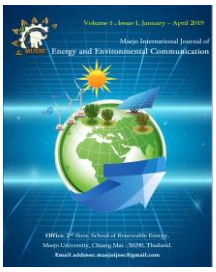
Maejo International Journal of
Energy and Environmental Communication



ARTICLE

Desalination of Polymer and Chemical industrial wastewater by using green photosynthetic microalgae, *Chlorella* sp.

Prakash Bhuyar[1], Dang Diem Hong[2], Emelina Mandia[3], Mohd Hasbi Ab. Rahim[1], Gaanty Pragas Maniam[1], Natanamurugaraj Govindan[1]*

[1]Biotechnology Laboratory, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia.
[2]Algae Biotechnology Department, Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Str., Cau Giay, Hanoi, Vietnam
[3]Microalgae Systematics and Applied Phycology Research Laboratory Science and Technology Research Center, Biology Department, De La Salle University 2401 Taft Avenue, Manila, Philippines

*Corresponding author, *E-mail address*: natanam@ump.edu.my

ARTICLE INFORMATION

Received 19th July 2019
Accepted 13th October 2019

Keywords:
Microalgae
Chlorella sp.
Industrial waste water
Salinity reduction
TDS

ABSTRACT

In this investigation microalgae *Chlorella* sp. were isolated and identified from the industrial wastewater. Microalgae species was mass cultivated by using BG11 medium. After 30 days, mixture of *Chlorella* with different wastewater A, B, C, D, E and F with different ration of *Chlorella*: wastewater which were 1:6, 1:1, and 2:1. Incubated at room temperature at illuminated area. Dissolved oxygen, TDS, salinity, pH, optical density, oxygen saturation and conductivity were measured for day 0, 10, 20 and 30. For microalgae in wastewater A and C, value of pH, salinity, dissolved oxygen, oxygen saturation, conductivity, salinity and TDS did not change while absorbance value decreased from day 0 to day 30. For wastewater B, D, E and F, the absorbance and pH value increase for all concentration from day 0 to day 30. The highest oxygen saturation after 30 days for wastewater B, D, E and F was at concentration 1:6, 2:1 and 1:1 respectively. The highest dissolved oxygen for wastewater B, D and E was at concentration 1:1 and F was at 2:1. The lowest conductivity, salinity and TDS for wastewater B, C, D and F were all at the concentration of 2:1. The result showed that, Chlorella managed to reduce the salinity for wastewater B, C, D and F, at concentration of 2:1 which were 3.67 %, 4.53 %, 5.4 % and 4.91 % respectively.

1. Introduction

Pollution has become the main concern nowadays. One of the pollutions is water pollution. Water pollution has become one of the biggest issues as water is the home/habitat to various marine organisms and the source of clean water for human use and consumption. Water is considered polluted when they contain higher chemical or biological agents than the natural content. Wastewater contains high salinity that can affect marine living things when they are released to the sea or river. Besides that, it also affects the ecosystem and degrades the quality of our lifestyles. It can affect the quality of our water supply such as for drinking, farming, and industrial use and also for recreation. According to Pei Xu and his group salinity which means that it contains salts (Xu et al., 2013).

Wastewater especially industrial wastewater is known to have high salinity as they contain dissolved mineral salts for example calcium, magnesium, potassium, sodium, sulfates and chloride. There are several ways of how these salts reach the wastewater. Some of the coastal cities around the worlds such as Hong Kong use the simple treated sea water to flush the toilets (Liu et al., 2016). Other example of how the salts can reach the wastewaters is from the industrial sector. The process such dye manufacturing can produce high inorganic salts. These inorganic salts will flow to the sewage and become part of wastewaters. As the high salinity of wastewater can bring harm to the ecosystem and to human, action need to be taken to overcome the high salinity of the wastewaters.

In the agricultural production (Bernard and Rout, 2018), high salinity of the soil water will lead to the osmosis process where the water molecule will pass through the semipermeable membrane from the less concentrated area to the more concentrated area. In this matter, the water molecule from plant will flow to the soil. This will result in dehydration of the plant and finally results in the death of the plants. According to the Queensland Government, it will affect plant nitrogen uptake that will lead to the reducing growth and death of the plants. The lack of nitrogen uptake will increase the chloride concentration in plant that will poison the plants. It is estimated that 80% of wastewater was used for irrigation in developing countries (Zhang and Shen, 2017). Thus, high salinity in wastewater will cause reduction of crop production in these countries.

Wastewater can also be treated to drinking water quality. Countries such as Singapore and Mexico already demonstrate the use of drinking water from the wastewater that has been treated. When the salinity of the wastewater is high, the process of reducing the salinity takes time and the cost of treatment will increase. When the high salinity water is used as drinking water, it will affect human bodies especially the kidney.

Algae are classified under kingdom Protista. They look like plant, but they are not plant. Just like plant, they grow through photosynthesis and can be eukaryotic or prokaryotic (Raven et al., 2014; Bhuyar et al., 2018). They are unicellular organisms that grow everywhere on Earth typically in freshwater and marine system. The reason they look like plant but not plant is because they lack roots, stem and leaves. Microalgae are one of the types of algae. They use nitrogen and phosphorus for their growth (Bhuyar et al., 2020).

Microalgae are used to treat the waste water due to its ability to use the inorganic nitrogen and also the phosphorus for their growth and their capability in removing heavy metals and toxic organic compound. It also does not lead to the secondary pollution from metabolite the product of the microalgae (Schirrmeister et al., 2016).

They usually need water to grow. Algae are responsible and contributed for 40% to 50% which is half of the total Earth photosynthetic primary production (Gangl et al., 2015). Microalgae have been found to be useful in pharmaceuticals, wastewater treatment, food, agriculture and biofuels (Yaakob et al., 2014). The use of the microalgae to treat waste water has been investigated by Oswald since 1960s (Abdulsada, 2014; Bhuyar et al., 2020). The treatment process that use algae or microalgae is known as phycoremediation. Microalgae are one of the living things that have high tolerance to the salinity. They can survive in the high salinity water. That is the reason why we can normally find them in the waste water.

It is difficult to treat high salinity of waste water by using biological treatment system. This is because of the high salinity. There are a few ways or methods that are used to treat high salinity of waste water. For example, the traditional activated sludge process where the salt tolerant microorganisms are used to treat the high salinity waste water. Other than that, physicochemical method such as ion exchange, reverse osmosis, and electrodialysis are also used to treat the high salinity waste water. There are a few phases for the wastewater treatment. They are pretreatment, primary treatment, secondary treatment, tertiary treatment and disinfection (Doble and Kumar, 2005).

Nowadays microalgae refer to most popular environmentally friendly applications (Ramaraj et al., 2016; Tsai et al., 2017; Unpaprom et al., 2017). *Chlorella* are abundantly available everywhere on the earth. Due to limited literature is available on the desalination study by microalgae, *Chlorella* can be used as best water bioremediation. In this study, *Chlorella* sp. were used to reduce the salinity of industrial wastewater. Microalgae usually *Chlorella* are widely used in wastewater treatment as they can reduce organic matter, nitrogen and phosphorus and to remove heavy metals. High salinity wastewater can give negative impact to the environment. The algae that are abundantly found in the wastewater are *Chlorella*, Ankistrodesmus, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria, Micractiniumm and also Golenkinia. The genera that are mostly tolerant to the wastewater and their pollution are *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium* (Sekhar et al., 2014). Scendesmus has been proved to be able to absorbs salt and make use of the salts in their metabolism such as for their growth. Even so, the drawbacks of using Algae in treating wastewater is that the harvesting of the biomass (El Nadi et al., 2014).

