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Evaluation of oil-producing algae as potential biodiesel feedstock

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HIGHLIGHTS

- Seven oil-producing algae were cultured indoors.
- *Chlorella* sp. NJ-18 was selected for further study because it had the highest lipid productivity.
- Nitrogen and phosphorus were optimized for *Chlorella* sp. NJ-18 indoors.
- *Chlorella* sp. NJ-18 could obtain high lipid productivity and preferred fatty acids in the outdoors.
- A semi-continuous cultivation mode was a good strategy for higher biomass and lipid productivity.

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ABSTRACT

This study attempted to connect the dots between laboratory research and the outdoors. *Chlorella* sp. NJ-18 was selected among seven oil-producing algae cultivated in this study because it had the highest lipid productivity. The nitrogen and phosphorus concentrations for cultivating this *Chlorella* strain were optimized indoors. This strain was incubated outdoors in a 70 L photobioreactor, containing the favorable nitrogen (8.32 mM urea) and phosphorus (0.18 mM monopotassium phosphate) concentrations. Semi-continuous cultivation was performed by harvesting 30 L biomass and replacing it with fresh medium. The maximum biomass and lipid productivity acquired outdoors were 91.84 and 24.05 mg L^{-1} d^{-1}, respectively. Furthermore, biomass productivity could be maintained at a high level throughout the cultivation process when using the semi-continuous mode, whereas it decreased dramatically in batch cultures. More than 95% of the total fatty acids obtained were C16 and C18, which are the main components for biofuel.

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1. Introduction

Fossil fuels such as petroleum, coal, and natural gas are the main energy resources used to date; such energy sources are non-renewable and have limited storage capacity (Peer et al., 2008). Moreover, the use of fossil oil may cause serious environmental problems. Exploring renewable energy has become an inevitable trend to realize sustainable economic and environmental development. Biodiesel is ideal renewable energy that has efficient fuel performance, high flash point, and lubricity (Knoth and Krahl, 2005; Chisti, 2007; Oh et al., 2010), but is non-toxic, with less emissions of CO or sulfur oxides (Smith et al., 2009).

Microalgae have the following advantages over other feedstock for producing biodiesel: (1) high photosynthetic efficiency and higher lipid productivity; (2) ability to grow in brackish or saline water, deserts, and arid or semi-arid lands that are unfavorable for agriculture; (3) ability to utilize nitrogen and phosphorus in wastewater such as agricultural run-off, industrial effluent, and urban wastewater; and (4) ability to absorb carbon dioxide released by the burning fossil fuels and other sources (Knoth and Krahl, 2005; Chisti, 2007; Hu et al., 2008; Smith et al., 2009). Therefore, microalgae have been considered the most viable feedstock for biodiesel.

Screening for candidate microalgae is the first step in biodiesel production. Griffiths and Harrison (2009) suggested that the key criterion for choosing oleaginous algae is lipid productivity. Therefore, lipid productivity was considered to be the most important criterion in the screening process. The available nutrients (e.g., nitrogen and phosphorus resources) and the environment (e.g., light intensity and temperature) greatly influence the growth rate and lipid accumulation of microalgae (Roessler et al., 1994; Chen and Chen, 2006; Converti et al., 2009). Li et al. (2010) studied the effect of nitrogen and phosphorus concentrations on the

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growth and lipid accumulation of *Scenedesmus* sp., and found that its high lipid content (30% and 53%, respectively, of its biomass) could be obtained under nitrogen and phosphorus limitation. Becker (1994) reported that urea is the best nitrogen source for culturing *Chlorella*. Choochote et al. (2010) similarly found that *Chlorella* sp. could grow very well with urea. Furthermore, the use of urea as a nitrogen source can cut the cost of microalgae cultivation, which is an advantage during industrial production. Consequently, the initial establishment of optimal nitrogen and phosphorus concentrations is necessary to improve lipid productivity and reduce the cost of biodiesel production. The large-scale outdoor cultivation of microalgae is another problem commercial biodiesel production to date (Rodolfi et al., 2008). Therefore, the outdoor cultivation of algae using natural sunlight is necessary and advantageous. However, not all oleaginous algae are able to acclimate to the variable outdoor environment. Thus, evaluating the capacity of oil-producing microalgae to grow well and accumulate lipids outdoors is pivotal. Hsieh and Wu (2009) suggested that cultivation using the semi-continuous mode could improve the lipid productivity, but to the best of our knowledge, this strategy has seldom been previously performed outdoors using large bioreactors.

