Korean J. Chem. Eng., 32(9), 1842-1846 (2015)
DOI: 10.1007/s11814-015-0012-3

pISSN: 0256-1115
eISSN: 1975-7220

Effect of light intensity on the correlation between cell mass concentration and optical density in high density culture of a filamentous microorganism

Jong Hyun Yoon*, Jong-Hwan Shin*, Joon Ho Park*, and Tai Hyun Park*,,†**

*School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Korea
**Advanced Institutes of Convergence Technology, Suwon 443-270, Korea
(*Received 26 October 2014 • accepted 13 January 2015*)

Abstract−We investigated the effect of light intensity on the correlation between optical density (OD) and cell mass concentration in high cell density culture of *Anabaena variabilis*. When the light intensity was gradually increased to maintain specific irradiation rate above 10 mmol/s/g dry cell, the dry cell mass concentration to OD ratio changed when the cell density became higher than OD 10, while the correlation was linear when light intensity remained constant after 2.5 days. When the OD was below 10, the dry cell concentration to OD ratio in unicells was the same as that of filaments, indicating that filament length, unicell size, and unicell dry cell concentration did not affect the dry cell concentration to OD ratio. Scanning electron microscope pictures revealed the differences in morphological structures between filaments below and above OD 10. Therefore, we propose that the morphological structures of filaments affected the dry cell concentration to OD ratio.

Keywords: High Cell Density, Optical Density (OD), Dry Cell Mass Concentration, Cell Morphology, Filamentous Microorganism

INTRODUCTION

The quantification of cell concentration in culture media is essential for the determination of the kinetics and stoichiometry of microbial growth. The methods used in the quantification of cell concentration are as follows: direct cell count, determination of dry cell mass concentration, optical density (turbidity) measurement, and intracellular component measurement. Among these, optical density measurement provides a fast, inexpensive, simple, and generally accurate method of estimating cell concentration [1]. The calibration curve relates optical density (OD) to determination of dry cell mass concentration. Such calibration curves can depend to some extent on the physiological state of the cells [2]. Optical density measurement has been mainly used for measuring cell concentrations in suspensions of relatively small and unicellular microorganisms [3]. However, it could be also applied to larger and multicellular systems: cyanobacteria [4,5], plant cells [6], and insect cells [7].

Microalgae undergo significant physiological and chemical changes in response to variations in light, temperature, nutrient availability, and cell density [8-13]. However, there have been few researches on the effect of light intensity on OD and cell morphology as reported in this work. *Anabaena variabilis* has been used for various applications [14,15] and was used as a model filamentous microorganism in this study. To better reflect the actual dry cell concentration of cells with morphologies other than unicellular morphology, it is critical to evaluate the correlations between OD and dry cell concentration for each morphology. This investigation shows that changes in morphologies, specifically filamentous microorganisms, actually do impact the correlation between OD and cell concentration, and help our understanding about the correlation between OD and dry cell concentration in high cell density culture of filamentous microorganism.

MATERIALS AND METHODS

1. Microorganism and Maintenance

Anabaena variabilis (ATCC 29413) in an Erlenmeyer flask (500 ml) containing 100 ml of the BG11 medium (ATCC, Washington DC, USA) was cultured in a shaking incubator (VS-8480, Vision, Korea) at 30 °C and 190 rpm [5].

2. Cultivation Conditions

Anabaena variabilis was cultivated at 30 °C in photobioreactor (working volume 260 ml) described previously [16]. The culture pH was maintained at 7.0 and the culture medium was the BG11 medium with 100 mg/l K_2HPO_4. Light was provided continuously with ten fluorescent lamps placed on one side of the photobioreactor. The light intensity was adjusted to various levels by changing either the distance between fluorescent lamps and the bubble column or the number of fluorescent lamps used.

3. Determination of Specific Irradiation Rate

As a parameter of the controlled incident light, a specific irradiation rate (q_i) was defined as follows [17,18]:

$$q_i = \frac{I_i \times A}{C \times V} = \frac{2I_0 \cdot h \cdot r}{C \cdot V} \quad (1)$$

†To whom correspondence should be addressed.
E-mail: thpark@snu.ac.kr
‡This article is dedicated to Prof. Hwayong Kim on the occasion of his retirement from Seoul National Univerisity.

where I_i=light intensity illuminated at a right angle on the surface area, μmol/s/m^2; A=illuminated surface area of the bubble column, m^2; C=cell concentration, g/*l*; V=volume of medium, *l*; I_0= incident light intensity on the surface of the bubble column, μmol/s/m^2; h=height of the bubble column, m, and r=radius of the bubble column, m.