The effect of microalgae on the compound such as N and P has been studied by multiple people but there are not many people who study about the effect of microalgae on the high salinity wastewater.

2. Methodology

2.1 Algae collection

The algae from the seawater were isolated. The debris or large particles will be filtrated in order to separate the large particles from the sample. The sample was isolated and cultivated at 20°C. Illumination was supplied by artificial light. The sample was cultivated in BG 11 media.

2.2 Media preparation

Algae media (BG 11) was used to cultivate the algae (Xiang et al., 2018). The composition of the media would be Sodium nitrate (1g/L), Dipotassium phosphate (0.25 g/L), Magnesium sulphate (0.513 g/L), Ammonium chloride (0.05 g/L), Calcium chloride (0.058 g/L), Ferric chloride (0.003 g/L) and distilled water (1 L). 15 g/L agar was added to form solidified media for isolation (Bhuyar et al., 2019).

2.3 Collection of the wastewater

Wastewaters were collected from six different industrial located in Pahang state of Malaysia. Wastewaters were collected from Organic Chemical company (UMP-W-201), LDPE Polymer production company (UMP-W- 301), Fertilizers Production Company (UMP-W- 401), Dye Manufacturing company (UMP-W-501), Mineral water treatment Plant (UMP-W – 601) and Petroleum Industry (UMP- W-701). The wastewaters were kept in white plastic bottles. Three bottles of each industrial effluent wastewater were kept. The wastewaters were labeled with A, B, C, D, E and F.

Label	Code	Wastewater
A	UMP-W-201	Organic Chemical Company
B	UMP-W- 301	LDPE Polymer production Company
C	UMP-W- 401	Fertilizers Production Company
D	UMP-W-501	Dye Manufacturing Company
E	UMP-W - 601	Mineral water Treatment Plant
F	UMP- W-701	Petroleum Industry

2.4 Isolation & identification of microalgae

The microalgae plated on BG-11 Agar to obtain single colonies. Approximately, 15-20 ml of the agar medium per one petri dish was used. The microalgae were spread evenly onto the surface of the agar. The volume of diluted microalgae that was use for the spreading is 1ml. The agar was stored or incubated at 20-25°C for 14 days. The grown algae were streaked on a new agar plates to produce unialgal plates. After 14 days, the algae were taken and observed under microscope and the images were compared with algae in the algae manual book by referring to the study done by Selvaraj and his group (Selvarajan et al., 2015).

2.5 Mass cultivation

BG 11 media were prepared to be used in order to cultivate the algae. The grown algae were transferred into two 1 L Erlenmeyer flask with each contain 800 mL of BG 11 medium. The respective microalgae culture was kept for mass production by maintaining culture conditions (Andersen, 2005).

2.6 Wastewater and algae mixture preparations

The filtrated wastewater and cultivated algae were mixed in a certain ratio. Three different ratios of microalgae: wastewater was used which were 1:6 (75 mL:225 mL), 1:1 (150:150) and also 2:1 (200:100). The total mixture of wastewater and microalgae were 300 ml. The volume of the wastewater and microalgae were measured using measuring cylinder. The mixture of wastewater and microalgae were kept in the 500 mL conical flask.

2.7 Microalgae cultivation in wastewater

The algae were incubated for 30 days. The following parameters such as pH, oxygen saturation (%) dissolved oxygen (DO) in mg/mL, salinity, total dissolved solids (TDS), conductivity and optical density were measured every 10 days. pH, salinity and TDS were measured by using OAKTON PC 650 pH/ Conductivity Meter while the optical density was measured by spectrophotometer at wavelength of 700 nm. DO was measured by YSI 5100 Dissolved Oxygen Meter.

For the pH, salinity and TDS measurement using OAKTON PC 650 pH/ Conductivity Meter, the probe was immersed in distilled water before taking the measurement. The probe was washed with distilled water and immersed in distilled water before taking the reading for different sample.

Nomenclature and Abbreviation

FTIR	Fourier Transform Infrared Spectroscopy
HDPE	High Density Polyethylene
LAF	Laminar Airflow
LDPE	Low Density Polyethylene
SEM	Scanning Electron Microscopy
UV	Ultraviolet

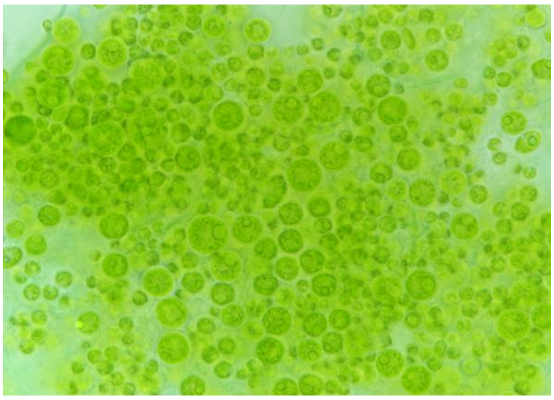


Figure 3.1. Morphological image of microalgae under fluorescent microscope at 100 X magnification.

For the measurement of DO using YSI 5100 Dissolved Oxygen Meter, the probe was immersed in distilled water before taking the measurement. The probe was washed with distilled water and immersed in distilled water before taking the reading for different sample. For the optical density measurement, the optical density was measured at wavelength of 700 nm. The blank used for each mixture of microalgae and wastewater must be the from the same source of wastewater.

3. Results and Discussion

3.1 Identification and Isolation of microalgae

The microalgae that have been collected from Pantai Gelora that were isolated were observed under fluorescent microscope to identify them according the microalgae manual book. The identification was based on their morphology, color, shape and the physical of the microalgae. According to the previous study the microalgae, the microalgae was identified as *Chlorella* sp.

3.2 Wastewater Treatment with Microalgae Analysis

3.2.1 Optical Density of the Microalgae

The optical density of microalgae in wastewater A, B, C, D, E and F at three different concentrations for 30 days were measured with spectrophotometry at 700 nm. The results of absorbance were plotted as shown in Figure 3.2 until (a), (b), (c), (d), (e), (f) respectively.

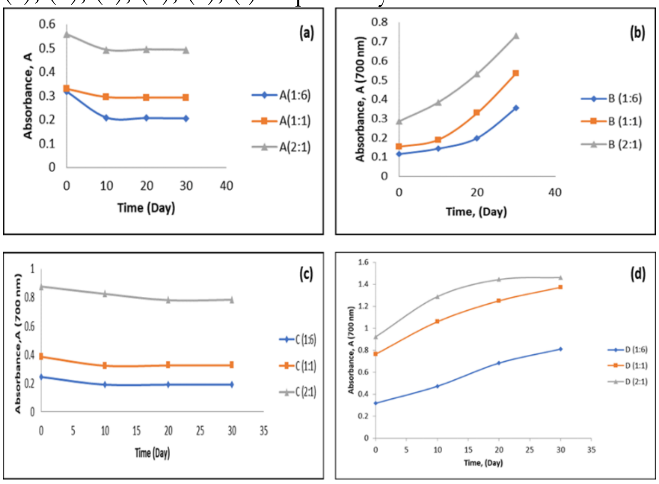


Figure 3.2: The Graph of Optical Density of Microalgae AT 700 nm in wastewater (a): A at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (b): B at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (c): C at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (d): D at three different concentrations (1:6, 1:1, and 2:1) for 30 Days.

