

Salinity Reduction From Poly-Chem-Industrial Wastewater by Using Microalgae (*Chlorella sp.*) Collected From Coastal Region of Peninsular Malaysia

Prakash Bhuyar¹, Dang Diem Hong², Emelina Mandia³, Mohd Hasbi Ab. Rahim¹, Gaanty Pragas Maniam¹, Natanamurugaraj Govindan^{1*}

¹Algae Biotechnology Laboratory, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia

²Algae Biotechnology Department, Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Str., Cau Giay, Hanoi, Vietnam

³Microalgae Systematics and Applied Phycology Research Laboratory Science and Technology Research Center, Biology Department, De La Salle University 2401 Taft Avenue, Manila, Philippines

*Corresponding author: Govindan N, Algae Biotechnology Laboratory, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia; Tel:+6095492465; E-mail: [natanam\[at\]ump.edu.my](mailto:natanam[at]ump.edu.my)

Received: May 11, 2020; Accepted: May 30, 2020; Published: June 10, 2020



All articles published by Gnoscience are Open Access under the Creative Commons Attribution License BY-NC-SA.

Abstract

The potential of microalgae as a source of bioremediation based on wastewater has received increasing interest throughout the world. A freshwater microalga Chlorella vulgaris was investigated for its ability to reduce salinity from six different poly-chem industrial wastewaters which were diluted in microalgae in three different proportions (namely, 1:6, 1:1 and 2:1). The results revealed that C. vulgaris grew in wastewater of chemical and fertilizer company, obtained value of pH, salinity, DO, salinity and TDS did not change while absorbance value decreased from day 0 to day 30 due to highly acidic nature. For polymer, dye, RO, and petroleum industry wastewater, the absorbance and pH value increase for all concentration. The lowest salinity and TDS for poly, dye, RO and petroleum industry were all at the concentration of 2:1. The result showed that, Chlorella sp. managed to reduce the salinity for wastewater polymer, dye, RO, and petroleum industry, at concentration of 2:1 which were 3.67 %, 4.53 %, 5.4 %, and 4.91 % respectively. The results suggest that the potential of removing nutrient from wastewater by microalgae cultivation as production feedstock.

Keywords: *Chlorella vulgaris; Microalgae; Salinity reduction; Phycoremediation*

Citation: Bhuyar P, Hong DD, Mandia E, et al. Salinity reduction from poly-chem-industrial wastewater by using microalgae (*chlorella sp.*) collected from coastal region of peninsular Malaysia. J Bio Med Open Access. 2020;1(1):105.

Graphical Abstract



1. Introduction

Over recent decades, wastewater generation issue has been increased tremendously, mostly caused by activities of anthropogenic, like industrialization, global urbanization, and agricultural practices [1–3]. The repeated wastewater disposal in the absence of enough and appropriate treatment may lead to some severe pollution problems. Eutrophication phenomenon is a part of the serious problems related to the effluents discharge continuously into the water bodies [4,5]. The peripheral effluents from wastewater contain nutrients, commonly phosphorus and nitrogen. This situation is accountable for the depletion of oxygen can produce pollutants such as ammonia, and key species becoming vanish, contributing in the total deterioration of freshwater ecosystems [6]. Thus, effective wastewater treatment methods are needed. Algae have the potentials for the salinity reduction in wastewater before dispensing into public usage [7–10].

Industrial system is one of the sources where the wastewater comes from large amount. Despite of how industrial wastewater is treated, the 'end product' is called an effluent. To obey with the environmental protection laws, some of the specific elements must be removed from the wastewater [11]. This includes organic matter, pathogens, inorganics (such potassium, sodium, calcium, nickel, lead, and zinc), and nutrients (most notably nitrogen and phosphorus) in tertiary wastewater treatment. The treated industrial wastewater can then be discharged safely into water bodies, applied to soil or land, or even can be reused in some plant operations [12,13].

Industrial wastewater has multiplex components and high degree of toxic compounds. In general, wastewater from industries are remediated by using a sort of hazardous chemicals for the pH correction, removal of sludge, as well as the removal of color and odor [14,15]. Referred to published reports, industrial wastewater is appropriate for the growth of microalgae. Several studies have exemplified the microalgae-formed remediation feasibility of various

specific industrial wastewater like chemical fertilizer industry, animal manure from livestock industry, and palm oil industry [5,11].

Phosphorus, sodium, magnesium, and nitrogen are nutrients that are aquatic ecosystems natural parts. Besides that, nitrogen is also the most plentiful component in the air we breathe. Both nutrients encourage the algae growth and other aquatic plants, which supply food and domain for shellfish, fish, and compact organisms that live in water [5]. Too much phosphorus and sodium contents in water cause pollution of nutrient in groundwater (millions of people in Malaysia use it as drinking water source) leads to salinity increment. This can be harmful, even at the low-level contents. Newborn are unsafe to a nitrogen-formed compound (nitrates) in drinking water. The excess of nitrogen in the atmosphere can create pollutants like ozone and ammonia that can harm our capability to breathe, cause visibility limitation and affect the growth of plant [13,15-17].

