Influence of crude glycerol on the biomass and lipid content of microalgae

Hee-Jeong Choi* and Sung-Whan Yu
Department of Environmental Engineering, Catholic Kwandong University, Gangneung, Korea
(Received 16 October 2014; accepted 28 January 2015)

The growth of the algae Chlorella vulgaris, Botryococcus braunii and Scenedesmus sp. under mixotrophic conditions in the presence of different concentrations of crude glycerol was evaluated with the objective of increasing the biomass growth and algal oil content. A high biomass concentration was characteristic of these strains when grown on crude glycerol compared to autotrophic growth, and 5 g/L glycerol yielded the highest biomass concentration for these strains. Mixotrophic conditions improved both the growth of the microalgae and the accumulation of triacylglycerols (TAGs). The maximum amount of TAGs in the algae biomass was obtained in the 5 g/L glycerol growth medium. The fatty acid profiles of the oil for the cultures met the necessary requirements and the strains are promising resources for biofuel production.

Keywords: biomass; glycerol; microalgae; mixotrophic; oil content

Introduction

Algae cultures have primarily been developed as an important source of aquaculture feeds, human food supplements and pharmaceuticals[1] and algae have also been proposed as good candidates for fuel production.[2,3] Algae strains that are robust and highly productive are selected for the conversion of biomass into energy,[4] and strains with relatively high lipid content are highly attractive for biodiesel fuel production.[3,5] Microalgae have high growth rates and produce lipids for biofuel production, which is essential for increasing biomass production and the amount of lipids, which in turn decreases the cost of biodiesel production.[6] Microalgae as a source of renewable energy have received considerable interest; however, further optimization of mass culture conditions is necessary for microalgal biofuel production to become economically viable and sustainable.[7]

Crude glycerol is a major by-product accumulated during the production of biodiesel.[8] With the burgeoning biodiesel manufacturing industry, the market is inundated with crude glycerol. Consequently, biodiesel producers must seek new uses for this waste stream. One possibility for utilization of crude glycerol is to use it as a substrate for fermentation, for example, of the oleaginous microalga Schizochytrium limacium,[9] which can produce significant amounts of total lipids and docosahexaenoic acid, when grown on glycerol or other carbon sources.[10,11] Thus, biodiesel-derived glycerol can be considered a potential substrate for mixotrophic cultivation of oleaginous microalgae, which would reduce the production cost of microalgal biodiesel.[12]

However, there are still only a few reports examining the effects of carbon sources, particularly on biomass production and algal cell components under mixotrophic conditions.[12–14] In this study, the effects of various concentrations of crude glycerol on the biomass growth and oil content of Chlorella vulgaris, B. braunii and Scenedesmus sp. under mixotrophic conditions were evaluated.

Materials and methods

Microalgae cultures and medium

Seed cultures of Chlorella vulgaris (KMMCC-355), B. braunii (KMMCC-1681) and Scenedesmus sp. (KMMCC-1235) [15] were cultivated in Jaworski’s medium (JM) under light-emitting diode (LED) lamps at ambient temperature. The microalgae were cultured in 200 mL conical flasks containing 100 mL of JM (pH 7.2 ± 0.3), in which 10 mL C. vulgaris, B. braunii and Scenedesmus sp. were added. The cultures were maintained at a dark and light cycle of 8 and 16 h, respectively.

