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AN INNOVATIVE CULTURE TECHNIQUE FOR MICROALGAE USING HOLLOW FIBER MEMBRANES

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ABSTRACT

The microalgal culture has been carried out utilizing the photobioreactor filled with hollow fibers of the gas exchange membrane, in place of the conventional air bubbling system which has been adopted to supply carbon dioxide in most conventional microalgal culture. The microalgal growth rate for *Chlorella vulgaris Beijerinck.* was measured using three culture systems in order to examine the usefulness of hollow fiber membranes for the microalgal culture. The microalgal growth rate within photobioreactor filled with hollow fibers was found three times greater than that of the bubbling culture system. On the other hand, the effective mass transfer coefficient of the carbon dioxide has been also measured within the present photobioreactor. It was found that the difference in dissolution behavior is indiscernible between the hollow fiber and bubbling culture systems in view of the culture period, since the carbon dioxide concentration in the culture medium reached in equilibrium after 2 hours regardless of culture systems. Moreover, it was found that the carbon dioxide supply can be significantly reduced by adopting the hollow fiber culture system of end-sealed hollow fibers while keeping the high microalgal growth rate.

NOMENCLATURE

a_l	= specific surface area [1/m]
A_{int}	= interface area between the liquid and membrane phases [m^2]
c	= total carbon concentration [kg/m^3]
c_M	= microalgal concentration [kg/m3]
c_{feed}	= CO_2 concentration of mixed air [%]
d_m	= outer diameter of the hollow fiber [m]
D	= inner diameter of the hollow fiber membrane device [m]
D_l	= carbon dioxide diffusion coefficient [m^2/s]
h_{eff}	= effective mass transfer coefficient [m/s]
Lx	= light intensity [$\mu mol/m^2s$]
n_i	= unit vector pointing outward from the liquid side to membrane side [-]
Q	= volume flow rate of the feed gas[m^3/s]
t	= time [s]
T	= temperature [℃]
t_m	= membrane thickness [m]
V	= representative elementary volume [m^3]
V_l	= total volume of liquid phase [m^3]

INTRODUCTION

The biomass fuel, which is carbon neutral to the environment by absorbing carbon dioxide, was successfully produced from the microalgae by several attempts [1, 2]. A report published by Y. Chisti [1] indicates that the production efficiency of the biomass fuel from microalgae is about ten times as high as that from palm. Various microalgal reactors have been proposed in view of the microalgal growth rate, cost and contamination etc.[2]. Especially, open ponds are the most widely applied in industrial processes [3], which are composed of long single or multiple loop channels placed outdoors. On the other hand, photobioreactors [4-10] have drawn attention since they can maintain a high biomass production and reduce the risk of contamination by controlling temperature and light intensity at favorable conditions for microalgal species.

Culture conditions, namely, light, minerals, temperature and CO_2 concentration are important factors to affect the microalgal growth rate. Microalgae culture systems are illuminated by artificial light or solar light [11-13]. The optimal temperature for microalgae cultures is generally between 20 and 25 ◦C [14]. On the other hand, it is also well-known that the carbon dioxide plays an important role in microalgal culture [15]. Chiu et al. [16] fed the mixed air controlled at high CO_2 concentration 10-15%v/v into microalgal culture of *Chlorella sp.*. Furthermore, Hirata et al. [17] revealed

that *Chlorella sp.* can grow in up to 40% CO_2 concentration at 30◦C.

In most conventional microalgae culture, an air bubbling has been adopted to supply the carbon dioxide. On the other hand, Carvalho et al. [18] mentioned that the membrane makes it possible to diffuse the carbon dioxide into the microalgae culture in low gas pressures. Subsequently, photobioreactors using membranes have been reported for cultures of mammalian cells [19], Chlamydomonas reinhardtii [20], P. tricornutum in seawater [21] and Anabaena Variabilis [22].Under these situations, Sano et al.[23] proposed a hollow fiber culture system for supplying the carbon dioxide to the microalgae. They found that the microalgal growth rate can be enhanced by exploiting the gas exchange membranes in the microalgal culture as compared with a non-membrane photobioreactor. However, in their system, almost CO_2 gas flew out from the end of hollow fibers.