4. Determination of Cell Concentration

Cell concentrations were determined by measuring optical density (OD) at 680 nm and were plotted versus dry cell concentration (g/*l*) on a standard curve [5].

5. Measurement of Light Intensity

The light intensity was measured by a quantum sensor (LI-COR, Model LI-190SA, Lambda Instrument Corp., USA) and a light meter (LI-COR, Model LI-250, Lambda Instrument Corp., USA).

6. Physical Disruption of Filaments to Unicells

Filaments were broken by sonication (UCX750, Sonics, USA) at an amplitude of 20% and at 4 °C. The depth of the probe in the cell suspension was the minimum penetration which did not cause splattering [19]. After 20 seconds of total sonication time (10×2 second pulse) the cell suspension was removed from the sonicator. In this cell suspension, unicells existed mainly and 2-3 cell lengths did a little, when they were observed through the microscope. Therefore, these cells were called unicells here.

7. Microscopic Estimation of the Number of Cells

After physical disruption of filaments to unicells, cells were directly enumerated microscopically with or without dilution after mounting on a haemocytometer (Marienfeld GmbH, Marienfeld, Germany).

8. Elemental Analysis

Elemental analysis of washed and dried samples was performed with elemental analyzer (EA1110, CE Instrument, Italy) [20].

9. Extraction of Chlorophyll a

Cells from 1 ml of suspension culture were centrifuged at 10,000 g for 10 min at 4 °C, resuspended in 1 ml of 95% *N,N*-dimethylformamide (Sigma Aldrich, St. Louis, MO, USA), left in the dark for 5 min, and then centrifuged at 12,000 g [21]. The supernatant was analyzed in a spectrophotometer (Genesys, Spectronic Instruments, USA).

10. Specimen Preparation for Scanning Electron Microscope (SEM)

Cells from the culture were fixed at 4 °C for 2-4 hours in modified Karnovsky's fixative [22], which consists of 2.5% glutaraldehyde (Sigma Aldrich, St. Louis, MO, USA) and 2% paraformaldehyde (Sigma Aldrich, St. Louis, MO, USA) in 0.15 M sodium cacodylate buffer, pH 7.4 (Sigma Aldrich, St. Louis, MO, USA). Then, cells were washed three times in 0.05 M sodium cacodylate buffer (pH 7.2) at 4 °C for 10 min, and followed by post-fixation with 1% osmium tertroxide in 0.05 M sodium cacodylate buffer (pH 7.2) at 4 °C for 2 hours. After the fixed cells were washed twice in distilled water at room temperature, they were dehydrated using a series of graded ethyl alcohols (30%, 50%, 70%, 80%, 90%, and 3 changes of 100% for 10 min each). After the dehydrated cells were washed twice in 100% hexamethyldisilazane (Sigma Aldrich, St. Louis, MO, USA), they were mounted on the stubs with adhesive tabs. After the specimens were dried, they were coated with gold and examined using SEM (JSM-5410LV, JEOL, Japan).

RESULTS AND DISCUSSION

1. Correlation between Cell Mass Concentration and OD

High cell density culture of *Anabaena variabilis* was performed at controlled light and nutrient supply in previous work [16]. Each

