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Photosynthetic performance of a cyanobacterium in a vertical flat-plate photobioreactor for outdoor microalgal production and fixation of CO_2

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Abstract

A vertical flat-plate photobioreactor was developed for the outdoor culture of microalgae using sunlight as the light source. The ability for biomass production and CO_2 fixation was evaluated by using a cyanobacterium, *Synechocystis aquatilis* SI-2. The average areal productivity was 31 g biomass m^{-2} d^{-1}, which corresponded to a CO_2 fixation rate of 51 g CO_2 m^{-2} d^{-1}, sustainable in the northern region of Japan during the winter time (January and February). The relationships between the efficiency of solar energy utilization of the reactor and its effect factors (cell concentration and irradiation) were investigated.

Introduction

Increased CO_2 concentration in the atmosphere has become of world-wide concern in recent years. As one of the strategies, a biological method has been proposed to decrease CO_2 emissions to the atmosphere, including biological conversion of CO_2 by high CO_2-tolerant microalgae (Kodama *et al.* 1993). Various new types of photobioreactor, such as the tubular-type (Watanabe *et al.* 1995), flat-plate type with a short light path to efficiently utilize sunlight (Zhang *et al.* 1999), and the type in which light is uniformly introduced into the reactor with optical fibers or plates (Hirata *et al.* 1996, Ogbonna *et al.* 1999) have been studied.

The flat-plate type photobioreactor constructed with a single plate was used in most of the past studies. To investigate the areal productivity from a large scale reactor system, we used a vertical flat-plate photobioreactor (VFPP) including several reactor plates in parallel and studied effects on the productivity of the reactor orientation and distance between reactor plates (Zhang *et al.* 1999). The biomass productivity from an east-west-facing orientation was higher than that when the reactor plates were placed in a north-south-facing orientation, because the former received more solar energy than the latter; changing the number of the reactor plates from three to five on the same land area. However, the overall land area-based productivity was the same, indicating that narrowing the distance between the reactor plates too much was not an effective method for increasing the areal yield of outdoor culture because of severe mutual shading of the plates. To improve the areal productivity from the VFPP, a 72-l VFPP, which was about 2.5-times as tall as that used in the previous work (Zhang *et al.* 1999), was constructed and tested for the mass production of microalgae in this present work. The effects of culture parameters of the outdoor experiment on the biomass productivity were studied during the experimental period.

Materials and methods

Strain and culture medium

A high-CO_2 tolerant cyanobacterium, *Synechocystis aquatilis* SI-2, and modified SOT inorganic medium

(Zhang *et al.* 1999) were used as the test organism and culture medium, respectively.

Photobioreactor

The reactor plate was constructed from sheets of transparent acrylic plastic (8 mm in thickness) and consisted of a culture chamber with a water jacket attached to one side for temperature control. The culture temperature was regulated to 40 ± 3 °C with a thermostat throughout the experimental period. Several straight baffle strips were inserted into both the culture chamber and water jacket to provide rigidity, in addition to promoting circulation and mixing. The size of the reactor plate was 1.7 m (height) × 1 m (length) × 0.015 m (width) with a working volume of 24 l per plate. The liquid level of the culture was kept at 1.65 m high. The ratio of the illuminated surface area of the plate to the working volume was about 138 m^{-1}. A perforated tube extending along the bottom of the culture chamber, through which a stream of compressed air enriched with 10% (v/v) CO_2, at an aeration rate of 0.05 vvm (a superficial gas velocity of 1.3 mm s^{-1}) was passed for supplying CO_2 and mixing the culture. This tube was perforated along its length direction at intervals of 10 mm with a needle of 0.7 mm in diameter. Three reactor plates (giving a total working volume of 72 l) were installed in parallel in a space of 1 m^2, each panel being separated by 0.5 m (Zhang *et al.* 1999). The active illuminated surfaces of the reactor plate were set to face east and west orientation.