In the present study, *Chlorella* sp. NJ-18 was selected as the best strain among the seven oil-producing algae. The influence of different nitrogen and phosphorus sources on the biomass and lipid accumulation of this strain was investigated to evaluate its potential as biodiesel feedstock. Furthermore, the algal strain was cultured outdoors in 70 L photobioreactors with optimized nitrogen and phosphorus concentrations to test its adaptability to the outdoor environment. Semi-continuous cultivation was likewise conducted outdoors to achieve higher biomass and lipid productivity.

2. Methods

2.1. Microalgae and medium

The seven oil-producing algae used in this study were provided by the laboratory of XuDong Xu in the Key Laboratory of Algal Biology, Institute of Hydrobiology. These strains were *Chlorella* sp. NJ-18, *Chlorella* sp. NMX37 N, *Chlorococcum* sp., *Chlorella* sp. XJ-12, *Chlorella sorokiniana* XJ-7, *Chlorella* sp. 1, and *Chlorella* sp. 2.

All the nutrient concentrations were prepared based on the BG11 medium (as shown in Section 2.2.) with the exception of nitrogen and phosphorus. Sodium nitrate and urea were selected as the nitrogen sources, whereas potassium dihydrogen phosphate was the source of phosphorus. Untreated tap water was used in all experiments.

2.2. Experimental design

Indoor experiments: In the first stage, seven oil-producing algae were cultivated in BG11 medium, and the potential feedstock algae were selected based on their lipid productivity. The BG 11 medium contained 40 mg L^{-1} $KH_2PO_4{\cdot}3H_2O$, 150 mg L^{-1} $NaNO_3$, 75 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 36 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 6.0 mg L^{-1} citric acid, 6.0 mg L^{-1} ferric ammonium citrate, 1.0 mg L^{-1} ethylenediaminetetraacetic acid, (EDTA), 20 mg L^{-1} Na_2CO_3, and 1.0 ml L^{-1} A_5 solution. The A_5 Co solution contained 2.86 g L^{-1} H_3BO_3, 1.81 g L^{-1} $MnCl_2{\cdot}H_2O$, 222 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, 79 mg L^{-1} $CuSO_4{\cdot}5H_2O$, 390 mg L^{-1} $Na_2MoO_4{\cdot}2H_2O$, and 49.4 mg L^{-1} $Co(NO_3)_2{\cdot}6H_2O$.

In the second stage, the selected algal strain (*Chlorella* sp. NJ-18) was cultivated in 800 ml of the culture medium in 1 L Erlenmeyer flasks. A light density of 60 μmol m^{-2} s^{-1} (light/dark ratio = 12:12) was provided by cool white fluorescent tubes, and the temperature was maintained at 25 C by an air conditioner. The nitrogen concentrations were set at 0, 4.16, 8.32, and 16.64 mM, with sodium nitrate and urea as the nitrogen sources. Phosphorus concentrations of 0, 0.04, 0.18, 0.31, and 0.44 mM were used.

Outdoor experiments: This study was conducted in Beijing, China (40 22′N, 116 20′E). A 70 L airlift photobioreactor (22 cm in diameter, 185 cm in height) was used in the experiments. The optimal nitrogen and phosphorus sources were chosen based on the indoor experiments.

Semi-continuous cultivation: The system was initiated as a batch culture with an initial biomass concentration of 0.05 g L^{-1} and a working volume of 70 L in the photobioreactors. The semi-continuous cultivation was performed by harvesting 30 L of biomass, which was replaced with the same volume of fresh medium (containing the optimal nitrogen and phosphorus concentrations) when the dry weight reached approximately 1 g L^{-1}.