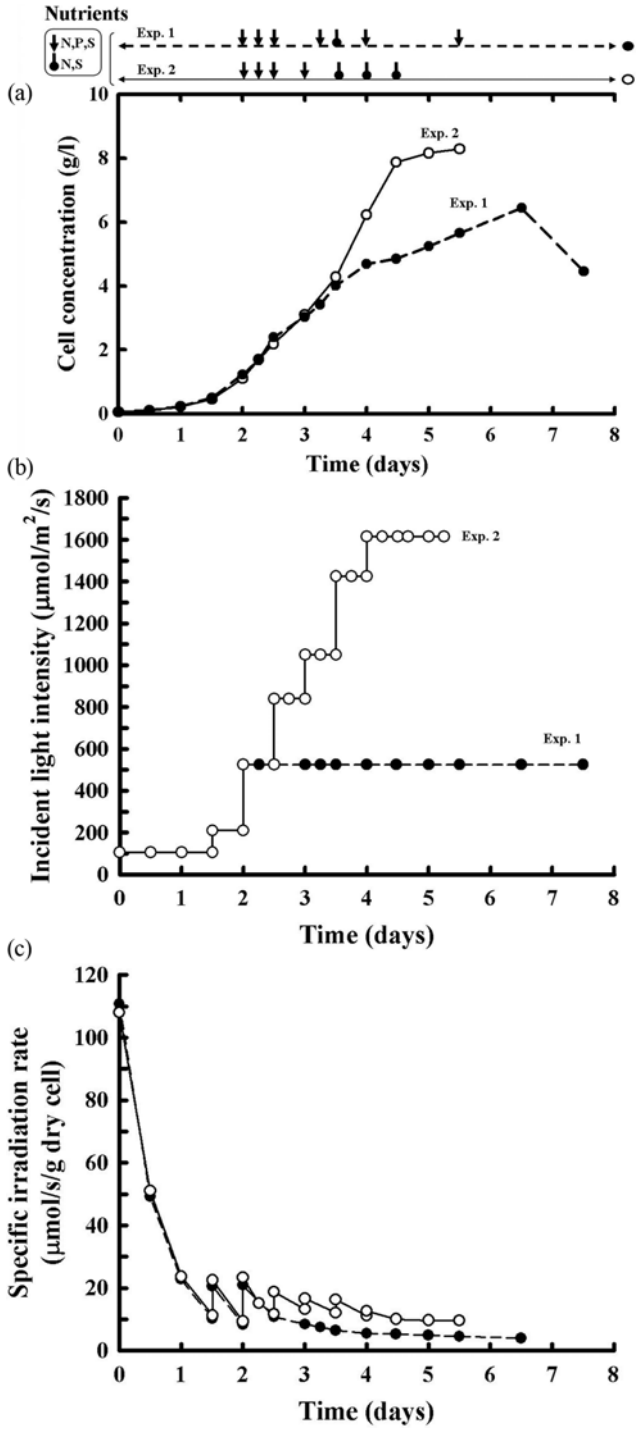

Fig. 1. The profile of cell growth in high cell density culture. (a) Cell growth profile. Downward arrows in the diagram indicate timing of nutrient addition. Abbreviations: N - nitrate; P - phosphate; S - sulfate. (b) Profile of incident light. (c) Profile of specific irradiation rate.

concentration of nitrate, phosphate, and sulfate was controlled by intermittent additions based on growth yield (Fig. 1(a)). During the cell growth, the specific irradiation rate was controlled above 10 μmol/s/g dry cell by increasing incident light intensity stepwise (Fig. 1(b) and (c)). Experiments 1 and 2 differed in the period controlling light intensity: 2.5 days in experiment 1 and 4.5 days in experiment 2. The culture profiles were divided into an exponential growth phase and a deceleration phase. During the exponential growth phase, the specific growth rate in experiment 2 was similar to that in experiment 1. However, during the deceleration phase, the average cell growth rate in experiment 2 was larger than that in experiment 1 (Fig. 1).

In experiment 1, the OD showed a linear relationship to cell concentration in the OD range of 0.100-16.0 (Fig. 2(a)). The relationship between dry cell concentration (Y) and OD (X) could be approximated by Eq. (4).

$$Y=0.39\ X\ (r^2=0.9963) \quad (4)$$

In experiment 2, linear relationships between the OD and cell concentration were obtained in the OD range of 0.100-10.0 and 10.0-15.2, respectively (Fig. 2(b)). The ratio of dry cell concentration to OD in experiment 1 was the same value as that in the OD range of 0.100-10.0 in experiment 2. However, in 2, the ratio of dry cell concentration to OD in the OD range of 10.0-15.2 was higher than that in the OD range of 0.100-10.0. The relationship between dry cell concentration (Y) and OD (X) in the OD range of 10.0-15.2 could be approximated by Eq. (5).