Experimental design

Optical panel photobioreactor (OPPBR) construction and operation

A schematic diagram of the OPPBR is shown in Figure 1(a). The three OPPBRs were operated in parallel at a 15 L working volume and each was equipped with an optical panel (OP). The initial concentrations of the inoculated microalgae were 0.357 g/L ± 0.7 g/L of C. vulgaris, 0.342 g/L ± 0.7 g/L of B. braunii and 0.367 g/L ± 0.6 g/L
of Scenedesmus sp. The experiments were conducted at neutral pH (7.2 ± 0.3) under dark and light cycles of 8 and 16 h, respectively. The temperature was maintained at 23 °C ± 1 °C using LEDs for 20 days. The OPPBRs were aerated continuously at an aeration rate of 0.5 L/min. CO2 at the equivalent aeration rate of 0.02vvm was used for cultivation. CO2 with a flow rate of 0.74 L/min was introduced into the reactors. The OPPBR was designed such that the light source (22 LEDs), an LED panel (bar type), was placed in the OPPBR. A v-grooved OP was inserted underneath this in the photobioreactor (PBR). The thickness of the OP was 6 mm (Figure 1(b)).[16] For greater functionality, the incident light was uniformly distributed across both sides of the OP in the reactor. A LED light source was used because it is efficient and provides the required wavelength light from 430 nm to 670 nm, which is selective for microalgal growth. Moreover, the light intensity representing the amount of light used for photosynthesis was 200–250 μmol/(m2 s). The mixotrophic conditions for algae cultivation were achieved with crude glycerol purchased as a by-product of biodiesel production. The corresponding amount of crude glycerol (0, 2, 5 and 10 g/L) was added to the JM growth medium to prepare the desired mixotrophic medium.

Optical panel in photobioreactor

The characteristics of the OP are listed in Table 1.[16] The OP exhibited 93% transmittance and 1.19 specific gravity.

Table 1. Characteristics of optical panel (OP). Reprinted with permission from Springer: [16] Choi HJ, Lee SM. Effect of optical panel thickness for nutrient removal and cultivation of microalgae in the photobioreactor. Bioprocess Biosyst Eng. 2014;34:697–705.

| Parameters               | Method     | Unit   | v-cut OP |
|--------------------------|------------|--------|----------|
| Specific gravity         | ISO 1183   |        | 1.19     |
| Transmittance            | ISO 13,468 | %      | 93       |
| Heat distortion temperature | ISO 75   | °C     | 94       |
| Melt flow rate           | ISO 1133   | g/10 min | 1.5     |
| Tensile strength         | ISO 527    | MPa    | 75       |
| Mold shrinkage           | MRC Method | %      | 0.2–0.6  |

The OP dimensions were 210 mm (L) × 290 mm (H) × 6 mm (W) as shown in Figure 1(b) and were constructed from a transparent panel of pure PMMA (poly-methylmethacrylate) sheets. This material has good transparency and its light absorption in the visible region is negligible.[17] In this study, a v-cut OP (Figure 1(b)) was introduced to evaluate and assess the quantitative effects of crude glycerol on cell growth, biomass productivity and lipid content. With the v-cut technology, the light was guided into the v-grooves that have x-, y- and z-direction dimensions, such as enlarged horizontal and vertical grooves. The vertical v-grooves are widely spaced when they are close to the light source and narrow when distant from the light source. The enlarged horizontal v-grooves are arranged in straight lines along the x-direction from the end edge of the OP and have maximum enlarged portions located on the other edge of the OP.[16] In addition, the v-cut provides a uniform distribution of light in the PBR.

Analytical methods

Measurement of cell weight and specific growth rate

The effect of crude glycerol concentrations (0, 2, 5 and 10 g/L) added during the initial growth phase was evaluated in relation to the growth of the algal biomass and lipid accumulation.

To determine the biomass concentration, a sample of microalgae in growth medium was centrifuged for 10 min at 628 g, washed with distilled water and then dried in an oven at 105 °C for 24 h to constant weight. The biomass productivity P (g/(L-day)) was calculated from the variation in biomass concentration X (g/L) within a cultivation time t (in days), according to the following equation:

\[ P = (X_t - X_0) / (t_t - t_0) \]  \hspace{1cm} (1)

The specific growth rate \( \mu \) (in days) was calculated using Equation (2):

\[ \mu = \ln(X_t / X_0) / (t_t - t_0) \]  \hspace{1cm} (2)
where $X_t$ and $X_0$ are the biomass concentration (g/L) on days $t_1$ and $t_0$, respectively.

