Korean J. Chem. Eng., **30**(8), 1614-1619 (2013)
DOI: 10.1007/s11814-013-0081-0

Optimum conditions for cultivation of *Chlorella* sp. FC-21 using light emitting diodes

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(*Received 29 September 2012 • accepted 9 May 2013*)

Abstract−The purpose of this study was to determine the optimum conditions for the cultivation of *Chlorella* sp. FC-21 under light-emitting diodes (LEDs). Specific growth rates and *Chlorella* cell concentrations were measured when they were grown under different LED wavelengths (red, blue, white, and mixed). The red LEDs were the most effective light source as determined by increases in specific growth rates and cell concentrations. Cell concentrations increased as light intensity was increased; however, the specific growth rate decreased as the initial cell concentration rose due to the shading effect of cells in the reactor. To determine if aeration is beneficial during cell cultivation, micro-air bubbles were aerated at 0.70 vvm in the reactor under red LED illumination. Aeration led to two- and ten-times greater specific growth rates. Our findings show that red LEDs with aeration are optimal for cultivation of *Chlorella* sp. FC-21.

Key words: Light-emitting Diodes, *Chlorella* sp. FC-21, Red LEDs, Carbon Dioxide, Photobioreactor

INTRODUCTION

Phytoplankton, also termed microalgae, are estimated to produce 1×10^{11} tons/year of organic materials worldwide. They are used as food for fish and shellfish in the aquaculture industry [1] and for feedstock for biofuels, proteins, and natural pigments [2,3]. The use of microalgae as a material from which biodiesel can be produced has drawn considerable scientific attention because the algae grow quickly, are inexpensive, and are renewable [4,5]. They are also easily biodegraded by microorganisms, and thus cause less environmental contamination than fossil fuels.

Currently, the primary raw materials for biodiesel are vegetable fats and oils that are composed of triacylglycerol (TAG) [6,7]. As the use of biodiesel expands, alternatives to vegetable oil sources (i.e., oilseed crops) are needed so that crop lands can be devoted to food grains. Microalgae as a fuel source are attractive because they are not used as human food and grow more rapidly than other oilseed crops. Biodiesel production per unit area using microalgae has reached 136,900 L/ha/yr [8], which is higher than corn, 172 L/ha/yr [8]; soy beans, 446-636 L/ha/yr [9]; and coconut, 5,366-5,950 L/ha/yr [8,9].

Current systems used for mass cultivation of microalgae are open raceway ponds and closed photobioreactors [8-10]. Open raceway ponds, which are widely used worldwide, have a waterway that is 0.1-0.3 m deep. They are easy to construct, maintain, and restore, but they inefficiently use carbon dioxide from the atmosphere and incompletely mix culture medium, resulting in low biomass concentration and possible contamination by other microalgae or microorganisms. In contrast, a closed photobioreactor is free of outside contamination, and temperature can be easily controlled; however, initial construction costs are higher than for open raceway ponds. Of several types of photobioreactors, the tubular photobioreactor is the most widely used. The goal when designing a tubular photobioreactor is to obtain the highest ratio of surface area to the reactor volume [8,10,11]. Thus, despite the high initial capital costs, the productivity per unit area makes the tubular photobioreactor the most economic microalgae culture system [8].

Microalgae grow via a photosynthetic reaction that requires light, carbon dioxide, water, and other nutrients. The most common light sources are sunlight and fluorescent lighting. Carbon dioxide is obtained from the atmosphere or from other sources such as discharges from gas- or coal-fired power plants or steelworks [12]. High concentrations of carbon dioxide (~15% v/v) inhibit microalgae growth [13].

Light source and light intensity for both open raceway ponds and closed photobioreactors are believed to the most important factors for the photosynthetic growth of microalgae [13]. Sunlight is a common light source for open raceway ponds, but fluorescent lighting is widely used in closed photobioreactors. Fluorescent light, however, is less efficient, produces more heat, is more expensive to maintain, and uses more electricity than light-emitting diodes (LEDs) and optical fibers [14]. Commercial LEDs are available in different wavelengths and prior studies indicate that microalgae grow well under LEDs. Wang et al. [15] reported good cultivation of *Spirulina platensis* under red LEDs with a 660-nm wavelength. *Haematococcus pluvialis* grown under blue LEDs (wave length, 460 nm) showed increased production efficiency of astaxanthin [16]. Lee and Palsson [17] also sought optimum cultivation conditions for *Chlorella pyrenoidosa* with various LED wavelengths.

