J. Microbiol. Biotechnol. (2005), **15**(3), 461–465

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Biological Fixation of CO_2 by *Chlorella* sp. HA-1 in a Semi-Continuous and Series Reactor System

LEE, JAE-YOUNG[1], TAE-SOON KWON, KITAE BAEK[2], AND JI-WON YANG*

Environmental Remediation Engineering Laboratory, Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science & Technology (KAIST), 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[1]*Transportation Key Technology Team, Korea Railroad Research Institute (KRRI), 360-1 Woalam-dong, Uiwang-City, Kyonggi-Do 437-757, Republic of Korea*
[2]*Department of Environmental Engineering, Kumoh National Institute of Technology (KIT), 188 Shinpyeong-dong, Gumi, Gyeongbuk 730-701, Republic of Korea*

Received: January 26, 2004
Accepted: December 18, 2004

Abstract Characteristics of biological CO_2 fixation by *Chlorella* sp. HA-1 were investigated in a semi-continuous and series reactor system using an internally illuminated photobioreactor to overcome shortcomings of physicochemical technologies such as adsorption and membrane separation. High CO_2 fixation rate was achieved in the semi-continuous reactor system, in which the dilution ratios of the culture medium were controlled. The average CO_2 fixation rate was maintained almost constantly when the dilution ratio increased by 0.1 increment from the initial value of 0.5. The total removal efficiency of CO_2 was enhanced by employing a series reactor system. The average CO_2 fixation rate increased until 4.013 g CO_2 day^{-1} in a series operation of four reactors, compared to 0.986 g CO_2 day^{-1} in a batch operation mode. The total CO_2 fixation rate was proportional to the number of reactors used in the series reactor system. In the series reactor system of semi-continuous operation, a large amount of CO_2 was removed continuously for 30 days. These results showed that the present reactor systems are efficient and economically feasible for a biological CO_2 fixation.

Key words: Semi-continuous operation, series reactor system, CO_2 fixation, *Chlorella* sp.

Biological CO_2 fixation is an efficient approach to reduce CO_2 concentration in the atmosphere, because of useful merits such as low investment cost, no need of a pre-treatment process for a flue gas, and reuse of biomass produced by CO_2 conversion [1–4]. Until now, most efforts have been focused on the development of a new photobioreactor [5–9]. In practice, various photobioreactors have been designed to offer economics of scale by enhancing the CO_2 fixation rate [9]. However, the amount of fixed CO_2 by conventional operation methods is relatively low, compared to the large amount of CO_2 released continuously in the flue gas. For example, the total volume of reactor needed to fix the total amount of CO_2 (135.6 ton/h) released from a 150 MW power station is about 150,000 m^3 in the microalgal density of 5 g l^{-1} after 10 h [1].

Therefore, the development of new operation systems as well as the design of efficient photobioreactors is needed to improve economics of scale. Many researchers have used a traditional batch or a simple continuous reactor for the biological fixation of CO_2 [10–13]. These reactor systems have certain drawbacks to maximize the amount of fixed CO_2 and to maintain a constant CO_2 fixation rate for a long time. In a batch reactor system, the CO_2 fixation rate decreased rapidly when the cell growth phase reached a stationary phase followed by a death phase. As the cell concentration increased in a batch reactor system, the cell growth rate decreased, because of the inhibition of illumination by shading effect [10–12]. Therefore, a batch reactor system must be improved to continuously remove a large amount of CO_2 for a long period. In a continuous reactor system, a high cell density was maintained, but the CO_2 fixation rate was not relatively high, compared to a batch reactor system [12, 13].

Consequently, it is necessary to develop new operation systems that simultaneously give a high CO_2 conversion and a constant CO_2 fixation rate during the CO_2 fixation process. In this study, an efficient biological CO_2 fixation system was developed by combining a semi-continuous and a series reactor system. The characteristics of CO_2 fixation and feasibility of each operation system were compared for field application.