All the experiments were conducted in triplicate. Aeration with 2% CO_2 was provided for each set-up.

The light and temperature in each bioreactor were measured using a Quantitherm light meter/thermometer (Hansatech, U.K.)

2.3. Measurement of dry weight

The algal density was determined by measuring the OD_{680} (the optical density of algal at 680 nm).The relationships between the dry weight (DW, g L^{-1}) and the OD_{680} values of the algae were described using the following equations:

$$DW = 0.564OD_{680}, \quad R^2 = 0.9906 \ (\textit{Chlorella} \text{ sp. XJ} - 12)$$

$$DW = 0.286OD_{680}, \quad R^2 = 0.9986 \ (\textit{Chlorella} \text{ sp. NMX37N})$$

$$DW = 0.302OD_{680}, \quad R^2 = 0.9971 \ (\textit{Chlorella} \text{ sp. NJ} - 18)$$

$$DW = 0.268OD_{680}, \quad R^2 = 0.9917 (\textit{C.sorokiniana} \text{ XJ} - 7)$$

$$DW = 0.233OD_{680}, \quad R^2 = 0.9972 \ (\textit{Chlorella} \text{ sp. } 1)$$

$$DW = 0.280OD_{680}, \quad R^2 = 0.9807 \ (\textit{Chlorella} \text{ sp. } 2)$$

$$DW = 0.382OD_{680}, \quad R^2 = 0.9828 (\textit{Chlorococcum} \text{sp.})$$

2.4. Measurement of total lipid

Approximately 50–100 mg of dried algae (B_1; g) was lyophilized using a vacuum freeze dryer (Alpha 1–2 LD plus; Christ); the total lipid was extracted from the dried algae using a Soxhlet apparatus, with chloroform–methanol (2:1, v/v) as the solvent (Cheung et al., 1998; Hsieh and Wu, 2009). The supernatant was transferred into a pre-weighted beaker (B_2; g) and blow-dried in a fume cupboard. The supernatant was dried to a constant weight in an oven at 105 °C and weighted (B_3; g). The lipid content (C, %) and biomass productivity (P, mg L^{-1} d^{-1}) were calculated using the following formulas:

$$C\ (\%) = (B_3 - B_2)/B_1 \times 100$$

$$P(\text{mg L}^{-1}\ d^{-1}) = DW_2 - DW_1/T_2 - T_1$$

where DW_1 and DW_2 are the dry weight (g L^{-1}) of the algal sample at inoculation and harvesting, respectively; and T_1 and T_2 represent the inoculation and harvesting time (d), respectively.

2.5. Analysis of fatty acid profiles

The methanolysis of fatty acids was performed with 1 M H_2SO_4 in methanol at 100 °C for 60 min. The fatty acid methyl esters were

analyzed by gas chromatography–mass spectrometry (GC–MS) using a GC–MS apparatus (Ultra Trace, Thermo-Scientific, USA) equipped with a DB-23 capillary column (0.25 mm × 60 m; 0.25 mm, film thickness; Aglient Technologies, USA) and a flame ionization detector (Garcia-Ayuso and Castro, 2001).

2.6. Statistical analysis

All values were expressed as the mean ± standard deviation. The data were analyzed by one-way ANOVA using the SPSS statistical software (version 17.0). $P < 0.01$ was considered to denote a statistically significant difference.

3. Results and discussion

3.1. Biomass and lipid content of seven oil-producing algae

Seven oil-producing algae were cultivated in BG11 medium. The dry weight, biomass productivity, lipid content, and lipid productivity are shown in Fig. 1 and Table 1. The highest biomass productivity was 122.64 mg L^{-1} d^{-1} for *Chlorella* sp. NJ-18, followed by *Chlorella* sp. NMX37 N (114.97 mg L^{-1} d^{-1}) and *Chlorella* sp. 2 (113.67 mg L^{-1} d^{-1}). Furthermore, the highest lipid content (25.03%) and lipid productivity (30.69 mg L^{-1} d^{-1}) were obtained for *Chlorella* sp. NJ-18. Given that lipid productivity is the key criterion for oleaginous algae (Griffiths and Harrison, 2009), *Chlorella* sp. NJ-18 was selected as a potential feedstock for producing biodiesel. This algal strain was used for further experiments in the present study.

