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Effect of nutrient regimes on desalination rate and lipid production of *Scenedesmus obliquus* in saline water

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ABSTRACT

The effect of nutrient regimes on desalination efficiency and lipid production of *Scenedesmus obliquus* was studied to optimize the technology using this species to simultaneously desalinate and produce lipids. *S. obliquus* was cultured in five nutrient regimes for 16 days. Removal of NaCl and lipid production was 559 mg L^{-1} and 152 mg L^{-1}, respectively, when the initial concentration of N was 46 mg L^{-1} and the value of P was 8.5 mg L^{-1}. When the P concentration was constant, a moderate N/P ratio (8.5) favored NaCl removal. In regimes with a N/P ratio of 8.5, NaCl removal increased from 505 mg L^{-1} to 559 mg L^{-1} as concentrations of N and P rose. The highest lipid production and lipid content of 215 mg L^{-1} and 27.5%, respectively, were found in the regime with 93 mg L^{-1} N and 10.88 mg L^{-1} P. Redundancy analysis results showed that the lipid content of *S. obliquus* was positively related to initial N concentration, and that increasing initial P concentration clearly enhanced the proportion of C16-C18 fatty acid and level of unsaturation. The optimum nutrient regime was 46 mg L^{-1} N and 5.44 mg L^{-1} P, when the removal of NaCl was 505 mg L^{-1} and the utilization of N and P was 80.43% and 97.61%, respectively. Lipid production and content were 152 mg L^{-1} and 20%, respectively, representing 66.2% unsaturated fatty acid and 78.3% C16-C18 fatty acid. The study indicated that nutrient regime adjustment can simultaneously promote lipid accumulation and desalination.

Keywords: Scenedesmus obliquus; Biological desalination; Nutrient regime; Lipid production

1. Introduction

Saline water, including seawater and brackish water on land, comprises 97.47% of global water resources. Consequently, advanced desalination has become the core technology in developing and utilizing saline water to solve water shortage problems. The commonly used technologies for desalination include distillation [1–3], membrane method [4–6] and electrochemical method [7–10]. Because of the huge energy consumption, neither the distillation nor the electrochemical method have been applied extensively. The membrane method is an emerging desalination technology that is used widely. However, it is still a challenge to obtain sufficient cheap freshwater, because this technology requires high investment and its operating cost is also high. Searching for a low-cost and highly efficient desalination technology is still urgent.

Recently, biological desalination attracted widespread attentions because it has low operating cost without high investment. It is an alternative technology when the water quality requirement is not high and funds are limited. There are three branches of biological desalination: microorganism desalination (e.g. purple nonsulfur bacteria; [11]), algae desalination, and plant desalination. Because cultivation of microorganisms needs abundant organic carbon sources, microorganism desalination is considered as an uneconomical technology. The efficiency of plant desalination is low and thus this method is unsuitable for general application. In comparison, algae desalination has

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obvious advantages. (1) Additional organic carbon source is not necessary for algae growth and thus the cultivation cost of algae desalination is much lower than that of microorganism desalination. Moreover, algal biomass can be used to produce by-products, such as biodiesel and polysaccharides [12], and thus the desalination cost could be reduced because of the high value of these by-products. (2) Algae growth is much quicker than that of plants. Thus, algae desalination is more efficient than plant desalination. (3) The equipment for algae cultivation for desalination is so simple that even a bag made from plastic film can be used.

Minas et al. [13] believed that desalination with blue-green algae was an emerging technology that removed Na^+ from seawater. Kokabian and Gude [14] proved the feasibility of algae desalination and achieved a 40% desalination rate from wastewater. Yao et al. [15] indicated that *S. obliquus* can efficiently transfer Na^+ from brackish water, and the highest amount of NaCl they removed was about 3.15 g L^{-1}. These examples demonstrate that algae can exhibit considerable efficiency in desalination. In addition, Gan et al. [16] reported that *S. obliquus* could be used to achieve simultaneous biological desalination and lipid production. Their study showed that the optimum initial NaCl concentration was 4.8 g L^{-1}. Under these conditions, lipid production was about 100 mg L^{-1} and the removal of NaCl reached 1.0 g L^{-1}. However, BG-11 medium was used in their experiment and the nutrient concentration was too high that reducing the cost of nutrient is still a challenge. It is still potential to improve desalination efficiency and lipid production by adjusting nutrient dosages.

