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Potential application of microalgae in produced water treatment

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ABSTRACT

The current study examines pollutant removal efficiency from the produced water of a local petroleum industry by five different local microalgae species. The five microalgae strains *Monoraphidium, Chlorella, Neochloris, Scenedesmus, Dictyosphaerium, Chlorella* and *Dictyosphaerium* species showed a significant amount of biomass generation within all different concentrations of produced water. Although the biomass yield of *Neochloris* strain was low, it was able to remove a higher amount of organic carbon than the other microalgae strains. Although biomass yield varied significantly among the microalgae strains, nitrogen removal efficiency was similar for all strains. Similar results were also obtained for most of the BTEX components. On an average, *Dictyosphaerium* sp. produced 0.5 g/L biomass density on different strength of produced water. Total nitrogen removal efficiency reached up to 63.76% when *Scenedesmus sp.* was grown in produced water. Only in case of phosphorus and various metals, removal efficiencies were better by *Dictyosphaerium* specie; reached up to 88.83%. Despite low biomass generation, *Neochloris* sp. removed 41.61% of total organic carbon from the different concentrations of produced water. Although benzene and ethylbenzene removal efficiency was 100% for all the different produced water, small amount of toluene and xylene remained in the produced water. Thus, the results indicate that microalgae strains can be used to remediate produced water effluents-derived from petroleum industries.

Keywords: Microalgae; Produced water; Oil and gas; Nutrients; Water treatment

1. Introduction

Due to scarcity of good quality water, reusing of low quality and contaminated water is highly increasing in Qatar. The main source of water in Qatar is desalination stations. Most of the desalinated water is for human usage. Agriculture in Qatar depends mainly on underground water; it is available but always saline and found in insufficient quantities [1]. Due to the increasing demand for water among industries and irrigation, using other alternative water resources such as produced water during oil and gas extraction would be of importance.

The quantity of produced water and pollutants concentration vary depending on the nature and location of the oil products [1]. It represents the major waste stream related to oil and gas processes. Large amount of produced water generated in Qatar has the potential to enhance the water resources. The crucial goal of produced water management is to eliminate dissolved harmful components and use it for beneficial uses that can efficiently improve environmental impact and water shortage. An exclusive characteristic of produced water comparing to other wastewater resources is the large variation and complexity in water chemistry. This would play a vital role in the remediation processes [1–4]. Produced water pollutants can cause an adverse effect on the surrounding environment.

In general, produced water effluents deriving from oil well contains various concentrations of hydrocarbons such as phenols, BTEX (benzene, toluene, ethylbenzene, and xylene), heavy metals and many inorganic salts [5]. These complex constituents of produced water exhibit toxicity

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to our surrounding environment. To mitigate the growing environmental pollution, bioremediation processes are used to remove and minimize the toxicity of produced water pollutants [6].

To remediate produced water pollutants, several technologies have been already established. Most of these treatment technologies involve energy inputs to remove contaminants from the produced water [4,7]. Physical and chemical treatment processes are commonly used to remove contaminants from the produced water. Both of these treatment processes ultimately raise the cost of final petroleum products [8]. Among many current treatment solutions, biological treatments can be utilized as a cost-effective way of treating produced water [8]. Furthermore, studies indicated that the growth of microorganisms could be optimized to enhance their bioremediation capability [9–13]. Mendes et al. [14] reported that *Cyanobium* could decrease the concentration of phosphate within the produced water.

Current development in treatment process introduces eco-technology approaches, where biological treatment process can reach higher removal rate of pollutants from the produced water [15]. 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 are able to utilize some of these pollutants as sources of nutrients [14]. A recent study has indicated that microalgae species *Parachlorella kessler* can utilize BTEX as a sole carbon source [15]. In another study, the toxicity test using water-soluble fraction (WSF) gasoline provides an important foundation for BTEX effect on microalgae growth [8]. However, a higher BTEX concentration with a longer period causes 50% growth inhibition on microalgae cultures [16]. Gasoline components with high BTEX content have lower toxicity than heavier hydrocarbons on microalgae growth. 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 [8]. Apart from nitrogen and phosphorus, there are other trace elements that 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 increases the production of microalgae biomass. Furthermore, cultivated microalgae biomass can be used as alternative feedstock for energy generation [10,17].

Based on the quality of the biomass, it has many different commercial applications such as animal feed, fertilizer, biofuels, and functional bioactive compounds [18–21]. 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.

Therefore, the aim of the present study was to assess the removal efficiency of Qatari local microalgae strains for various metals and organic pollutants from the produced water.

2. Materials and methods

2.1. Sample collections and characterization

Produced water samples were collected from different sources in Qatar and were initially filtered with 0.45 micron Millipore Steritop BTF-Durapore PVDF membrane to remove the suspended particulate matters. The samples were transferred in a big representative container, after consideration of all safety issues due to any possible leaking.

Water samples were then analyzed for their physical characteristics where pH and salinity were measured by Thermo Scientific Orion Star A325 Portable pH/Conductivity/Temperature Multiparameter. The total phosphorus content was estimated by colorimetric method (EPA Method 365.2, USEPA, 1983). While, HACH DR 3900 Benchtop VIS Spectrophotometer was used for the analysis of total nitrogen. 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, while Capillary Column Elite-1 L 60m was used. BTEX were analyzed by US-EPA 5021 method. Finally, the heavy metals were determined by PerkinElmer NexION 300D ICP–MS.