**Extraction of lipids**

The algal biomass for lipid extraction was prepared by centrifugation and drying. After oven drying, the algae were pulverized and subjected to Soxhlet extraction. All Soxhlet extractions were performed for 72 h using 500 mL of solvent per 1 g of pulverized dry algae with a cycle time of 10–15 min. Soxhlet extraction with hexane was used because the Bligh and Dyer [18] extraction method is suitable for extraction of all lipids, including triglycerides, phospholipids and other pigments.[19] The lipid content does not reflect the exact amount of triacylglycerols (TAGs; consisting of a glycerol moiety with each hydroxyl group esterified to a fatty acid) because only triglycerides are used in the synthesis of biodiesel and other components are undesirable. The excess hexane was evaporated by rotary evaporation until the total volume reached 30–40 mL. The solutions were diluted to 50 mL and used to determine the TAG content. The amount of TAG was determined using a Fourier transform infrared spectrometer Spectrum RX 1 (Perkin Elmer), according to the carbonyl stretching absorption at 1740 cm$^{-1}$.[20] The amount of TAG in the extract solutions was determined using a standard graph, and the amount of TAG was calculated in the dry algae (% w/w). The experiments were performed five times, and mean values and the standard deviation were calculated.

**Measurement of fatty acids composition**

The fatty acid composition of the algae oil was determined using the standards EN ISO 5508 and EN ISO 5509. The analysis was performed with a Clarus 500 (Perkin Elmer) gas chromatograph. The conditions for analysis were as follows: capillary column Alltech AT-FAME (30 m–0.25 mm–0.25 μm), initial oven temperature of 210 °C held for 5 min then increased at 20 °C/min from 210 to 230 °C and held at 230 °C for 12 min. Nitrogen was used as the carrier gas. The injector temperature was 250 °C. The fatty acids were identified by comparing their retention times with the standards. The experiments were performed five times and the mean values and standard deviations were calculated.

**Statistical analysis**

Data presented in tables and figures are mean values ± SE (standard error) from five replications. Where error bars are not visible, errors were smaller than or equal to the symbols. The differences between mean values were calculated using Tukey’s test at the 0.05 level by Origin software (v.7.5, OriginLab, Northampton, MA, USA).

---

**Results and discussion**

**Effect of glycerol concentration on the growth of algal species**

Figure 2 shows the effect of different glycerol concentrations on the growth of *C. vulgaris* compared to growth on JM medium. During the first five days, the microalgae grew similarly in all growth media with different amounts of glycerol. A slight difference in biomass was observed in the growth medium with initial concentrations of 2 and 10 g/L glycerol. The maximum biomass concentrations obtained in the medium containing 2, 5 and 10 g/L of glycerol were 1.61, 1.91 and 1.72 g/L, respectively. By comparison, the highest biomass concentration in the medium with 5 g/L glycerol was 39.42% higher than that achieved with *C. vulgaris* in the autotrophic medium, which was 1.37 g/L ($P \leq 0.05$). All media with glycerol yielded higher biomass than that accumulated in autotrophic conditions.

The growth kinetics of the *Scenedesmus* sp. strain in JM medium with different glycerol concentrations (Figure 3) showed that in the first four days, the microalgae grew similarly in autotrophic conditions and in the medium containing 10 g/L of glycerol. In the media with 2 and 5 g/L of glycerol, the growth was also similar in the first four days. However, after four days, a significantly faster ($P \leq 0.05$) growth rate was observed in the medium with 5 g/L glycerol than in the one with 2 g/L glycerol. The highest biomass concentration (1.92 g/L) was obtained during the stationary growth phase. This biomass concentration was 60.00% higher than that in the autotrophic medium, which was 1.20 g/L. These data indicate that *Scenedesmus* sp. grew better under mixotrophic conditions, using crude glycerol, than in autotrophic conditions.

The growth curve of *B. braunii* cultures is shown in Figure 4. During the first five days, the microalgae grew similarly in all growth media with different amounts of glycerol. The biomass concentrations for 2 and 5 g/L glycerol were similar. The highest achieved biomass
concentrations (2.23 and 2.32 g/L) were similar ($P > 0.05$) for these two concentrations of glycerol (2 and 5 g/L, respectively) and were found to be 51.70% and 57.82% higher ($P < 0.05$) than the corresponding biomass concentrations obtained without glycerol (the highest concentration was 1.47 g/L). Therefore, glycerol has a strong impact on the growth of *B. braunii* when compared to autotrophic growth in JM medium.