*Corresponding author
Phone: 82-42-869-3964; Fax: 82-42-869-3910;
E-mail: jwyang@kaist.ac.kr

MATERIALS AND METHODS

Algal Strain and Medium

Chlorella sp. HA-1 was obtained from NIES (National Institute of Environmental Studies, Japan). *Chlorella* sp. HA-1 has resistance against a high CO_2 concentration and can be cultivated without a serious growth inhibition due to contamination by other microorganisms [10]. The medium used in this study was M4N medium, which was composed of KNO_3 5.0 g, KH_2PO_4 1.25 g, $MgSO_4 \cdot 7H_2O$ 2.5 g, $FeSO_4 \cdot 7H_2O$ 0.003 g, and trace metals (H_3BO_3 2.86 mg, $MnSO_4 \cdot 7H_2O$ 2.5 mg, $ZnSO_4 \cdot 7H_2O$ 0.22 mg, $CuSO_4 \cdot 5H_2O$ 0.08 mg, Na_2MoO_4 0.021 mg) solution 1.0 ml in 1 l of distilled water [13]. For a seed culture broth, the microalgae were activated by pre-cultivation in a 10-l cowboy reactor under continuous illumination at 10% (v/v) CO_2 concentration.

Semi-Continuous and Series Reactor System

Figure 1A shows an overall semi-continuous reactor system. An internally illuminated photobioreactor (55 cm in length, 9 cm in diameter, and 3 l in volume) was designed to enhance an illuminating surface-to-volume ratio [16]. Fluorescent lamps (FL20SD/18, Kumho, Korea) were used for a continuous illumination, and CO_2 concentration was 10% (v/v). The gas flow rate was 1 VVM (volume to volume per minute), and the gas was supplied from the bottom of the reactor. The cultivation temperature was controlled at 30°C using water jacket, and the pH of the medium was not adjusted. In a semi-continuous reactor system, an appropriate volume of cell suspension was replaced with fresh medium (called dilution), when the cell concentration was saturated [17]. Dilution ratio is defined as:

$$D=\frac{V_n}{V_r}$$

where D is the dilution ratio, V_r is the working volume of the reactor, and V_n is the volume of newly added medium.

The schematic diagram of a series reactor system is shown in Fig. 1B. CO_2 with 10% concentration was supplied as an input gas from the bottom of the first reactor, and then output gas from the first reactor was introduced into the bottom of the second reactor by air pump. Four reactors were connected in series in the same manner as described above. The gas flow rates of all reactors were 1 VVM, and the temperature was maintained at 30°C.

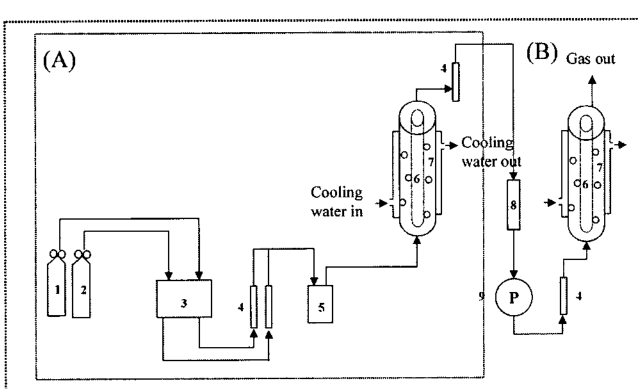

Fig. 1. Schematic diagram of a semi-continuous (**A**) and series operation (**B**) mode using an internally illuminated photobioreactor.
1, CO_2 bomb; 2, air bomb; 3, gas mixer; 4, flow meter; 5, humidifier; 6, fluorescent lamp; 7, photobioreactor; 8, silica gel; and 9, air pump.

Analysis

Cell growth was monitored by measuring the optical density (OD) at 660 m using a UV spectrophotometer (HP 8452A, Hewlett Packard, U.S.A.), which was converted into dry cell weight (DCW). DCW was obtained by drying 50 ml of the cell suspension at 105°C for 1 day after filtering through the pre-dried and pre-weighted 0.45 µm filter paper. The amount of fixed CO_2 was indirectly calculated by element analysis (CE EA-11 10, U.S.A.) of *Chlorella* sp. HA-1, because CO_2 was used as a sole carbon source during the algal photosynthesis.

