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Effect of Liquid Circulation Velocity and Cell Density on the Growth of *Parietochloris incisa* in Flat Plate Photobioreactors

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Abstract For more accurately describing the durations of the light and the dark phases of microalgal cells over the whole light-dark cycle, and probing into the relationship between the liquid circulation time or velocity, the aeration rate and cell density, a series of experiments was carried out in 10 cm light-path flat plate photobioreactors. The results indicated that the liquid flow in the flat plate photobioreactor could be described by liquid dynamic equations, and a high biomass output, higher content and productivity of arachidonic acid, 70.10 $gm^{-2}d^{-1}$, 9.62% and 510.3 mg/L, respectively, were obtained under the optimal culture conditions.

Keywords: liquid circulation velocity, microalgae, light-dark cycle, *Parietochloris incisa*, flat plate photobioreactor

INTRODUCTION

The photolimitation and photoinhibition phenomena are functions of the light regime to which cells are exposed [1], with the light regime being a function of the light profile inside a culture and the mean radial velocity of the cells. So, the cell density and liquid fluid dynamic are two major factors impacting on a high density culture of microalgae. The fluid dynamic is a very important parameter in bioreactor design and microbial fermentation, which can markedly affect the cultures. In the field of microalgal research, some excellent researchers have reported that the turbulence, mixing rate or stirring speed have very decisively affects on the growth of microalgae [2-5], but as is usual with microbial cultures, the fluid dynamics in much microalgal research are not discussed in detail, with this major parameter being absent or not recognized at all.

As an essential aspect, a major issue in the cultivation of photoautotrophic microalgae concerns the sustained trapping of high irradiance at as high an efficiency as possible; the more effectively this task is accomplished, the sounder will be the economic basis for algal biotechnology [1,6]. The efficient utilization of light by an individual cell in a culture is associated with many constrains. The higher the population density and the longer the light path, the more complex the basic requirements for the efficient utilization of light will become, *i.e.*, an even distribution of light to all cells.

Therefore, if the light received by each cell over time can be described by a more accurate method, a function relationship of the light intensity, liquid circulation velocity and optimal culture density can be set up, and a higher density culture can be obtained. Since an individual cell in a mass culture is illuminated intermittently, a function of the duration of cell-exposure to the light and dark zones in the bioreactor, as well as the relative length of such exposure in each zone is very important [7,8].

This research employed the parameter of liquid circulation time or velocity for describing the duration of cell-exposure to the light and the dark phases, and a basic relationship between the liquid circulation velocity and aeration or mixing rate was described using a mathematics equation.

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MATERIALS AND METHODS

Organism

Parietochloris incisa (*Trebouxiophyceae, Chlorophyta*) was isolated from Mount Oyama in Toyoma prefecture, Japan.

Medium

The culture medium was prepared from autoclaved stock solutions, containing double BG-11 nutrients as following: KNO_3, 3.0 g/L; KH_2PO_4, 61.0 mg/L; Na_2CO_3, 40.0 mg/L; $MgSO_4 \cdot 7H_2O$, 150.0 mg/L; $CaCl_2 \cdot 2H_2O$, 72.0 mg/L; $C_6H_8O_7 \cdot H_2O$, 12.0 mg/L; 0.25 M of Na_2EDTA solution, 2.2 mL; $C_6H_{11}FeNO_7$, 12.0 mg/L; $CuSO_4 \cdot 5H_2O$, 1.58 mg/L; $ZnSO_4 \cdot 7H_2O$, 4.44 mg/L; $MnCl_2 \cdot 4H_2O$, 37.2 mg/L; $Na_2MoO_4 \cdot 2H_2O$, 7.8 mg/L; HBO_3, 57.2 mg/L and $Co(NO_3)_2 \cdot 6H_2O$, 0.988 mg/L.

Photobioreactor and Culture Conditions

Flat-plate glass photobioreactors (70 × 50 cm, and a light-path of 10 cm), with culture suspension heights of 50 cm were used for all cultures. The temperature in the outdoor cultures was maintained at 25°C (± 1°C), and sunlight used for illumination (*ca.* 1,800 $\mu molm^{-2}s^{-1}$ at noon). The culture suspensions were vigorously agitated and supplied with sufficient carbon source by charging mixed gases (CO_2 and compressed air) through an aeration tube that extended to the bottom of the reactor. The pH of the microalgal suspensions was regularly determined using a microprocessor pH meter, and favorably stabilized at 7.5~8.0 by adjusting the CO_2 level. Nutrients were added every three days in the batch culture mode so as to avoid nutrient deficiency, with a certain amount of new medium added every day to replenish the water losses due to evaporation.

