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A POTENTIAL APPLICATION OF MICROALGAE IN PRODUCED WATER TREATMENT

BY

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ABSTRACT

Produced water, deriving from petroleum industry as a result of oil production, constitutes of high concentration of pollutants, such as dissolved nitrogen, phosphorus, dissolved organic carbon, heavy metals and monocyclic aromatic compound like BTEX (benzene, toluene, ethylbenzene, and xylene). Thus, removal of these pollutants from produced water is essential. Many conventional treatments are currently present, which often used for the produced water treatment. Most of the time, these treatments processes are costly and these processes increase the overall cost of oil production. As an alternative solution, microscopic microalgae can be used to remove these pollutants from the produced water effluents. These microalgae can bio-remediate produced water effluents while utilizing some of these pollutants as sources of nutrients. The current study examines pollutant removal efficiency of different microalgae species from produced water effluents. After initial screening, five species of microalgae strains *Monoraphidium, Chlorella, Neochloris, Scenedesmus, Dictyosphaerium* were chosen for the study. *Chlorella* and *Dictyosphaerium* species show a significant amount of biomass generation within all different concentration of produced water. Although the biomass yield of *Neochloris* strain was low, it was able to remove a higher amount of organic carbon than other microalgae strains. Although biomass generation was significantly varied within the microalgae strains, nitrogen removal efficiency by all the strains were similar. Also similar results were also found for most of the BTEX component. Only in the case of phosphorus and various metals, removal efficiency was better by *Dictyosphaerium* microalgae species. However, the variation of produced water concentration has no significant effect on the pollutants removal efficiency of microalgae strains. Thus, the results indicate that microalgae strains can grow in produced water effluents-deriving from petroleum industries and remove pollutants.

Key words: Microalgae, produced water, oil and gas, nutrients, treatment.

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CHAPTER 1: INTRODUCTION

Oil exploration is one of the most significant activities of petroleum industries around the world (Oliveira, 2005). In spite of its significance, a large amount of wastewater is generated during the exploration process. These type of petroleum wastewater is commonly known as produced water (Azetsu-Scott, 2007). These produced water pollutants can cause an adverse effect on the surrounding environment. Thus, to remediate produced water pollutants, many technologies have been already established. Most of these treatment technologies involve energy inputs to remove contaminants from the produced water (Arthur, 2005). Physical and chemical treatment processes are commonly used to remove contaminants from the produced water. Both of these physical and chemical treatment processes ultimately raise the cost of final petroleum products (Fakhru'l-Razi, 2009). The biological treatment process can be utilized to reduce the total cost of the whole treatment process. Where this biological process can use microalgae to treat wastewater effluents. Among many current treatment solutions, biological treatments can be utilized as a cost-effective way of treating produced water (Fakhru'l-Razi, 2009). Furthermore, studies indicated that microorganisms can be optimized to enhance their bioremediation capability (Bose, 2011). One of the unique ways of biological treatment is to use microalgae species because of their ability to utilize the pollutants within the produced water, as sources of nutrient for their growth. Species like *Cyanobium* can decrease the concentration of phosphate within the produced water (Mendes, 2010).

In general, produced water effluents deriving from oil well contains a various concentration of hydrocarbons, phenols, BTEX (benzene, toluene, ethylbenzene, and xylene), heavy metals and many inorganic salts (Campos, 2002). These complex constituents of produced water exhibit toxicity to our surrounding environment. Environmental pollution threat increases with the increasing amount of produced water generation. To mitigate the growing environmental pollution, bioremediation processes are used to remove and minimize the toxicity of produce water pollutants (Kao & Wang, 2000). Bioremediation processes include microorganisms like bacteria, fungi, and microalgae species. Microalgae species are photosynthetic organisms and hence can utilize carbon dioxide to produce biomass. This biomass can be used as a source of animal feed, bio-fertilizer and alternative feedstock for biofuel (Das, 2015; Silva, 2007; Illman,2000).

The current aim of the study is to find out possible approaches of using biological treatment, where local microalgae strains will be chosen for the treatment process and to identify optimum pollutants removal efficiency by local algae strains. Thus, we hypothesize that the locally isolated microalgae strains can be used for the produced water treatment. During this study several locally isolated microalgae strains would be chosen to assess their removal efficiency for various metals and organic pollutants from the produced water.

Thesis objectives:

a) Characterize the physical and chemical characteristics of the collected produced water samples:the parameters are pH, salinity, total organic carbon (TOC), total nitrogen, total phosphorus, heavy metals, and BTEX contents.
b) Screening algae strains for produced water treatment
c) Examine the growth rate and biomass yield of the selected strains with normal BG-11 media
d) Examine algae strains with different concentration of produced water to identify the suitable microalgae strain that can achieve maximum pollutants removal (heavy metals, total organic carbon total nitrogen, and phosphorus) from the collected produced water.
e) Perform the required statistical analysis for all data.

CHAPTER 2: LITERATURE REVIEWS

Produced water:

Oil and gas industry produces a significant amount of industrial wastewater as a byproduct of hydrocarbon extraction (Costa, 2012). These types of industrial wastewater develop along with the hydrocarbon extraction phase. Water is present naturally within the wells that sit right below the oil reservoir (Ebenezer, 2012). The oil extraction process reduces hydraulic pressure within the wells, which later requires additional pressure buildup to continue the overall hydrocarbon extraction process. Additional water is pumped into production wells to maintain the hydraulic pressure of the wells and to obtain the optimum level of oil and gas extraction from the wells. At the later stage of hydrocarbon exploration, both sources of water reach the production well, and is known as produced water (Zhang, 2010). This type of petroleum wastewater mainly comprised of mixed hydrocarbons and chemical additives (Strømgren, 1995). Usually, the composition of produced water depends on many different factors. Previous studies indicated that most of the factors derive from the hydrocarbon type, geological process and the age of the wells (Rodney, 2003). The quality of produced water worsens due to the presence of different components, such as microbe, dissolved organic and inorganics within the generated produced water (Thomas, 2009).

Produced Water constitutes:

Overall the produced water consists of dissolved organic and inorganics, dissolved gases and suspended particles (Forrestal, 2015). These pollutants can be classified by various anions and cations. Among the cations sodium (Na^{+}), calcium (Ca^{2+}), Magnesium (Mg^{2+}), Iron (Fe^{2+}), Barium (Ba^{2+}) Potassium (K^{+}), Strontium (Sr^{+2}), Aluminum (Al^{3+}), Lithium (Li^{+}) and among anions chloride (Cl^{-}). Among these ions, the most prevalent within the produced water are sodium cations (Na^{+}) and chloride anions (Cl^{-}) (Scientific, 2014). Some other common anions are carbonate (CO_3^{2-}), bicarbonate (HCO_3^{-}) and sulfate (SO_4^{2-}). Also calcium carbonate ($CaCO_3$), iron sulfide (FeS_2) and calcium sulfate ($CaSO_4$) may precipitate and produce particle flocks and cause higher turbidity of produced water. These precipitate occurs during the changes of pH, while the higher pH reduced the solubility of some of these constitutes (Martinez, 2016). As the produced water derives from the petroleum field, some of natural gas such as methane, ethane, propane, butane, hydrogen sulfide and carbon dioxide are also found (Jackson & Reddy, 2007).

The solubilies of organic and inorganics within the produce water differ based on many chemical and physical factors (Gupta, 2012). In most cases, dissolved organics comprise a complex oil such as BTEX, aliphatic hydrocarbons, phenols, carboxylic acid and some low molecular weight aromatic compounds are found as dissolved oil compounds (Khosravi, 2009). Production chemicals complexes are also found in dissolved forms that include solvents and co-solvents. These production chemicals are

used for inhibition of corrosion, wax deposition, hydrate formation and emulsion breaking to further enhance the separation of water and oil during the production phase (Hansen & Davies, 1994). Furthermore, because of the bacterial activity some forms of gases like oxygen, carbon dioxide and hydrogen sulfide are also present in dissolved form in the produced water (Gevertz, 2000).

Dissolved inorganic minerals can vary depending on the age of the wells. Within the old production wells, inorganic compounds mainly the incorporation of heavy metals, major cations and anions, and also radioactive materials (Fakhru'l-Razi, 2009). Whereas metals usually include the following compounds such as, iron, aluminum, barium, cadmium, copper, chromium, lithium, manganese, lead, strontium, titanium, zinc, arsenic, mercury, silver and beryllium (Fakhru'l-Razi, 2009). Depending on the oil extraction site, the produced water can have a wide range of salinity. The conductivity of the produced water is also affected by the presence of these inorganic minerals (Roach, 1993).

Some of the suspended solids found in produced water are clay, sand, silt, and carbonates. The formation of these suspended solids are generated due to temperature, pressure and chemical changes. Precipitation and corrosion are mainly responsible for suspended solids development (Fakhru'l-Razi, 2009). Suspended small oil droplets like polyaromatic hydrocarbons (PAHs) and heavy alkyl phenols are also found in produced water which have a tendency of scattering on top of the produced water (Veil JA, 2004). In some cases, bacterial agglomeration was also found in the produced water that causes clogging within the production line (Cline, 1998).

In an estimation, it was found that a total of 250 million barrels of produced water are generated per day globally, whereas the daily oil production is only about 80 million BPD; therefore, produced water generation is 3 times more than the oil production (Khatib, & Verbeek, 2003). Furthermore, the amount of the produced water is expected to rise to 95% of oil production as the oil and gas fields become old (Kaur, 2009).

Treatment of produced water:

With a significant increase of produced water generation, it is necessary to treat the produce water that will meet the current standard with a feasible solution. Furthermore, to reach a zero produced water level, this needs an advance treatment solution, where produced water can be reused and recycled in other energy and agriculture sectors. Where wastewater effluents from petroleum industry can be used for biofuel and biofertilizer production (Chisti, 2007). Currently many different methods are applicable which also increase the overall oil production cost. In general, the primary objective of these treatment process is to remove dispersed oil and grease, dissolve oil and production chemicals, suspended solids, dissolved gases, reduce salinity and water hardness, reduce impurities such as heavy metals and reduce toxicity (Arthur, 2005). These treatments are usually done by a physical, chemical and biological process, where the process can work separately or even with a combination (A. Fakhru'l-Razi, 2009).

Physical treatment processes:

One of the processes of removing the pollutants from produced water is adsorption by activated carbon (Naas, 2010). Activated carbon can have a large surface area with variable pore dimensions and different active sites (Bhattacharya, 2006). Studies have shown that various types of activated carbon derived from peach stones and olive stones can remove volatile organic like BTEX from wastewater (Daifullah,2003). In another study, heavy metals like Cr, Cd, Co, Cr, Ni, Cu, Pb were successfully recovered by apricot seed derived activated carbon, at low pH condition (Kobya, 2005).

Although the contaminant removal efficiency of activated carbon can reach up to 70 to 85%, the removal efficiency decreases with the presence of suspended particles within the produced water (Fakhru'l-Razi, 2009). Furthermore, pollutant removal efficiency of activated carbon gets dramatically reduced after few batches of treatment. Thus to regain the pollutant removal efficiency, activated carbon needs to be regenerated (Lu, 2011). During the activated carbon regeneration process, various chemicals like organic solvents, acids, bases and redox agents are used (McGhee, 1991) which can increase the overall cost of the treatment process.

Different filtration media can be used to achieve an optimum removal efficiency of total organic carbon from the produced water (Renou, 2008). Usually, sand, gravel and some seeds can be used in this treatment process. A recent study found that the removal efficiency for iron can reach up to 90% with few pretreatment adjustments of pH and oxygen concentration (Aziz, 2004). This treatment process requires a longer exposure

period, which is considered a negative point of this treatment process (Fakhru'l-Razi, 2009). Furthermore, more efficient filtration like ultrafiltration and reverse osmosis membrane filtration can also be utilized in different water treatment facilities. Although membrane filtration process is an extremely effective treatment process, these processes are not always feasible to use in some cases (Pearce, 2008).

This treatment process uses a conical shaped particle separator technic. Which is usually to remove suspended particulate matter within the produced water. It is one of the pretreatment processes where denser contaminants are separated. Although it does not require any chemical, it generates a large volume of sludge which may need further treatment (Svarovsky, 1992).

Chemical treatment processes:

One of the conventional chemical treatments processes of produced water is precipitation method (Li, 2000). In this process only suspended and colloidal particles are successfully removed with efficiency up to 97% (Liu, 2000). Different types of mixed flocculants and coagulants are used which mainly comprise inorganic metals like iron, magnesium and aluminum (FMA) polymers. These FMA mix polymers were found to be effective in removing contaminants (Zhou, 2000). Other studies also applied flocculants like anionic polymer and ferric chloride ($FeCl_3$) in a ballasted flocculation unit to remove particulate metals, phosphorus and carbonaceous compounds. But these flocculants were less efficient in removing nitrogen and hydrophilic compounds (Gasperi, 2012).

Strong oxidants and catalysts are used for decomposing the organic impurities with in the produced water (Huang, 1993). In general, chlorine, ozone, peroxide and oxygen are used as oxidants to break down multiple pollutants. The only problem with this process is the cost of extensive energy consumptions that requires for oxidants and operation process (Renou, 2008). Furthermore, a final treatment is also required for removing the particulate matter after the oxidation process (Ebenezer, 2012). Recent development in water treatment introduced an effective solution where organic pollutants are quickly oxidized by adding oxidant or mixture of oxidants. This process is known as Advanced oxidation processes (AOP) (Yang, 2016). These process use ozone, iron, and hydrogen peroxide chemical oxidizer. Furthermore, hydroxyl radicals like zinc oxide, titanium dioxide, and iron oxide are also introduced in this treatment process (Muruganandham, 2014).

Biological processes:

Among other conventional treatment approaches, biological treatment is considered as one of the least expensive pollutants removal process (Günther, 2000). Either aerobic or anaerobic condition are maintained in the biological treatment processes. In general, three types of microorganisms are present in the produced water such as algae, fungi, and bacteria with a size of 0.2 to 10 microns (Rodney, 2003). These microorganisms can be utilized to treat the produce water where these organisms use the pollutants as source of nutrients to grow (Lu, 2009). These microorganisms also bio-remediate the toxic substances to less toxic substances such as chlorinated hydrocarbons,

and metals (Perelo, 2010). While working with bacteria and fungi, many different reactors like sequencing batch reactors, and biological aerated filters were used (Fakhru'l-Razi, 2009).

A significant efficiency of removal was achieved while using the single batch reactor to remove aniline, nitrogen and phosphorus from wastewater under anoxic operating conditions (Jiang, 2016). With initial 250 mg/l aniline and 5.5 mg/l dissolved oxygen (DO), this treatment showed the highest efficiency of 95.80 % removal of chemical oxygen demand (COD) 87.13% of total Nitrogen and 0.95% of total Phosphorus removal (Jiang, 2016). Salinity and C/N ratio have a huge effect on wastewater treatment (Mohan, 2016). The total COD removal was quite high (93%) during the whole experiment, but higher salinity caused lower nitrification (Mannina, 2016). Treating nitrite containing waste water depends highly on denitrification and nitrite accumulation (Mohan, 2016). With the increase of C/N ratio, denitrification and nitrite accumulation can increase but with higher salinity it will decrease (Mohan, 2016; Mannina, 2016). Therefore, it is crucial to maintain a certain salinity by proper homogenization of the inlet wastewater with batch reactors. Similarly, C/N ratio plays a great role in removing nitrogen from municipal wastewater in Biological Aerated Filtration (BAF) system (Lin, 2016; Ryu, 2008). Using a different hydraulic retention time, the nitrogen removal efficiency was increased to 95-96% (Ryu, 2008). The denitrification performance under Total COD and Total Kjeldahl Nitrogen (TKN) ratio of 3:6 gave the best result, and the BAF system was proven to be the best technique to remove nitrogen from wastewater (Lin, 2016; Ryu, 2008). When the COD/N ratio was 5,

the removal efficiency reached to 83.7% for COD, 93.1% for ammonium and 84.6% for total nitrogen removal (Lin, 2016). The reduction of COD/N ratio will reduce the performance of the whole system dramatically. Unlike bacterial treatment process, photobioreactors and raceway pond are used in microalgae based treatment process (Wagenenet, 2015; Rogers, 2014). The pollutants removal efficiency from produced water also varies with inoculation species of microorganisms and on the operational systems (Pichtel, 2016).

Fate of produced water:

Discharged produced water within the environment has great impacts on living and non-living resources (Furuholt, 1996). In general, these effects derive from the organic and inorganics toxicity. Although the discharges of treated produced water are allowed with a particular concentration and volume, over time released of produced water might cause chronic toxicity on surrounding ecosystem. In most cases, impacts of chronic toxicity are difficult to measure (Hansen & Davies, 1994).

