Effect of Cell Movement by Random Mixing between the Surface and Bottom of Photobioreactors on Algal Productivity

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Effect of algae movement, as a result of random mixing, between the surface and bottom zones of shallow, moderately deep and deep photobioreactors (incident light intensities per unit volume were 8125, 4062 and 2031 \( \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), respectively) on the reactor productivity was investigated. The results showed that at low cell concentrations, movement of cells between the surface and bottom zones of shallow and moderately deep reactors had no significant effect on \( \text{Chlorella pyrenoidosa} \) C-212 growth and productivity. However, as the cell concentration in the reactors increased, cell movement between the two zones resulted in increased productivity of the shallow reactor but decreased productivity of the moderately deep reactor. On the other hand, in the deep reactor, random movement of cells between the two zones resulted in decreased \( \text{Chlorella} \) growth rate regardless of the cell concentration. This may be attributed to the fact that at high cell concentration or in a deep reactor, if the cells move between the surface and bottom of the reactor, they spend too long a time in the dark part of the reactor where there is no cell growth, and endogenous respiration as well as cell death may lead to a decrease in cell concentration. When \( \text{Spirulina platensis} \) M-135 cells were cultivated in the deep reactor, even at high cell concentration, movement of cells between the surface and bottom zones of the reactor led to an increase in the reactor productivity. The reasons for the difference in the results obtained with these two strains of algae could be attributed to the difference in their light requirements since it was found that the saturation light intensity and specific decrease in cell concentration when incubated in the dark were lower for \( \text{Spirulina} \) than for \( \text{Chlorella} \) cells.

[Key words: \( \text{Chlorella pyrenoidosa} \) C-212, \( \text{Spirulina platensis} \) M-135, random mixing, cell movement, flashing light, algal productivity]

Depending on the location and season, the intensity of solar radiation exceeds the saturation light intensities for photosynthetic growth of most algae species. In addition, the incident light is rapidly attenuated in dense algal cultures so that the bottom of the ponds may be completely dark while the light utilization efficiencies at the surface are very low. The phenomenon of flashing light effect (under certain frequencies, there is light integration in a cell suspension subjected to alternating light and dark periods, leading to increased light utilization efficiency) is well documented (1-3). It has been hoped that this flashing light effect can be exploited to improve the productivities of algal cultivation systems and much work has been done on this with some conflicting results. For example, Bosca et al. (4) reported that regardless of the intensity of light, the flashing light effect occurs and has positive effect on reactor productivity while Grobbelaar (5) noted that at the dark/light ratio of 1, no effect of dark/light cycles on photosynthetic rates of \( \text{Chlorella} \) and \( \text{Scenedesmus} \) species was observed.

Accurate determination of the flashing light effect involves the use of very low cell concentrations to avoid light shading and exposing the cells to alternating dark and constant light intensity at a specified frequency. While these conditions are good for data analyses, they differ from the conditions observed in algal reactors. The results cannot, therefore, be directly applied to actual cultivation systems where the cell concentrations are usually high and the cells experience varying light intensities ranging from the light-sufficient state to the state of absolute darkness as they move from the surface to the bottom of the pond. Furthermore, probability analysis of cell movement in turbulent flow suggests that cells in the same reactor under turbulent flow would experience different durations of light and dark periods (6).

Apart from moving cells in and out of the light zone, other main beneficial effects of mixing in algal cultures include: (a) degassing photosynthetically accumulated oxygen which may inhibit cell growth (7, 8), (b) improving mass transfer between cells and the environment, (c) eliminating thermal stratification and (d) preventing cells from settling to the bottom of the pond. Different levels of mixing intensities are required to satisfy each of these objectives and for nutrient-sufficient cultures of algae, a minimum degree of mixing is sufficient to satisfy objectives (a) to (d) while excessive mixing can even lead to carbon dioxide degassing and thus, lowers the carbon dioxide storage capacity of the pond (9). Mixing in most algal cultures is random but many workers have claimed that utilization of the flashing light effect in algal ponds requires modulated mixing whereby cells are alternately moved to the surface and bottom of the ponds at an optimum frequency. Laws et al. (10) have reported on the use of airplane wing-shaped foils to induce vortex circulation which leads to an ordered pattern of vertical mixing with consequent improvements in the productivity of the flowing algal culture. However, it is not clear if the improvement is due primarily to the light modulation experienced by the cells or whether other effects of mixing were involved.

It is difficult to accurately analyze the flashing light effects in actual cultivation ponds since aside from the light intensity gradient at high cell densities, cell circulation frequencies between the light and dark zones vary.

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among individual cells and also as the cultivation progresses. However, knowledge of the effects of cell movement in and out of the light zone on the reactor productivity is necessary in order to determine the economic benefits of installing and operating mixing systems in algal cultures. In this work, the effects of cell movement by random mixing (which is the type of mixing in most algal cultivation systems) between the surface and bottom of photobioreactors on the reactor productivity were investigated using reactors of different depths.