Measurement of Liquid Circulation Velocity

The liquid circulation time was initially measured using a double pH probe with 3 M H_2SO_4 and 30% NaOH as indicators at different aeration rates, and the liquid circulation velocity then calculated according to the data on the liquid circulation time [9].

Dry Cell Mass Measurement

Duplicate volumes of algal suspensions were vacuum filtered through pre-weighted Whatman glass fiber filters (GF/A). The cells were washed twice with an equal volume of acidified water (pH 4.0) to reduce mineral salt precipitates in the algal residues and simultaneously prevent cell lysis. The residues were then thoroughly dried overnight in an oven at 105°C to constant weight, and then weighted after naturally cooling to room-temperature in a desiccator.

Fatty Acid Analysis

Algal suspensions were centrifuged at 3,500 rpm for 5 min, the precipitates re-suspended in distilled water and then re-centrifuged. The above process was repeated three times, and the pellets then frozen in at -80°C and fully dried in a vacuum freeze dryer. 25 mg of algal powder, a magnetic bar and 0.25 mg heptadecanoic acid (C17), the latter as an internal standard, were added to a small Teflon bottle with a cap, with 2 mL H_2SO_4 methanol solution (2% H_2SO_4) then added to break the lipid molecular and modify a CH_3- group. After charging with argon gas, the sample was heated in an 80°C sand bath on a hotplate with magnetic stirring for 1 h. Then 1 mL each of distilled water and hexane were added into the tube, well mixed by vortex, and then centrifuged at 3,500 rpm for 5 min. The upper phase was transferred into another stoppered vial, concentrated by the passing of nitrogen, and stored in a freezer.

Gas chromatographic (hp Hewlett 5890, Packard series II) analysis was performed on a supecowax 10 (Supelco, Bellefonte, PA, USA) fused silica capillary column (30 mm × 0.32 mm), with FID and injector temperatures of 230°C and a split ratio of 1:100. Fatty acid methyl esters were identified by authentic standards (Sigma, St Louis, MO, USA), and the fatty acids calculated by comparison of each peak area with that of a standard C17 and its peak area.

Growth Measurements

The growth and productivity were estimated by daily measurement of the biomass concentration, and reflected as dry weight. The specific growth rate μ (d^{-1}) was calculated using the follow equation:

$$\mu = (\ln X_2 - \ln X_1)/(t_2 - t_1)$$

Where X_2 and X_1 represented the cell densities in terms of dry weights (g/L) at times t_2 and t_1, respectively.

The biomass volumetric output rate (P_v) and areal output rate (P_a) were calculated by:

$$P_v = (X_2 - X_1)/(t_2 - t_1) \qquad P_a = (X_2 - X_1)V_t/(t_2 - t_1)A$$

Where V_t and A represented the actual culture volume at time t and the effective illuminated surface area of the culture, respectively.

RESULTS

Relationships between Circulation Time, Liquid Circulation Velocity and Aeration Rate

The liquid was vigorously agitated by a sufficient gas current supplied through an aeration tube that extended to the bottom in the flat plate photobioreactor, forming a circulated liquid flow. When different aeration rates were selected, according to the data of experiments, the relationship of the circulation time (T_c), liquid circulation velocity (V_c) and aeration rate (X) in the flat plate photobioreactor could be expressed by the mathematical equations:

$$T_c = 2.47\,X^{-0.834},\ R^2 = 0.92 \quad (1)$$

$$V_c = -0.42X^4 + 1.49X^3 - 1.20X^2 + 0.90X + 0.14,\ R^2 = 0.997 \quad (2)$$

It was clear that the circulation time decreased and the liquid circulation velocity increased with increasing aeration rate (Fig. 1). At an aeration rate of 2.18 vvm, the liquid circulation velocity came close to its maximum. The results show that the liquid flow in the flat plate photobioreactor had a very regular pattern, and could also be described using similar liquid dynamic equations to those used in internal air-left photobioreactors [9,10].