Produced water pollutants may also comprise of oil droplets and many light aromatic hydrocarbons. Some of these hydrocarbon late degraded by bacterial species. Consequently, such conditions raised biochemical oxygen demands within marine environments (Fakhru'l-Razi, 2009). Some treatment chemicals also increase the partitioning within particles, which might eventually increase the chemicals accumulation within the sediments beneath. In another case, it was suggested that heavy metals from the produced water will not be significant solely because of the dilution factors (Fakhru'l-

Razi, 2009). On the other hand, the increase of heavy metals concentration with times will eventually reach a level of bioaccumulation and bio-magnification within the ambient marine ecosystem (Kumar, 2015).

Microalgae Based Treatment:

Current development in treatment process introduces an Eco-technology approaches, where biological treatment process can reach higher removal rate of pollutants from the produced water (Comninellis, 2008). Thus, these Eco-technology approaches define the use of microalgae-based treatment as a sustainable solution for the treatment process. In general, these microalgae can bio-remediate produced water effluents, where these microalgae able to utilize some of these pollutants as sources of nutrients (Mendes, 2010). A recent study has indicated that microalgae species like *Parachlorella kessler* can utilize BTEX as a sole carbon source (Takáčová, 2015). In another study, the toxicity test using water soluble fraction (WSF) gasoline provides an important foundation for BTEX effect on microalgae growth (Durako, 1993; Zieman, 1984). However, a higher BTEX concentration with a longer period of contact time causes 50% growth inhibition on microalgae cultures (Paixão, 2007). Gasoline components with high BTEX content have lower toxicity than heavier hydrocarbons on microalgae growth (Masten, 1994). Nevertheless, microalgae use nutrients like nitrogen and phosphorus, which are also limiting factors for their growth. On the other hand, produced water comprise of high concentration of nitrogen and phosphorus (Fakhru'l-Razi, 2009). Apart from nitrogen and phosphorus, there are other trace elements which

are essential for the growth of microalgae. Therefore, growing microalgae in the produced water has the potential to be used as efficient treatment process. The overall treatment process will also increase the production of microalgae biomass. Furthermore, cultivated microalgae biomass can also be used as alternative feedstock for energy generation (Chisti, 2007). A list of microalgae strains, that were previously used in wastewater treatment, is given in the Table 2.1.

Table 2.1: Microalgae strains uses for different water treatments from recent studies.

Microalgae uses in water treatment for bioremediation of pollutants		
Strain name	**Type of water**	**References**
Monoraphidium sp.	Produced water	(Mendes, 2011)
Chlorella sp.	Produced municipal wastewater	(Mendes, 2011); (Wang,2010)
Neochloris sp.	Industry effluent (Textile dyeing industry)	(Gopalakrishnan, 2014)
Scenedesmus sp.	Produced water Wastewater	(Johnson, 2015); (Di Caprio, 2015)
Dictyosphaerium sp.	Wastewater	(Zhou, 2014)

Microalgae:

Microalgae are unicellular organisms which utilize light as sources of energy to produce biomass (John, 2011). These microalgae also carry chlorophyll-*a* as a photosynthetic pigment. Microalgae can exist in an environment where adequate sunlight and moisture are present. During the photosynthesis process, microalgae produced

oxygen and consume carbon dioxide. Microalgae can grow as single cell and have no tissue differentiation in a microalgae colony (Barsanti, 2014). Almost one-half of earth oxygen is produced by the various microalgae species present within our aquatic environment (Cardozo, 2007).

A unique characteristic of some microalgae species is the ability to grow not only in phototropich but also in heterotopich condition (Liang, 2009). In the absences of light, microalgae species can use some of its developed carbon sources to survive heterotrophic condition. The evidence can easily found in a mixotrophic growth system (Xu, 2006). Normally the growth rate of microalgae is much faster than the traditional terrestrial crop species.

Chemical composition of microalgae:

Microalgae cell mostly comprise of protein, carbohydrates, and lipids (Demirbas, 2011). Also a smaller fraction of the cell content has nucleic acid and different photosynthetic pigments such as chlorophyll. Where the majority of the microalgae firstly consist of protein content ranging from 40 to 60 percent, secondly carbohydrates ranging from 20 to 30 percent, thirdly with a range of 10 to 20 percent of lipids (Singh, 2011). These chemical composites are usually differing with the changes of environmental conditions. Some recent studies encountered limiting nutrients within growth medium, which also effect on the metabolic system during cell division (Fernandes, 2013). These effect on metabolic system intern increases lipid and carbohydrate contents instead of

overall production of protein content (Gouveia, 2011). Such flexible nature of the composites has been useful in many applications to maximize the outcome of microalgae byproducts (Chisti, 2007). Based on the quality of the biomass it has many different commercial applications such as feed, fertilizer, biofuels, and functional bioactive compounds (Slade & Bauen, 2013). An example of a different chemical composition is shown in Table 2 (Becker, 2007).

Table 2.2: General composition of different algae (% of dry biomass) (Becker, 2007)

Name of microalgae strains	**Protein % in dry biomass**	**Carbohydrates % in dry biomass**	**Lipids % in dry biomass**
Anabaena cylindrica	43–56	25–30	4–7
Aphanizomenon flos-aquae	62	23	3
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	6
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Scenedesmus obliquus	50–56	10–17	12–14
Spirogyra sp.	6–20	33–64	11–21
Arthrospira maxima	60–71	13–16	6–7
Spirulina platensis	46–63	8–14	4–9
Synechococcus sp.	63	15	11

Protein

Among the other chemical composites, protein can reach more than half of the weight of microalgae cell (Spolaore, 2006). The protein content usually differs within species. These protein sources, rich in amino acids, also been used as an animal feed in aquaculture, poultry industry and many other livestock sectors (Hemaiswarya, 2011).

Carbohydrate

Most microalgae have carbohydrate similar to the plant like species, which usually consist of glucose (Chng, 2016). Where a larger amount of starch ranging up to 60 percent from the microalgae cell. Nitrogen, phosphorus and sulfur deficiency usually regulate the overall product of starch within the microalgae cells (Brányiková, 2011).

Lipid

Microalgae cells produce two types of lipids. These lipids can be classified by their polarity such as polar and non-polar lipids (Wang, 2009). Polar lipids mainly consist of fatty acids, glycolipids, and phospholipids, which they produce during the optimum growth condition. Furthermore, polar lipids such as fatty acids are valuable products which can be used for their nutritional properties. Also, longer chain fatty acids (i.e., omega -3 fatty acid) can have additional benefits (Tsai, 2016). On the other hand, due to several stress conditions, microalgae accumulate a higher concentration of non-polar lipids such as sterols, diacylglycerol, and monoacylglycerol within their cells. Previous

studies also found that, non-polar lipids can constituent as high as 80% of the total lipid. (Chisti, 2007).

Microalgae cultivation system

Microalgae species require a particular environmental condition to grow. Apart from nutritional requirement microalgae cultivation requires four main abiotic conditions that include optimum light intensity, appropriate temperature, water alkalinity (pH) and mixing. However, these abiotic conditional requirements may vary from one microalgae species to another. Below a general overview of microalgae growth condition is given:

Open system

Raceway pond is an open algal cultivation system with closed loop recirculation channels which are not very deep. Paddlewheel produces the circulation and mixing. Culture is fed continuously in front of the paddle wheel during daylight, and broth can be harvested on a daily basis. To prevent sedimentation paddle wheel needs to operate all the time at a specific rotation rate (Chisti, 2007).

Closed system

To produce a high density micro-algal biomass, photo-bioreactor is often used successfully (Carvalho, 2006). It consists of either vertical or horizontal transparent array of tubes/ solar collector to capture sunlight. The continuous broth is circulated using either submergible pump or compressed air. To increase the reflectance of the light to the photo-bioreactor, the ground below can be painted white.

Light

Firstly, light is one of the most important element in microalgae system (Wang, 2008). In the cultivation system, microalgae can be grown using either sunlight or artificial light sources. Based on the light intensity, the microalgae growth rate can vary significantly (Soulies, 2016). Biomass density of microalgae increases with the increase in sunlight intensity. Although increasing light intensity may also reduce photosynthesis due to light saturation which is also known as photo-inhibition (Lee, 1999). Thus, microalgae cultivation system requires optimum light intensity to reduce the photo-inhibition effect. Additionally, mutual shading or light saturation is also a common phenomenon when biomass density within growth medium increases with time (Pruvost, 2016.

Temperature

Secondly, like many other microorganisms, microalgae require a higher temperature to obtain optimum growth condition (Farrell, 1967). In general, appropriate temperature ranges between 10 to 30 degree Celsius. Although optimum temperature may also vary within different species (Pulz, 2001). Although this change can be controlled in a closed photobioreactor, but not possible in an open cultivation system (Huesemann, 2016). Therefore, appropriate microalgal strain should be selected so that it can cope with the fluctuations of the temperature. The lipid content of microalgae can be highly influenced by the effect of temperature (Renaud, 2002). Increase in temperature from 20 ^{0}C to 25^{0}C can increase the lipid content and an increase from 25 ^{0}C to 30 ^{0}C can bring it

down for certain strains (Converti, 2009). On the other hand, the rise of temperature affected the protein content in some microalgae but no consistent change in the carbohydrate (Renaud, 2002). Also, a strong relationship was found between specific growth rate and temperature for microalgae (White, 1991).

pH

Thirdly, one of the most important abiotic factors is the pH of the growth medium (Madigan, 2003; Van Vooren, 1999). Where most of the microalgae species Optimum pH range for most of the microalgae range from 7 to 9 Usually, pH of growth medium tends to increase with the increase of microalgae growth. These occur because of the consumption of dissolved CO_2 within the culture medium (Touloupakis, 2016). To reduce the effect of increasing pH, most of the time CO_2 is injected in the cultivation system (Zheng, 2016). Cultivation of blue-green microalgae as a source of lipid can be affected by pH. Biomass and lipid productivities of some microalgae changed with the change in pH; optimum culture pH was found as 7.5 (Moheimani, 2013)., Higher culture pH can influence the biomass harvesting by assisting incell flocculation up to 90%, especially for freshwater microalgae (Wu, 2012). During wastewater treatment using freshwater microalgae, the increase in pH increased the chlorophyll, pigments, lipid, and fatty acid content as well (de-Bashan, 2002).

Mixing

Finally, both open and close microalgae system mixings are necessary to stimulate high growth rate. Where microalgae cell can have an equal amount of light for photosynthesis. Appropriate mixing also applies to the nutrients uptake within the cultivation system. Furthermore, without the mixing microalgae, biomass may form flocks and precipitates, ultimately causing the microalgae culture to crash (J.B.K. Park, 2011).

Microalgae usually require two types of inorganic nutrients: macro and micro. Apart from carbon, nitrogen and phosphorus are the macronutrients and these two can be added in the culture using Redfield ratio C:N:P as 105:16:1 (Geider, 2002). Within the culture medium, carbon sources can be given either as carbon dioxide or bicarbonate (Richmond, 1986). However, some of the microalgae can utilize dissoveld organic as source of carbon. On the other hand, micronutrients consist of iron, manganese, calcium, copper, selenium, zinc, cobalt, molybdenum, and nickel (Richmond, 2008). In general, BG-11 growth medium has been used widely to grow different freshwater microalgae strains (Jena, 2011).

CHAPTER 3: MATERIALS AND METHODOLOGY

During all experimental period, the growth parameters such as light and temperature were kept at 25 ^{0}C with 12 hours of daylight exposure period. Completely randomized design was chosen with three replicates. All experiments were conducted in 250 mL Erlenmeyer flask and in a continuous shaker (Innova 44 incubator shaker series) with 120 RPM to maintain a homogeneous culture during the entire seven days growth period. Finally, the pollutants (TOC, TN, TP, heavy metals, BTEX) removal rate were analyzed before and after the final treatments. The figure 3.1 shows the methodology steps throughout my study.

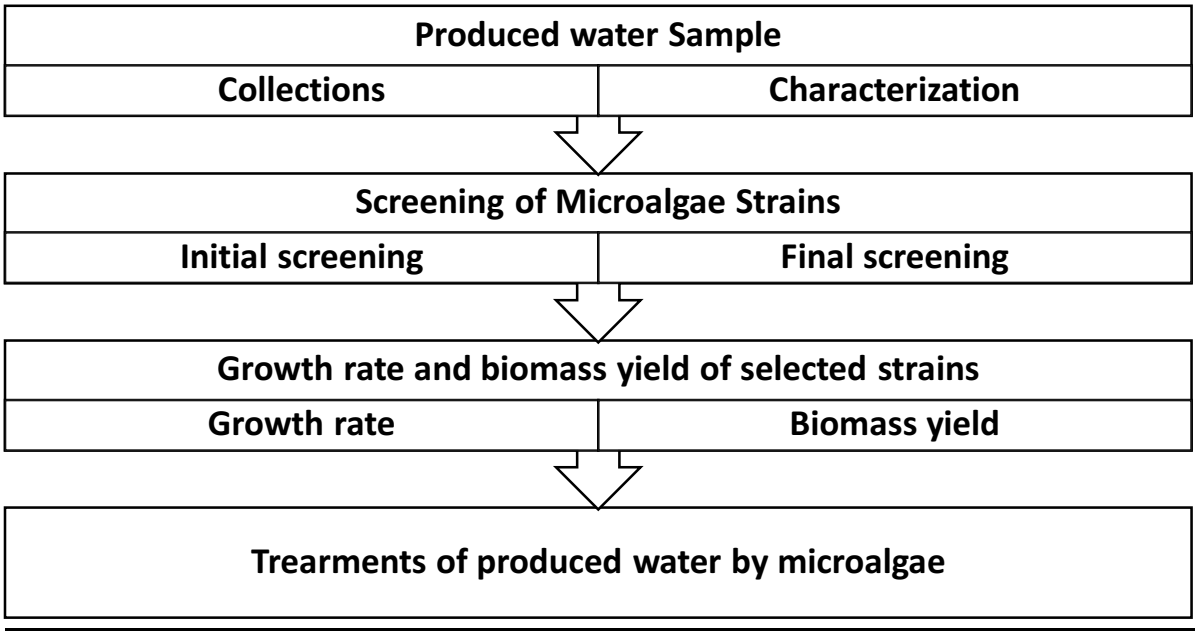


Figure 3.1: The methodology steps of this study.

Sample collections and characterization

The produced water samples were collected from different sources in Qatar. The collected produced waters initially were analyzed for their physical characteristics where the pH and salinity were analyzed by Thermo Scientific Orion Star A325 Portable pH/Conductivity/Temperature Multiparameter. Chemical characteristics such as the total phosphorus content was analyzed by colorimetric method (EPA Method 365.2, USEPA, 1983). HACH DR 3900 Benchtop VIS Spectrophotometer was used for the total nitrogen analysis. The Formacs High Temperature TOC Analyzer was used to quantify the total organic carbon in the produced water samples. The analysis of BTEX was carried out using PerkinElmer Clarus 680 Gas Chromatograph / flame ionization detector with Headspace Turbo Matrix 40Trap, Capillary Column Elite-1 L 60m was used. BTEX were analyzed by US-EPA 5021 method. Finally, the heavy metals were analyzed by PerkinElmer NexION 300D ICP-MS.

Screening of Microalgae Strains

The studies were conducted with microalgae strains collected from Qatar University Algal Technologies Program's culture collection (QUCCCM) lab. The selection of microalgae strains was based on two screening method. Initial screening of microalgae strains was performed by preliminary growth study in MicroWell Plates (Pavlic, 2006; Laurent, 1992) and final screening was performed by the growth of five selected microalgae strains in two different pH buffer solutions. Later produced water

pollutants removal rate by the selected microalgae strains were examined with different concentration of produced water.

Initial screening criteria:

Initially, total nine strains were taken for pre-screening. The pre-screening was done based on the microalgae growth potential, which was analyzed in transparent '96-well MicroWell Plates'. All of the strains were screened in 100 µL MicroWell Plates with 10% microalgae culture inoculums. Microalgae strains growth potential was examined at 750nm wavelength by a BioTek Synergy H4 Hybrid Microplate Reader. During seven days of growth period, the MicroWell Plates were kept in growth chambers (Versatile environmental test chamber, MLR-351/MLR-351H, SANYO, Japan), at 25°C temperature and with a 16-hour light/8-hour dark cycle.

Final screening criteria:

Base on the growth potential measured by optical density in initial screening, five microalgae strains were selected. During the final screening two different alkali solutions were added to adjust the pH of the produced water; these solutions were Sodium hydroxide and sodium bicarbonate. Previous studies also found that the biomass growth increased with sodium bicarbonate pH adjusted alkali solution (White, 2013).

Pretreatment:

The collected produced waters were initially filtered with 0.45 micron Millipore Steritop BTF-Durapore PVDF membrane to remove the suspended particulate meter.

