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Effects of urban wastewater dilution on growth and biochemical properties of *Scenedesmus* sp.

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ABSTRACT

Microalgal cultivation coupled with urban wastewater treatment can promote the large-scale production of algal biodiesel. However, most studies conducted to date have focused on effects of the nitrogen lipid production and nutrient removal, while ignoring the effects of dilution levels on the growth and biochemical properties. In this study, synthetic urban wastewater (SUW) was adopted to cultivate *Scenedesmus* sp. to explore the effects of dilution levels on the growth and biochemical properties, especially the lipid and protein properties. Microalgae reached maximum cell density (4.12×10^6 cell/mL) at 100% dilution, maximum specific growth rate (0.5815 d^{-1}) at 100% dilution, maximum total lipid content (25.4%) at 25% dilution, and maximum protein content (49.97%) at 75% dilution after 5 d of cultivation. The maximum removal rate was COD 69.5 mg/L/d (100% dilution), NH_3-N 7.33 mg/L/d (100% dilution) and TP 0.325 mg/L/d (75% dilution). Overall, SUW is a feasible medium for *Scenedesmus* sp. and 100% dilution is suggested.

Keywords: *Scenedesmus* sp.; Nutrients concentration; Microalgae biodiesel; Synthetic urban wastewater (SUW)

1. Introduction

Microalgae are small natural factories that can convert sunlight, carbon dioxide, and nutrients into biofuels and other useful materials [1–3]. Microalgae have recently received a great deal of attention because of their greater properties related to other oil producing plants [3–6]. In addition, the amount of CO_2 generated when burning microalgae biodiesel is lower than the amount absorbed by the microalgae

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when compared with fossil diesel and other types of biodiesel [7]. Thus, microalgal biodiesel can help prevent global warming. However, the main obstacle to industrial scale production is the high cost of nutrient, harvest, and lipid extraction.

Some researchers have started to focus on microalgal cultivation coupled with urban wastewater treatment [8–14], as this can reduce the costs required for mass production of urban wastewater and has the potential to be effective, cost-competitive, and energy saving [15–18]. This is because microalgae have the ability to use organic and inorganic nutrients in wastewater to produce lipids, proteins, and other useful organics [19,20]. However, some studies have concentrated on the effects of nitrogen on lipid production or nutrient removal [1,8,21,22]. To the best of our knowledge, no studies have examined the effects of dilution levels on the growth and biochemical properties, especially the lipid and protein properties, of *Scenedesmus* sp. cultivated in urban wastewater.

Therefore, in this study, four different diluted levels of synthetic urban wastewater (SUW) were investigated (100, 75, 50, and 25%). Additionally, the BG11 group was added as a comparison group. *Scenedesmus* sp. FACHB–933 was mixotrophically cultivated in five groups over 5 d, and the changes in algal growth rate, wastewater nutrient removal rate, and biomass composition were explored and discussed.

2. Materials and methods

2.1. Materials and photobioreactor

The cultivated strain, *Scenedesmus* sp. FACHB–933, was provided by the Freshwater Algae Culture Collection at the Institute of Hydrobiology. SUW used in this study contained the following compounds: $C_6H_{12}O_6$ 260.2 mg/L, $CH_3COONa{\cdot}3H_2O$ 260.2 mg/L, NH_4Cl 191 mg/L, $NaHCO_3$ 200 mg/L, KH_2PO_4 11 mg/L, $K_2HPO_4{\cdot}3H_2O$ 18 mg/L, $MgSO_4{\cdot}7H_2O$ 10 mg/L, $FeSO_4{\cdot}H_2O$ 10 mg/L, $CaCl_2{\cdot}2H_2O$ 10 mg/L, and 1 ml trace elements mother liquor. The composition of the trace element mother liquor was H_3BO_3 0.15 g/L, $CuSO_4{\cdot}5H_2O$ 0.03 g/L, KI 0.18 g/L, $MnCl_2{\cdot}4H_2O$ 0.12 g/L, $Na_2MoO_4{\cdot}2H_2O$ 0.06 g/L, $ZnSO_4{\cdot}7H_2O$ 0.12 g/L, $CoCl{\cdot}6H_2O$ 0.15 g/L, $FeSO_4{\cdot}7H_2O$ 10 g/L, $CaCl_2{\cdot}2H_2O$ 10 g/L, EDTA 10 g/L. This medium was developed based on a study by Jia et al. with brief modification [23]. The BG11 medium was prepared in accordance with Stanier et al. [24].