Throughout the experimental period, the growth parameters such as light and temperature were kept at 25°C with 12 h of daylight exposure period. All experiments were conducted in 250 mL Erlenmeyer flask with continuous shaking (Innova 44 incubator shaker series) with 120 RPM to maintain a homogeneous culture during the entire growth period. Finally, the pollutants (TOC, TN, TP, heavy metals, BTEX) removal rate were calculated before and after the final treatments.

2.2. Screening of microalgae strains

Selection of microalgae strains was based on two screening methods. Initial screening was performed by preliminary growth study in MicroWell Plates [22,23] and the final screening was performed by the growth of five selected microalgae strains in two different pH buffer solutions (3 M NaOH and 1 M $NaHCO_3$). Later produced water pollutants removal rate by the selected microalgae strains were examined with different concentration of produced water.

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

Based on the growth potential measurements using 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.

2.3. Growth rate and biomass yield of selected strains with normal growth media

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 3 M of NaOH to match the pH of standard growth media [24]. Whereas, in the second sets of the experiments, the pH value was also raised from 4.22 to 7.10 using sodium bicarbonate solution. Previously, it was found that sodium bicarbonate solution can increase the utilization of nitrate as well as the photosynthetic efficiency of microalgae strains [25,26]. Both experiments were carried out simultaneously with triplicates. The growth periods for the two 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. 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 d [27]. Table 1 shows the growth medium composition and the trace elements solution.

2.4. Algae strains with different concentrations of produced water

Growth of Algae strains was monitored everyday by taking optical density measurement of the cultures at 750 nm 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 dilutions of culture media were prepared. These dilution solutions were then filtered using pre-weighted 0.45 µM GC–F filter papers. After the filtration process, the filter paper was washed again with 0.5 M ammonium format to remove any salt. Five different dilutions of cultures were made. In order 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 2. 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 1
BG11 growth medium composition that was used in the study

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

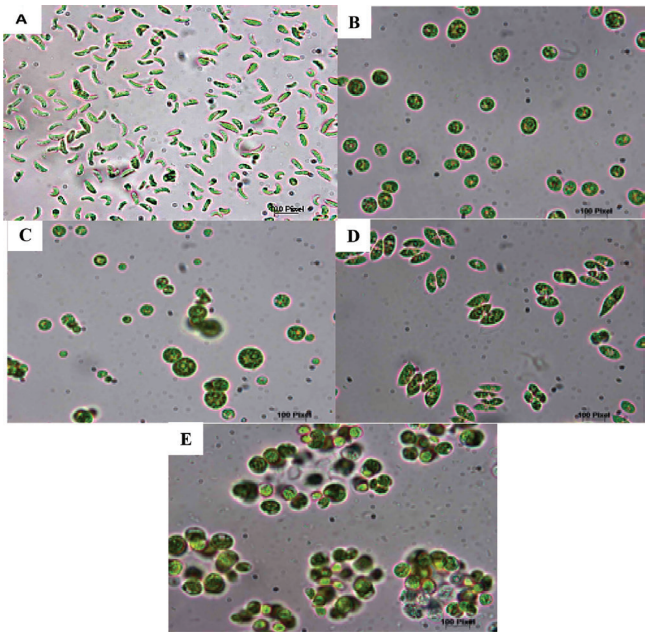

Fig. 1. List of the QUCCCM axenic strains as appeared under light microscope. The fresh water algal strains include (A) *Monoraphidium sp.*, (B) *Chlorella sp.*, (C) *Neochloris sp.*, (D) *Scenedesmus sp.*, and (E) *Dictyosphaerium sp.*

2.5. Statistical analysis

Experiments were conducted with at least triplicate treatments. Analysis of variance (ANOVA) was conducted to determine whether there was any significant difference in the mean values obtained for the selected parameters.

Table 2
Growth of five microalgae strains with different concentration of produced water

Treatment	Microalgae inoculum (mL)	Milli-Q water (mL)	Produced water (mL)	Total Volume (mL)
100% (control)	–	–	100.0	100.0
50%	10.0	45.0	45.0	100.0
60%	10.0	36.0	54.0	100.0
75%	10.0	22.5	67.5	100.0
100%	10.0	–	90.0	100.0

The mean values were considered to be different when the p-value was lower than the significance level (p = 0.05). Differences between pairs of values were analyzed using Least Significant Difference (LSD) intervals. All statistical tests were performed using Microsoft Excel® 2016.

3. Results and discussion

3.1 Produced water characteristics

The composition of filtrated produced water is shown in Table 3. The 0.45 micron Millipore filter reduced the turbidity as well as nitrogen, phosphorus, organic carbon, BTEX and trace metals of the produced water.