Microalgae are being used to treat the wastewater 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 [18]. Microalgae are one of the living things that have high tolerance to the salinity and, they can survive in the high salinity water. The use of the microalgae to treat wastewater has been investigated by Oswald since 1960s [11]. The treatment process that use microalgae is known as Phycoremediation.

The main approaches of this study are about bioactivity of microalgae. The isolated microalgae were studied about their mechanisms in reducing salinity from industrial wastewater. The optimization of these salts in industrial wastewater seems to be great significance to raise the compounds removal efficiency. The demand of wastewater treatment is high, especially in various industrial wastewaters. The effect of microalgae on the compound such as N and P has been previously studied by multiple researchers but there are not reported salinity reduction of wastewater. Therefore, the aims of this study were to isolate and identify the potential microalgae species for the reduction of residual nutrients such as sodium, magnesium, and phosphorus from industrial wastewater and study the effectiveness of microalgae in reducing the amounts of salinity from industrial wastewater samples by studying growth, pH, salinity, dissolved oxygen, and total dissolved solids.

2. Materials and Methods

2.1 Microalgae sample collection

Algae samples were collected from Teluk Cempedak east coast region of Peninsular Malaysia. The 5 µm plankton net was used to trap the microalgae at the subsurface level. The algae from the seawater were brought to laboratory by sampling bags. 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. Artificial illumination was provided for photosynthesis.

2.2 Algae culture media preparation

BG-11 culture medium is a widely used medium for freshwater algae that can be used as a biomarker for the ecological screening of Cd-contaminated waters including *Chlorella vulgaris* [19]. The modified BG-11 medium was prepared

based on methods used by Stanier et al. [20]. After preparation, the BG-11 medium was autoclaved and adjusted to pH 10.1 prior to use.

2.3 Identification and screening microalgae

Algae strains were isolated and differentiated based on the morphological examinations of colonies like color, shape and size of colonies once it grew well on the agar plate. The morphological structures were observed through Olympus BX53 Fluorescence Research Microscope. Cells and sub microscopic cellular components were identified with high degree specificity. Each isolate in the colonies was labelled and picture was photographed at magnification of 100X. The microalgae were morphologically identified based on the manual 'Microalgae Identification for Aquaculture'. The identification was based on their morphology, color, shape and physical characteristic of microalgae.

2.4 Pre-cultivation of microalgae

The microalgae were pre-cultured in a 500 ml Erlenmeyer flask of BG-11 culture medium. pH was adjusted at 10.1 as it was the optimum growth of the chosen strain of microalgae, *C. vulgaris* [9]. The culture was cultivated under light condition with a regimen of 24 h at $25 \pm 2^\circ\text{C}$ of temperatures with air (sole source of inorganic carbon) was supplied to culture by Atman AT-702 air pump. The culture was shaken manually twice a day. The culture was then transferred into a new 2 L Erlenmeyer flask and BG-11 medium was added until the total culture volume reached 1 L. The culture was examined daily for growth approximately 2-3 weeks.

2.5 Wastewater collection (Poly-chem industry)

Wastewaters were collected from six different industrial located in Pahang state of Malaysia. Wastewaters were collected from Chemical company (A), Polymer production Unit (B), Fertilizers Company (C), Dye Manufacturer (D), RO Plant (E) and Petroleum Industry (F). The wastewaters were kept in white plastic bottles sampling was done in triplicates. The effluent wastewater samples were filtered through a 0.22- μm pore size membrane. The industrial effluent wastewater samples were kept in white-colored plastic bottles to prevent light penetration which may help in the growth of algae [17, 21,22]. The wastewaters were then autoclaved at 121°C for 15 minutes. The wastewaters were labeled with A, B, C, D, E and F easily accessible codes.

2.6 Wastewater and algae mixture preparations

The filtrated wastewater samples and *Chlorella* sp. microalgae were mixed in certain amounts for each kind of industrial wastewater. Three different ratios of (microalgae: wastewater) used which were 1:6 (75: 225 mL), 1:1 (150: 150 mL) and 2:1 (200: 100 mL). The total mixture of wastewater and microalgae were 300 ml. The volume of the wastewater and microalgae were measured using measuring cylinder to reach the ratio goal. The mixture of wastewater and microalgae were kept in the 500 mL conical flask for further wastewater treatment.

2.7 Microalgae cultivation in wastewater

The cultures were shaken twice a day in 30 days incubation period. The results were observed and examined every ten days interval for 30 days. All treatments were in a biological incubator and the cultural conditions were the same as the

pre-culture conditions. The following parameters such as pH, dissolved oxygen (DO) in mg/mL, salinity, total dissolved solids (TDS) and optical density for growth were measured every 10 days.