From Figure 3.2(a), the absorbance value of microalgae in wastewater A (UMP-W-201) (1:6) decrease from 0.319 A to 0.206 A in 30 days. There was no increasing in the value of absorbance in 30 days. The low pH of the wastewater which was in acidic pH was not suitable for the growth of Chlorella and causes the microalgae to die. The value of absorbance obtained after the 30 days might be due to the suspended particles and dead microalgae. The absorbance value for Chlorella in wastewater A at ratio 1:1 and 2:1 also decrease in 30 days. There was no increase of absorbance for microalgae in wastewater A for all three concentrations. Even so, the absorbance of Chlorella in wastewater A for concentration 2:1 after 30 days was the highest out of three microalgae in different ratio of Chlorella and wastewater which was 0.493 compared to 0.293 (wastewater A 1:1) and 0.206 (wastewater A 1:6). This might be due to the volume of *Chlorella* added in wastewater A (2:1) was the highest compared to wastewater A (1:1) and (1:6). As for the *Chlorella* in wastewater B Figure 3.2(b) (UMP-W- 301) (1:6), the absorbance increased from 0.114 A to 0.354 A in 30 days. This was due to the pH of the water were suitable for *Chlorella* to grow. The nutrients in the wastewater also enough for the *Chlorella* to grow as the amount of the *Chlorella* added was the less and the volume of wastewater was higher than the volume of microalgae added. For the microalgae in wastewater B with ratio of 1:1 and 2:1, the absorbance increased from 0.153 A to 0.535 A and 0.286 A to 0.73 A respectively from day 0 to day 30.

For the *Chlorella* in wastewater C Figure 3.2 (c) (UMP-W- 401) with the ratio of (1:6), the absorbance also decreases from 0.242A to 0.189 A from 0 day to 30 days. While for the Chlorella in wastewater C with ratio 1:1 and 2:1, the absorbance decreases from 0.385 A to 0.326 A and 0.878 A to 0.785 A respectively. There was no increase in absorbance for *Chlorella* in all three concentration of wastewater. The decrease of absorbance was due to the death of *Chlorella* in all three concentrations. The death of those microalgae which was the *Chlorella* was due to the low pH of the wastewater (Xiang., 2018). Low pH environment or highly acidic environment was not suitable for the growth of *Chlorella* and will kill the *Chlorella*. According to Figure 3.2(d), absorbance for microalgae in wastewater D (UMP-W-501) with ratio 1:6, 1:1 and 2:1 increased from 0 day to 30 days. Absorbance increased from 0.318 A to 0.812 A, 0.768 A to 1.373 A and 0.92 A to 1.462 A respectively for ratio 1:6, 1:1 and 2:1. There was only a slight increased for *Chlorella* in wastewater D with ratio 2:1 from day 20 to day 30 which were 1.443 A to 1.462 A. The slight increase of the absorbance value was due to the *Chlorella* reach the stationary phase of their growth where the rate of cell dead is equal to the rate of cell growth. Lack of nutrient and many wastes in the mixture might be the cause of stationary phase.

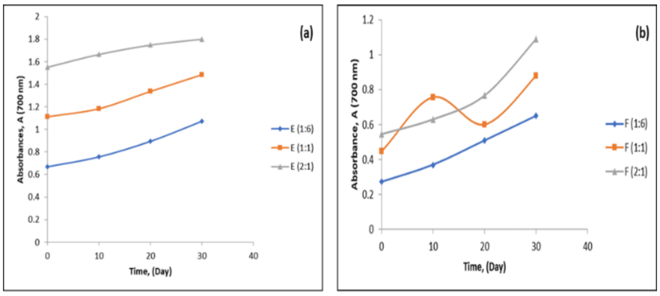


Figure 3.3: The Graph of Optical Density of Microalgae at 700 nm in wastewater (a): E at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (b): F at three different concentrations (1:6, 1:1, and 2:1) for 30 Days.

At the same time, the absorbance of microalgae in wastewater E Figure 3.3(a) (UMP-W-201) for the ratio of 1:6, 1:1 and 2:1 was 0.67 A to 1.071 A, 1.114 A to 1.484 A and 1.55 A to 1.8 A respectively. There was no decrease of absorbance from day 0 to day 30 for *Chlorella* in wastewater E. Even so, for ratio 2:1, there was only a slight increase of absorbance from day 20 to day 30. This is due to the *Chlorella* almost reach the stationary phase. Lastly for the optical density, absorbance of *Chlorella* in wastewater F Figure 3.3(b) (UMP- W-701) at ratio 1:6, 1:1 and 2:1, was 0.271 A to 0.649 A, 0.448 A to 0.879 A and 0.545 A to 1.09 A respectively from day 0 to day 30. There was only a slight increase of absorbance for ratio 2:1 from day 20 to day 30 which were from 0.966 A to 1.09 A due to the stationary phase. The absorbance of *Chlorella* in all wastewater with ratio of (1:6) was lower than *Chlorella* in the wastewater with ratio of (1:1) and (2:1) because the volume of *Chlorella* added was less. Thus, the light absorbed by the *Chlorella* in the wastewater with ratio (1:6) was lesser compared to *Chlorella* in wastewater with ratio 1:1 and 2:1. The results show that for *Chlorella* in wastewater A and C, they showed decrease of absorbance value from day 10 to day 30. This is due to the death of *Chlorella* in wastewater A and C since they have low pH. The pH was not suitable for Chlorella to survive. For wastewater B, D, E and F the absorbance increases for all concentration from day 0 to day 30.

3.3 pH of the Mixture of Microalgae and Wastewater

The pH of microalgae and wastewater mixture A, B, C, D, E and F at three different concentrations for 30 days were measured by using OAKTON PC 650 pH/ Conductivity Meter. The results of pH were plotted as shown in Figure 3.4 until (a), (b), (c), (d), (e), (f) respectively.

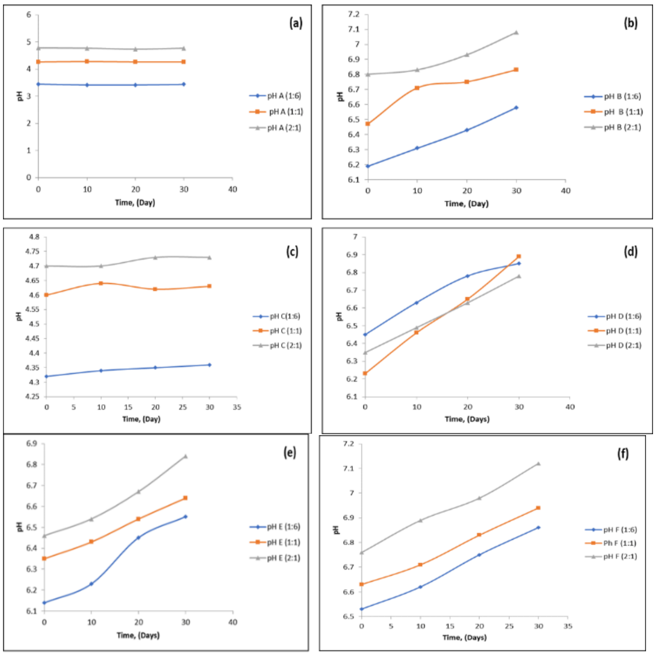


Figure 3.4: The Graph of pH of Microalgae and Wastewater (a): A at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (b): B at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (c): C at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (d): D at three different concentrations (1:6, 1:1, and 2:1) for 30 Days, (e): E at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (f): F at three different concentrations (1:6, 1:1, and 2:1) for 30 Days.