A slight decrease of 1.87 g/L in the biomass was observed in the medium containing 10 g/L of glycerol.

The maximum biomass productivity and maximum specific growth rates of the studied microalgae in media with glycerol concentrations ranging from 0 to 10 g/L are shown in Table 2. *C. vulgaris* achieved 0.227 g/(L·day) maximum biomass productivity and 0.342 1/day maximum specific grow rate in the medium containing 5 g/L of glycerol, whereas those in the presence of 2 and 10 g/L of glycerol were slightly different. The maximum biomass productivity (0.231 g/(L·day)) and maximum specific growth rate (0.306 1/day) were achieved with 5 g/L glycerol for *Scenedesmus* sp. *B. braunii* showed no significant differences in the highest values of the maximum biomass productivity (0.258 g/(L·day) and 0.271 g/(L·day)) and maximum specific growth rate (0.228 1/day and 0.301 1/day) in the presence of 2 and 5 g/L glycerol, respectively. In all the three microalgal species, however, the maximum biomass productivity and maximum specific growth rate in autotrophic conditions were lower compared to those in mixotrophic conditions. These results are in agreement with previous reports that mixotrophic growth significantly increases the microalgal (*Chlorella* sp.) cell concentration and volumetric productivity in batch systems.[12,21] A possible explanation could be sought in the fact that the anabolism in mixotrophic cultures is accelerated due to adenosine triphosphate formed both in photochemical reactions and in heterotrophic reactions.

In the present study, the glycerol concentration in mixotrophic systems influenced the biomass concentration and growth rate of *C. vulgaris*, *Scenedesmus* sp. and *B. braunii*. The highest biomass concentrations were obtained in mixotrophic conditions in the presence of 5 g/L of glycerol for *C. vulgaris*, *Scenedesmus* sp. and *B. braunii* and were, respectively, 39.42%, 60.00% and 57.82% greater than the corresponding ones in autotrophic conditions. In terms of biomass concentration, *Scenedesmus* sp. showed the highest increase (in percentage) in mixotrophic medium enriched with glycerol. *B. braunii*, however, also showed good growth enhancement, with absolute biomass values even slightly exceeding those of *Scenedesmus* sp. in mixotrophic conditions. Thus, the results of this study suggest that the investigated algae species may be excellent biofuel producers because organic by-products stimulate the growth rate of these strains.

**Total fatty acids in algae species**

The results from the measurements of the total TAG content in the algal biomass harvested from the cultures grown in the presence of different glycerol concentrations are presented in Table 3. The highest TAG content was 15.91%, 16.24% and 16.41% for *C. vulgaris*, *Scenedesmus* sp. and *B. braunii*, respectively, cultured with 5 g/L glycerol. In other words, in the biomass grown in the 5 g/L glycerol medium, the relative TAG content was 12.20%, 13.11% and 9.30% higher than that in autotrophically grown *C. vulgaris*, *Scenedesmus* sp. and *B. braunii*, respectively. In fact, the three microalgal species under all the tested mixotrophic conditions had 2% to 13% higher lipid content than in autotrophic conditions. The 2 and 10 g/L glycerol media yielded similar TAG content for *C. vulgaris* and *Scenedesmus* sp. ($P > 0.05$). However, for *B. braunii*, the biomass grown in the 2 g/L glycerol medium had approximately 6% higher total TAG content than that cultured in the 10 g/L glycerol medium. In the medium that proved optimal for growth, i.e. the 5 g/L glycerol-containing medium, it was observed that the three investigated species possessed almost identical TAG contents.
The lipid content increased from 22% with 1 g/L glycerol to 32% with 2 g/L glycerol. However, in their study, the highest amount (10 g/L) of glycerol had an inhibitory effect on the growth of algae and TAG content. Although available data are difficult to compare due to differences in species, strains, cultivation conditions and methods used, evidence seems to be accumulating that microalgae may prove useful for utilization of by-product glycerol and biofuel production.