In this study, a hollow fiber culture system proposed by Sano et al. has been improved for both enhancing the microalgal growth rate and reducing the carbon dioxide usage. The effective mass transfer coefficient of the carbon dioxide was measured within the present photobioreactor of end-sealed hollow fibers. The carbon dioxide consumption within the present photobioreactor is compared against that of other cultures containing a bubbling system, so as to examine the usefulness of hollow fiber membranes. Subsequently, we shall evaluate the microalgal growth rate for *Chlorella vulgaris Beijerinck.* within the present photobioreactor.

MATERIALS

1 Culture of chlorella vulgaris Beijerinck.

Chlorella vulgaris Beijerinck (Kokuritsu Kenkyujo) has been selected in this study. C-type medium widely used for the microalgal culture of *Chlorella sp.* was adopted to the culture medium. All components of C-type medium are listed in table 1 and table 2. The culture conditions, namely, temperature and light intensity for *Chlorella vulgaris Beijerinck.* were previously determined by a set of careful cultivations carried out in 100 mL glass beakers illuminated by the light emitting diode (Red and Blue, AG3-BP, AGRIng) under the several temperature 20-30℃. Thus, temperature 26℃ and the light intensity 40 $\mu molm^{-2}s^{-1}$ were adopted in this study. The microalgal concentration was measured through its correlation to absorbance of the dry weight for *Chlorella vulgaris Beijerinck.* at 690 nm by using an absorption spectrometer (AS ONE, ASV11D).

2 Hollow fiber devices

The microalgal culture within a hollow fiber membranes is schematically shown in Figure 1, in which only outer fiber is shown for clarity. Several thousands of small silicon tubes are arranged in order to allow to the carbon dioxide diffuse into the microalgal culture through gas exchange membranes. A hollow fiber device

Table 1 Ingredient list of C nutrient medium

Ingredient	Concentration [mg/mL]	Quantity [mL]	Mass [g]
$Ca(NO_3)_2 \cdot 4H_2O$	10	15	
KNO_3	10	10	
β–Na_2glycerophosphate $\cdot 5H_2O$	10	10	
$MgSO_4 \cdot 7H_2O$	10	10	
Vitamin B_{12}	0.001	0.1	
Biotin	0.001	0.1	
Thiamine HCl	0.1	0.1	
PIV metals		1.5	
Tris (hydroxymethyl) aminomethane			500
Distilled water		963	

Table 2 Ingredient list of PIV metals

Ingredient	Concentration [mg/mL]	Quantity [mL]	Mass [g]
Distilled water		485.8	
$Na_2EDTA \cdot 2H_2O$			0.5
$FeCl_3 \cdot 6H_2O$	10	9.8	
$MnCl_2 \cdot 4H_2O$	10	1.8	
$ZnSO_4 \cdot 7H_2O$	10	1.1	
$CoCl_2 \cdot 6H_2O$	10	0.2	
$Na_2MoO_4 \cdot 2H_2O$	1	1.25	

M40-3000 produced by Nagayanagi Kogyo (Japan) was applied for the photobioreactor of *Chlorella vulgaris Beijerinck* by reference to Sano et al. [23]. Geometrical details of the membrane module provided by the manufacturer are listed in Table 3. The CO_2 diffusion process through a silicon membrane takes place as CO_2 molecules are adsorbed on the membrane interface, and then diffuse through the membrane, finally dissolved to the liquid side. The carbon dioxide penetrates through the membrane due to the partial pressure difference between gas phase (inside hollow fibers) and culture medium phase (outside hollow fibers).