Measurements of the algal productivity and irradiation

An LI-400 quantum meter (LICOR Inc., Lincoln, USA) was used to measure the photosynthetically-active radiation (PAR) on a horizontal plane with a flat cosine-corrected quantum sensor (LI-190SA) and above the VFPP panel with a 4π (spherical) sensor (LI-193SA). The irradiations from the sensors were named as horizontal and 4π irradiation (Richard & Bruce 1992), respectively. Conversion from the PAR value to total solar irradiation was made by using the conversion of 1 J m^{-2} s^{-1} to 4.57 μmol m^{-2} s^{-1} (Thimijan & Heins 1983). The daylight duration was about 9.5 h each day during the experimental period.

The cell concentration was measured by the method of Zhang *et al.* (1999). The areal (land space) biomass productivity (g biomass m^{-2} d^{-1}) of each reactor plate (east, center and west) was calculated on the basis of the same land area for each plate

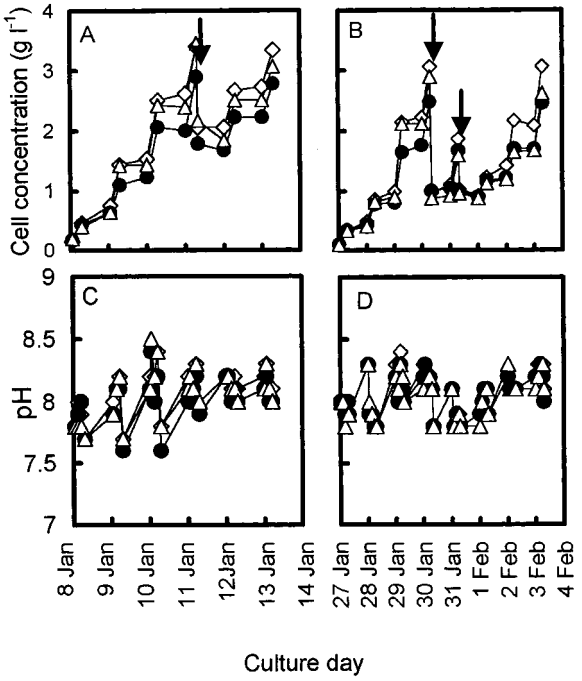

Fig. 1. Growth of *Synechocystis aquatilis* SI-2 cells and variation of pH in the different culture plates during the two culture periods. The outdoor semi-batch culture was done twice during January and February. (A) and (B) are the growth of the cells, (C) and (D) variation of pH during the two periods, respectively. ◊ east plate; ● center plate; △ west plate; arrows indicate times at which part of the culture suspension was replaced with fresh medium.

(= 0.5 m^2) because when more plates were installed to build a larger scale culture system at the same distance of 0.5 m, the installation area of each inside plate would be 0.5 m^2. The daily biomass productivity of each reactor plate from 16:00 for 24 h (night biomass loss included) was used to calculate the values for biomass productivity in Table 1 and the biomass energy in Figure 2. The biomass energy of *S. aquatilis* SI-2 calculated from the combustion heat was 0.0214 MJ g dry $cells^{-1}$. The areal CO_2 fixation rate was calculated by using the equation:

$$0.45P \times 44 \times 12^{-1}(\text{g } CO_2 \text{ m}^{-2} \text{ d}^{-1}), \quad (1)$$

where 0.45 was the carbon content of dried cells (g carbon g $biomass^{-1}$), P the productivity (g biomass m^{-2} d^{-1}) , and 44 and 12 the molecular weights of carbon dioxide and carbon, respectively.

Results and discussion

Two semi-batch culture experiments were conducted during January and February, 1999 to estimate the

productivity of the VFPP system (Figure 1). The inoculation cell concentration was 0.1–0.2 g l^{-1}, half or more of the culture was replaced with the same volume of a fresh medium at the arrows shown in Figure 1. The east and west plates received more light than the center plate, resulting in generally higher productivity by the outer plates. The highest daily volumetric productivity of 1.0 g l^{-1} d^{-1} by the center plate, which was calculated from the change in cell concentration during one day shown in Figure 1, was obtained on 10 January, a sunny day.