$$Y=0.82\ X-4.3\ (r^2=0.9648) \quad (5)$$

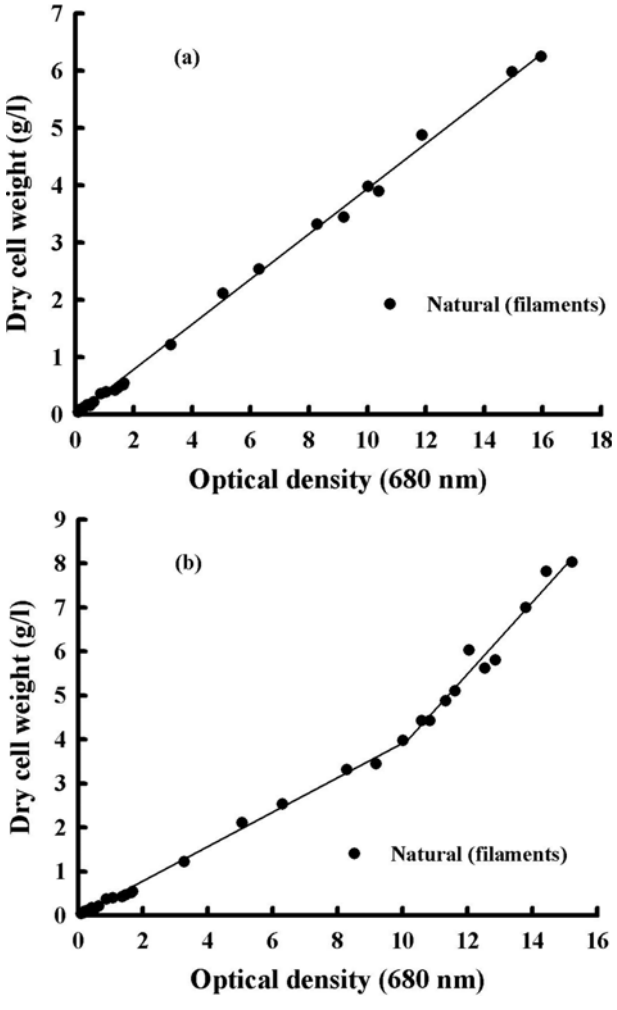

Fig. 2. The ratio of dry cell concentration to OD in Exp. 1 (a) and Exp. 2 (b). Solid line is the regression line between OD and dry cell concentration.

2. The Change of the Ratio of Dry Cell Concentration to OD in Unicells

If the lengths of filaments affect the ratio of dry cell concentration to OD in experiment 2, the ratio of dry cell concentration to OD in filaments was different from the ratio in unicells, which were made of filaments by sonication [23]. Probable diagrams of the ratio of dry cell concentration to OD are Fig. 3(a) and (b). If the length of filaments did not affect the ratio of dry cell concentration to OD, the ratio of dry cell concentration to OD in filaments was the same as the ratio in unicells (Fig. 3(c)). In previous studies, there were some factors affecting the ratio in unicells: unicell size, unicell dry weight, and pigment content [2,24,25].

Cyanobacteria have many pigments: chlorophyll a, phycocyanin, allophycocyanin, phycoerythrocyanin, and various carotenoids. The absorption maximum of chlorophyll a is about 680 nm, while that of the other pigments is not about 680 nm. Therefore, the effect

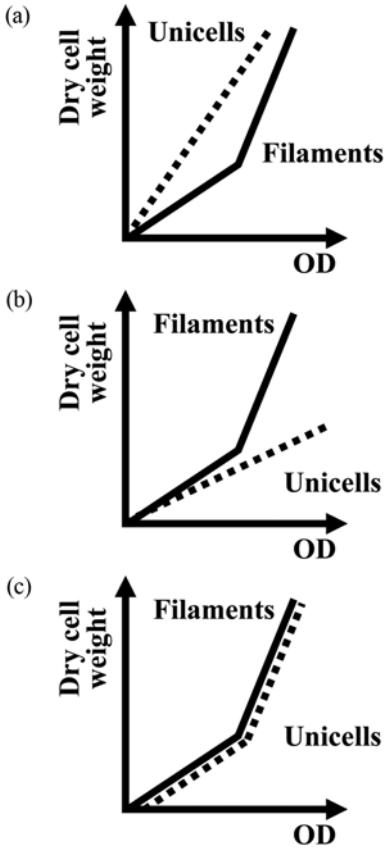

Fig. 3. Probable diagrams of the ratio of dry cell concentration to OD in filaments and unicells.

of chlorophyll a on the OD of cells was investigated (data not shown). The absorbance of chlorophyll a was the value measured in vitro.