3.2. Screening of microalgae strains

In order to test the survival, and biomass yield of the microalgae strains in produced water culture, *Monoraphidium* sp., *Chlamydomonas* sp., *Chlorella* sp., *Scenedesmus* sp., *Neochloris* sp., *Oorococcus* sp., *Chlorococcum* sp., *Oocystis* sp., *and Dictyosphaerium* sp. were cultured for a growth period of 7 days. Fig. 2 shows the growth curves of the nine microalgae strains in filtrated produced water. Among these nine microalgae strains, only five strains (e.g., *Chlorella* sp., *Dictyosphaerium* sp., *Scenedesmus* sp., *Neochloris* sp., and *Monoraphidium sp*.) were able to survive; however, *Chlorella* sp. and *Dictyosphaerium* sp. species were found to have better biomass yield compared to the rest of the microalgae (Fig. 2). The rest of the species *Oorococcus* sp., *Chlorococcum, Oocystis,* and *Chlamydomonas,* were not able to survive in the produced water (Fig. 2).

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. The variation in the biomass densities among these strains could be attributed to the combination of several parameters: low light intensity, insufficient nutrient, different growth rate, and 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 [11]. Although some microalgae require high light intensity, some microalgae can grow at low light intensity very efficiently because of their different light harvesting pigment structures. Nutrient requirements also vary among microalgae species and therefore, some of the required nutrients could have been limiting for the microalgae and let to low growth rate. During the experiment, all the cultures were maintained at a fixed temperature (25°C) which could limit the growth of some microalgae.

Table 3
Chemical characteristics of collected produced water

Parameters of the produced water		Unit	Concentration of contaminants
Total nitrogen (TN)		mg/L	27.6
Total organic carbon (TOC)		mg/L	317.
Total phosphorus (TP)		mg/L	180.
BTEX	Benzene	mg/L	16.1
	Toluene	mg/L	3.21
	Ethylbenzene	mg/L	1.05
	Xylene	mg/L	3.11
	Salinity	p.s.u.	4

p.s.u: Practical Salinity Unit

Presence of the organics (especially BTEX) and other heavy metals could have been toxic to these species. Additionally, pH of the produced water was very low which could have negative effect on the growth. It is well known that some microalgae can survive in extreme culture pH; for example, *Galadaria sulphuria* can grow at a culture pH of 3, *Spirulina* sp. can grow in culture having pH 10 and above. It was clear that the above 4 microalgae strains, which couldn't survive in the produced water, could not be used for the remediation of the produced water. Therefore, the other five strains were selected for the remainder of this study.

Using 250 mL Erlenmeyer flasks filled with 100 mL produced water as a growth medium and during seven days of the growth period, the microalgae growth was much faster than the initial microplate screening and this might be due to the large size of the flask which allows mixing and enhance O_2 exchange (Fig. 1). The *Chlorella* sp. obtained the highest biomass yield compared to the other four species. Due to the alteration of the growth, medium most of the microalgae species were able to acclimatize during the first and second days with no increase in biomass concentration. From the third day onwards all the five strains started to grow rapidly and *Chlorella* sp. had the highest growth rate while *Monoraphidium* sp. had the lowest growth rate.

The overall microalgae biomass did not significantly increased by increasing the pH using sodium bicarbonate solution, However, *Chlorella* sp., *Neochloris* sp., and *Scenedesmus* sp. species were able to generate higher biomass compared to other two microalgae species (Fig. 2). Addition of Na_2CO_3 to the produced water could have changed the water chemistry and enhance the growth of microalgae starting from the 2nd day onwards. At high pH, some of the trace metals were expected to precipitate which were essential for microalgae growth and the available carbonate in the culture might be utilized by the examined microalgae.

3.3. Growth of microalgae strains in different concentrations of produced water

The growth profiles of five different microalgae species, in different strength produced waters, is shown in Fig. 3. The starting inoculum of microalgae was equal for all studied species and all concentration treatments (0.04 g/L). Initial growth period for all species had a noticeable lag phase. *Dictyosphaerium* sp. had the highest biomass concentration; after a day of lag period, its biomass density reached 0.54 g/L. *Chlorella* sp. obtained the 2nd highest biomass density followed by *Scenedesmus* sp. and *Monoraphidium* sp. The growth of the latter two species stayed in prolonged stationary phase after two days of growth. For *Neochloris* sp., the biomass increased for two consecutive days and then diminished. The reason