2.8 Determination of growth rate, pH, DO, TDS and salinity

The optical density measurement was done to observe the growth rate of microalgae, 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. The 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. For the pH, salinity and TDS 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. Dissolved oxygen (DO) was measured by YSI 5100 Dissolved Oxygen Meter. For the measurement of DO the probe was immersed in distilled water before taking the measurement [4,23]. The probe was washed with distilled water and immersed in distilled water before taking the reading for different sample to avoid the human error.

3. Results and Discussion

3.1 Identification and Isolation of microalgae

The microalgae that have been collected from Teluk Cempedak that were isolated were observed under fluorescent microscope to identify according the microalgae manual book. The identification was based on their morphology, color, shape and the physical of the microalgae. According to the algae manual and morphological identification the microalgae was identified as *Chlorella* sp. as observed in Fig. 1.

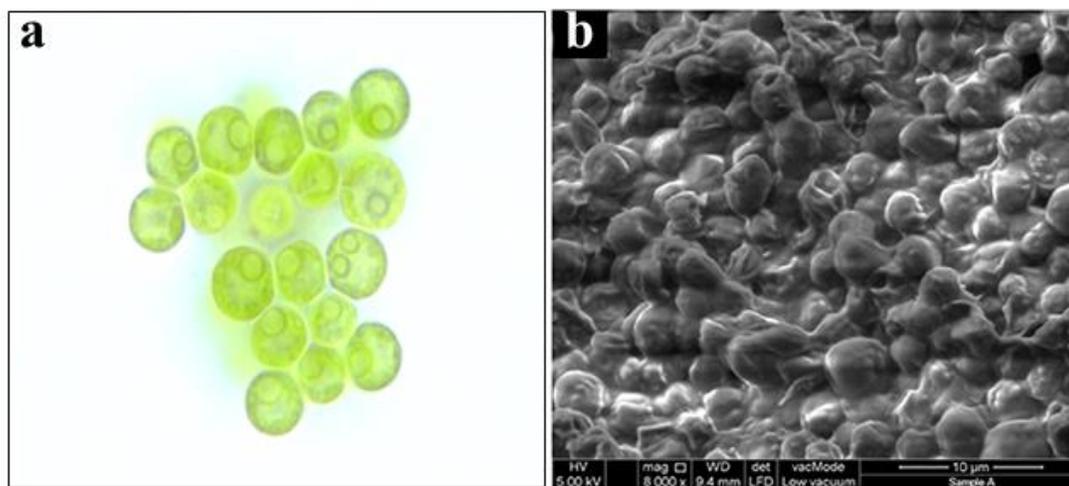


Fig. 1. Morphological image of *Chlorella* sp. green microalgae (a) under fluorescent microscope at 100 X magnification and (b) scanning electron microscopic photograph.

The microalgae that have been isolated were identified by referring to the study done by Selvarajan [24]. The microalgae observed under the fluorescent microscope Fig. 1a by using fluorescent microscope and Fig. 1b by using scanning electron microscopy 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 [25]. *Chlorella* belongs to 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 [12]. *Chlorella* can be used as food source, biofuel and 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 [6] as *Chlorella* can with stand highly saline environment [26].

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) [27]. The *Chlorella* can use the oxygen released from the photosynthesis for their respiration and produce carbon dioxide (CO_2).

3.2 Analysis of growth rate

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 [20,28]. At these wavelengths, *Chlorella* absorbs the lightest when the light passes through it. The absorbance will increase when the growth of *Chlorella* increased.