RESULTS AND DISCUSSION

Semi-Continuous Reactor System

Cell growth rate in the semi-continuous reactor system was strongly influenced by the dilution ratio. In this study,

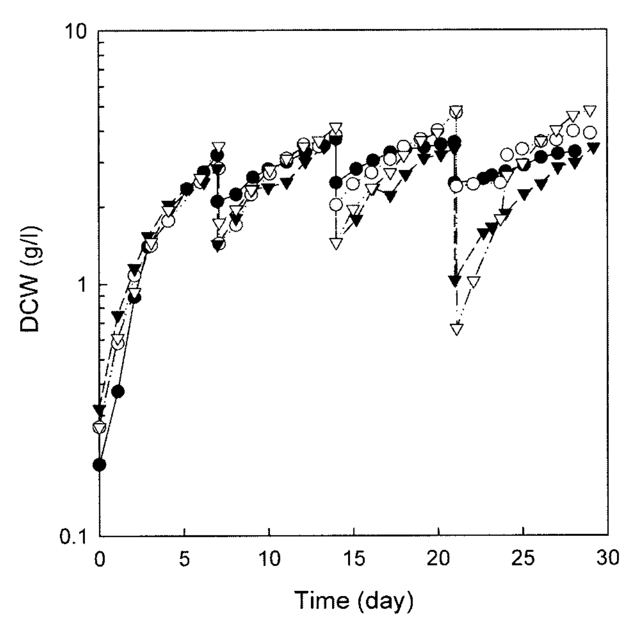

Fig. 2. Cell growth curves with dilution ratios in a semi-continuous reactor system.
● 0.3-0.3-0.3, ○ 0.5-0.5-0.5, ▼ 0.5-0.6-0.7, ▽ 0.5-0.7-0.9.

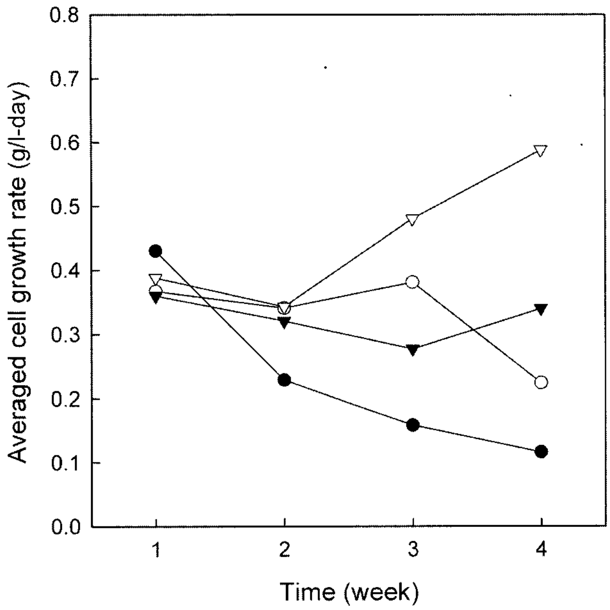

Fig. 3. Comparison of average cell growth rates with dilution ratios in a semi-continuous reactor system.
● 0.3-0.3-0.3, ○ 0.5-0.5-0.5, ▼ 0.5-0.6-0.7, ▽ 0.5-0.7-0.9.