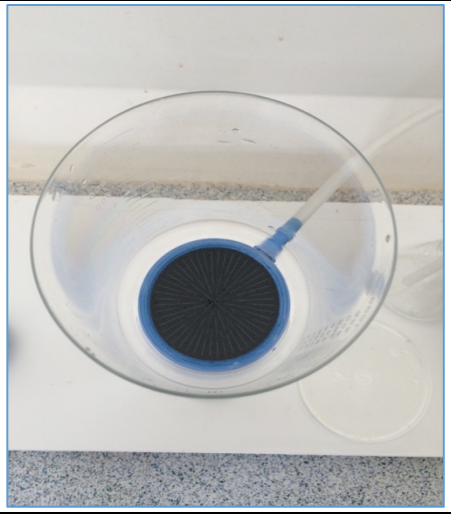
Before filtration	After filtration
	
	

Figure 3.2: produced water filtered with 0.45 micron to remove suspended particulate meter.

pH adjustments:

To increase the initial pH of the produced water during screening two types of buffer solutions were used. In the first set of the experiments, pH values were raised from 4.22 to 7.1 with 3M of NaOH to match the pH of standard growth media (Al-Shatri, 2015).

Whereas, in the second sets of the experiments, the pH value was also raised from 4.22 to 7.1 with sodium bicarbonate solution. Previously, it was found that sodium bicarbonate solution can also increase the utilization of nitrate and also increase the photosynthetic efficiency of microalgae strains (White, 2013). Both experiments were carried out simultaneously with triplicates. The growth periods for both these experiments were seven days. In these experiments, only produced water was used as a growth medium where 10 ml of algae culture inoculum was added to 90 ml of the filtered produced water. For both experiments, 250 ml Erlenmeyer flasks were used to screen the selected microalgae strains (Figure 3.3).

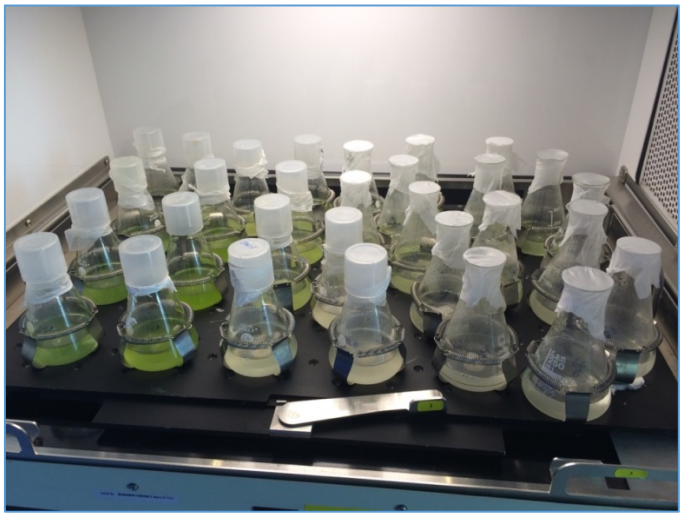


Figure 3.3: Screening experiment in Innova 44 incubator shaker

Examine growth rate and biomass yield of selected strains with normal growth media

Growth Medium:

Five different microalgae strains were grown with BG11 (Blue-Green Medium) using fresh water algae. This was done to identify their optimum growth condition in the BG11 medium for 15 days (Shi, 2007). Table 3.1 shows the growth medium composition and the trace elements solution.

Table 3.1: BG11 Growth medium composition.

Compound	**Concentration (g/L)**
$NaNO_3$	1.5
K_2HPO_4	0.04
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_2O$	0.036
Citric acid	0.006
Ammonium ferric citrate green	0.006
$EDTANa_2$	0.001
Na_2CO_3	0.02
Compound	**Concentration (mg/L)**
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.22
$Na_2MoO_4.2H_2O$	0.39
$CuSO_4.5H_2O$	0.08
$Co(NO_3)_2.6H_2O$	0.05

Figure 3.4 shows the list of the QUCCCM axenic strains identification via ribotyping. The fresh water algal strains includes *Monoraphidium sp., Chlorella sp., Neochloris sp., Scenedesmus sp.,* and *Dictyosphaerium sp.*

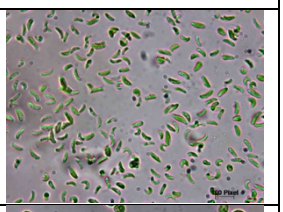
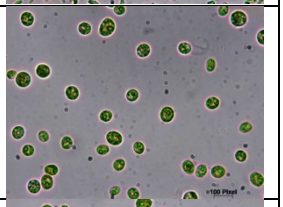
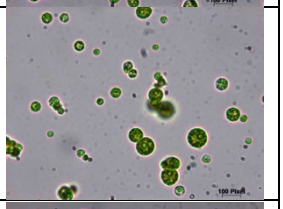
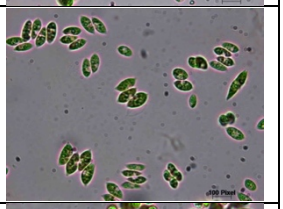
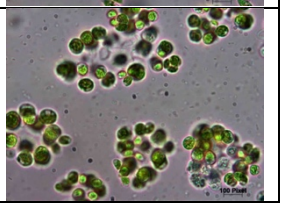
Group: Chlorophyta (green microalgae)		
Strain name	**Molecular Classification**	**Light Microscope Picture**
QUCCCM1	*Monoraphidium* sp.	
QUCCCM10	*Chlorella* sp.	
QUCCCM28	*Neochloris* sp.	
QUCCCM63	*Scenedesmus* sp.	
QUCCCM66	*Dictyosphaerium* sp.	

Figure 3.4: List of the QUCCCM axenic strains identification via ribotyping

Relation between Optical density & Dry biomass:

Growth of these strains was monitored everyday by taking optical density measurement of the cultures at 750nm wavelength using a Jenway 6850 UV/Vis. Spectrophotometer. Previously, calibration curve of biomass density and optical density for each culture was established.

To measure microalgae biomass density in sterilized culture media, different dilution of culture media were prepared. These dilution solutions were then filtered using preweighted- 0.45µM GC-F filter papers. After the filtration process, the filter paper was washed again with 0.5M ammonium formate to remove any salt. Finally, the filter paper was dried in an oven at 80^0C for 6 hours. Using the difference of two weights (i.e., filter papers weight with and without dry biomass), biomass concentration was determined for each dilution solution. Five different dilutions of cultures were made. As shown in Table 3.2.

Table 3.2: Dilution preparation process.

Microalgae Culture strength	**Culture volume**	**Deionize water**	**Total volume**	
100 %	20 ml	0 ml	20 ml	Reading Optical Density at 750nm
80 %	16 ml	4 ml	20 ml	
50 %	10 ml	10 ml	20 ml	
25 %	5 ml	15 ml	20 ml	
10 %	2 ml	18 ml	20 ml	

The following dilution equation (1) was used to quantify initial inoculum for the experiments as illustrated in Figure 3.5.

$$\boldsymbol{m1\ v1 = m2\ v2} \qquad (1)$$

Here, ***m1*** is the measured optical density reading of culture solution at 750nm. Whereas, ***m2*** is the required concentration of culture media. Similarly, ***v2*** is the final volume taken during the experiment and ***v1*** is unknown, which is showing the amount of culture needed to obtain desirable optical density reading at 750nm.

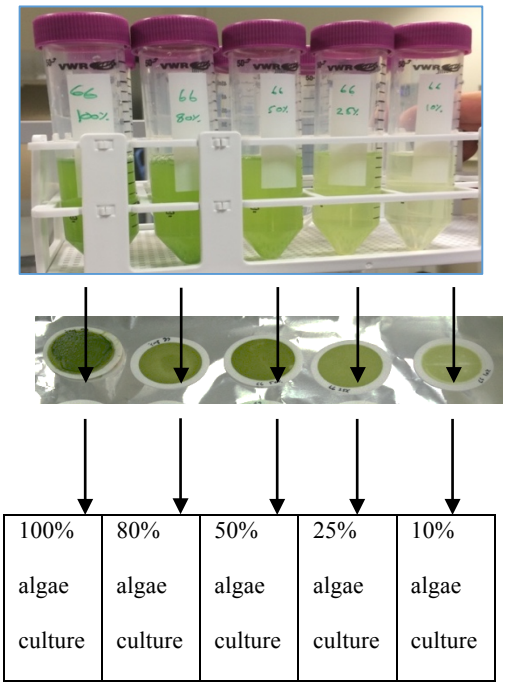


Figure 3.5: different dilution of *Dictyosphaerium sp.* experiment to find the relation between OD/BIOMASS

Examine algae strains with different concentration of produced water

To evaluate the pollutants removal rate from different concentrations of the collected produced water, five microalgae strains were inoculated. Along with the blank, four different concentrations of the produced water were selected for these experiments as described in Table 3.3. For all different concentrations of the produced water, 10% culture inoculum was added. As a control, 100 mL volume of each treatment was taken in the flask and placed in the orbital shaker together with other flasks that had no inoculum of the microalgae. All treatments were triplicated.

Table 3.3: Five Microalgae strains with different concentration of produced water.

Five treatments (for each Microalgae strains)				
Treatment	**Microalgae Inoculum (mL)**	**Milli-Q water (mL)**	**Produced water(mL)**	**Total Volume(mL)**
100 % (control)	-	-	100	100
50%	10	45	45	100
60%	10	36	54	100
75%	10	22.5	67.5	100
100%	10	-	90	100

CHAPTER 4: RESULTS AND DISCUSSION

Section A: Produced water characteristics

The collected produced water was analyzed before and after the filtering with 0.45 micron Millipore. The filtration was done in order to reduce the turbidity of the produced water as shown in Table 4.1. Due to filtration process, nitrogen, phosphorus, organic carbon, BTEX and trace metals were reduced.

Table 4.1: Chemical characteristics of collected produced water

Parameters of the produced water		Unit	Concentration of contaminants before filtration	Concentration of contaminants after filtration
Total Nitrogen (TN)		ppm	35.8	27.6
Total organic carbon (TOC)		ppm	389.1	317
Total phosphorus (TP)		ppb	277.8	180
BTEX	Benzene	ppm	21	16.1
	Toluene	ppm	3.8	3.2
	Ethylbenzene	ppm	1.2	1.1
	Xylene	ppm	3.4	3.1
Salinity		p.s.u.	4	4

Section B: Screening of Microalgae Strains

Initial screening in micro-well plate:

Initial growth experiments were conducted with *Monoraphidium sp, Chlamydomonas sp, Chlorella sp, Scenedesmus sp, Neochloris sp, Oorococcus sp, Chlorococcum sp, Oocystis sp, and Dictyosphaerium sp* on collected produced water. These experiments were simply to test the survivability of the microalgae strains. Each of these microalgae were inoculated in 100 µL MicroWell Plates with 10% inoculums for seven days. Optical density was measured to quantify the microalgae growth. Figure 4.1 shows the optical density values with time for the nine microalgae strains in a fully concentrated produced water.

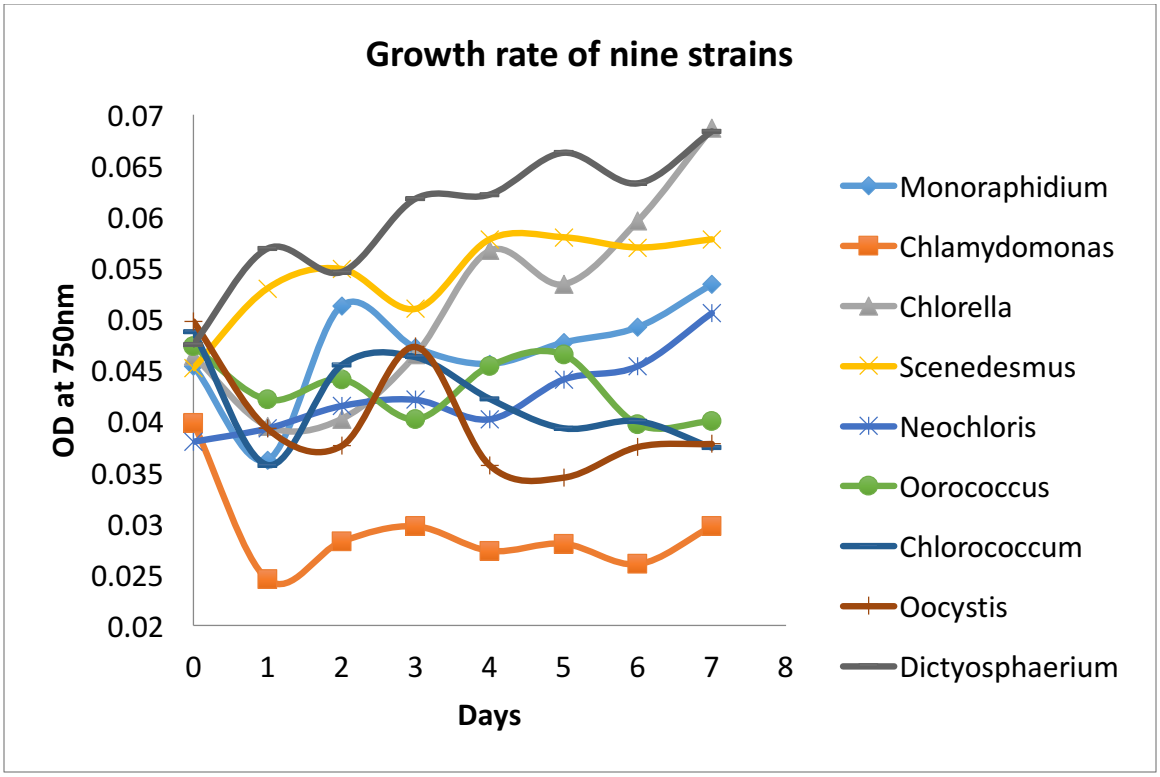


Figure 4.1: Initial screening of microalgae in fully concentrated produced water.

Among these nine microalgae strains, only five strains were able to survive and with an increasing in optical density. The results of the growth experiments are shown in Figure 4.1. Growth result indicated an increase optical density for *Chlorella, Dictyosphaerium* sp., *Scenedesmus* sp., *Neochloris* sp., and *Monoraphidium sp*. Where *Chlorella* and *Dictyosphaerium* sp. species were found to have better biomass yield compared to the rest of the microalgae. Apart from these two strains, *Scenedesmus* sp., and *Neochloris sp.* and *Monoraphidium* sp. also had net positive biomass yield, although

much lesser compared to *Chlorella* and *Dictyosphaerium* sp. Rest of the species *Oorococcus* sp.*, Chlorococcum, Oocystis,* and *Chlamydomonas,* were not able to survive in the produced water. Presence of BTEX and other heavy metals could have been toxic to these species and therefore these strains had net negative biomass yield. Also the pH of the produced water was very low which could be another reason for these strains not to survive in this experiment. It is knowing that some microalgae can survive can thrive in extreme culture pH; for example, *Galadaria sulphuria* can grow at a culture pH of 3, *Spirullina* sp. can grow in culture having pH 10 and above. It was clear that the above 4 microalgae strains were not suitable for growth in the produced water and hence could not be used for the remediation of the produced water. Therefore, other five strains were selected for the remainder of this study.

2nd screening with two different pH adjusted buffer solution for optimum growth condition

During seven days of the growth period, 250mL Erlenmeyer flasks with 100mL volume of the produced water medium were used. Due to the larger scale and appropriate mixing, the microalgae growth was much faster than the initial microplate screening. Figures 4.2 and 4.3 showing the growth of five microalgae species in 100 mL volume.

Microalgae growth in sodium hydroxide pH adjusted buffer solution:

In Figure 4.2, overall experimental results show that the *Chlorella* sp. obtained highest biomass yield compared to other four species. Due to the alteration of growth

medium most of the microalgae species were able to acclimatize and during this period (i.e., first 2 days), the biomass concentration did not increase. Starting from second-day onwards *all* the five strains started to grow, although all these strains had different growth rates. While *Chlorella* sp. had the highest growth rate, *Monoraphidium* sp. had the lowest growth rate. All data sets were represented with mean values and with standard error.