What is important for biodiesel production is the lipid content and effectiveness of microalgal growth. In some cases, improved accumulation of oil but slower growth of microalgae may result in lower oil yields compared to faster growing microalgae with less oil accumulation. The results of this study showed that *B. braunii* grown in the presence of 5 g/L glycerol accumulated the highest concentration of TAG and that growth in this medium was also higher than in other glycerol-containing media. *B. braunii* grown in 2 g/L glycerol gave higher $P_{\text{max}}$ and almost the same TAG content as *C. vulgaris* and *Scenedesmus* sp. grown in 5 g/L glycerol, which had lower biomass concentrations. *C. vulgaris* and *Scenedesmus* sp. grown in 5 g/L glycerol accumulated higher contents of TAG than in other glycerol concentrations. Therefore, to obtain high TAG content, the recommended mixotrophic condition for the microalgae species is 5 g/L glycerol.

### Composition of total fatty acids

The fatty acid profiles of the algae oil are shown in Table 4. For this experiment, we selected the samples with the highest oil content, i.e. *C. vulgaris*, *Scenedesmus* sp. and *B. braunii* biomass in the 5 g/L glycerol-containing medium. For comparison, the profiles of the fatty acids of the autotrophic cultures and rapeseed oil, commonly used for biodiesel fuel production, are also provided. The content of saturated fatty acids in *C. vulgaris*, *Scenedesmus* sp. and *B. braunii* was 34.94%, 20.23% and 21.39%, respectively, and the amount of unsaturated fatty acids was 65.06%, 79.77% and 78.61%, respectively, under mixotrophic conditions. The most pronounced effect of glycerol was that on the fatty acid profiles of *C. vulgaris*. The saturated fatty acid content changed from 16.91% in autotrophic conditions to 34.94% in the 5 g/L glycerol-containing medium. The saturated fatty acid content in the biomass of the other two species did not increase in mixotrophic conditions. *Scenedesmus* sp. and *B. braunii* showed changes in the saturated fatty acids content by 6.96% and 5.11%, respectively.
### Table 4. Composition of total fatty acid profiles of the algae oil.

| Fatty acids | Composition (%) of total fatty acids | C. vulgaris | Scenedesmus sp. | B. braunii | Rapeseed |
|-------------|--------------------------------------|-------------|-----------------|------------|----------|
|             |                                      | 0           | 5               | 0          | 5        |          |
| Saturated   |                                      | 0.31 ± 0.002 | 0.31 ± 0.003 | ND         | 0.23 ± 0.002 | ND       | 5.40 |
| 14:0        |                                      | (11.09 ± 0.84) | (15.54 ± 0.67) | (7.62 ± 0.48) | (15.04 ± 0.75) | (7.78 ± 0.54) | (13.23 ± 0.38) | 3.22 |
| 16:0        |                                      | (0.47 ± 0.04) | (1.71 ± 0.11) | (0.93 ± 0.07) | (0.48 ± 0.03) | (1.55 ± 0.14) | (1.45 ± 0.08) | ND |
| 18:0        |                                      | (4.11 ± 0.12) | (5.76 ± 0.07) | (2.81 ± 0.09) | (2.85 ± 0.06) | (3.99 ± 0.12) | (5.84 ± 0.13) | 2.18 |
| 20:0        |                                      | (0.57 ± 0.003) | (4.13 ± 0.024) | (0.73 ± 0.002) | (0.47 ± 0.004) | (1.37 ± 0.003) | (0.77 ± 0.002) | ND |
| 22:0        |                                      | (0.24 ± 0.002) | (5.27 ± 0.024) | (1.09 ± 0.012) | (0.71 ± 0.007) | (1.81 ± 0.007) | (0.04 ± 0.001) | ND |
| 24:0        |                                      | (0.13 ± 0.002) | (2.22 ± 0.008) | (0.09 ± 0.001) | (0.1 ± 0.004) | (0.05 ± 0.003) | (0.06 ± 0.002) | ND |
| Unsaturated |                                      | (83.09 ± 2.31) | (65.06 ± 2.48) | (86.73 ± 3.58) | (79.77 ± 3.46) | (83.22 ± 2.61) | (78.61 ± 1.56) | 94.60 |
| 16:1        |                                      | (2.28 ± 0.27) | (2.10 ± 0.38) | (2.07 ± 0.07) | (2.10 ± 0.07) | (1.66 ± 0.03) | (2.49 ± 0.04) | ND |
| 18:1        |                                      | (53.01 ± 1.14) | (34.54 ± 2.06) | (62.27 ± 2.51) | (47.05 ± 1.67) | (50.43 ± 1.21) | (48.36 ± 1.65) | 16.92 |
| 20:1        |                                      | (40.99 ± 1.35) | (5.92 ± 0.31) | (12.72 ± 2.15) | (17.65 ± 1.95) | (14.65 ± 2.11) | (19.73 ± 1.23) | 66.32 |
| 22:1        |                                      | (6.04 ± 1.11) | (7.42 ± 1.24) | (5.44 ± 0.48) | (11.12 ± 0.87) | (10.94 ± 1.38) | (7.03 ± 1.41) | 11.14 |
| 24:1        |                                      | (0.02 ± 0.001) | (3.05 ± 0.021) | (1.49 ± 0.056) | (0.65 ± 0.004) | (0.12 ± 0.007) | (0.05 ± 0.001) | ND |