Lipid content and fatty acid composition are key factors affecting biodiesel production. It was reported that both nutrients and salinity affected lipid content and fatty acid composition [17–19]. A number of studies have analyzed the effects of nutrients on lipid content and the fatty acid components of microalgae [20–22], but they all used freshwater. Whether the effects of N and P on lipid production and fatty acid composition of microalgae in saline water are different from those in freshwater still needs further verification. The aim of this work was to study the effects of nutrient regimes on desalination, oil production, and fatty acid composition in *S. obliquus*. The results of this work will contribute to optimizing the technology using *S. obliquus* to simultaneously desalinate and produce lipids.

2. Materials and methods

2.1. Algae species

The freshwater Chlorophyta *S. obliquus* (FACHB416) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. This unicellular strain was purified by cultivation for more than 3 months under axenic culture conditions in BG-11 medium. The BG-11 medium was prepared with the following composition (per liter): 1500 mg $NaNO_3$, 40 mg K_2HPO_4, 75 mg $MgSO_4{\cdot}7H_2O$, 36 mg $CaCl_2{\cdot}2H_2O$, 6 mg citric acid, 6 mg ammonium ferric citrate, 1 mg $EDTANa_2$, 20 mg Na_2CO_3, 2.86 mg H_3BO_3, 1.86 mg $MnCl_2{\cdot}4H_2O$, 0.22 mg $ZnSO_4{\cdot}7H_2O$, 0.39 mg $Na_2MoO_4{\cdot}2H_2O$, 0.08 mg $CuSO_4{\cdot}5H_2O$, and 0.05 mg $Co(NO_3)_2{\cdot}6H_2O$ [23].

2.2. Experiment design and culture conditions

The algae were cultured in 120 mL liquid culture medium in 250-mL conical flasks at 25°C. The light intensity was 50 μmol photons m^{-2} s^{-1} with a light:dark cycle of 12 h:12 h. The medium was based on BG-11 medium and the concentrations of N and P were adjusted to varying levels, as shown in Table 1, using $NaNO_3$ and K_2HPO_4. Concentrations of N and P were based on the amounts of N and P consumed in our previous work [16]. For each regime, 16 replicates were prepared, to create enough biomass for subsequent lipid extraction. All the samples were cultured for 16 days. The flasks were shaken by hand two to three times daily to prevent the cells from adhering to the inner walls of the flasks.

2.3. Analysis of cell density

Cell density of *S. obliquus* in three random replicates was measured every two days with a spectrophotometer (UV-1780, Shimadzu, Japan). The relationship between light absorption value at 665 nm (OD_{66}) and cell density was obtained in earlier work [16] and is shown in Eq. (1):

$$\text{Cell density } (10^4 \text{ cells mL}^{-1}) = 1002.2\ OD_{665} + 45.3, \quad R^2 = 0.999 \tag{1}$$

2.4. Removal of NaCl and nutrient residue

At the end of the experiment, every two replicates were combined as a mixed sample. For each mixed sample, 30 mL culture medium was centrifuged at 16000 × *g* for 6 min. The supernatant was filtered through a membrane (pore size = 0.45 μm). The filtrate was used to analyze the removal of NaCl and nutrient residue. The total nitrogen (TN) concentration and total phosphorus (TP) concentration was measured by the spectrophotometric method [24]. Cellular N:P was counted by the ratio of ΔN:ΔP; ΔN was determined by subtraction with TN concentrations measured in the initial BG-11 culture medium and residual medium. ΔP was determined in the same way as ΔN. The removal of NaCl was assessed using total dissolved solid (TDS) [16], which was analyzed by a conductivity meter (LA-EC20, Hach, USA).

2.5. Lipid extraction and fatty acid analysis

The remaining mixed samples were centrifuged at 10000 × *g* for 6 min. After rinsing twice with deionized water, the pellet was dried at 60°C for lipid extraction. The lipid was

Table 1
N/P ratios and concentrations of N and P in the culture medium

	N (mg L^{-1})	P (mg L^{-1})	N/P
A	46	5.44	8.5
B	93	10.88	8.5
C	138	16.32	8.5
D	54	10.88	5.0
E	131	10.88	12.0

extracted by the methanol–chloroform method [25]. Next, 100 mg dried algae for each mixed sample was weighed accurately and put into 50-mL centrifuge tubes. Injected into 12 mL 1:2 chloroform:methyl alcohol, the tubes were ultrasonically extracted by ultrasonic cleaner at 25°C and 70 Hz. The sample was again centrifuged at 10000 × *g* for 6 min and the supernatant filtered through a 0.45 μm filter membrane. The extraction procedure was repeated twice and the filtrate was combined in a clean tube. The extracting solution was added to a 16 mL 5% NaCl solution and mixed well. The lower liquid layer was transported into a round-bottomed flask for rotary evaporation. Afterwards, the sample was dried to a constant weight by N-EVAP at 45°C. The fatty acid content was measured by the difference method.