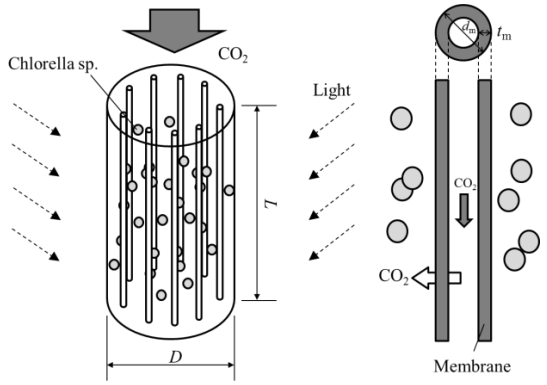

Figure 1: Photobioreactor using a hollow fibers

Table 3 Geometrical details of the membrane module

	N[-]	d_m [mm]	t_m[mm]	D[mm]	L [mm]
M40-3000	3000	0.25	0.04	31	135

METHODS

1 Microalgae culture using a hollow fiber membrane device

The present hollow fiber culture system for microalgae is schematically illustrated in Figure 2. This experimental set-up was placed in a constant temperature room, in which the temperature was kept to 26℃. The inside and outside temperatures on photobioreactor were

monitored to adjust the microalgal culture condition 26℃ by using a fan. The hollow fiber membrane device was placed in the tunnel illuminated by six LED lights (AG3-BP, AGRIng). The light intensity was adjusted to 40 $\mu molm^{-2}s^{-1}$ at the surface of the cylinder for photobioreactor using a hollow fiber membrane device, as shown in Figure 3. The apparatus was covered with a black curtain to block the light from outside.

Mixed air was fed into one end of the hollow fiber module from a mixed air tank (CO_2 concentration 20%v/v). This mixed air flows inside hollow fibers, and then penetrates through the membrane due to the partial pressure difference between gas phase (inside hollow fibers) and culture medium phase (outside hollow fibers). In this study, the other end of the hollow fiber module was sealed to reduce the carbon dioxide consumption within the hollow fiber culture system. Therefore, two culture systems, which one of the end was closed or opened (hereinafter called the closed and opened culture systems), were adopted to compare about enhancing the microalgal growth rate of the microalgae and reducing the carbon dioxide consumption.

The air volume flow rate was set to 50 ml/min in the opened culture system. In this experiment, an inside pressure of hollow fibers may be considered as an atmosphere pressure since the mixed air leaves to the ambient air from a hollow fiber module. However, the air volume flow rate of the closed culture system was not captured by a flowmeter since the permeation of the mixed air through the membranes is very small. Therefore, the secondary pressure of the mixed air tank was set to an almost atmosphere pressure in order to compare with the opened culture system.

Moreover, in this study, an air bubbling was carried out within a non-membrane photobioreactor, which is a cylindrical acrylic tank as large as hollow fiber membrane devices, in order to examine the usefulness of the present photobioreactor filled with hollow fiber membranes. The mixed air was fed by an air bubbling from the bottom of a cylindrical tank. The air volume flow rate was set to 50 ml/min in order to compare the results against other hollow fiber culture systems. In this study, the microalgal growth rate for *Chlorella vulgaris Beijerinck.* was estimated in all cultivation systems.

2 Mass transfer through membranes

The overall mass transfer coefficient of the carbon dioxide from the gas phase to the culture medium phase was estimated by exploiting the present photobioreactor, as illustrated in Figure 2. In this experiment, pure water and pure CO_2 gas were utilized in place of the culture solution and the mixed air for an accurate measurement of the total CO_2 concentration. Pure water was filled in the liquid phase of a present photobioreactor (namely shell side of the membrane), and then pure CO_2 gas was fed inside hollow fibers. Total carbon concentration was measured using a multi-water quality meter (MM60, ToaDKK) with a carbonic acid electrode (CE-2041, ToaDKK).