In this study, we investigated the use of red, blue, and white plus mixed types of LEDs as light sources for the cultivation of *Chlorella* sp. FC-21. Our goal was to find the optimum LED wavelength for the cultivation of *Chlorella* sp. FC-21 as well as the optimum conditions for mass production of this organism for use as a raw

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material for possible biomass production.

MATERIALS AND METHODS

1. Algal Strain and Medium

Chlorella sp. FC-21 (KMMCC-193) was obtained from KMMCC (Korea Marine Microalgae Culture Center, Pusan, Korea), which was collected from the lake of Andongho in Korea. *Chlorella* sp. FC-21 was routinely maintained at 20 °C in 250 mL Erlenmeyer flasks containing 100 mL Jaworski's medium kept in an incubator under continuous illumination (2,700 lux). Microalgae used in this study were grown on Jaworski's medium, which was composed of $Ca(NO_3)_2 \cdot 4H_2O$ (20 mg/L), KH_2PO_4 (12.4 mg/L), $MgSO_4 \cdot 7H_2O$ (50 mg/L), $NaHCO_3$ (15.9 mg/L), $Na_2HPO_4 \cdot 12H_2O$ (36 mg/L), $NaNO_3$ (80 mg/L), EDTA FeNa (2.25 mg/L), $EDTANa_2$ (2.25 mg/L), H_3BO_3 (2.48 mg/L), $MnCl_2 \cdot 4H_2O$ (1.39 mg/L), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1.00 mg/L), cyanobalamin (0.04 mg/L), thiamine HCl (0.04 mg/L), and biotin (0.04 mg/L). The seed culture was prepared by using 250-mL Erlenmeyer flasks at a constant temperature of 23 °C in an illuminated incubator (model HB-201S, Han-Baek Scientific, Puchon, Korea). All media and cultivation apparatus were sterilized with steam at 121 °C, 0.11 MPa for 15 min.

2. Cultivation of *Chlorella* sp.

A stock culture of *Chlorella* sp. (approximately 1×10^5 cells/mL) was incubated in an Erlenmeyer flask containing 500 mL working volume of Jaworski's medium at 23 °C and 56 μmol/m²/s.

Six days after incubation, microalgal cells were harvested by using a filtering apparatus. Cells were then resuspended in a glass reactor at the density of 8.0×10^5 cells/mL or other initial densities.

3. Photobioreactor

Red, blue, and white LEDs were purchased from S-tech Inc. (Ilsan, Kyongi, Korea). The LEDs have narrow spectral output peaks at wavelengths of 660 nm (red) and 450 nm (blue), respectively. These LEDs were powered by DC power supplies (Whawoo Tech. Co., Ilsan, Korea).

A photobioreactor system was prepared in a dark room to cultivate *Chlorella* sp. FC-21 under LEDs with different wavelengths. A black acrylic panel was used to house the LEDs, bioreactor, and testing apparatus. The dimensions of each reactor (height, length, and width) were 60, 60, and 58 cm, respectively. Each LED unit was placed on top of the reactor to provide light from top to bottom of the reactor. The light intensity of the LED unit was varied by opening a door to the reactor.

The light intensity was measured by a silicon photo cell (model 0560, Testoterm GmbH & Co., Germany) and a quantum sensor (model MQ-306, Apogee Instruments, Ilsan, Korea). The experiment was conducted with continuous light at 68.4 W.

The *Chlorella* sp. was incubated in a cylindrical glass reactor at a working volume of 500 mL. Cultures were placed on a glass reactor at 23±1 °C under continuous LEDs or fluorescent lights. Cultures in the photobioreactor were aerated continuously with one of the mixtures at a rate of 0.70 vvm (volume gas/volume media/min).

A semicontinuous cultivation system was set up in a single photobioreactor as explained in Fig. 1. The photobioreactor contained 500 mL cultured microalgae. The culture was started as a batch culture. When cell density reached maximum, half of volume of the culture broth was replaced with fresh Jaworski's medium. In the photobioreactor, the culture was aerated with various air flow rates. Before fresh medium was inserted, the culture broth was sampled to estimated cell numbers in solution.