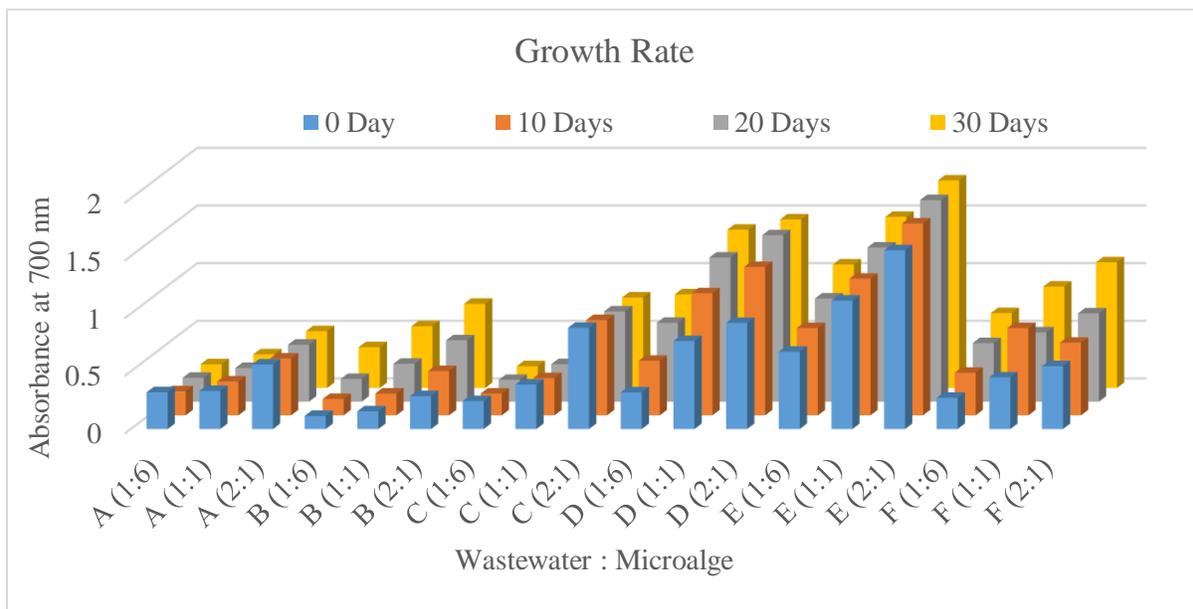


Fig. 2. The growth rate of microalgae cultivated in wastewater for 30 days incubation absorbance at 700 nm.

From Fig. 2, the absorbance value of microalgae in wastewater A all the ratios decrease from 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. 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 with the ratio of 1:6, the absorbance also decreases while the ratio 1:1 and 2:1, the absorbance decreases. There was no increase in absorbance for *Chlorella* in all three concentration of wastewater C. 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 acidic pH of the wastewater. Low pH environment or highly acidic environment was not suitable for the growth of *Chlorella*.

According to Fig. 2. absorbance for microalgae in wastewater D 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 [29].

At the same time, the absorbance of microalgae in wastewater E 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. This is due to the *Chlorella* almost reach the stationary phase. Lastly for the optical density, absorbance of *Chlorella* in wastewater F 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. 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 [20]. 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 analysis

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. According to the study, the uptake efficiency of nitrogen and phosphate increase directly proportional to the increase of pH [15]. In real condition, the increase of pH during algal blooms due to hydroxide that was form as the algae consumes all carbonate alkalinity [30]. 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.

Based on Fig. 3, 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. According to the data obtained, 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 was 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 [19].

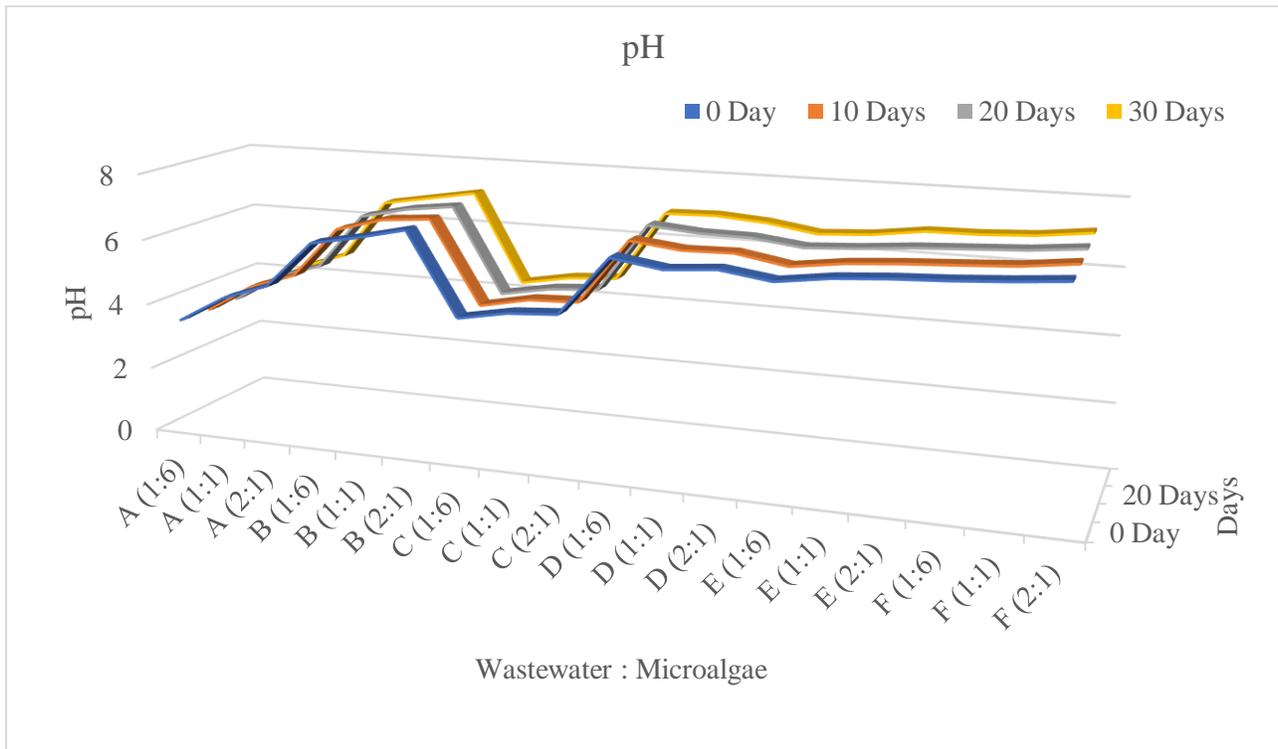


Fig. 3. The Graph representing the pH of microalgae and wastewater at different proportions.