Fatty acid methyl esterification was obtained by BF_3 catalysis which directly translated the extracted lipid into fatty acid methyl esters (FAMEs) [26]. The dried lipid was dissolved by 4 mL 14% w/v BF_3- methanol solutions and then was refluxed by boiling water bath for 15 min. After cooling, 2 mL n-heptane and 4 mL NaCl saturated solution was added and the mixture was fully shocked. After stratification, the supernate was filtrated using 0.2 μm polyvinylidene fluoride syringe microfilters. FAMEs were analyzed using GC-FID (GC-2014C, Shimadza, Japan) equipped with a DB-5 ms capillary column (60 m, 0.25 mm × 0.25 μm, Agilent, USA). The injection volume was 1 μL, the injector temperature was 280°C, and the detector temperature was 290°C. The initial oven temperature was 120°C holding for 3 min; it was then raised to 220°C at a rate of 4°C min^{-1}. After being maintained for 5 min, the temperature was raised to 280°C with a rate of 3°C min^{-1} and then was held for 20 min.

2.6. Statistics

All data in the current study are presented as means ± standard deviations. The differences among regimes were tested by ANOVA implemented in SPSS 10.0. Redundancy analysis (RDA) was carried out to evaluate the relationships between lipid production, fatty acid composition, and nutrient concentrations. RDA was conducted by CANOCO 4.5. A significant difference was considered at the level of $P < 0.05$.

3. Results

3.1. Growth of S. obliquus

Cell density of *S. obliquus* varied slightly in the first two days (Fig. 1). Later, cell density increased sharply and reached a stationary phase on day 16. The maximum cell density, from 1.25 × 10^7 to 1.40 × 10^7 cells mL^{-1}, was not significantly different among different regimes.

3.2. Removal of NaCl and nutrient residue

The removal of NaCl and nutrient residue of *S. obliquus* under varying nutrient concentrations is shown in Table 2. The removal of NaCl was in the order of C>B>D>A>E. In the regimes with an N/P value of 8.5, the removal of NaCl increased from 505 mg L^{-1} to 559 mg L^{-1} with increasing concentrations of N and P.

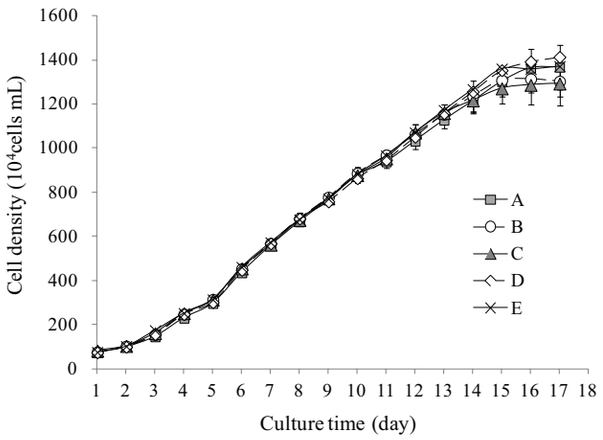

Fig. 1. Growth curves of *Scenedesmus obliquus* in varying nutrient regimes (with A, B, C, D, and E represent the different regimes).

The maximum use of N was up to 80.43%, and appeared in regime A in which the concentration of P was 5.44 mg L^{-1} and the N/P ratio was 8.5. P was almost exhausted in all regimes except for regime C. The use of P was highest (99.17%) in regime E.

3.3. Lipid production of S. obliquus

The highest and lowest lipid contents were 27.5% and 18.0%, respectively, in regimes B and D, respectively (Fig. 2). In regimes A, B, and C with the same N/P ratio, lipid content first increased and then decreased as nutrient concentrations rose. The change in pattern of lipid production was similar to that of lipid content. The maximum and minimum lipid production was 215 mg L^{-1} and 140 mg L^{-1}, respectively, in regimes B and D.

3.4. Fatty acid composition of S. obliquus

The major fatty acids in *S. obliquus* were palmitic acid (C16:0) and α-linolenic acid (C18:3). The difference in fatty acid composition among the five different nutrient regimes was insignificant. The proportion of unsaturated fatty acid in regimes A and B reached 66%, less than in regimes C, D, and E. The proportion of C16-C18 fatty acid in regimes C and D (> 86%) was clearly higher than in regimes A and B.