Note: Values are mean ± SE, N = 5; same letters denote statistically significant differences (P < 0.05).
ND: not detected.
*Data from 20-day cell growth in medium.
The quality parameters of biodiesel are influenced by the fatty acid composition of the oil.[24] The results from the present study showed that the content of saturated fatty acids in algal oil was higher than that in rapeseed oil (5.40%) and the content of unsaturated fatty acids was lower (94.60% in rapeseed oil). A high proportion of polyunsaturated fatty acids for biodiesel are undesirable because they adversely impact the stability of the biodiesel.[19] Compared to rapeseed oil, algal oil is not as rich in polyunsaturated fatty acids. Furthermore, in our study, the content of linolenic acid (18:3) in the three algae species was shown to correspond to the requirements of the EN 14214 standard, which states that the content of linolenic acid methyl ester in biodiesel fuel should not exceed 12%. Thus, the algae strains used in this study could be considered promising, since the biodiesel fuel produced from them may potentially meet the requirements for the linolenic acid methyl ester content. Based on these preliminary laboratory-scale experiments, the study may further be extended for real application of the optimized data at a larger scale or even at a pilot plant level production of biodiesel using these microalgae species.

Conclusions
The growth of the algae strains C. vulgaris, Scenedesmus sp. and B. braunii under mixotrophic conditions in the presence of different concentrations of crude glycerol was investigated with the objective of increasing the biomass growth and algae oil content. The highest biomass concentration was obtained in the presence of 5 g/L of glycerol and was 39.42%, 60.00% and 57.82% higher than for the autotrophically grown C. vulgaris, Scenedesmus sp. and B. braunii, respectively. The content of TAG in the three species cultured under mixotrophic conditions was also higher than that under autotrophic conditions. In particular, in the biomass obtained in the 5 g/L glycerol-containing medium, the TAG content was 12.20%, 13.11% and 9.30% higher than the respective values for the above species in autotrophic conditions. The content of saturated fatty acids in C. vulgaris, Scenedesmus sp. and B. braunii biomass was 34.94%, 20.23% and 21.39%, and of unsaturated fatty acids, respectively, 65.06%, 79.77% and 78.61% under mixotrophic conditions. The algae strains grown on glycerol produced higher biomass concentrations with higher lipid content compared to the autotrophically grown cultures. The fatty acid content of the oils from these species suggests their potential use as biodiesel feedstock.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology [grant number 2013006899].