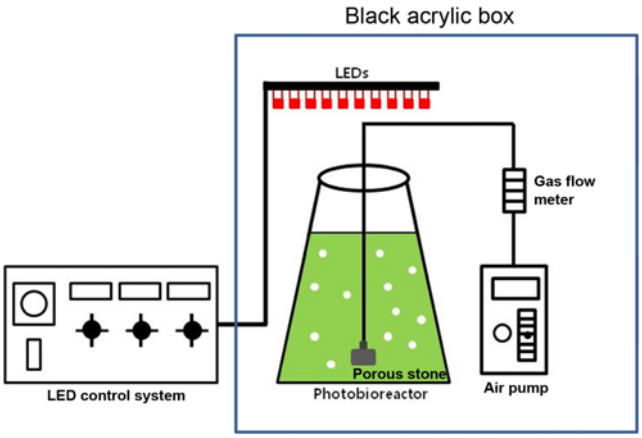

Fig. 1. Schematic diagram of the photobioreactor for the experiments on microalgae growth for batch and semicontinuous microalgal culture. The airstream was adjusted by a gas flow meter. External illumination of light was provided by a continuous LED light.

4. Microalgal Cell Counting and Dry Weight

A direct microscopic count was conducted on the sample of microalgal suspension with a Brightline hemacytometer (BOECO, Hamburg, Germany) and an optical microscope (AE2000; MOTIC, China).

Several different cell concentrations were prepared to determine the relationship between cell concentration (cells/mL) and cell dry weight (g/mL). The sample of microalgal suspension was filtered through a 0.45-μm membrane filter. Dry weight was measured after drying the microalgal filtrate at 105 °C for 16 hrs [18].

5. Measurement of Growth Rate

Specific growth rate (μ_{max}, d^{-1}) was calculated as follows:

$$\mu_{max} = t^{-1} \cdot (\ln (x_f / x_0)) \quad (1)$$

where x_f and x_0 were the final and initial biomass concentrations, respectively, and t was the cultivation time in days [19].

RESULTS AND DISCUSSION

1. Evaluation of Cell Concentration and Biomass

Cell concentration and biomass were measured by direct counting of cells and by cell dry weight, respectively. Thus, relationships between cell concentration and biomass were established by linear regression as follows:

$$\text{Biomass (g/L)} = 2.0 \times 10^{-8}; \text{ Cell concentration (cells/mL)} \quad (R^2 = 0.994;\ p < 0.001) \quad (2)$$

We used the cell concentration values to calculate the related biomass of *Chlorella* sp. FC-21 in each experiment according to our Eq. (2).

2. Effect of Different LEDs on Microalgal Culture

Five sets of tests were conducted using LEDs and a fluorescent lamp. Specific test conditions are summarized in Table 1. To investigate the effect of different LEDs on the growth of *Chlorella* sp. FC-21 (Test set 1), four types of LEDs (red, blue, white, mixed (red

Table 1. Biomass production and specific growth rates of *Chlorella* sp. FC-21 with different colored LEDs and fluorescent lights and differing test conditions

Test set	LEDs	Light intensity		Specific growth rate (day^{-1})	Initial cell concentration (cells/mL)	Maximum cell concentration (cells/mL)	Biomass (g/L)[b]
		Lux	μmol/m^2/s				
1	RED	1700	50	0.30	8.0×10^5	2.2×10^7	0.44
	BLUE	1900	165	0.27	8.0×10^5	1.5×10^7	0.30
	WHITE	6900	102	0.28	8.0×10^5	1.7×10^7	0.34
	Mixed (R1 : W1 : B1)	4000	115	0.26	8.0×10^5	1.4×10^7	0.28
2	RED	2600	67	0.33	8.0×10^5	7.9×10^6	0.16
	WHITE	6200	90	0.27	8.0×10^5	6.9×10^6	0.14
	Mixed (R3 : B1)	2300	70	0.22	8.0×10^5	5.9×10^6	0.12
3	RED	4400	116	0.42	8.5×10^5	4.6×10^6	0.09
	WHITE	9000	140	0.33	8.5×10^5	3.3×10^6	0.07
	Mixed (R3 : B1)	3600	120	0.25	8.5×10^5	3.0×10^6	0.06
4	RED	4400	116	0.74	2.0×10^4	3.5×10^6	0.07
	RED	4400	116	0.53	1.0×10^5	4.1×10^6	0.08
	RED	4400	116	0.44	2.0×10^5	4.3×10^6	0.09
	RED+air[a]	4400	116	1.09	2.0×10^5	5.1×10^7	1.02
5	Fluorescent light	4500	56	0.17	8.5×10^5	3.0×10^6	0.06
	Fluorescent light+air[a]	4500	56	0.48	8.5×10^5	3.0×10^6	0.06

