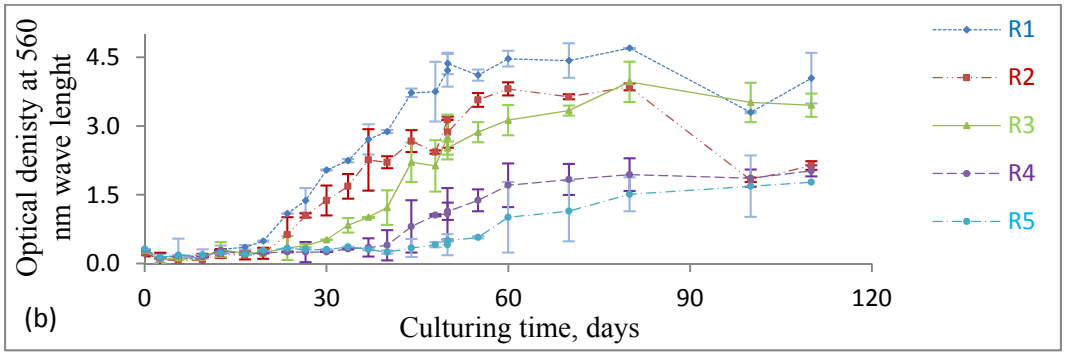
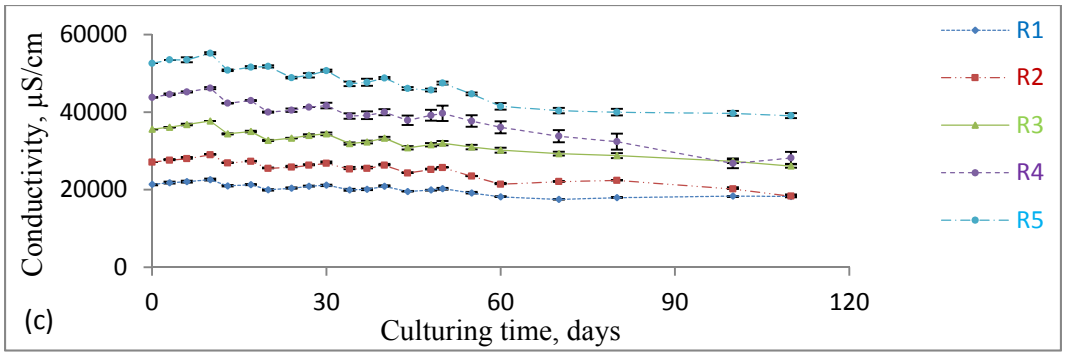


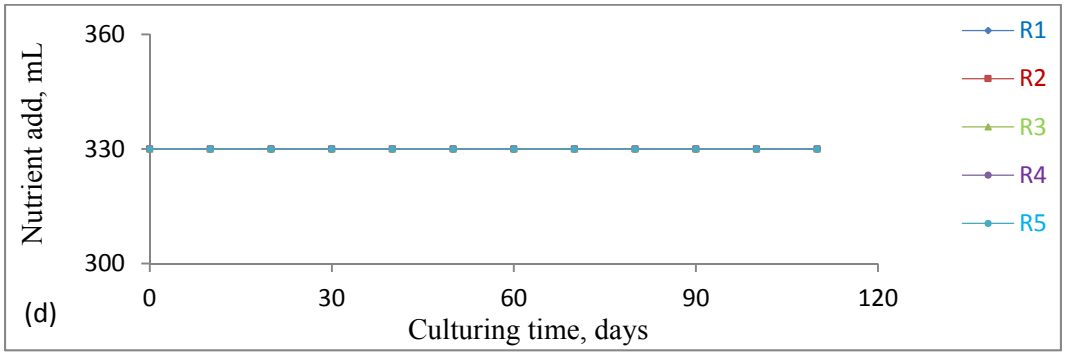

**Figure 4.12. Residuals vs. predicted maxima biomass**

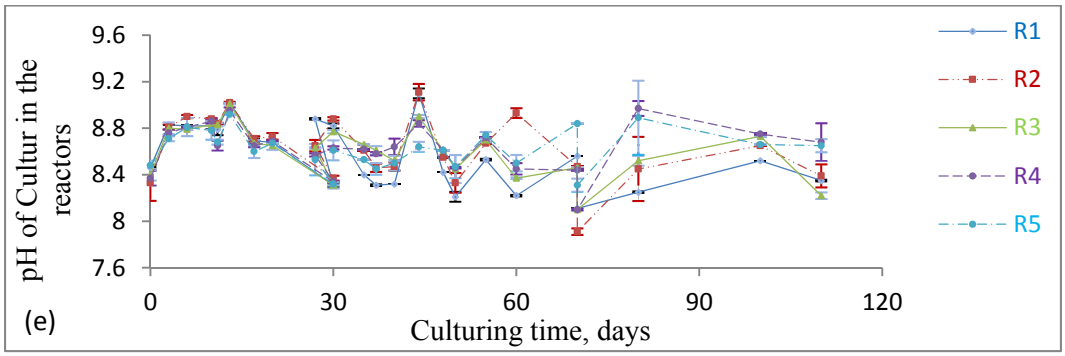
**Figure 4.13. BGNDRF species growing characteristics with time: a) dry weight; (b) optical density; (c) conductivities of medium in reactors; (d) nutrient added into reactors; (e) pH; (f) temperature; (g) air flow rate, (Mean ± SE).**

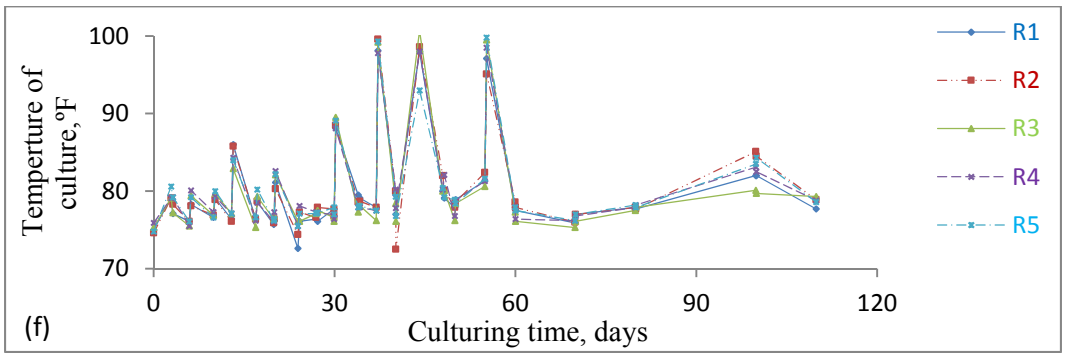


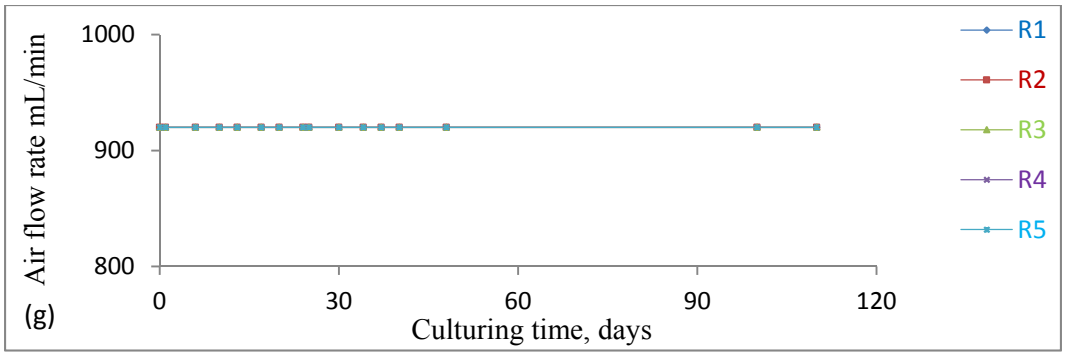




Conductivity, µS/cm
60000
40000
20000
0
0
30
60
90
120
Culturing time, days
R1
R2
R3
R4
R5
(c)


Nutrient add, mL
360
330
300
0
30
60
90
120
Culturing time, days
R1
R2
R3
R4
R5
(d)


pH of Cultur in the reactors
9.6
9.2
8.8
8.4
8
7.6
0
30
60
90
120
Culturing time, days
R1
R2
R3
R4
R5
(e)


Temperture of culture, ºF
100
90
80
70
0
30
60
90
120
Culturing time, days
R1
R2
R3
R4
R5
(f)




Note: R1 (21000 µS/cm), R2 (27100 µS/cm), R3 (35500 µS/cm), R4 (48500 µS/cm), R5 (52800 µS/cm)

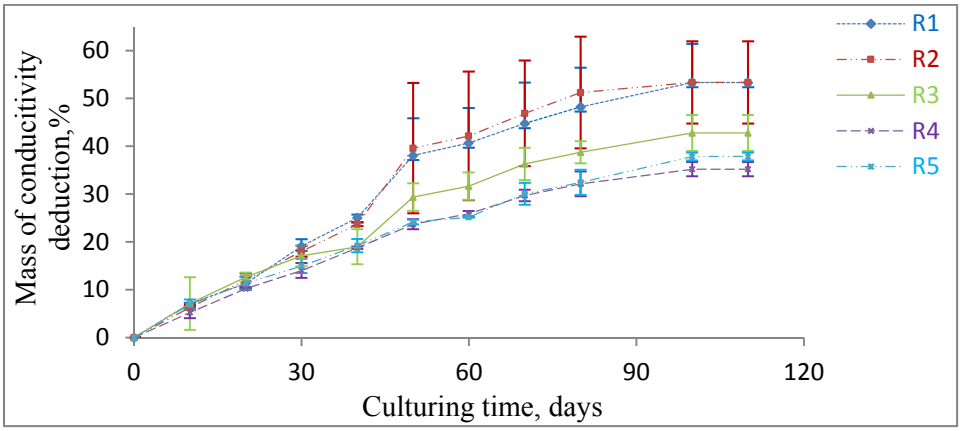



**Figure 4.14. Mass of conductivity deduction vs. culturing time (Mean ± SE).**

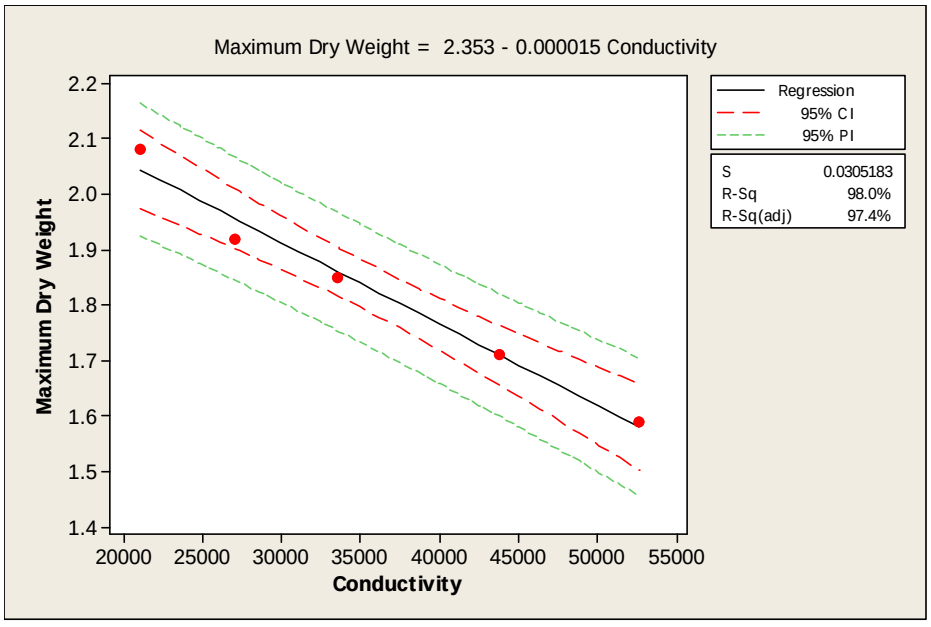



**Figure 4.15. Maximum dry weights vs. initial conductivity**

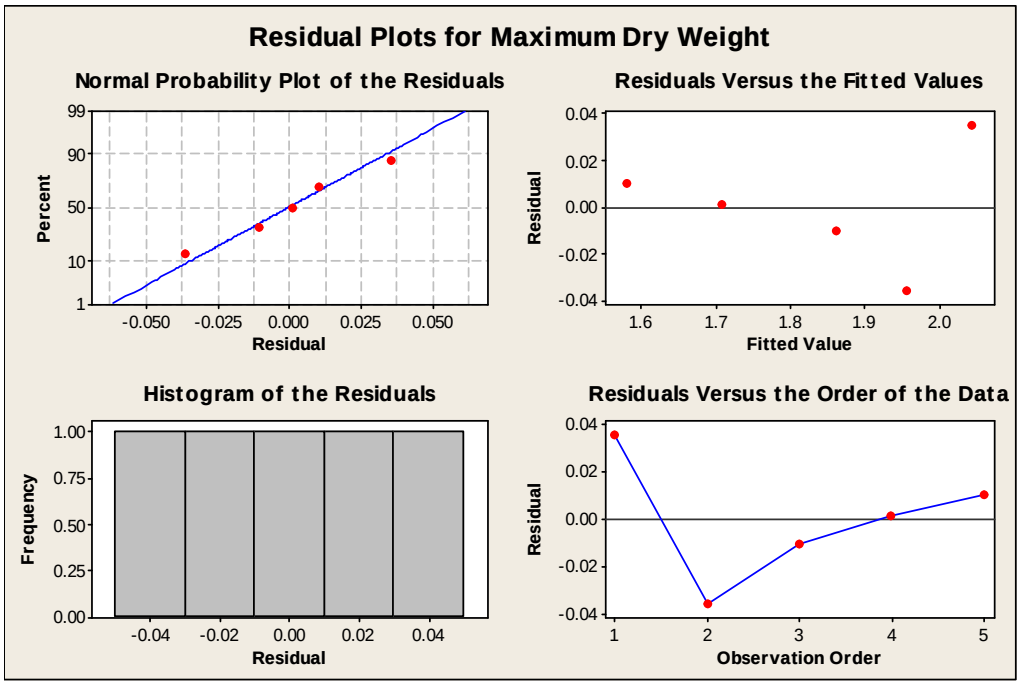



**Figure 4.16. Residual Plots for Maximum Dry Weight**

# Chapter 7 – APPENDIX

# Appendix A

## Units of Measure

| | |
|---|---|
| ºC | Degree(s) Celsius |
| ºF | Degree(s) Fahrenheit |
| ft | Feet |
| g | Gram(s) |
| g/L | Gram(s) per liter |
| g/L/d | Gram(s) per liter per day |
| g. $MJ^{-1}$ | Gram(s) per mega joule(s) |
| GPD | Gallon(s) per day |
| Kg | kilogram |
| KWh | kilowatt hour |

