Bull. Soc. Sea Water Sci., Jpn., 73, 30 − 34 (2019)

Bulletin of the Society of
Sea Water Science, Japan

(Note)

Promotion of Cyanobacteria Growth Induced by Fine Bubble Injection

Sawako KATOH[1], Yoshinari WADA[1], Mitsunori KATAYAMA[1], Chikako NORO[1],
Kazuaki YOSHIMUNE[1], Toshihiko HIAKI[1] and Masakazu MATSUMOTO[1]*

In this study, a cultivation technique enabling growth promotion of cyanobacteria with low efficiency CO_2 absorption and conversion to organic compounds was developed utilizing the gas-liquid interfaces surrounding fine air bubbles as new reaction fields for photosynthesis. Cyanobacteria CO_2 absorption efficiency is increased in the regions near the fine bubble surfaces because of the accelerated CO_2 mass transfer caused by minimizing the bubble diameter, as well as cyanobacteria accumulation due to the negative charge on the fine bubble surface; hence, the rate of photosynthesis is increased and cyanobacteria growth is promoted. Fine air bubbles with an average bubble diameter (d_{bbl}) of 80 μm were continuously supplied to culture medium containing cyanobacteria in a semi-batch photobioreactor equipped with a self-supporting bubble generator, a fluorescent lamp, and a reaction vessel and the cyanobacteria were grown at a reaction temperature of 30 ℃. The light intensity was changed by the number of fluorescent lamps located inside and outside of the reaction vessel. Additionally, the d_{bbl} was varied along a range of 40 to 6,000 μm at a constant light intensity. Consequently, when the light intensity range was 37 - 527 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at a d_{bbl} of 80 μm, the specific growth rate of the cyanobacteria (r_{sg}) showed a maximum value at a light intensity of 365 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Furthermore, at a light intensity of 365 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the r_{sg} noticeably increased with a decrease in d_{bbl} and the r_{sg} at a d_{bbl} of 40 μm was approximately 2.5-fold greater than that at 1,500 μm. These results indicate that fine bubble injection promoted cyanobacteria growth in a semi-batch photobioreactor under CO_2-limited conditions.

Key Words : Fine bubbles, Photosynthesis, Cultivation, Cyanobacteria, Light Intensity

1. Introduction

The increase of atmospheric CO_2, which is considered a major greenhouse gas, plays a crucial role in global warming and climate change[1]. To control and reduce CO_2 emissions, CO_2 capture and storage technologies have been comprehensively developed for sequestration of CO_2 in combustion flue gas using chemical or biological strategies[2]. In particular, biological CO_2 fixation by photosynthetic microorganisms is receiving increased attention as a potential means to reduce CO_2 emissions, as well as achieve effective utilization of microalgal biomass[2]. As photosynthetic microorganisms, cyanobacteria convert solar energy to chemical energy in the form of ATP (Adenosine triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate) via photosynthesis in the cells, which is then used to fix CO_2 into organic compounds[3]. Thus, the synthetic metabolic pathways of cyanobacteria are able to generate various compounds directly from CO_2 using solar energy. Various chemicals have already been produced using cyanobacteria strains derived from *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942, as reported in several recent reviews[4-6]. For example, Hirokawa et al.[3] constructed an isopropanol-producing strain derived from *Synechococcus* sp. PCC 7942[3, 7], as isopropanol is readily converted to propylene via dehydration[8]. However, biological CO_2 fixation technology is still under development, as CO_2 capture and conversion efficiency is too low to be applicable in industry[1].