Many studies have shown that mixing an algal culture enhanced its growth and hence its biomass productivity (Laws *et al.* 1983, Hu *et al.* 1996). However, the cost for such a high aeration rate accounted for over half of the whole operating cost in pilot operation. In this work, we investigated the relationship between biomass productivity and the aeration rate and found that 0.05 vvm was an optimum point for this style of the reactor under indoor condition (unpublished data). The same aeration rate of 0.05 vvm was applied and tested during the outdoor culture period. A low aeration rate could be much expected to limit the CO_2 supply, reduce the productivity, and raise the pH value. No extreme pH change was apparent with an aeration rate of 0.05 vvm during the experimental period, only a slight variation in pH occurred, even with a daily concentration variation of over 1 g l^{-1} (Figure 1, e.g., on 10 January), which indicates that the supply of carbon to the culture and the demand for cell growth were in balance, and this low aeration rate was sufficient for cell growth in such irradiation conditions.

The cell concentration, biomass productivity, CO_2 fixation rate and daily irradiation obtained during two culture periods are given in Table 1. The overall productivity was mainly related to both the irradiation and cell concentration. At the beginning of the culture, the biomass productivity was strongly affected by the initial cell concentration; for example, although the daily irradiation was the same on 27 and 28 January (7.0 MJ m^{-2} d^{-1}), with an initial cell concentration of 0.4 g l^{-1} on 28 January, the productivity was twice the productivity on 27 January when the initial cell concentration was 0.1 g l^{-1} (Table 1). When the cell concentration had reached 1–2 g l^{-1}, the effect of the initial cell concentration on the productivity became insignificant.

High productivity was obtained when the cell concentration was maintained between 1 and 2 g l^{-1}. A maximum areal biomass productivity of 45 g m^{-2} d^{-1} from the center plate was recorded on 10 January, a sunny day (Table 1). Excluding data when the cell concentration was lower than 0.5 g l^{-1} in the mornings, the average productivity from the center plate was about 31 g biomass m^{-2} d^{-1} (the average 4π irradiation was 8.3 MJ m^{-2} d^{-1}). The average and maximum CO_2 fixation rates of the center plate, which were calculated according to Equation (1), were 51 g CO_2 m^{-2} d^{-1} and 74 g CO_2 m^{-2} d^{-1}, respectively.

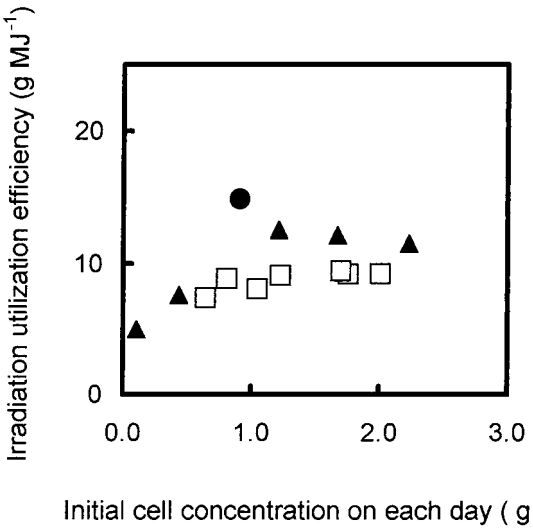

Fig. 2. Irradiation utilization efficiency (IUE) for various weather conditions and initial cell concentration. IUE: biomass productivity (g biomass m^{-2} d^{-1})/(4π irradiation) (MJ m^{-2} d^{-1}). ● cloudy (4π irradiation below 3 MJ m^{-2} d^{-1}); ▲ patchy cloud (3–7 MJ m^{-2} d^{-1}); □ sunny (over 7 MJ m^{-2} d^{-1}).

