Int. J. Environ. Res., 10(4):555-560, Autumn 2016
ISSN: 1735-6865

Treatment of Petrochemical Wastewater by the Green Algae *Chlorella vulgaris*

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Received 3 June 2016; Revised 18 Aug. 2016; Accepted 22 Aug. 2016

ABSTRACT: In recent years, cultures of algae were developed for both wastewater treatment and bio-fuel production. In this research, green microalgae *Chlorella vulgaris* were applied for bioremediation of an Iranian petrochemical wastewater samples. The efficiency of C. *vulgaris* and nonionic surfactants were examined in laboratory scale for elimination of COD, BOD, Total Nitrogen (TN), Total Phosphors (TP), and total petroleum hydrocarbons (TPH). In the preliminary study, the growth of the algae were monitored in pure as well as 25%, 50% and 75% diluted wastewater samples The effectiveness of two surfactants of Tween 20 and Tween 60 were investigated in 15 day experiments. Removal of all parameters was significantly increased using surfactants. Nitrogen and Phosphors removal were observed in all experiment up to 100%. Maximum TPH degradation of 27% was observed by Tween 60 compare to 10% for control sample. Hence, using *Chlorella vulgaris* and surfactants can be recommended for treatment of wastewaters from petroleum industries.

Key words: Petrochemical wastewater, Microalgae, Chlorella vulgaris, Bioremediation

INTRODUCTION

Wastewaters in the petroleum industries are complex mixture of toxic recalcitrant organic and inorganic chemicals and are mainly difficult to manage. Particularly, petrochemical wastewaters contain high concentration level of nitrogen and phosphors which can cause eutrophication effect in the freshwater and marine ecosystems. Bioremediation is extensively suggested for treatment of petrochemical wastewaters in the literature (El-Ashtoukhy et al., 2013; Yang et al., 2015).

Chlorella vulgaris is widely used for bio-fuel production since they are lipid accumulator (Mallick et al., 2012; Pragya et al., 2013). On the other hand, the alga is capable to remove chemicals in wastewater systems and successfully employed for palm oil mill effluents (POME) remediation (Kamarudin et al., 2013), textile wastewater treatment (Lim et al., 2010) and Nitrogen and phosphorus removal from municipal wastewater (Silva-Benavides and Torzillo, 2012). Furthermore, it demonstrated excellent for wastewater treatment and bio-fuel production at the same time (He et al., 2013, Ma et al., 2016). A wide range of N (55-88%) and P (12-100%) removal has been reported when municipal wastewater was used as the waste stream (Khan et al. 2008., Ruiz-Marin et al, 2010. Li et al., 2011). In an effort, Abou Shanab al (2013) strived to integrate the treatment of piggery wastewater (TN: 56±2 mg/L and TP:13.5 ±0.6 mg/L) and biofuel production. They reported that six microalgal species including *Ourococcus multisporus*, *Nitzschia cf. pusilla*, *Chlamydomonas mexicana*, *Scenedesmus obliquus*, *Chlorella vulgaris*, and *Micractinium reisseri* were capable of efficiently treating wastewater and producing high oil content for biodiesel production. Among the studied species, *C. Mexicana* was proven to have the highest removal rates, i.e., N (62%), phosphorus (28%), and inorganic carbon (29%). Hence and due to the higher lipid

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productivity and lipid content (0.31±0.03g/L and 33±3%, respectively), compared with the other species, the authors suggested that *Chlorella Spp* could be a suitable candidate for integrated wastewater treatment and biodiesel production. Thus, it recognized as a cost effective and environmental friendly technique.

Several parameters can affect *Chlorella spp* biomass production including light, CO_2, Nutrients, etc. However, the role of surfactant on enhancement of bioremediation of organic contaminants by *Chlorella vulgaris* is still unclear. The novelty of this research lies in the application of nonionic surfactants**.**The objective of the present study was to examine the effectiveness of *Chlorella vulgaris* on bioremediation of petrochemical wastewater and to determine efficiency of two commercial surfactants on removal of organic substances from wastewaters.