Several studies have shown that when cultured in photobioreactors, microalgae can reach a much higher biomass concentration than when cultivated in raceway [3]. Therefore, in this study, a photobioreactor composed of a support system, an observation system, and a control system was used. The support system was designed to support experimental devices and provide constant temperature (via an air conditioner) and stable light (via six fluorescent tubes). The observation system was responsible for monitoring the temperature and light density of the culture system. The control system consisted of a time switch to provide semicontinuous illumination by turning on or off the fluorescent lights. The pattern of the culture system is shown in Fig. 1.

2.2. Cultivation conditions

Five liters of synthetic wastewater was prepared and diluted to a gradient of 100, 75, 50, and 25%. BG11 culture was prepared simultaneously as a positive control. All solutions were decanted into the designed Erlenmeyer flasks with an additional tap for injecting the algal suspension, and the initial pH was adjusted to 7.0 ± 0.2. High-pressure steam sterilization was then applied at 120˚C and 100 kPa for 30 min before injecting the algae. The initial algal concentration was set to 0.2×10^6 cells/ml.

During cultivation, the rotation rate of magnetic stirrers was adjusted to 180 rpm to prevent microalgal cells from settling on the bottoms and walls of the flasks. Six fluorescent tubes served as the light source of the photobioreactor, and light was provided from 6 am to 6 pm. at a stable intensity of 2,500 lux. The temperature of the entire bioreactor was maintained at 25 ± 2˚C.

2.3. Growth assessment

The cell density was determined by UV spectrophotometry at 680 nm based on comparison with a calibration curve combining the density with the

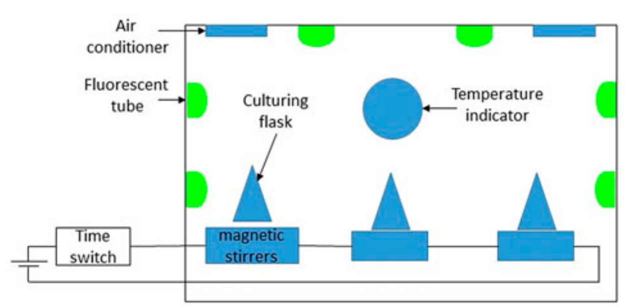

Fig. 1. Front view pattern of the designed photobioreactor.

absorbance of the algal suspension every 24 h. The equation used to calculate the density was as follows:

$$y = 7.9018x \ (R^2 = 0.993) \quad (1)$$

where x and y represent the OD_{680} value and the cell density, respectively. The concentration was calculated based on the following equation to assess the specific growth rate.

$$\mu = (\ln N_1 - \ln N_2)/(T_2 - T_1) \quad (2)$$

where N_1 and N_2 are the concentrations at days T_1 and T_2.

The daily variations in COD, NH_3-N, and TP value were measured spectrophotometrically to reflect the nutrient removal conditions indirectly. The potassium dichromate oxidation method was adopted to determine the COD using DR2800 (HACH Inc., USA) according to the Chinese standard methods (GB11914-1989). Ammonia nitrogen was measured using Nessler's reagent colorimetric method according to Song et al. and TP was determined using the ammonium persulfate digestion and ascorbic reduction method [25,26]. The nutrient removal ratio (% of the initial value) was employed to determine the algal removal efficiency, and the removal rates (mg/L/d) were used to show the algal removal ability.

2.4. Lipid extraction and fatty acids methyl ester (FAME) analysis

Lipids were extracted and measured as previously described [27]. Briefly, FAME was abstracted through a two-step *in situ* process [28]. Next, the FAME composition was determined by one-step transesterification [29], which was followed by Trace GC Ultra and DSQIIMS (Thermo Fisher Scientific, Somerset, NJ, USA) analysis to determine the composition as previously described [27].

2.5. Protein and amino acid analysis

To analyze the protein composition, the dry algal powder was treated with hydrochloric acid at 110℃ for 22 h. The treated samples were then added to a U–3000 CLC–ODS chromatographic column to determine the amino acid composition.

