Biotechnology and Bioprocess Engineering 15: 277-284 (2010)
DOI 10.1007/s12257-009-0127-7

RESEARCH PAPER

Statistical Optimization of Culture Media for Growth and Lipid Production of *Botryococcus braunii* LB572

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Received: 3 June 2009 / Revised: 27 July 2009 / Accepted: 9 August 2009

Abstract *Botryococcus braunii* has an outstanding ability to produce lipid; however, it is a slow-growing green microalgae. Statistical optimization of growth media was performed to faster growth and to increase lipid concentration. The effect of media composition on the growth of *B. braunii* LB572 was examined using fractional factorial design and central composite design. The media components examined include sodium carbonate, potassium phosphate, calcium chloride, magnesium sulfate, ferric citrate, and sodium nitrate. The results indicated that potassium phosphate and magnesium sulfate were major impact factors. The optimum concentrations of potassium phosphate and magnesium sulphate were found to be 0.058 and 0.09 g/L, respectively, for growth and 0.083 and 0.1 g/L, respectively, for lipid production. These values were validated using bubble column photobioreactors. Lipid productivity increased to 0.19 g/L/day in lipid-optimized media, with an average biomass productivity of 0.296 g/L/day and 64.96% w/w. In growth-optimized media, lipid productivity was 0.18 g/L/day, with an average biomass productivity of 0.304 g/L/day and 59.56% w/w.

Keywords: Fractional factorial design, central composite design, lipid, fatty acids, microalgae, *Botryococcus braunii* LB572

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1. Introduction

Biodiesel has received considerable attention in recent years because it is a biodegradable, renewable, and non-toxic fuel [1]. Microalgae are considered very good candidates for fuel production because of their advantages over other energy crops: higher photosynthetic efficiency, higher biomass production, and faster growth [1]. Moreover, microalgae are eukaryotic photosynthetic microorganisms that can be used to produce high-value compounds (*i.e.* carbohydrates, hydrocarbons, and natural oils). Microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel [2] and microalgae with high oil productivities are desired for producing biodiesel [3]. Depending on the species, microalgae produce many different lipids, hydrocarbons, and other complex oils. Some species have high lipid content and lipid synthesis - especially of the non-polar triacylglycerides (TAGs), which are the best substrate to produce biodiesel that can be modulated by varying growth conditions; therefore, there is interest in using microalgae for oil production. The total content of lipid in microalgae may vary between 1~85% of the dry weight [4]. *B. braunii* is a green microalga that produces hydrocarbons up to 75% of its dry biomass and it has already been proposed as a future renewable source of fuel [5]. In light of this, the potential biotechnological applications of microalga growth and lipid production characteristics need to be explored.

Fractional factorial design (FFD) and central composite design (CCD) are a collection of mathematical and statistical techniques widely used to determine the effects of several variables. These methods have been successfully applied to the optimization of medium composition for growth and metabolite production. In the present study, a central composite experimental design combined with frac-

tional factorial design was used to determine the influence of medium constituents on lipid production and to identify the optimal of culture conditions for enhanced growth. Cultivations were validated by using bubble column photobioreactors.

2. Materials and Methods

2.1. Microalgal strain

B. braunii (LB572) were obtained from the University of Texas, USA. Stock cultures of *B. braunii* were maintained both in agar slants and in liquid cultures of BG-11 medium, which consisted of (g/L): 1.5, $NaNO_3$ (sodium nitrate); 0.04, K_2HPO_4 (potassium phosphate); 0.075, $MgSO_4 \cdot 7H_2O$ (magnesium sulfate); 0.0036, $CaCl_2 \cdot 2H_2O$ (calcium chloride); 0.006, $C_6H_8O_7 \cdot x$Fe$\cdot x NH_3$ (ferric-ammonium citrate); 0.006, $C_6H_8O_7$ (citric acid); 0.001, EDTA-2Na (EDTA, disodium salt); and 0.02, Na_2CO_3 (sodium carbonate), at pH of 7.4.