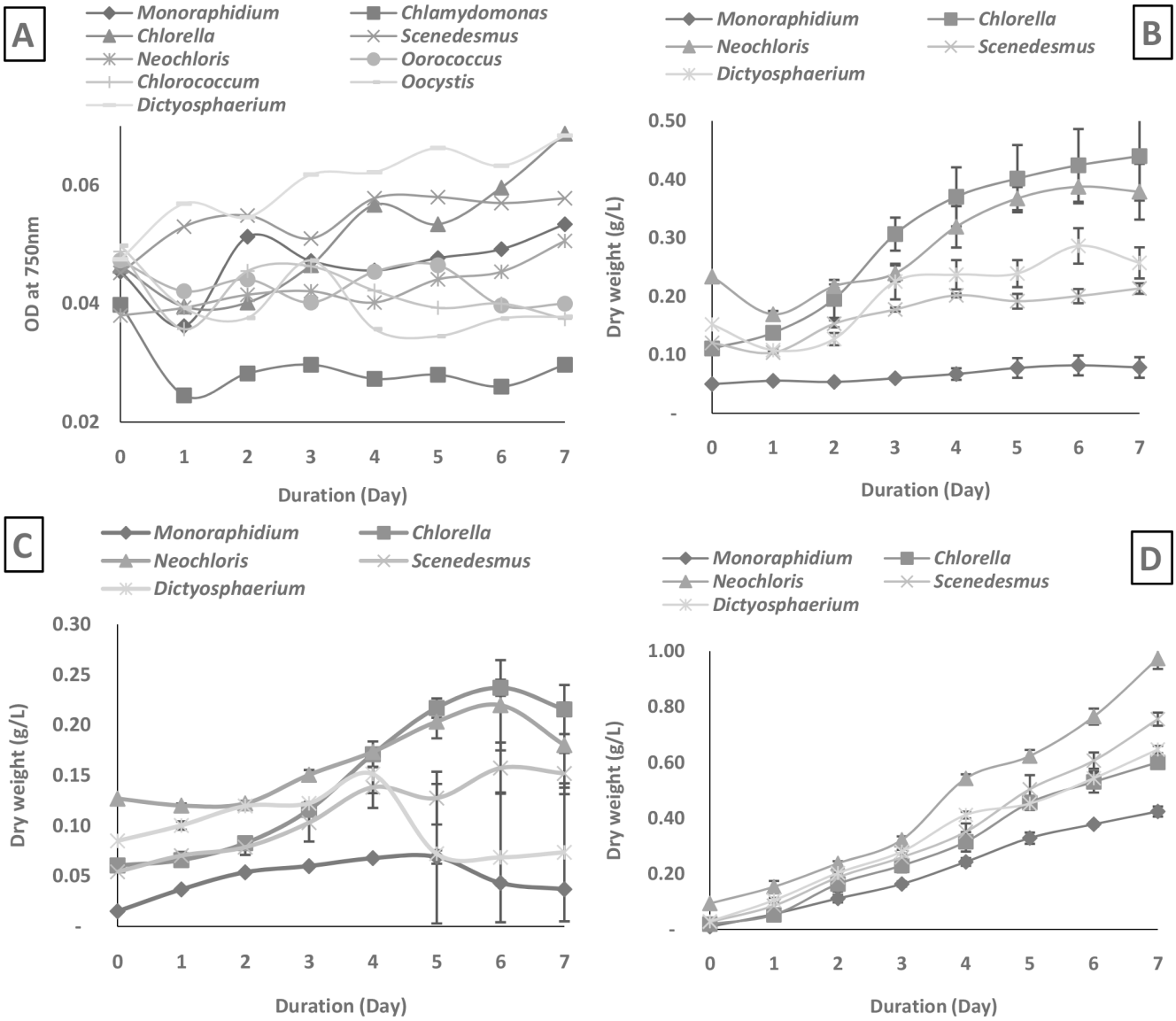

Fig. 2. (a) Initial screening of microalgae in fully concentrated produced water, (b) screening of microalgae in NaOH treated produced water, (c) screening of microalgae in Na_2CO_3 treated produced water and (d) microalgae species growth rate in standard BG11 growth medium; necessary amount of NaOH and Na_2CO_3 were added in the produced water to adjust the pH of the produced water to 7.1.

behind the diminishing growth of *Neochloris* sp. could be the formation of large clumps after four days of growth; this was also observed for the growth of *Neochloris* sp., and *Monoraphidium* sp. in microplate experiment. 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 these strains to have higher biomass yields. 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, it can be concluded that some of the micronutrients (e.g., nitrogen and phosphorus) were very limited in the cultures. It was also possible that a fraction of some, or all the nutrients could 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 factor; 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 was affected by the salinity of the produced water.

As a result, of diluting the produced water to 75% strength with distilled water, both the salinity and the concentrations of contaminants decreased which could have improved the growth conditions for these microalgae. *Dictyosphaerium* sp. in 75% still had the highest biomass concentration (0.5 g/L) which was slightly less than the biomass concentration obtained in 100% produced water