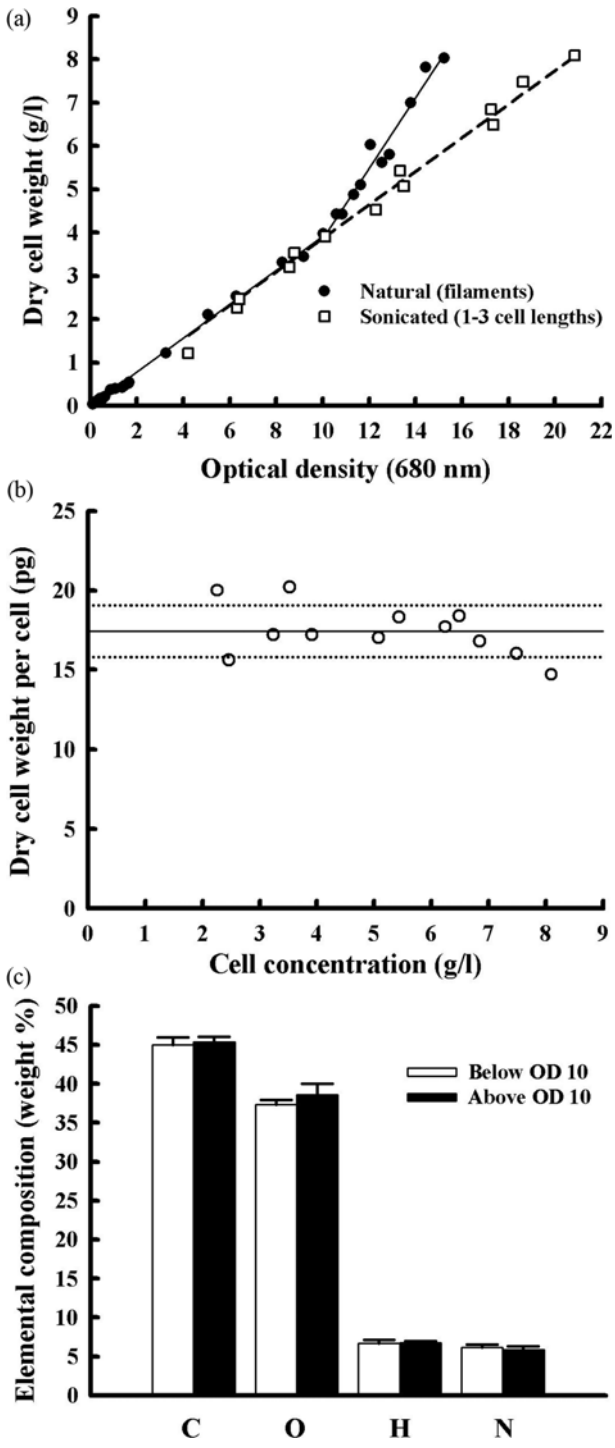

Fig. 4. Characteristics of filaments in Exp. 2. (a) The ratio of dry cell concentration to OD in natural and sonicated forms. Solid and dashed lines are the regression line between OD and dry cell concentration. (b) Constant unicell weight between various cell concentrations. The solid line is the average unicell weight and the dotted line is the standard deviation of unicell weight. (c) Elemental composition of cells above or below OD 10 (4 g dry cell/*l*).

Since the effect of chlorophyll a on the OD of cells was below 12%, chlorophyll a did not mainly affect the ratio of dry cell concentration to OD. After filaments were broken into unicells, the ratio of dry cell concentration to OD in unicells was not one of Fig. 3 (Fig. 4(a)). Therefore, filament length, unicell size, and unicell dry weight did not affect the ratio of dry cell weight to OD.