2.2. Culture system

Optimization of *B. braunii* using the statistical method was performed in 250 mL flasks. The medium and flasks were sterilized in an autoclave for 20 min at 121°C to prevent contamination. Growth was conducted in a shaking incubator (Model VS-8480SF, Vision Scientific Co. Ltd, Buchon-Si, Gyeonggi-do, Korea) equipped with artificial lighting. Fluorescent lamps (FL20D, OSRAM, Seoul, Korea) were used as a light source for growth. Illuminated continuously at 30 μE/m^2/s and the cultures were maintained at 22°C.

Composite design results were validated in bubble column photobioreactors. Column photobioreactors were made of Pyrex glass tubes (650 mm in height, 35 mm of internal diameter). Fluorescent lamps were used as light source for growth. Illuminated continuously at 50 μE/m^2/s and the photoreactors were maintained at 22°C. Sterile-air (*via* air-filter) containing 2% (v/v) CO_2 was aerated into the column (flow rate of 0.2 vvm) through an air sparger at the bottom of the column.

2.3. Analytical methods

2.3.1. Biomass estimation

The dry weight of the algal cells was measured by filtering an aliquot of the culture suspension through pre-weighed Nylon filters (Model R04SP0470S, GE Osmonics Labstore, Minnetonka, MN, USA). The cultures were harvested by centrifugation at 5,000 rpm for 10 min and the cells were washed twice with distilled water. Then, the pellet was dried at 80°C for 24 h and reweighed. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight (g/L).

2.3.2. Lipid extraction (modified bligh and dyer method)

The lipids were extracted using a mixture of chloroform/methanol (1:2 v/v). The quantity of lipid residue was measured gravimetrically and expressed as dry weight percentage.

2.3.3. Fatty acid extraction

Samples were dissolved in 2 mL of a freshly prepared mixture of acetyl chloride and methanol (5:100, v/v) using nonadecanoic acid (19:0) as an internal standard. The reaction continued at 100°C for 1 h under pure nitrogen and darkness. Fatty acid methyl esters (FAMEs) were extracted by hexane. FAMEs were analyzed using a gas chromatograph equipped with an automatic injector, a flame ionization detector (FID), and a HP-INNOWax column (length = 30 m, diameter = 0.25 mm, and film thickness = 0.25 μm). Chromatographic conditions: carrier gas, helium; ow rate, 3 mL/min; sample input temperature, 250°C; initial temperature, 110°C; initial time, 1 min; rate, 5°C/min; and resulting in a total heating time of 28 min. The FAMEs were identified by comparing their fragmentation patterns with those of standards (F.A.M.E. Mix C4-C24, SUPELCO, Bellefonte, PA, USA).

2.4. Statistical method

Statistical optimization for growth media was performed to determine optimal growth rate and concentration of lipid. To optimize a culture medium the variables that affect lipid production need to be identified. Six variables (Na_2CO_3, $NaNO_3$, K_2HPO_4, $MgSO_4$, $CaCl_2$, and ferric-ammonium citrate) were evaluated with 2 center points in 18 runs of a resolution III design. The orthogonal experimental design of coded levels and actual levels for fractional factorial design are shown in "Variables" columns in Table 1.

For optimizing growth medium, fresh cell weight was selected as the response, which can be calculated using the equation (1).

$$y = \beta_0 + \sum_{i}^{k} \beta_i x_i + \sum\sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

Where, y is predicted response, β_i is the coefficient of the equation, β_0 is the intercept of the plane, x_i and x_j are coded levels of variables, and ε is the error term. The effects of each variable were determined by the statistical software MINITAB (V 13, Minitab Inc., State College, PA, USA) [8]. To fit an empirical second-order polynomial model a central composite design used to optimize the levels of variables with significant influence on *B. braunii* cell growth, can be written as a function (response surface). Four vari-

