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RESEARCH PAPER

Effect of Dilution on Nitrogen Removal from Ammonia Plant Effluent using *Chlorella vulgaris* and *Spirulina platensis*

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ABSTRACT
In this study, the removal of nitrogen from effluent of ammonia plant by *Chlorella vulgaris* and *Spirulina platensis* was investigated. For this purpose, microalgae were cultivated in three diluting percentage of the wastewater (1, 3, and 5%) at 29±1 °C and light intensity at surface of culture were adjusted to 150 µmol photon / (m^2. s). The results showed that *Spirulina platensis* is more capable than *Chlorella vulgaris* to grow in high levels of total nitrogen concentration. Also, maximum biomass production rate happened in 1% diluted samples for *Chlorella vulgaris* and 3% for *Spirulina platensis*. Furthermore, *Chlorella vulgaris* reduce total nitrogen concentration up to 55%. This value for *Spirulina platensis* was about 96%. However, for both species the removal of nitrogen in 1% diluted wastewater was maximum. According to the results of diluted wastewater of ammonia plant, it is a suitable culture medium for microalgae and it can be used to remove the nitrogen before entering the wastewater in nature.

KEYWORDS: Ammonia plant, Wastewater treatment, Diluting percentage, Nitrogen removal, Microalgae.

INTRODUCTION

Environmental pollution, caused by urban, agricultural and industrial wastewater, with both organic and inorganic sources, constitutes a relatively large amount of contaminants released in water and import adverse effects on human and animal food cycles (Halling-Sørensen & Jorgensen, 1993). Depletion of receiving waters from dissolved oxygen which cause toxicity to aquatic life, the phenomenon of Eutrophication and Methemoglobinemia disease have all been reported to be of the adverse effects of the presence of nitrogen and phosphorus in wastewater. Diverse engineering methods such as biological processes, striping, ion exchange, chlorination, reverse osmosis, distillation, sedimentation and membrane processes have been widely applied to remove nitrogen compounds (Halling-Sørensen & Jorgensen, 1993; Thomson & Tracy, 2005). These processes generally require high expenses, complex operations and large volumes of waste sludge production. Therefore, more research is needed to develop the technologis for nutrients removal.

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Microalgae culture system plays an important role in wastewater treatment. Through removal of nutrients, organic matter and heavy metals as well as absorption of carbon dioxide, microalgae have grown and become a source of biofuels, which is a big step towards protecting the environment (Markou & Georgakakis, 2011; Mata et al., 2010; Habibi et al., 2019; Habibi et al., 2018; Gharabaghi et al., 2015). On the other hand, microalgae are able to reduce BOD and COD (Kshirsagar, 2013; Mata et al., 2012). Microalgae can also be exploited for applications as energy, valuable food materials, cosmetics, fertilizers and also in pharmaceutical industries (Chew et al., 2017; Mata et al., 2010; Mtaki et al., 2021).

Researchers have studied diverse species of microalgae for municipal and industrial wastewater treatment (Cai et al., 2013). *Chlorella* (Chan et al., 2014; Ruiz et al., 2011; Ruiz et al., 2013; Ruiz-Marin et al., 2010; Yeh et al., 2012), *Spirulina* (Chan et al., 2014; Jiang et al., 2015; Markou et al., 2012) and *Scenedesmus* (González et al., 1997; Ji et al., 2013; Mata et al., 2012) constitute a large part of studied microalgae species for nutrient removal. Wang and et al. (Wang et al., 2013) showed that *Chlorella sp.* is capable to remove more than 83% of nitrogen and 90% phosphor from the municipal wastewater. Hanumantha Rao et al. (2011) studied the growth of *Chlorella vulgaris* in industrial wastewater and reported 75% removal of total Nitrogen. Also, Ruiz and et al. (Ruiz et al., 2011; Ruiz et al., 2013) presented a kinetic model to remove Nitrogen and Phosphor from urban wastewater by *chlorella vulgaris. They* reached nitrogen and phosphor removal to about 100%. Chan et al. (Chan et al., 2014) compared the growth of *Chlorella vulgaris*, *Spirulina maxima*, and mixed cultures of naturally growing algae in Collingwood Wastewater. They reported high reduction percentage of phosphate and ammonia level to be about 90.4% and 86.2% respectively. In other work, Kosaric et al. (1974) investigated the capability of *Spirulina maxima* in industrial wastewater. They showed *Spirulina* could completely remove 40 mg/L of nitrogen and 3 mg/L phosphor from wastewater. Furthermore, Phang et al. (Phang et al., 2000) showed that *Spirulina platensis* removes ammoniacal-nitrogen and phosphate completely from the industrial wastewater. Markou and et al. (Markou et al., 2012) reported complete Nitrogen removal of industrial wastewater by *Spirulina platensis* after 16 days.