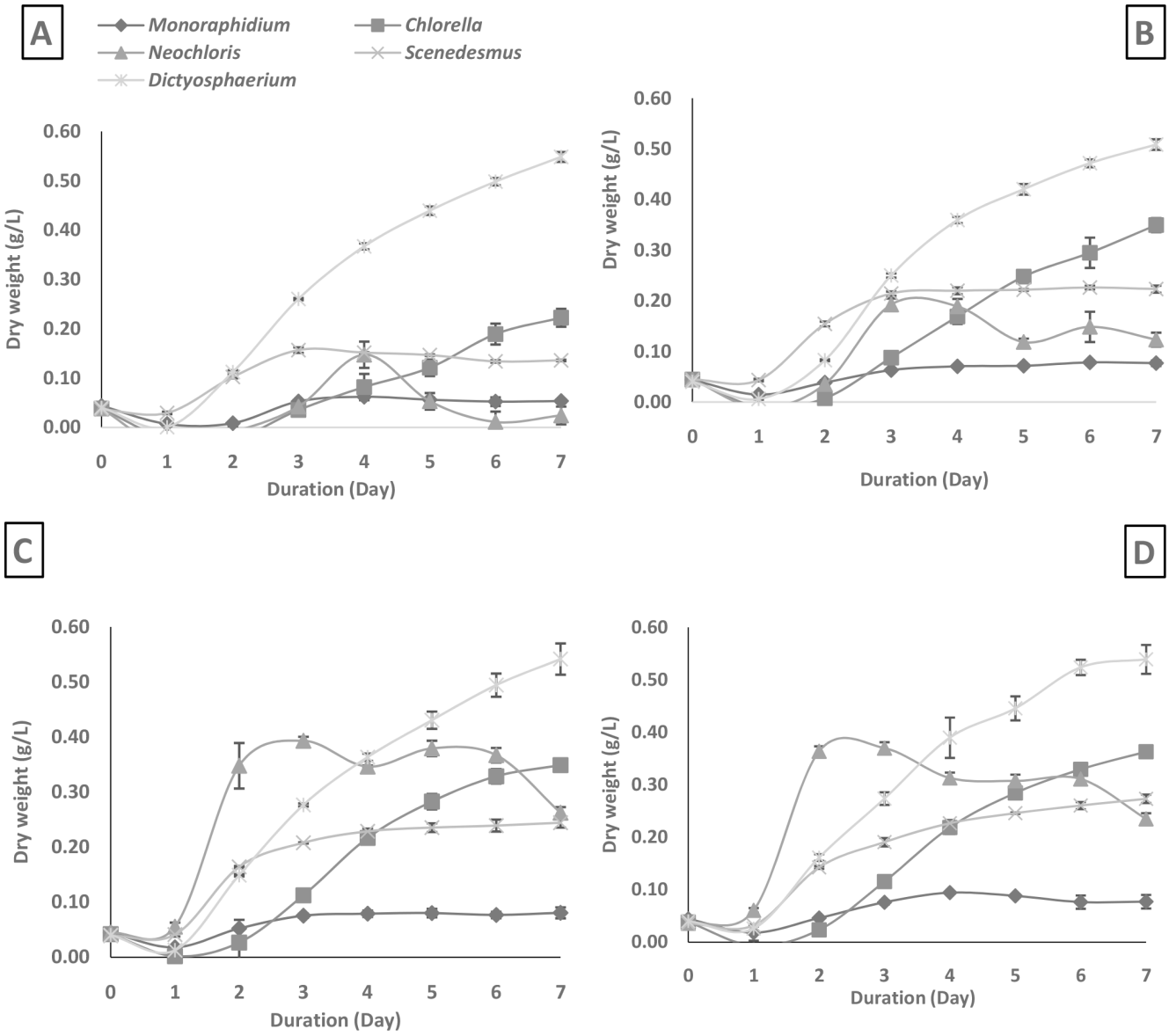

Fig. 3. The growth of different microalgae in (a) the stock produced water (100%), and in different dilutions (b) 75% (c) 60% and (d) 50% of the stock original solution.

culture. The species of *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 in 75% produced water culture. Reduced salinity and other pollutants could have improved the growth of these strains. Although an increase was observed for *Neochloris* sp. after the second day, the 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'.

At 60% produced water treatment, the only microalgae showed better growth is *Neochloris* sp. The species was able to reach 0.39 g/L within three days (Fig. 3) and from the fourth day, *Neochloris* sp. started forming larger clumps. The salinity might play a limiting factor in determining the growth of *Neochloris* sp. At 75% of produced water, the rest of the microalgae species showed similar biomass growth at 60% treatment. The highest biomass had been reported to *Dictyosphaerium* sp. followed by *Chlorella sp., Scenedesmus* sp. and *Monoraphidium* sp. (Fig. 3).

Biomass yield of these five strains in 50% produced water is shown in Fig. 3; the yields were almost identical with the yields obtained for 60% produced water (Fig. 3). The highest biomass was achieved 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.

In general, the results showed that the *Dictyosphaerium* sp. could achieve almost similar growth rate in all concentrations of produced water. *Chlorella* sp. was able to grow in different concentrations of produced water, but

the biomass yield was higher at 75% and 50% produced water concentrations. Similar results were obtained for *Scenedesmus* sp. On the other hand, *Neochloris* sp., showed better growth at 60% and 50% concentrations of produced water than at higher concentrations. Only *Monoraphidium* sp. remained in the stationary phase in all the concentration of produced water. Among the different concentration levels of produced water, 50% and 60% produced water concentration had higher microalgae biomass concentrations compared to higher concentrations (Fig. 2). Similar results were obtained for *Nannochloropsis* sp. in a previous study by Arriada and Abreu [28]. Arriada and Abreu 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. where variation in produced water concentration had a minimum effect on biomass yield. This could be due to the fact that *Dictyosphaerium* sp. could grow in a wide 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 occurred due to the physiological adjustment of the strains in a newly introduced medium. Such phenomenon was also observed by Lee [29]; exposer to higher irradiance could - introduce a lag period. Arriada and Abreu [28] 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–suggesting additional macro-nutrients should be added into produced water for the production of high density biomass. Nonetheless, low biomass yield could also be used for treating the produced water.

3.4. Pollutants removal efficiency of microalgae strains from different concentrations of produced water

Concentrations of total nitrogen, total phosphorus, total organic carbon, and BTEX were analyzed before and after the treatment of the produced water, by the microalgae species. 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. The results showed no significant difference (p 0.05) while comparing the total nitrogen removal efficiency from different concentration of produced water, although significant differences were observed among the microalgal strains. 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 was encountered with *Scenedesmus* sp. and *Monoraphidium* sp. where total nitrogen uptaking achieved to 63.76% and 62.98%, respectively (Fig. 3). On the other hand, microalgae species like *Dictyosphaerium* sp., *Chlorella* sp. and *Neochloris* sp. removed 61.17%, 58.89% and 55.23%, respectively (Fig. 3). The presence of microbial community within the produced water could have also utilized part of the nitrogen in the control experiments. Finally, the statistical analysis showed significant nitrogen removal efficiency among microalgae but not due to different concentrations of the produced water. *Scenedesmus* sp., *Monoraphidium* sp. and *Dictyosphaerium* sp. were equal in utilizing nitrogen without significant differences (Fig. 4).