Table 1. FFD of variables (coded and actual levels) with biomass yield and lipid production as responses

Run order	Variables														Responses	
	$NaNO_3$ (g/L)		KH_2PO_4 (g/L)		$MgSO_4$ (g/L)		$CaCl_2$ (g/L)		Citric acid (g/L)		Ferric-ammonium citrate (g/L)		Na_2CO_3 (g/L)		Yield of biomass	Lipid production
	Coded level	Actual level	Coded level	Actual level	Coded level	Actual level	Coded level	Actual level	Coded level	Actual level	Coded level	Actual level	Coded level	Actual level	(g/L)	(g/L)
1	1	2.25	1	0.06	−1	0.0184	1	0.0405	−1	0.003	−1	0.003	−1	0.01	0.1168	0.0733
2	−1	0.75	−1	0.02	−1	0.0184	1	0.0405	−1	0.003	1	0.009	1	0.03	0.0345	n.d.
3	1	2.25	−1	0.02	−1	0.0184	−1	0.0135	1	0.009	−1	0.003	1	0.03	0.2316	0.1867
4	1	2.25	1	0.06	1	0.055	1	0.0405	1	0.009	1	0.009	1	0.03	0.0650	n.d.
5	0	1.5	0	0.04	0	0.0366	0	0.027	0	0.006	0	0.006	0	0.02	0.1011	0.067
6	−1	0.75	1	0.06	1	0.055	−1	0.0135	−1	0.003	−1	0.003	1	0.03	0.1128	0.08
7	1	2.25	−1	0.02	1	0.055	−1	0.0135	−1	0.003	1	0.009	−1	0.01	0.0191	n.d.
8	−1	0.75	1	0.06	−1	0.0184	−1	0.0135	1	0.009	1	0.009	−1	0.01	0.1310	0.0867
9	−1	0.75	−1	0.02	1	0.055	1	0.0405	1	0.009	−1	0.003	−1	0.01	0.0319	n.d.
10	−1	0.75	−1	0.02	−1	0.0184	−1	0.0135	−1	0.003	−1	0.003	−1	0.01	0.1359	0.0867
11	−1	0.75	−1	0.02	1	0.055	−1	0.0135	1	0.009	1	0.009	1	0.03	0.0535	n.d.
12	−1	0.75	1	0.06	−1	0.0184	1	0.0405	1	0.009	−1	0.003	1	0.03	0.1149	0.073
13	1	2.25	−1	0.02	−1	0.0184	1	0.0405	1	0.009	1	0.009	−1	0.01	0.1165	0.067
14	0	1.5	0	0.04	0	0.0366	0	0.027	0	0.006	0	0.006	0	0.02	0.1011	0.067
15	1	2.25	1	0.06	1	0.055	−1	0.0135	1	0.009	−1	0.003	−1	0.01	0.1905	0.14
16	1	2.25	−1	0.02	1	0.055	1	0.0405	−1	0.003	−1	0.003	1	0.03	0.1362	0.0933
17	1	2.25	1	0.06	−1	0.0184	−1	0.0135	−1	0.003	1	0.009	1	0.03	0.2232	0.1667
18	−1	0.75	1	0.06	1	0.055	1	0.0405	−1	0.003	1	0.009	−1	0.01	0.0390	n.d.

n.d.: not detected

ables were used for CCD: K_2HPO_4, $MgSO_4$, ferric-ammonium citrate, and Na_2CO_3. The experimental design in the actual and coded levels of variables using CCD is shown in Table 2.

The response functions (biomass yield and lipid production in the culture) were optimized by a second-order model.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

MINITAB calculated the optimal values using equation (2) [8].

3. Results and Discussion

3.1. Fractional factorial design

Fractional factorial design is the most widely used design type in screening experiments. Using FFD, 18 runs were carried out to identify important factors that attribute to cell growth. The effects of various independent variables on 2 targeted parameters (biomass yield and lipid production) are shown in the "Responses" columns of Table 1.