References
[1] Pulz O, Grass W. Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol. 2004;65:635–648.
[2] Borowitzka MA, Moheimani NR. Sustainable biofuels from algae. Mitig Adapt Strateg Glob Chang. 2013;18:13–25.
[3] Makarevičienė V, Andreulevičiūtė V, Skorupskiätė V, Kasperovičienė J. Cultivation of microalgae Chlorella sp. and Scenedesmus sp. as a potential biofuel feedstock. Environ Res Eng Manag. 2011;3(57):21–27.
[4] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng. 2006;10:87–96.
[5] Rodolfi L, Zittelli GC, Bassin N, Padovani G, Biondi N, Bonini G, Tredicic MR. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng. 2009;102:100–112.
[6] Xu H, Miao X, Wu Q. High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters. J Biotechnol. 2006;126:499–507.
[7] Pittman JK, Dean AP, Osumboko O. The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol. 2011;102(1):17–25.
[8] Johnson DT, Taconi KA. The glycerol glut: options for the value-added conversion of crude glycerol resulting from biodiesel production. Environ Prog. 2007;26:338–348.
[9] Chi Z, Pyle D, Wen Z, Fearn C, Chen S. A laboratory study of producing docosahexaenoic acid from biodiesel-water glycerol by microagal fermentation. Process Biochem. 2007;42:1537–1545.
[10] Pyle DJ, Garcia RA, Wen Z. Docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: effects of impurities on DHA production and algal biomass composition. J Agric Food Chem. 2008;56:3933–3939.
[11] O’Grady J, Morgan JA. Heterotrophic growth and lipid production of Chlorella protothecoides on glycerol. Bio- process Biosyst Eng. 2011;34:121–125.
[12] Kong WB, Yang H, Cao YT, Song H, Hua SF, Xia CG. Effect of glycerol and glucose on the enhancement of biomass, lipid and soluble carbohydrate production by Chlorella vulgaris in mixotrophic culture. Food Technol Biotechnol. 2013;51(1):62–69.
[13] Zhang H, Weihtang LY, Yang W, Shen G. Mixotrophic cultivation of Botryococcus braunii. Biomass Bioenergy. 2011;35:1710–1715.
[14] Andrade MR, Costa JAV. Mixotrophic cultivation of microalga Spirulina platensis using molasses as organic substrate. Aquaculture. 2007;264:130–134.
[15] Thompson AS, Rhodes JC, Pettman I. Culture collection of algae and protozoa, catalogue of strains. 5th ed. Ambleside (UK): Culture Collection of Algae and Protozoa; 1988.
[16] Choi HJ, Lee SM. Effect of optical panel thickness for nutrient removal and cultivation of microalgae in the photobioreactor. Bioprocess Biosyst Eng. 2014;34:697–705.
[17] Choi HJ, Lee JM, Lee SM. A novel optical panel photobioreactor for cultivation of microalgae. Water Sci Technol. 2013;67(1):2543–2548.
[18] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37:911–917.
[19] Sobczuk TM, Chisti Y. Potential fuel oils from the microalga Choricystis minor. J Chem Technol Biotechnol. 2010;85:100–108.
[20] Stehfest K, Toepel J, Wilhelm C. The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. Plant Physiol Biochem. 2005;43:717–726.
[21] Mobin S, Alam F. Biofuel production from algae utilizing wastewater. 19th Australasian Fluid Mechanics Conference; 2014 Dec 8–11; Melbourne, Australia.
[22] Chen YH, Walker TH. Biomass and lipid production of heterotrophic microalgae Chlorella protothecoides by using biodiesel-derived crude glycerol. Biotechnol Lett. 2011;33:1973–1983.
[23] Liang Y, Sarkany N, Cui Y, Blackburn JM. Batch stage study of lipid production from crude glycerol derived from yellow grease or animal fats through microalgal fermentation. Bioreour Technol. 2010;101:6745–6750.
[24] Andruleviciute V, Makareviciene V, Skorupskaitė V, Gumbyte M. Biomass and oil content of Chlorella sp., Haematococcus sp., Nannochloris sp. and Scenedesmus sp. under mixotrophic growth conditions in the presence of technical glycerol. J Appl Phycol. 2014;26:83–90.