MATERIAL & METHODS

Algae and chemicals: *Chlorella vulgaris* was purchased from the Culture Collection of Algae and Protozoa (CCAP, Oban, UK) and was cultured in modified BG11 medium containing (g/L): $NaNO_3$ (1.5); K_2HPO_4 (0.04); $MgSO_4 \cdot 7H_2O$ (0.075); $CaCl_2$ (0.036); citric acid (0.006); Na_2CO_3 (0.02); Na_2EDTA (0.001); and ferric ammonium citrate (0.006)to create stock biomass media. Nonionic surfactants of Tween® 20 and Tween® 60 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were obtained from Merck (Darmstadt, Germany).

Bioremediation procedure: Petrochemical wastewater samples were collected from Asaluyeh area, Iran. The specification of the wastewater is presented in Table 1. Bioremediation experiments were performed in laboratory conditions at 25°C and were carried out in 250 ml Erlenmeyer flasks, each containing 200 ml of sterilized wastewater sample 10 ML of algal media were added to every flasks and the alga was grown for 15 days in triplicate. As a preliminary study, the growth of the microalgae were tested in pure as well as 25%, 50% and 75% diluted wastewater samples and was monitor by optical density at a wavelength of 680 nm. A stock solution 0.001 ml/L of surfactants (Tween® 20 and Tween® 60) was prepared. 5, 10 and 15 ml/L of stock solution were used for wastewaters samples which were 20% diluted.

Chemical analysis: Chemical Oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), and were measured by a Hach DR 5000 Spectrophotometer (Hach Company, Loveland, CO, USA). Five-day biochemical oxygen demand (BOD) was determined following Standard method (APHA, 2012). Total petroleum hydrocarbons (TPH) were extracted by carbon tetrachloride followed by a Florisil column cleanup and analyzed with a Perkin-Elmer Spectrum One FTIR spectrophotometer (Perkin-Elmer Life and Analytical Sciences, Shelton, CT, USA) according to ASTM D7066-04 (ASTM, 2016). A calibration standard mixture (v/v) of n-hexadecane (37.5%), isooctane (37.5%) and benzene (25%) were used and the spectrum was recorded between the 3,100– 2,800 cm^{-1} range. The absorbance value was measured at 2,926 cm^{-1} (Kostenko, et al., 2013). Quality assurance/Quality control (QA/QC) was performed for all chemical analysis.

Statistical analysis: Data analyses were applied using SPSS 17.0 (SPSS, Chicago, IL, USA) one-way analysis of variance (ANOVA) were carried out and the criterion selected for significance was $p < 0.05$. The homogeneity and normality of variance were checked by Levene's test and the Kolmogorov–Smirnov test, respectively.

RESULTS & DISCUSSION

The growth of *Chlorella vulgaris* in several dilution ration of petrochemical wastewater is illustrated in Fig 1. The maximum growth in 25% diluted wastewater were in day 15 while highest level of growth were observed later in dilution ration of 100% and 75% as shown in Fig 1. Thus, 15 day experiments can be suitable time for the culture of C. *vulgaris.*

Table 1. The Characteristics of the petrochemical wastewater samples

No.	Parameter	Range
1	pH	5.5 - 8.0
2	TN	4.4 - 18.0
3	TP	2.5 - 3.7
4	TPH	50 – 150
5	COD	600 – 2300
6	BOD	200 – 1500
7	COD/BOD ratio	1.62 - 3.43

