Effect of light/dark cycle on nitrate and phosphate removal from synthetic wastewater based on BG11 medium by *Scenedesmus* sp.

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Abstract

In this study, microalgae growth in the synthetic wastewater and their ability to remove nutrients under different light levels was investigated. For this purpose, a comparative study was conducted on freshwater microalgae *Scenedesmus* sp. to evaluate their performance to remove nitrate and phosphate from both slaughterhouse and dairy synthetic treated wastewaters, under different light/dark cycles (12/12, 16/8 and 24/0 h), in Erlenmeyer flasks. The best light/dark cycles in Erlenmeyer flasks for nitrate and phosphate removal and growth were obtained at 24/0 h. Moreover, nitrate and phosphate removal under light conditions at 24/0 h light/dark cycles were tested in a designed open raceway pond. The maximum nitrate removal in slaughterhouse and dairy synthetic wastewater was 78% and 99.7%, and the phosphate removal was 31% and 68%, respectively. Furthermore, the highest biomass productivity in dairy and slaughterhouse synthetic wastewater during 9 days was 0.65 g L⁻¹ and 1.5 g L⁻¹, respectively. Thus, *Scenedesmus* sp. could be potential candidates by showing their intrinsic merit, for the reduction of nitrate and phosphate residue levels from dairy and slaughterhouse synthetic wastewaters in open raceway ponds.

Keywords *Scenedesmus* sp. · Microalgae · Synthetic wastewater · Nutrient removal

Abbreviations

DSW	Dairy synthetic wastewater
SSW	Slaughterhouse synthetic wastewater
12/12 DSW	12/12 light/dark cycle dairy synthetic wastewater
16/8 DSW	16/8 light/dark cycle dairy synthetic wastewater
24/0 DSW	24/0 light/dark cycle dairy synthetic wastewater
12/12 SSW	12/12 light/dark cycle slaughterhouse synthetic wastewater

16/8 SSW	16/8 light/dark cycle slaughterhouse synthetic wastewater
24/0 SSW	24/0 light/dark cycle slaughterhouse synthetic wastewater

Introduction

Water is considered as a necessity for human life and different kinds of living creatures and is one of the most essential requirements of human activities (Park and Craggs 2010). Today with the rapid population growth, industrialization of the countries, demands for agricultural consumptions and energy generation and limitation of water resources, the optimized and hygienic water resources are essential necessities. Due to dry climatic conditions, increasing population and industries development, the importance of this issue is two-fold for Iran (Sayadi et al. 2016; Bazrafshan et al. 2012; Piazadeh et al. 2014). Among different pollutants present in the industrial and agricultural wastewater, elimination of nitrogen and phosphorous pollutants has a significant importance because of its bad effects on the environment (Rezaei and Sayadi 2015). To understand the importance of nitrogen and

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phosphorous elimination, it is noted that many of the developed countries have enacted hard regulations and standards for the amount of nitrogen and phosphorous (USEPA 2011).

Among industrial wastewater, dairy and slaughterhouse synthetic wastewaters are highly polluted. The constituent compounds of slaughterhouse wastewater and household sewage are similar, but the concentration of nutrient in the slaughterhouse wastewater is more (Mittal 2006). Slaughterhouse wastewater is highly rich in organic and nitrogen compounds and has a very high organic load of blood and proteins, which will cause certain contamination to the environment and its creatures. This factor even has a great influence on algae growth and makes the fish death double (Merzouki et al. 2005; Bustillo-Lecompte et al. 2016). Therefore, extended studies and researches have been conducted on the purification of slaughterhouse wastewater, some are still being conducted. In addition, the dairy industry is generally considered to be the most source of sewage in many countries, because of its pollution, which basically has an organic origin large water consumption and generation, which is the main source of pollution of this type of industry (Vourch et al. 2008). In general, wastewater in dairy industry contains high amounts of organic matter such as proteins, carbohydrates, fats, and suspended solids. Therefore, their effluents are characterized by having high concentrations of COD, BOD, and nutrients (nitrate and phosphate) (Daufin et al. 2001; Perle et al. 1995).