In this study, the total amino acid composition was used to represent the algal protein content. In addition, the essential amino acid percentage (EAAP) was defined to reflect the proportion of essential amino acids (EAAs) in the total amino acids. The following equation was used to calculate the EAAP:

$$\text{EAAP} = \text{Pe/Pt} \times 100\% \quad (3)$$

where Pe is the percentage content of EAAs in the test sample, and Pt represents the percentage of total amino acids in the test sample.

2.6. Statistical analysis

Experiments were performed in triplicates, and groups were compared by the least significant difference test using IBM SPSS Statistics 19.0. Graphs of the data were generated using Origin Pro 9.1.

3. Results and discussion

3.1. Effect of wastewater on algal growth

The detailed growth trend is shown in Fig. 2. The initial concentration of each group was adjusted to 0.2×10^6 cells/ml. After 5 d of culture, the growth rate improved greatly with the support of SUW compared with BG11 medium. All densities of microalgae cultivated in SUW were significantly higher than those cultivated in BG11 medium via statistical analysis. Moreover, the growth rate of the *Scenedesmus* sp. was greatest in the 100% dilution, while the growth rates showed few evident changes in the other dilutions (75, 50, and 25%). This may be explained by that fact that there would be no significant effect on algal growth rates unless the nutrients in the SUW reached a definite concentration. Overall, the SUW could

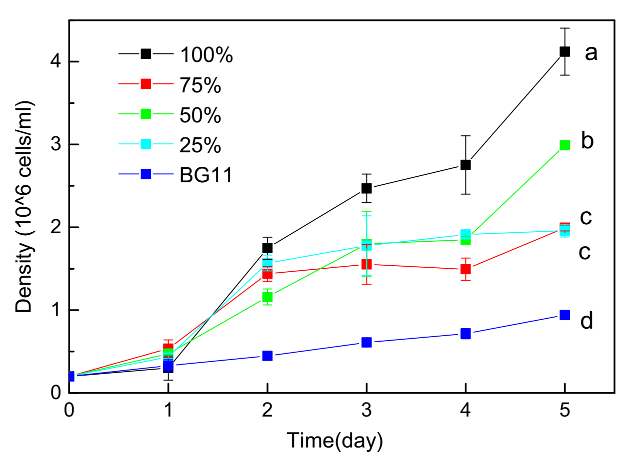

Fig. 2. Microalgal growth under different types of media (error bars indicate the standard deviation of three repeats, small letters show the LSD test result at the 0.05 level).

promote the growth of *Scenedesmus* sp. more than the BG11 medium, and high dilutions, especially 100% dilution, were the best, which is in accordance with the results of previous studies [30–34].

The specific growth rate (d^{-1}) was investigated, and the results are presented in Fig. 3. All SUW groups showed higher specific growth rates than the BG11 group. The 100% wastewater showed the highest specific growth rate, while the growth rates of the other three dilutions were similar, which was concordant with cell density. Moreover, all specific growth rates (d^{-1}) of the SUW groups were greater than 0.479 d^{-1} after 5 d of cultivation, which was superior to the results of similar studies [31,33].

3.2. Nutrient removal performances

Recent studies have demonstrated that large-scale wastewater treatment through microalgae is feasible [34–36]. During mixotrophic cultivation, nutrients needed by microalgae are taken up from wastewater, which leads to its purification. Nutrient removal can be directly determined by variations in the COD, NH_3-N, and TP value. The undiluted SUW used in this study had an initial NH_3-N value of 50 mg/L, COD of 400 mg/L, and TP of 5 mg/L. Variations in the nutrient removal ratio (% of the initial value) are shown in Fig. 3, and their removal rates (mg/L/d) are listed in Table 1.