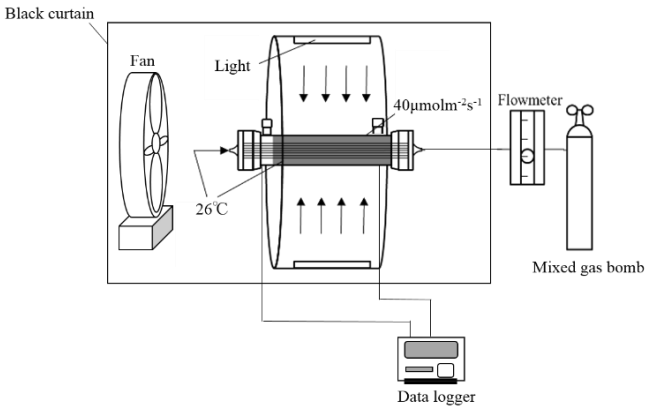

Figure 2 Hollow fiber culture system

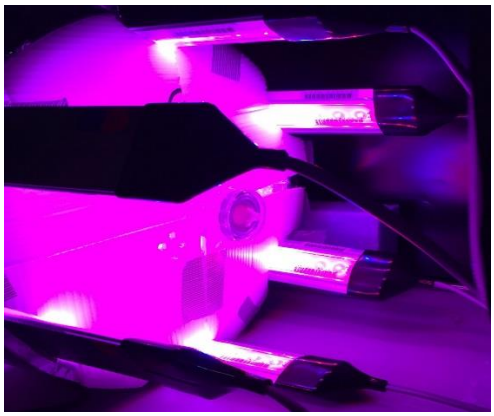

Figure 3 Picture of the hollow fiber culture system

Sano et al. [23] introduce the volume averaging theory for describing the mass transport phenomenon through the gas exchange follow fiber membrane within the microalgal culture. According to Goldman et al. [24] and Becker [25], the resistance at the gas-liquid interface is much larger than that at the boundary layer of the cells. We obtain an unsteady mass balance equation for the carbon dioxide passing through membranes as follows:

$$V_l \frac{\partial \bar{c}}{\partial t} = \int_{A_{\text{int}}} D_l \frac{\partial c}{\partial x_j} n_{l\,j} dA = A_l h_{eff} (\bar{c}^* - \bar{c}) \tag{1}$$

where the bulk average of a certain variable ϕ in the liquid phase is defined as

$$\bar{\phi} \equiv \frac{1}{V_l} \int_{V_l} \phi dV \tag{2}$$

where V_l is the volume space which the liquid phase occupies, thus the porosity $\varepsilon_l \equiv V_l / V$ is the volume fraction of the liquid space. Note that $n_{l\,j}$ is the unit vector pointing outward from the liquid side to membrane side, while A_{lint} is the interfacial area between the liquid and membrane phases. D_l and h_{eff} are the

carbon dioxide diffusion coefficient in the liquid phase and the effective mass transfer coefficient, respectively. Moreover, $\bar{c}^*$ indicates carbon dioxide equilibrium concentration.

For the initial CO_2 concentration $\bar{c} = \bar{c}\big|_{t=0}$, which is already measured, Eq.(1) can be solved to give

$$\frac{\bar{c}-\bar{c}\big|_{t=0}}{\bar{c}^*-\bar{c}\big|_{t=0}} = 1-\exp\left(-\frac{A_l h_{eff}}{V_l}t\right) = 1-\exp\left(-\frac{a_l h_{eff}}{\varepsilon_l}t\right) \quad (3)$$

The effective mass transfer coefficient is estimated by fitting the measured temporal development of the total CO_2 concentration in the liquid phase to the foregoing equation.

RESULTS

1 Mass transfer through membranes

A careful experiment for measuring the total CO_2 concentration using three culture systems was carried out under culture conditions in this study. Figure 4 shows the temporal development of the bulk average of the total CO_2 concentration within the opened culture system, when cultivate conditions were set to T=26℃, V_l=75ml, and Q=50ml/min. As can be seen from Figure 4, the total CO_2 concentration in the liquid phase suddenly rises after feeding CO_2 gas to the hollow fibers, and then achieves the critical value for the equal amount of pure CO_2 gas. On the other hand, all measured values of the total CO_2 concentration within an air bubbling system are plotted in the same figure. The dissolution rate of the carbon dioxide within an air bubbling system is higher than that of the opened culture system using hollow fiber membranes, since the additional mass transport resistance exists when using gas exchange membranes.

Figure 5 shows the temporal development of the bulk average of the total CO_2 concentration within the closed type culture system, when cultivate conditions were set to T=26℃, V_l=75ml, and $Q \cong$ 0ml/min. In this experiment, the CO_2 volume flow rate was not measured since the permeation was so low in the closed type culture system. However, as can be seen from Figure 5, the dissolution rate of the carbon dioxide within the closed culture system is almost the same as compared with that within the opened culture system. Since the pressure inside hollow fibers was set to an atmospheric pressure in both systems. Note that the carbon dioxide consumption can be significantly reduced by adopting the closed culture system using hollow fiber membranes as compared with other cultivate systems.