Until now, different methods have been considered to absorb these pollutants which include: ACS (activated sludge system), chemical oxidation, membrane process, chemical treatment, ionic exchange, etc. (Shahriari Moghadam et al. 2016; Chan et al. 2009; Seow et al. 2016). Purified wastewater from these systems still has a high nitrate and phosphate load. The kind of dairy and slaughterhouse wastewaters quality and refinery's mean output are shown in Table 1 (Bustillo-Lecompte et al. 2016; Gurel and Buyukgungor 2011).

Given the amount of industrial wastewater in Iran, still there is no efficient wastewater treatment process, considering the high amount of nitrate and phosphate in their wastewater, of course there should have been significant investments for supplementary purification of the output wastewater (Piadeh et al. 2014). For removing nitrate and phosphate from their wastewater, microalgae are used. The application

of microalgae for removal of nitrate and phosphate from wastewater has many advantages such as not causing environmental dangers regarding the natural ecosystems, not adding secondary pollution in case of using productive biomass and the ability of microalgae at recycling nutritious effective materials presented in the secondary wastewater.

Many kinds of researches have shown efficient ability of microalgae in wastewater treatment (Martinez et al. 2000; Markou and Georgakakis 2011; Mousavi et al. 2009). Despite nutrients sources, sufficient areas with adequate photoperiods and light sources are required for photosynthesis (Jorquera et al. 2010; Khoeyi et al. 2012). Considerable efforts have been made in the algae cultivation systems to increase the algal biomass productivity by controlling various environmental factors, such as temperature, pH and different light/dark cycles (Hindersin et al. 2014; Guedes et al. 2011; Simionato et al. 2013).

In the report by Hernández et al. (2016), the microalgal–bacterial consortium successfully removed nutrients from slaughterhouse wastewater. In addition, as reviewed by Kothari et al. (2012), Shanab et al. (2013), Farooq et al. (2013), Aravinthan et al. (2014), Maroneze et al. (2014) and Ashok et al. (2014), the microalgae *Chlorella* and *Scenedesmus* species are widely employed in different wastewater treatment.

The objective of this study is to investigate the feasibility of *Scenedesmus* sp. microalgae cultivation in a treated wastewater based on synthetic medium, and their influence to remove nitrate and phosphate. Since the treatment systems can not completely treat wastewater, the following experiments were carried out on synthetic wastewater, similar to the actual wastewater discharged from the Treatment plant. In this study, the first part of the experiment is for the growth of *Scenedesmus* sp. microalgae under different light/dark cycle, in 250 cc Erlenmeyer flasks and the second part is the use of the results of aforementioned part (highest removal of nitrate and phosphate in the optimum light/dark cycle) to remove nitrate and phosphate from two different synthetic treated wastewaters (slaughterhouse and dairy) in the new designed open raceway pond.

Materials and methods

Microalgae strains and culture conditions

The microalgae *Scenedesmus* sp. obtained from the University of Agricultural Sciences and Natural Resources, Institute of Genetics and Biotechnology Tabarestan (Sari, Iran). Stock cultures were grown in Erlenmeyer flasks (working volume of 50 mL) of BG11 culture medium (Zamani et al. 2011). The broth was grown at 23 ± 2 °C, under continuous irradiance cool white fluorescent light (Pars Company Iran,

Table 1 Various levels of nitrate, phosphate and organic load in treated wastewater

Type of synthetic wastewater	Nitrate (mg L ⁻¹)	Phosphate (mg L ⁻¹)	Organic load (glucose) (mg L ⁻¹)
Dairy	30	20	90
Slaughterhouse	100	10	30

36W, FPL), at light intensity of $28 \pm 2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of photosynthetic active radiation (PAR), which is measured by a photo-radiometer (Model LP 80, LI-COR, Netherlands). Different light/dark cycles of 12:12, 16:8, and 24:0 h. were also considered.

Synthetic treated wastewater composition

In this work, treated dairy and slaughterhouse wastewaters were used (Kothari et al. 2012; Gurel and Buyukgungor 2011). The composition of the synthetic treated wastewater was based on the recipe of the modified BG 11 culture medium (Table 2).