In the first 2 d, the COD, NH_3-N, and TP value of each group decreased sharply. However, the removal efficiency had a negative correlation with the concentrations (Fig. 4), with the initial nutrients being present in high concentration, then consumed with a relatively low removal efficiency [34]. After 2 d, the

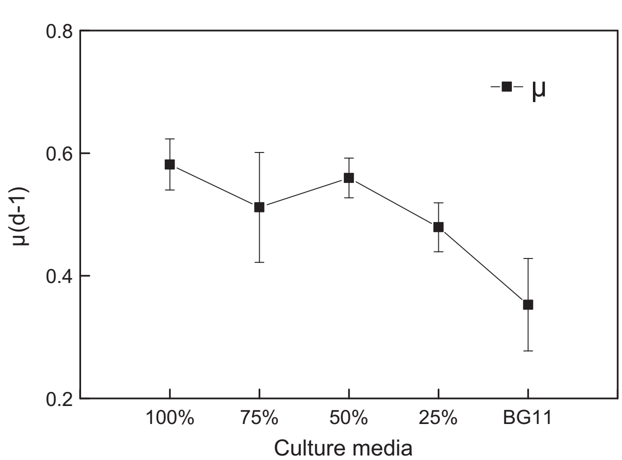

Fig. 3. Specific growth rate of the first 5 d (error bars denote standard deviations of three repeats).

Table 1
Nutrient removal rates (mg/L/d) under different SUW concentrations

	COD	NH_3-N	TP
100%	69.5 ± 2.27a	7.33 ± 0.151a	0.298 ± 0.084a
75%	56.3 ± 1.81b	5.90 ± 0.659b	0.325 ± 0.064a
50%	33.5 ± 1.31c	4.47 ± 0.518c	0.194 ± 0.010b
25%	12.4 ± 1.50d	2.34 ± 0.389d	0.161 ± 0.019c

Note: Different letters show significant difference ($p < 0.05$).

COD, NH_3-N, and TP removal ratios all declined at different levels, which were likely caused by ruptured algal [33,37]. As a result, the microalgal growth rate decreased from day 2 to day 4 (Fig. 2). Additionally, the endocellular contents released from the dead algal cells resulted in increasing nutrient levels. The nutrient removal ratios then began to recover to a higher level, and the algal growth rates later increased again (Figs. 2 and 4). These phenomena can explain the unstable nature of the nutrient removal ratio. Overall, rupture caused the algal growth rate to decrease, which led to further leaded increases in nutrient.

After 5 d of cultivation, nutrients in the SUW had declined remarkably. The COD, NH_3-N, and TP removal rates peaked at 92.9, 76.3, and 85.1% with 75, 25, and 25% SUW, respectively. The 100% group also had a high removal of organics, which were reflected by COD removal rates immediately below the 75% group. At the same initial NH_3-N concentration of wastewater, Li et al. only reached 31.1% N removal rates after 13 d of cultivation [38], while Sacristan et al. reached COD removal percentages of 36.5–77.3% when culturing *Scenedesmus acutus* with different municipal wastewater groups [39], which were lower than the results observed in this study. As shown in Table 1, *Scenedesmus* sp. had different removal abilities in different dilutions. The undiluted wastewater had significantly higher nutrients removal ability than other dilutions ($p < 0.05$). Overall, these findings indicate that 100% SUW is a desired solution to cultivate the strain for wastewater treatment.

3.3. Typical products output

3.3.1. Lipid content and FAME composition

Wastewater lipids were extracted and measured (Table 2). The lipid content of dry biomass was 21.5–25.4%, which is higher than the reported average microalgae lipid content of 20% [27]. Moreover, Song et al. used three kinds of synthetic wastewater and achieved a final lipid content ranging from 16 to 19% of the dry weight [27]. The lipid content of the SUW