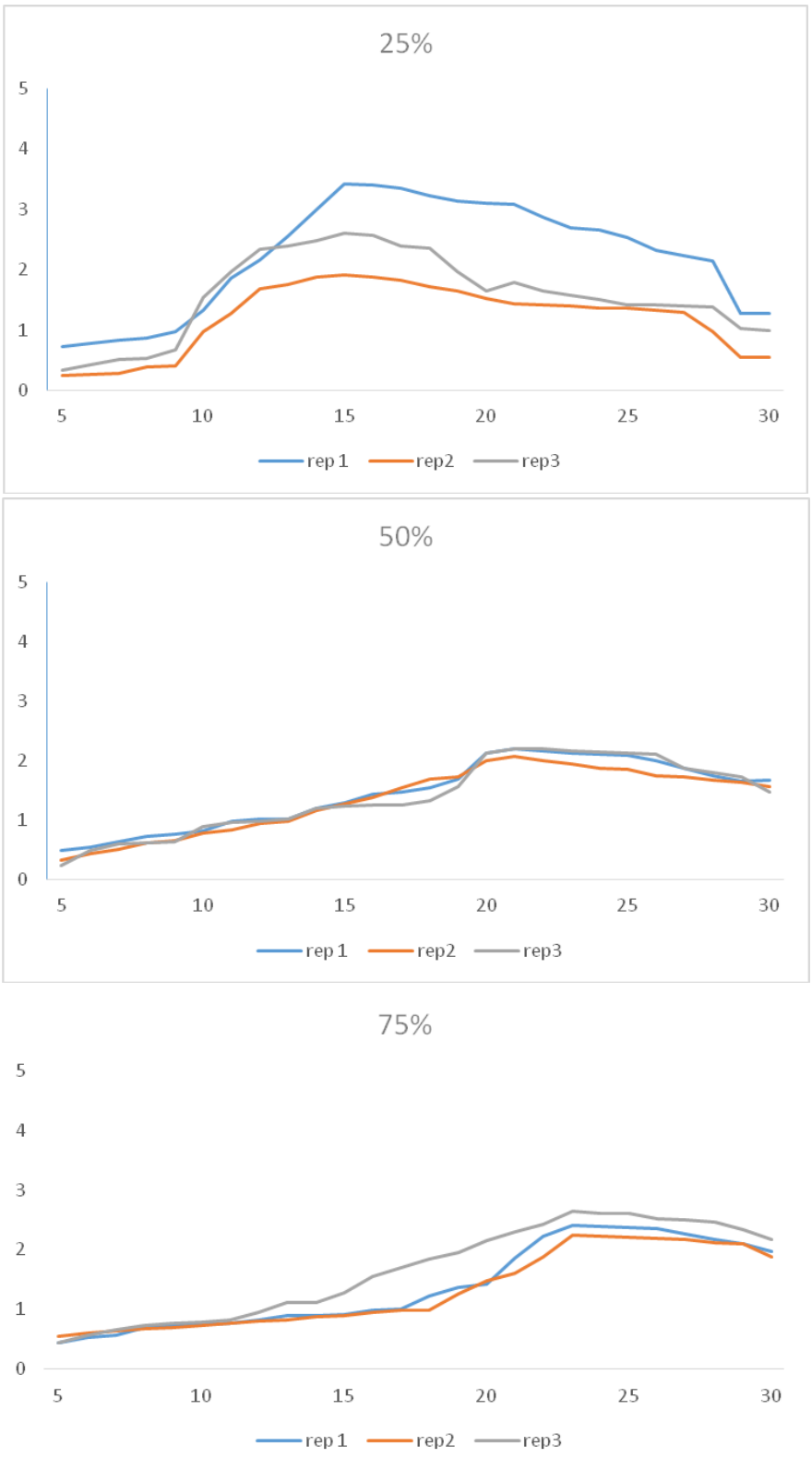
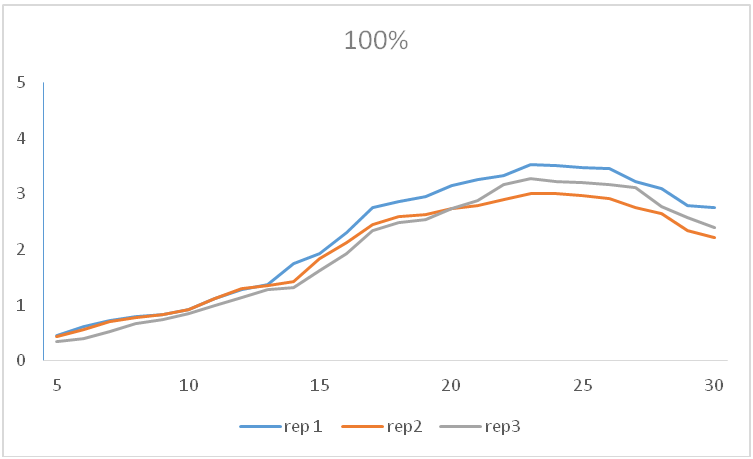