Effect of Liquid Circulation Velocity and Cell Density on the Growth of *P. incisa* in 10 cm Light-path Photobioreactors

At the surface of the bioreactor, the aeration rate and

Table 1. Effects of the liquid circulation velocity and cell density on the growth, volumetric and aeral biomass output rates of *P. incisa*

Liquid circulation velocity (m/s)	Average L/D cycle[a] (ms)	Initial cell density (g/L)	Net increase biomass (%)	Growth rate[b] (d^{-1})	Volumetric output rate ($gL^{-1}d^{-1}$)	Aeral output rate[c] ($gm^{-2}d^{-1}$)
0.65 (0.7 vvm)	154	0.23	367	0.267	0.156	15.15
		0.85	116	0.129	0.164	15.92
		2.00	39	0.055	0.130	12.62
		3.30	17	0.031	0.113	10.97
1.36 (1.3 vvm)	74	0.23	456	0.286	0.176	17.09
		0.85	138	0.144	0.201	19.51
		2.00	51	0.069	0.168	16.31
		3.30	28	0.042	0.158	15.34
2.18 (1.9 vvm)	46	0.23	524	0.305	0.214	20.78
		0.85	165	0.162	0.245	23.50
		2.00	85	0.102	0.286	27.47
		3.30	39	0.052	0.205	19.90
2.35 (3.0 vvm)	43	0.39	395	0.267	0.257	24.95
		0.91	165	0.162	0.250	24.27
		1.80	98	0.114	0.293	28.45
		3.38	53	0.071	0.297	28.83

[a] the time of light/dark cycle was calculated as the light-path divided by the liquid circulation velocity;
[b] the culture time was 6 days; illuminated area of one side for each photobioreactor was 0.206 m^2.

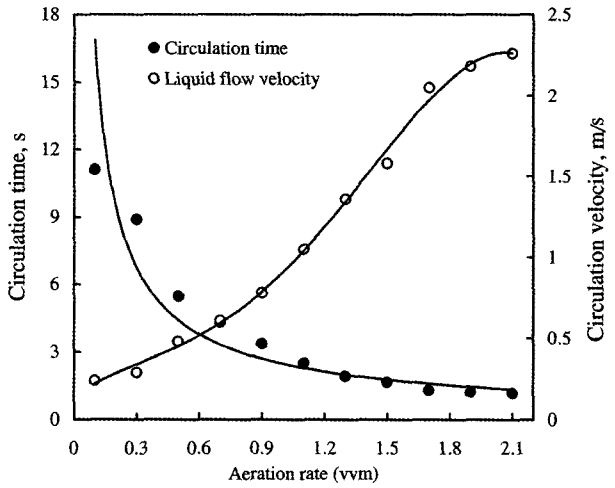

Fig. 1. Effect of aeration rate on the circulation time and liquid circulation velocity in a flat plat photobioreactor.

liquid circulation velocity were the same when used to express the affects of mixing on the growth of microalgae, but with large discrepancies in the results (Table 1). According to Eq. (2), when the aeration rate was 2.18 vvm, the liquid velocity and the trend of the times of the light/dark cycles reached their maxima, and even greater aeration could not support higher liquid velocities. Additionally, as the liquid circulation velocity is more closely related with the time of light-dark cycles, the effect of mixing on the growth could be more easily understood. When the liquid circulation velocity was below 1.36 m/s, the optimal culture density was only about 0.85 g/L, whereas at 2.18 m/s, this was higher than 2.0 g/L. Despite the marked difference in the aeration rates at 1.9 vvm and 3.0 vvm, there was no distinct difference with reference to the time of the light-dark cycle; therefore, the culture results were nearly the same, *i.e.* with similar optimal culture densities, the growth and biomass output rates showed no distinct differences.

Many experiments have indicated that the mixing rate and aeration mode had a very distinctive effect on the growth of microalgae when their nutritional and environmental requirements were satisfied [11-14]. However, using only the mixing rate or liquid turbulent flow to explain the affect on microalgal growth does not seem very logical, although these parameters can be used in qualitative analyses. Using only the mixing rate has been know to have some affect, but for much more accuracy of increasing the effect, higher non optimized mixing rates can be used. For example, in order to investigate the effect of the aeration rate on the growth of microalgae, some experiments have tried to use high aeration rates, even greater than 4 vvm, but this not only wasted energy, but also hurt the cells. Using the concept of liquid dynamic, the effect of the mixing rate on the growth of microalgae can be more logical and clearly understood.