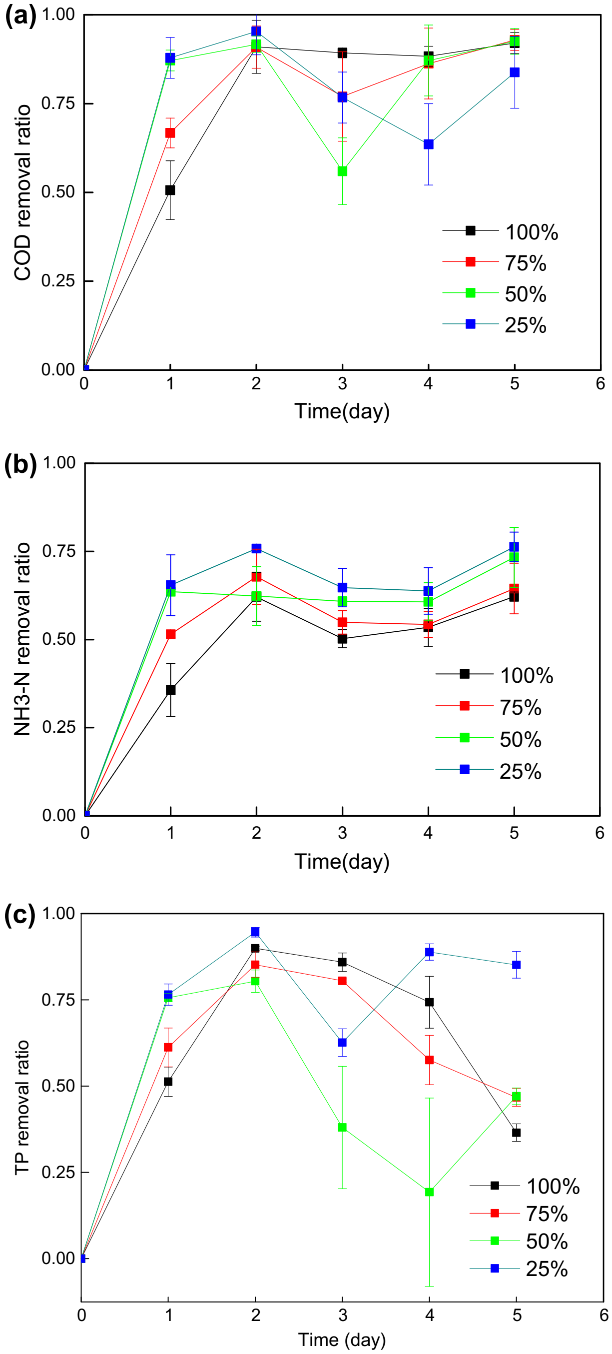

Fig. 4. Nutrient removal ratio with time under four dilutions of SUW (a) COD, (b) NH_3-N, and (c) TP.

group was generally higher than that of the BG11 group, indicating that wastewater may promote the production of lipids as well. Moreover, the highest lipid content was obtained at 25% dilution, while the 100% dilution produced the lowest lipid content. These findings indicated a negative correlation

Table 2
Fatty acid properties of *Scenedesmus* sp. at different SUW concentrations (% of total FAME) and lipid contents (% of dry biomass)

Fatty acid profile	100%	75%	50%	25%	BG11
C16–C18	94.136	91.685	95.943	95.82	99.82
PUFA	6.762	8.452	10.339	12.134	6.532
MUFA	43.778	41.561	22.038	27.943	21.274
SFA	43.642	45.04	66.96	56.658	71.217
Lipid content	21.5	23.3	23.9	25.4	18.2

between lipid content and wastewater concentration because of the low nitrogen concentration [1,21]. However, the 100% dilution produced the highest lipid content from the dry biomass on the fifth day because of its high biomass.

Four important biodiesel properties, C16–C18 fatty acids, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), were also investigated (Table 2). The content of C16–C18 series in SUW was higher than that of the BG11 groups, while the contents of the 50 and 25% dilutions were 95.94 and 95.82%, respectively, which were slightly higher than those of the 100 and 75% dilutions. These levels were superior to those of several previous studies. For example, under similar cultivation conditions, Zhu et al. found that *Chlorella zofingiensis* could generate 80.3% C16–C18 series, while Li et al. found that *Chlorella* sp. could generate 82.7% [21,40]. The SFA ranged from 43.6 to 67.0%, while the MUFA ranged from 22.0 to 43.8% and the PUFA ranged from 6.8 to 12.1%. The fatty acid compositions varied among dilutions in certain orders. For example, PUFA and SFA showed a negative correlation with concentration, while MUFA showed the opposite. Additionally, the 100% dilution was low in SFA and PUFA, but high in MUFA. It is widely agreed that the composition of the most favorable biodiesel would consist of low levels of saturated and polyunsaturated FAs to decrease the cold flow and oxidative stability problems [41,42]. Hence, 100% SUW was the best medium for producing high-quality biofuel.