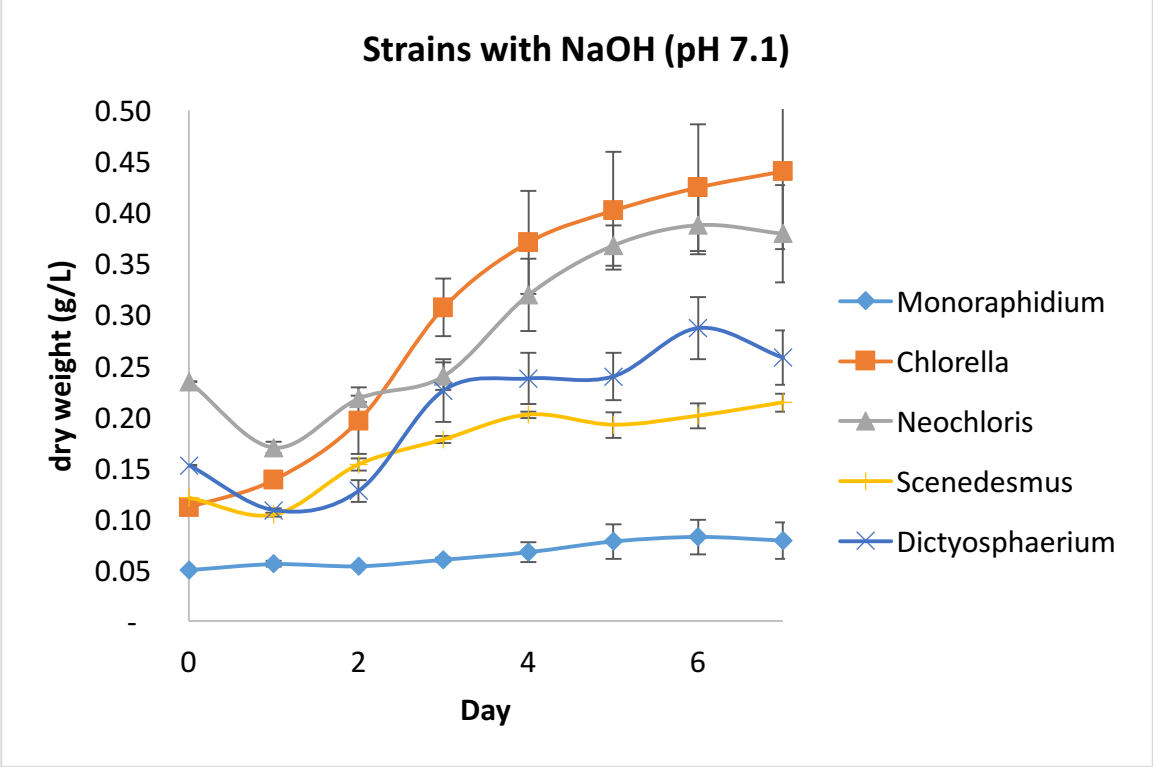


Figure 4.2: Screening of microalgae in fully concentrated produced water pH using NaOH solution.

Microalgae growth in sodium bicarbonate pH adjusted buffer solution:

While increasing the pH with sodium bicarbonate solution, the overall microalgae biomass did not increase as much as it increased in the previous section, for all the strains., However, *Chlorella* sp., *Neochloris* sp., and *Scenedesmus* sp. species were the able to generate higher biomass compared to other two microalgae species (see figure 4.3). Addition of Na_2CO_3 in the produced water could have changed the water chemistry and white suspended materials were visible for all the cultures from 2nd day onwards (see figure 3.3). pH of microalgae culture tends to increase when no CO_2 is added in the culture. At high pH, some of the trace metals were expected to precipitate which were essential for microalgae growth. In this case, the available carbonate in the culture was utilized by the microalgae; as a result, the pH could have increased too high which could have resulted lesser final biomass density for all the strains.

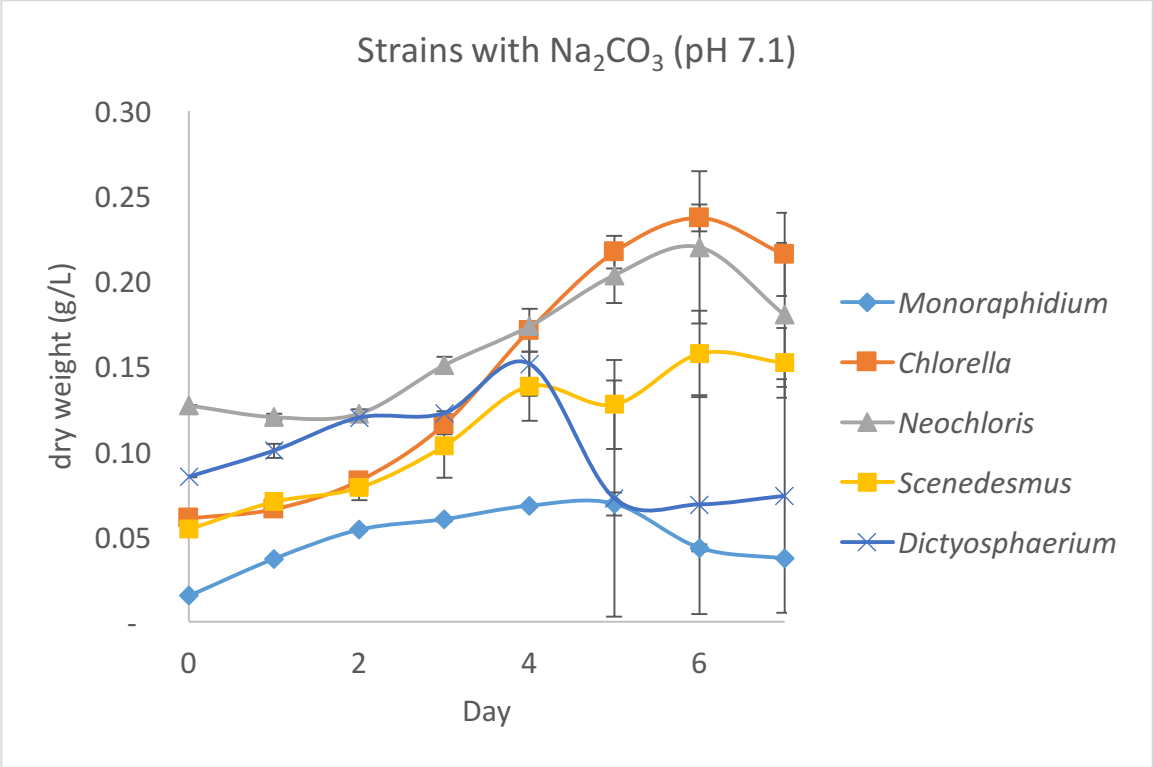


Figure 4.3: Screening of microalgae in fully concentrated produced water pH using Na_2CO_3 solution.

Finally, the results from screening experiments showed that five species could grow on the produced water. Furthermore, the second screening with two different pH buffer solutions showed that sodium hydroxide solution was the ideal solution to promote microalgae growth.

Section C: Examine growth rate and biomass yield with normal growth media

Five selected strains were tested for their optimum growth rate in axenic BG-11 (Blue-Green Medium) growth medium as shown in Figure 4.4. The growth curves of these five strains are shown in Figure 4.4. Biomass densities of *Neochloris* sp., *Scendesmus* sp., *Chlorella* sp., *Dictyospaerium* sp, and *Monoraphidium* sp. were 0.97, 0.76, 0.60, 0.60 and 0.42 g/L respectively. A large variation in the biomass density among these strains can be attributed by the combination of several parameters: low light intensity, insufficient nutrient, different growth rate, low temperature. Light intensity in the shaker was 100 μmol $E/m^2/s$ which could have affected the growth of all these strains. Just for comparison, outdoor light intensity in Qatar on mid-day can be as high as 2250 μmol $E/m^2/s$ (Das, 2015). Although some microalgae require high light intensity, some microalgae can utilize low light intensity very efficiently because of their different light harvesting pigment structures (Horton,1996). Nutrient requirement will also vary among microalgae species and therefore some of the required nutrients could have been limiting for the microalgae with low growth rate. During the experiment, all the cultures were maintained at a fixed temperature (25 ^{0}C) which could limit the growth of some microalgae. Lastly, more importantly, even after providing all the optimum conditions, the growth rate of different microalgae will vary based on their individual growth characteristics. All data sets were shown with mean values and with standard error.

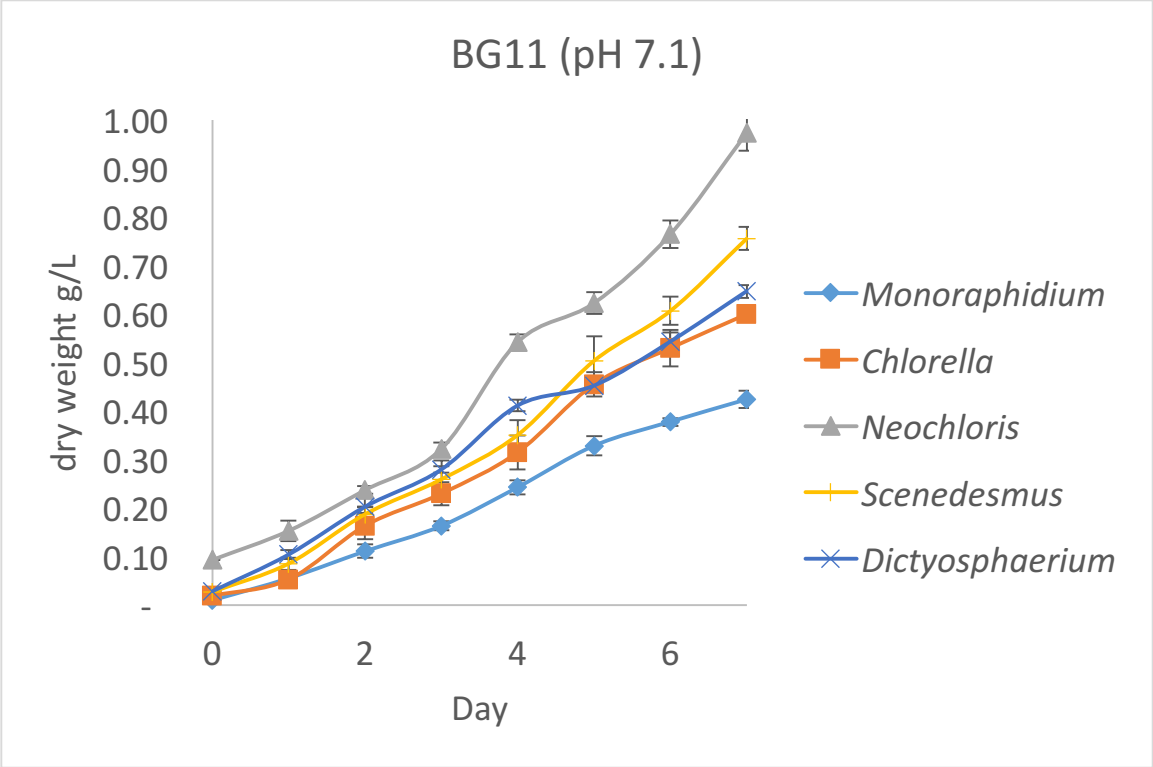


Figure 4.4: Microalgae species growth rate in standard BG11 (Blue-Green Medium).

Section D: Examine microalgae strains with different concentration of produced water

Effect of produced water on the growth of microalgae species:

During the final experiment setups, the growth rate and biomass production for five different microalgae species were observed. Initially, as shown in Figures (7.1 to 7.4) for all the growth trials similar amount of microalgae biomass (0.04 g/L) was inoculated. This similar amount of biomass selected for different concentrated produced water

mediums. Four different concentration 100%, 75%, 60% and 50% of the produced water were implemented for the experiments. Initial growth period for all species had a noticeable lag phase with four different produced water concentration. These experiments determined the rate of microalgae biomass growth in produced water. All data sets were represented with mean values and with standard error.

Growth in 100% produced water concentration:

The growth of *Dictyosphaerium* sp obtained the highest biomass concentration, where after a day of lag period biomass growth up to 0.54 g/L (Figure 4.5). Similar observation was seen with the *Chlorella* sp, although the biomass did not grow as *Dictyosphaerium* sp. Whereas, *Scenedesmus* sp and *Monoraphidium* sp stayed in prolonged stationary phase after two days of growth. A different result was observed with *Neochloris* sp., where biomass grows for two consecutive days and later the biomass concentration tends to decrease (Figure 4.5). The decreasing result was found for *Neochloris* sp. due to the formation of large clumps after four days of growth. Similar to microplate experiment, *Neochloris sp*. and *Monoraphidium* sp. could not grow in the produce water. The other three strains had better biomass yields compared to the yields obtained in the microplate experiment which was probably due to better mixing and higher light intensity. Adjusting the pH of the culture could have also allowed theses strains to have higher biomass yields. The microalgae used the amount of available nutrient in the produce water. Since, no nutrients were added, growth of these microalgae was controlled by the nutrients-present in the produced water. From the characteristics of

the produced water (see Table 4.1), it can be concluded that some of the micronutrients (e.g., Nitrogen and Phosphorus) were very limited in this experiment. It was also possible that a fraction of some, or all the nutrients can come to these cultures as residuals from the inoculum; this could also support the growth of some of the microalgae to some extent. Another important parameter was the salinity of the cultures; while the control experiment was conducted with DI water, 100% produced water cultures had salinity of 4 p.s.u. Therefore, it was also possible that growth of these strains were affected by the salinity of the produced water. Effect of salinity on the growth was evident for *Neochloris sp*. and *Monoraphidium* sp.

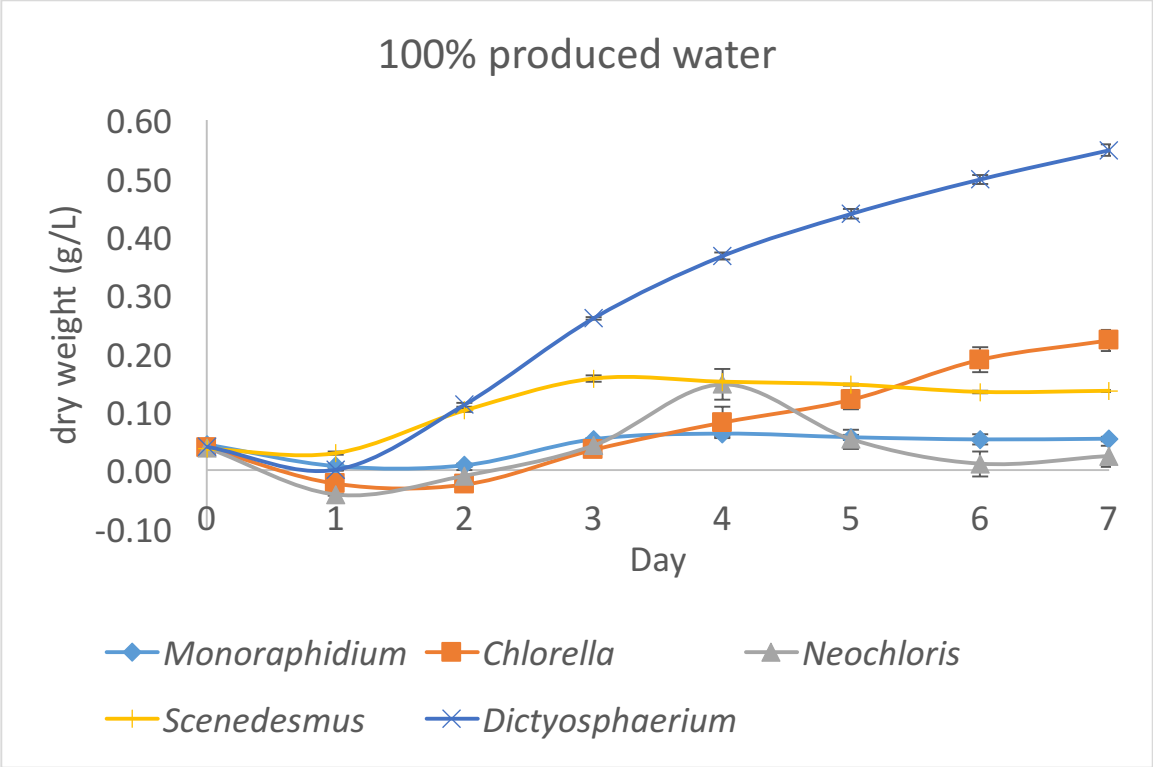


Figure 4.5: Microalgae species growth rate in 100% produced water concentration.

Growth in 75% produced water concentration:

Microalgae biomass during these experiments showed a better result. The only exception found with *Monoraphidium* sp, which remained in the stationary phase throughout the experiment (Figure 4.6). As a result of diluting the produced water to 75% strength with DI water, both the salinity and the concentrations of contaminants decreased which could have improved the growth conditions for these microalgae. *Dictyosphaerium* sp. still had the highest biomass concentration (0.5 g/L) which was

slightly lesser than the biomass concentration obtained in 100 % produced water culture. Therefore, the dilution of produced water had no effect on the growth of *Dictyosphaerium* sp. After the growth period, the species like *Chlorella* sp., *Scenedesmus* sp. and *Neochloris* sp. had biomass densities of 0.35 g/L, 0.22 g/L and 0.12 g/L respectively. Reduced salinity and other pollutants could have improved the growth of these strains. Although a rapid increase was observed for *Neochloris* sp. after the second day, its biomass concentration continued to decrease. *Neochloris* sp. could have reached the 'stationary phase' on day 3 and therefore its biomass concentration started to decrease in the 'death phase'.