The most common issue with wastewater treatment by microalgae is the separation of microorganisms from the culture medium. Microalgae harvesting processes are centrifugation, filtration, coagulation, flotation, electrophoresis, and sedimentation (Christenson & Sims, 2011). Among the diverse microalgae species, *Spirulina* is preferred in terms of enhanced ability to grow under heterotrophic and mixotrophic conditions, potential to grow at a very high NH^{4+}-N concentration (up to 130 mg/L), functioning in a wide range of PH, role as bioadsorbent for heavy metals, easier harvesting and application as a food supplement for fish and mammals (Olguín et al., 2003).

In this study, total nitrogen removal from diluted ammonia plant effluent of 1, 3 and 5% using *Chlorella vulgaris* (*C. vulgaris*) and *Spirulina Platensis* (*S. plantensis*) was investigated. Also, biomass production rate for both species was obtained.

MATERIALS AND METHODS

Microorganisms *C. vulgaris* and *S. platensis* was provided by the Iranian Research Organization for Science and Technology and pre-cultivated in BG11 and zarrouk medium, respectively (Raoof et al., 2006; Stanier et al., 1971). Composition of different media are presented in Table 1. Microorganisms were grown at 29±1 $^{\circ}$C in 250-ml Erlenmeyer flask with light flux density of 150 µmol photon / (m^2. s) for 14 days.

Table 1. Composition of BG11 [24] and Zarrouk's [25] Media.

Ingredients	BG11 (g)	Zarrouk (g)
NaNO3	1.5	2.5
K2HPO4.3H2O	0.04	0.5
MgSO4.7H2O	0.075	0.2
CaCl2.2H2O	0.038	0.04
FeSO4.7H2O	0.038	0.01
Ferric ammonium citrate	0.006	-
EDTA Na	0.001	0.08
NaHCO3	-	16.8
Citric acid	0.006	-
NaCl	-	1
K2SO4	-	1
H3BO3	0.00286	0.00286
MnCl2.4H2O	0.00181	0.00181
ZnSO4.7H2O	0.00022	0.00022
CuSO4.5H2O	0.00008	0.00008
Na2MoO4.2H2O	0.00039	0.00001
Distilled water	1Litr	1Litr

Wastwater effluent was collected from Khorasan Petrochemical Company (Bojnord, North Khorasan province, Iran) comprises urea, ammonia, and melamine production units. The characteristics of the collected wastewater are listed in Table 2. In order to cultivate microalgae and investigate the removal of total nitrogen, 5 ml of seed culture were added to 250 ml of diluted wastewater (1, 3 and 5%) and aerated with air in 250 ml Erlenmeyer flasks. The temperature conditions of 29±1 ºC and light intensity of 150 µmol photon / (m^2. s) was maintained. Noteworthy, initial algae in the logarithmic phase of growth, was used as seed culture in all treatments.

Table 2. Physico-chemical parameters of wastewater.

parameter	Wastwater effluent
$N-NH_4^+$	1867
$N-NO_3^-$	903
SO_4^{2-}	330
TDS	3050
PO_4^{3+}	8
Ca^{2+}	22
Mg^{2+}	20
pH	9.5

All values are in mg/L except for pH.

Collected samples from photobioreactor were centrifuged at 2500×g for 15 mins. TN was determined using a total nitrogen analyzer (EXPLORER, Netherland). phosphate ions were analyzed using the chloroacetone (at 690 nm) standard method (Baird & Bridgewater, 2017).