Based on Fig. 3, 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 CO₂ 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 [12] 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* [19]. According to [13,31], the increase of pH might be because of the consumption, absorption of CO₂ and the algal release at the stationary phases [17]. Total uptake of phosphate and nitrogen increased directly proportional to the pH [7].

3.4 Dissolved oxygen (mg/mL)

The dissolved oxygen of microalgae and wastewater mixture were measured by using YSI 5100 Dissolved Oxygen Meter. The results of dissolved oxygen were plotted as shown in Fig. 4.

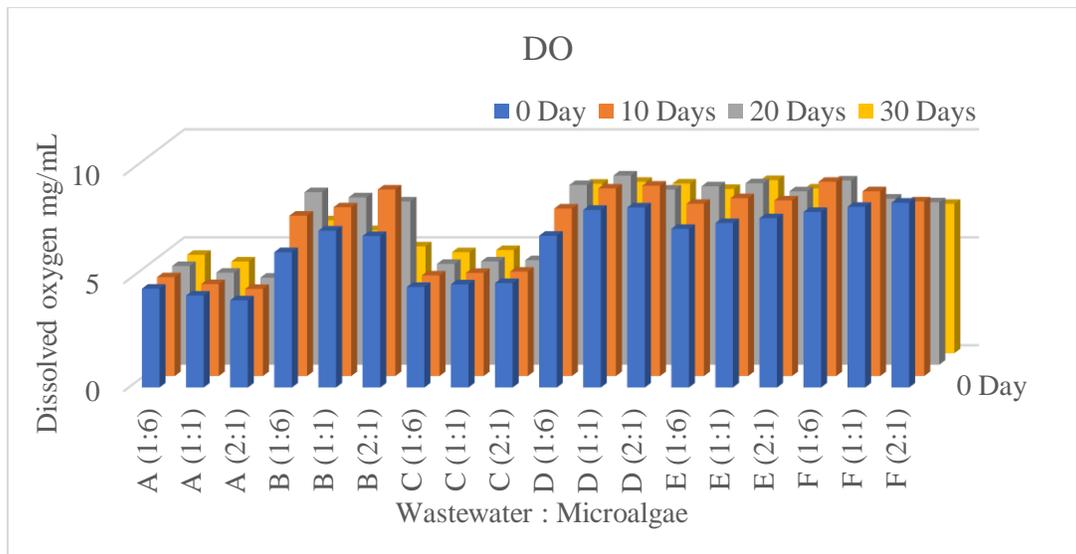


Fig. 4. The dissolved oxygen (mg/mL) level of microalgae and wastewater proportions.

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 4, 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 4, 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. 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, 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. The DO value increased from day 0 to day 10 then decreased as it reached day 30 for the F. 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 [32]. 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 [33]. 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.

3.5 Total dissolved solids (TDS)

TDS of microalgae and wastewater mixture were measured by using OAKTON PC 650 pH/ Conductivity Meter.