| | |
|---|---|
| L | Liter(s) |
| $m^3$ | Cubic meter(s) |
| mg/L | Milligram(s) per liter |
| MJ | Mega Joule |
| MGD | Million gallon(s) per day |
| ppm | Part per million |
| Rpm | Revolutions per minute |
| µm | Micrometer(s) |
| µS/cm | Micro-Siemens per centimeter |
| % | Percentage |

# Appendix B

## Elements/Ions in Anaerobic Digested Sludge (EPA, 2006)

| Elements/ions | Range (mg/L) |
|---|---|
| Ammonia Nitrogen | 1,500–3,000 |
| Sodium | 3,500–5,500 |
| Calcium | 1,500–4,500 |
| Magnesium | 1,000–1,500 |
| Potassium | 2,500–4,500 |

Anaerobic digested sludge also contains other elements such as copper, chromium VI, chromium, nickel and zinc.

# Appendix C

## BBM Recipe

| | | |
|---|---|---|
| To 940 ml of distilled water | Salt | g/400 ml dH2O |
| Add 10 ml of each of the | $NaNO_3$ | 10.0 |
| Following stock solutions: | $CaCl_2.2H_2O$ | 1.0 |
| | $MgSO_4.7H_2O$ | 3.0 |
| | $KH_2PO_4$ | 3.0 |
| | NaCl | 1.0 |

Next, add 1 ml of each of the trace element stock solution:
(1) EGTA: 50 g
KOH: 31 g
1.0 L $dH_2O$
(2) $FeSO_4.7H_2O$: 4.98 g
1.0 ml $H_2SO_4$
999ml $dH_2O$
(3) $H_3BO_3$:11.42 g
1.0 $dH_2O$
(4) $ZnSO_4.7H_2O$: 8.82 g
$MnCl_2.4H_2O$: 1.44 g
MoO3: 0.71 g
CuSO4.5$H_2O$: 1.57 g
$Co(NO_3).6H_2O$: 0.49 g
1.0 L $dH_2O$

# Appendix D

## F/2 Recipe

For one liter of F/2
1. To approximately 950 mL of non-pasteurized seawater, add each of the components in the order specified in the table (except vitamins) while stirring continuously.
2. Bring total volume to 1 L with non-pasteurized seawater.
3. Cover and autoclave medium.
4. When cooled add sterile vitamins.
5. Store at refrigerator temperature.

| Component | Amount | Stock Solution |
|---|---|---|
| NaNO3 | 1 mL | 7.5 g/100 mL dH20 |
| NaH2PO4•H2O | 1 mL | 0.5 g/100 mL dH20 |
| Na2SiO3•9H2O | 1 mL | 3 g/100 mL dH20 |
| Trace Metals Solution | 1 mL/L | |
| Vitamin B12 | 1 mL/L | |
| Biotin Vitamin Solution | 1 mL/L | |
| Thiamine Vitamin Solution | | |

# Appendix E

## Data Record

### *Nutrient (mL)*

**Reactors: D1-D4; Seed microalgae: *D. salina***

| Day | D1-1 (SADS) | D1-2 (SADS) | D1 (average) | D2-1 (SADS) | D2-2 (SADS) | D2 (average) |
|---|---|---|---|---|---|---|
| 0 | 80 | 80 | 80 | 80 | 80 | 80 |
| 2 | 80 | 80 | 80 | 80 | 80 | 80 |
| 4 | 80 | 80 | 80 | 80 | 80 | 80 |
| 6 | 80 | 80 | 80 | 80 | 80 | 80 |
| 9 | 80 | 80 | 80 | 80 | 80 | 80 |
| 11 | 100 | 100 | 100 | 100 | 100 | 100 |
| 13 | 100 | 100 | 100 | 100 | 100 | 100 |
| 16 | 100 | 100 | 100 | 100 | 100 | 100 |
| 18 | 250 | 250 | 250 | 250 | 250 | 250 |
| 23 | 100 | 100 | 100 | 100 | 100 | 100 |
| 25 | 100 | 100 | 100 | 100 | 100 | 100 |
| 27 | 120 | 120 | 120 | 120 | 120 | 120 |
| 30 | 120 | 120 | 120 | 120 | 120 | 120 |
| 32 | 130 | 130 | 130 | 130 | 130 | 130 |
| 34 | 130 | 130 | 130 | 130 | 130 | 130 |
| 37 | 195 | 195 | 195 | 195 | 195 | 195 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 39 | 140 | 140 | 140 | 140 | 140 | 140 |
| 41 | 240 | 240 | 240 | 240 | 240 | 240 |

| Day | D3-1 (BBM) | D3-2 (BBM) | D3 (average) | D4-1 (BBM) | D4-2 (BBM) | D4 (average) |
|---|---|---|---|---|---|---|
| 0 | 80 | 80 | 80 | 80 | 80 | 80 |
| 2 | 80 | 80 | 80 | 80 | 80 | 80 |
| 4 | 80 | 80 | 80 | 80 | 80 | 80 |
| 6 | 80 | 80 | 80 | 80 | 80 | 80 |
| 9 | 80 | 80 | 80 | 80 | 80 | 80 |
| 11 | 100 | 100 | 100 | 100 | 100 | 100 |
| 13 | 100 | 100 | 100 | 100 | 100 | 100 |
| 16 | 100 | 100 | 100 | 100 | 100 | 100 |
| 18 | 250 | 250 | 250 | 250 | 250 | 250 |
| 23 | 100 | 100 | 100 | 100 | 100 | 100 |
| 25 | 100 | 100 | 100 | 100 | 100 | 100 |
| 27 | 120 | 120 | 120 | 120 | 120 | 120 |
| 30 | 120 | 120 | 120 | 120 | 120 | 120 |
| 32 | 130 | 130 | 130 | 130 | 130 | 130 |
| 34 | 130 | 130 | 130 | 130 | 130 | 130 |
| 37 | 195 | 195 | 195 | 195 | 195 | 195 |
| 39 | 140 | 140 | 140 | 140 | 140 | 140 |
| 41 | 240 | 240 | 240 | 240 | 240 | 240 |

**Reactors: S1-S4; Seed microalgae: *S. platensis***

| Day | S1-1 (SADS) | S1-2 (SADS) | S1 (average) | S2-1 (SADS) | S2-2 (SADS) | S2 (average) |
|---|---|---|---|---|---|---|
| 0 | 20 | 20 | 20 | 20 | 20 | 20 |
| 6 | 20 | 20 | 20 | 20 | 20 | 20 |
| 7 | 40 | 40 | 40 | 40 | 40 | 40 |
| 8 | 30 | 30 | 30 | 30 | 30 | 30 |
| 9 | 30 | 30 | 30 | 30 | 30 | 30 |
| 10 | 30 | 30 | 30 | 30 | 30 | 30 |
| 14 | 90 | 90 | 90 | 90 | 90 | 90 |
| 17 | 90 | 90 | 90 | 90 | 90 | 90 |
| 20 | 90 | 90 | 90 | 90 | 90 | 90 |
| 24 | 120 | 120 | 120 | 120 | 120 | 120 |
| 27 | 200 | 200 | 200 | 200 | 200 | 200 |
| 31 | 200 | 200 | 200 | 200 | 200 | 200 |
| 34 | 240 | 240 | 240 | 240 | 240 | 240 |

| Day | S3-1 (f/2) | S3-2 (f/2) | S3 (average) | S4-1 (f/2) | S4-2 (f/2) | S4 (average) |
|---|---|---|---|---|---|---|
| 0 | 20 | 20 | 20 | 20 | 20 | 20 |
| 6 | 20 | 20 | 20 | 20 | 20 | 20 |
| 7 | 40 | 40 | 40 | 40 | 40 | 40 |
| 8 | 30 | 30 | 30 | 30 | 30 | 30 |
| 9 | 30 | 30 | 30 | 30 | 30 | 30 |
| 10 | 30 | 30 | 30 | 30 | 30 | 30 |
| 14 | 90 | 90 | 90 | 90 | 90 | 90 |
| 17 | 90 | 90 | 90 | 90 | 90 | 90 |
| 20 | 90 | 90 | 90 | 90 | 90 | 90 |
| 24 | 120 | 120 | 120 | 120 | 120 | 120 |
| 27 | 200 | 200 | 200 | 200 | 200 | 200 |
| 31 | 200 | 200 | 200 | 200 | 200 | 200 |
| 34 | 240 | 240 | 240 | 240 | 240 | 240 |

## *Dry Biomass (g/L)*

**Reactors: D1-D4; Seed microalgae: *D. salina***

| Day | D1-1 | D1-2 | D1 (average) | D2-1 | D2-2 | D2 (average) |
|---|---|---|---|---|---|---|
| 0 | 0.76 | 0.76 | 0.76 | 0.83 | 0.83 | 0.83 |
| 2 | 0.43 | 1.08 | 0.755 | 0.79 | 0.87 | 0.83 |
| 4 | 0.67 | 0.69 | 0.68 | 0.72 | 0.76 | 0.74 |
| 6 | 0.42 | 0.78 | 0.6 | 0.64 | 0.68 | 0.66 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | 0.5 | 0.5 | 0.5 | 0.55 | 0.51 | 0.53 |
| 11 | 0.66 | 0.64 | 0.65 | 0.68 | 0.62 | 0.65 |
| 13 | 0.69 | 0.63 | 0.66 | 0.79 | 0.75 | 0.77 |
| 16 | 0.87 | 0.83 | 0.85 | 0.86 | 0.84 | 0.85 |
| 18 | 0.97 | 0.93 | 0.95 | 0.91 | 0.93 | 0.92 |
| 23 | 0.88 | 1.43 | 1.155 | 1.08 | 1.04 | 1.06 |
| 25 | 1.1 | 1.14 | 1.12 | 0.98 | 1.36 | 1.17 |
| 27 | 1.22 | 1.3 | 1.26 | 1.31 | 1.27 | 1.29 |
| 30 | 1.15 | 1.28 | 1.215 | 1.25 | 1.23 | 1.24 |
| 32 | 1.16 | 1.26 | 1.21 | 1.28 | 1.36 | 1.32 |
| 34 | 1.2 | 1.32 | 1.26 | 1.39 | 1.49 | 1.44 |
| 37 | 1.31 | 1.41 | 1.36 | 1.41 | 1.55 | 1.48 |
| 39 | 1.27 | 1.15 | 1.21 | 1.38 | 1.46 | 1.42 |
| 41 | 1.13 | 1.11 | 1.12 | 1.41 | 1.34 | 1.375 |
| | | | | | | |
| **Day** | **D3-1** | **D3-2** | **D3 (average)** | **D4-1** | **D4-2** | **D4 (average)** |
| 0 | 0.76 | 0.76 | 0.76 | 0.73 | 0.73 | 0.73 |
| 2 | 0.41 | 1.08 | 0.745 | 0.71 | 0.81 | 0.76 |
| 4 | 0.58 | 0.68 | 0.63 | 0.66 | 0.7 | 0.68 |
| 6 | 0.35 | 0.7 | 0.525 | 0.54 | 0.58 | 0.56 |
| 9 | 0.33 | 0.32 | 0.325 | 0.31 | 0.41 | 0.36 |
| 11 | 0.47 | 0.49 | 0.48 | 0.44 | 0.42 | 0.43 |
| 13 | 0.28 | 0.3 | 0.29 | 0.46 | 0.48 | 0.47 |
| 16 | 0.6 | 0.58 | 0.59 | 0.56 | 0.46 | 0.51 |
| 18 | 0.64 | 0.66 | 0.65 | 0.55 | 0.53 | 0.54 |
| 23 | 0.6 | 0.62 | 0.61 | 0.73 | 0.39 | 0.56 |
| 25 | 0.73 | 0.71 | 0.72 | 0.42 | 0.44 | 0.43 |
| 27 | 0.93 | 0.89 | 0.91 | 0.67 | 0.76 | 0.715 |
| 30 | 1.08 | 0.98 | 1.03 | 0.61 | 0.76 | 0.685 |
| 32 | 0.78 | 0.94 | 0.86 | 0.67 | 0.69 | 0.68 |
| 34 | 0.9 | 0.94 | 0.92 | 0.72 | 0.74 | 0.73 |
| 37 | 0.88 | 1.19 | 1.035 | 0.78 | 0.88 | 0.83 |
| 39 | 0.9 | 1.17 | 1.035 | 0.76 | 0.92 | 0.84 |
| 41 | 1.1 | 1.14 | 1.12 | 0.746 | 0.744 | 0.745 |