3.2. Effect of different nitrogen sources and concentrations on the biomass and lipid accumulation of Chlorella sp. NJ-18 cultured indoors

Nitrogen is an important and indispensable element for the growth of algae. Hsieh and Wu (2009) and Choochote et al. (2010) reported that *Chlorella* sp. can grow very well in the presence of urea. However, Li et al. (2008) found that *Neochloris oleoabundans* grows faster with sodium nitrate than urea. Sodium nitrate and urea were chosen as nitrogen sources to determine the optimal nitrogen source for *Chlorella* sp. NJ-18 in the present study.

The growth of *Chlorella* sp. NJ-18 under different nitrogen sources and concentrations is presented in Fig. 2. The cell concentrations gradually increased when the nitrogen content was from 0 to 8.32 mM, but began to decrease when the available nitrogen exceeded 8.32 mM (Fig. 2), regardless of the nitrogen source. The biomass productivity obtained with urea (174.71 mg L^{-1} d^{-1}) was higher than that with sodium nitrate (164.15 mg L^{-1} d^{-1}). This result suggested that high nitrogen concentration might be inhibitive for the growth of *Chlorella* sp. NJ-18 (Li et al., 2008).

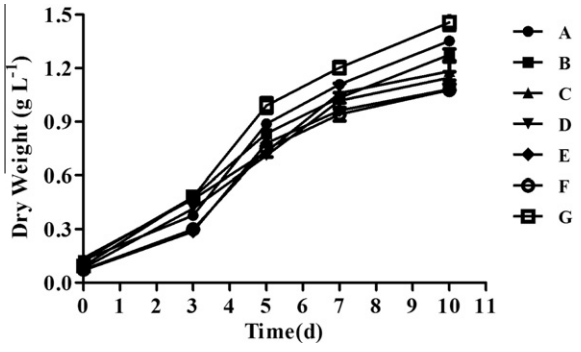

Fig. 1. Dry weights of seven microalgae: (A) *Chlorella* sp. NMX37N; (B) *Chlorella* sp. 2; (C) *Chlorella* sp. 1; (D) *Chlorella* sp. XJ-12; (E) *Chlorococcum* sp.; (F) *C. sorokiniana* XJ-7; and (G) *Chlorella* sp. NJ-18.

Table 1
The biomass productivity, lipid content and lipid productivity of seven microalgae.

	Biomass productivity (mg L^{-1} d^{-1})	Lipid content (%)	Lipid productivity (mg L^{-1} d^{-1})
Chlorella sp. XJ-12	106.58 ± 3.37	23.90 ± 0.42	25.47 ± 1.68
Chlorella sp. NMX37N	114.97 ± 3.24	23.06 ± 0.60	26.52 ± 0.91
Chlorella sp. NJ-18	122.64 ± 2.14	25.03 ± 0.61	30.69 ± 1.44
Chlorella sorokiniana XJ-7	101.24 ± 2.57	21.82 ± 1.03	22.10 ± 1.25
Chlorella sp. 1	107.20 ± 3.58	23.45 ± 0.06	25.14 ± 0.84
Chlorella sp. 2	113.67 ± 3.30	19.87 ± 0.10	22.67 ± 0.39
Chlorococcum sp.	106.58 ± 3.37	22.82 ± 1.02	23.83 ± 0.64