The effective mass transfer coefficient is tabulated in Table 4, which was estimated by fitting the measured total CO_2 concentration developments to the CO_2 concentration profile obtained from Eq.(3). The difference in dissolution characteristic was indiscernible between the opened and closed culture systems. In the same table, the experimental data reported by Carvolho et al. [18] are tabulated for reference.

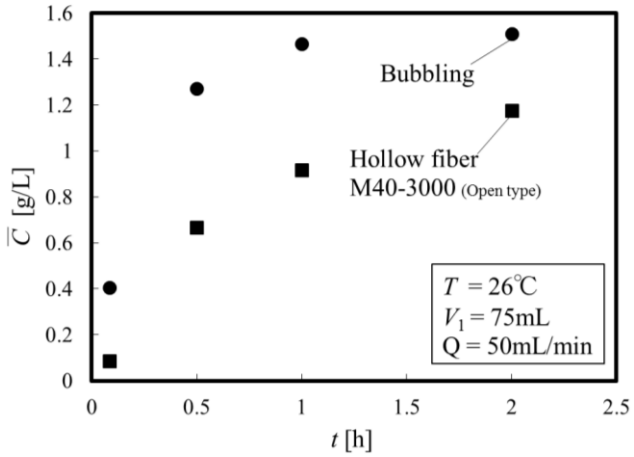

Figure 4 Development of total CO_2 concentration within the opened culture system and bubbling culture system.

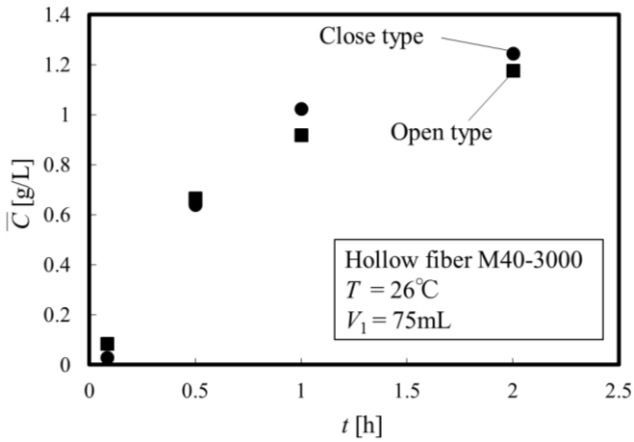

Figure 5 Development of total CO_2 concentration within the opened and closed culture systems.

Table 4 Effective mass transfer coefficient.

device	$a_l h_{eff}/\varepsilon_l$[min^{-1}]	A [m^2]	a_l[m^{-1}]	h_{eff}[m/s]	reference
M40-3000 (Open type)	1.69×10^{-2}	0.30	3160	7.10×10^{-8}	present study
M40-3000 (Close type)	1.73×10^{-2}	0.30	3160	7.31×10^{-8}	present study
HIFO	1.48×10^{-2}	1.70	1700	1.45×10^{-7}	Carvolho et al. [18]
HIFI	1.33×10^{-2}	0.14	140	1.59×10^{-6}	Carvolho et al. [18]

2 Microalgae culture using a hollow fiber membrane device

Figure 6 shows the increment of the microalgae cell concentration ΔC_M in the culture period of 5 days (note that culture medium was kept in photobioreactor for a day before experiments in this study), in which cells may increase linearly, although it is known that the microbial growth rate can be expressed an exponential function. As can be seen in Figure 6, the microalgal growth rate within the opened culture system using hollow fibers is found to be three times greater than that of a non-membrane photobioreactor (the bubbling culture

system). Sano et al. [23] mentioned that the high cultivation rate of microalgae using hollow fiber membranes would be due to reduction of shear stress associated with supplying gas, since carbon dioxide concentration in the culture medium reached in an equilibrium irrespective of supply methods after two hours. The results similar to those indicated by Sano et al. [23] were obtained by exploiting a new experimental apparatus and microalgae, which indicates the reproducibility of their experimental results.