**Reactors: S1-S4; Seed microalgae: *S. platensis***

| **Day** | **S1-1** | **S1-2** | **S1 (average)** | **S2-1** | **S2-2** | **S2 (average)** |
|---|---|---|---|---|---|---|
| 0 | 0.0326 | 0.0366 | 0.0346 | 0.0427 | 0.0487 | 0.0457 |
| 6 | 0.548 | 0.538 | 0.543 | 0.66 | 0.662 | 0.661 |
| 10 | 0.941 | 0.945 | 0.943 | 0.69 | 0.67 | 0.68 |
| 14 | 1.13 | 1.15 | 1.14 | 1.445 | 1.466 | 1.4555 |
| 17 | 1.166 | 1.187 | 1.1765 | 1.223 | 1.263 | 1.243 |
| 20 | 1.641 | 0.43 | 1.0355 | 1.689 | 1.67 | 1.6795 |
| 24 | 1.05 | 0.97 | 1.01 | 1.995 | 1.98 | 1.9875 |
| 27 | 1.25 | 1.23 | 1.24 | 1.3 | 1.32 | 1.31 |
| 31 | 1.315 | 0.7182 | 1.0166 | 1.43 | 1.41 | 1.42 |
| 34 | 0.9528 | 0.744 | 0.8484 | 0.928 | 0.923 | 0.9255 |

| **Day** | **S3-1** | **S3-2** | **S3 (average)** | **S4-1** | **S4-2** | **S4 (average)** |
|---|---|---|---|---|---|---|
| 0 | 0.031 | 0.031 | 0.031 | 0.0137 | 0.0137 | 0.0137 |
| 6 | 0.502 | 0.551 | 0.5265 | 0.446 | 0.466 | 0.456 |
| 10 | 0.88 | 0.9 | 0.89 | 0.6 | 0.62 | 0.61 |
| 14 | 0.39 | 1.09 | 0.74 | 0.28 | 0.32 | 0.3 |
| 17 | 0.739 | 0.999 | 0.869 | 0.613 | 0.653 | 0.633 |
| 20 | 0.884 | 0.886 | 0.885 | 0.66 | 0.64 | 0.65 |
| 24 | 0.8996 | 0.908 | 0.9038 | 0.67 | 0.666 | 0.668 |
| 27 | 0.6528 | 1.4102 | 1.0315 | 0.525 | 0.538 | 0.5315 |
| 31 | 0.692 | 1.689 | 1.1905 | 0.546 | 0.55 | 0.548 |
| 34 | 0.845 | 0.865 | 0.855 | 0.674 | 0.452 | 0.563 |

## *Optical Density at 560 nm*

**Reactors: D1-D4; Seed microalgae: *D. salina***

| Day | D1-1 | D1-2 | D1 (average) | D2-1 | D2-2 | D2 (average) |
|---|---|---|---|---|---|---|
| 0 | 0.1715 | 0.1715 | 0.1715 | 0.1715 | 0.1715 | 0.1715 |
| 2 | 0.02273 | 0.02277 | 0.02275 | 0.2104 | 0.2106 | 0.2105 |
| 4 | 0.2898 | 0.2892 | 0.2895 | 0.2071 | 0.2079 | 0.2075 |
| 6 | 0.2291 | 0.2289 | 0.229 | 0.1425 | 0.1435 | 0.143 |
| 9 | 0.3 | 0.301 | 0.3005 | 0.4318 | 0.4312 | 0.4315 |
| 11 | 0.6223 | 0.6228 | 0.62255 | 0.9007 | 0.9013 | 0.901 |
| 13 | 0.98 | 0.984 | 0.982 | 1.198 | 1.222 | 1.21 |
| 16 | 1.237 | 1.245 | 1.241 | 1.351 | 1.353 | 1.352 |
| 18 | 1.397 | 1.396 | 1.3965 | 1.4677 | 1.4673 | 1.4675 |
| 23 | 1.59 | 1.61 | 1.6 | 1.737 | 1.725 | 1.731 |
| 25 | 1.9371 | 1.9379 | 1.9375 | 2.0108 | 2.0102 | 2.0105 |
| 27 | 1.877 | 1.873 | 1.875 | 2.02 | 2.034 | 2.027 |
| 30 | 1.898 | 1.929 | 1.9135 | 2.1212 | 2.098 | 2.1096 |
| 32 | 1.955 | 1.996 | 1.9755 | 2.1668 | 2.1662 | 2.1665 |
| 34 | 2.001 | 2.003 | 2.002 | 2.257 | 2.261 | 2.259 |
| 37 | 2.019 | 2.009 | 2.014 | 2.251 | 2.266 | 2.2585 |
| 39 | 1.9482 | 1.9488 | 1.9485 | 2.311 | 2.315 | 2.313 |
| 41 | 0.1715 | 0.1715 | 0.1715 | 0.1715 | 0.1715 | 0.1715 |

| Day | D3-1 | D3-2 | D3 (average) | D4-1 | D4-2 | D4 (average) |
|---|---|---|---|---|---|---|
| 0 | 0.0855 | 0.0855 | 0.0855 | 0.08 | 0.08 | 0.08 |
| 2 | 0.0978 | 0.0982 | 0.098 | 0.0725 | 0.0725 | 0.0725 |
| 4 | 0.062 | 0.06 | 0.061 | 0.0705 | 0.0695 | 0.07 |
| 6 | 0.1398 | 0.1382 | 0.139 | 0.107 | 0.113 | 0.11 |
| 9 | 0.2442 | 0.2448 | 0.2445 | 0.2606 | 0.2594 | 0.26 |
| 11 | 0.2711 | 0.2699 | 0.2705 | 0.33 | 0.332 | 0.331 |
| 13 | 0.395 | 0.407 | 0.401 | 0.378 | 0.378 | 0.378 |
| 16 | 0.489 | 0.475 | 0.482 | 0.414 | 0.42 | 0.417 |
| 18 | 0.5649 | 0.5661 | 0.5655 | 0.3439 | 0.3431 | 0.3435 |
| 23 | 0.789 | 0.787 | 0.788 | 0.5 | 0.502 | 0.501 |
| 25 | 0.8769 | 0.8781 | 0.8775 | 0.7163 | 0.7168 | 0.71655 |
| 27 | 1.002 | 1.012 | 1.007 | 0.8664 | 0.8686 | 0.8675 |
| 30 | 1.0519 | 1.0531 | 1.0525 | 0.8827 | 0.8843 | 0.8835 |
| 32 | 1.1932 | 1.1938 | 1.1935 | 1.1412 | 1.1398 | 1.1405 |
| 34 | 1.359 | 1.351 | 1.355 | 1.371 | 1.377 | 1.374 |
| 37 | 1.5561 | 1.5569 | 1.5565 | 1.3671 | 1.3699 | 1.3685 |
| 39 | 1.6651 | 1.6659 | 1.6655 | 1.418 | 1.42 | 1.419 |
| 41 | 0.0855 | 0.0855 | 0.0855 | 0.08 | 0.08 | 0.08 |

**Reactors: S1-S4; Seed microalgae: *S. platensis***

| Day | S1-1 | S1-2 | S1 (average) | S2-1 | S2-2 | S2 (average) |
|---|---|---|---|---|---|---|
| 0 | 0.036 | 0.036 | 0.036 | 0.0405 | 0.0405 | 0.0405 |
| 3 | 0.037 | 0.038 | 0.0375 | 0.165 | 0.167 | 0.166 |
| 6 | 0.082 | 0.078 | 0.08 | 0.243 | 0.241 | 0.242 |
| 8 | 0.081 | 0.079 | 0.08 | 0.241 | 0.243 | 0.242 |
| 10 | 0.057 | 0.056 | 0.0565 | 0.3537 | 0.3533 | 0.3535 |
| 14 | 0.124 | 0.126 | 0.125 | 0.7517 | 0.7513 | 0.7515 |
| 17 | 0.191 | 0.195 | 0.193 | 1.138 | 1.136 | 1.137 |
| 20 | 0.2054 | 0.2056 | 0.2055 | 1.183 | 1.185 | 1.184 |
| 24 | 0.271 | 0.279 | 0.275 | 1.657 | 1.594 | 1.6255 |
| 27 | 0.316 | 0.305 | 0.3105 | 1.486 | 1.484 | 1.485 |
| 31 | 0.641 | 0.609 | 0.625 | 1.6505 | 1.6095 | 1.63 |
| 34 | 0.754 | 0.656 | 0.705 | 1.361 | 1.27 | 1.3155 |

| Day | S3-1 | S3-2 | S3 (average) | S4-1 | S4-2 | S4 (average) |
|---|---|---|---|---|---|---|
| 0 | 0.028 | 0.028 | 0.028 | 0.0255 | 0.0255 | 0.0255 |
| 3 | 0.137 | 0.133 | 0.135 | 0.131 | 0.132 | 0.1315 |
| 6 | 0.1585 | 0.1575 | 0.158 | 0.182 | 0.18 | 0.181 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 0.159 | 0.157 | 0.158 | 0.182 | 0.18 | 0.181 |
| 10 | 0.198 | 0.2 | 0.199 | 0.195 | 0.185 | 0.19 |
| 14 | 0.407 | 0.411 | 0.409 | 0.211 | 0.215 | 0.213 |
| 17 | 0.418 | 0.414 | 0.416 | 0.203 | 0.201 | 0.202 |
| 20 | 0.437 | 0.431 | 0.434 | 0.2358 | 0.2352 | 0.2355 |
| 24 | 0.542 | 0.548 | 0.545 | 0.252 | 0.258 | 0.255 |
| 27 | 0.564 | 0.566 | 0.565 | 0.2701 | 0.2699 | 0.27 |
| 31 | 0.524 | 0.526 | 0.525 | 0.2107 | 0.2094 | 0.21005 |
| 34 | 0.609 | 0.592 | 0.6005 | 0.205 | 0.195 | 0.2 |

## *pH*

**Reactors: D1-D4; Seed microalgae: *D. salina***

| Day | D1-1 | D1-2 | D1 (average) | D2-1 | D2-2 | D2 (average) |
|---|---|---|---|---|---|---|
| 0 | 6.72 | 6.72 | 6.72 | 6.72 | 6.72 | 6.72 |
| 2 | 6.9 | 6.9 | 6.9 | 6.82 | 6.86 | 6.84 |
| 4 | 7.88 | 7.9 | 7.89 | 7.61 | 7.65 | 7.63 |
| 6 | 6.89 | 6.85 | 6.87 | 6.82 | 6.86 | 6.84 |
| 9 | 8.35 | 8.33 | 8.34 | 8.3 | 8.26 | 8.28 |
| 11 | 8.7 | 8.72 | 8.71 | 8.59 | 8.55 | 8.57 |
| 13 | 8.87 | 8.65 | 8.76 | 8.7 | 8.66 | 8.68 |
| 16 | 8.38 | 8.46 | 8.42 | 8.48 | 8.48 | 8.48 |
| 18 | 8.45 | 8.63 | 8.54 | 8.59 | 8.57 | 8.58 |
| 23 | 8.44 | 8.62 | 8.53 | 8.38 | 8.56 | 8.47 |
| 25 | 8.57 | 8.43 | 8.5 | 8.33 | 8.29 | 8.31 |
| 27 | 8.25 | 8.43 | 8.34 | 8.18 | 8.25 | 8.215 |
| 30 | 8.4 | 8.42 | 8.41 | 8.29 | 8.27 | 8.28 |
| 32 | 8.52 | 8.5 | 8.51 | 8.34 | 8.32 | 8.33 |
| 34 | 8.55 | 8.53 | 8.54 | 8.34 | 8.48 | 8.41 |
| 37 | 8.52 | 8.54 | 8.53 | 8.36 | 8.14 | 8.25 |
| 39 | 8.43 | 8.45 | 8.44 | 8.55 | 8.56 | 8.555 |
| 41 | 6.72 | 6.72 | 6.72 | 6.72 | 6.72 | 6.72 |

| Day | D3-1 | D3-2 | D3 (average) | D4-1 | D4-2 | D4 (average) |
|---|---|---|---|---|---|---|
| 0 | 6.85 | 6.85 | 6.85 | 6.82 | 6.82 | 6.82 |
| 2 | 6.86 | 6.86 | 6.86 | 6.79 | 6.83 | 6.81 |
| 4 | 7.26 | 7.22 | 7.24 | 7.07 | 7.09 | 7.08 |
| 6 | 6.8 | 6.8 | 6.8 | 6.76 | 6.82 | 6.79 |
| 9 | 8.25 | 8.27 | 8.26 | 8.28 | 8.26 | 8.27 |
| 11 | 8.71 | 8.73 | 8.72 | 8.74 | 8.76 | 8.75 |
| 13 | 8.65 | 8.55 | 8.6 | 8.72 | 8.78 | 8.75 |
| 16 | 8.31 | 8.29 | 8.3 | 8.2 | 8.2 | 8.2 |
| 18 | 8.41 | 8.43 | 8.43 | 8.38 | 8.48 | 8.43 |
| 23 | 8.52 | 8.38 | 8.45 | 8.43 | 8.45 | 8.44 |
| 25 | 8.43 | 8.41 | 8.42 | 8.43 | 8.42 | 8.425 |
| 27 | 8.37 | 8.39 | 8.38 | 8.52 | 8.34 | 8.43 |
| 30 | 8.44 | 8.42 | 8.43 | 8.46 | 8.46 | 8.46 |
| 32 | 8.48 | 8.46 | 8.47 | 8.46 | 8.49 | 8.475 |
| 34 | 8.47 | 8.52 | 8.495 | 8.41 | 8.43 | 8.42 |
| 37 | 8.48 | 8.42 | 8.45 | 8.38 | 8.48 | 8.43 |
| 39 | 8.44 | 8.44 | 8.44 | 8.42 | 8.4 | 8.41 |
| 41 | 6.85 | 6.85 | 6.85 | 6.82 | 6.82 | 6.82 |