3.3.2. Protein and amino acid properties

The compositions of amino acids determined by liquid chromatography are presented in Table 3. The protein contents of the SUW and BG11 group were 46.59, 49.97, 47.21, 45.34, and 37.01%, respectively. The protein content of the four dilutions was higher than those of the BG11 medium. The 75% dilution produced

Table 3
Amino acid compositions and contents (mg/100 mg dry biomass) under different SUW concentrations

	100%	75%	50%	25%	BG11	*L. filiformis* [43]	*S. quadricauda* SDEC-13 [2]
Thr	2.30	2.42	2.32	2.21	2.13	0.6	1.65
Val	2.78	3.08	2.76	2.57	2.20	0.3	0.86
Met	1.15	1.30	1.15	1.14	0.65	0.5	0.96
Ile	1.96	2.18	1.93	1.76	1.41	0.8	2.40
Leu	4.57	5.00	4.63	4.39	3.02	0.5	1.70
Phe	2.44	2.58	2.48	2.43	2.01	1.0	1.97
Lys	2.94	2.91	3.10	3.16	2.11	0.5	1.88
Asp	4.11	4.18	4.26	4.22	3.54	1.5	3.17
Ser	1.92	1.92	2.06	2.03	1.73	0.6	1.50
Glu	5.68	6.18	5.58	5.50	4.96	1.4	3.48
Pro	3.92	4.10	4.10	4.02	2.31	0.7	1.76
Gly	3.00	3.28	3.02	2.89	2.91	0.7	2.10
Ala	4.50	5.28	4.32	3.82	3.62	0.1	1.30
Cys	0.29	0.37	0.30	0.33	0.22	0.6	1.56
Tyr	1.82	1.90	1.83	1.93	1.37	0.2	0.58
His	0.93	0.93	0.99	0.59	0.81	0.6	1.14
Arg	2.28	2.35	2.39	2.34	2.01	0.5	1.56
EAA	18.14	19.47	18.36	17.67	13.53	4.2	11.42
Protein	46.59	49.97	47.21	45.34	37.01	11.10	29.57
EAAP	0.3893	0.3897	0.3890	0.3898	0.3656	0.3784	0.3862

the highest protein content among the four dilutions. Specifically, this dilution produced 49.97 mg in 100 mg dry biomass, which was higher than that reported for *Scenedesmus quadricauda* SDEC–13 in campus sewage and approached the level of biomass reported for *Chlorella vulgaris* in diluted monosodium glutamate wastewater [2,14]. Moreover, all of the protein contents of the SUW were much higher than those reported for *Laurencia filiformis* [43]. The 100% dilution gained more protein in the dry biomass harvested on the fifth day, because of its high biomass. These results show the SUW promoted the protein content of the *Scenedesmus* sp. with the 100% dilution being better.

The EAA levels indicated the percentage of EAA in 100 mg dry biomass. The 75% dilution produced the highest EAA value of 19.47%. However, the 100% dilution harvested the highest EAA content because of its high biomass. BG11 showed a value of 13.53%, which was much lower than that in SUW. Overall, these results show that the synthesis of EAA can also be promoted in SUW. Moreover, the EAA gained in the SUW was higher than the reference values of *L. filiformis* and *S. quadricauda* SDEC–13 [2,43]. The EAAP is the percentage of EAA in the protein, which shows the quality of the protein. The EAAP results revealed no significant differences among different dilutions. Based on these findings, the concentration of the SUW had no effect on the EAAP. However, the SUW promoted the EAAP compared with the BG11 medium, with the SUW having a higher EAAP value.

4. Conclusion

Urban sewage can be used as a good medium for microalgal cultivation with light or no dilution. When cultivated with SUW, *Scenedesmus* sp. attained a better growth rate and higher lipid and protein content. These results indicate that the SUW is superior to BG11 medium because of its high growth rate and better performance based on the quality and quantity of the proteins and lipids. Moreover, treatment by *Scenedesmus* sp. showed a higher removal rate at 100% dilution. As a result, SUW treatment coupled with the *Scenedesmus* sp. cultivation is feasible. The 100% dilution was best among tested dilutions based on the analysis of the biochemical properties in the different dilutions. The 100% dilution resulted in the highest lipid content, protein content, and EAA value because of its high biomass, although its promotion was not largest. Simultaneously, its lipid content indicated that 100% dilution was the ideal medium for biofuel. Overall, the 100% dilution is suggested for the cultivation of *Scenedesmus* sp. because of its good effects on growth and biochemical properties.

Acknowledgments

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