The Harvesting Regime for *P. incisa* in 10 cm Light-path Photobioreactors

To investigate the effect of the liquid circulation velocity on the biomass output rate and the biomass productivity in more detail, a mode of semi-continuous culturing was performed (Fig. 2 and Table 2).

When the growth of the cells reached their steady state, the output rate significantly increased, with the subsequent increase in the dilution rates with different liquid

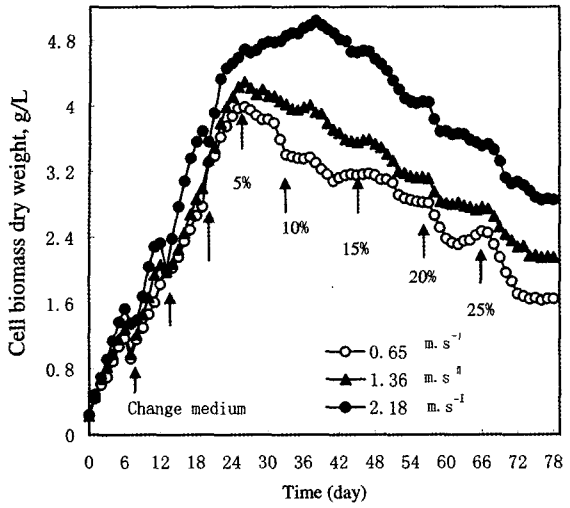

Fig. 2. The growth of *P. incisa* under different liquid circulation velocities.

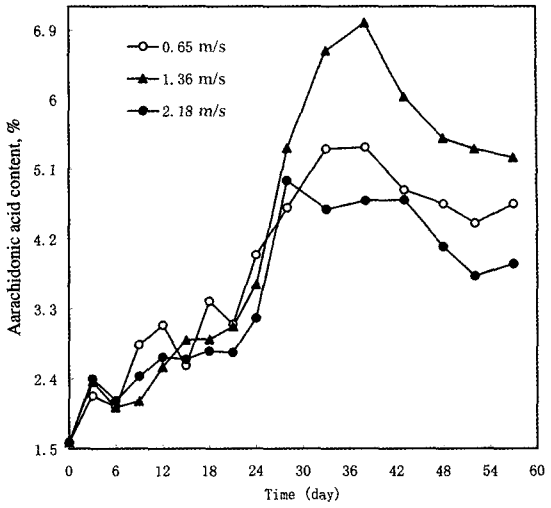

Fig. 3. Changes of the arachidonic acid content in *P. incisa* under different liquid circulation velocities.

Table 2. Effects of the liquid circulation velocity and harvesting regime on the estimated steady state volumetric and aeral biomass output rates of *P. incisa*

Liquid flow velocity (m/s)	Daily harvest (%)	Harvesting volume (L)	Volumetric biomass output ($gL^{-1}d^{-1}$)	Areal biomass output ($gm^{-2}d^{-1}$)
0.65	5	1.0	0.184	17.87
	10	2.0	0.319	30.97
	15	3.0	0.448	43.50
	20	4.0	0.490	47.57
	25	5.0	0.415	40.29
1.35	5	1.0	0.205	19.90
	10	2.0	0.373	36.19
	15	3.0	0.527	51.17
	20	4.0	0.552	53.59
	25	5.0	0.545	52.91
2.18	5	1.0	0.239	23.20
	10	2.0	0.484	46.99
	15	3.0	0.643	62.43
	20	4.0	0.722	70.10
	25	5.0	0.715	69.42

Illuminated area of one side for each photobioreactor is 0.206 m^2.

circulation velocities, reaching their maximum at a 20% dilution rate. Also, aeral output rates of 47.57, 53.59 and 70.10 $gm^{-2}d^{-1}$ were obtained with the liquid circulation velocities of 0.65, 1.36 and 2.18 m/s, respectively. At the optimal culture density, the relationship between the output rate and liquid circulation velocity was very reasonable. With liquid circulation rates between 0.65 and 1.36 m/s (aeration rate 0.7 and 1.3 vvm), the growth and biomass output rates showed only a slight significant difference; however, when the aeration rate was increased to 1.9 vvm only (the liquid circulation velocity was 2.18 m/s), the growth and biomass output rates increased very significantly.