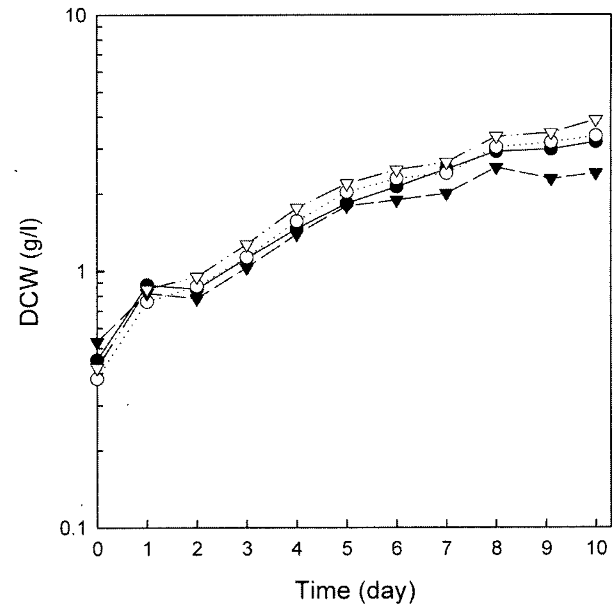

Fig. 4. Cell growth curves of four reactors in a series reactor system.
● 1st Reactor, ○ 2nd Reactor, ▼ 3rd Reactor, ▽ 4th Reactor.

effects of the dilution ratio were investigated in separate reactors by periodically changing the dilution ratios. As shown in Figs. 2 and 3, the average cell growth rate reduced gradually, when the dilution ratio was constantly maintained at 0.3 during four weeks. The decrease in the average cell growth rate was caused mainly by the shading effect, which is the shrinkage of light intensity to penetrate into the reactor due to high concentration of microalgae [1−16]. In a semi-continuous reactor system, the shading effect was diminished as cell concentration decreased after each dilution. However, the dilution ratio of 0.3 was not sufficient to reduce the shading effect.

At the constant dilution ratio of 0.5, the average cell growth rates during four weeks were 0.367, 0.341, 0.381, and 0.224 g l^{-1} day^{-1} in increasing order for each week: The average cell growth rate was sustained at a constant level during the first three weeks, but decreased rapidly at the beginning of the fourth week, because the cell concentration after the third dilution was still too high. Therefore, it is necessary to change the dilution ratio, considering the final cell concentration at each period.

When the dilution ratios increased during four weeks by the increment of 0.1 and 0.2 from the initial value of 0.5, the average cell growth rates were 0.360, 0.321, 0.277, 0.340 g l^{-1} day^{-1} and 0.388, 0.343, 0.480, 0.588 g l^{-1} day^{-1} for each week, respectively. The average cell growth rates did not significantly change with the dilution ratio increment of 0.1, but gradually increased with the increment of 0.2. From the practical point of view, however, the dilution ratio increment of 0.1 seemed to be an adequate value at the initial dilution ratio of 0.5 to maintain the CO_2 fixation rate at a certain level. To apply a semi-continuous reactor system in a field-scale operation, the optimum dilution ratio should be carefully decided, because the loss of substrate can be considered to be due to a large reactor volume.

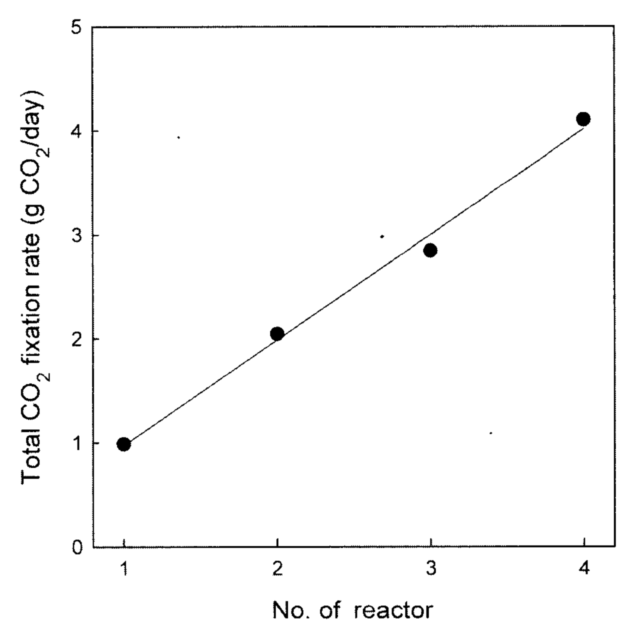

Fig. 5. Correlation of total CO_2 fixation rate and the number of connected reactors.