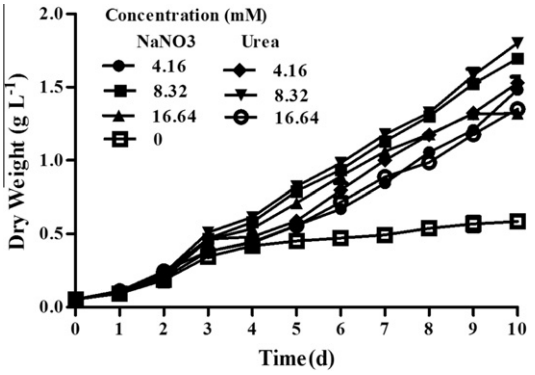

Fig. 2. Effect of different sodium nitrate and urea concentrations on the growth of *Chlorella* sp. NJ-18 (0 mM sodium nitrate and urea were used as the respective controls).

The lipid content was 45.6% under the nitrogen-deficient condition, which was much higher than that of the other treatments (Table 2). Courchesne et al. (2009) discussed that the cell density was lower with nitrogen limitation and individual cells were exposed to more light energy, thereby increasing the metabolic flux to promote lipid accumulation. Higher lipid concentrations were similarly obtained when comparing a lower nitrogen concentration (4.16 mM) to a higher concentration (8.32 mM), which had lipid content of 32.95% and 26.21%, respectively, using sodium nitrate, and 29.1% and 26.74%, respectively, using urea (Table 2). The highest lipid productivity (46.29 mg L^{-1} d^{-1}) was obtained with 8.32 mM urea as the nitrogen source in this study (Table 2). However, the lipid productivity of *Chlorella* sp. NJ-18 was the lowest when cultivated in the nitrogen-deficient condition. Therefore, the nutrition limitation strategy was not a feasible method for improving lipid productivity with this algal strain.

More than 95% of the total fatty acids obtained with the optimal nitrogen concentration (8.32 mM) were C16 and C18 fatty acids. No significant differences were observed between urea and sodium nitrate (see Table S1 in the Table S1 in the supplementary material). The highest lipid productivity (46.29 mg L^{-1} d^{-1}) obtained with 8.32 mM urea was higher than that obtained with sodium nitrate (41.78 mg L^{-1} d^{-1}). Griffiths and Harrison (2009) suggested that lipid productivity is the most obvious and important characteristic when choosing an algal species for biodiesel production. Furthermore, urea should be the most favorable nitrogen source for *Chlorella* sp. NJ-18 because of its reduced cost of industrial cultivation.

Table 2
Effect of different concentrations of sodium nitrate and urea on the growth and lipid accumulation of *Chlorella* sp. NJ-18 (0 mM was the contrast for both sodium nitrate and urea).

Nitrogen concentration (mM)	0	4.16	8.32	16.64
Sodium nitrate biomass (g L^{-1})	0.59 ± 0.00	1.48 ± 0.00	1.70 ± 0.02	1.32 ± 0.02
Biomass Productivity (mg L^{-1} d^{-1})	53.16 ± 0.25	142.77 ± 0.47	164.15 ± 2.28	126.69 ± 1.67
Lipid (%)	45.60 ± 0.42	32.95 ± 0.65	26.21 ± 0.23	25.27 ± 0.18
Lipid productivity (mg L^{-1} d^{-1})	24.57 ± 0.12	37.73 ± 0.12	41.78 ± 0.58	40.92 ± 0.54
Urea biomass (g L^{-1})	–	1.53 ± 0.04	1.80 ± 0.02	1.35 ± 0.02
Biomass productivity (mg L^{-1} d^{-1})	–	147.53 ± 4.10	174.71 ± 1.67	129.62 ± 2.36
Lipid (%)	–	29.15 ± 0.23	26.74 ± 0.24	23.89 ± 0.20
Lipid productivity (mg L^{-1} d^{-1})	–	43.34 ± 1.20	46.29 ± 0.44	30.71 ± 0.59

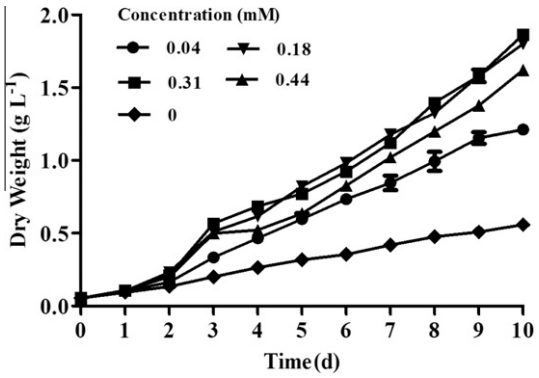

Fig. 3. Effect of different phosphorus concentrations on the growth of *Chlorella* sp. NJ-18.