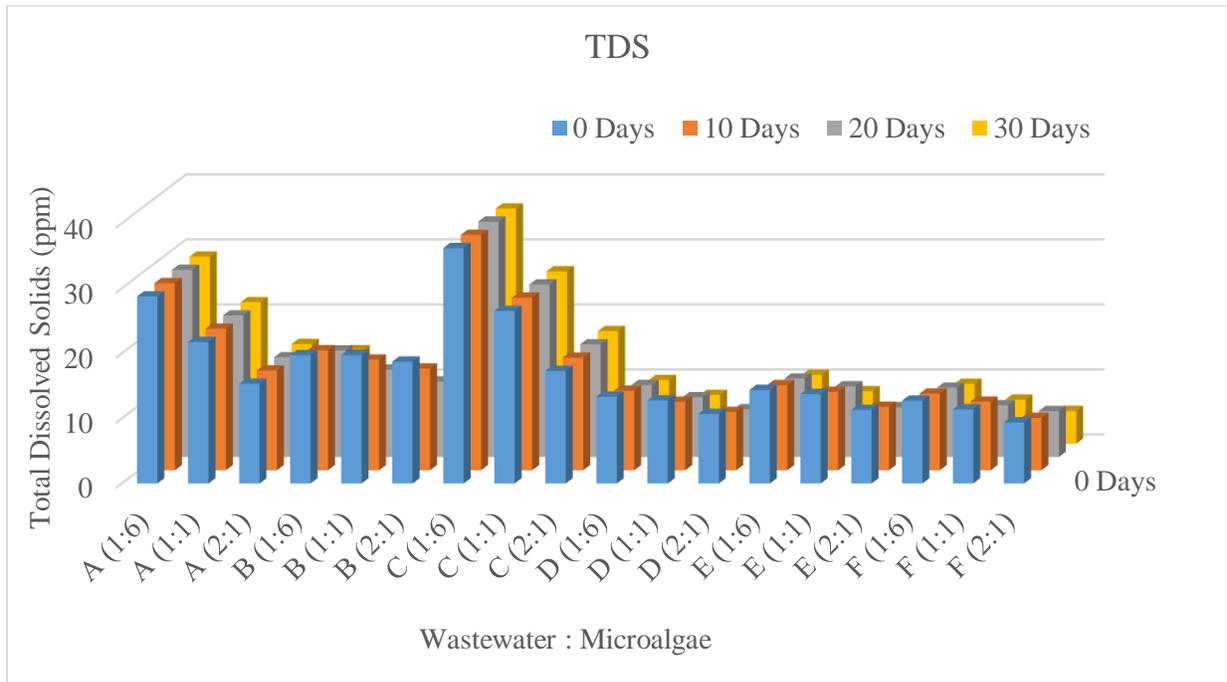


Fig. 5. The total dissolved solids (ppm) of microalgae and wastewater proportions.

Total dissolved solids (TDS) are the dissolved mineral, salts, ions or metals that dissolved in water. According to Fig. 5 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 5, TDS (ppm) for wastewater B, C, D and F decreased from day 0 to day 30 for all concentration.

Decrease of TDS in the wastewater was due to the utilizing of dissolved solids for growth and metabolism through the mechanisms of bio-absorption/adsorption. Many researchers reported the decreased of TDS for water treatment using microalgae [33]. 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.

3.6 Salinity (psu) Measurement

Salinity (psu) of microalgae and wastewater mixture were measured by using OAKTON PC 650 Conductivity Meter. The results of dissolved oxygen were plotted as shown in Fig. 6. 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 [34], catalytic wet peroxide oxidation (CWPO) process [35], using salt tolerant microorganisms [10] and others.

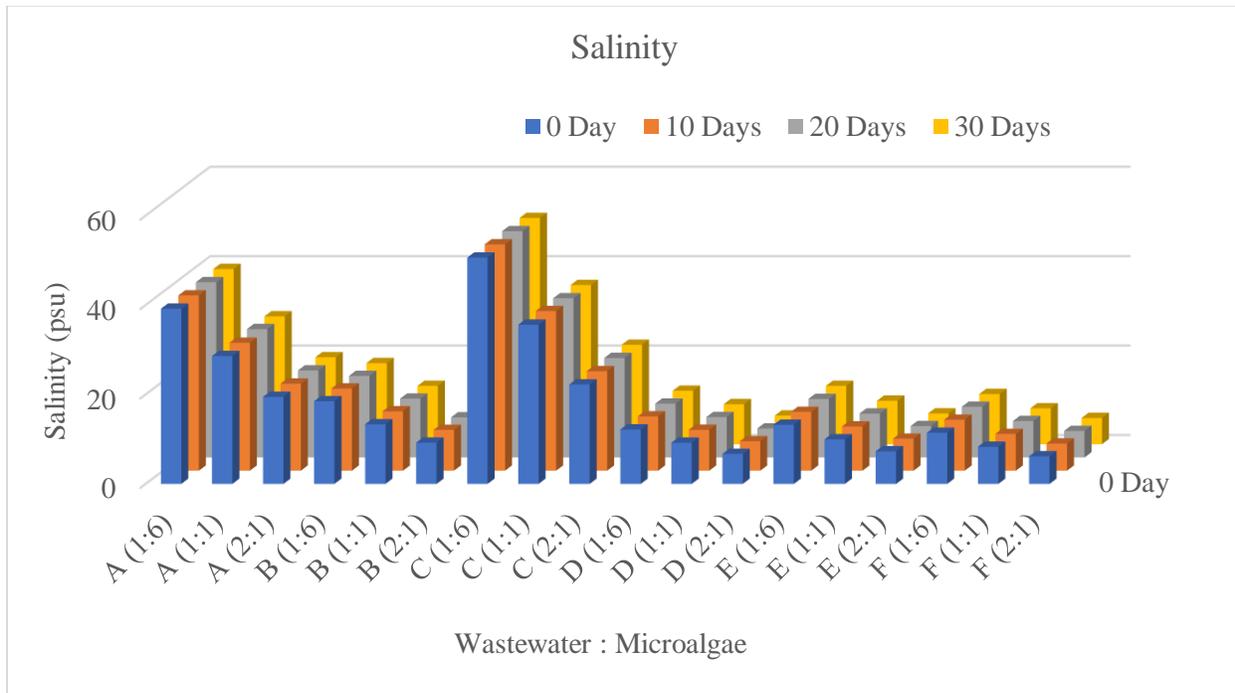


Fig. 6. The Graph representing salinity (psu) of microalgae and wastewater proportions for 30 days treatment period.