Table 3 shows the cell growth FFD effects and coefficients. The *t*-value measures how large a coefficient is in relationship to its standard error (*i.e.* a 'signal-to-noise' type measure). The *t*-values are obtained by dividing each coefficient by its standard error [8]. The *p*-value is the likelihood of getting a larger *t*-value (in absolute value) by chance alone. This probability is based on the assumption that the random error associated with the model is normally distributed. A small *p*-value suggests that the coefficient is a large signal compared to noise because it is too large to have arisen by chance alone.

Based on the small *p*-values (< 0.04), K_2HPO_4, $MgSO_4$, ferric-ammonium citrate, and Na_2CO_3 were identified as the impact factors of growth and lipid production of the microalgae. However, FFD cannot determine the exact optimal values of individual factors.

3.2. Central composite design

The next experiment (using central composite design) was performed to find more precise factor values to produce a desired response. Central composite designs are useful designs for acquiring data to fit a polynomial. To arrive at the central composite design for 4 factors, a 25-factorial design with 5 replications of the center points and 8 axial points was performed. The results are shown in Table 4. Growth and lipid production were taken as the response. Both K_2HPO_4 and $MgSO_4$ were identified as factors impacting growth and lipid production of the microalgae.

Table 2. Orthogonal experimental design for coded levels and actual levels of variables for CCD

Run order	K_2HPO_4		$MgSO_4$		Ferric-ammonium citrate		Na_2CO_3	
	Coded level	Actual level	Coded level	Actual level	Coded level	Actual level	Coded level	Actual level
1	1	0.06	−1	0.0375	−1	0.003	1	0.03
2	−1	0.02	−1	0.0375	−1	0.003	−1	0.01
3	0	0.04	0	0.075	0	0.006	0	0.02
4	1	0.06	−1	0.0375	1	0.009	−1	0.01
5	1	0.06	1	0.1125	−1	0.003	−1	0.01
6	−1	0.02	−1	0.0375	1	0.009	1	0.03
7	−1	0.02	1	0.1125	1	0.009	−1	0.01
8	−1	0.02	1	0.1125	−1	0.003	1	0.03
9	1	0.06	1	0.1125	1	0.009	1	0.03
10	0	0.04	0	0.075	0	0.006	0	0.02
11	−1	0.02	1	0.1125	−1	0.003	−1	0.01
12	1	0.06	1	0.1125	1	0.009	−1	0.01
13	−1	0.02	−1	0.0375	−1	0.003	1	0.03
14	0	0.04	0	0.075	0	0.006	0	0.02
15	1	0.06	1	0.1125	−1	0.003	1	0.03
16	−1	0.02	1	0.1125	1	0.009	1	0.03
17	1	0.06	−1	0.0375	1	0.009	1	0.03
18	−1	0.02	−1	0.0375	1	0.009	−1	0.01
19	1	0.06	−1	0.0375	−1	0.003	−1	0.01
20	0	0.04	0	0.075	0	0.006	0	0.02
21	−2	0	0	0.075	0	0.006	0	0.02
22	0	0.04	2	0.15	0	0.006	0	0.02
23	0	0.04	0	0.075	2	0.012	0	0.02
24	0	0.04	0	0.075	−2	0	0	0.02
25	0	0.04	0	0.075	0	0.006	2	0.04
26	0	0.04	0	0.075	0	0.006	0	0.02
27	0	0.04	−2	0	0	0.006	0	0.02
28	0	0.04	0	0.075	0	0.006	0	0.02
29	0	0.04	0	0.075	0	0.006	−2	0
30	2	0.08	0	0.075	0	0.006	0	0.02

Table 3. FFD determined effects and coefficients of various factors on cell growth