Experimental setup

The experiments were conducted in 500-mL conical flask with 300 mL working volume of synthetic wastewater. The prepared inoculation liquid (microalgae) was, then, added for the removal of phosphate and nitrate. The initial microalgae biomass was kept at 0.05 g L^{-1} for every experiment. The cultivation medium pH was maintained at 7.2 ± 0.2 . Moreover, temperature was settled at $25 \pm 2^\circ \text{C}$. Artificial illumination was provided by fluorescent lamps (Pars Company Iran, 36W, FPL) with a photoperiod of 12/12 h, 16/8 h and 24/0 h light/dark cycles. To supply gas requirements and prevent sedimentation and also to create wider surface to be contacted with the synthetic wastewater, and to receive more desirable light, equal and continuous aeration during all periods in synthetic wastewater was established for a period of 8 days (Jaysudha and Sampathkumar 2014; Taskan 2016). To examine the removal of

phosphate and nitrate during the experiment, in every 2 days, the samples were monitored. The growth rate of algae, the concentrations of nitrate and phosphate were measured. Then, the effect of light/dark cycle on growth of microalgae with optimal source was investigated. The effect of nitrate and phosphate concentration on growth of microalgae was evaluated in a new open raceway pond.

Photobioreactor

Ponds were made of Poly(methyl methacrylate) (PMMA) sheets with 80 cm length and 40 cm width. It is a transparent plastic material of 0.6 cm thickness and easy to work with. The raceway photobioreactor includes four open loop channels in which water circulated by two paddle wheels coupled with an electromotor (Fig. 1), the illuminated area of culture is 0.4 m^2 and the total working volume was equivalent to 100 L. The paddle wheel diameter is 0.4 m and rotation speed is 17 RPM. The required light for culture was supplied by cool white florescent lamps (Pars Company Iran, 36W, FPL), light intensity was $28 \pm 2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ under light/dark cycles of 12:12, 16:8, and 24:0 h. Airflow rate of 0.5 L s^{-1} from the underside of the raceway pond was provided by a pump (HAILEA ACO-7703).

Analytical methods

The optical density (OD) of the broth was determined by measuring the absorbance at 550 nm (Delavari et al. 2014), in a double beam UV/Vis spectrophotometer (1800, Shimadzu, Japan). To measure biomass dry weight, a 10 mL sample of algal suspension was filtered through a pre-dried and pre-weighed 47 mm Whatman paper filter (GF/F, nominal pore size $0.7 \mu\text{m}$) and washed twice with 20 mL distilled water (Delavari et al. 2014).

Calculation of the specific growth rate was obtained, based on the dry weight using the following equation (Tang et al. 2011):

$$\mu = \frac{\ln\left(\frac{X_t}{X_0}\right)}{t}, \quad (1)$$

where μ is the specific growth rate (day^{-1}), X_0 and X_t represent the biomass concentration at the beginning and any given time t , respectively.

The biomass productivity rate (P in $\text{mg L}^{-1} \text{day}^{-1}$) was estimated using the following equation (Ryu et al. 2009):

$$P = \frac{X_f - X_0}{t_f}, \quad (2)$$

Table 2 Composition of BG11 medium used in *Scenedesmus* sp. microalgae cultivation

Component	Slaughterhouse (mg L^{-1})	Dairy (mg L^{-1})
NaNO_3	140	42
K_2HPO_4	24	48
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	75	75
$\text{CaCl}_2 \cdot \text{H}_2\text{O}$	36	36
Na_2CO_3	20	20
Citric acid	6	6
EDTA	1	1
Trace metals		
H_3BO_3	2.86	2.86
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81	1.81
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.222	0.222
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.39	0.39
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.079	0.079
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.049	0.049
$\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$	0.03	0.09

Fig. 1 **a** Picture of open raceway pond, **b** schematic diagram of the raceway pond; left: side view, right: top view. Raceway open pond include: (1) input sedimentation, (2) output sedimentation, (3) motor gearbox DC, (4) axle motor, (5) flow, (6) paddle wheel, and (7) baffle separation

where X_f is the biomass concentration at the end of cultivation (t_f) and X_0 is the biomass concentration at the beginning (t_0).