3. The Change of 3D Structure of Filaments

After physical disruption of filaments to unicells in experiment 2, the ratio of dry cell concentration to OD in the whole range was changed to the same value in the OD range of 0.100-10.0 in natural form (Fig. 4(a)). Since the ratio of dry cell concentration to OD

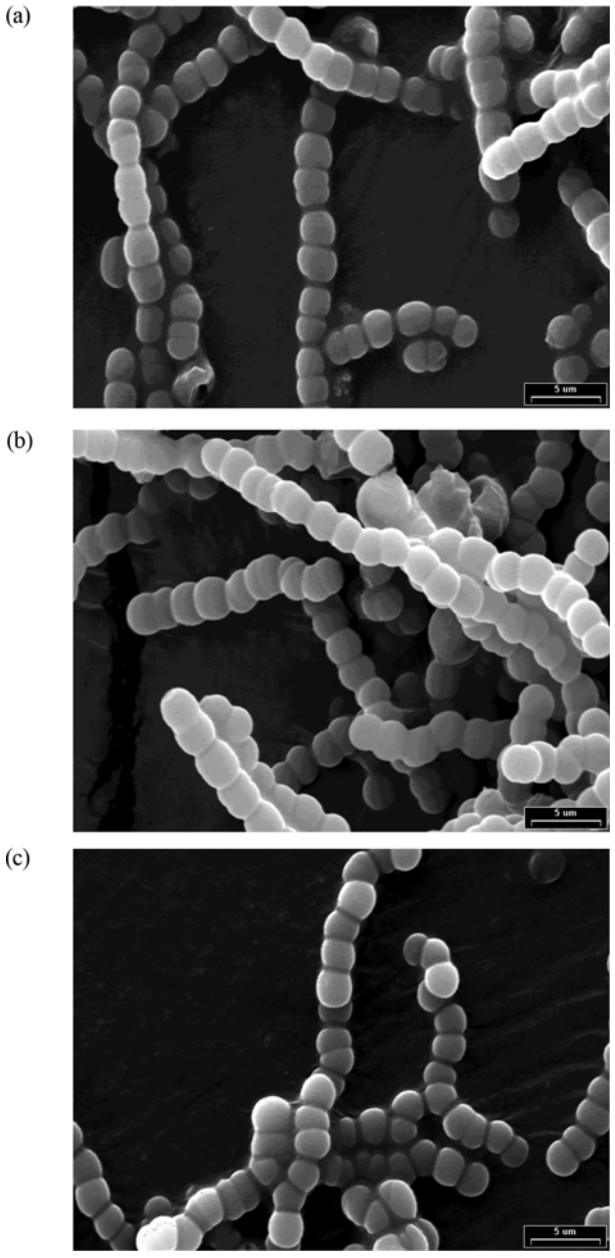

Fig. 5. SEM pictures of cell morphology. (a) Common morphology of cells below 4.0 g/*l* (OD 10). (b) Morphology of cells above 4.0 g/*l* (OD 10) in Exp. 1. (c) Morphology of cells above 4.0 g/*l* (OD 10) in Exp. 2.

in unicells in experiment 2 (Fig. 4(a)) was the same as that below OD 10 in filaments in experiment 2 (Fig. 2(b)) or that in filaments in experiment 1 (Fig. 2(a)), we assumed that the unicell weight in the filaments was the same regardless of cell growth.

To measure unicell weight in filaments, filaments in experiment 2 were broken into unicells by sonication. Cells were enumerated microscopically and were dried for measuring the dry cell weight. Data on the cell numbers per dry cell weight were analyzed by means of ANOVA with Microsoft Office Excel 2003 software. ANOVA analysis showed no difference between various cell concentrations for the cell numbers per dry cell weight (data not shown). This result suggests that there was no difference between various cell concentrations for unicell weight because the reciprocal of the cell numbers per dry cell weight is unicell weight (Fig. 4(b)). The unicell weight was 17±2 pg.

As mentioned, the ratio of dry cell concentration to OD in unicells was similar to that in natural form and the unicell weight was constant regardless of cell growth. These results suggest that the elemental composition of cells will be the same above or below OD 10. Actually, the content of carbon, oxygen, hydrogen, and nitrogen above OD 10 was similar to those below OD 10 (Fig. 4(c)).

From these results, we assumed that the morphological structures of filaments affected the ratio of dry cell concentration to OD.