3.3. Effect of different phosphorus concentrations on the biomass and lipid accumulation of Chlorella sp. NJ-18 grown indoors

Phosphorus is another essential nutrient that influences the growth and lipid accumulation of microalgae (Chen and Chen, 2006). Phosphorus is mostly incorporated into nucleic acids, phospholipids, and coenzymes; it may be stored in the cell as polymetaphosphate (Wu et al., 2010). Different concentrations of phosphorus were tested with the optimal nitrogen concentration (8.32 mM urea as nitrogen source). The biomass concentration achieved with phosphate repletion was higher than that with phosphate starvation (Fig. 3). The growth rate was directly proportional to phosphorus concentrations within the range of 0–0.31 mM (Fig. 3). Only 0.69 g L^{-1} of biomass was obtained under the phosphorus-deficient condition (Table 3). The highest biomass productivity of 181.05 mg L^{-1} d^{-1} was obtained with a phosphorus concentration of 0.31 mM (Table 3).

The lipid content (37.20%) with the phosphorus-deficient condition was much higher than that with other concentrations (Table 3). The result agreed with the report by Khozin-Goldberg and Cohen (2006), wherein the lipid content of *Monodus subterraneus* increased with the decreasing phosphate content in the medium. However, the lipid productivity (19.13 mg L^{-1} d^{-1}) was lowest under the phosphorus-deficient condition, which is similar to the nitrogen-deficient condition. The lipid content was inversely proportional to the phosphate concentrations, as shown in Table 3. The lipid content (27.53%) with 0.18 mM phosphate was higher than that with 0.31 mM phosphate (26.46%). The most favorable phosphorus concentration for growth was 0.31 mM, but the highest lipid productivity (49.40 mg L^{-1} d^{-1}) was obtained with 0.18 mM phosphorus when biomass productivity and lipid content were considered Therefore, 0.18 mM was found to be the optimal phosphorus concentration for the growth and lipid accumulation for *Chlorella* sp. NJ-18.

3.4. Effect of outdoor conditions and semi-continuous cultivation on the biomass and lipid accumulation of Chlorella sp. NJ-18

Chlorella sp. NJ-18 was cultivated in a 70 L photobioreactor to evaluate the alga's adaptability in the outdoors, with the optimal nitrogen and phosphorus concentrations (8.32 mM urea as nitrogen source and 0.18 mM monopotassium phosphate) that were obtained from the abovementioned experiments.

Temperature and light intensity are two important factors for the outdoor cultures, which could greatly affect the growth and lipid accumulation of the algae (Oh et al., 2010; Feng et al., 2011). The outdoor environment was not as constant as that indoors (Fig. S1 in the Supplementary Material), and the temperature and light intensity changed dramatically within a day. The light intensity varied from 59 to 411 μmol m^{-2} s^{-1}, and the temperature ranged from 27 to 38 °C. The light intensity was relatively low on day 6 because of rainfall (Fig. S1b in the Supplementary Material) and the cell growth was nearly stopped (Fig. 4). However, the cells recovered on the following day, thereby indicating that *Chlorella* sp. NJ-18 had the strong ability to adapt to unstable conditions. The highest biomass productivity obtained in the 70 L photobioreactor for this study was 91.84 mg L^{-1} d^{-1} (Table 4), which was 1.57 times higher than the biomass productivity of *Chlorella zofingiensis* (58.4 mg L^{-1} d^{-1}) cultured in a 60 L flat outdoor photobioreactor (Feng et al., 2011).