[a]CO_2 concentration in air is about 0.03%

[b]Biomass was measured when the cells grew to the plateau stage in culture

1, blue 1, white 1) were installed in each reactor, and cells were incubated in glass reactors for 270 hrs at 23±1 ℃. Electrical input was 68.4 W for each LED type.

Although all LEDs had the same electrical input, their light intensities were significantly different, ranging from 50 μmol/m^2/s for red to 165 μmol/m^2/s for blue. White and mixed were 102 and 115 μmol/m^2/s, respectively.

As the cells grew to plateau stage, the biomass under red, blue, white, and mixed type LEDs with initial cell inoculums of 8.0×10^5 cells/mL was 0.44, 0.30, 0.34, and 0.28 g/L, respectively. When illuminated by red LEDs, *Chlorella* sp. grew most rapidly with a specific growth rate of 0.30 day^{-1} as shown in Fig. 2. In other tests, the specific growth rates decreased in the following order: 0.28 day^{-1} (white), 0.27 day^{-1} (blue), and 0.26 day^{-1} (mixed). The specific growth rate of 0.30 day^{-1} was similar or greater than of other studies [20-23]. For example, the specific growth rates ranged between 0.40 day^{-1} (*Chlorella vulgaris*) and 0.38 day^{-1} (*Chlorella emersonii*) under similar light intensities [20]. Aeration with 2% CO_2 slightly increased the specific growth rate to 0.49 day^{-1} but 5% CO_2 reduced the specific growth rate to 0.13 day^{-1} [22]. This result indicates that the red LEDs are the most suitable light source for the cultivation of *Chlorella* sp.. Others also found that red LEDs were the most effective light source for cultivating *S. platensis* [15] and *C. pyrenoidosa* [17]. Red is a complementary color of green, which is the typical color of *Chlorella* sp. FC-21. Thus, red light generated from red LED could be more effectively used for photosynthesis than other wavelengths of LED lights [23-24].

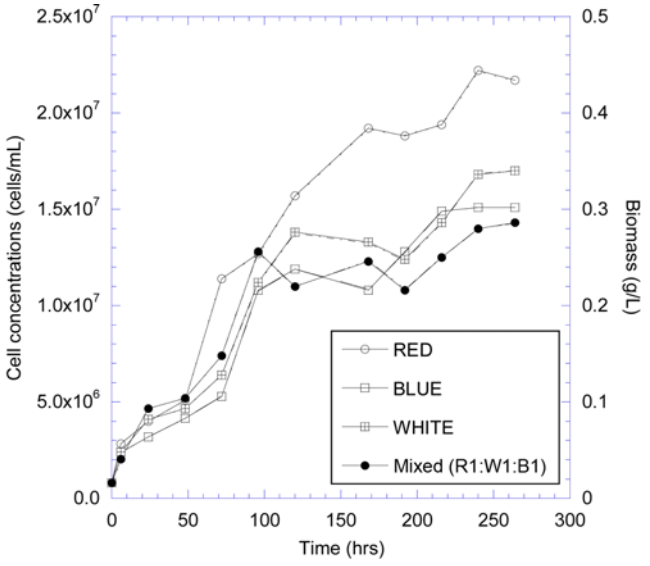

Fig. 2. Cell growth curves of Chlorella sp. FC-21 in a photobioreactor grown under LEDs of different wavelengths (red, blue, white, mixed).

3. Effect of Light Intensity on Specific Growth Rate

Blue and mixed LEDs (i.e., red, white, and blue) were not used in the second and third experiments because they were less effective for the cultivation of *Chlorella* sp. in the first study. Instead, red, white, and the newly designated mixed LEDs (i.e., 3 red and 1 blue) were used for the cultivation of *Chlorella* sp. in the second and third tests. Also, in those tests the light intensity of the LEDs installed in the reactors was increased by installing additional black acrylic panels around the reactors to prevent light loss. Light intensity, specific growth rate, and maximum biomass concentration are summarized in Table 1.