Factors	Effect	Coefficient	*t*-value	*p*-level
Constant		0.006959	105.59	0.006
($NaNO_3$)	0.001087	0.000543	8.24	0.077
(KH_2PO_4)	0.002961	0.00148	22.46	0.028
($MgSO_4$)	−0.002124	−0.001062	−16.11	0.039
($CaCl_2$)	0.000656	0.000328	4.97	0.126
(Citrate)	0.001847	0.000924	14.01	0.045
(Fe-Cit)	−0.002522	−0.001261	−19.14	0.033
(Na_2CO_3)	0.00335	0.001675	25.42	0.025
($NaNO_3$)(KH_2PO_4)	0.001913	0.000956	14.51	0.044
($NaNO_3$)($MgSO_4$)	0.000289	0.000145	2.2	0.272
($NaNO_3$)($CaCl_2$)	0.00019	0.000095	1.44	0.386
($NaNO_3$)(Citrate)	0.000856	0.000428	6.49	0.097
($NaNO_3$)(Fe-Cit)	−0.000925	−0.000463	−7.02	0.09
($NaNO_3$)(Na_2CO_3)	0.001713	0.000857	13	0.049
(KH_2PO_4)($CaCl_2$)	−0.000868	−0.000434	−6.59	0.096

Table 4. Estimated regression coefficients of CCD

Factors	Fresh cell weight			Lipid production		
	Coefficient	*t*-value	*p*-level	Coefficient	*t*-value	*p*-level
Constant	0.411	13.514	0	0.0933	13.018	0
(K_2HPO_4)	0.079	2.598	0.022	0.017767	2.479	0.028
($MgSO_4$)	0.060667	1.995	0.067	0.003317	0.463	0.651
(Fe-Cit)	0.019167	0.63	0.539	0.015567	2.172	0.049
(Na_2CO_3)	−0.006667	−0.219	0.83	−0.000017	−0.002	0.998
(K_2HPO_4)(K_2HPO_4)	−0.115	−2.021	0.064	−0.011642	−0.868	0.401
($MgSO_4$)($MgSO_4$)	−0.1655	−2.909	0.012	−0.028292	−2.11	0.055
(Fe-Cit)(Fe-Cit)	−0.011	−0.193	0.85	0.015008	1.119	0.283
(Na_2CO_3)(Na_2CO_3)	0.0605	1.063	0.307	0.021708	1.619	0.129
(K_2HPO_4)($MgSO_4$)	0.0215	0.289	0.777	0.02	1.139	0.275
(K_2HPO_4)(Fe-Cit)	−0.0165	−0.221	0.828	−0.01995	−1.136	0.276
(K_2HPO_4)(Na_2CO_3)	−0.0105	−0.141	0.89	0.0067	0.382	0.709
($MgSO_4$)(Fe-Cit)	0.026	0.349	0.733	0.02665	1.518	0.153
($MgSO_4$)(Na_2CO_3)	0.058	0.779	0.45	0.0267	1.521	0.152
(Fe-Cit)(Na_2CO_3)	0.007	0.094	0.927	0.04005	2.281	0.04

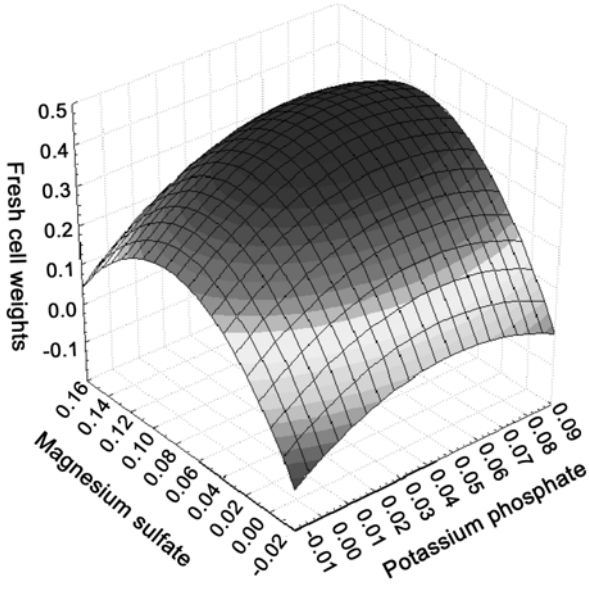

Fig. 1. Three-dimensional response surface plot for growth as a function of potassium phosphate and magnesium sulfate.