In this study, a cultivation technique was developed to promote cyanobacteria growth under light irradiation, as a biological CO_2 fixation technology; this technique utilizes the gas-liquid interfaces surrounding fine bubbles as new reaction fields where photosynthesis can proceed. Minimizing the bubble diameter in gas-liquid systems results in the following: i) acceleration of mass transfer and gas absorption by increasing the gas-liquid interfacial area, ii) an increase in the average residence time of the bubbles by decreasing the buoyancy, and iii) the occurrence of interactions near the gas-liquid interface caused by the electrification of the fine bubbles[9-11]. Accordingly, in the regions surrounding the gas-liquid interfaces of the fine bubbles, the contact efficiency of cyanobacteria and CO_2, i.e., the CO_2 absorption efficiency of cyanobacteria, remarkably improves, because the CO_2 mass transfer is accelerated by minimizing the bubble diameter and cyanobacteria accumulate via the negative charge on the fine bubble surface. Thus, the rate of photosynthesis increases and cyanobacteria growth should be promoted. In this paper, we report the

[1] College of Industrial Technology, Nihon University, 1-2-1 Izumi-cho, Narashino, Chiba 275-8575, Japan
* Corresponding author E-mail : matsumoto.masakazu@nihon-u.ac.jp Tel : 047-474-2850

effects of light intensity and average bubble diameter (d_{bbl}) on the specific growth rate of cyanobacteria (r_{sg}) in a semi-batch photobioreactor equipped with a self-supporting bubble generator.

2. Experimental

2.1 Preparation of culture medium containing cyanobacteria

Synechococcus sp. PCC 7942, a model species of filamentous cyanobacteria, was obtained from the Pasteur Culture Collection of Cyanobacteria (Paris, France). The culture medium for cyanobacteria growth was prepared by mixing BG11 medium and TAPS (*N*-Tris (hydroxymethyl) methyl-3-amino-propanesulfonic acid) buffer solution [pH 9.0]. The culture medium was inoculated with a pre-cultured cyanobacteria suspension at an optical density at 730 nm (OD_{730}) of 0.8 to 2.0.

2.2 Semi-batch photobioreactor equipped with a self-supporting bubble generator

Fig. 1 shows a schematic of the semi-batch photobioreactor consisting of a gas flow controller (FCC-3000-G1, KOFLOC Co.), a pH meter (S220, Mettler Toledo), a self-supporting bubble generator (Tech Ind.), a dispersing-type bubble generator (hole size of dispersing plate: 65-300 μm), internal and external fluorescent lamps, and a reaction vessel. The reaction vessel (usable volume of 1.5 L) consists of an acrylic tube with an inner diameter of 100 mm, 260 mm in length, and a wall thickness of 3.0 mm. Fine bubbles with a d_{bbl} of 40 or 80 μm were generated using a self-supporting bubble generator by the shear of the impeller and negative pressure owing to high-rotation[11-12]; the rotation rate was sustained at 2,100 min^{-1} and the air flow rate (F_{air}) was controlled at 2.23 or 4.46 $mmol \cdot L^{-1} \cdot min^{-1}$. In contrast, bubbles with a d_{bbl} of 200, 800, 1,500, or 6,000 μm were obtained using a dispersing-type generator at a F_{air} of 2.23 $mmol \cdot L^{-1} \cdot min^{-1}$. The d_{bbl} was determined based on the bubble size distribution measured with a laser particle size analyzer (SALD-2100, SHIMADZU Co., Ltd.) and by image analysis using a digital microscope (VH-5000, KEYENCE, Co.).

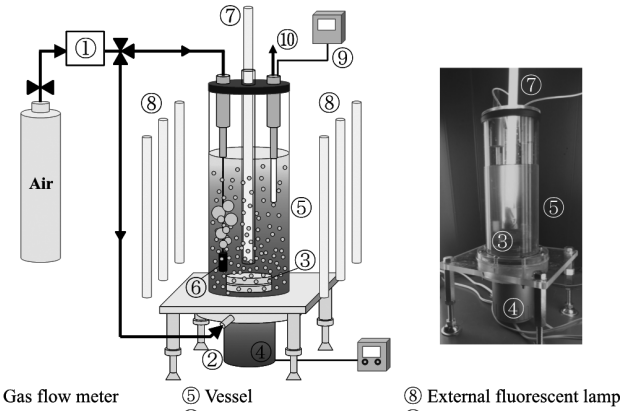

Fig. 1 Outline of the semi-batch photobioreactor equipped with a self-supporting bubble generator