Specific growth rates of *Chlorella* sp. linearly increased as light intensities of each LED type increased (Fig. 3). However, red LEDs showed the greatest increase in specific growth rate. As shown in Fig. 3, the slope for red LEDs was 0.00182, which is greater than

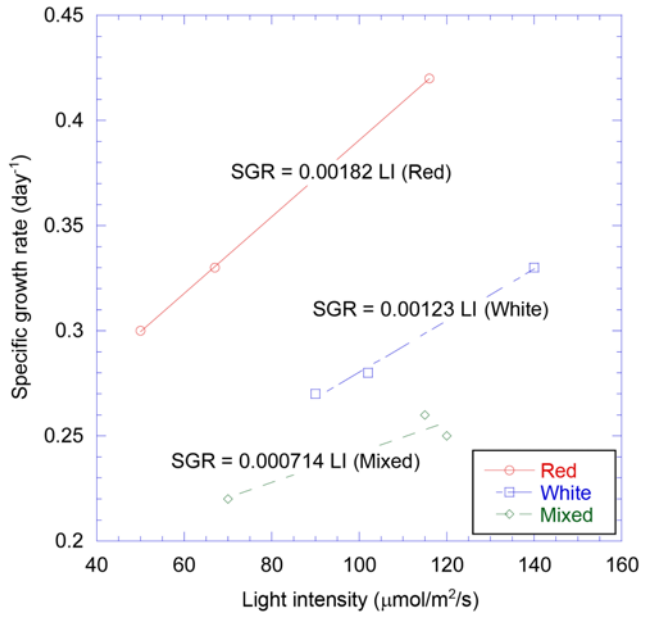

Fig. 3. Graph of relationships between specific growth rates (SGR) and light intensities of red, white, and mixed LEDs.

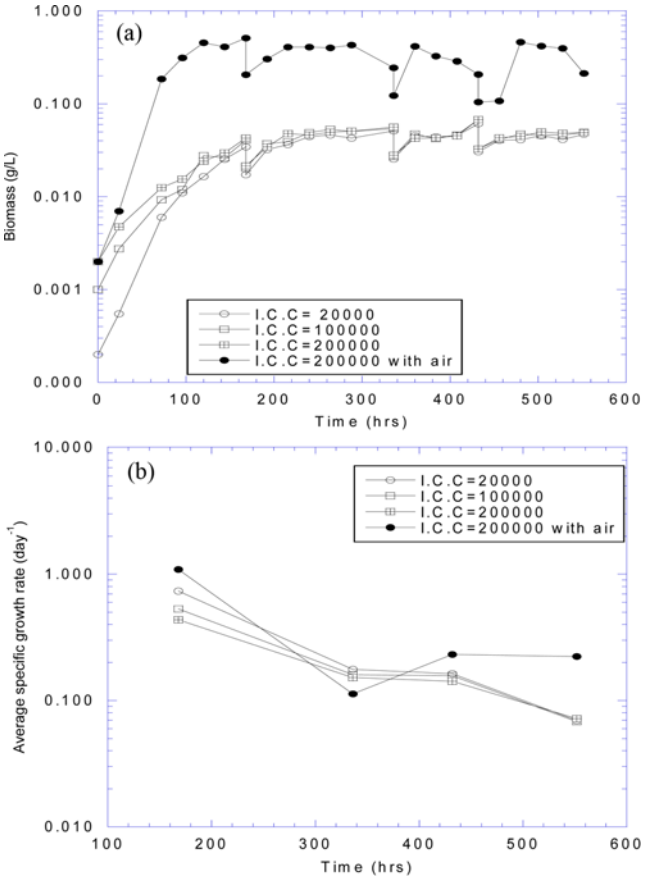

Fig. 4. Effects of initial cell concentrations of *Chloreella* sp. FC-21 on specific growth rates (a) and maximum cell concentrations (b), and cell growth curves with dilution ratio of 0.5 (50% dilution) in a semi-continuous reactor system under red LED illumination at a light intensity of 116 μmol/m²/s (I.C.C=initial cell concentration, cells/mL).

the 0.00123 and 0.00071 found for white and mixed LEDs, respectively.