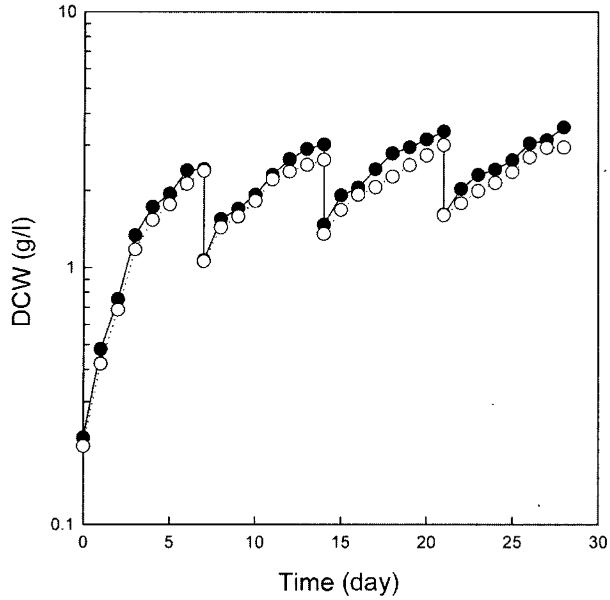

Fig. 6. Cell growth curves of two reactors in a series reactor system of semi-continuous operation.
● 1st Reactor, ○ 2nd Reactor.

Series Reactor System

Figure 4 shows the cell growth curves of four reactors in a series reactor system. The average cell growth rates of each reactor were 0.308, 0.331, 0.250, and 0.365 g l^{-1} day^{-1}, respectively. The total CO_2 fixation rate was significantly enhanced up to 4.013 g CO_2 day^{-1}, which is much higher than the 1.320 g CO_2 day^{-1} in a batch reactor system. The relationship between the total CO_2 fixation rate and the number of reactors connected in a series reactor system is described in Fig. 5. As expected, the total CO_2 fixation rate was proportional to the number of reactors connected in the series reactor system. Therefore, the series reactor system could be used to improve the total CO_2 fixation rate without enlarging the photobioreactor and to overcome difficulties that arise in the maintenance and operation of a large-scale photobioreactor.

Series Reactor System of Semi-Continuous Operation

In order to exploit the advantages of a semi-continuous and a series reactor system simultaneously, two reactor systems were combined. Figure 6 shows the cell growth curves of two reactors in the series reactor system of semi-continuous operation. As shown in the figure, the average cell growth rates were constantly maintained during the cultivation period, because of the semi-continuous reactor system. The total amount of fixed CO_2 increased as the number of reactors increased, due to the series reactor system. As a result, the combination of a semi-continuous and a series reactor system could continuously improve the efficiency of total CO_2 removal for a long time.

In order to overcome limitations of conventional operation systems of biological CO_2 fixation, a semi-continuous and a series reactor system were combined, which could continuously remove a large amount of CO_2 for a long period (Table 1). The problem of decrease of CO_2 fixation rate due to the shading effect could be eliminated by dilution in a semi-continuous reactor system, and a high removal rate of CO_2 was achieved by employing a series reactor system. Consequently, the series reactor system of semi-continuous operation could be used as an efficient operation method to remedy drawbacks of a conventional operation in a large-scale photobioreactor.

Acknowledgments

The authors gratefully acknowledge the financial support from KEMCO (Korea Energy Management Corporation) & Lafarge Halla Co. under Grant No. 1999-C-CD02-P-01 and also from National Research Laboratory Program of Korean Ministry of Science and Technology.

REFERENCES

1. Karube, I., T. Takeuchi, and D. J. Barnes. 1992. Biotechnological reduction of CO_2 emissions. *Adv. Biochem. Eng. Biotechnol.* **46:** 63–78.
2. Ral, L. C., H. D. Kumar, F. H. Mohn, and C. J. Soeder. 2000. Services of algae to the environment. *J. Microbiol. Biotechnol.* **10:** 119–136.
3. Jin, E., J. E. W. Polle, H. K. Lee, S. M. Hyun, and M. Chang. 2003. Xanthophylls in microalgae: From biosynthesis to biotechnological mass production and application. *J. Microbiol. Biotechnol.* **13:** 165–174.
4. Lee, J. S., D. K. Kim, J. P. Lee, S. C. Park, J. H. Koh, and S. J. Ohh. 2001. CO_2 fixation by *Chlorella* KR-1 using flue gas and its utilization as a feedstuff for chicks. *J. Microbiol. Biotechnol.* **11:** 772–775.