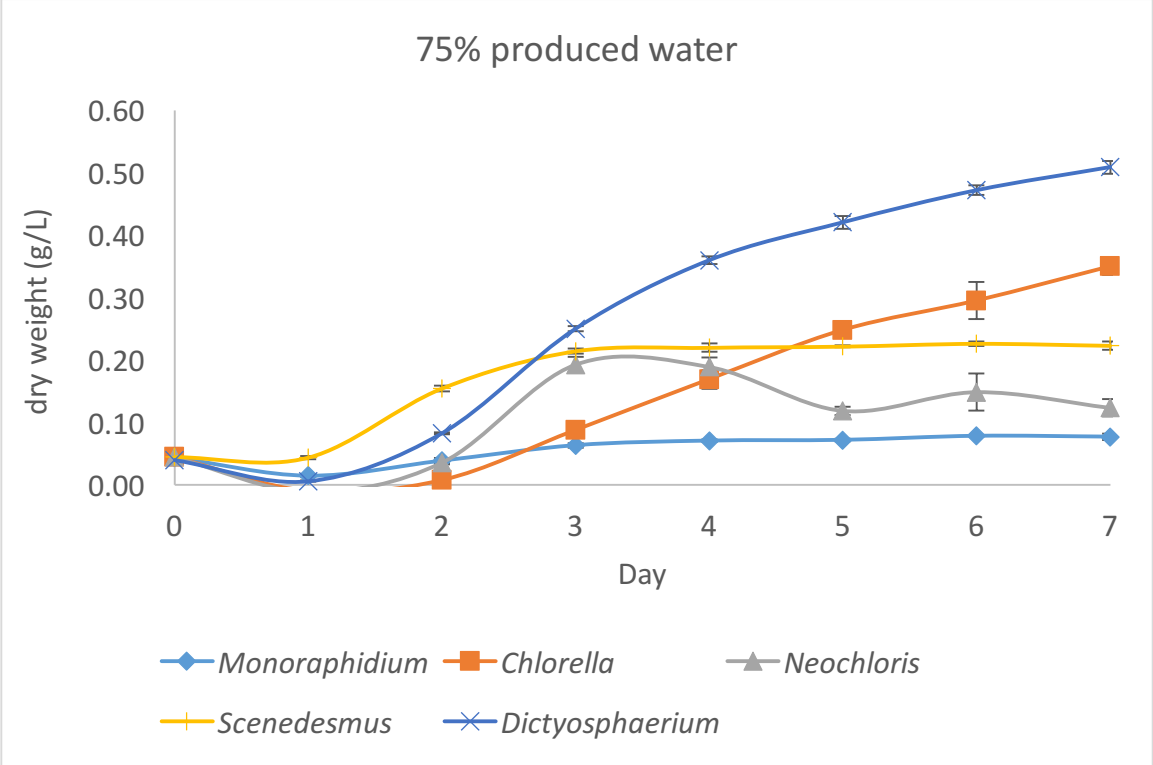


Figure 4.6: Microalgae species growth rate in 75% produced water concentration.

Growth in 60% produced water concentration:

With 60% produced water concentration *Neochloris* sp. were able to reach to 0.39 g/L within three days (Figure 4.7). From fourth day, *Neochloris* sp. started forming larger clumps. Salinity of these cultures reduced further with an increase in dilution; it had a strong influence on the growth of *Neochloris* sp. The rest of the microalgae species showed similar growth as it was found previously found with 75% produced water. *Dictyosphaerium* sp. had the highest biomass concentration of 0.54 g/L, *Chlorella* sp. had 0.35 g/L, *Scenedesmus* sp. had 0.24 g/L. The lowest biomass concentration was observed for *Monoraphidium* sp. (Figure 4.7).

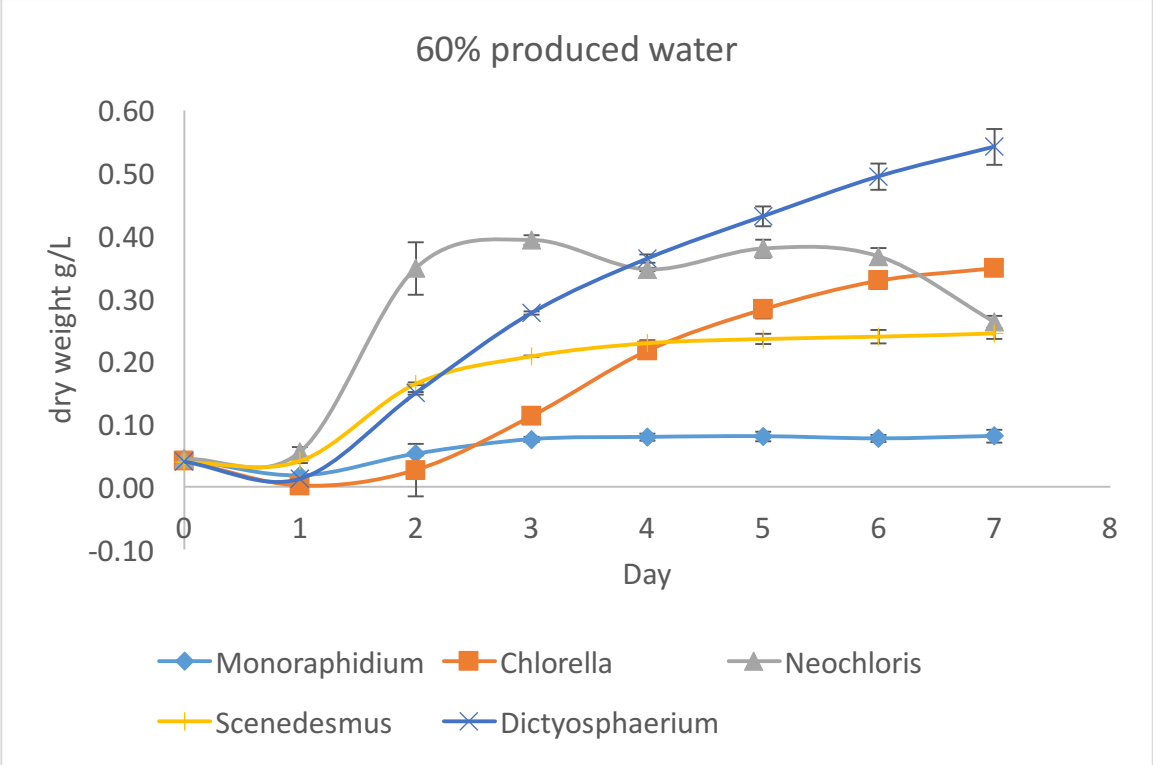


Figure 4.7: Microalgae species growth rate in 60% produced water concentration.

Growth in 50% produced water concentration:

Biomass yield of these five strains in 50 % produced water was shown in Figure 4.8; the yields were almost identical with the yields obtained for 60% produced water (see Figure 4.7). The highest biomass by *Dictyosphaerium* sp. with a 0.54 g/L biomass concentration. Consequently, *Chlorella* with 0.36 g/L, *Scenedesmus* sp. with 0.27 g/L, *Neochloris* sp. with 0.24 g/L and lastly *Monoraphidium* sp. with 0.08 g/L biomass concentration.

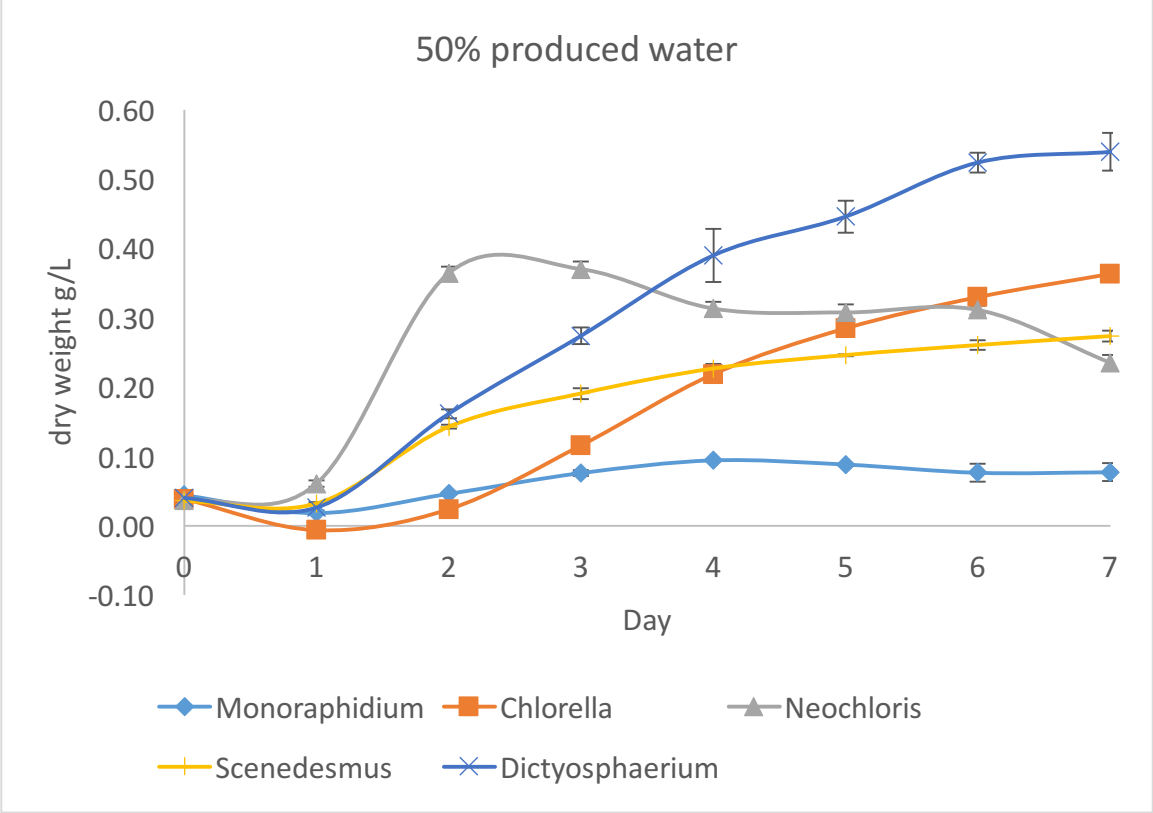


Figure 4.8: Microalgae species growth rate in 50% produced water concentration.

Finally, overall result showed that the *Dictyosphaerium* sp. can grow in all concentrations of produced water. Whereas *Chlorella* sp. were able to grow in different concentrations of produced water but the biomass yield was higher for 75% and 50 % produced water concentrations. Similar results were obtained for *Scenedesmus* sp. *Neochloris* sp. on the other hand, shown better growth with 60% and 50% concentration produced water. Only *Monoraphidium* sp. remained in the stationary phase in all the concentration of produce water and were not able to grow.

Among the different concentration level of produced water, 50% and 60% produced water concentration had higher microalgae biomass concentrations (Figure 4.7 & 4.8); similar results were obtained for *Nannochloropsis* sp. in the previous study by Arriada et al. (2014). Arriada also suggested that 50% produced water concentration would be better for use as a microalgae culture media. The previous finding was supporting the case for most of the microalgae species. The only exception found with *Dictyosphaerium* sp. microalgae species, where variation in produced water concentration had a minimum effect of biomass generation. This could be the fact that *Dictyosphaerium* sp. could grow in a wider range of salinity; whereas, the other four strains in this study required low to zero salinity for growth.

Initially within all microalgae species lag phase was observed due to the transfer from standard growth medium to produced water medium. This lag phase occurs due to the physiological adjustment of a newly introduced medium. Such cases also have been reported by Lee et al. (2016) in previous studies. It was also mentioned that exposer to higher irradiance could also introduce a lag period.

Arriada et al. (2014) added regular nutrients for supporting the microalgae growth. On the other hand, this experiment showed that the available contaminants within produced water could be used as nutrient for supporting microalgae growth. However, the biomass concentration of these microalgae were much lower compared to BG-11 growth medium-suggesting additional macro-nutrients should be added in produced water for producing high density biomass in it. Nonetheless, low biomass yield could also be used for treating the produced water.

Section E: Contaminants removal efficiency of microalgae strains from different concentration of produced water

Concentrations of total nitrogen, total phosphorus, total organic carbon, and BTEX was analyzed before and after the treatment with five microalgae species. The results are shown in Table 4.1.

Total Nitrogen removal efficiency:

Total nitrogen removal efficiency was analyzed to determine all forms of nitrogen which can appear such as nitrites, nitrates, ammonia, ammonium salts and also as an organic nitrogen compound. Before the treatment, total nitrogen concentration in the filtered produced water was 27.6 mg/L (Table 4.1).

The results showed no significant difference while comparing the total nitrogen removal efficiency from different concentration of produced water. Although among the microalgal strains, a significant difference was observed (Table, 7.2). Although some of

the microalgae species were not able to grow on produced water medium, their average removal efficiency rate was higher than other species. Such case has found with *Scenedesmus* sp. and *Monoraphidium* sp. recovering up to 63.76% and 62.98% from the produced water (Figure 4.9). On the other hand, microalgae species like *Dictyosphaerium* sp., *Chlorella* sp. and *Neochloris* sp. removed 61.17%, 58.89% and 55.23% TN respectively (Figure 4.9). Presence of bacteria community within the produced water could have also reduced the nitrogen concentration in the control experiments. Finally, the statistical analysis showed a significant nitrogen removal efficiency between microalgae but not in different concentration of the produced water. Furthermore, no significant difference was found within *Scenedesmus* sp., *Monoraphidium* sp. and *Dictyosphaerium* sp. (Table 4.2).

Table 4.2: Average total nitrogen removal efficiency:

PW level	Microalgae Species						
	Monoraphidium	*Chlorella*	*Neochloris*	*Scenedesmus*	*Dictyosphaerium*	No Algae	Mean
100%	64.40	54.16	69.90	58.83	63.80	11.80	53.81
75%	58.95	62.33	54.39	66.54	61.29	11.80	52.55
60%	67.07	61.46	46.03	67.59	58.73	11.80	52.11
50%	61.49	57.60	50.62	62.07	60.88	11.80	50.74
Mean	62.98	58.89	55.23	63.76	61.17	11.80	

Nitrogen removal effecency among different microalgae species were statictically significant with a p-value <0.05 probability. least significant difference for total nitrogen removal effecency by different microalgae species is 2.74.

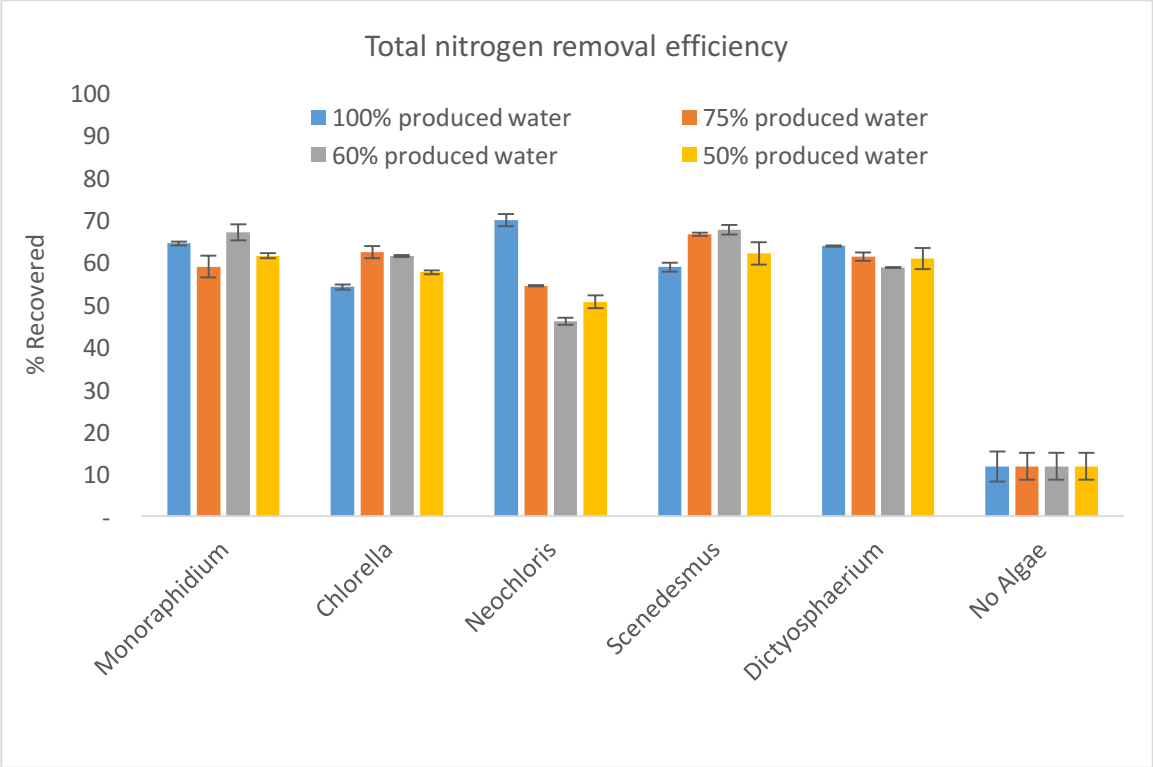


Figure 4.9: Total Nitrogen removal efficiency by five microalgae species from different of produced water concentrations.