4. Effects of Initial Cell Concentration and Aeration

To determine the effect of initial cell concentration (ICC) on specific growth rate, reactors containing initial concentrations of 2.0×10^4, 1.0×10^5, and 2.0×10^5 cells/mL were prepared and cultivated under red LEDs with a light intensity of 4,400 lux and 116 μmol/m²/s. Specific growth rates for each test were calculated from Eq. (1).

As shown in Fig. 4, the cell growth rate gradually slowed as the ICC was increased. The specific growth rate was 0.74 day^{-1} when the ICC was 2.0×10^4 cells/mL. However, the specific growth rate was just 0.44 day^{-1} when the ICC was 2.0×10^5 cells/mL. This decrease was caused primarily by the shading effect that occurs when high concentrations of cells block light on [25-28]. In our studies, maximum cell concentrations were slightly higher when the ICC increased.

To overcome the shading effect, cultures in 100 ml flasks were aerated with four different flow rates to move cells inside the reactor and to enable better LED light penetration. The greatest specific growth rate (1.51 day^{-1}) was observed at 0.7 vvm (Fig. 5(a)), and also the maximum biomass concentration (1.02 g/L) was obtained at 0.7 vvm (Fig. 5(b)). Thus, an air flow rate of 0.7 vvm was used during the study when aeration was required.

In addition to proper choice of wavelength of LED light, aera-

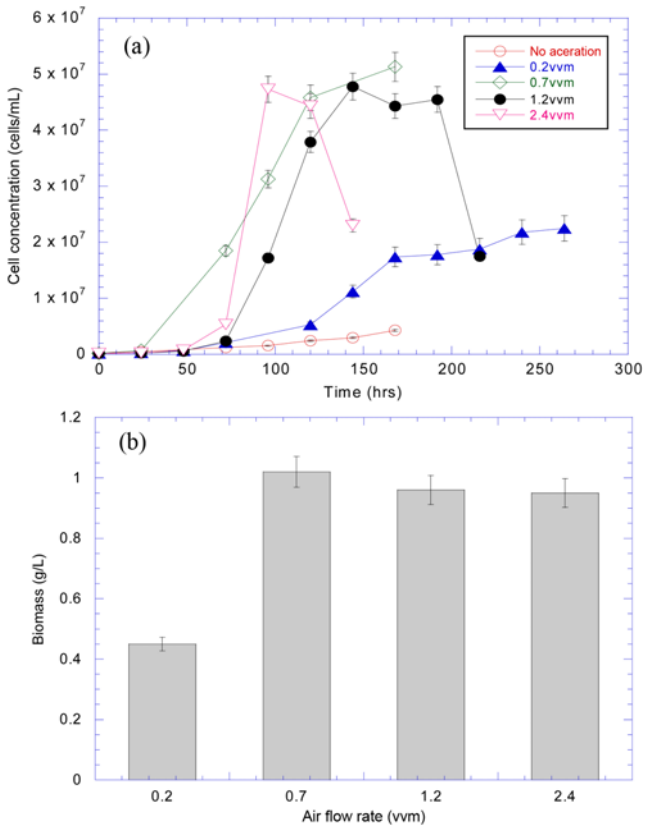

Fig. 5. Cell growth curves of Chlorella sp. FC-21 in a photobioreactor grown under different air flow rates (a) and produced weight of biomass (b). All photobioreactors were illuminated by red LEDs at the light intensity of 116 μmol/m²/s.

tion is essential to promote algal growth because light penetration depth is limited under algal growth condition [29,30]. As a result, the best growth condition could be obtained when led LED light as a light source and aeration for the better light contact of microalgae are provided.

5. Effects of Dilution on Cell Growth with Semi-continuous Cultivation

As noted, the red LEDs were found to be the most effective light source, and aeration with the red LEDs further increased specific growth rates. Subsequently, we conducted tests using the semi-continuous cultivation method to determine which ICC grew best with aeration of 0.70 vvm under red LED illumination (light intensity, 116 μmol/m^2/s). In the semi-continuous reactor system, the appropriate volume of cell suspension was replaced with fresh medium (termed dilution) when the cell concentration was saturated [31]. We used 50% dilution, which we previously found to produce good growth in semi-continuous reactors [32]. Biomass concentrations and average specific growth rates are shown in Fig. 4.