2.3 Culturing cyanobacteria in the semi-batch photobioreactor

Fine air bubbles with a d_{bbl} of 80 μm were continuously supplied to 1 L of culture medium containing cyanobacteria in the reaction vessel. The culture was irradiated using fluorescent lamps located on the inside and outside of the reaction vessel and the cyanobacteria were grown at a reaction temperature of 30 ℃. The light intensity for cyanobacteria growth, as an operational parameter, was changed by varying the number of the internal and external fluorescent lamps in a range of 0 - 1 and 2 - 12, respectively. The light intensity was determined based on the average intensity values at four points near the center and four points near the bottom of the reaction vessel measured using a light meter (LI-250A, LI-COR, Inc.). Following photosynthesis and the progression of cyanobacteria growth for a specified length of time, the OD_{730} of 1 mL samples was measured using a UV-Vis spectrophotometer (UV-1800, SHIMADZU Co., Ltd.). Additionally, the OD_{730} was measured for samples grown in the supply of air bubbles with a d_{bbl} of 40, 200, 800, 1,500, or 6,000 μm under irradiation with an internal fluorescent lamp and eight external fluorescent lamps.

3. Results and Discussion

3.1 Optimization of light intensity for cyanobacteria growth in a semi-batch photobioreactor

As light intensity is the rate-limiting factor in photosynthesis and cyanobacteria growth depends on the light intensity and level of CO_2 absorption in the culture medium, light intensity optimization is necessary in order to effectively promote cyanobacteria growth by minimizing bubble diameter. **Table 1** shows the light intensity measurement results in a semi-batch photobioreactor equipped with a self-supporting bubble generator, with a varying number of internal and external lamps. The light intensity under irradiation with one internal lamp and two external lamps or with two external lamps was 253 or 37 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the difference in light intensity values between both conditions, i.e., the light intensity of one internal lamp, was 216 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Additionally, it was assumed that the light intensity of one internal lamp is approximately equivalent to 10 external lamps, as the light intensity under irradiation of one internal lamp and

Table 1 Light intensity of the cultivation system in the semi-batch photobioreactor under different configuration of fluorescent lamps

Number of fluorescent lamps [-]		Light intensity [μmol·m^{-2}·s^{-1}]
internal	external	
0	2	37
1	2	253
1	4	285
1	6	324
1	7	349
1	8	365
1	10	429
1	12	527

10 external lamps showed a value of 429 μmol·m^{-2}·s^{-1}.

Fig. 2 shows the comparison of changes in OD_{730} over time when fine air bubbles with a d_{bbl} of 80 μm were continuously supplied to culture medium containing cyanobacteria with various light intensity values. The tendency of the OD_{730} to increase with photosynthesis progression was observed at all light intensity values. At a light intensity of 365 μmol·m^{-2}·s^{-1} under illumination with one internal lamp and eight external lamps, the OD_{730} markedly increased from 5 h onward. Furthermore, when a light intensity of 527 μmol·m^{-2}·s^{-1} was irradiated using one internal lamp and 12 external lamps, the increase in OD_{730} with reaction time (t) was more gradual than that observed at a light intensity of 365 μmol·m^{-2}·s^{-1}. The r_{sg}, calculated based on the gradient of the change in OD_{730} over time, was plotted by light intensity, as shown in **Fig. 3**. The maximum r_{sg} value was observed at a light intensity of 365 μmol·m^{-2}·s^{-1}. Generally, although light has been recognized as a key environmental factor for microalgal growth, excessive exposure to light leads to high light stress through photo-oxidative damage including over-production of active oxygen species, resulting in reduced microalgae growth[13]. Consequently, under a light intensity range of 37 to 365 μmol·m^{-2}·s^{-1}, light was considered the rate-limiting factor for photosynthesis, as cyanobacteria growth was enhanced by increasing the light intensity. Above 365 μmol·m^{-2}·s^{-1}, cyanobacteria growth inhibition was induced by high light stress, because the absorbed light energy exceeded the energy consumed and dissipated within the cells.