The highest lipid content in the 70 L photobioreactors from this study was 27.89%, which was much higher than that of *C. sorokiniana* (12.8%) and of *Chlorella minutissima* (12.8%) cultured in large photobioreactors, as reported by Zheng et al. (2012) and Oh et al. (2010), respectively.

Our selected algal strain was cultured in a 70 L photobioreactor under outdoor conditions for several times. The results showed that some cells began to settle down at the bottom of the photobioreactors when the biomass reached 1 g L^{-1}. These cells consequently could not receive sufficient sunlight. Therefore, the semi-continuous mode of cultivation was performed by harvesting

Table 3
Effect of different phosphorus concentrations on the growth and lipid accumulation of *Chlorella* sp. NJ-18.

Concentration(mg L^{-1})	0	0.04	0.18	0.31	0.44
Biomass (g L^{-1})	0.69 ± 0.07	1.28 ± 3.62	1.80 ± 0.02	1.86 ± 0.02	1.62 ± 0.02
Biomass productivity (mg L^{-1} d^{-1})	63.81 ± 6.65	123.00 ± 3.62	178.03 ± 1.66	181.05 ± 1.67	156.59 ± 1.96
Lipid content (%)	7.20 ± 0.70	9.41 ± 0.52	7.53 ± 0.49	6.46 ± 0.45	24.92 ± 0.83
Lipid productivity (mg L^{-1} d^{-1})	9.13 ± 0.69	4.49 ± 1.05	9.40 ± 0.47	47.09 ± 0.47	40.32 ± 0.51

Data are represented as mean ± standard.

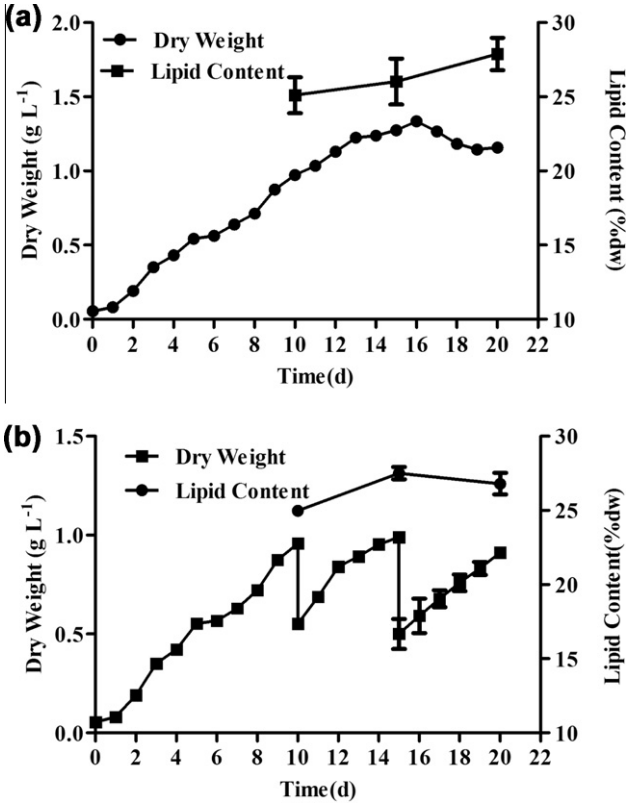

Fig. 4. Effect of (a) batch and (b) semi-continuous cultivation on the growth and lipid accumulation of *Chlorella* sp. NJ-18.

Table 4
The biomass and lipid productivity of *Chlorella* sp. NJ-18 in batch and semi-continuous culture mode under outdoor conditions (0–10, 10–15 and15–20 are the biomass productivity and lipid productivity from day 0 to 10, day 10 to 15 and day 15 to 20, respectively).