The irradiation utilization efficiency (IUE), which is defined in this study as the efficiency of solar irradiation conversion to biomass energy, was calculated to describe the impact of climatic conditions on the biomass productivity (Figure 2). When the cell concentration in the culture was lower than 1.0 g l^{-1}, IUE dropped with decreasing initial cell concentration, because the incident irradiation, which was transmitted through the culture plate without being absorbed by the algal cells was increased. With increasing cell concentration to over 1 g l^{-1}, IUE was consistently high, because almost all the light impinging on the plate surface was absorbed for photosynthesis. Little variation in IUE was observed during sunny conditions in the cell concentration range of 1–2 g l^{-1}. However, the IUE was also influenced by climatic conditions (= irradiation); IUE was higher in cloudy conditions than in patchy cloud and sunny conditions. In contrast, the productivity was higher in sunny conditions than in cloudy conditions (Table 1). The highest IUE

Table 1. Biomass productivity and CO_2 fixation rate in the culture plates under outdoor conditions.

Culture date	Initial C[a]	Biomass productivity[b]			CO_2 fixation rate[c]			4π irradiation[d]	Horizontal irradiation[e]
		E	C	W	E	C	W		
8 Jan.	0.2	15	12	10	24	20	16	–	–
9 Jan.	0.6	46	31	48	75	51	80	9.6	4.4
10 Jan.	1.2	51	45	47	84	75	78	10.0	4.7
11 Jan.	2.0	42	40	49	70	65	80	10.9	4.9
12 Jan.	1.7	29	21	16	48	35	26	6.6	3.0
13 Jan.	2.2	32	27	27	53	44	44	6.7	3.2
27 Jan.	0.1	12	12	12	20	20	20	7.0	3.5
28 Jan.	0.4	25	21	23	42	35	39	7.0	3.5
29 Jan.	0.8	62	42	62	102	69	103	11.3	5.3
30 Jan.	1.8	44	40	37	73	66	62	10.3	4.9
31 Jan.	1.0	43	32	34	71	53	56	10.8	5.4
1 Feb.	0.9	9	8	8	15	13	13	2.4	1.3
2 Feb.	1.2	46	26	25	75	43	41	6.0	2.9
3 Feb.	1.7	43	36	47	71	60	77	8.6	4.4
Average [f]	–	39	31	35	65	51	58	8.3	4.0
Maximum	–	62	45	62	102	74	103	11.3	5.3

[a]Initial cell concentration of the center reactor (C) on each day (g l^{-1}).
[b,c]E, C, W indicate the areal productivity or CO_2 fixation rate for the east (E), center (C), and west (W) plate reactors (g biomass m^{-2} d^{-1}).
[d]Measured with an LI-193SA spherical sensor (MJ m^{-2} d^{-1}).
[e]Measured with an LI-190SA plate sensor (MJ m^{-2} d^{-1}).
[f]The average was calculated from the data obtained with an initial cell concentration of over 0.4 g l^{-1}.

value was recorded on a cloudy day (1 February, Figure 2). The productivity on this day was the lowest during the experimental period (Table 1). Grobbelaar *et al.* (1995) have shown that algal cells acclimated to the average light within a culture suspension having a progression from high light (HL) following inoculation or dilution to low light (LL) acclimation as the culture suspension became denser. LL-acclimated cells were more efficient in utilizing a low level of light, had a low maximum light-saturated photosynthetic rate (P_{max}) and had a large antenna size. The opposite was true for HL acclimated cells. Since our cultures were mostly dense, except for sometimes after dilution, it is reasonable to assume that the cells in the cultures were LL-acclimated. This would explain why the highest IUE value was measured on a overcast day, because the relatively less light was utilized efficiently and there was less radiant energy wasted due to the low P_{max} rate.

The percentage of bio-energy obtained by photosynthesis per 4π irradiation shown in Figure 3, was calculated from the value of productivity of the center culture plate listed in Table 1, in which the cell concentration was over 0.5 g l^{-1}, and the combustion heat

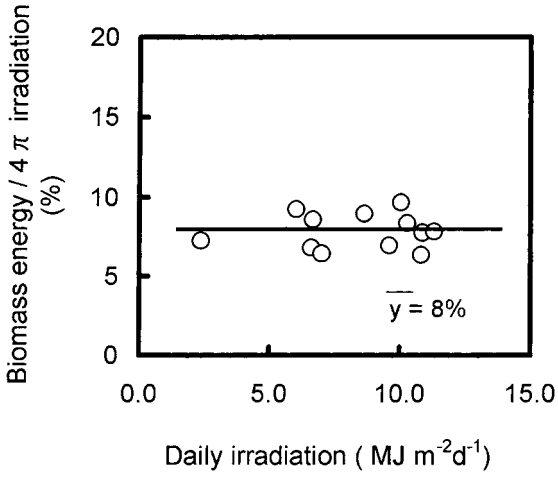

Fig. 3. Distribution of the ratio of biomass energy to 4π irradiation during cultivation. 4π Irradiation was used for the X-axis.