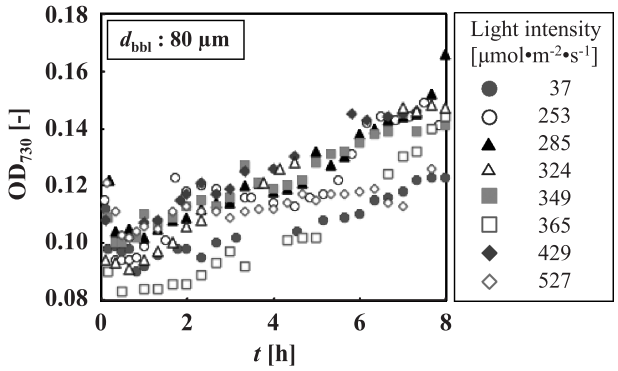

Fig. 2 Light intensity-dependant changes of OD_{730} in continuous bubbling (d_{bbl} : 80 μm)

3.2 Effects of minimizing bubble diameter on cyanobacteria growth in the semi-batch photobioreactor

When CO_2 absorption from the air bubbles is the rate-limiting factor of photosynthesis, the effects of minimizing the bubble diameter on cyanobacteria growth become more pronounced. Therefore, air bubbles with different d_{bbl} values were continuously supplied to culture medium containing cyanobacteria, under a constant light intensity of 365 μmol·m^{-2}·s^{-1}, and the r_{sg} values, calculated based on the gradient of the change in OD_{730} over time, were plotted against the d_{bbl}, as shown in **Fig. 4**. The r_{sg} increased with decreasing d_{bbl}; the r_{sg} value at a d_{bbl} of 40 μm reached 0.0690 g·g^{-1}·h^{-1}. These findings might be due to the acceleration

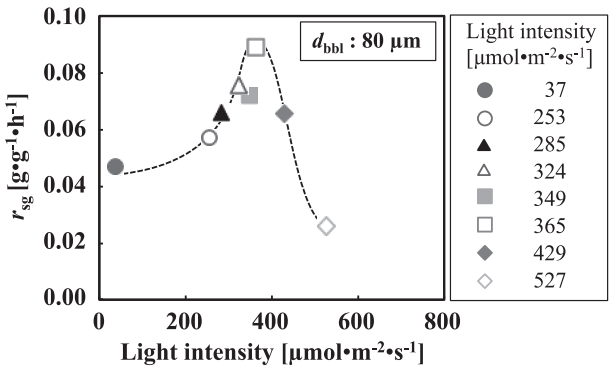

Fig. 3 Association between light intensity and r_{sg} in the semi-batch photobioreactor

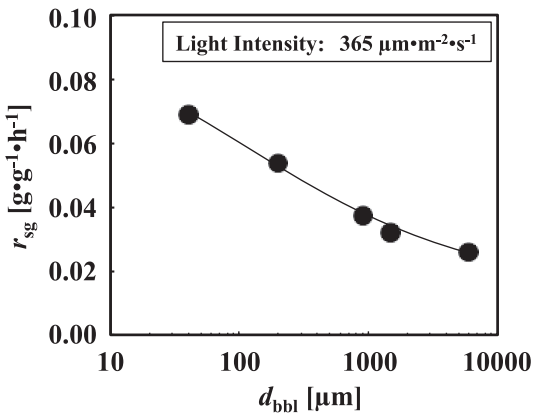

Fig. 4 Association between d_{bbl} and r_{sg} in the semi-batch photobioreactor

of CO_2 absorption and the electrification of the bubble surface caused by downsizing the air bubbles[9, 14].