**Reactors: S1-S4; Seed microalgae: *S. platensis***

| Day | S1-1 | S1-2 | S1 (average) | S2-1 | S2-2 | S2 (average) |
|---|---|---|---|---|---|---|
| 0 | 8.59 | 8.59 | 8.59 | 8.69 | 8.69 | 8.69 |
| 3 | 8.35 | 8.37 | 8.36 | 8.4 | 8.42 | 8.41 |
| 6 | 8.62 | 8.66 | 8.64 | 8.17 | 8.15 | 8.16 |
| 8 | 8.89 | 8.87 | 8.88 | 8.77 | 8.81 | 8.79 |
| 10 | 8.56 | 8.54 | 8.55 | 8.61 | 8.65 | 8.63 |
| 14 | 8.66 | 8.64 | 8.65 | 8.3 | 8.28 | 8.29 |
| 17 | 8.79 | 8.69 | 8.74 | 8.47 | 8.49 | 8.48 |
| 20 | 9.22 | 8.62 | 8.92 | 8.43 | 8.39 | 8.41 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 24 | 8.98 | 8.64 | 8.81 | 8.95 | 8.71 | 8.83 |
| 27 | 9.02 | 9 | 9.01 | 8.54 | 8.6 | 8.57 |
| 31 | 8.9 | 8.92 | 8.91 | 8.6 | 8.58 | 8.59 |
| 34 | 8.87 | 8.86 | 8.865 | 8.69 | 8.59 | 8.64 |

| Day | S3-1 | S3-2 | S3 (average) | S4-1 | S4-2 | S4 (average) |
|---|---|---|---|---|---|---|
| 0 | 8.51 | 8.51 | 8.51 | 8.73 | 8.73 | 8.73 |
| 3 | 8.47 | 8.45 | 8.46 | 8.4 | 8.4 | 8.4 |
| 6 | 8.34 | 8.36 | 8.35 | 8.4 | 8.38 | 8.39 |
| 8 | 8.53 | 8.49 | 8.51 | 8.53 | 8.53 | 8.53 |
| 10 | 8.49 | 8.45 | 8.47 | 8.46 | 8.44 | 8.45 |
| 14 | 8.45 | 8.46 | 8.455 | 8.26 | 8.26 | 8.26 |
| 17 | 8.51 | 8.31 | 8.41 | 8.28 | 8.32 | 8.3 |
| 20 | 8.46 | 8.68 | 8.57 | 8.51 | 8.39 | 8.45 |
| 24 | 8.54 | 8.36 | 8.45 | 8.52 | 8.38 | 8.45 |
| 27 | 8.75 | 8.57 | 8.66 | 8.58 | 8.64 | 8.61 |
| 31 | 8.57 | 8.61 | 8.59 | 8.58 | 8.56 | 8.57 |
| 34 | 8.6 | 8.62 | 8.61 | 8.53 | 8.57 | 8.55 |

## *Temperature (°F)*

**Reactors: D1-D4; Seed microalgae: *D. salina***

| Day | D1-1 | D1-2 | D1 (average) | D2-1 | D2-2 | D2 (average) |
|---|---|---|---|---|---|---|
| 0 | 76.5 | 76.5 | 76.5 | 77 | 77 | 77 |
| 2 | 63 | 63.2 | 63.1 | 63.1 | 62.9 | 63 |
| 4 | 75.6 | 75.4 | 75.5 | 75.5 | 75.5 | 75.5 |
| 6 | 74.5 | 74.3 | 74.4 | 74.3 | 74.5 | 74.4 |
| 9 | 75.4 | 75.2 | 75.3 | 76.6 | 76.2 | 76.4 |
| 11 | 73.5 | 73.3 | 73.4 | 75.6 | 74.6 | 75.1 |
| 13 | 76.9 | 76.8 | 76.85 | 73.7 | 73.1 | 73.4 |
| 16 | 77.1 | 78.5 | 77.8 | 77.4 | 73.5 | 75.45 |
| 18 | 75.6 | 75.8 | 75.7 | 77.7 | 76.9 | 77.3 |
| 23 | 72.6 | 72.4 | 72.5 | 75.6 | 75.4 | 75.5 |
| 25 | 75.1 | 74.9 | 75 | 72.9 | 70.5 | 71.7 |
| 27 | 75.2 | 75.4 | 75.3 | 71.9 | 74.9 | 73.4 |
| 30 | 73.7 | 74.1 | 73.9 | 75.2 | 75.2 | 75.2 |
| 32 | 73.4 | 73.4 | 73.4 | 74.3 | 74.5 | 74.4 |
| 34 | 74.9 | 74.7 | 74.8 | 76 | 76.2 | 76.1 |
| 37 | 75.2 | 74.8 | 75 | 75.7 | 76.1 | 75.9 |
| 39 | 75.1 | 74.9 | 75 | 76 | 76.2 | 76.1 |
| 41 | 74.6 | 74.2 | 74.4 | 74.6 | 75.8 | 75.2 |

| Day | D3-1 | D3-2 | D3 (average) | D4-1 | D4-2 | D4 (average) |
|---|---|---|---|---|---|---|
| 0 | 75.9 | 75.9 | 75.9 | 77.3 | 77.3 | 77.3 |
| 2 | 62.3 | 62.3 | 62.3 | 62.4 | 61.9 | 62.15 |
| 4 | 74.2 | 74.6 | 74.4 | 75.1 | 75.3 | 75.2 |
| 6 | 73.1 | 73.3 | 73.2 | 74.9 | 73.9 | 74.4 |
| 9 | 75.5 | 74.9 | 75.2 | 76.9 | 76.5 | 76.7 |
| 11 | 75.3 | 75.3 | 75.3 | 75.1 | 75.3 | 75.2 |
| 13 | 75.4 | 77.4 | 76.4 | 76.9 | 76.3 | 76.6 |
| 16 | 77 | 77.2 | 77.1 | 76.5 | 77.1 | 76.8 |
| 18 | 74.2 | 74.4 | 74.3 | 74.9 | 74.7 | 74.8 |
| 23 | 73.8 | 73.6 | 73.7 | 73.8 | 73.6 | 73.7 |
| 25 | 73 | 73.4 | 73.2 | 74.1 | 74.1 | 74.1 |
| 27 | 74.9 | 74.7 | 74.8 | 76 | 76.2 | 76.1 |
| 30 | 75.7 | 75.9 | 75.8 | 76.1 | 76.1 | 76.1 |
| 32 | 76 | 76.2 | 76.1 | 76.3 | 76.7 | 76.5 |
| 34 | 76.6 | 76.6 | 76.6 | 74.8 | 74.8 | 74.8 |
| 37 | 76.7 | 76.5 | 76.6 | 75.3 | 74.7 | 75 |
| 39 | 75.7 | 75.9 | 75.8 | 73.6 | 74 | 73.8 |
| 41 | 76 | 74.4 | 75.2 | 71.5 | 74.5 | 73 |

**Reactors: S1-S4; Seed microalgae: *S. platensis***

| Day | S1-1 | S1-2 | S1 (average) | S2-1 | S2-2 | S2 (average) |
|---|---|---|---|---|---|---|
| 0 | 73.9 | 73.9 | 73.9 | 75.3 | 75.3 | 75.3 |
| 3 | 73.9 | 73.9 | 73.9 | 75.3 | 75.3 | 75.3 |
| 6 | 86.6 | 86.4 | 86.5 | 89.1 | 88.9 | 89 |
| 8 | 75.1 | 75.3 | 75.2 | 75.4 | 75.2 | 75.3 |
| 10 | 73 | 73 | 73 | 76.4 | 76.8 | 76.6 |
| 14 | 77.7 | 77.6 | 77.65 | 80 | 81 | 80.5 |
| 17 | 75.2 | 74.8 | 75 | 72.3 | 72.3 | 72.3 |
| 20 | 82.5 | 82.9 | 82.7 | 83 | 83.2 | 83.1 |
| 24 | 75 | 75.2 | 75.1 | 78.3 | 77.9 | 78.1 |
| 27 | 77.3 | 76.7 | 77 | 78.5 | 77.9 | 78.2 |
| 31 | 79.5 | 78.9 | 79.2 | 80.1 | 80.1 | 80.1 |
| 34 | 92.6 | 92 | 92.3 | 91.3 | 91.1 | 91.2 |

| Day | S3-1 | S3-2 | S3 (average) | S4-1 | S4-2 | S4 (average) |
|---|---|---|---|---|---|---|
| 0 | 75 | 75 | 75 | 73.5 | 73.5 | 73.5 |
| 3 | 75 | 75 | 75 | 73.5 | 73.5 | 73.5 |
| 6 | 86.6 | 86.8 | 86.7 | 87.3 | 87.5 | 87.4 |
| 8 | 74.3 | 74.5 | 74.4 | 73.9 | 73.9 | 73.9 |
| 10 | 73.2 | 74.2 | 73.7 | 75.8 | 76 | 75.9 |
| 14 | 81.6 | 81 | 81.3 | 79.1 | 79.9 | 79.5 |
| 17 | 75.7 | 75.3 | 75.5 | 74.8 | 75 | 74.9 |
| 20 | 82.7 | 82.7 | 82.7 | 82.9 | 82.9 | 82.9 |
| 24 | 76 | 76.2 | 76.1 | 76.7 | 77.1 | 76.9 |
| 27 | 75.4 | 74.8 | 75.1 | 76.4 | 76.4 | 76.4 |
| 31 | 79 | 79.6 | 79.3 | 78 | 78.2 | 78.1 |
| 34 | 93.4 | 93.6 | 93.5 | 92.7 | 92.5 | 92.6 |