Based on Figure 3.4(a), the pH for the mixture of Chlorella and wastewater A at ratio 1:6, 1:1 and 2:1 was 3.45 to 3.44, 4.27 to 4.27 and 4.78 to 4.77 respectively from day 0 to day 30. There was not much different of pH for each mixture ratio as the *Chlorella* was dead. *Chlorella* cannot survive in the mixture because the mixture was acidic and not suitable for their growth. As for *Chlorella* in wastewater C figure 3.4(c), the pH decreases from day 0 to day 30 for all three concentrations. However, the pH increased from concentration 1:6 to 2:1. This was due to the volume of microalgae added to the wastewater. As more *Chlorella* added to the wastewater, the pH increased. Based on {figure 3.4(b)}, pH increased for *Chlorella* in wastewater B for all three concentrations. According to the data obtained in the Table A.2, the increase of pH from day 0 to day 30 for Chlorella in wastewater B for ratio of 1:6, 1:1 and 2:1 were 6.19 to 6.58, 6.47 to 6.83 and 6.8 to 7.08 respectively. *Chlorella* managed to grow at this pH as the suitable pH for them to grow is 6.5 to 8 (Rachlin and Grosso, 1991). Based on Figure 3.4 (d), (e), (f), Figure 4.12, Figure 4.13, pH for *Chlorella* in wastewater D, E and F increased as the day increased. The increase of pH also contributes to the growth of *Chlorella* as the growth of *Chlorella* in wastewater B, D, E and F also increase from day 0 to day 30. There was only a slight increase of pH during day 20 to day 30. This was due to the *Chlorella* achieve stationary state during that time. The pH increased because of the assimilation of the photosynthetic CO_2 during the photosynthesis process. Other than that, as the *Chlorella* absorb or utilize the nitrogen that exists in the wastewater, the pH increased as OH- ion was produced during the process of absorbing/utilizing nitrogen (Andersen, 2005) pH 3 to 6.2 which are acidic and pH 8.3 to 9 which are alkaline are not suitable for the growth of Chlorella as they can hold back the growth of *Chlorella* 8 (Rachlin and Grosso, 1991). According to Zhang et al. (2014), the increase of pH might be because of the consumption, absorption of CO_2 and the algal release at the stationary phases (Park et al., 2012; Gao et al., 2012). Total uptake of phosphate and nitrogen increased directly proportional to the pH. A study was done to investigation the efficiency of microalgae to take up nitrogen and phosphate at pH 5, 7, 9, 10 and 11.

3.3.3 Oxygen Saturation (%) of the Mixture of Microalgae and Wastewater

The oxygen saturation of microalgae and wastewater mixture A, B, C, D, E and F at three different concentrations for 30 days were measured by using YSI 5100 Dissolved Oxygen Meter. The results of oxygen saturation were plotted as shown in Figure 3.5 until Figure (a-f), respectively.

Oxygen saturation is the measurement of the oxygen that is carried as a maximal concentration that can be dissolved in the water. Based on Figure 3.5(a), the saturation of oxygen in the wastewater did not really change in the mixture of *Chlorella* and wastewater A for all three-concentration ratio of wastewater. This was due to the death of the *Chlorella*. Oxygen saturation in the mixture of *Chlorella* and wastewater C Figure 3.5(c) also gave the same results for all three-concentration ratio.

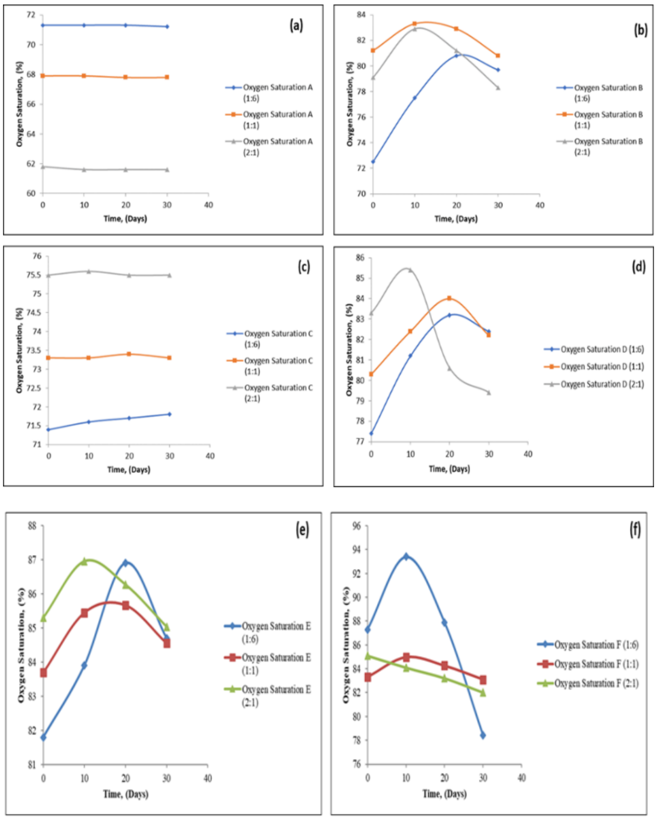


Figure 3.5: The Graph of oxygen saturation (%) of Microalgae and Wastewater (a): A at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (b): The Graph of oxygen saturation (%) of Microalgae and Wastewater B at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (c): C at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (d): D at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (e): E at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (f): F at three different concentrations (1:6, 1:1, and 2:1) for 30 Days.

There was only ± 0.01 of change between the value in oxygen saturation for wastewater A and C. These happened as there was no photosynthesis and respirations occur since *Chlorella* in wastewater A and C was dead. For the mixture of *Chlorella* and wastewater B (Figure 3.5(b), the oxygen saturation increased from day 0 to day 20 and decrease to day 30 for concentration ratio of 1:6. While for the concentration ratio of 1:1 and 2:1 oxygen saturation increased from day 0 to 10 and decreased to day 30. Based on Figure 3.5(d), the oxygen saturation for the mixture of *Chlorella* and wastewater D increased from day 0 to day 20 for concentration ratio of 1:6 and 1:1. At day 20 to day 30, the oxygen saturation decreased. On the contrary, for the concentration ratio of 2:1, the oxygen saturation decreased from day 10 to 30. The abrupt decreased of oxygen saturation from day 10 to day 30 might be due to the utilization of oxygen by *Chlorella* for respiration process. The increased of oxygen saturation might be due to the production of oxygen by *Chlorella* from photosynthesis process. As for *Chlorella* and wastewater E mixture Figure 3.5(e), the oxygen saturation increased from day 0 to day 20 and decreased from day 20 to day 30 for concentration ratio 1:6 and 1:1 while for concentration ratio 2:1, the value increase from day 0 to day 10 but decreased from day 10 to day 30. Lastly, for oxygen saturation *Chlorella* and wastewater F Figure 3.5(f), it increased from day 0 to day 10 but decreased from day 10 to day 30 for all concentration. The results show that for *Chlorella* in wastewater A and C, they do not show any change of oxygen saturation value for all concentration. Wastewater B at concentration 1:1 showed the highest value of oxygen saturation compared to concentration 1:6 and 2:1. As for wastewater D, they showed the highest oxygen saturation at concentration 1:6. Wastewater E showed the highest oxygen saturation at 2:1. Compared to other wastewater, wastewater F showed the highest oxygen saturation at concentration 1:1.