Total phosphorus removal efficiency:

The filtered produced water had 180 µg/L total phosphorus which could be a combination of organic and inorganic phosphorus. (Table 4.1). The highest average phosphorus removal efficiency from the different concentration of the produced water was found for *Dictyosphaerium* sp. spwith 88.83% and *Chlorella* sp. with 73.23%, followed by *Neochloris* sp. with 57.22%, *Scenedesmus* sp. with 54.41% and *Monoraphidium* sp. with 35.23% of phosphorus removal efficiency (Table 4.3 and Figure

4.10). Due to the presence of naturally occurring bacterial community, the concentration of total phosphorus was also reduced (Figure 4.10). Statistical analysis found a significant removal efficiency among the microalgae and the produced water concentration. Where removal efficiency is significant in 100% and 50% produced water than the others. On the other hand, *Dictyosphaerium* sp. had the highest significant removal efficiency than the others, where *Chlorella* sp. come in second. *Dictyosphaerium* sp. had the highest biomass growth in all the produced water cultures and therefore it could utilize maximum amount of phosphorus-present in the produced water. Furthermore, no significant difference was found in the total phosphorus removal efficiencies between *Scenedesmus* sp and *Neochloris* sp (Table 4.3).

Table 4.3: Average total phosphorus removal efficiency:

PW level	Microalgae Species						
	Monoraphidium	*Chlorella*	*Neochloris*	*Scenedesmus*	*Dictyosphaerium*	No Algae	Mean
100%	41.27	77.36	69.22	58.00	87.65	10.64	57.36
75%	31.38	67.33	51.19	51.19	88.07	10.64	49.97
60%	27.71	71.03	55.41	55.41	90.13	10.64	51.72
50%	40.57	77.81	53.06	53.06	89.46	10.64	54.10
Mean	35.23	73.38	57.22	54.41	88.83	10.64	

Phosphorus removal effecency among different microalgae species and produced water level were statictically significant with a p-value <0.05. least significant difference for total phosphorus removal effecency by different microalgae species is 4.48 and different produced water level is 3.66.

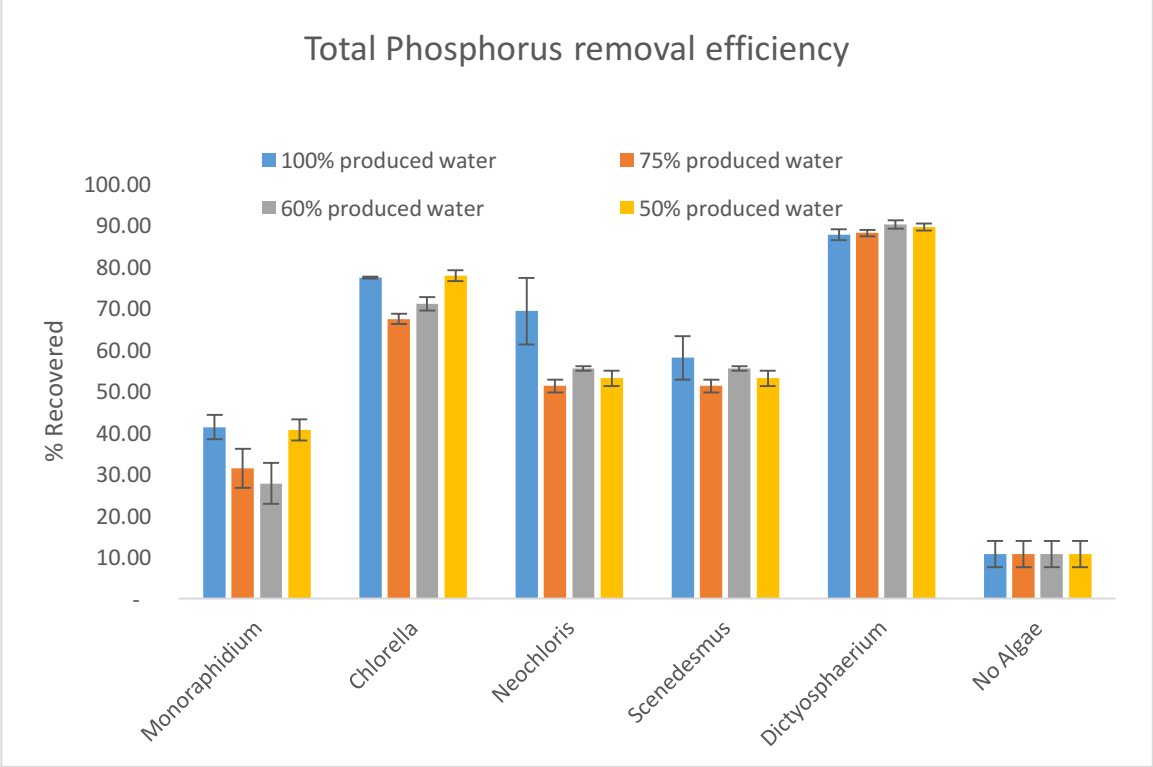


Figure 4.10: Total phosphorus removal efficiency by five microalgae species from different of produced water concentrations.

Xin et al. (2010) found that *Scenedesmus* sp. could remove 99% of nitrogen and phosphorus from the wastewater as long as the nitrogen and phosphorus stay within 5:1 to 8:1ratio. In another study, *Chlorella* sp. was found to have a similar 99% nitrogen and 90% phosphorus removal efficiencies (Yang, 2011). *Neochloris* sp. could also remove

100% phosphorus and 78% nitrogen from the wastewater (Wang, 2011). Wang et al. (2011) also found that phosphorus removal by *Neochloris* sp. was independent while nitrogen removal efficiency was dependent on the phosphorus concentration within the wastewater. In the produced water, the ratio of N:P was 151:1 which had more nitrogen than phosphorus as compared to Redfield ratio (i.e., 16:1) In this study, apart from *Dictyosphaerium* sp., all other strains had much lower biomass yield and hence the residual phosphorus concentration was much higher for their cultures. Therefore, not only the N:P ratio, but also biomass yield were responsible for phosphorus removal efficiencies.

Total organic carbon removal efficiency:

After flirtation, the produced water had 317 mg/L of total organic carbon (Table 4.1). The results showed wide variations in removal efficiencies among species and also with different concentrations of the produced water.

Overall, among other microalgae species, *Neochloris* sp. showed a better result in TOC removal efficiency. The total average organic carbon among different concentrations of the produced water by *Neochloris* sp. showed 41.61% removal efficiency, whereas *Chlorella* sp., *Dictyosphaerium* sp., and *Monoraphidium* sp. recovered 30.75%, 28.78%, and 27.20% respectively. *Scenedesmus* sp. had the lowest average removal efficiency 20.82% after the treatment (Table 4.4 and Figure 4.11). In the control where no microalgae biomass was added, the total organic carbon was also reduced because of bacterial community presence within the produced water (Figure

4.11). the statistical results showed a higher significant removal efficiency was found with *Neochloris* sp. and also 100% produced water concentration. Whereas TOC removal efficiency in *Chlorella, Dictyosphaerium* sp., and *Monoraphidium* sp. had no significant difference among each other.

Table 4.4: Average organic carbon removal efficiency:

PW level	Microalgae Species						
	Monoraphidium	*Chlorella*	*Neochloris*	*Scenedesmus*	*Dictyosphaerium*	No Algae	Mean
100%	41.56	37.04	45.20	23.02	35.03	17.95	33.30
75%	27.62	33.77	36.24	26.87	31.44	17.95	28.98
60%	26.59	31.28	46.37	13.08	22.78	17.95	26.34
50%	13.01	20.94	38.63	20.30	25.89	17.95	22.78
Mean	27.20	30.75	41.61	20.82	28.78	17.95	

Organic carbon removal effecency among different microalgae species and produced water level were statictically significant with a p-value <0.05. least significant difference by different microalgae species is 4.37 and different produced water level is 3.56.

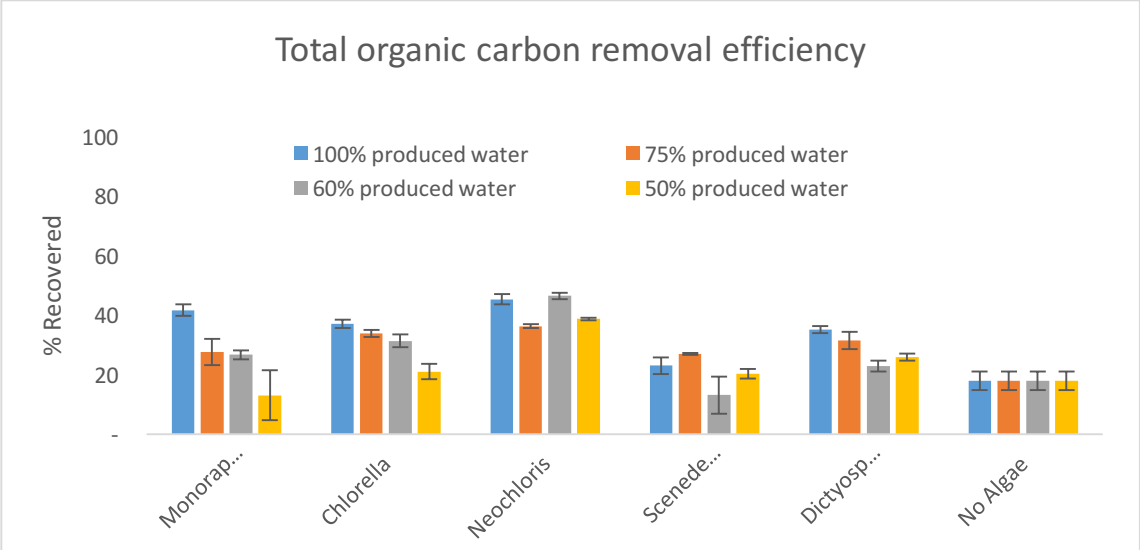


Figure 4.11: Total organic carbon removal efficiency by five microalgae species from different of produced water concentrations.

Recent studies found that the total organic carbon removal rate is low than the nitrogen and phosphorus. Even it was found that no organic carbon was recovered by microalgae from the wastewater (Lee, 2002). Lee also state that while nitrogen removal was 98.6%, there was no removal of organic carbon from the wastewater. Some of the microalgae have the ability to use dissolved organic carbon as a source of carbon and this phenomenon is known as mixotrophy. It was possible that all the strains, used in this study, were mixotrophic and hence some part of the TOC in the produced water was removed due to consumption. Although all the organic compounds were not characterized, it was possible that there were many compounds that microalgae couldn't utilize as a carbon source. Bio-based materials (i.e., activated carbon) are often used to

remove the suspended and soluble organic carbon. Similarly, it was possible that all the microalgae could absorb a fraction of the TOC-present in the produced water. Microalgae are also known to produce extracellular organic matter (EOM) which mainly comprised of carbohydrate (Pivokonsky, 2006). Such carbohydrates could have also contributed in the residual TOC concentrations; however, contribution from EOM in the residual TOC was low, for all the microalgae studied here.

Benzene removal efficiency:

Benzene concentration in the produced water was 16.1 mg/L (Table 4.1). The overall results indicating no significant difference in removal efficiency between the treatments with different microalgae species and also for various concentration of produced water. Statistically benzene removal efficiency had no significant variation among the treatments. From the Figure 4.12, it was clear that benzene in control flask was either evaporated in the presence of light or removed the micro-organisms present in the produced water. Similar removal efficiencies were also found for all the microalgae cultures. Ability for microalgae to consume benzene was not reported in the past. Therefore, it was possible that evaporation and bacterial mineralization were responsible for the complete removal of benzene from the produced water. Additionally, it was also possible that a fraction of the benzene could have been adsorbed on the surface of the microalgae.

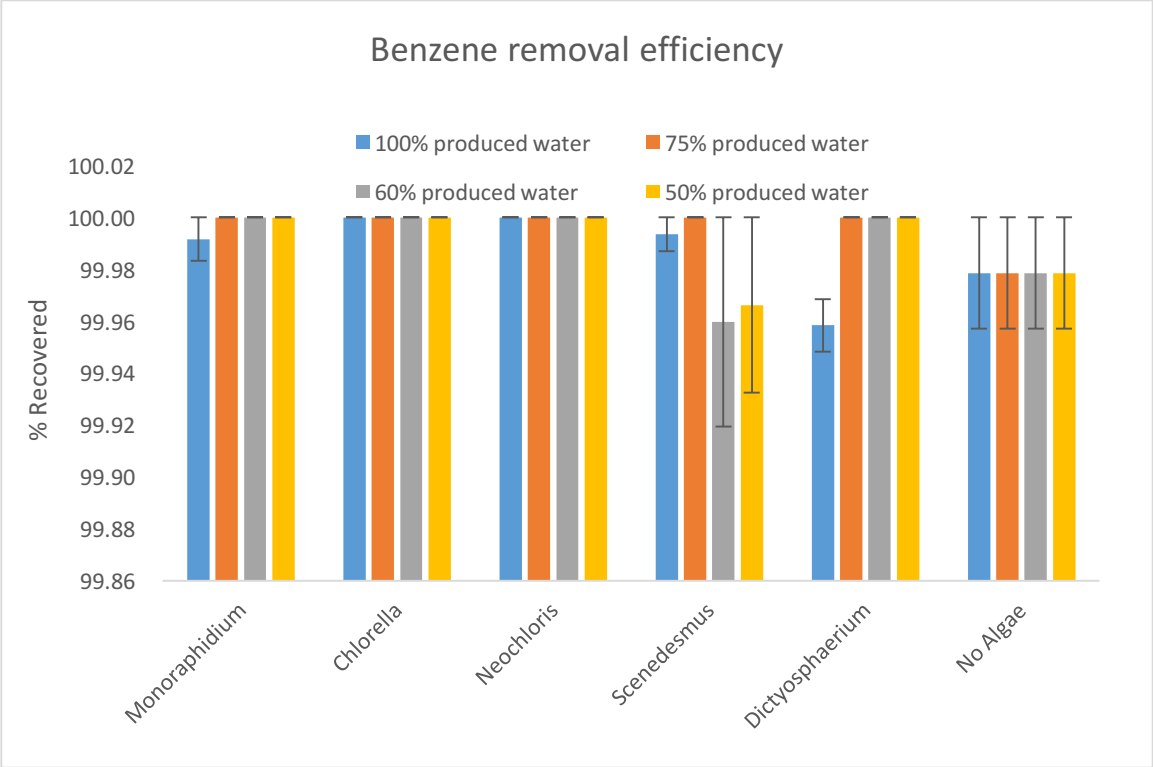


Figure 4.12: Benzene removal efficiency by five microalgae species from different of produced water concentrations.

Toluene removal efficiency:

Initially toluene concentration was found as 3.21ppm (Table 4.1). After treating the produced water with five microalgae at different concentrations of produced water *Dictyosphaerium* sp. had the highest toluene removal efficiency (Figure 4.13). Toluene removal efficiencies for *Neochloris* sp., *Chlorella*, *Monoraphidium* sp., and *Scenedesmus* sp. were 97.35 %, 96.71 %, 94.89 % and 94.02 % respectively. Surprisingly, even higher (97.31 %) toluene removal efficiency was observed in the control. Statistical analysis

showed both factors, i.e., microalgae species and produced water level, influenced the toluene removal efficiency significantly. *Dictyosphaerium* sp., *Neochloris* sp. and *Chlorella* sp. had no significant deference in toluene removal efficiency. Whereas, toluene removal efficiency from 100% and 75% produced water concentration level have the highest significance (Table 4.5).

Table 4.5: Average toluene removal efficiency:

PW level	**Microalgae Species**						
	Monoraphidium	**Chlorella**	**Neochloris**	**Scenedesmus**	**Dictyosphaerium**	**No Algae**	**Mean**
100%	96.30	97.62	98.02	97.03	98.17	97.31	97.41
75%	95.31	97.09	97.63	94.47	98.44	97.31	96.71
60%	94.15	96.37	97.15	92.99	97.20	97.31	95.86
50%	93.80	95.77	96.62	91.59	97.17	97.31	95.38
Mean	94.89	96.71	97.35	94.02	97.74	97.31	

Toluene removal effecency among different microalgae species and produced water level were statictically significant with a p-value <0.05. least significant difference by different microalgae species is 1.07 and different produced water level is 0.88.