## *Conductivity (µS/cm)*

**Reactors: D1-D4; Seed microalgae: *D. salina***

| Day | D1-1 | D1-2 | D1 (average) | D2-1 | D2-2 | D2 (average) |
|---|---|---|---|---|---|---|
| 0 | 30,499 | 30,499 | 30,499 | 25,634 | 25,634 | 25,634 |
| 2 | 30,450 | 30,550 | 30,500 | 25,660 | 25,540 | 25,600 |
| 4 | 30,120 | 30,080 | 30,100 | 25,000 | 25,200 | 25,100 |
| 6 | 29,525 | 29,475 | 29,500 | 24,750 | 24,650 | 24,700 |
| 9 | 28,860 | 28,740 | 28,800 | 24,270 | 24,330 | 24,300 |
| 11 | 28,040 | 28,160 | 28,100 | 24,200 | 24,000 | 24,100 |
| 13 | 27,420 | 27,380 | 27,400 | 23,100 | 19,900 | 21,500 |
| 16 | 25,875 | 25,725 | 25,800 | 25,880 | 25,720 | 25,800 |
| 18 | 25,150 | 25,250 | 25,200 | 21,770 | 21,630 | 21,700 |
| 23 | 23,520 | 23,480 | 23,500 | 20,950 | 20,050 | 20,500 |
| 25 | 22,910 | 22,890 | 22,900 | 20,100 | 19,900 | 20,000 |
| 27 | 22,470 | 22,530 | 22,500 | 19,770 | 19,710 | 19,740 |
| 30 | 21,660 | 21,740 | 21,700 | 19,170 | 19,130 | 19,150 |
| 32 | 21,150 | 21,250 | 21,200 | 18,730 | 18,690 | 18,710 |
| 34 | 20,830 | 20,770 | 20,800 | 18,200 | 18,180 | 18,190 |
| 37 | 20,450 | 20,350 | 20,400 | 18,060 | 18,000 | 18,030 |
| 39 | 19,530 | 19,590 | 19,560 | 17,230 | 17,150 | 17,190 |
| 41 | 18,100 | 18,120 | 18,110 | 16,790 | 16,670 | 16,730 |

| Day | D3-1 | D3-2 | D3 (average) | D4-1 | D4-2 | D4 (average) |
|---|---|---|---|---|---|---|
| 0 | 31,030 | 31,030 | 31,030 | 26,030 | 26,030 | 26,030 |
| 2 | 30,780 | 30,820 | 30,800 | 25,880 | 25,720 | 25,800 |
| 4 | 30,000 | 30,200 | 30,100 | 25,100 | 25,300 | 25,200 |
| 6 | 29,450 | 29,550 | 29,500 | 24,890 | 24,710 | 24,800 |
| 9 | 29,450 | 29,550 | 29,500 | 24,000 | 24,200 | 24,100 |
| 11 | 28,660 | 28,540 | 28,600 | 23,450 | 23,150 | 23,300 |
| 13 | 27,500 | 27,700 | 27,600 | 22,660 | 22,540 | 22,600 |
| 16 | 26,400 | 26,200 | 26,300 | 21,440 | 21,360 | 21,400 |
| 18 | 25,580 | 25,420 | 25,500 | 20,660 | 20,740 | 20,700 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23 | 23,770 | 23,630 | 23,700 | 19,100 | 19,020 | 19,060 |
| 25 | 23,700 | 22,300 | 23,000 | 18,100 | 18,700 | 18,400 |
| 27 | 22,810 | 22,790 | 22,800 | 18,200 | 18,220 | 18,210 |
| 30 | 22,000 | 22,200 | 22,100 | 17,400 | 17,520 | 17,460 |
| 32 | 21,650 | 21,550 | 21,600 | 16,920 | 16,980 | 16,950 |
| 34 | 21,040 | 20,960 | 21,000 | 16,620 | 16,660 | 16,640 |
| 37 | 20,650 | 20,750 | 20,700 | 16,300 | 16,360 | 16,330 |
| 39 | 19,620 | 19,580 | 19,600 | 15,520 | 15,540 | 15,530 |
| 41 | 20,000 | 20,000 | 20,000 | 15,150 | 15,110 | 15,130 |

**Reactors: S1-S4; Seed microalgae: *S. platensis***

| Day | S1-1 | S1-2 | S1 (average) | S2-1 | S2-2 | S2 (average) |
|---|---|---|---|---|---|---|
| 0 | 36,800 | 36,800 | 36,800 | 24,800 | 24,800 | 24,800 |
| 3 | 36,000 | 36,400 | 36,200 | 25,000 | 25,200 | 25,100 |
| 6 | 35,750 | 35,650 | 35,700 | 25,000 | 24,800 | 24,900 |
| 8 | 35,150 | 35,450 | 35,300 | 26,670 | 26,530 | 26,600 |
| 10 | 34,680 | 34,720 | 34,700 | 24,810 | 24,790 | 24,800 |
| 14 | 33,920 | 33,880 | 33,900 | 24,250 | 24,350 | 24,300 |
| 17 | 34,520 | 34,480 | 34,500 | 24,875 | 24,725 | 24,800 |
| 20 | 33,630 | 33,570 | 33,600 | 24,100 | 24,300 | 24,200 |
| 24 | 33,745 | 33,655 | 33,700 | 24,625 | 24,575 | 24,600 |
| 27 | 26,920 | 26,880 | 26,900 | 19,440 | 19,600 | 19,520 |
| 31 | 36,800 | 36,800 | 36,800 | 24,800 | 24,800 | 24,800 |
| 34 | 36,000 | 36,400 | 36,200 | 25,000 | 25,200 | 25,100 |

| Day | S3-1 | S3-2 | S3 (average) | S4-1 | S4-2 | S4 (average) |
|---|---|---|---|---|---|---|
| 0 | 37,200 | 37,200 | 37,200 | 24,900 | 24,900 | 24,900 |
| 3 | 38,050 | 38,150 | 38,100 | 26,400 | 26,800 | 26,600 |
| 6 | 37,750 | 37,650 | 37,700 | 26,950 | 26,850 | 26,900 |
| 8 | 38,000 | 38,000 | 38,000 | 26,850 | 27,350 | 27,100 |
| 10 | 37,850 | 37,950 | 37,900 | 27,130 | 27,070 | 27,100 |
| 14 | 37,825 | 37,775 | 37,800 | 27,410 | 27,390 | 27,400 |
| 17 | 39,110 | 39,090 | 39,100 | 28,770 | 28,630 | 28,700 |
| 20 | 38,750 | 38,850 | 38,800 | 28,930 | 28,870 | 28,900 |
| 24 | 39,550 | 39,250 | 39,400 | 30,480 | 30,320 | 30,400 |
| 27 | 38,130 | 38,270 | 38,200 | 30,640 | 30,560 | 30,600 |
| 31 | 37,200 | 37,200 | 37,200 | 24,900 | 24,900 | 24,900 |
| 34 | 38,050 | 38,150 | 38,100 | 26,400 | 26,800 | 26,600 |

## *Air flow rate (mL/min)*

**Reactors: D1-D4; Seed microalgae: *D. salina***

| Day | D1-1 | D1-2 | D1 (average) | D2-1 | D2-2 | D2 (average) |
|---|---|---|---|---|---|---|
| 0 | 368 | 368 | 368 | 368 | 368 | 368 |
| 2 | 299 | 299 | 299 | 460 | 460 | 460 |
| 4 | 115 | 115 | 115 | 299 | 299 | 299 |
| 6 | 596 | 596 | 596 | 345 | 345 | 345 |
| 9 | 506 | 506 | 506 | 920 | 920 | 920 |
| 11 | 736 | 736 | 736 | 713 | 713 | 713 |
| 13 | 667 | 667 | 667 | 667 | 667 | 667 |
| 16 | 598 | 598 | 598 | 664 | 664 | 664 |
| 18 | 713 | 713 | 713 | 667 | 667 | 667 |
| 23 | 460 | 460 | 460 | 644 | 644 | 644 |
| 25 | 644 | 644 | 644 | 644 | 644 | 644 |
| 27 | 690 | 690 | 690 | 782 | 782 | 782 |
| 30 | 690 | 690 | 690 | 782 | 782 | 782 |
| 32 | 874 | 874 | 874 | 736 | 736 | 736 |
| 34 | 1196 | 1196 | 1196 | 966 | 966 | 966 |
| 37 | 805 | 805 | 805 | 989 | 989 | 989 |
| 39 | 1150 | 1150 | 1150 | 1035 | 1035 | 1035 |
| 41 | 943 | 943 | 943 | 1104 | 1104 | 1104 |

| Day | D3-1 | D3-2 | D3 (average) | D4-1 | D4-2 | D4 (average) |
|---|---|---|---|---|---|---|
| 0 | 368 | 368 | 368 | 368 | 368 | 368 |
| 2 | 368 | 368 | 368 | 483 | 483 | 483 |
| 4 | 253 | 253 | 253 | 690 | 690 | 690 |
| 6 | 460 | 460 | 460 | 345 | 345 | 345 |
| 9 | 805 | 805 | 805 | 805 | 805 | 805 |
| 11 | 598 | 598 | 598 | 667 | 667 | 667 |
| 13 | 575 | 575 | 575 | 598 | 598 | 598 |
| 16 | 644 | 644 | 644 | 644 | 644 | 644 |
| 18 | 667 | 667 | 667 | 690 | 690 | 690 |
| 23 | 621 | 621 | 621 | 598 | 598 | 598 |
| 25 | 690 | 690 | 690 | 644 | 644 | 644 |
| 27 | 805 | 805 | 805 | 782 | 782 | 782 |
| 30 | 805 | 805 | 805 | 782 | 782 | 782 |
| 32 | 851 | 851 | 851 | 851 | 851 | 851 |
| 34 | 966 | 966 | 966 | 874 | 874 | 874 |
| 37 | 897 | 897 | 897 | 966 | 966 | 966 |
| 39 | 1012 | 1012 | 1012 | 989 | 989 | 989 |
| 41 | 1058 | 1058 | 1058 | 1012 | 1012 | 1012 |

**Reactors: S1-S4; Seed microalgae: *S. platensis***

| Day | S1-1 | S1-2 | S1 (average) | S2-1 | S2-2 | S2 (average) |
|---|---|---|---|---|---|---|
| 0 | 368 | 368 | 368 | 368 | 368 | 368 |
| 3 | 828 | 828 | 828 | 1012 | 1012 | 1012 |
| 6 | 1104 | 1104 | 1104 | 943 | 943 | 943 |
| 8 | 920 | 920 | 920 | 1242 | 1242 | 1242 |
| 10 | 874 | 874 | 874 | 1380 | 1380 | 1380 |
| 14 | 1265 | 1265 | 1265 | 1196 | 1196 | 1196 |
| 17 | 805 | 805 | 805 | 1380 | 1380 | 1380 |
| 20 | 1334 | 1334 | 1334 | 1357 | 1357 | 1357 |
| 24 | 1380 | 1380 | 1380 | 1380 | 1380 | 1380 |
| 27 | 1380 | 1380 | 1380 | 1495 | 1495 | 1495 |
| 31 | 1380 | 1380 | 1380 | 1495 | 1495 | 1495 |
| 34 | 368 | 368 | 368 | 368 | 368 | 368 |

| Day | S3-1 | S3-2 | S3 (average) | S4-1 | S4-2 | S4 (average) |
|---|---|---|---|---|---|---|
| 0 | 368 | 368 | 368 | 368 | 368 | 368 |
| 3 | 805 | 805 | 805 | 805 | 805 | 805 |
| 6 | 805 | 805 | 805 | 851 | 851 | 851 |
| 8 | 1334 | 1334 | 1334 | 1242 | 1242 | 1242 |
| 10 | 805 | 805 | 805 | 1288 | 1288 | 1288 |
| 14 | 1334 | 1334 | 1334 | 1495 | 1495 | 1495 |
| 17 | 1265 | 1265 | 1265 | 1242 | 1242 | 1242 |
| 20 | 1357 | 1357 | 1357 | 1380 | 1380 | 1380 |
| 24 | 1357 | 1357 | 1357 | 1380 | 1380 | 1380 |
| 27 | 1426 | 1426 | 1426 | 1380 | 1380 | 1380 |
| 31 | 1380 | 1380 | 1380 | 1380 | 1380 | 1380 |
| 34 | 368 | 368 | 368 | 368 | 368 | 368 |

## *Nutrient (mL)*

**Reactors: R1-R5; Seed microalgae: BGNDRF; Nutrient: SADS**

| Day | R1-1 | R1-2 | R1 (average) | R2-1 | R2-2 | R2 (average) |
|---|---|---|---|---|---|---|
| 0 | 330 | 330 | 330 | 330 | 330 | 330 |
| 3 | 330 | 330 | 330 | 330 | 330 | 330 |
| 6 | 330 | 330 | 330 | 330 | 330 | 330 |
| 10 | 330 | 330 | 330 | 330 | 330 | 330 |
| 13 | 330 | 330 | 330 | 330 | 330 | 330 |
| 17 | 330 | 330 | 330 | 330 | 330 | 330 |
| 20 | 330 | 330 | 330 | 330 | 330 | 330 |
| 24 | 330 | 330 | 330 | 330 | 330 | 330 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 27 | 330 | 330 | 330 | 330 | 330 | 330 |
| 30 | 330 | 330 | 330 | 330 | 330 | 330 |
| 34 | 330 | 330 | 330 | 330 | 330 | 330 |
| 40 | 330 | 330 | 330 | 330 | 330 | 330 |
| 44 | 330 | 330 | 330 | 330 | 330 | 330 |
| 50 | 330 | 330 | 330 | 330 | 330 | 330 |
| 53 | 330 | 330 | 330 | 330 | 330 | 330 |
| 60 | 330 | 330 | 330 | 330 | 330 | 330 |
| 70 | 330 | 330 | 330 | 330 | 330 | 330 |
| 80 | 330 | 330 | 330 | 330 | 330 | 330 |
| 90 | 330 | 330 | 330 | 330 | 330 | 330 |
| 100 | 330 | 330 | 330 | 330 | 330 | 330 |
| 110 | 330 | 330 | 330 | 330 | 330 | 330 |