3.2.4 Dissolved Oxygen (mg/mL) of the Mixture of Microalgae and Wastewater

The dissolved oxygen of microalgae and wastewater mixture A, B, C, D, E and F at three different concentrations for 30 days were measured by using YSI 5100 Dissolved Oxygen Meter. The results of dissolved oxygen were plotted as shown in Figure 3.6 until Figure (a), (b), (c), (d), (e), (f) respectively.

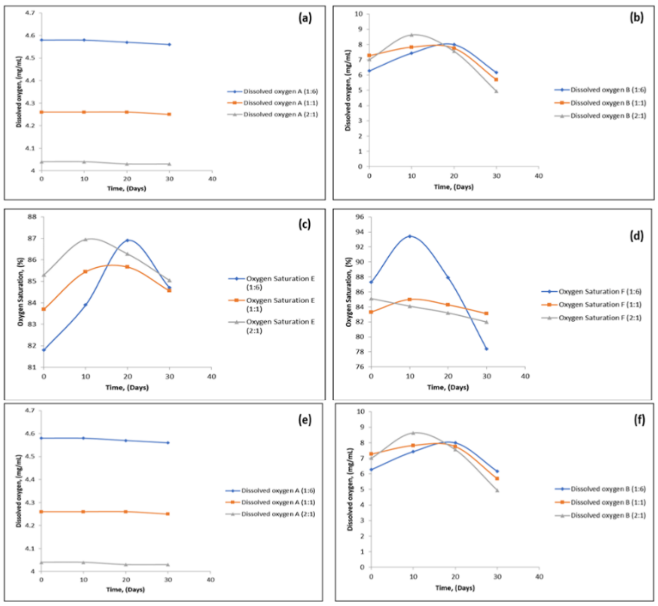


Figure 3.6: The Graph of dissolved oxygen (mg/mL) of Microalgae and Wastewater (a): A at three different concentrations (1:6, 1:1, and 2:1) for 30 days. (b): B at three

different concentrations (1:6, 1:1, and 2:1) for 30 days. (c): C at three different concentrations (1:6, 1:1, and 2:1) for 30 days. (d): D at three different concentrations (1:6, 1:1, and 2:1) for 30 days. (e): E at three different concentrations (1:6, 1:1, and 2:1) for 30 days. (f): F at three different concentrations (1:6, 1:1, and 2:1) for 30 days.

Dissolved oxygen (DO) is the amount of oxygen in the water. Water received oxygen from atmosphere and the aquatic plants. Low level of DO can be caused by several factors such as algal bloom. As the DO level decrease, it can affect other marine living things. According to Figure 3.6 (a) and (c), the DO of *Chlorella* and wastewater A mixture and *Chlorella* and wastewater C mixture shows no change of value from day 0 to day 30. This is due to the death of Chlorella in the wastewater that prevents the process of respiration and photosynthesis to occur that involve the utilization of oxygen and carbon dioxide. For *Chlorella* in wastewater B Figure 3.6(b), the value of DO increased from day 0 to day 20 and decreased abruptly to from day 20 to day 30 for concentration ratio of 1:6. As for concentration ratio of 1:1 and 2:1, the DO increased from day 0 to day 10 and decreased from day 10 to 30. As shown in Figure 3.6 (d), the DO value for *Chlorella* in wastewater D increased from day 0 to day 20 and decreased from day 20 to day 30 for concentration ratio of 1:6 and 1:1. While for concentration ratio 2:1, DO value only increased from day 0 to day 10 and started to decrease from day 10 to day 30. On the contrary, for *Chlorella* in wastewater E Figure 3.6(e), DO value increased from day 0 to day 20 and decreased from day 20 to day 30 for concentration ratio 1:6 and 1:1. As for concentration ratio 2:1, DO value increase from day 0 to day 10 and decreased from day 10 to day 30. As for wastewater the F Figure 3.6 (f), the DO value increased from day 0 to day 10 then decreased as it reached day 30. The increased of DO value is due to the decreased of salinity and at the same time, it also due to the photosynthesis process that produced oxygen that will dissolve in the water. Salts ion attracted molecules of water. Fewer hydrogen and oxygen ions will be available in order to disassociate gas molecules (Kielmas M., 2018). Decreased of DO indicated that the excessive growth of microalgae in the water. DO also become lower as the decomposition of the industrial wastes utilized oxygen and decreased the concentration of oxygen in the water. In Malaysia, the value surface water DO is from 3.0 to 5.0 mg/mL (Hossain, 2013). The DO value for wastewater A, B, C, D, E and F exceeded Malaysia standard DO value. From the results obtained, *Chlorella* in wastewater A and C does not show any changes of dissolved oxygen for all concentration. Wastewater B showed the highest dissolved oxygen at concentration 1:1 compared to concentration 1:6 and 2:1. As for wastewater D, they showed the highest dissolved oxygen at concentration 1:1. Wastewater E also showed the highest dissolved oxygen at concentration 1:1. Compared to other wastewater, wastewater F showed highest dissolved oxygen at concentration 2:1.

3.2.5 Conductivity (μS/cm) of the Mixture of Microalgae and Wastewater

Conductivity (μS/cm) of microalgae and wastewater mixture A, B, C, D, E and F at three different concentrations for 30 days were measured by using OAKTON PC 650 pH/ Conductivity Meter. The results of dissolved oxygen were plotted as shown in Figure 3.7 and Figure (a), (b), (c), (d), (e), (f) respectively.

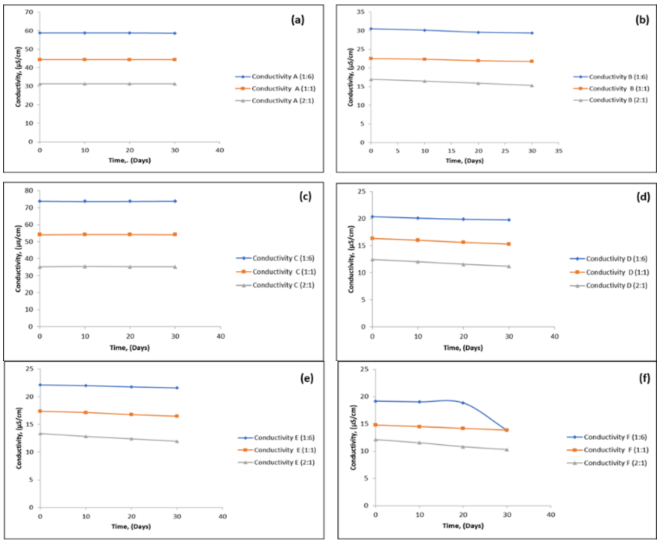


Figure 3.7: The Graph of conductivity (μS/cm) of Microalgae and Wastewater (a): A at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (b): B at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (c): C at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (d): D at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (e): E at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (f): F at three different concentrations (1:6, 1:1, and 2:1) for 30 Days.