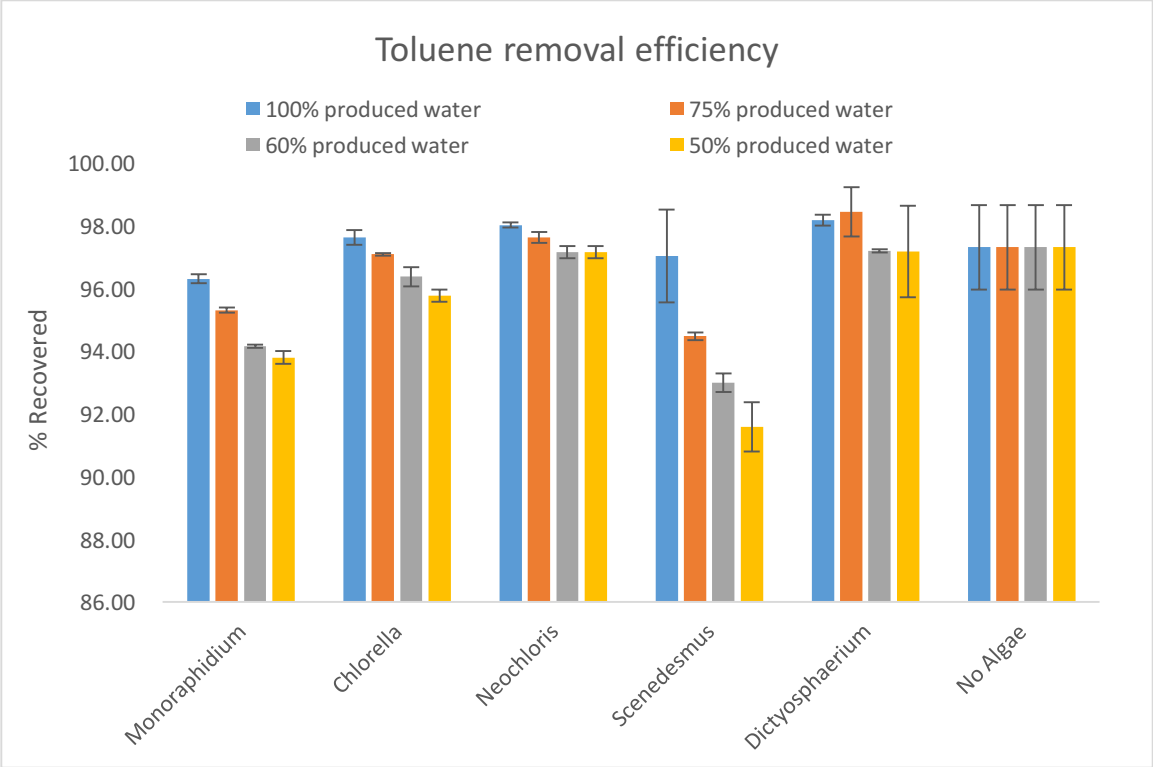


Figure 4.13: Benzene removal efficiency by five microalgae species from different of produced water concentrations.

Ethylbenzene removal efficiency:

Among other BTEX constituent's ethylbenzene concentration was found to be the lowest 1.05 mg/L (Table 4.1). Removal efficiency of ethylbenzene between different concentration didn't have any significant difference. Whereas, ethylbenzene removal efficiency had significant variation among different microalgae. Overall average ethylbenzene recovered from different produced water concentrations, where *Neochloris* sp. recovered 100%, *Dictyosphaerium* sp. recovered 98.12%, *Monoraphidium* sp.

97.60%, *Scenedesmus* sp. recovered 93.94%, *Chlorella* sp. recovered 90.09% and control recovered with 86.71% (Figure 4.14). Statistical significance showed ethylbenzene have higher removal efficiency can be obtained by *Neochloris* sp*, Dictyosphaerium* sp*, Monoraphidium* sp and with *Scenedesmus* sp species (Table 4.6).

Table 4.6: Average ethylbenzene removal efficiency:

PW level	**Microalgae Species**						
	Monoraphidium	**Chlorella**	**Neochloris**	**Scenedesmus**	**Dictyosphaerium**	**No Algae**	**Mean**
100%	97.42	71.43	100.00	100.00	100.00	86.71	92.59
75%	92.97	94.62	100.00	91.19	100.00	86.71	94.25
60%	100.00	94.32	100.00	93.63	100.00	86.71	95.78
50%	100.00	100.00	100.00	90.93	92.49	86.71	95.02
Mean	97.60	90.09	100.00	93.94	98.12	86.71	

Ethylbenzene removal effecency among different microalgae species were statictically significant with a p-value <0.05. least significant difference by different microalgae species is 7.52.

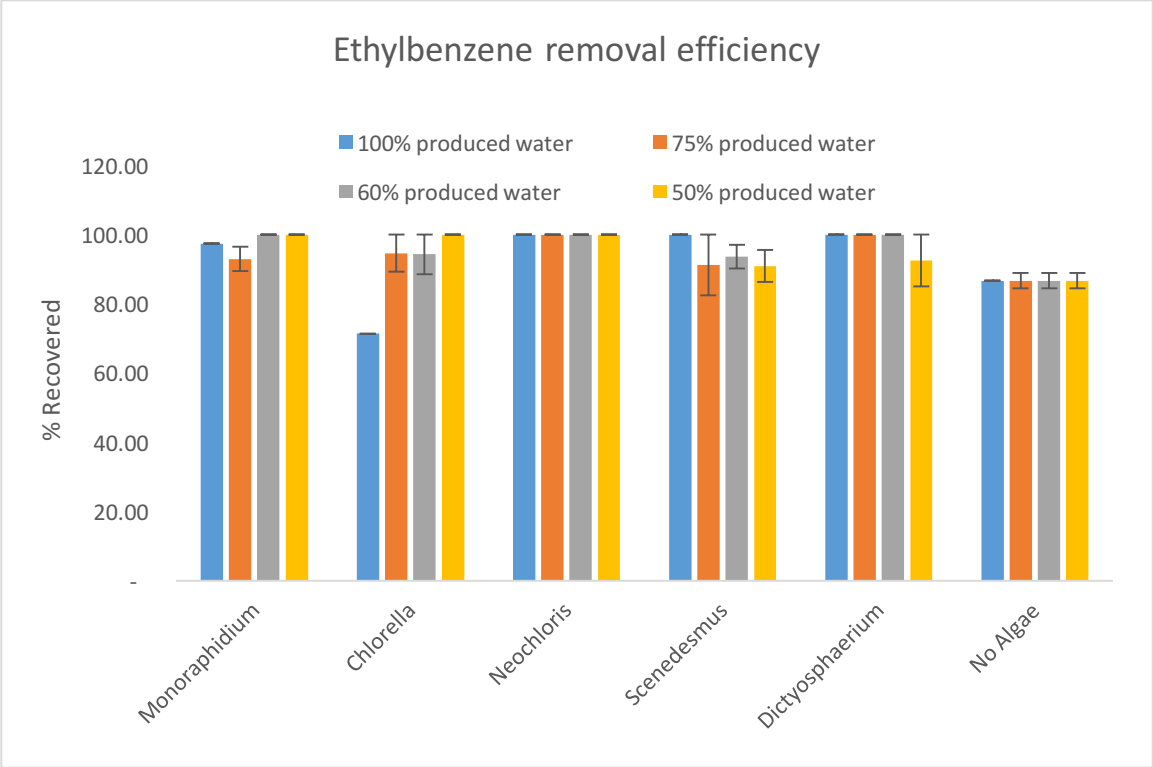


Figure 4.14: Ethylbenzene removal efficiency by five microalgae species from different of produced water concentrations.

Xylenes removal efficiency:

Produced water had an initial 3.11 mg/L concentration of xylenes (Table 4.1). Among all the microalgal treatment including control, no significant difference in xylenes removal efficiency was observed with different levels of produced water. Although no microalgae biomass was added in control, Xylenes removal efficiency was as high as 96.41% (Figure 4.15). The removal efficiency for *Dictyosphaerium* sp, Chlorella sp,

Neochloris sp, *Scenedesmus* sp and *Monoraphidium* sp were 95.96 %, 95.76 %, 94.59 %, 89.50 % and 88.40 % respectively. Finally, the xylenes removal efficiency was also found significant among microalgae species. Although apart from *Monoraphidium* sp. all other species can have similar xylenes removal efficiency from produced water.

Table 4.7: Average xylenes removal efficiency:

PW level	Microalgae Species						
	Monoraphidium	Chlorella	Neochloris	Scenedesmus	Dictyosphaerium	No Algae	Mean
100%	92.74	90.27	96.96	90.77	100.00	96.41	94.52
75%	83.08	99.16	93.89	89.69	94.29	96.41	92.75
60%	85.41	100.00	97.88	96.19	92.81	96.41	94.78
50%	92.35	93.63	89.62	81.36	96.75	96.41	91.69
Mean	88.40	95.76	94.59	89.50	95.96	96.41	

Xylenes removal effecency among different microalgae species were statictically significant with a p-value <0.05. least significant difference by different microalgae species is 6.53.

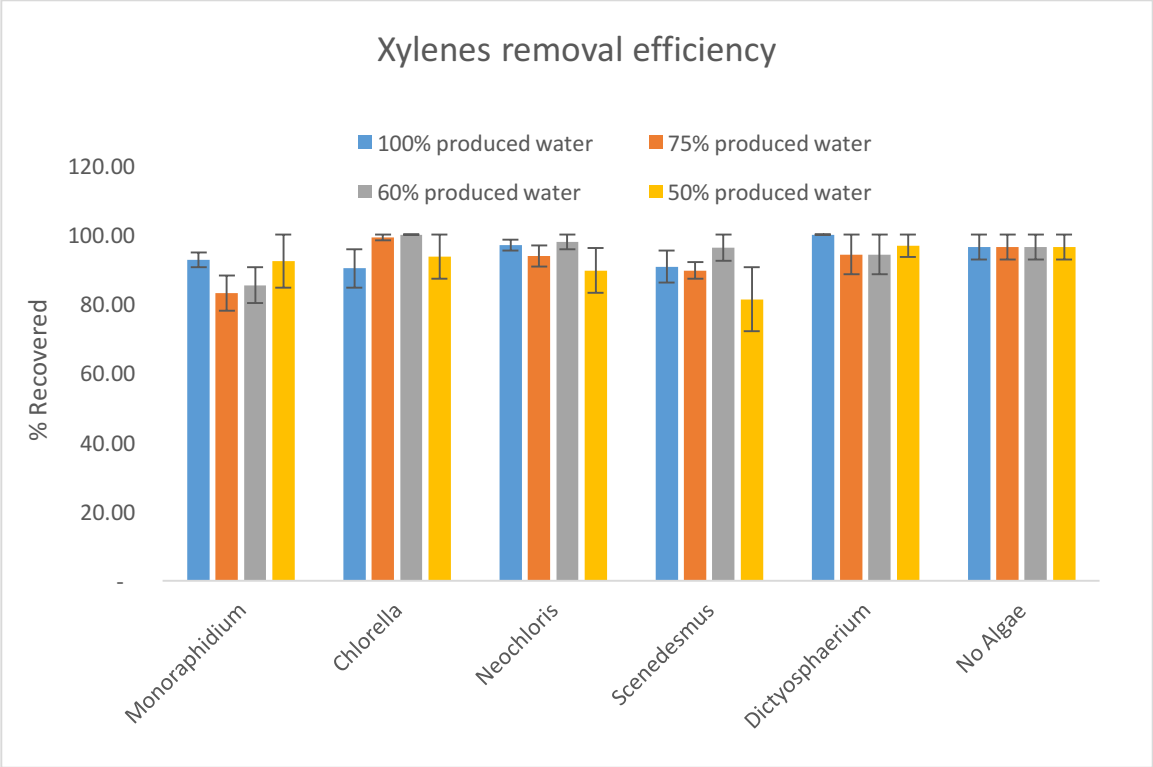


Figure 4.15: Xylenes removal efficiency by five microalgae species from different of produced water concentrations.

Studies have shown wide verities of approaches were biological treatment should an excellent alternate solution to remediate BTEX constitutes. In biological system different species like bacteria, fungi and algae can be used to treat BTEX (El-Naas, 2014). These biological treatments can be approach aerobic degradation (You, 2013). Most of the time these biodegradation processes are affected by the physical, chemical and biological condition of the produced water. Among them, the concentration of

inorganic nutrients, pH, temperature and adaptation of microbial community are the most important (Singh, 2010). A pervious study has found that under aerobic condition microorganisms are highly receptive to BTEX constituted (An, 2001). Among the monoaromatic hydrocarbons of BTEX, toluene was found to have a faster biodegradation due to their structure configure that allows the microorganism to oxidize the aromatic ring (Andreoni, 2007). BTEX have at list one possible aerobic degradation pathway. During the biodegradation process benzene is degraded to catechol, toluene into 3-methylcatechol, ethylbenzene to 3-ethylcatechol and finally the three types of xylenes usually to 3-methylcatechol. Later each of these constitutes cleaved by a dioxygenase (El-Naas, 2014).

Andreoni, (2007) also reported that biodegradation of BTEX requires dissolved oxygen to cleavage the aromatic nucleus as the acceptor of the electron during the biodegradation process. Zhang, (2013) reported that with a pH range of 7.2 to 7.4 *Mycobacterium cosmeticum* species can biodegrade 82 to 100 % of BTEX. Singh, (2010) also reported that a mixture of bacterial community in a batch system can reach up to 100% benzene, 80% toluene,100% ethylbenzene and 70% xylene degradation in a 7.5 pH solution. Whereas these studies found benzene, toluene, ethylbenzene and xylenes removal reached up to 100%, 97.75%, 100%, 95.96% by different algae species. Among all microalgae species *Dictyosphaerium* sp. was able to recover more compounds of BTEX then other species.

Recent studies have also shown a promising sign where BTEX can be use as a sole carbon source (Takáčová, 2015). Takáčová findings concluded that, among BTEX

constitutes toluene was biodegraded 63%, benzene and xylenes was biodegraded 40 %, and ethylbenzene with 30% by *Parachlorella kessleri* in a photo bioreactor. in this study, it showed even better result where almost 100% of benzene and ethylbenzene was recovered by microalgae, where 97.38% toluene and 95.96% xylenes recovered with microalgae. Also experimental result showed removal efficiency of BTEX also occurred in control due to the presence of bacterial community as it was also reported by previous study (Hendrickx, 2006).

Metal removal efficiency:

Metal removal efficiencies by different microalgae in different strength produced waters are shown in Table 4.8. From the table it is clear that *Dictyosphaerium* sp. had a higher removal efficiency of Mg, Cr, Ni, Cu, Sr and B. *Neochloris sp* were able to recover highest amount of Al, Mn and Fe. Similarly, *Scenedesmus* sp. removed highest amount of K and V*, Chlorella sp.* removed highest amount of Fe and Cd*,* and *Monoraphidium* sp. removed highest amount of Zn and Ba. Due to less biomass generation within produced water, the lowest amount of metals removal was found for *Monoraphidium sp.*

Table 4.8 Trace metals concentration in produced water.

Trace metal ™	Concentration of metals Before filtration (ppb)	Concentration of metals After filtration ppb	Maximum metals removal efficiency by microalgae	Microalgae species
K	73,618.87	67,740.06	11.27%	*Scenedesmus* sp.
Mg	41,715.72	39,257.55	13.9%	*Dictyosphaerium* sp.
Sr	11,198.61	10,573.55	21.23%	*Dictyosphaerium* sp.
B	4,259.05	3,747.13	20.23%	*Dictyosphaerium* sp.
Mn	318.56	318.56	87.80%	*Neochloris* sp.
Cu	224.97	180.78	91.65%	*Dictyosphaerium* sp.
Fe	287.94	100.19	100%	*Neochloris* sp.*;* *Chlorella* sp.
Ba	55.69	43.35	13.06%	*Monoraphidium* sp.
Cr	24.09	17.20	19.36%	*Dictyosphaerium* sp.
Al	114.41	13.68	100%	*Neochloris* sp.
Zn	25.09	7.87	100%	*Monoraphidium* sp.
Ni	7.83	3.71	92.29%	*Dictyosphaerium* sp.
V	1.87	1.46	36.26%	*Scenedesmus* sp.
Cd	0.09	0.06	97.37%	*Chlorella* sp.

Initially, after analyzing metals, some elements were found over 1 ppm or mg/L level. Such elements are K, Mg, Sr, and B as shown in Table 4.8. Where rest of the elements concentrations like Mn, Cu, Fe, Ba, Cr, Al, Zn, Ni, V and Cd was below 1 ppm (Table 4.8). After the microalgae biomass growth results showed a 100 % removal efficiency of Al, Fe and Zn from the produced water. Also recovered concentrations of other element were found according (Cd > Ni > Cu > Mn > V > Sr > B > Cr > Mg > Ba). On the other hand, the lowest removal efficiency was found for K with 11.27 % (Table 4.8).

Microalgae require some of these elements like Zn, Fe, Cu, Mn, B, Mg, and K as micronutrients (Stanier, 1971). Some studies also found that at higher concertation of these elements may increase toxicity (Cai, 2013). In our findings showed that *Dictyosphaerium sp* was able to grow better than other species and able to recover more element. Another study also found some similarity where *Dictyosphaerium sp* were resistance to Cr within growth medium (Pereira, 2013). A study by López-Rodas also found that *Dictyosphaerium sp* which was able to grow within metal reach water. All of these findings were found to support our experimental result were produced water constitutes had less effect on *Dictyosphaerium sp.* Furthermore, metals removal efficiency also depends on the morphological structure of microalgae species, where microalgae may present in unicellular, colonial and filamentous shape (Shehata, 1999).

Nutrients availability within the growth medium is one of the most important factors that has a direct impact on microalgae growth. Usually, these nutrients are divided into two main groups, starting from macronutrients to micronutrients. In macronutrients includes nitrogen, phosphorus and carbon sources. On the other hand, micronutrients include potassium K, magnesium Mg, calcium Ca, iron Fe, boron B, manganese Mn, zinc Zn, molybdenum Mo, copper Cu and cobalt Co (shown in Table 3.1). Each of these elements has their function for the growth of microalgae spices (Miazek, 2015).