The nutrients analyzed were nitrate and phosphate, assessed using standard methods in water and wastewater (APHA 2000). For determination of residual nitrate and

Fig. 2 Effects of the light/dark cycles on pH and microalgae growth in both synthetic wastewater: **a** pH, **b** growth rate

Fig. 3 Effect of the light/dark cycles on nutrient removal in both synthetic wastewater: **a** nitrate concentration, **b** phosphate concentration

phosphate, the microalgae was separated by centrifugation at $2500\times g$ for 10 min, and the result was filtered to measure only dissolved nutrients ($0.45\ \mu\text{m}$). The concentration of nitrate and phosphate was measured by spectrophotometer UV-1800 (Shimadzu, Japan) with wavelength 690 nm for phosphate and 220 nm for nitrate.

Results

Effects of the light/dark cycle on microalgae production and nutrient removal

Microalgae growth and nutrient removal were evaluated during different light/dark cycles, as shown in Figs. 2 and 3. Figure 2 shows the effects of the light/dark cycles on pH and microalgae growth in both synthetic wastewater versus time. The results show that microalgae production increases with a longer illumination period. The highest microalgae concentration of $1.35\ \text{g L}^{-1}$ and $0.78\ \text{g L}^{-1}$ were obtained (in) from slaughterhouse synthetic wastewater (SSW) and dairy synthetic wastewater (DSW), respectively, during a light/dark cycle of 24:0. This demonstrated that continuous light exposure did not inhibit the photosynthesis of *Scenedesmus* sp. The effects of various light/dark cycles on nutrient removal

Table 3 Overview of growth parameter by *Scenedesmus* sp. in both synthetic wastewaters

Sample ID	μ_{max} (day^{-1})	P_{max} ($\text{g L}^{-1}\ \text{day}^{-1}$)	% Removal nitrate per day	% Removal phosphate per day
12/12 SSW	0.32	0.033	3.69 (11)	9.09 (11)
12/12 DSW	0.55	0.027	9.09 (11)	4.54 (11)
16/8 SSW	0.43	0.044	4.57 (11)	14.28 (7)
16/8 DSW	0.51	0.044	14.28 (7)	5.45 (11)
24/0 SSW	0.69	0.12	9.09 (11)	11.11 (9)
24/0 DSW	0.6	0.066	14.28 (7)	11.11 (9)

are illustrated in Fig. 3. The nitrate removal rates during the light/dark cycles of 12:12, 16:8, and 24:0 were different, the highest nitrate and phosphate removal by *Scenedesmus* sp. from DSW and SSW were obtained during a light/dark cycle of 24:0 (Table 3). For these light/dark cycles, almost all the nitrate and phosphate were consumed during 9 days of operation. However, during the shorter illumination periods (light/dark cycles of 12:12 and 16:8), the nitrogen removal rates were half of those during longer illumination periods (light/dark cycles of 24:0). Thus for an efficient removal of nitrate and phosphate, the illumination periods should be at least 16 and 24 h, respectively. Therefore, for efficient

Fig. 4 Dry weight of *Scenedesmus* sp. versus time in dairy and slaughter house synthetic wastewater

Table 4 Mean specific growth rate (d^{-1}) trend in the treatment of *Scenedesmus* Sp.

Time (day)	1–3	3–5	5–7	7–9
Type synthetic wastewater				
Dairy	0.58	0.42	0.23	0.05
Slaughterhouse	0.69	0.5	0.28	0.21

removal of both nitrogen and phosphorous, the illumination period should be at least 24 h.

Growth of microalgae in the open raceway pond

The growth of microalgae *Scenedesmus* sp. in both synthetic wastewaters was measured in terms of biomass concentrations and specific growth rates are presented in Fig. 4 and Table 4, respectively. The growth rate in first days of cultivation was slow, then microalgae growth rate in synthetic wastewater has increased. The maximum biomass produced in DSW and SSW by *Scenedesmus* sp. was 0.65 g L^{-1} and 1.5 g L^{-1} , respectively. Furthermore, maximum biomass productivity in SSW was almost 56% more than DSW for the period of 9 days. (Fig. 4).

According to the results in Table 4 which were calculated by Eq. 1, the growth rate increased by reducing the concentration of nutrient in the treatment synthetic wastewater. As demonstrated, the highest growth rate of *Scenedesmus* sp. in DSW and SSW was 0.58 and $0.69 \text{ (day}^{-1}\text{)}$, respectively.