| Day | R3-1 | R3-2 | R3 (average) | R4-1 | R4-2 | R4 (average) |
|---|---|---|---|---|---|---|
| 0 | 330 | 330 | 330 | 330 | 330 | 330 |
| 3 | 330 | 330 | 330 | 330 | 330 | 330 |
| 6 | 330 | 330 | 330 | 330 | 330 | 330 |
| 10 | 330 | 330 | 330 | 330 | 330 | 330 |
| 13 | 330 | 330 | 330 | 330 | 330 | 330 |
| 17 | 330 | 330 | 330 | 330 | 330 | 330 |
| 20 | 330 | 330 | 330 | 330 | 330 | 330 |
| 24 | 330 | 330 | 330 | 330 | 330 | 330 |
| 27 | 330 | 330 | 330 | 330 | 330 | 330 |
| 30 | 330 | 330 | 330 | 330 | 330 | 330 |
| 34 | 330 | 330 | 330 | 330 | 330 | 330 |
| 40 | 330 | 330 | 330 | 330 | 330 | 330 |
| 44 | 330 | 330 | 330 | 330 | 330 | 330 |
| 50 | 330 | 330 | 330 | 330 | 330 | 330 |
| 53 | 330 | 330 | 330 | 330 | 330 | 330 |
| 60 | 330 | 330 | 330 | 330 | 330 | 330 |
| 70 | 330 | 330 | 330 | 330 | 330 | 330 |
| 80 | 330 | 330 | 330 | 330 | 330 | 330 |
| 90 | 330 | 330 | 330 | 330 | 330 | 330 |
| 100 | 330 | 330 | 330 | 330 | 330 | 330 |
| 110 | 330 | 330 | 330 | 330 | 330 | 330 |

| Day | R5-1 | R5-2 | R5 (average) |
|---|---|---|---|
| 0 | 330 | 330 | 330 |
| 3 | 330 | 330 | 330 |
| 6 | 330 | 330 | 330 |
| 10 | 330 | 330 | 330 |
| 13 | 330 | 330 | 330 |
| 17 | 330 | 330 | 330 |
| 20 | 330 | 330 | 330 |
| 24 | 330 | 330 | 330 |
| 27 | 330 | 330 | 330 |
| 30 | 330 | 330 | 330 |
| 34 | 330 | 330 | 330 |
| 40 | 330 | 330 | 330 |
| 44 | 330 | 330 | 330 |
| 50 | 330 | 330 | 330 |
| 53 | 330 | 330 | 330 |
| 60 | 330 | 330 | 330 |
| 70 | 330 | 330 | 330 |
| 80 | 330 | 330 | 330 |
| 90 | 330 | 330 | 330 |
| 100 | 330 | 330 | 330 |
| 110 | 330 | 330 | 330 |

## *Dry Biomass (g/L)*

**Reactors: R1-R5; Seed microalgae: BGNDRF; Nutrient: SADS**

| Day | R1-1 | R1-2 | R1 (average) | R2-1 | R2-2 | R2 (average) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0.3005 | 0.2429 | 0.2717 | 0.5505 | 0.4395 | 0.495 |
| 6 | 0.066 | 0.055 | 0.0605 | 0.19 | 0.28 | 0.235 |
| 10 | 0.2032 | 0.1234 | 0.1633 | 0.2837 | 0.2829 | 0.2833 |
| 13 | 0.3184 | 0.1482 | 0.2333 | 0.2969 | 0.4347 | 0.3658 |
| 17 | 0.4207 | 0.3423 | 0.3815 | 0.7706 | 0.618 | 0.6943 |
| 20 | 0.5277 | 0.3273 | 0.4275 | 0.4025 | 0.3409 | 0.3717 |
| 24 | 0.7402 | 0.8801 | 0.81015 | 0.3857 | 0.4209 | 0.4033 |
| 27 | 0.9514 | 0.8516 | 0.9015 | 0.5718 | 0.5682 | 0.57 |
| 30 | 1.2963 | 1.1371 | 1.2167 | 0.6964 | 0.7403 | 0.71835 |
| 34 | 0.5025 | 0.4675 | 0.485 | 0.6303 | 0.6363 | 0.6333 |
| 40 | 1.3764 | 1.2802 | 1.3283 | 1.2985 | 1.3481 | 1.3233 |
| 44 | 1.6498 | 1.5718 | 1.6108 | 1.2996 | 1.3621 | 1.33085 |
| 50 | 1.8316 | 1.7284 | 1.7800 | 1.6241 | 1.4310 | 1.52755 |
| 53 | 1.6714 | 1.7801 | 1.7258 | 0.9990 | 1.1260 | 1.0625 |
| 60 | 2.0116 | 2.0099 | 2.0108 | 1.4173 | 1.3777 | 1.3975 |
| 70 | 2.007 | 1.939 | 1.9730 | 1.8255 | 1.6830 | 1.75425 |
| 80 | 2.0020 | 1.923 | 1.9625 | 1.9401 | 1.8999 | 1.92 |
| 90 | 2.1984 | 1.98 | 2.0892 | 1.7110 | 1.8990 | 1.805 |
| 100 | 1.9129 | 1.7654 | 1.8392 | 1.7833 | 1.7835 | 1.7834 |
| 110 | 1.9263 | 1.8386 | 1.8825 | 1.8036 | 1.6448 | 1.7242 |

| Day | R3-1 | R3-2 | R3 (average) | R4-1 | R4-2 | R4 (average) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0.1712 | 0.2523 | 0.21175 | 0.312 | 0.202 | 0.257 |
| 6 | 0.2821 | 0.3245 | 0.3033 | 0.2213 | 0.1653 | 0.1933 |
| 10 | 0.352 | 0.418 | 0.385 | 0.301 | 0.12 | 0.2105 |
| 13 | 0.4513 | 0.2521 | 0.3517 | 0.3771 | 0.501 | 0.43905 |
| 17 | 0.664 | 0.5761 | 0.62005 | 0.5429 | 0.4417 | 0.4923 |
| 20 | 0.5164 | 0.467 | 0.4917 | 0.4006 | 0.6003 | 0.50045 |
| 24 | 0.81 | 0.73 | 0.77 | 0.5992 | 0.6994 | 0.6493 |
| 27 | 0.8644 | 0.799 | 0.8317 | 0.144 | 0.116 | 0.13 |
| 30 | 0.9665 | 0.9669 | 0.9667 | 0.4312 | 0.4354 | 0.4333 |
| 34 | 0.9902 | 0.9963 | 0.9933 | 0.4409 | 0.4390 | 0.4400 |
| 40 | 1.0122 | 0.994 | 1.0031 | 0.6347 | 0.642 | 0.63835 |
| 44 | 1.311 | 1.39 | 1.3505 | 0.7928 | 0.7988 | 0.7958 |
| 50 | 1.6464 | 1.492 | 1.5692 | 1.0184 | 0.9201 | 0.96925 |
| 53 | 1.3518 | 1.5498 | 1.4508 | 0.8585 | 0.8599 | 0.8592 |
| 60 | 1.4712 | 1.5538 | 1.5125 | 1.1617 | 1.1599 | 1.1608 |
| 70 | 1.8045 | 1.6005 | 1.7025 | 1.4834 | 1.3851 | 1.43425 |
| 80 | 1.905 | 1.797 | 1.851 | 1.5319 | 1.5321 | 1.532 |
| 90 | 1.8188 | 1.6196 | 1.7192 | 1.6849 | 1.5831 | 1.634 |
| 100 | 1.4013 | 1.5003 | 1.4508 | 1.8108 | 1.6912 | 1.751 |
| 110 | 1.048 | 1.2185 | 1.13325 | 1.2221 | 1.4195 | 1.3208 |

| Day | R5-1 | R5-2 | R5 (average) |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 3 | 0.3078 | 0.3862 | 0.347 |
| 6 | 0.384 | 0.448 | 0.416 |
| 10 | 0.3775 | 0.4759 | 0.4267 |
| 13 | 0.5159 | 0.4147 | 0.4653 |
| 17 | 0.2674 | 0.2666 | 0.267 |
| 20 | 0.6602 | 0.5599 | 0.61005 |
| 24 | 0.5257 | 0.4643 | 0.495 |
| 27 | 0.4853 | 0.4898 | 0.48755 |
| 30 | 0.551 | 0.5524 | 0.5517 |
| 34 | 0.8034 | 0.8100 | 0.8067 |
| 40 | 0.6321 | 0.578 | 0.60505 |
| 44 | 0.8162 | 0.7604 | 0.7883 |
| 50 | 0.8762 | 0.9373 | 0.90675 |

| | | | |
|---|---|---|---|
| 53 | 0.7181 | 0.8785 | 0.7983 |
| 60 | 0.5223 | 0.4477 | 0.485 |
| 70 | 1.2566 | 1.2534 | 1.255 |
| 80 | 1.2967 | 1.3951 | 1.3459 |
| 90 | 1.5204 | 1.473 | 1.4967 |
| 100 | 1.6111 | 1.569 | 1.59005 |
| 110 | 1.5197 | 1.3369 | 1.4283 |

## *Optical Density at 560 nm*

**Reactors: R1-R5; Seed microalgae: BGNDRF; Nutrient: SADS**

| Day | R1-1 | R1-2 | R1 (average) | R2-1 | R2-2 | R2 (average) |
|---|---|---|---|---|---|---|
| 0 | 0.22 | 0.22 | 0.22 | 0.23 | 0.23 | 0.23 |
| 3 | 0.12 | 0.1 | 0.11 | 0.087 | 0.093 | 0.09 |
| 6 | 0.106 | 0.104 | 0.105 | 0.063 | 0.057 | 0.06 |
| 10 | 0.115 | 0.105 | 0.11 | 0.078 | 0.082 | 0.08 |
| 13 | 0.33 | 0.27 | 0.3 | 0.21 | 0.23 | 0.22 |
| 17 | 0.359 | 0.351 | 0.355 | 0.176 | 0.174 | 0.175 |
| 20 | 0.4855 | 0.4955 | 0.4905 | 0.227 | 0.223 | 0.225 |
| 24 | 1.11 | 1.07 | 1.09 | 0.31 | 0.95 | 0.63 |
| 27 | 1.57 | 1.18 | 1.375 | 1.07 | 1.03 | 1.05 |
| 30 | 2.01 | 2.07 | 2.04 | 1.072 | 1.678 | 1.375 |
| 34 | 2.248 | 2.242 | 2.245 | 1.52 | 1.84 | 1.68 |
| 37 | 2.347 | 3.075 | 2.711 | 1.59 | 2.94 | 2.265 |
| 40 | 2.89 | 2.87 | 2.88 | 2.22 | 2.2 | 2.21 |
| 44 | 3.723 | 3.727 | 3.725 | 2.46 | 2.88 | 2.67 |
| 47 | 3.75 | 3.75 | 3.75 | 2.44 | 2.42 | 2.43 |
| 48 | 4.306 | 3.204 | 3.755 | 3.132 | 3.138 | 3.135 |
| 50 | 4.367 | 4.363 | 4.365 | 2.482 | 3.248 | 2.865 |
| 55 | 4.15 | 4.07 | 4.11 | 3.37 | 3.77 | 3.57 |
| 60 | 4.45 | 4.49 | 4.47 | 3.832 | 3.789 | 3.8105 |
| 70 | 4.0273 | 4.8258 | 4.42655 | 3.64 | 3.641 | 3.6405 |
| 80 | 4.702 | 4.7 | 4.701 | 3.8532 | 3.8538 | 3.8535 |
| 100 | 3.3 | 3.3 | 3.3 | 1.829 | 1.825 | 1.827 |
| 110 | 3.65 | 4.49 | 4.07 | 1.9752 | 2.2998 | 2.1375 |

| Day | R3-1 | R3-2 | R3 (average) | R4-1 | R4-2 | R4 (average) |
|---|---|---|---|---|---|---|
| 0 | 0.29 | 0.29 | 0.29 | 0.3 | 0.3 | 0.3 |
| 3 | 0.093 | 0.097 | 0.095 | 0.125 | 0.115 | 0.12 |
| 6 | 0.124 | 0.126 | 0.125 | 0.178 | 0.172 | 0.175 |
| 10 | 0.139 | 0.131 | 0.135 | 0.15 | 0.15 | 0.15 |
| 13 | 0.196 | 0.415 | 0.3055 | 0.25 | 0.31 | 0.28 |
| 17 | 0.227 | 0.223 | 0.225 | 0.18 | 0.24 | 0.21 |
| 20 | 0.236 | 0.234 | 0.235 | 0.238 | 0.222 | 0.23 |
| 24 | 0.152 | 0.528 | 0.34 | 0.257 | 0.253 | 0.255 |
| 27 | 0.37 | 0.41 | 0.39 | 0.04 | 0.46 | 0.25 |
| 30 | 0.521 | 0.523 | 0.522 | 0.257 | 0.253 | 0.255 |
| 34 | 0.767 | 0.893 | 0.83 | 0.315 | 0.325 | 0.32 |
| 37 | 1 | 1.02 | 1.01 | 0.192 | 0.51 | 0.351 |
| 40 | 0.82 | 1.62 | 1.22 | 0.176 | 0.624 | 0.4 |
| 44 | 1.82 | 2.6 | 2.21 | 0.24 | 1.38 | 0.81 |
| 47 | 2.15 | 2.11 | 2.13 | 1.057 | 1.053 | 1.055 |
| 48 | 1.58 | 2.68 | 2.13 | 1.089 | 1.081 | 1.085 |
| 50 | 2.21 | 2.83 | 2.52 | 0.52 | 1.62 | 1.07 |
| 55 | 2.66 | 3.07 | 2.865 | 1.181 | 1.579 | 1.38 |
| 60 | 2.82 | 3.434 | 3.127 | 1.2 | 2.3015 | 1.75075 |
| 70 | 3.332 | 3.34 | 3.336 | 1.534 | 2.034 | 1.784 |
| 80 | 3.688 | 4.238 | 3.963 | 1.458 | 2.42 | 1.939 |
| 100 | 3.213 | 3.816 | 3.5145 | 1.698 | 1.998 | 1.848 |
| 110 | 3.251 | 3.655 | 3.453 | 1.862 | 2.18 | 2.021 |

| Day | R5-1 | R5-2 | R5 (average) |
|---|---|---|---|
| 0 | 0.315 | 0.315 | 0.315 |

| | | | |
|---|---|---|---|
| 3 | 0.143 | 0.137 | 0.14 |
| 6 | 0.186 | 0.174 | 0.18 |
| 10 | 0.191 | 0.189 | 0.19 |
| 13 | 0.221 | 0.229 | 0.225 |
| 17 | 0.207 | 0.203 | 0.205 |
| 20 | 0.286 | 0.284 | 0.285 |
| 24 | 0.334 | 0.336 | 0.335 |
| 27 | 0.309 | 0.301 | 0.305 |
| 30 | 0.306 | 0.304 | 0.305 |
| 34 | 0.36 | 0.371 | 0.3655 |
| 37 | 0.32 | 0.3 | 0.31 |
| 40 | 0.26 | 0.24 | 0.25 |
| 44 | 0.15 | 0.53 | 0.34 |
| 47 | 0.41 | 0.41 | 0.41 |
| 48 | 0.4 | 0.42 | 0.41 |
| 50 | 0.192 | 0.63 | 0.411 |
| 55 | 0.57 | 0.57 | 0.57 |
| 60 | 0.242 | 1.766 | 1.004 |
| 70 | 0.497 | 1.794 | 1.1455 |
| 80 | 1.111 | 1.912 | 1.5115 |
| 100 | 1.177 | 2.198 | 1.6875 |
| 110 | 1.77 | 1.779 | 1.7745 |