3.5. Relationship between fatty acid composition and nutrient concentration

Fig. 3 shows the RDA result demonstrating relationships between lipid production, fatty acid composition, and nutrient concentrations. The first and second axes explain 85.2% and 12.6% of the correlations, respectively. The first axis was determined by the initial concentrations of N and P, while the second axis was determined by the initial N/P ratio. Both lipid production and lipid content were positively related to the initial N/P ratio. The proportion of unsaturated fatty acids and C16-C18 fatty acids was positively related to initial concentrations of P.

Table 2
Removal of NaCl (mg L^{-1}) and nutrient residue (mg L^{-1}) of *Scenedesmus obliquus* under varying nutrient regimes

	Initial NaCl	Removal of NaCl	Initial N	Rest of N	Initial P	Rest of P	Cellular N:P
A	4800	505 ± 5	46	9 ± 4.7	5.44	0.13 ± 0.05	7.1
B		548 ± 16	93	77 ± 11.8	10.88	0.28 ± 0.03	8.0
C		559 ± 11	138	114 ± 15.9	16.32	5.70 ± 0.12	12.2
D		518 ± 4	54	24 ± 0.7	10.88	0.12 ± 0.00	4.6
E		490 ± 3	131	104 ± 2.6		0.09 ± 0.00	11.0

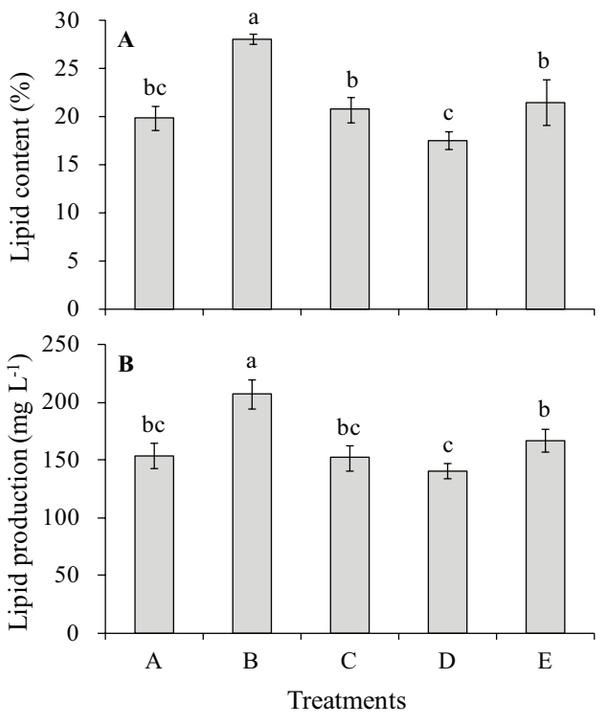

Fig. 2. Lipid content and lipid production of *Scenedesmus obliquus* under varying nutrient regimes (a, b, and c indicate the significance level).

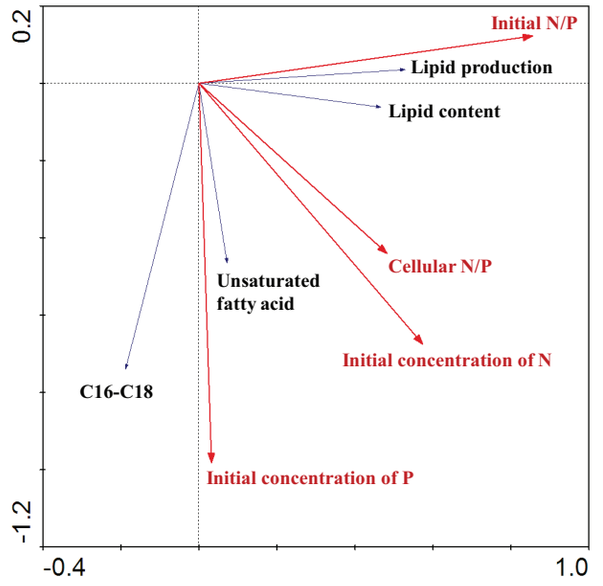

Fig. 3. Redundancy analysis of the relationship between lipid production, fatty acid composition, and nutrient concentrations under varying nutrient regimes.