Nutrient removal from synthetic wastewater in the open raceway pond

In the present investigation, nitrate and phosphate are the important nutrients that support the growth of microalgae in the treated wastewater. To understand the better nutrient assimilation, nitrate and phosphate were monitored every

Fig. 5 Nitrate removal by *Scenedesmus* sp. versus time in open raceway pond

Fig. 6 Phosphate removal by *Scenedesmus* sp. versus time in open raceway pond

2 days in dairy and slaughterhouse synthetic wastewater. The percentage of nitrate removed by *Scenedesmus* sp. from DSW and SSW is depicted in Fig. 5.

The nitrate removal ability of *Scenedesmus* sp. is shown in Fig. 5, nitrate could be removed almost completely within 9 days of experiment, even, while supplied with 30 mg L^{-1} nitrate. On the other hand, the nitrate removal efficiency of *Scenedesmus* sp. during 9 days with 100 mg L^{-1} nitrate was 78%. Results from this experiment showed that nitrate was removed efficiently during the 9 days (Fig. 3). Nitrate concentrations at the end of culture for *Scenedesmus* sp. in DSW and SSW were 0.07 mg L^{-1} and 22 mg L^{-1} , respectively.

The phosphate concentrations in DSW and SSW during the 9 days of experiment are shown in Fig. 6. Phosphate was efficiently removed from both synthetic wastewaters by *Scenedesmus* sp. decreasing during the culturing time from an initial concentration of about 10 mg L^{-1} to less than 6.9 mg L^{-1} for SSW and 20 mg L^{-1} to less than 6.4 mg L^{-1} for DSW. The mean decreased phosphate concentration rate from DSW is measured as $1.5 \text{ mg L}^{-1} \text{ d}^{-1}$ and SSW is measured as $0.34 \text{ mg L}^{-1} \text{ d}^{-1}$. The phosphate contaminants

significantly decreased for DSW within the 9 days, while the phosphate concentration from SSW was 31%.

Discussion

The effects of the light/dark cycle on microalgae growth, pH, and the efficiency of removing nitrate and phosphate were evaluated. Microalgae growth increased with increasing illumination periods up to 20 h radiation. The enhancement of microalgae production with the use of this photoperiod also is reported in the previous studies (Eduardo et al. 2009; Lee and Lee 2001). When the illumination period is insufficient, microalgae are unable to generate enough energy via photosynthesis to fulfill their metabolic requirements, resulting in low nitrogen and phosphorous removal rates. When microalgae are illuminated sufficiently (at least 20 h, according to this study), photosynthesis occurs more efficiently, resulting in efficient nitrogen and phosphorous removal.

It is evident (Fig. 2) that pH is dependent on microalgae growth of the medium, and light/dark cycle affects the photosynthesis directly. Therefore, this is a strong factor which controls the growth of microalgae. In this experiment, three different light/dark cycles (12:12, 16:8, and 24:0) had significantly different effect on the growth and pH of the medium in all the treatments. The results of this experiment show that light/dark cycle has the most important role on *Scenedesmus* sp. growth. As the results obtained before, *Scenedesmus* sp. grow well at light/dark cycle of 24:0 h. From day 3 to day 5, the growth rate in two synthetic wastewaters was faster than the rest of the days, for this reason with reduce nutrients (including: nitrate and carbon), pH of medium increased, which have been reported in the Handbook of microalgal culture (Richmond 2007).

The results show that the algae growth is directly related to the decreasing of the nitrate and phosphate concentrations. The removal of nitrate and phosphate sources into wastewater causes the increase of the growth rate of microalgae (Hongyang et al. 2011). Similar results were obtained also by Abinandan et al. (2015) from *Chlorella* and *Scenedesmus* cultivation in Rice Mill Effluent.

Adding the microalgae to the new culture medium (wastewater) could cause stress for microalgae cells. Generally, this process needs some time to adapt, eliminate stress and regulate metabolic activity (Tam et al. 1994). As result shows in Fig. 4, the growth rate was slow in the first 3 days of experiment, because the microalgae would not adapt to the both synthetic wastewaters. This result agrees with those reported by Ding et al. (2015), who stated that higher nutrient concentration in culture medium needs more time for adapting microalgae.