The Arachidonic Acid Production of *P. incisa* in 10 cm Light-path Photobioreactors

P. incisa has been regard as a potential alternative for investigation, due to its high arachidonic acid (AA) content, for exploitation of its ability to be produced outdoors; therefore, cultures of *P. incisa* were grown under different liquid circulation velocities (Fig. 3) and nitrogen starvation conditions (Fig. 4).

The arachidonic acid content increased with culture time during the cell-growing phase, reaching its maximum when the cells were in a steady state, but then declined after the stationary phase. Maximal productivities of 375.8, 228.6 and 165.3 mg/L were obtained with liquid circulation velocities of 1.36, 0.65 and 2.18 m/s, respectively. The results indicated that only an appropriate liquid circulation velocity could promote arachidonic acid synthesis, but higher or lower liquid circulation velocities were unable to support a higher arachidonic acid content synthesis.

Higher contents and productivities of arachidonic acid, 9.62% and 510.3 mg/L, respectively, were obtained under nitrogen starvation and a liquid circulation velocity of 1.36 m/s; strongly suggesting higher arachidonic acid productivity could be achieved under optimal culture conditions.

DISCUSSION

It is well known that optimal mixing or aeration rates can increase the productivity of microalgae, as this can increase the efficiency of photosynthesis. In microalgal cultures, especially cultured in high density and under high light intensity, the cells can only grow faster with an appropriate light regime for each cell. Kok found the ra-

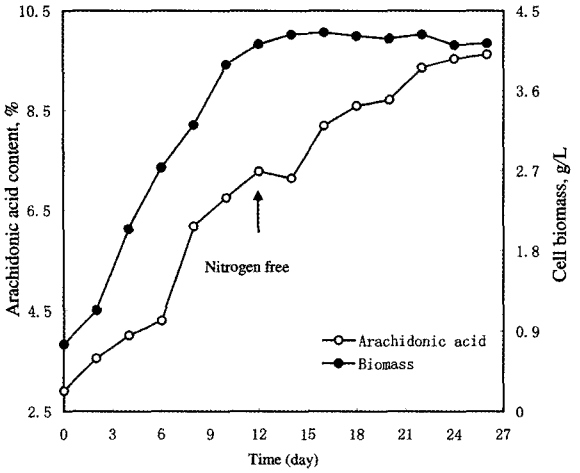

Fig. 4. Effect of nitrogen starvation on productivity of arachidonic acid under 1.36 m/s liquid circulation velocity.

tios of light-dark to be a key factor in the photosynthetic efficiency of microalgal cells [15], and other research suggests the mixing rate or turbulence was an advantage to the light-dark cycles that increased the efficiency of light utilization [7,16-18]. A total or rough estimation could be made using the mixing or turbulence concept; however, the detailed extent of this affect on cell growth could not be explained. Especially, little research has been conducted on the time of light-dark cycles for quantity analysis in large-scale photobioreactors.

According to the light attenuation mathematics models of *Porphyridium cruentum* and *Spirulina platensis*, which could represent the relationship of microalgal cell density, photon flux density and the depth of light penetration, with a cell density of 2.5 g/L the light zone (99% light penetration) was only about 0.23 mm [9,19,20]. Therefore, in a longer light-path flat plate photobioreactor; assumed the cells moved back and forth across the axis of the light-path, the time of light-dark cycles can be calculated exactly using the light-path divided by the liquid circulation velocity (Fig. 1 and Table 1). It is clear that the frequency of the light-dark cycle can be expressed more logically and with greater understanding using the liquid circulation velocity, which makes it easy to explain the beneficial effects of mixing on the microalgal productivity and light regime capability with an optimal cultural density (Fig. 2).

Our results also make it easy understand that the affect of light intensity on fatty acids synthesis in microalgal cells. In other word, not as same as biomass, the higher or lower circulation velocities don't support a high productivity of arachidonic acid, only with an appropriate circulation velocity the contents and productivities of arachidonic acid could be arrived the maximum (Figs. 3 and 4).

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