The increments of the microalgae cell concentration within both opened and closed culture systems are plotted in Figure 7. As shown in figure 7, the difference in the microalgal growth rates was almost the same between the opened and closed culture systems, since the difference in dissolution behavior of carbon dioxide was indiscernible. Figure 8 shows the microalgal growth rate among all culture systems carried out in this study. The hollow fiber membrane is usefulness for the microalgae culture in terms of the microalgal growth rate. Moreover, as has been explained above, the carbon dioxide consumption can be significantly reduced by adopting the closed culture system using hollow fiber membranes as compared with other culture systems.

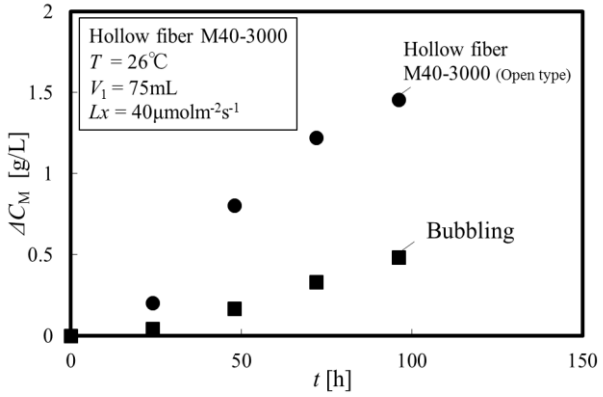

Figure 6 Development of cell concentration with in the opened culture and air bubbling culture.

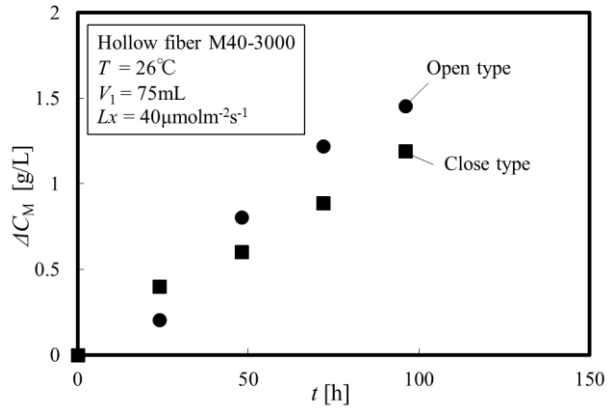

Figure 7 Development of cell concentration with in the opened and closed culture systems.

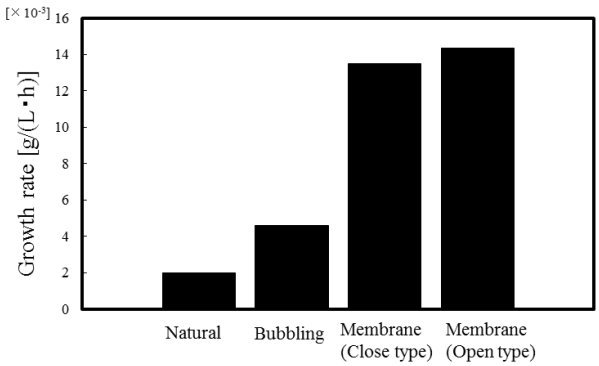

Figure 8 Microalgal growth rate

CONCLUSIONS

A hollow fiber culture system was proposed for both enhancing the microalgal growth rate and reducing the carbon dioxide consumption. The microalgal growth rate for *Chlorella vulgaris Beijerinck.* was measured using the present photobioreactor. The microalgal growth rate within the closed culture system was found three times greater than that of the bubbling culture system. The effective mass transfer coefficient of the carbon dioxide was measured within the present photobioreactor. It was found that the difference in dissolution behavior~~s~~ are indiscernible between the hollow fiber culture systems and the bubbling culture system in view of the culture period, since the carbon dioxide concentration in the culture medium reached in equilibrium after 2 hours irrespective of culture systems. Moreover, it was found that the carbon dioxide consumption can be significantly reduced by adopting the hollow fiber culture system of end-sealed hollow fibers while keeping the high microalgal growth rate. The present study clearly indicates that the hollow fiber membrane is usefulness for the microalgae culture in terms of both enhancement of microalgal growth rate and ~~the~~ reduction of carbon dioxide consumption.

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