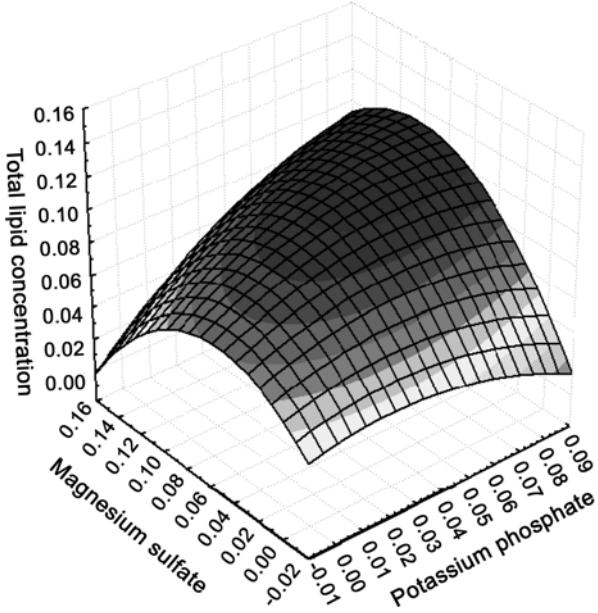

Fig. 2. Three-dimensional response surface plot for lipid production as a function of potassium phosphate and magnesium sulfate.

Central composite design is illustrated in a 3-dimensional graph of the calculated response surface for the most important factors as shown in Figs. 1 and 2.

Three-dimensional response surface plots of K_2HPO_4 and $MgSO_4$ against biomass concentration and lipid production can further explain the results of the statistical and mathematical analyses. In this work, the optimum concentrations of potassium phosphate and magnesium sulphate were found to be 0.058 and 0.09 g/L, respectively, for growth and 0.083 and 0.1 g/L, respectively, for lipid production.

3.3. Validation of growth and lipid production in column photobioreactors

To confirm statistical results, cell growth and lipid production in modified BG-11 media were compared with growth and lipid production in standard BG-11 media in 400 mL bubble column photobioreactors. Microalgal biomass was measured every 3 days to determine the growth phase. The lipid content and fatty acid composition was