According to a previous study examining the fundamental properties of fine bubbles in liquid phase, the gas-liquid interfacial area based on the unit gas volume of bubbles at a d_{bbl} of 10 μm is 100-fold greater than that at 1,000 μm. Furthermore, the inner pressure of fine bubbles with a d_{bbl} of 10 μm increases to 2.9×10^4 Pa in comparison with the surrounding pressure of fine bubbles. The mass transfer amount of bubbles, based on the unit time, to the liquid phase increases 6×10^4-fold when the d_{bbl} is reduced from 1,000 to 10 μm, assuming that the mass transfer resistance in the gas phase is negligible. The flotation rate of bubbles at a d_{bbl} of 10 μm in water is reduced to $1/1.7 \times 10^3$ and the mass transfer amount based on the unit flotation distance increases to 1.0×10^8 compared with the mass transfer amount at 1,000 μm[11, 15, 16]. In previous research[17] on CO_2 absorption, the physical properties of the liquid phase were evaluated by continuously feeding of CO_2 fine bubbles into distilled water, Tris-HCl [pH 7.8], $Ca(NO_3)_2$, or $Ca(NO_3)_2$/Tris-HCl [pH 7.8], and the gas absorption rate of CO_2 increased with decreasing the bubble diameter, irrespective of the different solutions. Additionally, Yang et al.[18] investigated the influence of air bubble diameter (1.5, 2.5, 3.5, or 6.0 mm) on the growth of microalgal strain *Chlorella* sp. SDEC-18, lipid productivity, and lipid properties in the photobioreactors equipped with four different finepore diffusers. They reported that the maximal lipid productivity was observed in the system with small bubbles, because the increase in the bubble diameter decreases the mass transfer coefficient. Moreover, the electrification of fine bubbles was examined based on the zeta potential measurement to determine the occurrence of interactions in the regions surrounding the gas-liquid interfaces[10, 11, 16, 19]. The zeta potential on the fine bubble surface at a d_{bbl} of 10 - 30 μm in distilled water was a negative value between -50 and -100 mV. Additionally, Martinez et al.[20] reported that the zeta potentials of active *Synechococcus* sp. and *Planktothrix* sp. cyanobacteria in $NaNO_3$ solutions, with a concentration of 0.001 to 0.1 mol·L^{-1}, show a maximum value of approximately +12 mV at a pH of 8 to 10. These findings indicate that an increase in the negative charge on the bubble surface, due to minimizing the bubble diameter, resulted in the accumulation of cyanobacteria with a positive charge in the regions surrounding the fine gas-liquid interfaces. Therefore, as the accumulated cyanobacteria and dissolved CO_2 contact with high probability near the fine bubble surface, cyanobacteria photosynthesis progresses efficiently. Consequently, fine bubble injection, which enables the acceleration of CO_2 mass transfer and the accumulation of cyanobacteria in the boundary area of the gas-liquid interfaces of the fine bubbles, can effectively promote cyanobacteria growth.

4. Conclusion

The growth of cyanobacteria with low CO_2 capture efficiency and conversion to organic compounds was enhanced using the gas-liquid interfaces surrounding fine air bubbles as new reaction fields for photosynthesis. The fine air bubbles were continuously supplied to culture medium containing cyanobacteria in a semi-batch photobioreactor equipped with a self-supporting bubble generator and the light intensity for photosynthesis was changed by the number of fluorescent lamps located inside and outside of the reaction vessel. Additionally, the average bubble diameter (d_{bbl}) was altered in a range of 40 - 6,000 μm at a constant light intensity. Consequently, when the light intensity was varied along the range of 37 to 527 μmol·m^{-2}·s^{-1} at a constant d_{bbl} of 80 μm, the maximum specific cyanobacteria growth rate (r_{sg}) was observed at a light intensity of 365 μmol·m^{-2}·s^{-1}. Moreover, at a constant light intensity of 365 μmol·m^{-2}·s^{-1}, the r_{sg} increased with decreasing d_{bbl} and the r_{sg} value at a d_{bbl} of 40 μm increased approximately 2.5-fold compared to that at 1,500 μm. These findings indicate that cyanobacteria growth in a semi-batch photobioreactor can be promoted by minimizing the bubble diameter under CO_2-limited conditions.