As shown in Fig. 4, the average specific growth rate gradually declined over 560 hrs with 50% dilution conditions. The decrease was caused primarily by the shading effect. For samples tested without aeration, the average specific growth rates gradually decreased. For example, at an ICC of 2×10^4 cells/mL, the average specific growth rates were 0.736 (168 hrs), 0.177 (336 hrs), 0.163 (432 hrs), and 0.069 (552 hrs) day^{-1}. This result indicates that 50% dilution is not adequate to sustain continuous cultivation. However, the average specific growth rates with aeration initially dropped precipitously from 1.09 to 0.113 day^{-1} but then slightly increased to 0.232 and 0.223 day^{-1}. These findings show that aeration is important for maintaining continuous cultivation of *Chlorella* sp. The specific growth rates obtained from the semi-continuous cultivation system ranged from 0.223 (after dilution) to 1.09 (before dilution) day^{-1}, which are comparable to those of *Chlorella vulgaris* (0.40 day^{-1}) and *Chlorella emersonii* (0.38 day^{-1}) [20] but higher than that of *Chlorella* sp. [22] where these values were obtained from the batch types culturing experiments.

6. Concentrations of NO_3^--N and PO_4^{3-} in the Culture Medium

Jaworski's medium contains various nutrients necessary for the growth of *Chlorella* sp. FC-21 including NO_3^--N and PO_4^{3-} for the growth of *Chlorella* sp. In this study, we tried to determine how NO_3^--N and PO_4^{3-} are consumed by cells growing under red LEDs so that we could set the appropriate dilution time. In addition, we wanted to obtain basic data for the removal of NO_3^--N and PO_4^{3-} in wastewater using *Chlorella* sp. FC-21. Concentrations of NO_3^--N and PO_4^{3-} with different initial concentrations are shown in Fig. 6.

NO_3^--N concentrations continuously declined until the first dilution, when additional NO_3^--N was supplied by fresh medium. Similar changes were observed for the second and third dilutions, but NO_3^--N was never completely depleted except during the test with aeration. In that test, 17.6 mg/L of NO_3^--N quickly decreased and disappeared after 100 hrs due to fast cell growth. At 168 hrs, the first dilution was done and NO_3^--N increased to 9.2 mg/L; again it was quickly consumed by cells within 48 hrs.

Concentrations of PO_4^{3-} also decreased, but high concentrations remained in the solution before the first dilution. The concentrations of PO_4^{3-} were 7.7 mg/L (ICC, 2×10^4 cells/mL), 6.6 mg/L (ICC, 1×10^5 cells/mL), 7.0 mg/L (ICC, 2×10^5 cells/mL), and 5.0 mg/L (ICC, of 2×10^5 cells/mL with aeration). Concentrations of PO_4^{3-} rose after the second and third dilutions, and then slowly decreased, but never dropped below 5.0 mg/L. This suggests that NO_3^--N is more important for cell than PO_4^{3-}. These findings show that dilution should be done within 48 hrs, especially at 50% dilution, to maintain high cell growth rates under red LED illumination with aeration.