4. SEM Pictures of Cell Morphology

To prove this hypothesis, it was necessary to examine the cell morphology using SEM. Fig. 5(a) shows that the cells below 4.0 g/*l* (OD 10) are put together to make slight curved filaments. In Fig. 5(b), the cell morphology above 4.0 g/*l* (Exp. 1) is similar to that below 4.0 g/*l*. However, in Fig. 5(c), cells above 4.0 g/*l* (Exp. 2) exhibit sinuous branched filaments. The surface area of the sinuous structure is smaller than that of slight curved structure because the contact surface area between cells in the sinuous structure is larger than that in slight curved structure. Although the SEM image analysis gives us a better understanding of the correlations between OD and cell concentration, it does not provide us with enough information for a quantitative analysis. Further investigation is required to obtain the relationship between the OD and the cell morphology.

ACKNOWLEDGEMENTS

This article is dedicated to Professor Hwa Yong Kim in honor of his retirement from the School of Chemical and Biological Engineering, Seoul National University.

REFERENCES

1. C. M. Harris and D. B. Kell, *Biosensors.*, **1**, 17 (1985).
2. S. O. Hwang and R. S. Feldberg, *Biotechnol. Prog.*, **6**, 48 (1990).
3. T. H. Park, J.-H. Seo and H. C. Lim, *Biotechnol. Bioeng.*, **34**, 1167 (1989).
4. S. D. Archer, K. A. McDonald and A. P. Jackman, *Appl. Biochem. Biotech.*, **67**, 139 (1997).
5. J. H. Yoon, S. J. Sim, M.-S. Kim and T. H. Park, *Int. J. Hydrogen Energy*, **27**, 1265 (2002).
6. H. Tanaka, H. Aoyagi and T. Jitsufuchi, *J. Ferment. Bioeng.*, **73**, 130 (1992).
7. C. Bédard, M. Jolicoeur, B. Jardin, R. Tom, S. Perret and A. Kamen, *Biotechnol. Technol.*, **8**, 605 (1994).
8. M. R. Brown, G. A. Dunstan, S. W. Jeffrey J. M. LeRoi, *J. Phycol.*, **29**, 601 (1993).
9. Q. Hu, N. Kurano, M. Kawachi, I. Iwasaki and S. Miyachi, *Appl. Microbiol. Biotechnol.*, **49**, 655 (1998).
10. C.-G. Lee and B. Ø. Palsson, *Biotechnol. Bioeng.*, **44**, 1161 (1994).
11. H. Qiang, H. Guterman and A. Richmond, *J. Phycol.*, **32**, 1066 (1996).
12. M. A. Tedesco and E. O. Duerr, *J. Appl. Phycol.*, **1**, 201 (1989).
13. P. A. Thompson, M. X. Guo and P. J. Harrison, *J. Phycol.*, **28**, 481 (1992).
14. J. H. Yoon, J.-H. Shin, M.-S. Kim, S. J. Sim and T. H. Park, *Int. J. Hydrogen Energy*, **31**, 721 (2006).
15. J. H. Yoon, S. S. Choi and T. H. Park, *Bioresour. Technol.*, **110**, 430 (2012).
16. J. H. Yoon, J.-H. Shin and T. H. Park, *Bioresour. Technol.*, **99**, 1204 (2008).
17. S.-L. Choi, I. S. Suh and C.-G. Lee, *Enzyme. Microb. Technol.*, **33**, 403 (2003).
18. J. H. Yoon, J.-H. Shin, E. K. Ahn and T. H. Park, *J. Microbiol. Biotechnol.*, **18**, 918 (2008).
19. S. S. Golden, Mutagenesis of cyanobacteria by classical and gene-transfer-based methods, *In* Packer, L. & Glazer, A. N. [Ed.] *Methods in Enzymology.* Vol. 167. Academic Press, San Diego, pp. 714-27 (1988).
20. A. G. Fontes, J. Moreno, A. Vargas and J. Rivas, *Biotechnol. Bioeng.*, **40**, 681 (1992).
21. D. Chamoviz, G. Sandmann and J. Hirschberg, *J. Biol. Chem.*, **268**, 17348 (1993).
22. M. J. Karnovsky, *J. Cell Biol.*, **27**, 137A (1965).
23. A. I. Majerník, M. Lundgren, P. McDermott, R. Bernander and J. P. Chong, *J. Bacteriol.*, **187**, 1856 (2005).
24. A. L. Koch, *Biochim. Biophys. Acta*, **51**, 429 (1961).
25. E. O. Powell and P. J. Stoward, *J. Gen. Microbiol.*, **27**, 489 (1962).