References

1) P. H. Chen, H. L. Liu, Y. J. Chen, Y. H. Cheng, W. L. Lin, C. H. Yeh and C. H. Chang, Enhancing CO_2 bio-mitigation by genetic engineering of cyanobacteria, *Energy. Environ. Sci.*, **5**, 8318-8327 (2012)

2) B. Zhao and Y. Su, Process effect of microalgal-carbon dioxide fixation and biomass production: A review, *Renewable Sustainable Energy Rev.*, **31**, 121-132 (2014)

3) Y. Hirokawa, I. Suzuki and T. Hanai, Optimization of isopropanol production by engineered cyanobacteria with a synthetic metabolic pathway, *J. Biosci. Bioeng.*, **119**, 585-590 (2015)

4) L. Rosegaard, A. J. de Porcellinis, J. H. Jacobsen, N. U. Frigaard and Y. Sakuragi, Bioengineering of carbon fixation, biofuels, and biochemical in cyanobacteria and plants, *J. Biotechnol.*, **162**, 134-147 (2012)

5) B. Wang, J. Wang, W. Zhang and D.R. Meldrum, Application of synthetic biology in cyanobacteria and algae, *Front. Microbiol.*, **3**, 1-15 (2012)

6) Y. Yu, L. You, D. Liu, W. Hollinshead, Y. J. Tang and F. Zhang, Development of Synechocystis sp. PCC 6803 as a phototropic cell factory, Mar. Drugs, **11**, 2894-2916 (2013)

7) T. Kusakabe, T. Tatsuke, K. Tsuruno, Y. Hirokawa, S. Atsumi, J.C. Liao and T. Hanai, Engineering a synthetic pathway in cyanobacteria for isopropanol production directly from carbon dioxide and light, *Metab. Eng.*, **20**, 101-108 (2013)

8）C.L. Kibby and W.K. Hall, Studies of acid catalyzed reactions: XII. Alcohol decomposition over hydroxyapatite catalysts, *J. Catal.*, **29**, 144-159（1973）
9）M. Matsumoto, T. Fukunaga and K. Onoe, Polymorph control of calcium carbonate by reactive crystallization using microbubble technique, *Chem. Eng. Res. Des.*, **88**, 1624-1630（2010）
10）Y. Wada, M. Matsumoto and K. Onoe, Utilization of minute-bubble technique on crystallization: Application to antisolvent crystallization of sodium chloride, *J. Cryst. Growth*, **373**, 92-95（2013）
11）Y. Tsuchiya, Y. Wada, T. Hiaki, K. Onoe and M. Matsumoto, Effects of CO_2 fine bubble injection on reactive crystallization of dolomite from concentrated brine, *J. Cryst. Growth*, **469**, 36-41（2017）
12）M. Matsumoto, Y. Wada, A. Oonaka and K. Onoe, Polymorph control of glycine by antisolvent crystallization using nitrogen minute-bubbles, *J. Cryst. Growth*, **373**, 73-77（2013）
13）P. J. le B. Williams and L. M. L. Laurens, Microalgae as biodiesel & biomass feedstocks: Review and analysis of the biochemistry, energetics and economics, *Energy Environ. Sci.*, **3**, 554-590（2010）
14）M. Matsumoto, T. Fukunaga, M. Suzuki and K. Onoe, Reactive crystallization of $CaCO_3$ using CO_2/NH_3 bubbles -Effects of bubble size and molar ratio of CO_2/NH_3 on polymorphism-, *Bull. Soc. Sea Water Sci. Jpn.*, **64**, 11-18（2010）
15）H. Tsuge（Ed.）, Micro- and Nanobubbles: fundamentals and applications, Pan Stanford Publishing, Singapore（2014）
16）Y. Tsuchiya, Y. Wada, K. Masaoka, T. Sato, M. Okada, T. Hiaki, K. Onoe and M. Matsumoto, Reactive crystallization of dolomite by CO_2 microbubble injection into concentrated brine, *Bull. Soc. Sea Water Sci. Jpn.*, **71**, 103-109（2017）
17）M. Matsumoto, M. Isago and K. Onoe, The application of micro-bubbles for dissolution and crystallization of calcium carbonate in gas-liquid-solid system, *Bull. Soc. Sea Water Sci. Jpn.*, **58**, 475-486（2004）
18）Z. Yang, H. Pei, F. Han, Y. Wang, Q. Hou and Y. Chen, Effects of air bubble size on algal growth rate and lipid accumulation using fine-pore diffuser photobioreactors, *Algal Research*, **32**, 293-299（2018）
19）M. Takahashi, ζ potential of microbubbles in aqueous solutions: Electrical properties of the gas−water interface, *J. Phys. Chem. B*, **109**（46）, 21858-21864（2005）
20）R. E. Martinez, E. Gardés, O. S. Pokrovsky, J. Schott and E. H. Oelkers, Do photosynthetic bacteria have a protective mechanism against carbonate precipitation at their surfaces?, *Geochim. Cosmochim. Acta*, **74**, 1329-1337（2010）