The optical density (OD) of the broth was determined by measuring the absorbance at 550 nm for *C.vulgaris* and 560 nm for *S. platensis* in a double beam UV/Vis spectrophotometer (V-550 JASCO, USA) with a cell path length of 1cm. To measure cell dry weight, a 10 ml sample of algal suspension was filtered through a pre-dried and pre-weighed 47 mmWhatman paper filter (GF/F, nominal pore size 0.7 µm), and washed twice with 20 ml of distilled water. The filter was, then, dried at 105°C overnight then placed in a desiccator and weighed to the nearest 0.1 mg (Delavari Amrei et al., 2014; Delavari Amrei et al., 2015). The relationship between the biomass concentration (X, g/L) or cell dry weight and optical density (OD) is obtained as follow:

$$X_{Cv} = 0.7381 \times OD_{550nm} - 0.0456 \quad , \quad R^2 = 0.9958 \tag{1}$$

$$X_{Sp} = 0.8561 \times OD_{560nm} - 0.0592 \quad , \quad R^2 = 0.9934 \tag{2}$$

Also, specific growth rate of the culture can be calculated using Eq. (3):

$$\mu = \frac{\ln(\frac{X_t}{X_0})}{t} \tag{3}$$

where µ is the specific growth rate (1/day), X_t and X_0 are the biomass concentration at time t and at the beginning, respectively.

The biomass productivity rate (P, g/(L . day)) is estimated by Eq. (4):

$$P = \frac{(X_F - X_0)}{t_F - t_0} \tag{4}$$

where X_F is the biomass concentration at the end of the cultivation (t_F).

Percentage of nutrient removal is calculated by the following equation:

$$R\% = \frac{C_f - C_0}{C_0} \times 100 \tag{5}$$

where C_0 and C_f are the concentration of total nitrogen or phosphate ions in waste water at the beginning and the end of the cultivation, respectively.

RESULTS AND DISCUSSION

Time course of cell concentration of *C.vulgaris* is presented in Fig. 1. As can be seen in this figure, for diluted wastewater of 3%, algae grows until the 4th day of experiment, then the growth is reduced until the 6th day and, after that, starts to grow again. For sample diluted of 5%, algae could not be adapted until the 11th day of experiment. In fact, *C.vulgaris* could not grow in high concentration of total nitrogen. According to the result of Table 3, maximum specific growth rate of 0.55 (day^{-1}) happned for 1% diluted sample. Also, the results shows that biomass productivity rate for this sample is 0.10 g L^{-1} day^{-1} in 14th.

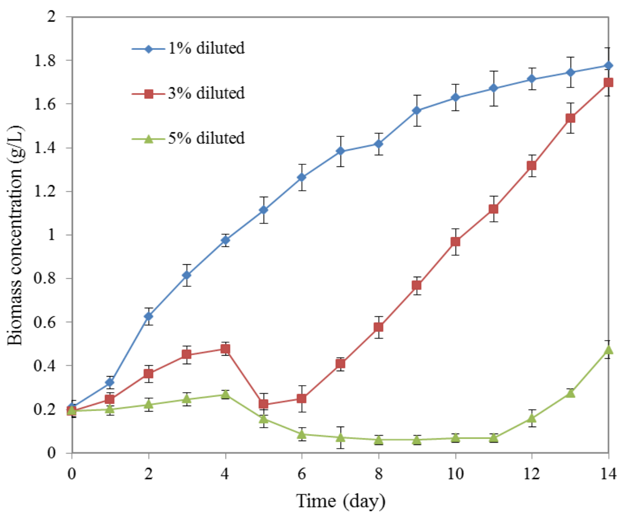

Fig. 1. Time course of cell concentration of *C. vulgaris* in different diluted samples.

Table 3. Growth parameters of *C. vulgaris* and nutrients removal percent by the algae.

Growth parameters and R%	Diluting percentage		
	1%	**3%**	**5%**
P (g L^{-1} day^{-1})	0.10	0.09	0.03
μ_{max} (day^{-1})	0.55	0.32	0.30
R% for nitrogen	55	38.5	37.3
R% for phosphate	100	98.3	17.7

As regards, pH is an important phenomenon that is mainly related to photosynthesis (Yeh et al., 2012). The pH value of culture for algal growth is shown in Fig. 2. In the 1% diluted sample, pH increased due to biomass production (Yeh et al., 2012). In treatment 2 and 3, the pH is decreased when the alge bigan to decline between the 4th and the 6thday, and then when it starts to grow the pH value decreases again. This may be caused by degration of algae residue (Jiang et al., 2015).