Table 1. Comparison of the operating efficiency of three reactor systems.

	Operation period (days)	The averaged cell growth rate (g/l-day)	Total amount of fixed CO_2 (g/day)
Batch [18]	7	0.413	1.320
Semi-Continuous (0.5-0.6-0.7)	30	0.360, 0.321, 0.277, 0.340 (per week)	1.038
Series (4 reactors)	7	0.308, 0.331, 0.250, 0.365 (per reactor)	4.013

5. Suh, I. S. and C.-G. Lee. 2003. Photobioreactor engineering: Design and performance. *Biotechnol. Bioprocess Eng.* **8:** 313–321.
6. Watanabe, Y. and D. O. Hall. 1996. Photosynthetic production of the filamentous cyanobacterium *Spirulina platensis* in a cone-shaped helical tubular photobioreactor. *Appl. Microbiol. Biotechnol.* **44:** 693–698.
7. Ogbonna, J. C., H. Yada, H. Masui, and H. Tanaka. 1996. A novel internally illuminated stirred tank photobioreactor for large-scale cultivation of photosynthetic cells. *J. Ferment. Bioeng.* **82:** 61–67.
8. Mori, Kei. 1995. Photoautotrophic bioreactor using visible solar rays condensed by fresnel lenses and transmitted through optical fibers. *Biotechnol. Bioeng. Symp.* **15:** 331–345.
9. Ogbonna, J. C. and H. Tanaka. 1997. Industrial-size photobioreactors. *Chemtech.* 43–49.
10. Watanabe, Y., N. Ohmura, and H. Saiki. 1992. Isolation and determination of cultural characteristics of microalgae which functions under CO_2 enriched atmosphere. *Energy Convers. Mgmt.* **33:** 545–552.
11. Negoro, M., N. Shioji, Y. Ikuta, T. Makita, and M. Uchiumi. 1992. Growth characteristics of microalgae in high-concentration CO_2 gas, effects of culture medium trace components, and impurities thereon. *Appl. Biochem. Biotech.* **34/35:** 681–692.
12. Hirata, S., M. Taya, and S. Tone. 1996. Characterization of *Chlorella* cell cultures in batch and continuous operations under a photoautotrophic condition. *J. Chem. Eng. Japan* **29:** 953–959.
13. Samson, R. and A. Leduy. 1985. Multistage continuous cultivation of blue-green alga *Spirulina maxima* in flat tank photobioreactors with recycle. *Can. J. Chem. Eng.* **63:** 105–112.
14. Yanagi, M., Y. Watanabe, and H. Saiki. 1995. CO_2 fixation by *Chlorella* sp. HA-1 and its utilization. *Energy Convers. Mgmt.* **36:** 713–716.
15. Yun, Y.-S. and J. M. Park. 1997. Development of gas recycling photobioreactor system for micro algal carbon dioxide fixation. *Kor. J. Chem. Eng.* **14:** 297–300.
16. Pulz, O. and K. Scheibenbogen. 1998. Photobioreactors: Design and performances with respect to light energy input. *Adv. Biochem. Eng. Biotechnol.* **59:** 124–148.
17. Kim, S.-J., G.-J. Kim, D.-H. Park, and Y.-W. Ryu. 2003. High-level production of astaxanthin by fed-batch culture of mutant strain *Phaffia rhodozyma* AJ-6-1. *J. Microbiol. Biotechnol.* **13:** 175–181.
18. Lee, J.-Y., T.-S. Kwon, H.-J. Kim, and J.-W. Yang. 2003. Effect of light on growth of *Chlorella* sp. HA-1 in large-scale photobioreactors for biological CO_2 fixation. *Kor. J. Biotechnol. Bioeng.* **18:** 340–345.