（平成 30 年 10 月 10 日受付，平成 30 年 11 月 26 日採用決定
Received October 10, 2018, Accepted November 26, 2018）

ファインバブルの導入によるシアノバクテリアの増殖促進

加藤 佐和子[1]，和田 善成[1]，片山 光徳[1]，野呂 知加子[1]，
吉宗 一晃[1]，日秋 俊彦[1]，松本 真和[1]*

要 旨

本研究では，ファインバブルの気−液界面を光合成が進行する新規な反応場として利用し，CO_2 吸収および有機化合物への転換効率の低いシアノバクテリアの増殖促進技術を開発した．ファインバブルの気 - 液界面近傍では，気泡の負の表面電位特性によってシアノバクテリアの濃縮が引き起こされ，気泡の微細化にともなう CO_2 物質移動の促進によってシアノバクテリアの CO_2 吸収効率が高まる．結果として，光合成速度の増大によるシアノバクテリアの増殖促進が期待できる．反応温度が 303 K において，自吸式微細気泡発生器，蛍光灯，反応槽からなる半回分式バイオリアクターを用いて，シアノバクテリアを含む培地に平均気泡径（d_{bbl}）が 80 μm のファインバブルを連続供給し，シアノバクテリアを増殖させた．光強度は反応槽の内部および外部に設置した蛍光灯の本数により変化させた．さらに，光強度が一定の条件下で d_{bbl} を 40 から 6000 μm の範囲で変化させた場合についても検討した．その結果，d_{bbl} が 80 μm で光強度を 37 - 527 μmol・m^{-2}・s^{-1} の範囲で変化させた場合，シアノバクテリアの比増殖速度（r_{sg}）は光強度が 365 μmol・m^{-2}・s^{-1} において極大値を示した．さらに，光強度が 365 μmol・m^{-2}・s^{-1} で d_{bbl} を変化させた場合，d_{bbl} の減少にともない r_{sg} が増大する傾向が得られ，d_{bbl} が 40 μm での r_{sg} は 1,500 μm の約 2.5 倍となった．これより，CO_2 の供給律速条件下では，ファインバブルの導入によりシアノバクテリアの増殖を促進できることが示唆された．

キーワード：ファインバブル，光合成，培養，シアノバクテリア，光強度

[1] 日本大学生産工学部（〒 275-8575 千葉県習志野市泉町 1-2-1）
* Corresponding author E-mail：matsumoto.masakazu@nihon-u.ac.jp Tel：047-474-2850