Salinity is the concentration of salts in water such as NaCl, Na₂SO₄, MgSO₄, CaSO₄, MgCl₂, KCl, and Na₂CO₃ [36]. Based on Fig. 6, 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. 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.

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 [5]. *Chlorella* is considered as salt tolerant microalgae as they can survive in an environment with 0.4 M of salt concentration [10]. 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 [37]. This is due to the existence of ionic salts such as Na⁺ and Cl⁻ that dissociates in water and can conduct electrical charges. Results revealed that, 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. Finally, optimum values required for operational parameters to achieve maximum reduction of salinity was illustrated in Table 1.

Table 1: Optimum Values of Operational Parameters to Achieve Maximum Efficiency to Treat Wastewater.

Parameters	Optimum range
Light intensity	50 $\mu\text{mol m}^{-2} \text{s}^{-1}$
pH	6.5 \pm 0.2 to 8 \pm 0.2
Temperature	25 \pm 2 $^{\circ}\text{C}$
Proportion	2: 1 (200 mL/100mL)
Optimal media	BG 11
Dissolved Oxygen	3.0 to 5.0 mg/mL
Incubation Time	30 ays

4. Conclusion

In this study, we have investigated the effect of optimal concentration of *C. vulgaris* for the reduction of salinity in the industrial wastewater. The result showed that, *Chlorella sp.* managed to reduce the salinity for wastewater polymer, dye, RO and petroleum industry, at concentration of 2:1 which were 3.67 %, 4.53 %, 5.4 %, and 4.91 % respectively. Total dissolved solids were removed more effectively throughout the process. The optimal ratio of *C. vulgaris* density concentration caused an enhancement of its removal efficiency. This concentration of microalgae and wastewater (2:1) reduces the most salinity compared to other concentration. Therefore, microalgae are one of the promising solutions in wastewater remediation. Further research will help to enhance the modified and more defined methods to treat the poly-chem industrial wastewater efficiently.

REFERENCES

1. Saengsawang B, Bhuyar P, Manmai N, et al. The optimization of oil extraction from macroalgae , *Rhizoclonium sp.* by chemical methods for efficient conversion into biodiesel. *Fuel*. 2020;274:117841.
2. Xiang X, Ozkan A, Kelly C, et al. Importance of microalgae speciation on biogas production and nutrient recovery from anaerobic digestion of lipid-extracted microalgae biomass. *Environ Eng Sci*. 2018;35:382–389.
3. Bhuyar P, Hong DD, Mandia E, et al. Desalination of Polymer and Chemical industrial wastewater by using green photosynthetic microalgae, *Chlorella sp.* *Maejo Int J Energy Environ Commun* 2019;1:9–19.
4. Bhuyar P, Rahim MHA, Sundararaju S, et al. Synthesis of silver nanoparticles using marine macroalgae *Padina sp.* and its antibacterial activity towards pathogenic bacteria. *Beni-Suef Univ J Basic Appl Sci*. 2020; 9:3.
5. Hwang JH, Church J, Lee SJ, et al. Use of microalgae for advanced wastewater treatment and sustainable bioenergy generation. *Environ Eng Sci*. 2016;33:882–897.
6. Ahmad F, Khan AU, Yasar A. The potential of *Chlorella vulgaris* for wastewater treatment and biodiesel production. *Pakistan J Bot*. 2013;45:461–465.
7. Bhuyar P, Hasbi Ab. Rahim M, Yusoff MM, et al. A selective microalgae strain for biodiesel production in