Among these micronutrients, metals are found with a small concentration. Microalgae utilize These metals by two different sorption mechanisms. Mechanisms like adsorption, microalgae directly adsorb metals on the cell surface and in absorption metals are used by cells for intercellular activity (Monteiro, 2012).

The finding from this study showed that some of these essential elements were present within the collected produced water. Thus, this could be one of the underlying reason for the microalgae species to grow within the produced water. Although some studies also concluded that these metals can induce toxic effect among many microalgae and also in some cases, the tolerable ranges are species specific (Napan, 2015).

In this experiment, 14 metals species within the produced water. Among them, only half of the metals considered as micronutrients. These micronutrients like potassium are essential in many enzymic reactions (Talling, 2010). Whereas, copper and iron as an essential for photosynthetic electron transport system (Andersen, 2005). In DNA transcription and phosphorus uptake zinc is used by microalgae (Sunda, 2012). On the other hand, metals like cadmium and chromium are nonessential metals, and that may have a negative effect on cell division and reduce the photosynthetic ability at high concentration (Monteiro, 2012). A previous study found that higher chromium concentration 0.75 ppb causes a significant reduction in Chlorophyll a intensity in *Scenedesmus* sp. (Millach, 2015). It was found that *Dictyosphaerium* sp and *Chlorella pyrenoidosa* can tolerate as high as 13 - 17 mg/L and 2mg/L chromium concentration respectively (Hörcsik, 2006; D'ors, 2010). Whereas result from this study found a lesser concentration of chromium. This could also be one of the reasons for higher biomass yield for *Scenedesmus* sp, *Dictyosphaerium* sp, and *Chlorella* sp. On the other hand, lack of iron may have reduced growth rate for *Neochloris* sp and after 72 hours.

CHAPTER 5: CONCLUSION

Below are the main conclusions that could be drawn from this study.

- *Dictyosphaerium* sp. microalgae showed a high growth potential within all produced water concentration level. On an average, *Dictyosphaerium* sp. produced 0.5 g/L biomass density on different strength produced water.
- Total nitrogen removal efficiency reached to 63.76% when Scenedesmus sp. was grown in produced water.
- Total phosphorus removal efficiency reached to 88.83% when *Dictyosphaerium* sp. was grown in produced water.
- Despite low biomass generation, *Neochloris* sp. removed 41.61% of total organic carbon from the different level of produced water concentrations.
- Although benzene and ethylbenzene removal efficiency were 100 % for all the different produced water, there were small amounts of toluene and xylene which remained in the produced water. Evaporation and bacterial mineralization could have been the possible reasons for such lower residual BTEX concentrations in the produced water.
- Among all the microalgae species *Dictyosphaerium* sp. was able to retrieve the maximum number of metals from the produced water. Although despite less biomass generation *Neochloris* sp. was able to recover 100% of Fe and Al from the produced water.

CHAPTER 6: RECOMMENDATIONS AND FUTURE WORK:

It was clear that salinity of the produced water had a strong effect on the growth of majority of the microalgae tested in this study. Strains that can tolerate a wider range of salinity can also be used in the future screening phase. Further studies with longer exposure period are needed to determine the adaptability of different microalgae culture. Also, acclimatization of microalgae culture needs to be explored to enhance the pollutants removal efficiency. Removal effeciecy may also vary with pH variation thus continuous pH fluctuation during the experimental period is needed. Findings from the current study where *Dictyosphaerium* sp perform better should be taken into consideration for future research where detail removal efficiency mechanisms can be specified and enhanced. Furthermore, to support the removal of organic compound, generated biomass should be analyze by Fourier transform infrared spectroscopy (FTIR).

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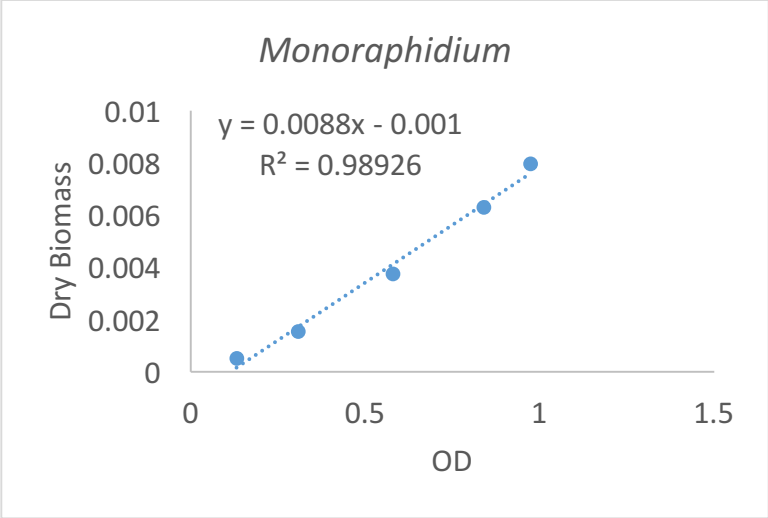
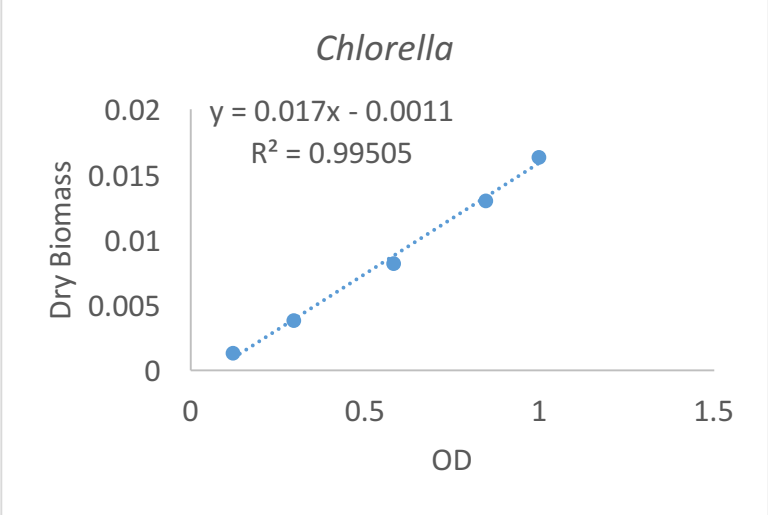
Appendix

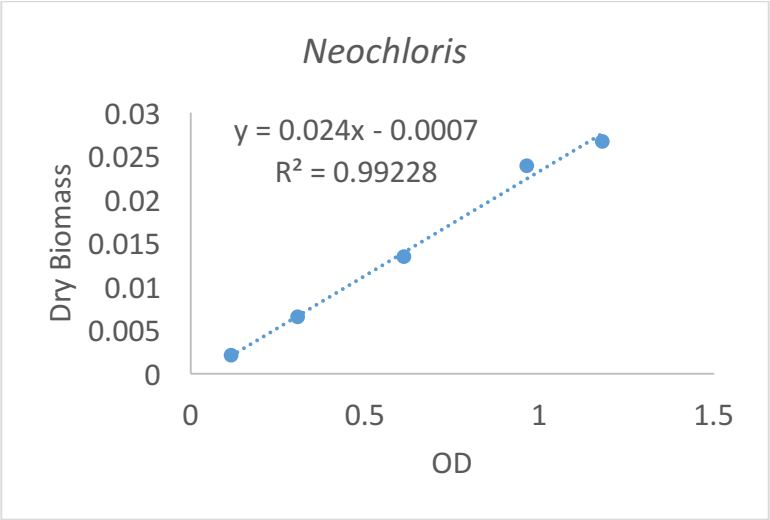
Appendix A Relation between Optical density & Dry biomass:



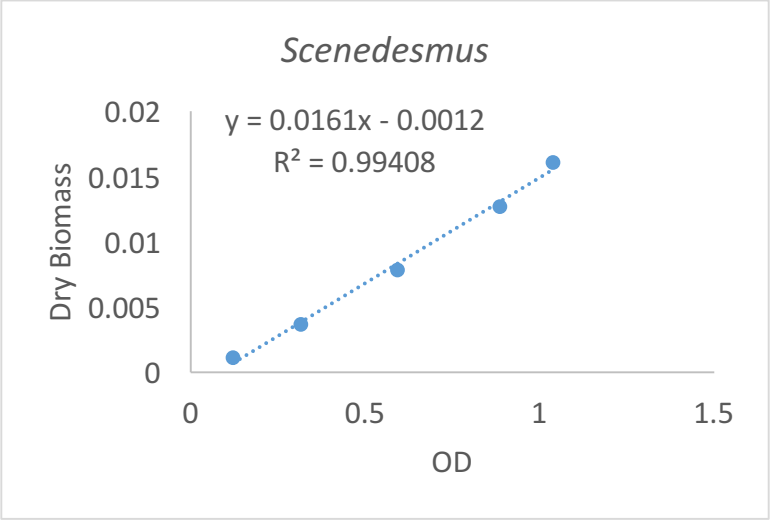
Monoraphidium sp. Relation between optical density and biomass density



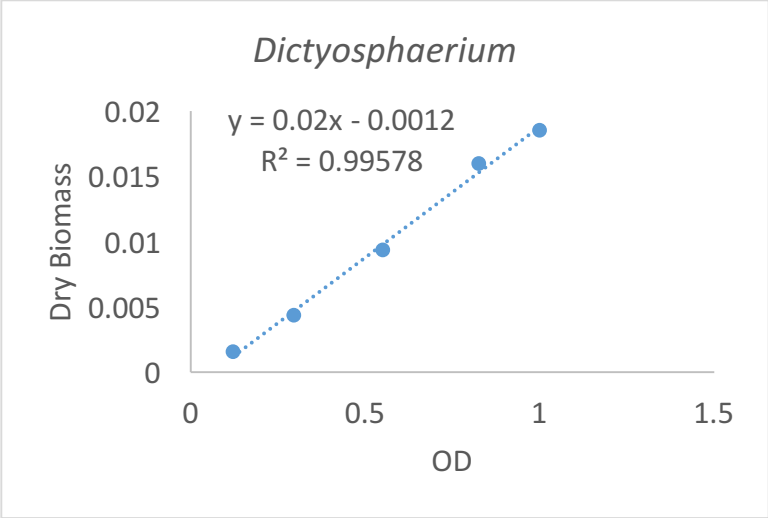
Chlorella sp. Relation between optical density and biomass density



Neochloris sp. Relation between optical density and biomass density



Scenedesmus sp. Relation between optical density and biomass density



Dictyosphaerium sp. Relation between optical density and biomass density

Appendix B Total Nitrogen (TN):

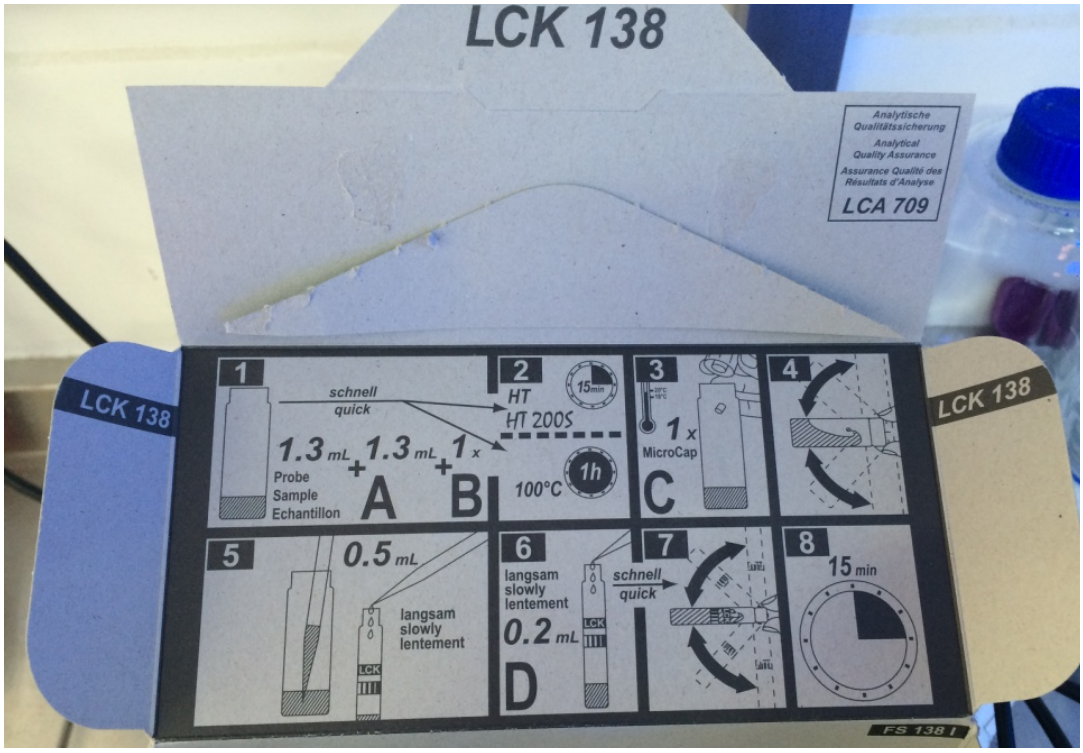
Method: HACH Analysis

Reagents:

- Four stocks are used A (1.3 ml), B (1 tablet), C (1 tablet), D (0.2 ml)

Steps:

1. Take 1.3 ml of sample + add 1.3 ml of stock A + add 1 tablet from stock B (heat the mixed sample for 1 hours at 100^{o}C)
2. Later after cooling down the mixture to room temperature add 1 tablet of stock C and shake gently till the tablet dissolves
3. Take 0.5 ml of sample and add it to LCK solution bottle (shake gently)
4. Finally add Stock D 0.2ml (shake gently)
5. Wait for 15 minuets and place the LCK solution bottle in HACH analyzer.



Total Nitrogen cuvette kits.

Appendix C Total Phosphorous (TP):

Method: Spectrophotometric Analysis

Reagents preparation steps:

1. Stock A Sulfuric acid (H_2SO_4):
   (15.5 ml H_2SO_4 add in 50ml DI water)
2. Stock B Antimony Potassium Tartrate solution:
   (0.3g Antimony Potassium Tartrate in 100 ml DI water)
3. Stock C Ammonium molybdate solution:
   (4g Ammonium molybdate in 100 ml DI water)
4. Stock D Ascorbic acid solution:
   (1.76g Ascorbic acid in 100 ml DI water)

5. Stock E Ammonium Persulfate solution:
   (16g Ammonium Persulfate in 50 ml DI water)

6. Stock F Sodium hydroxide:
   (2.4g Sodium hydroxide add in 100ml DI water)

7. Stock G Phosphate stock (to prepare calibration standards 0,100,200,500, and 1000 ppb):
   (1.0985g KH_2PO_4 add in 250ml DI water)

8. Mix Stocks A, B, C & D in centrifuge tube (Solution M)
   (Stock A 25ml+ Stock B 5ml+ Stock C 15ml+ Stock D 30ml + add 25 ml DI water)

Sample Digestion:

9. Add 5 ml of sample and standard in digestion tubes

10. Add (0.125 ml of Stock E + 0.1 ml of Stock A) in the tubes

11. Put all tubes in 121 ^oC for 30 minutes

Reading OD at 880 nm wavelength in spectrophotometer:

12. Add 1.5 ml of Solution M in the tube (after neutralizing the pH)

13. Read the optical density at 880 nm wavelength after 10 minutes

Appendix D Analysis of varience

Analysis of varience for different pollutants removal effecincy as affected by different dilution of produced water and different microalgae species.

MS												
S.O.V	*df*	***TN***	***TP***	***TOC***	***Benzene***	***Toluene***	***Ethyl benzene***	***Xylene***	***K***	***Mg***	***Sr***	***B***
Produced Water Level	3	28.85	184.23*	353.37*	0.00	14.61*	33.38	39.18	24.41	6.91	688.96*	7,106.08*
Microalgae	5	4,839.76*	9,189.84*	831.86*	0.00	27.73*	320.16*	150.88*	88.79*	246.13*	557.43*	1,394.85*
Interaction (MAXPW)	15	87.01*	52.19	93.07*	0.00	1.99	116.01	56.13	39.12*	24.47	536.69*	2,192.99*
Error	48	10.82	28.86	27.42	0.00	1.66	81.44	61.40	17.44	16.55	122.83	54.33
Total	71											

MS											
S.O.V	*df*	***Mn***	***Cu***	***Fe***	***Ba***	***Cr***	***Al***	***Zn***	***Ni***	***V***	***Cd***
Produced Water Level	3	175.86	300.19*	2,005.60	24,777.91*	1,529.11*	1,815.16	5,010.53	440.31	565.76	2,012.51
Microalgae	5	10,780.44*	276.49*	16,157.04	64.21	447.29	9,642.35	12,585.41*	7,068.51*	2,504.44*	22,928.38*
Interaction (MAXPW)	15	647.57*	38.44*	888.56	2,584.02*	753.49*	3,434.94	8,149.88*	2,234.29	712.97*	2,125.78*
Error	48	204.09	11.48	1,069.75	173.08	296.00	2,902.50	3,130.10	1,717.31	251.24	992.52
Total	71										