## *pH*

**Reactors: R1-R5; Seed microalgae: BGNDRF**

| Day | R1-1 | R1-2 | R1 (average) | R2-1 | R2-2 | R2 (average) |
|---|---|---|---|---|---|---|
| 0 | 8.46 | 8.46 | 8.46 | 8.33 | 8.33 | 8.33 |
| 3 | 8.82 | 8.84 | 8.83 | 8.82 | 8.8 | 8.81 |
| 6 | 8.81 | 8.83 | 8.82 | 8.9 | 8.9 | 8.9 |
| 10 | 8.81 | 8.83 | 8.82 | 8.89 | 8.87 | 8.88 |
| 11 | 8.8 | 8.78 | 8.79 | 8.85 | 8.84 | 8.845 |
| 13 | 9.01 | 9.03 | 9.02 | 9.04 | 9 | 9.02 |
| 17 | 8.7 | 8.68 | 8.69 | 8.7 | 8.72 | 8.71 |
| 20 | 8.7 | 8.66 | 8.68 | 8.71 | 8.75 | 8.73 |
| 24 | 8.34 | 8.28 | 8.31 | 8.39 | 8.35 | 8.37 |
| 27 | 8.88 | 8.88 | 8.88 | 8.66 | 8.64 | 8.65 |
| 30 | 8.81 | 8.83 | 8.82 | 8.89 | 8.87 | 8.88 |
| 35 | 8.4 | 8.4 | 8.4 | 8.6 | 8.62 | 8.61 |
| 37 | 8.3 | 8.32 | 8.31 | 8.47 | 8.45 | 8.46 |
| 40 | 8.31 | 8.33 | 8.32 | 8.47 | 8.47 | 8.47 |
| 44 | 9.11 | 9.19 | 9.15 | 9.09 | 9.13 | 9.11 |
| 48 | 8.43 | 8.41 | 8.42 | 8.56 | 8.54 | 8.55 |
| 50 | 8.23 | 8.19 | 8.21 | 8.32 | 8.34 | 8.33 |
| 55 | 8.51 | 8.55 | 8.53 | 8.68 | 8.66 | 8.67 |
| 60 | 8.23 | 8.21 | 8.22 | 8.94 | 8.92 | 8.93 |
| 70 | 8.58 | 8.54 | 8.56 | 8.49 | 8.45 | 8.47 |
| 80 | 8.12 | 8.1 | 8.11 | 7.9 | 7.92 | 7.91 |
| 90 | 8.24 | 8.26 | 8.25 | 8.46 | 8.44 | 8.45 |
| 100 | 8.5 | 8.54 | 8.52 | 8.66 | 8.64 | 8.65 |
| 110 | 8.37 | 8.33 | 8.35 | 8.4 | 8.38 | 8.39 |

| Day | R3-1 | R3-2 | R3 (average) | R4-1 | R4-2 | R4 (average) |
|---|---|---|---|---|---|---|
| 0 | 8.4 | 8.4 | 8.4 | 8.37 | 8.37 | 8.37 |
| 3 | 8.83 | 8.93 | 8.88 | 8.75 | 8.77 | 8.76 |
| 6 | 8.8 | 8.78 | 8.79 | 8.83 | 8.79 | 8.81 |
| 10 | 8.85 | 8.81 | 8.83 | 8.85 | 8.87 | 8.86 |
| 11 | 8.81 | 8.85 | 8.83 | 8.66 | 8.64 | 8.65 |
| 13 | 9.01 | 9.01 | 9.01 | 8.97 | 8.93 | 8.95 |
| 17 | 8.66 | 8.68 | 8.67 | 8.66 | 8.64 | 8.65 |
| 20 | 8.66 | 8.64 | 8.65 | 8.7 | 8.68 | 8.69 |
| 24 | 8.32 | 8.3 | 8.31 | 8.35 | 8.33 | 8.34 |
| 27 | 8.62 | 8.64 | 8.63 | 8.56 | 8.58 | 8.57 |
| 30 | 8.76 | 8.78 | 8.77 | 8.61 | 8.65 | 8.63 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 35 | 8.65 | 8.67 | 8.66 | 8.62 | 8.63 | 8.625 |
| 37 | 8.6 | 8.62 | 8.61 | 8.59 | 8.57 | 8.58 |
| 40 | 8.52 | 8.52 | 8.52 | 8.63 | 8.65 | 8.64 |
| 44 | 8.91 | 8.89 | 8.9 | 8.83 | 8.85 | 8.84 |
| 48 | 8.61 | 8.61 | 8.61 | 8.63 | 8.59 | 8.61 |
| 50 | 8.47 | 8.43 | 8.45 | 8.48 | 8.44 | 8.46 |
| 55 | 8.7 | 8.7 | 8.7 | 8.73 | 8.71 | 8.72 |
| 60 | 8.38 | 8.36 | 8.37 | 8.46 | 8.44 | 8.45 |
| 70 | 8.47 | 8.45 | 8.46 | 8.45 | 8.45 | 8.45 |
| 80 | 8.1 | 8.1 | 8.1 | 8.16 | 8.04 | 8.1 |
| 90 | 8.54 | 8.5 | 8.52 | 8.9 | 9.04 | 8.97 |
| 100 | 8.71 | 8.75 | 8.73 | 8.71 | 8.79 | 8.75 |
| 110 | 8.21 | 8.23 | 8.22 | 8.7 | 8.66 | 8.68 |

| Day | R5-1 | R5-2 | R5 (average) |
|---|---|---|---|
| 0 | 8.48 | 8.48 | 8.48 |
| 3 | 8.71 | 8.71 | 8.71 |
| 6 | 8.84 | 8.78 | 8.81 |
| 10 | 8.79 | 8.77 | 8.78 |
| 11 | 8.69 | 8.67 | 8.68 |
| 13 | 8.91 | 8.93 | 8.92 |
| 17 | 8.65 | 8.55 | 8.6 |
| 20 | 8.68 | 8.66 | 8.67 |
| 24 | 8.3 | 8.34 | 8.32 |
| 27 | 8.52 | 8.54 | 8.53 |
| 30 | 8.6 | 8.62 | 8.61 |
| 35 | 8.52 | 8.54 | 8.53 |
| 37 | 8.46 | 8.44 | 8.45 |
| 40 | 8.55 | 8.46 | 8.505 |
| 44 | 8.63 | 8.65 | 8.64 |
| 48 | 8.62 | 8.6 | 8.61 |
| 50 | 8.49 | 8.45 | 8.47 |
| 55 | 8.75 | 8.73 | 8.74 |
| 60 | 8.5 | 8.5 | 8.5 |
| 70 | 8.83 | 8.85 | 8.84 |
| 80 | 9.35 | 8.59 | 8.97 |
| 90 | 8.83 | 8.96 | 8.895 |
| 100 | 8.69 | 8.63 | 8.66 |
| 110 | 8.67 | 8.63 | 8.65 |

## *Temperature ($^{o}$F)*

**Reactors: R1-R5; Seed microalgae: BGNDRF**

| Day | R1-1 | R1-2 | R1 (average) | R2-1 | R2-2 | R2 (average) |
|---|---|---|---|---|---|---|
| 0 | 75 | 75 | 75 | 74.6 | 74.6 | 74.6 |
| 3 | 79.5 | 78.7 | 79.1 | 79.1 | 79.1 | 79.1 |
| 6 | 77.1 | 77.1 | 77.1 | 78.4 | 78.2 | 78.3 |
| 10 | 76.4 | 75.9 | 76.15 | 76.1 | 76.1 | 76.1 |
| 13 | 78.8 | 77.9 | 78.35 | 78.3 | 77.9 | 78.1 |
| 17 | 76.6 | 76.6 | 76.6 | 76.8 | 77 | 76.9 |
| 20 | 79.3 | 78.9 | 79.1 | 78.2 | 79.6 | 78.9 |
| 24 | 76.1 | 76.7 | 76.4 | 76.1 | 76.1 | 76.1 |
| 27 | 86 | 86 | 86 | 85.7 | 85.9 | 85.8 |
| 30 | 76.9 | 75.9 | 76.4 | 76.8 | 76 | 76.4 |
| 33 | 78.3 | 78.9 | 78.6 | 78.7 | 78.7 | 78.7 |
| 37 | 75.9 | 75.5 | 75.7 | 75.8 | 76 | 75.9 |
| 40 | 76.3 | 76.1 | 76.2 | 76.8 | 76 | 76.4 |
| 48 | 81.9 | 80.08 | 81.0 | 80.7 | 79.9 | 80.3 |
| 50 | 72.8 | 72.4 | 72.6 | 74.4 | 74.4 | 74.4 |
| 55 | 76.4 | 75.8 | 76.1 | 77.2 | 77.4 | 77.3 |
| 60 | 76.8 | 76.0 | 76.4 | 76.7 | 76.5 | 76.6 |
| 70 | 76.1 | 76.1 | 76.1 | 77.7 | 78.1 | 77.9 |
| 80 | 77.9 | 77.5 | 77.7 | 77.2 | 78.1 | 77.7 |
| 100 | 76.9 | 76.7 | 76.8 | 76.9 | 76.7 | 76.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 110 | 77.6 | 77.8 | 77.7 | 78.9 | 78.8 | 78.9 |

| Day | R3-1 | R3-2 | R3 (average) | R4-1 | R4-2 | R4 (average) |
|---|---|---|---|---|---|---|
| 0 | 75.3 | 75.3 | 75.3 | 75.9 | 75.9 | 75.9 |
| 3 | 79.3 | 78.9 | 79.1 | 79.5 | 78.7 | 79.1 |
| 6 | 77.1 | 77.3 | 77.2 | 79.2 | 79.2 | 79.2 |
| 10 | 75.6 | 75.4 | 75.5 | 75.7 | 75.3 | 75.5 |
| 13 | 79.5 | 79.3 | 79.4 | 80.3 | 79.9 | 80.1 |
| 17 | 76.9 | 76.9 | 76.9 | 77.3 | 77.3 | 77.3 |
| 20 | 79.4 | 80 | 79.7 | 79.8 | 78.8 | 79.3 |
| 24 | 77.1 | 77.1 | 77.1 | 77.5 | 76.7 | 77.1 |
| 27 | 82.4 | 83.4 | 82.9 | 84.3 | 84.3 | 84.3 |
| 30 | 75.7 | 74.9 | 75.3 | 76.5 | 75.9 | 76.2 |
| 33 | 79.1 | 79.5 | 79.3 | 78.8 | 78.2 | 78.5 |
| 37 | 76.9 | 75.9 | 76.4 | 76.1 | 76.1 | 76.1 |
| 40 | 76.4 | 77.2 | 76.8 | 77.1 | 77.5 | 77.3 |
| 48 | 82.1 | 83.1 | 82.6 | 82.9 | 82.3 | 82.6 |
| 50 | 76.1 | 76.1 | 76.1 | 75.7 | 75.3 | 75.5 |
| 55 | 76.8 | 75.4 | 76.1 | 78.4 | 77.8 | 78.1 |
| 60 | 77.1 | 77.1 | 77.1 | 77.2 | 77.4 | 77.3 |
| 70 | 77.8 | 77.2 | 77.5 | 77.2 | 77.2 | 77.2 |
| 80 | 76.4 | 76.8 | 76.6 | 77.6 | 76.4 | 77 |
| 100 | 76.1 | 76.1 | 76.1 | 76.9 | 75.9 | 76.4 |
| 110 | 79.6 | 79.0 | 79.3 | 78.9 | 78.7 | 78.8 |

| Day | R5-1 | R5-2 | R5 (average) |
|---|---|---|---|
| 0 | 74.8 | 74.8 | 74.8 |
| 3 | 80.6 | 80.6 | 80.6 |
| 6 | 79.5 | 78.9 | 79.2 |
| 10 | 76.1 | 76.1 | 76.1 |
| 13 | 78.9 | 79.5 | 79.2 |
| 17 | 76.8 | 76.4 | 76.6 |
| 20 | 80 | 80 | 80 |
| 24 | 77.7 | 76.5 | 77.1 |
| 27 | 84.7 | 83.3 | 84 |
| 30 | 76.8 | 76.4 | 76.6 |
| 33 | 80.7 | 79.7 | 80.2 |
| 37 | 76.8 | 75.6 | 76.2 |
| 40 | 76.9 | 75.9 | 76.4 |
| 48 | 82.7 | 81.8 | 82.25 |
| 50 | 75.5 | 75.5 | 75.5 |
| 55 | 77.6 | 76.6 | 77.1 |
| 60 | 77.5 | 76.7 | 77.1 |
| 70 | 77.8 | 76.6 | 77.2 |
| 80 | 77.4 | 78.4 | 77.9 |
| 100 | 77.4 | 76.6 | 77 |
| 110 | 78.7 | 78.5 | 78.6 |