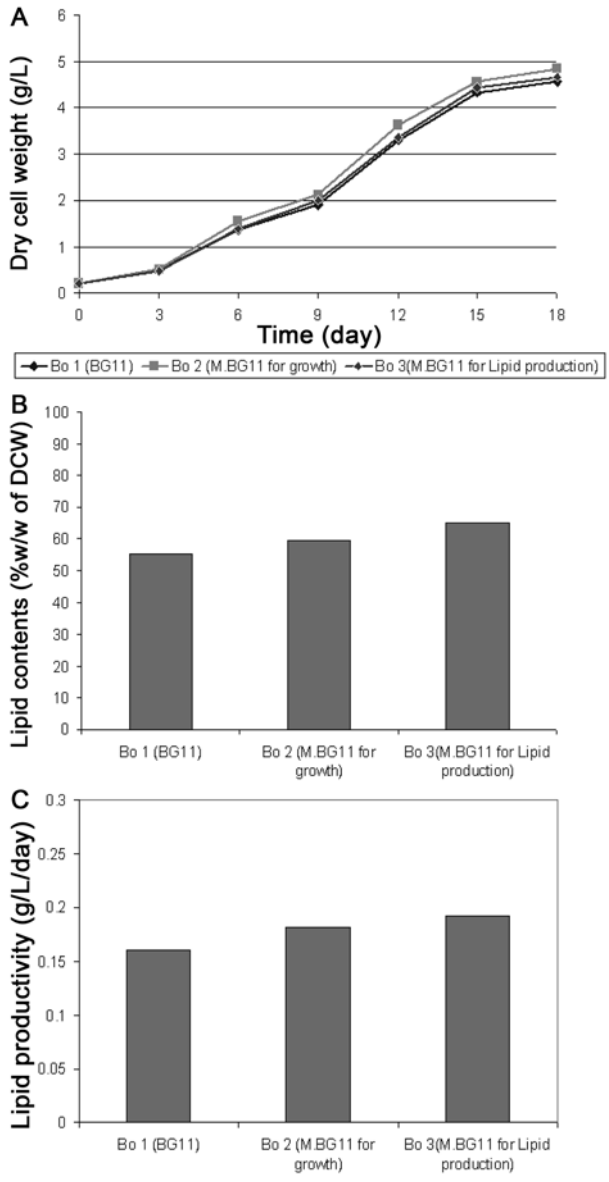

Fig. 3. Biomass and lipid yields of *B. braunii* LB572 grown in original and modified BG-11.

measured at the end of the exponential phase of cultivations (Day 15). Growth and lipid production data obtained in original and modified BG-11 media are given in Fig. 3.

From an initial density of 0.2 g/L, the culture in growth-modified BG-11 medium increased approximately 23-fold to 4.57 g/L by the end of the exponential phase (day 15); increased 22.15 times to 4.43 g/L in lipid production-modified BG-11; and increased 21.65~4.33 g/L in the original BG-11. As seen in Fig. 3, the BG-11 medium modified for lipid production displayed the highest lipid content (64.96%) and lipid productivity (0.19 g/L/day), followed by the sample with growth-modified BG-11 (59.56% and 0.18 g/L/day) and the original BG-11 medium (55.39% and 0.16 g/L/day). These results confirm that high concentrations of phosphate and sulfate in the range of the experimental variables are suitable for increasing biomass productivity and lipid productivity.

As shown in Fig. 3, the lipid productivity of *B. braunii* in this study (0.19 g/L/day) was higher than reported values for production in various freshwater microalgae. It was almost 1.43 times higher than the second highest lipid productivity, which was obtained with the phototrophic cultivation of *Neochloris oleoabundans* UTEX 1185 (0.133 g/L/day) [9]. Unusually high lipid productivity (1.214 g/L/day) of *Chlorella protothecoides* in heterotrophic fermentation was reported elsewhere [10]; however, heterotrophic fermentation consumes sugars (*i.e.* glucose) and releases CO_2; these features conflict with the mandate for solar energy capture and CO_2 mitigation. The biomass productivity, lipid cell content, and overall lipid productivity are key parameters affecting the economic feasibility of algal oil for biodiesel production; therefore, an ideal process should produce lipid at the highest productivity with the highest lipid cell content. Statistical optimization of biomass and of lipid products shown in this study in combination with previous results showing the oils of *B. braunii* increased up to 70% of dry cell weight [11] indicate that this species is a very promising source for biodiesel production. In addition, light delivery and distribution in high density cultures are always a challenge. In algal cultures, high cell density decreases the efficiency of light energy use due to mutual shading effects [12]. Thus, the low optimal light intensity for growth and lipid production of *B. braunii* LB572 is a distinct advantage.

3.4. Fatty acids profile of *B. braunii*

A gas chromatograph of fatty acid methyl esters of *B. braunii* cultured in original and modified BG-11 media is shown in Table 5.