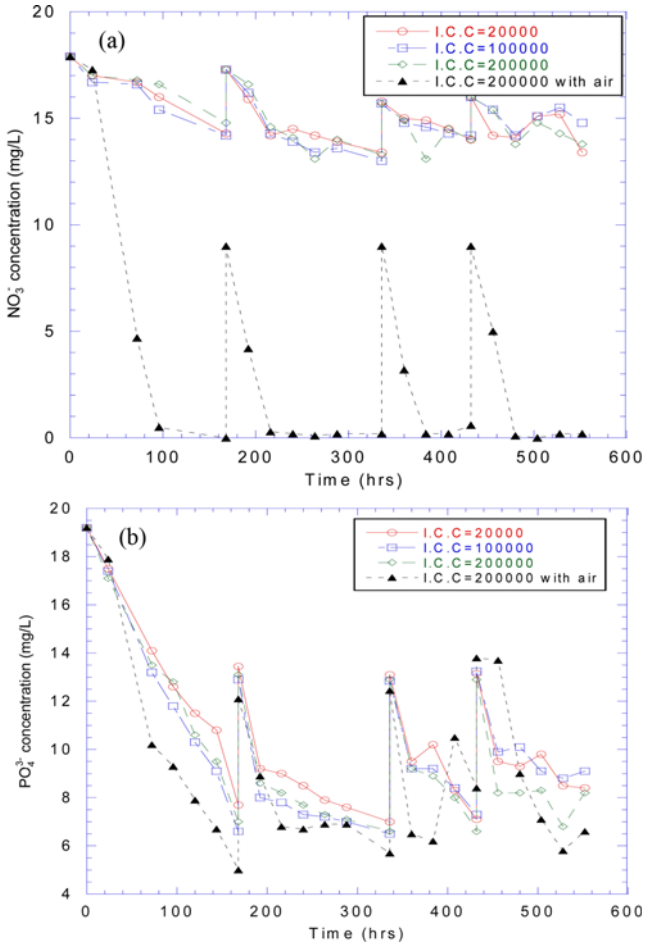

Fig. 6. Concentrations of NO_3^--N and PO_4^{3-} with dilution ratio of 0.5 (50% dilution) in a semi-continuous reactor system illuminated by red LEDs at a light intensity of 116 μmol/m^2/s (I.C.C=initial cell concentration, cells/mL).

7. Chlorella Growth under Fluorescent Lights

Fluorescent lighting is a widely used light source for microalgae growth in closed photobioreactors. In this study, we cultivated *Chlorella* sp. under fluorescent lamps and compared growth rates with those obtained from LED tests. The fluorescent lamp was adjusted to 4,500 lux (64 W), similar to the light intensity of test set 4 (Table 1). However, the fluorescents produced less light (56 μmol/m^2/s) than the red LEDs (116 μmol/m^2/s), indicating that red LEDs are more effective for photosynthetic reaction than fluorescent lighting. The specific growth rate was 0.17 day^{-1} under fluorescent lighting when the ICC was 8.5×10^5 cells/mL; however, red LEDs had a 2.5 times greater specific growth rate (see Table 1). Aeration also enhanced cell growth under fluorescent lighting with specific growth more than 2.8 times higher than in the study without aeration. This finding indicates that aeration is an important factor for cultivation of Chlorella regardless of light source.

CONCLUSIONS

Chlorella sp. FC-21 was cultivated under LEDs of different wavelengths or combined types to find the optimal LED wavelengths. We also did comparison studies using fluorescent lighting. Our findings indicate that red LEDs produce the greatest growth and that aeration is important for the optimum cultivation of *Chlorella* sp. FC-21.

Our findings are as follows:

1) Red LEDs produced the highest specific growth rates when we compared red, blue, white and mixed (red 1, white 1, blue 1; red 3, blue 1) LEDs at 68.4 W.

2) Under each lighting condition, the specific growth rate increased linearly as light intensity increased. In all tests, red LEDs produced the greatest specific growth rate increase per unit increase of light intensity.

3) Under red LEDs, specific growth rates gradually decreased as the ICC increased due to the shading effect of cell growth. However, the maximum cell concentration slightly increased as the ICC rose.

4) Specific growth rate of *Chlorella* sp. increased more than two times from 0.44 day^{-1} (without aeration) to 1.09 day^{-1} (with aeration) under red LED (light intensity, 116 $\mu mol/m^2/s$). The aeration enabled circulation of inner cells, which lacked light, and also provided carbon dioxide to the cells.

5) With repeat cell dilutions, the average specific growth rate consistently decreased, indicating that the 50% dilution used in this study did not avoid the shading effect. In the test with aeration, the highest specific growth rate (1.09 day^{-1}) was obtained before dilution, and the growth rate decreased to 0.11, 0.23, and 0.22 day^{-1} when dilution was repeated three times.

6) Under fluorescent lighting, the specific growth rate of *Chlorella* sp. was 0.17 day^{-1} at an ICC of 8.5×10^5 cells/mL. In contrast, red LEDs at an ICC of 2.0×10^5 cells/mL had a growth rate more than two times higher.

7) In semi-continuous reactors, 17.6 mg/L of NO_3^--N quickly diminished and disappeared after 100 hrs due to fast cell growth. After dilution, NO_3^--N levels increased to 9.2 mg/L but again were quickly consumed by cells within 48 hrs.

8) Our findings show that red LEDs are more effective than conventional fluorescent lights for the cultivation of *Chlorella* sp. FC-21, and also that aeration is important because it enables better light distribution for cell growth and also distributes carbon dioxide.

ACKNOWLEDGEMENTS

This work was funded by the Korea Ministry of Land Infrastructure and Transport under Grant C-D-2012-0938.

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