of dry cells. The value varied from 6% to 10%, with an average of 8%. These values would be higher if the flat cosine-corrected PAR values were to be used instead of the 4π PAR irradiation, where the value of 4π PAR value is about double that of the flat cosine-corrected PAR. We used the 4π irradiation values since the re-

Table 2. Comparison of productivity between the previous experiments and this work.

Exp. No.	Plate No. and orientation	Aeration rate (vvm)	Height of plate (m)	Irradiance (MJ $m^{-2}d^{-1}$)	Total productivity[a] (g m^{-2} d^{-1})	Productivity in M plate[b] (g m^{-2} d^{-1})	Productivity per unit irradiance[c] (g MJ^{-1})	Productivity per unit irradiance[d] (g MJ^{-1})	References
1	3 plates E-W	1	0.6	4.5	23	13	2.9	5.1	Zhang *et al*. (1999)
2	5 plates E-W	1	0.6	6.3	32	20	3.1	5.1	Zhang *et al*. (1999)
3	3 plates E-W	0.05	1.65	8.3	53	31	3.7	6.4	This work

[a]Total productivity in Nos. 1–3 was calculated according to $\Sigma p/A$, where p is daily productivity of each plate (g day^{-1}), and A is the installation land area (1m^2 in Exp. Nos. 1–3) (Zhang *et al.* 1999).
[b]Productivity of the middle plate in Nos. 1–3 was calculated according to p/A, where p is daily productivity of the inside plate (g day^{-1}), and A is the land area of each plate (0.5 m^2 in Nos. 1 and 3; 0.25 m^2 in No. 2).
[c]Productivity per unit irradiance in Nos. 1–3 was calculated according to P/I, where P is the total productivity, I is the irradiance in each experiment.
[d]Productivity in the middle plate per unit irradiance in Nos. 1–3 was calculated according to P'/I, where P' and I are the productivity and irradiance in the middle plate, respectively.

actor was exposed to irradiation from all directions. Pirt *et al.* (1980) have indicated that the theoretical maximum photosynthetic efficiency for an algal mass culture would be 18% for a horizontal raceway, while Laws *et al.* (1985) have measured an efficiency of up to 11% in a well-mixed shallow outdoor flume culture. Laws *et al.* (1985) have calculated the percentage of bio-energy fixed per total daily irradiation and found values between 8% and 11%. Since our data are close to those calculated by Laws *et al.* (1985), we are convinced that the VFPP system can render high areal productivity.

Compared to the productivity per unit irradiation from the center plate obtained in our previous work (Zhang *et al.* 1999), in which 3 or 5 vertical plates of 0.6 m in height were set in 1 m^2 of ground area at a separation distance of 0.5 m or 0.25 m and with a supply of 1 vvm of CO_2-enriched air, about 1.2 times higher values were obtained in the present study (Table 2). However, the increase in productivity per unit irradiation is not proportional to the increase in the illuminated area by increasing the height of the plate, which may be explained by the following factors: (1) The low aeration rate of 0.05 vvm in the present experiment lead to a decreased rate of growth due to the lower light flashing effect from reduced mixing (Hu *et al.* 1996). (2) Raising the level of the liquid would increase the shaded area on the surface of the center plate, which would lead to decreased illumination. We assume that if the experiments were done under the same aeration and weather conditions, the difference in productivity between the 1.65-m plate and 0.6-m plate would have been much greater than that obtained from the present experiments.

Our results indicate that the VFPP system has the potential to achieve a high yield under outdoor conditions. However, we realize that considerable work needs to be done before optimum configuration can be defined.

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