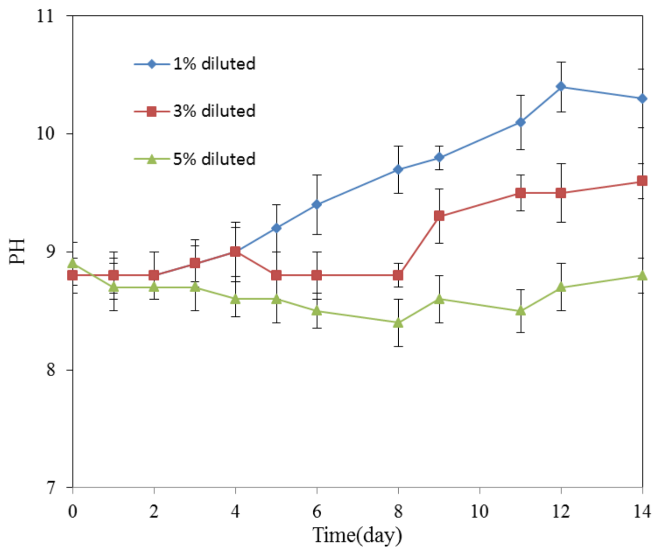

Fig. 2. pH value of *C. vulgaris* culture in different diluted samples.

Time course of cell concentration of *S. platensis* is presented in Fig. 3. It shows that *S. platensis* has more considering effect on the treatment of the samples. Growth parameters and nutrients removal percentage are presented in Table 4. The results shows that by increasing the amount of wastewater, the spesific growth rate decreased. It means that the presence of amonium has a disincentive effect on growth the of algae and it needs more time to adapt with its new media. According to Table 4, maximum specific growth rate for 1% diluted sample is more than others. Also, for the 3% diluted sample, biomass production is more than other samples in 14 days of expriment. pH value is shown in Fig. 4. The high increased in pH was in treatment 2 due to high growth rate.

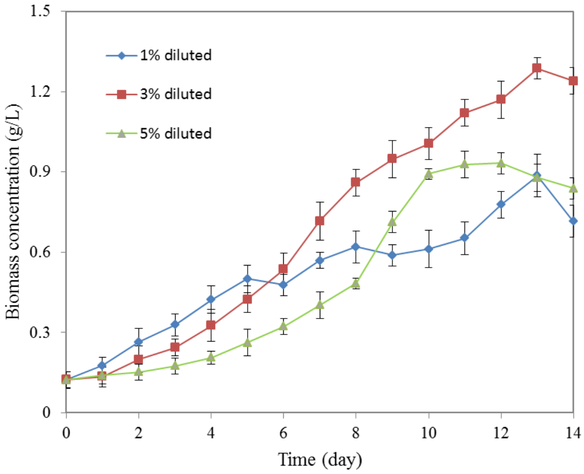

Fig. 3. Time course of cell concentration of *S. paltensis* in different diluted samples.

Table 4. Growth parameters of *S. platensis* and nutrients removal percent by the algae.

Growth parameters and R%	Diluting percentage		
	1%	3%	5%
P (g L^{-1} day^{-1})	0.04	0.07	0.05
μ_{max} (day^{-1})	0.38	0.25	0.19
R% for nitrogen	96.4	86.43	82.61
R% for phosphate	98	61.45	43.60

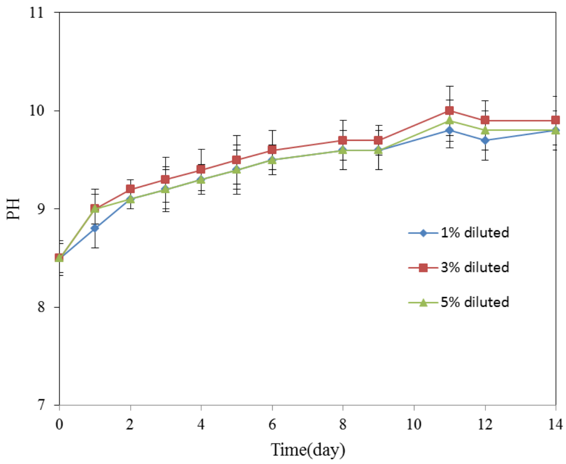

Fig. 4. pH value of *S. platensis* culture in different diluted samples.