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

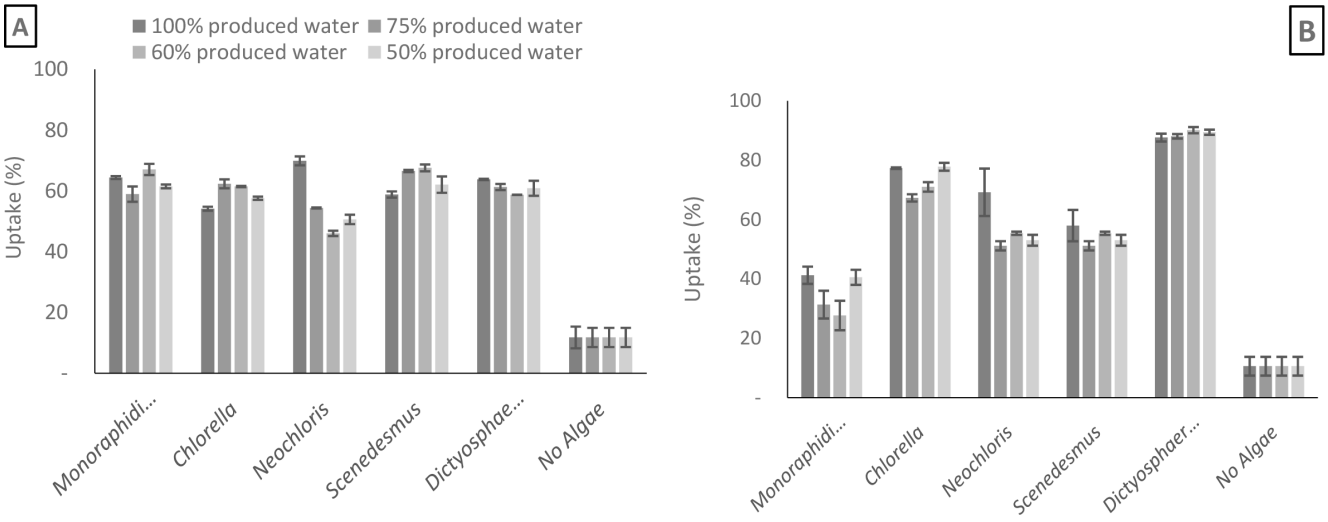

Fig. 4. (a) Total nitrogen removal efficiency by the five microalgae species from different of produced water concentrations and (b) Total phosphorus removal efficiency by five microalgae species from different of produced water concentrations. Least significant difference (LSD) (algae X PW) for nitrogen and phosphorous = 5.48 and 8.96.

and *Monoraphidium* sp. with 35.23% (Fig. 3). Again part of the phosphorous is expected to be utilized by microbial community (Fig. 4). Statistical analysis found significant removal efficiencies among the microalgae and the produced water concentration. *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 and this may explain why this species is the best in removing phosphorus.

Xin et al. [30], 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 8:1ratios. In another study, *Chlorella* sp. was found to have similar 99% nitrogen, and 90% phosphorus removal efficiencies [26,31]. *Neochloris* sp. could also remove 100% phosphorus and 78% nitrogen from the wastewater [32]. Wang and Lan [32], 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 current study produced water has the N:P ratio of 151:1 which had much more nitrogen than phosphorus 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 difference in N:P ratio, but also differences in biomass yields were responsible for the differences in phosphorus removal efficiencies.

After filtration, the produced water had 317 mg/L of total organic carbon (TOC). The results showed wide variations in removal efficiencies among species and due to different concentrations of the produced water. Overall, among other microalgae species, *Neochloris* sp. showed a better result in TOC removal efficiency. The maximum TOC removal efficiency (i.e., 41.61%) was achieved by *Neochloris* sp., whereas *Chlorella* sp., *Dictyosphaerium* sp., and *Monoraphidium* sp. removed 30.75%, 28.78%, and 27.20% TOC respectively. *Scenedesmus* sp. had the lowest average removal efficiency (20.82%) (Fig. 5). In the control where no microalgae biomass was added, the total organic carbon was also reduced because of the presence of bacterial community in the produced water (Fig. 5). *Neochloris* sp. was reported to have the highest removal efficiency of TOC from 100% produced water; around 47%. Whereas TOC removal efficiency was found to be similar among *Chlorella*, *Dictyosphaerium* sp. and *Monoraphidium* sp. which was around 40%, while it was 22% removal efficiency for *Scenedesmus*.

Recent studies found that the total organic carbon removal rate is lower than the nitrogen and phosphorus. Even it was found that no organic carbon was uptaken by microalgae from the wastewater [33]. Some of the microalgae have the ability to use dissolved organic carbon as a source of carbon and this phenomenon is known as mixotrophy. 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 [34]. Such carbohydrates could also have contributed to the residual TOC concentrations.