## *Conductivity (µS/cm)*

**Reactors: R1-R5; Seed microalgae: BGNDRF**

| Day | R1-1 | R1-2 | R1 (average) | R2-1 | R2-2 | R2 (average) |
|---|---|---|---|---|---|---|
| 0 | 21,400 | 21,400 | 21,400 | 27,100 | 27,100 | 27,100 |
| 3 | 21,700 | 21,900 | 21,800 | 27,500 | 27,700 | 27,600 |
| 6 | 22,000 | 22,200 | 22,100 | 28,400 | 27,600 | 28,000 |
| 10 | 22,500 | 22,700 | 22,600 | 30,000 | 28,000 | 29,000 |
| 13 | 21,100 | 20,900 | 21,000 | 26,800 | 27,000 | 26,900 |
| 17 | 21,200 | 21,400 | 21,300 | 27,300 | 27,300 | 27,300 |
| 20 | 21,900 | 21,700 | 21,800 | 28,100 | 27,900 | 28,000 |
| 23 | 20,500 | 20,300 | 20,400 | 25,900 | 25,700 | 25,800 |
| 27 | 21,000 | 20,800 | 20,900 | 26,200 | 26,400 | 26,300 |
| 30 | 21,100 | 21,300 | 21,200 | 26,700 | 26,900 | 26,800 |
| 33 | 19,940 | 19,980 | 19,960 | 25,200 | 25,400 | 25,300 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 38 | 20,000 | 20,200 | 20,100 | 25,400 | 25,600 | 25,500 |
| 40 | 20,700 | 21,100 | 20,900 | 26,100 | 26,500 | 26,300 |
| 44 | 19,430 | 19,630 | 19,530 | 24,200 | 24,400 | 24,300 |
| 48 | 19,830 | 20,090 | 19,960 | 25,100 | 25,300 | 25,200 |
| 50 | 20,200 | 20,400 | 20,300 | 25,500 | 25,900 | 25,700 |
| 55 | 19,200 | 19,140 | 19,170 | 23,600 | 23,400 | 23,500 |
| 60 | 20,000 | 20,200 | 20,100 | 24,200 | 24,400 | 24,300 |
| 70 | 18,300 | 18,060 | 18,180 | 21,300 | 21,500 | 21,400 |
| 80 | 19,410 | 19,210 | 19,310 | 25,600 | 25,600 | 25,600 |
| 90 | 19,860 | 20,020 | 19,940 | 26,100 | 26,300 | 26,200 |
| 100 | 20,700 | 20,700 | 20,700 | 23,400 | 23,200 | 23,300 |
| 110 | 20,300 | 20,500 | 20,400 | 23,200 | 23,400 | 23,300 |

| Day | R3-1 | R3-2 | R3 (average) | R4-1 | R4-2 | R4 (average) |
|---|---|---|---|---|---|---|
| 0 | 35,600 | 35,600 | 35,600 | 43,800 | 43,800 | 43,800 |
| 3 | 36,200 | 36,000 | 36,100 | 44,600 | 44,600 | 44,600 |
| 6 | 36,500 | 37,100 | 36,800 | 45,000 | 45,400 | 45,200 |
| 10 | 37,600 | 37,800 | 37,700 | 46,100 | 46,300 | 46,200 |
| 13 | 34,500 | 34,300 | 34,400 | 42,200 | 42,400 | 42,300 |
| 17 | 35,100 | 34,900 | 35,000 | 43,100 | 42,900 | 43,000 |
| 20 | 36,400 | 36,600 | 36,500 | 44,000 | 44,000 | 44,000 |
| 23 | 33,100 | 33,500 | 33,300 | 40,600 | 40,400 | 40,500 |
| 27 | 34,000 | 34,000 | 34,000 | 41,200 | 41,400 | 41,300 |
| 30 | 34,300 | 34,500 | 34,400 | 41,800 | 41,600 | 41,700 |
| 33 | 31,800 | 32,000 | 31,900 | 39,200 | 38,800 | 39,000 |
| 38 | 32,100 | 32,500 | 32,300 | 39,100 | 39,300 | 39,200 |
| 40 | 33,200 | 33,400 | 33,300 | 40,100 | 39,900 | 40,000 |
| 44 | 30,900 | 30,700 | 30,800 | 37,800 | 38,000 | 37,900 |
| 48 | 31,400 | 31,800 | 31,600 | 39,100 | 39,300 | 39,200 |
| 50 | 32,000 | 32,000 | 32,000 | 37,400 | 42,000 | 39,700 |
| 55 | 31,100 | 30,900 | 31,000 | 36,600 | 38,800 | 37,700 |
| 60 | 32,900 | 32,700 | 32,800 | 34,100 | 38,100 | 36,100 |
| 70 | 30,150 | 30,250 | 30,200 | 33,300 | 34,300 | 33,800 |
| 80 | 33,200 | 32,800 | 33,000 | 37,200 | 37,400 | 37,300 |
| 90 | 32,700 | 32,700 | 32,700 | 31,000 | 33,800 | 32,400 |
| 100 | 31,200 | 30,800 | 31,000 | 26,000 | 27,600 | 26,800 |
| 110 | 29,000 | 29,200 | 29,100 | 26,200 | 30,200 | 28,200 |

| Day | R5-1 | R5-2 | R5 (average) |
|---|---|---|---|
| 0 | 52,600 | 52,600 | 52,600 |
| 3 | 53,300 | 53,700 | 53,500 |
| 6 | 53,400 | 53,600 | 53,500 |
| 10 | 55,100 | 55,300 | 55,200 |
| 13 | 50,600 | 51,000 | 50,800 |
| 17 | 51,500 | 51,700 | 51,600 |
| 20 | 52,800 | 53,000 | 52,900 |
| 23 | 48,800 | 49,000 | 48,900 |
| 27 | 49,400 | 49,600 | 49,500 |
| 30 | 50,600 | 50,800 | 50,700 |
| 33 | 47,400 | 47,200 | 47,300 |
| 38 | 47,600 | 47,800 | 47,700 |
| 40 | 48,900 | 48,700 | 48,800 |
| 44 | 46,100 | 46,100 | 46,100 |
| 48 | 45,600 | 45,800 | 45,700 |
| 50 | 47,400 | 47,600 | 47,500 |
| 55 | 44,800 | 44,600 | 44,700 |
| 60 | 46,200 | 46,400 | 46,300 |
| 70 | 41,650 | 41,350 | 41,500 |
| 80 | 45,000 | 45,200 | 45,100 |
| 90 | 44,600 | 44,600 | 44,600 |
| 100 | 44,500 | 44,300 | 44,400 |
| 110 | 43,400 | 43,600 | 43,500 |

## *Air flow rate (mL/min)*

**Reactors: R1-R5; Seed microalgae: BGNDRF**

| Day | R1-1 | R1-2 | R1 (average) | R2-1 | R2-2 | R2 (average) |
|---|---|---|---|---|---|---|
| 0 | 920 | 920 | 920 | 920 | 920 | 920 |
| 3 | 920 | 920 | 920 | 920 | 920 | 920 |
| 6 | 920 | 920 | 920 | 920 | 920 | 920 |
| 10 | 920 | 920 | 920 | 920 | 920 | 920 |
| 11 | 920 | 920 | 920 | 920 | 920 | 920 |
| 13 | 920 | 920 | 920 | 920 | 920 | 920 |
| 17 | 920 | 920 | 920 | 920 | 920 | 920 |
| 20 | 920 | 920 | 920 | 920 | 920 | 920 |
| 23 | 920 | 920 | 920 | 920 | 920 | 920 |
| 27 | 920 | 920 | 920 | 920 | 920 | 920 |
| 30 | 920 | 920 | 920 | 920 | 920 | 920 |
| 35 | 920 | 920 | 920 | 920 | 920 | 920 |
| 37 | 920 | 920 | 920 | 920 | 920 | 920 |
| 40 | 920 | 920 | 920 | 920 | 920 | 920 |
| 44 | 920 | 920 | 920 | 920 | 920 | 920 |
| 48 | 920 | 920 | 920 | 920 | 920 | 920 |
| 50 | 920 | 920 | 920 | 920 | 920 | 920 |
| 55 | 920 | 920 | 920 | 920 | 920 | 920 |
| 60 | 920 | 920 | 920 | 920 | 920 | 920 |
| 70 | 920 | 920 | 920 | 920 | 920 | 920 |
| 70 | 920 | 920 | 920 | 920 | 920 | 920 |
| 80 | 920 | 920 | 920 | 920 | 920 | 920 |
| 100 | 920 | 920 | 920 | 920 | 920 | 920 |
| 110 | 920 | 920 | 920 | 920 | 920 | 920 |

| Day | R3-1 | R3-2 | R3 (average) | R4-1 | R4-2 | R4 (average) |
|---|---|---|---|---|---|---|
| 0 | 920 | 920 | 920 | 920 | 920 | 920 |
| 3 | 920 | 920 | 920 | 920 | 920 | 920 |
| 6 | 920 | 920 | 920 | 920 | 920 | 920 |
| 10 | 920 | 920 | 920 | 920 | 920 | 920 |
| 11 | 920 | 920 | 920 | 920 | 920 | 920 |
| 13 | 920 | 920 | 920 | 920 | 920 | 920 |
| 17 | 920 | 920 | 920 | 920 | 920 | 920 |
| 20 | 920 | 920 | 920 | 920 | 920 | 920 |
| 23 | 920 | 920 | 920 | 920 | 920 | 920 |
| 27 | 920 | 920 | 920 | 920 | 920 | 920 |
| 30 | 920 | 920 | 920 | 920 | 920 | 920 |
| 35 | 920 | 920 | 920 | 920 | 920 | 920 |
| 37 | 920 | 920 | 920 | 920 | 920 | 920 |
| 40 | 920 | 920 | 920 | 920 | 920 | 920 |
| 44 | 920 | 920 | 920 | 920 | 920 | 920 |
| 48 | 920 | 920 | 920 | 920 | 920 | 920 |
| 50 | 920 | 920 | 920 | 920 | 920 | 920 |
| 55 | 920 | 920 | 920 | 920 | 920 | 920 |
| 60 | 920 | 920 | 920 | 920 | 920 | 920 |
| 70 | 920 | 920 | 920 | 920 | 920 | 920 |
| 70 | 920 | 920 | 920 | 920 | 920 | 920 |
| 80 | 920 | 920 | 920 | 920 | 920 | 920 |
| 100 | 920 | 920 | 920 | 920 | 920 | 920 |
| 110 | 920 | 920 | 920 | 920 | 920 | 920 |

| Day | R5-1 | R5-2 | R5 (average) |
|---|---|---|---|
| 0 | 920 | 920 | 920 |
| 3 | 920 | 920 | 920 |
| 6 | 920 | 920 | 920 |
| 10 | 920 | 920 | 920 |
| 11 | 920 | 920 | 920 |
| 13 | 920 | 920 | 920 |
| 17 | 920 | 920 | 920 |
| 20 | 920 | 920 | 920 |

| | | | |
|---|---|---|---|
| 23 | 920 | 920 | 920 |
| 27 | 920 | 920 | 920 |
| 30 | 920 | 920 | 920 |
| 35 | 920 | 920 | 920 |
| 37 | 920 | 920 | 920 |
| 40 | 920 | 920 | 920 |
| 44 | 920 | 920 | 920 |
| 48 | 920 | 920 | 920 |
| 50 | 920 | 920 | 920 |
| 55 | 920 | 920 | 920 |
| 60 | 920 | 920 | 920 |
| 70 | 920 | 920 | 920 |
| 70 | 920 | 920 | 920 |
| 80 | 920 | 920 | 920 |
| 100 | 920 | 920 | 920 |
| 110 | 920 | 920 | 920 |