Conductivity is the ability of material to pass electric charge. In this context, the ability of the wastewater to pass electrical charge was measured. The major ion that involved in conducting electrical charges are chloride, sodium, and magnesium (Rusydi, 2018). Figure 3.7(a) and (c) showed that the conductivity value for wastewater A and C that contained *Chlorella* did not show any changes from day 0 to day 30 for all concentration ratios. This was due to the death of the *Chlorella*. As the *Chlorella* dead, there was no metabolism activity happen to reduce or increase the ions that cause conductivity. Even so, the conductivity value for wastewater B Figure 3.7(b) that contained *Chlorella* decreased from day 0 to day 30 for all concentration (1:6, 1:1 and 2:1). Based on Figure 3.7 (d), (e) and (f), the conductivity value for wastewater D, E and F that contained *Chlorella* decreased from day 0 to day 30 for all concentration (1:6, 1:1 and 2:1). As *Chlorella* growing, they will utilize and reduced the ions that responsible for conducting electrical charges. It has been known that microalgae can consume and utilizes those ions as source of nutrient for metabolism and growth purposes. As the growth of *Chlorella* increase, the value of conductivity will be reduced as *Chlorella* will utilized the ions that responsible for conducting electrical charges. The results showed that *Chlorella* in wastewater A and C did not show any changes in conductivity value. The lowest conductivity value for wastewater B, D, E and F were all at the concentration of 2:1.

3.2.6 Salinity (psu) of the Mixture of Microalgae and Wastewater

Salinity (psu) of microalgae and wastewater mixture A, B, C, D, E and F at three different concentrations for 30 days were measured by using OAKTON PC 650 pH/ Conductivity Meter. The results of dissolved oxygen were plotted as shown in Figure 4.32 and (a), (b), (c), (d), (e), (f) respectively

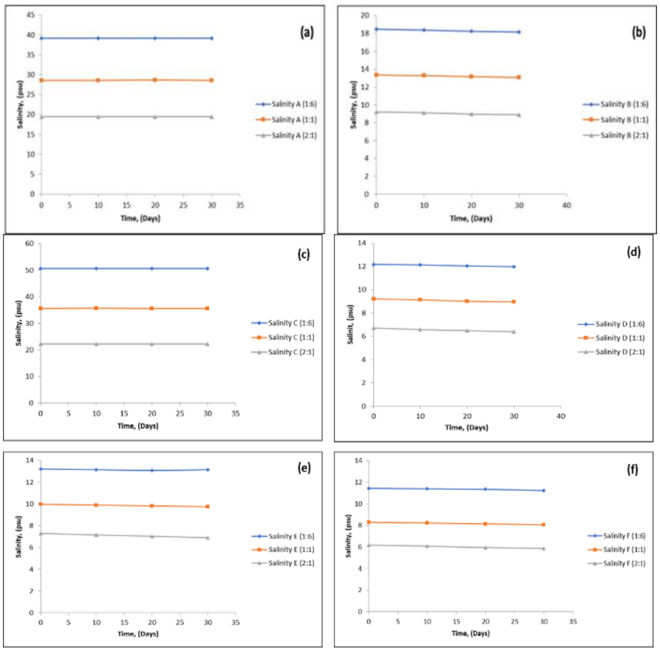


Figure 3.8: The Graph of salinity (psu) of Microalgae and Wastewater (a): A at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (b): B at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (c): C at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (d): D at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (e): E at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (f): F at three different concentrations (1:6, 1:1, and 2:1) for 30 Days.

Salinity is the concentration of salts in water such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3 (Tavakkoli et al., 2010). Based on Figure 3.8 (a) and (c), salinity was measured in ppm unit and the value of salinity for wastewater A and C with *Chlorella* showed no changes from day 0 to day 30 for all concentration. Based on Figure 3.8 (b), (d), (e) and (f), wastewater B, D, E and F with *Chlorella* shows reduction value for all concentration from day 0 to day 30.

The value of salinity for wastewater A and C shows no reduction of salinity as *Chlorella* already dead due to the unsuitable pH. Due to the death of *Chlorella*, there was no process of utilizing the salts for metabolisms and growth.

Table 1: The percentage of salinity reduction performed by *Chlorella* sp. in wastewater A, B, C, D, E and F at three different concentrations for 30 days.

Wastewater	Concentration ratio (microalgae: wastewater)	Percentage salinity reduced after 30 days (%)
A	1:6	0
	1:1	0
	2:1	0
B	1:6	1.89
	1:1	2.17
	2:1	3.64
C	1:6	0
	1:1	0
	2:1	0
D	1:6	1.77
	1:1	2.71
	2:1	4.53
E	1:6	1.21
	1:1	2.30
	2:1	5.4
F	1:6	1.79
	1:1	3.06
	2:1	4.91

3.2.7 Total Dissolved Solids (TDS) of the Mixture of Microalgae and Wastewater

TDS of microalgae and wastewater mixture A, B, C, D, E and F at three different concentrations for 30 days were measured by using OAKTON PC 650 pH/ Conductivity Meter. The results of dissolved oxygen were plotted as shown in Figure 3.9 and (a), (b), (c), (d), (e), (f) respectively.

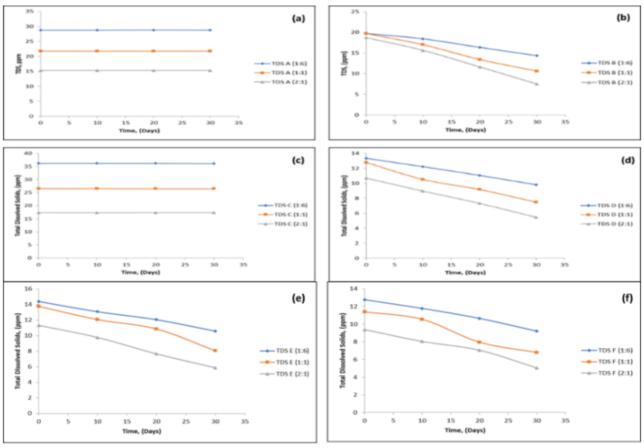


Figure 3.9: The Graph of total dissolves solids (ppm) of Microalgae and Wastewater (a): A at three different

concentrations (1:6, 1:1, and 2:1) for 30 Days (b): B at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (c): C at three different concentrations (1:6, 1:1, and 2:1) for 30 Days. (d): D at three different concentrations (1:6, 1:1, and 2:1) for 30 Days (e): E at three different concentrations (1:6, 1:1, and 2:1) for 30 Days (f): F at three different concentrations (1:6, 1:1, and 2:1) for 30 Days.

Total dissolved solids (TDS) are the dissolved mineral, salts, ions or metals that dissolved in water. According to Figure 3.9(a) and (c), total dissolved solids (TDS) for wastewater A and C that contained *Chlorella*, does not show changes from day 0 to day 30 for all concentration due to the death of *Chlorella*. As the *Chlorella* was dead, there was no action of utilizing the inorganic and salts molecules. Based on Figure 3.9 (b), (d), (e) and (f), TDS (ppm) for wastewater B, C, D and F decreased from day 0 to day 30 for all concentration.