Fig. 1. Growth of *Chlorella vulgaris* in several dilution ration of petrochemical wastewater (a) 25% , (b):50%, (c:)75% (d):100% and (E) BG11 medium

d)



e)

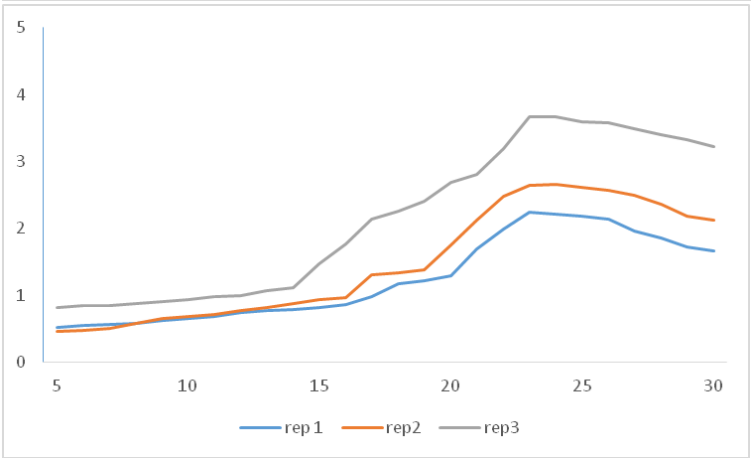


Fig. 1. Growth of *Chlorella vulgaris* in several dilution ration of petrochemical wastewater (a) 25% , (b):50%, (c:)75% (d):100% and (E) BG11 medium

Table 2. Removal of nutrients and organic contaminants

	%Removal				
Experiment	**TN**	**TP**	**COD**	**BOD**	**TPH**
Tween60 - 5cc	17.57	79.17	15.33	42.31	26
Tween60 - 10cc	36.49	100.00	38.00	100.00	27
Tween60 - 15cc	51.35	100.00	28.00	100.00	20
Tween20 - 5cc	9.46	75.00	20.67	32.69	16
Tween20 - 10cc	16.22	100.00	14.67	32.69	18
Tween20 - 15cc	40.54	100.00	16.00	100.00	14
*Control	43.24	54.17	11.33	51.92	7

* Control: Wastewater20%+algae

The removal of nitrogen, phosphorus, COD and BOD are presented in Table 2. *Chlorella vulgaris* shows excellent ability for phosphorus elimination in most experiments. In Tween 20, addition of 15 mL, were shown nitrogen and phosphorus removal of 40.5 and 100% respectively. By using 15 mL of Tween 60, nitrogen and phosphorus elimination were 51.3 and 100% respectively. Several reports were confirmed effectiveness of surfactants for removal of organic materials. Tween 20 and Tween 60 were extremely effective on BOD and COD removals as well. As can be seen, both surfactants were demonstrating better once used 15 mL. Consequently, low concentrations of surfactants are not enough for efficient degradation of organics from petrochemical wastewaters. Most of samples were shown significant BOD removals. Both

nonionic surfactants were significantly assisted remediation of hydrocarbons from petrochemical wastewater samples. Similar observations have been made by other researchers Sponza and Gök (2010); Tian et al., (2015). However, Tween 60 was more practical and can be recommended for biological treatment petrochemical wastewater using *Chlorella vulgaris*. The result of this research agreed with other reports including Christenson and Sims (2011) and Abdel-Raouf et al., (2012). Eralier in the year 2010, Lim et al made an attempt to treat textile wastewater medium using *C. vulgaris* and reportedly managed to remove N and P by 45% and 33%, respectively. Compare to other reports, our results were shown better removal of organic and inorganic compounds. Maximum 91% of TP removals were reported by Al-Mamun and co-workers. Ahmad et al., 2013 used *Chlorella vulgaris* for wastewater treatment and highest BOD removal of 100% was observed.

CONCLUSIONS

Bio-treatment of petrochemical wastewaters was carried out effectively by microalgae *Chlorella vulgaris*. There was sufficient evidence for the role of surfactants on bioavailability of petroleum hydrocarbons. The study concluded that combination of surfactants and *Chlorella vulgaris* is a superior approach for pre-treatment of wastewaters and can be suggested for removal of nutrients from petrochemical wastewaters.

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