	0–10	10–15	15–20
Batch culture			
Biomass productivity ($mg\ L^{-1}\ d^{-1}$)	91.84 ± 3.02	60.46 ± 0.42	–
Lipid productivity ($mg\ L^{-1}\ d^{-1}$)	21.91 ± 0.19	15.61 ± 0.66	–
Semi-continuous culture			
Biomass productivity ($mg\ L^{-1}\ d^{-1}$)	90.40 ± 1.96	87.43 ± 4.46	82.40 ± 3.27
Lipid productivity ($mg\ L^{-1}\ d^{-1}$)	22.57 ± 0.06	24.05 ± 1.83	22.08 ± 2.35

"–" The dry weight of the batch culture on day 20 was lower than that on day 15. Therefore, the productivity from day 15 to 20 was a negative value.
Data are represented as mean ± standard.

30 L of algae from the bottom of the reactor and replacing it with the same volume of fresh medium on day 10 and 15, when the dry weight was approximately 1 $g\ L^{-1}$. As shown in Table 4, the biomass productivity (87.43 and 82.40 $mg\ L^{-1}\ d^{-1}$) and lipid productivity (24.05 and 22.08 $mg\ L^{-1}\ d^{-1}$) in semi-continuous cultivation could keep high from day 10 to 15 and from day 15 to 20, respectively. However, the productivity was dramatically decreased in the batch culture from day 10 to 15. The biomass and lipid productivity values were only 60.46 and 15.61 $mg\ L^{-1}\ d^{-1}$, respectively, from day 10 to 15 in the batch culture. A significant difference ($P < 0.01$) was observed between the batch and semi-continuous cultures based on the ANOVA test. The dry weight of the batch culture on day 20 was lower than that on day 15. Therefore, the productivity from day 15 to 20 was a negative value (Table 4). The results could be attributed to the unit cell in the semi-continuous culture that received more sunlight. Furthermore, the added fresh medium may have been more suitable for growth than the batch culture.

Table 5
Fatty acid profiles of *Chlorella* sp. NJ-18 on day 20 in batch cultivation and semi-continuous mode under outdoor conditions.

	Batch cultivation	Semi-continuous cultivation
C14:0	0.89 ± 0.06	1.08 ± 0.10
C14:1	0.39 ± 0.02	0.06 ± 0.00
C16:0	27.82 ± 3.29	32.04 ± 4.33
C16:1	1.55 ± 0.39	1.17 ± 0.32
C18:0	1.74 ± 0.32	1.16 ± 0.18
C18:1	15.09 ± 2.10	14.91 ± 1.34
C18:2	36.79 ± 3.98	36.10 ± 2.40
C18:3	15.56 ± 2.90	13.44 ± 2.12
C18:4	0.04 ± 0.00	ND
C20:0	0.03 ± 0.00	0.05 ± 0.00
C20:4	0.04 ± 0.00	ND
C22:0	0.05 ± 0.00	ND

Data are represented as mean ± standard.

The suitability of the obtained fatty acids is a key characteristic in the choice of biodiesel feedstock (Griffiths et al., 2012). The fatty acid profiles obtained in batch and semi-continuous cultivation were similar (Table 5). The predominant fatty acids were C16 and C18, which are the main components of biofuels (Li et al., 2010). Moreover, the fatty acids profiles obtained in the two cultivation modes were similar to the main profiles for biodiesel. No significant differences were observed between the two cultivation modes. Therefore, semi-continuous cultivation could be carried out to obtain more biomass and lipid yield during large scale cultivation of *Chlorella* sp. NJ-18 under outdoor conditions.

4. Conclusions

Seven oleaginous algae were cultured in this study. The *Chlorella* sp. NJ-18 strain was selected for further study because of its relatively high lipid productivity. High lipid productivity was achieved with large outdoor photobioreactors using the optimized concentrations of the key nutrients (8.32 mM urea for nitrogen and 0.18 mM monopotassium phosphate) in the medium. Furthermore, higher lipid productivity was achieved, and the primary fatty acids C16 and C18 for biodiesel were obtained using semi-continuous cultivation. Consequently, *Chlorella* sp. NJ-18 is a potential feedstock for biodiesel, which could be cultivated outdoors in a large scale.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.02.008.

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