4. Discussion

Industrial wastewaters are prone to be highly saline. This wastewater contained high salinity due to the presence of high concentration of dissolved mineral such as calcium, magnesium, potassium, sodium, sulfates and chloride. There are several studies to decrease the salinity of wastewater such as by using sand bioreactors (Conroy, 2017), catalytic wet peroxide oxidation (CWPO) process (Zhuo et al., 2017), using salt tolerant microorganisms (Bhuyar et al., 2019) and others.

The microalgae that have been isolated were identified by referring to the study done by Selvarajam et al. (2015). The microalgae observed under the fluorescent microscope (Figure 3.1) have the same characteristics as *Chlorella* sp. From the observation, the microalgae were green in color thus it indicated that the microalgae are in the division of chlorophyta. *Chlorella* has round or oval shape with the diameter between 2-15 μm (Belcher and Swale, 1976). *Chlorella* come from the class Trebouxiophyceae, order Chlorellales, family Oocystaceae and genus *Chlorella* sp. This species usually found cluster or single form. They can be found in salt or fresh water as well as in soil (Beuckels et al., 2015). *Chlorella* can be used as food source, biofuel and also for the wastewater treatment as they can efficiently remove nitrogen, phosphorus, COD and at the same time BOD. This microalga can produce oxygen during their photosynthesis and the oxygen can be used by the bacteria present in the wastewater to converts the nutrients in wastewater into biomass. They can also utilize nutrients available in the wastewater for their growth and metabolism (Ahmad et al., 2013). *Chlorella* can stand highly saline environment (Adenan et al., 2013).

The *Chlorella* was exposed to the artificial light for 24 hours as the light play an important role in the growth of microalgae. The light energy will be converted into chemical energy. The phase of converting the light energy into chemical energy is called photochemical phase. *Chlorella* produced Adenosine triphosphate (ATP), Nicotinamide adenine dinucleotide phosphate oxidase (NADPH) and oxygen (O_2) (Al-Qasmi et al., 2012). The *Chlorella* can use the oxygen released from the photosynthesis for their respiration and produce carbon dioxide (CO_2).

Optical density was measured by UV-Vis Spectrophotometry in terms of absorbance at certain wavelength. As for the *Chlorella*, wavelength of 700 nm was used to measure the optical density of the microalgae each 10 days for 30 days. Wavelength of 700 nm and 750 nm are usually are used to measure the optical density or the absorbance of the microalgae (Belianin et al., 1975). At these wavelengths, *Chlorella* absorbs the most light when the light passes through it. The absorbance will increase when the growth of *Chlorella* increased.

According to the study, the uptake efficiency of nitrogen and phosphate increase directly proportional to the increase of pH (Zhang et al., 2014). In real condition, the increase of pH during algal blooms due to hydroxide that was form as the algae consumes all carbonate alkalinity (Gao et al., 2012). The results show that for *Chlorella* in wastewater A and C, they do not show changes of pH for all concentrations. For wastewater B, D, E and F, the pH value increase for all concentration from day 0 to day 30.

Decrease of TDS in the wastewater was due to the utilizing of dissolved solids for growth and metabolism through the mechanisms of bioabsorption/adsorption. Many researchers reported the decreased of TDS for water treatment using microalgae (Azarpira et al., 2014; Fawzy et al., 2010). As *Chlorella* utilized the dissolved solids, the TDS decreased and at the same time, the salinity decreased as the dissolved solids also plays a role in the salinity of wastewater. *Chlorella* in wastewater A and C show no changes in TDS value for all concentration. On the contrary, wastewater B, D, E and F reduced the most TDS at concentration of 2:1 compared to the other concentration.

As *Chlorella* grows, they will utilize and use the salts that present in the wastewater for their growth as a source of nutrient. As they utilize the salts, the salinity will decrease (Hwang et al., 2016). *Chlorella* is considered as salt tolerant microalgae as they can survive in an environment with 0.4 M of salt concentration (Hiremath and Mathad, 2010). From the study, it shows that the capability of *Chlorella* to consume salinity increased as the salinity availability increases. High salinity can cause osmotic stress to other organisms in the wastewater.

As salinity decreased, the concentrations of salts also decrease, and it will decrease the conductivity of the wastewater as the salts can conduct electrical charges in the wastewater (Golnabi et al., 2009). This is due to the existence of ionic salts such as Na+ and Cl- that dissociates in water and can conduct electrical charges. From table 1, wastewater B, C, D and F, concentration of 2:1 was the most suitable concentration to reduce the salinity as they reduced the most salinity which were 3.67 %, 4.53 %, 5.4 % and 4.91 % respectively.

4. Conclusion

In this study, microalgae that were collected from Pantai Gelora was brought to the lab and cultivated. Isolation was done by agar plating. The isolated microalgae will form unialga and will be observed under fluorescent microscope to compare with other result from previous study. From previous study, it was identified that the isolated microalgae were *Chlorella*. Only one type of microalgae was isolated from the study. *Chlorella* can be used to reduce the salinity of wastewater as they are tolerant to high saline environment. From the result obtained, *Chlorella* managed to reduce the salinity for wastewater B, C, D and F, at concentration of 2:1 which were 3.67 %, 4.53 %, 5.4 % and 4.91 % respectively. This concentration reduces the most salinity compared to other concentration. This concentration also reduced the most TDS compared to the other concentration.

Acknowledgements

The authors gratefully acknowledged Universiti Malaysia Pahang for the financial assistance through the Internal Research Grant No. RDU1903125 and Flagship Grant No. RDU182205. Author [Prakash Bhuyar] is thankful to UMP for providing Doctoral Research Scholarship (DRS) and Grant No. PGRS190351as a financial support.

References

Abdulsada Z.K., 2014. Evaluation of microalgae for secondary and tertiary wastewater treatment. Department of Civil and Environmental Engineering, Environmental Engineering Ottawa, Ontario, 114.

Adenan N.S., Yusoff F.M., Shariff M., 2013. effect of salinity and temperature on the growth of diatoms and green algae. Journal of Fisheries and Aquatic Science.

Abou-Elela S.I., Kamel, M.M., Fawzy M.E., 2010. Biological treatment of saline wastewater using a salt-tolerant microorganism. Desalination 250(1): 1-5.

Ahmad F., Khan A.U., Yasar A., 2013. The potential of *Chlorella vulgaris* for wastewater treatment and biodiesel production. Pakistan Journal of Botany 45: 461-465.

Al-Qasmi M., Raut N., Talebi S., Al-Rajhi S., Al-Barwani T., 2012. A review of effect of light on microalgae growth. In Proceedings of the World Congress on Engineering on July 4-6, 2012 in London, UK, 1-3.

Andersen R., 2005. Algal Cultural Techniques. California: Academic Press.

Azarpira H., Behdarvand P., Dhumal K., Pondhe G., 2014. Potential use of cyanobacteria species in phycoremediation of municipal wastewater. International Journal of Biosciences 4(4): 105-111.

Belcher H., Swale E., 1976. A beginner's guide to freshwater algae. Natural environment research council.

Belianin V.N., Spirov V.V., Furiaev E.A., 1975. Spectrophotometry of individual *Chlorella* cells. Biofizika, 20 (5), 848-852.

Beuckels A., Smolders E., Muylaert K., 2015. Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. Water Research, 77, 98–106.