3.6. Relationship between the lipid content and initial N and P concentration

The relationship between the lipid content of *S. obliquus* and initial N and P concentrations, including the results of the current study, are illustrated in Fig. 4. Lipid contents decreased as initial N and P concentrations rose. The data in this study were consistent with previous studies.

4. Discussion

Our results demonstrated that the removal of NaCl and lipid production was 505 mg L^{-1} and 152 mg L^{-1}, respectively, when the initial concentration of N was 46 mg L^{-1} and the value of P was 5.44 mg L^{-1}. Compared with the results of Gan et al. [16] using BG-11 culture medium, lipid production in the current study increased by 52.0%. This means that, in the current study, the consumption of N decreased by 171.7 mg but the lipid production increased by 201 mg, compared with previous work [16]. Clearly, the efficiency of simultaneous biological desalination and lipid production by *S. obliquus* was significantly improved by adjusting the nutrient addition strategy.

Scenedesmus can adsorb cations by means of specific groups such as $-COO^-$ and $-NH_2^-$ on the cell surface [29–31] and thus is able to desalinate. In addition, species in this genus also need to maintain intracellular osmotic pressure by absorbing Na^+ during growth. This is another important means of desalination. Gan et al. [16] reported that absorption and adsorption processes occurred simultaneously in the process of desalination by *S. obliquus*, but that the proportion of adsorption processes increased constantly with increasing salt concentration. Therefore, the amount of salt adsorbed by *Scenedesmus* at high salt concentration was mainly determined by the initial salt concentration. In the current study, the initial salt concentration was high and identical in different regimes. The initial salt concentration was high only relative to freshwater, but did not reach the salt concentration in seawater. Thus, it can be

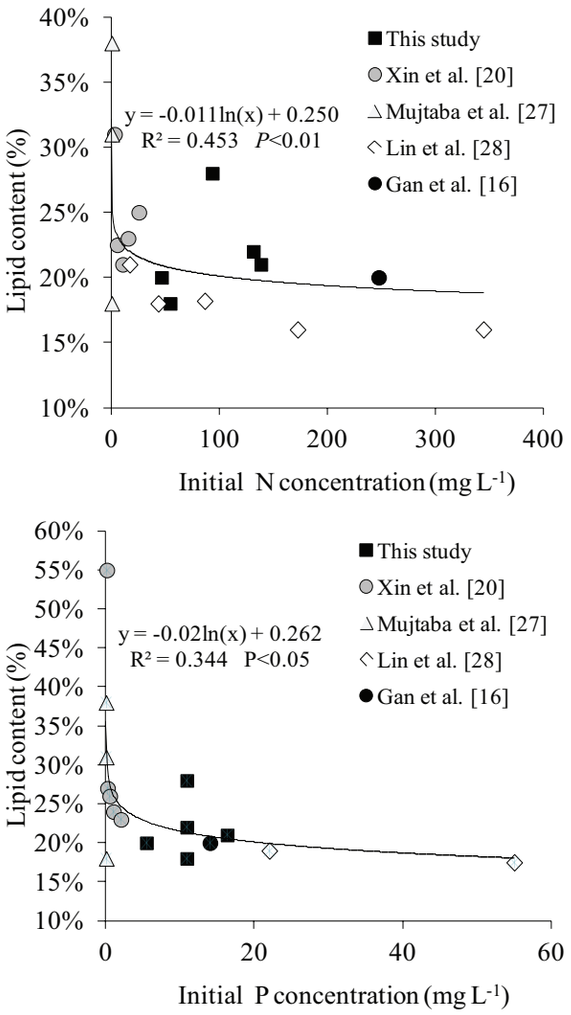

Fig. 4. Correlations between the lipid content of *Scenedesmus obliquus* and nutrient concentrations, based on data published previously and results in the current study.

deduced that there was no difference in the amount of salt adsorption because the cell density in different regimes was similar. However, there was still a significant difference in the removal of NaCl in the current study. This phenomenon indicated that there were differences in the amount of salt absorbed by *Scenedesmus* cells between the regimes. The lipid content of *S. obliquus* in regime B was higher than in other regimes, and the removal of NaCl was also high. Fatty acids played important roles in maintaining osmotic pressure and lipid content would increase as NaCl levels rose [32–34]. Therefore, the high amount of salt absorbed by *Scenedesmus* cells in regime B was a consequence of osmotic regulation via increasing fatty acid levels induced by the specific nutrient concentration.