Different componets of nitrogen such as ammonium, nitrate, nitrite, urea and phosphate are assimilated by microalgaes (Larsdotter, 2006). In this study, total amount of nitrogen and phosphate have been analysed and the resuls for *C. vulgaris* are shown in Figs. 5 and 6. The result shows that phosphate and nitrgen concentrations in 3 and 5% diluted sample decreases between the 4^{th} and the 6^{th} day, while there is no significent biomass production of *C. vulgaris* in this period (Fig. 1). It may be due to the pH of the media and equilibrium between ammonium and ammonia. Since most nitrogen in wastewater are ammonium, the amount of ammonium was not absorbed by algae and it changed to ammonia in gas phase at the high pH (Chan et al., 2014). It had been reported that ammonium can change to ammonia gas when it is aerated in high temperature even if pH was less than 9 (Cai et al., 2013; Markou & Georgakakis, 2011). Phosphore is in different shape in the aquatic solutions and it could precipitate with increasing the pH in the presence of potasium, sodium, and manyazium ions (González et al., 1997). In 3% diluted sample changes in phosphate concenteration are due to the change in forms of phosohore in its equilibrium. The maximum of nitrogen removal and phosphate removal by *C.vulgaris* in 1% diluted sample was 55% and 100%, respectively. Abinandan et al. (Abinandan et al., 2013) investigated the growth of *chlorella sp.* in different dilution of swage waste. Like their data, in this study the result showed shows that *C. vulgaris* is not capable to grow, and to remove in high amount of ammonium. Wang and et al. (Wang et al., 2013) reported 58% nitrogen removal and 97.3% phosphore growth *Chlorella sp.* in domestic wastewater. Considerable ammonia inhabitation to *C. vulgaris* growth was reported 30 mg/L by Ruiz et al. (Ruiz et al., 2013). In this study, the result showed that *C. vulgaris* were capable to absorb about 17 mg/L total nitrogen and 5.7 mg/L total phosphor. The growth of *C. vulgaris* in a medium containing 7.7 mg/L ammonium in the presence of 60 mg/L organic carbon was investigated by Ruiz et al. In other work, Kim et al. reported that after 9 days, biomass production and ammonium removal were 1.3g/L and 78%, respectively. Also, they showed pH increased to 10. In this work, the biomass production for treatment 1 was 1.26 g/L after 9 days and pH increased to 9.8 (Kim et al., 2010).

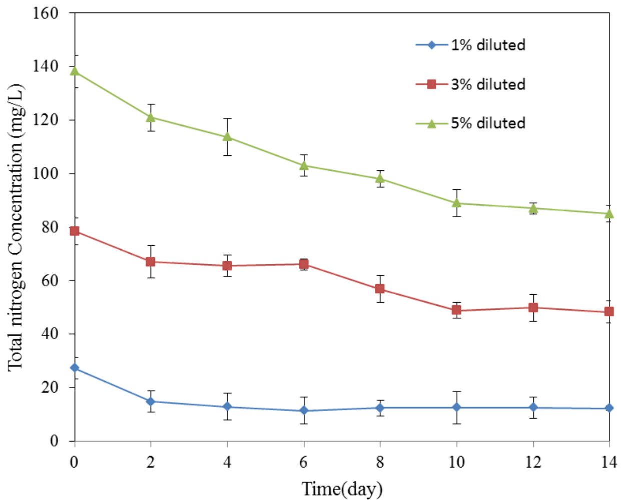

Fig. 5. Total nitrogen concentration in the treatments during the experiment by *C. vulgaris* (mg/L).

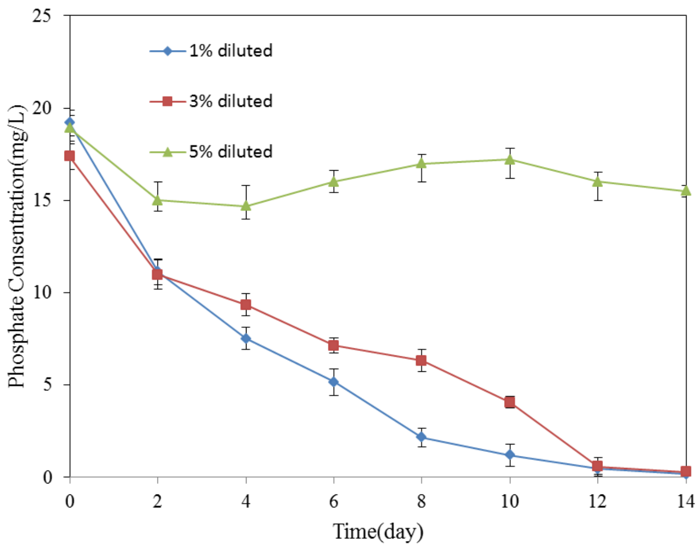

Fig. 6. Phosphate concentration in the treatments during the experiment by *C. vulgaris* (mg/L).