Bhuyar P., Sundararaju S., Rahim M.H.A., Ramaraj R., Maniam G.P., Govindan N., 2019. Microalgae cultivation using palm oil mill effluent as growth medium for lipid production with the effect of CO_2 supply and light intensity. Biomass Conversion and Biorefinery 1-9.

Bhuyar P., Muniyasamy S., Govindan N., 2018. Green revolution to protect environment – An identification of potential micro algae for the biodegradation of plastic waste in Malaysia. Expert Opinion Environmental Biology 7.

Bhuyar P., Rahim M. H. A., Sundararaju S., Ramaraj R., Maniam G. P., Govindan N., 2020. Synthesis of silver nanoparticles using marine macroalgae *Padina* sp. and its antibacterial activity towards pathogenic bacteria. Beni-Suef University Journal of Basic and Applied Sciences 9(1): 1-15.

Bhuyar P., Rahim M.H.A., Yusoff M.M., Gaanty P.M., Govindan N., 2019. A selective microalgae strain for biodiesel production in relation to higher lipid profile. Maejo International Journal of Energy and Environmental Communication1(1):8-14.

Bhuyar P., Rahim M.H., Sundararaju S., Maniam G.P., Govindan N., 2020. Antioxidant and antibacterial activity of red seaweed Kappaphycus alvarezii against pathogenic bacteria. Global Journal of Environmental Science and Management 6(1):47-58.

Camarena-Bernard C., Rout N.P., 2018. Native Microalgae from Eutrophic Water: Potential for Wastewater Treatment, Low-Cost Biomass, and Lipid Production. Industrial Biotechnology 14(5): 257-264.

Conroy K., 2017. Treating high salt content wastewater with sand bioreactors. (Electronic Thesis or Dissertation). The State Ohio University, pp 1-82

Doble M., Kumar A., 2005. Treatment of Solid Waste. Biotreatment of Industrial Effluents.

El Nadi M.H.A., El Sergany F.A.G.H., El Hosseiny O.M. 2014. Desalination using algae ponds under nature egyptian conditions. Journal of Water Resources and Ocean Science 3(3): 69-73.

Gangl D., Zedler J.A.Z., Rajakumar P.D., Martinez, E.M.R., Riseley A., Włodarczyk A., Robinson C., 2015. Biotechnological exploitation of microalgae. Journal of Experimental Botany 66(22): 6975-6990.

Gao Y., Cornwell J. C., Stoecker D. K., Owens M. S. 2012. Effects of cyanobacterial-driven pH increases on sediment nutrient fluxes and coupled nitrification-denitrification in a shallow fresh water estuary. Biogeosciences 7: 2697-2710.

Golnabi H., Matloob M.R., Bahar M., Sharifian M., 2009. Investigation of electrical conductivity of different water liquids and electrolyte solutions. Iranian Physical Journal 3(2): 24-28.

Hiremath S., Mathad P., 2010. Impact of salinity on the physiological and biochemical traits of *Chlorella vulgaris Beijerinck*. Journal of Algal Biomass Utilization 1(2): 51-59.

Hossain M.A., Sujaul I.M., Nasly M.A., 2013. Water quality index: An indicator of surface water pollution in eastern part of peninsular Malaysia. Research Journal of Recent Science 2(10): 10-17.

Hwang J.H., Church J., Lee S.J., Park J., Lee W.H., 2016. Use of microalgae for advanced wastewater treatment and sustainable bioenergy generation. Environmental Engineering Science 33(11): 882-897.

Park J., Seo J., Kwon E.E., 2012. Microalgae production using wastewater: effect of light-emitting diode wavelength on microalgal growth. Environmental Engineering Science 29(11): 995-1001.

Kielmas M., 2018. How Does Salinity Affect the Solubility of Oxygen in Water?

Liu X., Dai J., Wu D., Jiang F., Chen G., Chui H.K., van Loosdrecht M.C.M., 2016. Sustainable application of a novel water cycle using seawater for toilet flushing Engineering 2(4): 460-469.

Rachlin J. W., Grosso A., 1991. The effects of pH on the growth of *Chlorella vulgaris* and its interactions with cadmium toxicity. Archives of Environmental Contamination and Toxicology 20(4): 505-508.

Ramaraj R., Unpaprom Y., Kawaree R., 2016a. Direct transesterification of microalga *Botryococcus braunii* biomass for biodiesel production. Emergent Life Sciences Research 2: 1-7.

Ramaraj R., Unpaprom Y., Dussadee N., 2016b. Potential evaluation of biogas production and upgrading through algae. International Journal of New Technology and Research 2(3): 128-133.

Raven J. A., Giordano M., 2014. Algae. Current Biology, 24(13).

Rusydi A.F., 2018. Correlation between conductivity and total dissolved solid in various type of water: A review. In IOP Conference Series: Earth and Environmental Science 118(1): 012019.

Schirrmeister B.E., Sanchez-Baracaldo P., Wacey D., 2016. Cyanobacterial evolution during the Precambrian. International Journal of Astrobiology 15(3): 187-204.

Sekhar K.M., Rachapudi V.S., Mudalkar S., Reddy A.R., 2014. Persistent stimulation of photosynthesis in short rotation coppice mulberry under elevated CO_2 atmosphere. Journal of Photochemistry and Photobiology B: Biology 137: 21-30.

Selvarajan R., Felföldi T., Tauber T., Sanniyasi E., Sibanda T., Tekere M., 2015. Screening and evaluation of some green algal strains (Chlorophyceae) isolated from freshwater and soda lakes for biofuel production. Energies 8(7): 7502-7521.

Tavakkoli E., Rengasamy P., McDonald G.K., 2010. High concentrations of Na+ and Cl-ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. Journal of Experimental Botany 61(15): 4449-4459.

Tsai D.D.W., Chen P.H., Ramaraj R., 2017. The potential of carbon dioxide capture and sequestration with algae. Ecological engineering 98: 17-23.

Xiang X., Ozkan A., Kelly C., Radniecki, T., 2018. Importance of microalgae speciation on biogas production and nutrient recovery from anaerobic digestion of lipid-extracted microalgae biomass. Environmental Engineering Science 35(4): 382-389.

Xu P., Cath T.Y., Robertson A.P., Reinhard M., Leckie J.O., Drewes J.E., 2013. Critical review of desalination concentrate management, treatment and beneficial use. Environmental Engineering Science 13(8): 502-514.

Yaakob Z., Ali E., Zainal A., Mohamad M., Takriff M.S., 2014. An overview: Biomolecules from microalgae for animal feed and aquaculture. Journal of Biological Research (Greece) 21(1): 6.

Unpaprom Y., Ramaraj R., Whangchai K., 2017. A newly isolated green alga, Scenedesmus acuminatus, from Thailand with efficient hydrogen production. Chiang Mai Journal of Science 44: 1270-1278.

Zhang Q., Wang T., Hong Y., 2014. Investigation of initial pH effects on growth of an oleaginous microalgae *Chlorella* sp. HQ for lipid production and nutrient uptake. Water Science and Technology 70(4): 712-719.

Zhang Y., Shen Y., 2017. Wastewater irrigation: past, present, and future. Wiley Interdisciplinary Reviews: Water, e1234.

Zhuo Y., Sheng M., Liang X., Cao G., 2017. Treatment of high salinity wastewater using CWPO process for reuse. Journal of Advanced Oxidation Technologies 20.