Benzene concentration in the produced water was 16.1 mg/L. The overall results indicated that no significant difference in removal efficiency among different microalgae species and for various concentration of produced water. Benzene removal efficiency had no significant differences among the treatments. From Fig. 5 it is clear that benzene in control flask was either evaporated in the presence of light or removed by the micro-organisms present in the produced water. Similar removal efficiencies were also found for all the microalgae cultures. According to our knowledge, 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.

Initially toluene concentration was found as 3.21 mg/L. *Dictyosphaerium* sp. had the highest toluene removal efficiency (Fig. 5). 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 differences in toluene removal efficiency. Whereas, toluene removal efficiency from 100% and 75% produced water concentration level have the highest significance.

Among other BTEX constituent's ethylbenzene concentration was found to be the lowest 1.05 mg/L. Removal efficiency of ethylbenzene between different concentrations didn't have any significant difference. Whereas, ethylbenzene removal efficiency had significant variation among different microalgae. Overall average ethylbenzene removed from different produced water concentrations, where *Neochloris* sp., *Dictyosphaerium* sp., *Monoraphidium* sp, *Scenedesmus* sp., *Chlorella* sp., and the control removed 100%, 98.12%, 97.60%, 93.94%, 90.09% and 86.71%, respectively (Fig. 5).

Produced water had an initial 3.11 mg/L concentration of xylenes. Among all the microalgal treatment including control, no significant difference in xylene removal efficiencies were observed concentration of produced water. Although no microalgae biomass was added in control, xylenes removal efficiency was as high as 96.41% (Fig. 4). 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 efficiencies from produced water.

Studies have shown wide verities of approaches where biological treatment should be an excellent alternative solution to remediate BTEX constitutes. In biological system, different microorganisms such as bacteria, fungi and algae can be used to remove BTEX [35]. These

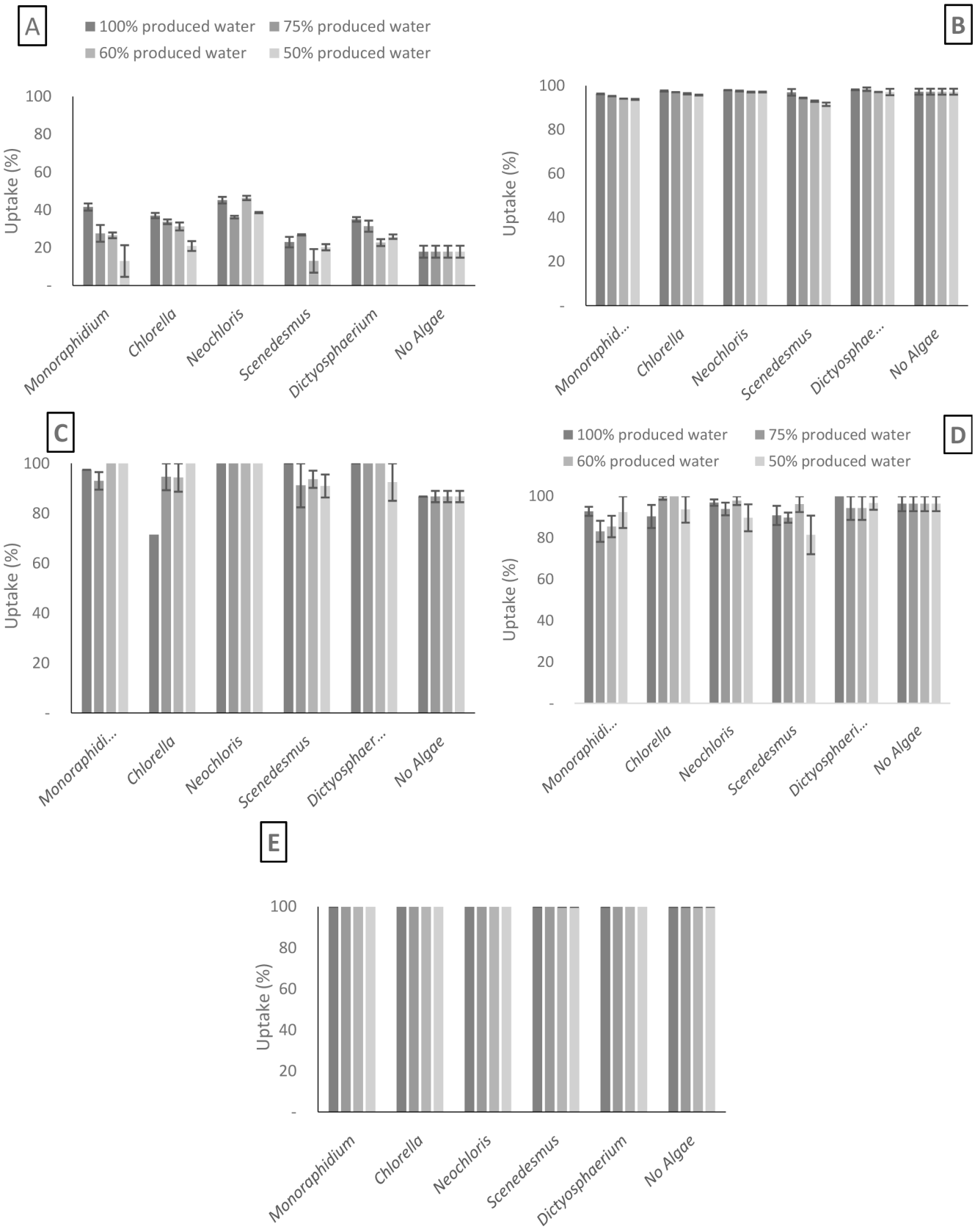

Fig. 5. The removal efficiency by five microalgae species from different of produced water concentrations for: (a) Total organic carbon, (b) Toluene, (c) Ethylbenzene , (d) Xylenes, and (e) Benzene. . Least significant difference (LSD) (algae x PW) for organic carbon, benzene, toluene, ethylbenzene, xylenes = 8.73, 1.11, 2.15, 15.05, 13.07, respectively.