In this research, the mean biomass productivities in DSW and SSW after 9 days were almost $0.072 \text{ g L}^{-1} \text{ d}^{-1}$

and $0.16 \text{ g L}^{-1} \text{ d}^{-1}$, respectively. Li et al. (2011b) reported that the mean biomass productivity of *Chlorella* sp. in raw municipal wastewater was only $0.07 \text{ g L}^{-1} \text{ d}^{-1}$. In another report by Mata et al. (2012), the highest productivity of *Scenedesmus obliquus* was ($0.1 \text{ g L}^{-1} \text{ d}^{-1}$) from brewery effluent during 9 days. The algal growth results indicated that dairy and slaughterhouse wastewaters (in this study synthetic wastewater was used) were a good source of nutrients for the growth of algae.

Microalgae require all three major nutrients for their growth: carbon, nitrogen and phosphorus (Petrovic and Simonic 2015; Christenson and Sims 2011). Thus, in this paper, for microalgae, the form of inorganic nitrogen and phosphorus, that are directly assimilated, are preferred to be nitrate and phosphate. As presented in Figs. 5 and 6, a decrease in nitrate and phosphate concentration were shown in DSW and SSW by *Scenedesmus* sp. during 9 days of cultivation. The highest nitrate removal percentage of 99.7% and 78% were reported from DSW and SSW, respectively. The lowest nitrate removal rate was observed in the both synthetic wastewater treatment during 3 first days, because *Scenedesmus* sp. are getting compatible with the new culture medium (wastewater).

Moreover, in this study the N-removal rates by *Scenedesmus* sp. were 3.3 and $8.6 \text{ mg NO}_3 \text{ L}^{-1} \text{ d}^{-1}$ from DSW and SSW, respectively. In a similar study conducted by Taskan (2016), an N-removal rate of $10.3 \text{ mg N L}^{-1} \text{ d}^{-1}$ from slaughterhouse wastewater is reported. In another study, such as Aravinthan et al. (2014) and Cabanelas et al. (2013), the N-removal rates were less when compared to this study, where N-removal rates of $4.85 \text{ mg ammoniacal-nitrogen L}^{-1} \text{ d}^{-1}$ and $9.8 \text{ mg TN L}^{-1} \text{ d}^{-1}$ were reported, respectively.

Phosphate, including chemical contaminants enters surface water and groundwater resources through wastewater. Therefore, wastes (output from refineries) containing phosphates must meet the discharge limits for phosphates, which are 5.0 mg L^{-1} (USEPA 2011). To meet effluent quality standards, secondary treatment of the refinery output effluent is required. Figure 6 shows the phosphate removal at various time intervals. In this study, the phosphate removal was 31% and 68% from DSW and SSW, respectively. Similar results have also been reported in several studies, such as Yan et al. (2013d) in a study conducted on the nitrogen and phosphorus removal, from synthetic wastewater by *Chlorella vulgaris*, reported 84.37% and 94.26% of nitrogen and phosphorus removal, respectively. Usha et al. (2016) reported that *Scenedesmus* sp. were able to remove 65% nitrate and 71.3% phosphate from pulp and paper mill effluent. In another report by Renuka et al. (2013b), the consortium of native filamentous strains removed 90% of the nitrate and 97% phosphate from sewage wastewater during 10 days. In this study, the increase in nutrient removal during culturing time could be due to the development and increase in the

number of cells and their size of microalgae species in the batch culture.

In conclusion, based on this study, the proposed new open raceway pond for cultivation of microalgae not only offers efficient method but also improves efficient utilization of sedimentation box for optimum biomass extraction. The maximum volumetric productivity of *Scenedesmus* sp. recorded under batch culturing was 0.65 g L^{-1} and 1.5 g L^{-1} from dairy and slaughterhouse synthetic wastewater, respectively.

Microalgae *Scenedesmus* sp. are an important marine organism that is used directly and indirectly in human nutrition and the production of various chemicals. The results indicate that the *Scenedesmus* sp. had a potential to remove the high concentrations of nitrate and phosphate with an increase in biomass production completely. However, there is a linear relationship between biomass production and concentration of nitrate. Moreover, *Scenedesmus* sp. can be safely regarded as a viable option for the tertiary treatment of synthetic treated dairy and slaughterhouse wastewater.

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Compliance with ethical standards

Conflict of interest The author(s) declared no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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