Biodiesel largely consists of fatty acid methyl esters, which are produced by the transesterification of biologically derived lipids [13]. The quality of biodiesel is considerably affected by the composition of its fatty acids [14]. Fatty acids recovered from *B. brauniii* (expressed as % weight of total fatty acids) are shown in Table 5. The pattern of fatty acids in *B. braunii* is very typical of *Chlorococcales* [15]: it is very rich in palmitic acid (16:0), oleic acid (18:1n9c), and linoleic acid (18:3). Oleic acid was the primary fatty acid (57.65~68.17%.) The next dominating fatty acids were linoleic acid (13.77~18.87%) and palmitic acid (9.08~10.83%), and palmatoleic acid and stearic acid existed as minor fatty acids. Oleic acid is the precursor of the non-isoprenoid hydrocarbons produced by *Botryococcus* and is involved in the formation of very long fatty acid

Table 5. Fatty acid composition of *B. braunii* in original and modified BG-11 media.

Fatty acids (% weight of total FA)	*B. braunii* LB572		
	Bo 1 (original BG-11)	Bo 2 (M.BG-11 for growth)	Bo 3 (M.BG-11 for Lipid production)
C6:0	0.28 ± 0.09	0.39 ± 0.05	0.30 ± 0.09
C8:0	nd	nd	nd
C14:0	0.45 ± 0.03	0.52 ± 0.09	0.65 ± 0.11
C14:1	nd	nd	nd
C16:0	9.08 ± 0.29	10.83 ± 0.12	10.06 ± 0.22
C16:1	0.38 ± 0.04	0.39 ± 0.05	0.34 ± 0.04
C18:0	0.35 ± 0.02	1.19 ± 0.17	0.87 ± 0.07
C18:1n9c	68.17 ± 1.57	57.65 ± 0.82	62.19 ± 1.31
C18:1n9t	3.81 ± 0.23	6.04 ± 0.14	5.71 ± 0.31
C18:3	13.77 ± 0.26	18.87 ± 0.20	16.04 ± 0.55
C20:1	1.25 ± 0.06	0.77 ± 0.03	0.92 ± 0.03
C22:0	2.13 ± 0.07	3.08 ± 0.05	2.73 ± 0.12
C22:1	0.33 ± 0.01	0.27 ± 0.08	0.19 ± 0.07

Values are given as means, with the standard deviation of 3 replicates.
nd: not detected

derivatives through chain elongation [16]. Oleic acid also is a main component of biodiesel, occupying up to 50% weight of total fatty acids in *B. braunii.* The addition of mythyl oleate was suggested for improving biodiesel fuel properties (*i.e.* oxidative stability and low melting temperature) [14]. Thus, based on fatty acid composition, *B. braunii* was the most appropriate for producing superior quality biodiesel, due to its high lipid content and oleic acid proportion.

4. Conclusion

To optimize the growth and lipid production of *B. braunii* LB572, 4 major factors were selected as targets for improvement. Validated in bubble column photobioreactors, the major factors were K_2HPO_4 and $MgSO_4$ (media components) and CO_2 concentration, flow rate of gas composition, and light intensity (operating parameters). The optimal values of these major factors for growth and lipid production were investigated using statistical methods. The optimum concentrations of potassium phosphate and magnesium sulphate were found to be 0.058 and 0.09 g/L, respectively, for growth and 0.083 and 0.1 g/L, respectively, for lipid production. After optimization, biomass concentration and lipid production increased significantly, and results were validated in bubble column photobioreactors. Lipid productivity increased to 0.19 g/L/day in lipid-optimized media (with an average biomass productivity of 0.296 g/L/day and 64.96% w/w). In growth-optimized media lipid productivity was 0.18 g/L/day (with an average biomass productivity of 0.304 g/L/day and 59.56% w/w). Therefore, these results suggest that *B. braunii* LB572 cultured in optimized culture media has increased growth and lipid production, and *B. braunii* LB572 is well-suited for production of biodiesel due to its high lipid productivity and proportion of oleic acid.

Acknowledgement This work was supported by a Korea Energy Management Corporation (KEMCO) Grant (2007-N-BI02-P-02-0-000), for which the authors are thankful.

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