The results for *S. platensis* showed that this species was more capable to grow in high level of ammonium. Figs. 7 and 8 represent the evolution of nitrogen and phosphate concentration by spirullina in diluted samples. The nitoregen and phosphate removal were 86.4% and 61.4%, respectively, for diluted sample 3%. In fact, ammonium and nitrate effect on the enzyme nitrate reductase that helps alge to consume nitrate. Producing this enzyme is decresed in the precense of ammonium (Jeanfils et al., 1993; Morris & Syrett, 1963; Morris & Syrett, 1965). Dunn et al. (2013) investigated the growth of Arthrospira in tannery wastwaters. They reported the inhibition of growth was at ammonium levels above 60 mg/L (Dunn et al., 2013). Also, Ogbonna et al. demonestrated the growth of *spirulina* and *C. sorokiniana* and nitrogen removal decreased gardually with increasing ammonium concentration. They showed the growth was utterly inhibited in a media containing 200 mg/L ammonium (Ogbonna et al., 2000). The microalgae *S. platensis* had maximum growh rate in the presence of 72 mg/L ammonium (run2) and the growth decreased in the presence of 120 mg/L ammonium at run3. According to Fig. 8 the microalgae consumed about 14 mg/L phosphate at run2.

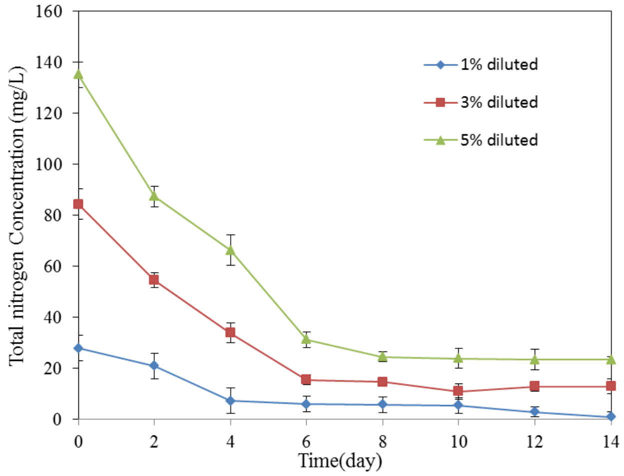

Fig. 7. Total nitrogen concentration in the treatments during the experiment by *S.platensis* (mg/ L).

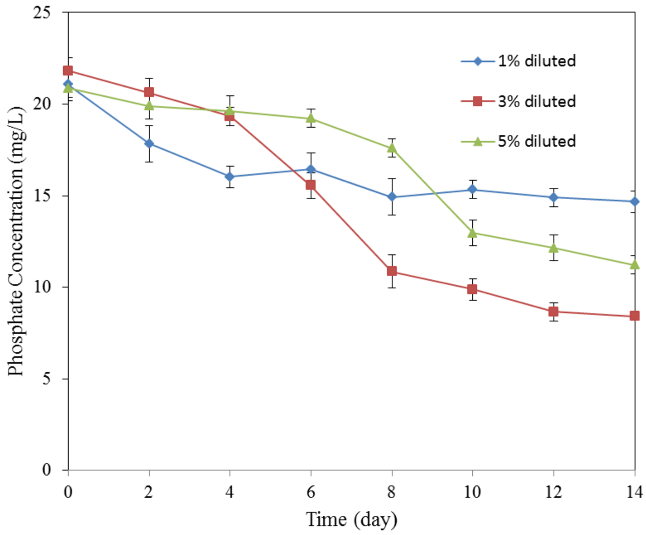

Fig. 8. Phosphate concentration in the treatments during the experiment by *S. platensis* (mg/L).

CONCLUSION

It was found that wastewater effluent from ammonia plant is a suitable medium for microalgae cultivation. Furthermore, microalgae is capable of reducing total nitrogen concentration in the wastewater remarkably. Also, *S. platensis* showed more nitrogen removal than *C. vulgaris*. Biomass productivity rate for *C. vulgaris* in the 1% diluted sample was the highest one. It is important to note that the high level of ammonium in the wastewater reduces algal growth remarkably. Therefore, it is important to cultivate algae in diluted wastewater.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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