biological treatments can approach aerobic degradation [36]. 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 [19]. A pervious study has found that under aerobic condition microorganisms are highly receptive to BTEX constitute [36]. Among the monoaromatic hydrocarbons of BTEX, toluene was found to have a faster biodegradation due to its structure configure that allows the microorganisms to oxidize the aromatic ring [38]. All constitutes of BTEX have at least 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 [35]. Andreoni and Gianfreda [38], 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 et al. [39], reported that with a pH range of 7.2–7.4 *Mycobacterium cosmeticum* species can biodegrade 82–100% of BTEX. Singh and Celin [19] 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%, and 95.96% by different algae species. Among all microalgae species *Dictyosphaerium* sp. was able to remove more compounds of BTEX then other species. Recent studies have also shown a promising sign where BTEX can be used as a sole carbon source [15]. The authors 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 results where almost 100% of benzene and ethylbenzene was uptaken by microalgae, where 97.38% toluene and 95.96% xylenes removed by microalgae. In addition, 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 [40].

Metal removal efficiencies by different microalgae in different concentrations of produced waters are shown in Table 4. 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 be removed 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 in *Monoraphidium sp.*

Initially, after analyzing metals, some elements were found over 1 mg/L level. Such elements are K, Mg, Sr, and B as shown in Table 4. Where the rest of the element concentrations like Mn, Cu, Fe, Ba, Cr, Al, Zn, Ni, V and Cd were below 1 ppm. After the microalgae biomass growth results showed a 100% removal efficiency of Al, Fe and Zn from the produced water. In addition, removal 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
Metals concentration in the produced water before and after filtration in the presence of different microalgae species

Metal	Initial metal concentration (µg/L)	Metals removal efficiency by microalgae (%)	Microalgae species
K	73.6×10^3	11.3	*Scenedesmus* sp.
Mg	41.7×10^3	13.9	*Dictyosphaerium* sp.
Sr	11.2×10^3	21.2	*Dictyosphaerium* sp.
B	4.26×10^3	20.2	*Dictyosphaerium* sp.
Mn	318.	87.8	*Neochloris* sp.
Cu	225.	91.7	*Dictyosphaerium* sp.
Fe	288.	100.0	*Neochloris* sp.; *Chlorella* sp.
Ba	55.7	13.1	*Monoraphidium* sp.
Cr	24.1	19.4	*Dictyosphaerium* sp.
Al	114.	100.0	*Neochloris* sp.
Zn	25.1	100.0	*Monoraphidium* sp.
Ni	7.83	92.3	*Dictyosphaerium*
V	1.87	36.3	*Scenedesmus*
Cd	0.09	97.4	*Chlorella*

Microalgae require some of these elements like Zn, Fe, Cu, Mn, B, Mg, and K as micronutrients [41]. Some studies also found that at higher concertation of these elements may increase toxicity [42]. In our findings showed that *Dictyosphaerium sp* was able to grow better than other species and able to remove more elements. Another study also found some similarity where *Dictyosphaerium sp* were resistance to Cr within growth medium [43]. 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. 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 4). Each of these elements has their function for the growth of microalgae spices [44]. 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 [45].

The new finding here in this study is 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 could induce toxic effect among many microalgae and in some cases, the tolerable ranges are species specific [46]. In this study, 14 metals were found within the produced water. Among them, only half of the metals considered as micronutrients. These micronutrients like potassium are essential in many enzyme reactions [47]. Whereas, copper and iron are essential for photosynthetic electron transport system [48]. Usually, in DNA transcription and phosphorus uptake zinc is used by microalgae [49]. 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 [45]. In a previous study, it was found that higher chromium concentration 0.75 ppb causes a significant reduction in Chlorophyll an intensity in *Scenedesmus* sp. [50]. It was found that *Dictyosphaerium* sp and *Chlorella pyrenoidosa* can tolerate as high asset al. 13–17 mg/L and 2 mg/L chromium concentration respectively [51,52]. 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 h.

4. Conclusion

Produced water constitutes of high concentration of pollutants, such as dissolved nitrogen, phosphorus, dissolved organic carbon, heavy metals and monocyclic aromatic compounds like BTEX (benzene, toluene, ethylbenzene, and xylene). Thus, removal of these pollutants from produced water is essential to reuse it for different purposes without any harmful effects on the environment. 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. *Dictyosphaerium* sp. showed a high growth potential within all produced water concentration levels. Total nitrogen removal efficiency reached up to 63.76% when *Scenedesmus* sp. was grown in produced water. In case of total phosphorus, removal efficiency reached up 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 concentrations of 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.

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