RDA results showed that the lipid content of *S. obliquus* was positively related to initial N concentration, but no significant relationship was found between lipid content and initial P concentration. Inversely, most researchers reported that nutrient limitation effectively promoted lipid accumulation in *S. obliquus*. It was also found that the initial N and P concentrations in the current study were moderate, and had a weak influence on the lipid content of *S. obliquus*. Therefore, the variation in lipid content of *S. obliquus* in different regimes was caused by varying N/P ratios, and this was confirmed by RDA (Fig. 3). Xin et al. [20] also indicated that the N/P ratio significantly affected lipid production of *S. obliquus* and found that the highest lipid production was observed in a moderate N/P ratio of 5:1–12:1.

Table 3
Fatty acid composition of *Scenedesmus obliquus* under varying nutrient regimes

Fatty acid	Content (%) in *S. obliquus*				
	A	B	C	D	E
C15:0	3.2	0	3.9	3.4	3.8
C16:0	26.2	26.7	24.7	23.6	24.5
C18:0	0	0	2.2	1.6	0
C18:1	4.7	0	5.1	5.4	5.6
C18:2	2.5	0	2.8	3.1	3.4
C18:3	44.9	51.3	52.5	52.9	49.8
C20:1	0	0	0.8	0.8	0
C20:4	9.1	11.6	1.6	2.4	3.6
C20:5	5.1	3.2	5.3	5.6	6.7
C22:0	4.4	7.2	1.1	1.2	2.6
Saturated	33.8	33.9	31.9	29.9	30.9
Unsaturated	66.2	66.1	68.1	70.1	69.1
C16-C18	78.3	78.0	87.3	86.6	83.3

Generally, microalgae synthesize carbohydrate by means of photosynthesis and store energy as starch [35]. Under conditions of adversity, starch is decomposed and transformed into lipid [36]. Because of this, salt stress can promote lipid accumulation in microalgae [13,37,38]. Under conditions of N limitation, protein synthesis is restrained and the surplus energy inside microalgae is stored in the form of lipid or carbohydrate [39,40]. In addition, part of the macromolecule compounds containing N is also decomposed and transformed into micromolecule compounds, such as amino acids and enzymes, which are vital for algal growth. During this process, some of the excess energy from the decomposition of macromolecule compounds containing N is also stored in the form of lipid or carbohydrate [41,42]. At the end of the current study, N concentration in all regimes was still considerable, indicating that N limitation did not appear during the experiment. This was why no significant correlation between N concentration and lipid content was found in the current study.

Results also indicated that fatty acid composition was also affected by nutrient concentrations. It has been reported that the combustion and viscosity of biodiesel rises as the carbon chain length of fatty acids increases [43], and C16–C18 fatty acid has been considered the optimum for biodiesel production [44]. The increase in unsaturation levels of fatty acids favors the production of biodiesel having good unctuosity and a low melting point [45]. The proportion of C16–C18 fatty acid in the current study was more than 78%, and the proportion of unsaturated fatty acid

exceeded 66%. Both indicators, which determine the quality of biodiesel in the current study, exceeded the values investigated by Ho et al. [46]. Moreover, the results also illustrate that increasing the initial P concentration clearly enhanced the proportion of C16-C18 fatty acid and the unsaturation level. This phenomenon was similar to a previous result obtained in freshwater [47].

5. Conclusions

This work suggests that nutrient regime adjustment can simultaneously promote lipid accumulation and desalination by *S. obliquus*. The growth of *S. obliquus* was not affected by varying nutrient regimes, but removal of NaCl and lipid production varied significantly. It was found that a moderate N/P ratio (8.5) favored the removal of NaCl. In the regimes with an N/P ratio of 8.5, the removal of NaCl increased from 505 mg L^{-1} to 559 mg L^{-1} as concentrations of N and P rose. The highest lipid production and lipid content of 215 mg L^{-1} and 27.5%, respectively, were found in the regime with 93 mg L^{-1} N and 10.88 mg L^{-1} P. RDA results show that the lipid content of *S. obliquus* is positively related to initial N concentration, and that increasing initial P concentration clearly enhanced the proportion of C16-C18 fatty acid and the unsaturation level. The optimum nutrient regime was 46 mg L^{-1} N and 5.44 mg L^{-1} P. Under these conditions, the removal of NaCl was 505 mg L^{-1} and the use of N and P was 80.43% and 97.61%, respectively. Lipid production and lipid content were 152 mg L^{-1} and 20%, respectively, of which 66.2% was unsaturated fatty acid and 78.3% was C16-C18 fatty acid.

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