- relation to higher lipid profile. Maejo Int J Energy Environ Commun. 2019;1:8–14.
8. Bhuyar P, Rahim MH, Sundararaju S, et al. Antioxidant and antibacterial activity of red seaweed; *Kappaphycus alvarezii* against pathogenic bacteria. Glob J Environ Sci Manag. 2020;6(1):47-58.
 9. Bhuyar PM, Muniyasamy S, Govindan N. Green revolution to protect environment – An identification of potential micro algae for the biodegradation of plastic waste in Malaysia. Expert Opin Environ Biol. 2018;7: 87-88.
 10. Abou-Elela SI, Kamel MM, Fawzy ME. Biological treatment of saline wastewater using a salt-tolerant microorganism. Desalination. 2010;250:1–5.
 11. Abdulsada ZK. Evaluation of microalgae for secondary and tertiary wastewater treatment. Dep Civ Environ Eng Environmen, 2014.
 12. Beuckels A, Smolders E, Muylaert K. Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. Water Res. 2015;77:98–106.
 13. Juneja A, Ceballos RM, Murthy GS. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. Energies. 2013;6:4607–4638.
 14. Rai MP, Gautom T, Sharma N. Effect of salinity, pH, light intensity on growth and lipid production of microalgae for bioenergy application. Online J Biol Sci. 2015;15:260–267.
 15. Zhang Q, Wang T, Hong Y. Investigation of initial pH effects on growth of an oleaginous microalgae *Chlorella* sp. HQ for lipid production and nutrient uptake. Water Sci Technol. 2014;70:712–719.
 16. Bhuyar P, Sathyavathi S, Math RK. Production of bioethanol from starchy tuber (*Amorphophallus commutatus*) and antimicrobial activity study of its extracts. African J Biol Sci. 2020;2:70–76.
 17. Park J, Seo J, Kwon EE. Microalgae production using wastewater: Effect of light-emitting diode wavelength on microalgal growth. Environ Eng Sci. 2012;29:995–1001.
 18. Schirrmeister BE, Sanchez-Baracaldo P, Wacey D. Cyanobacterial evolution during the Precambrian. Int J Astrobiol. 2016;15:187–204.
 19. Rachlin JW, Grosso A. The effects of pH on the growth of *Chlorella vulgaris* and its interactions with cadmium toxicity. Arch Environ Contam Toxicol. 1991;20:505–508.
 20. Bhuyar P, Sundararaju S, Hasbi Ab. Rahim M, et al. Microalgae cultivation using palm oil mill effluent as growth medium for lipid production with the effect of CO₂ supply and light intensity. Biomass Convers Biorefinery. 2019, 1-9p.
 21. Bhuyar P. Isolation, Partial Purification and Characterization of Protease Enzyme from Proteolytic Bacteria from Dairy Soil. Int J Res Appl Sci Eng Technol. 2017;5:4083–4095.
 22. Bhuyar P, Zagade S, Revankar R, et al. Isolation, Characterization and Partial Purification of Keratinase from Keratinolytic Bacteria. Sch J Appl Sci Res. 2018;1:40–45.
 23. Bhuyar P, Binti Mohd Tamizi NA, Hasbi Ab. Rahim M, et al. Effect of ultraviolet light on the degradation of Low-Density and High-Density Polyethylene characterized by the weight loss and FTIR. Maejo Int J Energy Environ Commun. 2019;1:26–31.
 24. Selvarajan R, Felföldi T, Tauber T, et al. Screening and evaluation of some green algal strains (*Chlorophyceae*) isolated from freshwater and soda lakes for biofuel production. Energies. 2015;8:7502–7521.

25. Corliss JO, Belcher H, Swale E. A beginner's guide to freshwater algae. *Trans Am Microsc Soc.* 1997;96:283.
26. Nurul Salma A, Fatimah Md Y, Mohamed S. Effect of Salinity and Temperature on the Growth of Diatoms and Green Algae. *J Fish Aquat Sci.* 2013;8:397–404.
27. Al-Qasmi M, Raut N, Talebi S, et al (2012) A review of effect of light on microalgae growth. *Lect Notes Eng Comput Sci.* 2012;2197:608–610.
28. Bhuyar P, Yusoff MM, Hasbi Ab. Rahim M, et al. Effect of plant hormones on the production of biomass and lipid extraction for biodiesel production from microalgae *chlorella sp.* *J Microbiol Biotechnol Food Sci.* 2020;9:4.
29. Jesus SS, Filho RM. Modeling growth of microalgae *Dunaliella salina* under different nutritional conditions. *Am J Biochem Biotechnol.* 2010;6:279–283.
30. Gao Y, Cornwell JC, Stoecker DK, et al. Effects of cyanobacterial-driven pH increases on sediment nutrient fluxes and coupled nitrification-denitrification in a shallow fresh water estuary. *Biogeosciences.* 2012; 9:2697–2710.
31. Bhimba V. Standardization of growth parameters for the productions of marine microalgae. Manonmaniam Sundaranar University, 2005, 109p.
32. Zhang Q, Gradinger R, Spindler M. Experimental study on the effect of salinity on growth rates of Arctic-sea-ice algae from the Greenland Sea. *Boreal Environ Res.* 1999;4:1–8.
33. Biosci IJ, Azarpira H, Behdarvand P, et al. Potential use of cyanobacteria species in phycoremediation of municipal wastewater. *Int J Biosci.* 2014;6655:105–111.
34. Nations U. Innovative Approaches to Quality Assurance in Health Care. *Bulletin of Roszdravnadzor,* 2017,1-26p.
35. Zhuo Y, Sheng M, Liang X, et al. Treatment of high salinity wastewater using CWPO process for reuse. *J Adv Oxid Technol.* 2017;20(2):1-11.
36. Tavakkoli E, Rengasamy P, McDonald GK. High concentrations of Na⁺ and Cl⁻ ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J Exp Bot.*2010;61:4449–4459.
37. Zhuo Y, Sheng M, Liang X, et al. Treatment of high salinity wastewater using CWPO process for reuse. *J Adv Oxid Technol.* 2017;20(2):1-9.

Citation: Bhuyar P, Hong DD, Mandia E, et al. Salinity reduction from poly-chem-industrial wastewater by using microalgae (*chlorella sp.*) collected from coastal region of peninsular Malaysia. *J Bio Med Open Access.* 2020;1(1):105.