MATERIALS AND METHODS

Bioreactor systems Each reactor was assumed to be made up of light and dark zones and the effects of random movement of cells between these two zones on the reactor productivity were examined using reactors of various depths. Since the average light/dark cycles experienced by cells depend not only on the actual depth of the reactor but also on the incident light intensity, shallow, moderately deep and deep reactors (cultivation ponds) are defined as those with incident light intensities per unit volume (assuming that the illumination area is constant) of 8125, 4062 and 2031 μmol·m⁻²·s⁻¹, respectively. Reactors each with illumination area of 187.5 cm² and depth of 4 cm, 8 cm or 16 cm were constructed with acrylic sheets as shown in Fig. 1. Two reactors were used in each case. One reactor (Fig. 1A) was not separated into surface and bottom compartments (hereafter called unseparated reactor) and the cells moved freely and randomly between the illuminated and dark zones. The second reactor (hereafter called separated reactor) was separated into surface and bottom halves by transparent acrylic sheet so that (depending on cell concentration) light passes through the surface compartment to the bottom compartment while cells are restricted to each half of the reactor (Fig. 1B). Mixing was achieved by means of gas sparging through glass ball filters inserted into the reactor and by means of magnetic stirrers (Fig. 1). During the cultivation, the mixing intensity (the aeration rate, the number of the glass ball filters and the magnetic stirring speed) was maintained equal and constant in both types of the reactors so that any difference in the growth characteristics of the algae can be attributed to the difference in the movement of cells within the reactor. Light fluorescent tubes for plant growth experiments (FL-40-w-PG, Matsushita Denki Sangyo, Tokyo) were used for illumination. In Fig. 1, all sides of the reactor are shown to be transparent for clarity. In the actual reactors, except for the illumination surface and the demarcating transparent acrylic sheet, black acrylic sheets were used in the construction of all other sides including the lid to ensure that light entered the reactor through the illumination surface only. In this paper, the surface and bottom compartments (zones) refer to those adjacent to, and remote from, the illumination surface, respectively (Fig. 1).

Microorganisms and growth conditions Chlorella pyrenoidosa C-212 and Spirulina platensis M-135 were obtained from the algal collection of the Institute of Applied Microbiology, University of Tokyo, Japan. C. pyrenoidosa was cultivated in a medium which contained (in g·l⁻¹) KNO₃, 0.5; KH₂PO₄, 1.25; K₂HPO₄, 0.1; MgSO₄·7H₂O, 1.8; FeSO₄·7H₂O, 0.03; EDTA, 0.04; and A₈ solution, 1.0 ml·l⁻¹. The composition of the medium used for S. platensis cultivation was (in g·l⁻¹) NaHCO₃, 16.8; K₂HPO₄, 0.5; NaNO₃, 2.5; K₂SO₄, 1.0; NaCl, 1.0; MgSO₄·7H₂O, 0.02; CaCl₂·2H₂O, 0.04; Na₂EDTA·2H₂O, 0.01; FeSO₄·7H₂O, 0.01 and 1.0 ml·l⁻¹ of A₈ solution. The A₈ solution was of the following composition (in g·l⁻¹): H₃BO₃, 2.86; MnSO₄·7H₂O, 2.5; ZnSO₄·7H₂O, 0.222; CuSO₄·5H₂O, 0.079 and Na₂MoO₄·0.021.

Incident light intensity, pH and temperature were 325 μmol·m⁻²·s⁻¹, 5.8 and 38°C for C. pyrenoidosa and 200 μmol·m⁻²·s⁻¹, 9.8 and 30°C for S. platensis, respectively. The aeration rate with 5% CO₂ in air for C. pyrenoidosa or with ordinary air for S. platensis was 0.3 vvm.

Determination of kinetic parameters The kinetic parameters were determined during the initial growth phase (when the cell concentrations were still very low) of batch cultures in 100 ml Roux bottles. It was assumed that at such very low cell concentrations, light shading was negligibly small and cell growth rate was represented by Eq. 1 which was rearranged to give Eq. 2.

\[
\mu = \frac{\mu_{\text{max}} \times I_c}{K_l + I_o}
\]

\[
\frac{1}{\mu} = \frac{K_l}{\mu_{\text{max}}} \times \frac{1}{I_c} + \frac{1}{\mu_{\text{max}}}
\]

where \(\mu\) = specific growth rate (h⁻¹), \(\mu_{\text{max}}\) = maximum specific growth rate (h⁻¹), \(I_c\) = incident light intensity (μmol·m⁻²·s⁻¹) and \(K_l\) = light saturation constant (μmol·m⁻²·s⁻¹). \(\mu\) was determined from the initial growth curves while \(\mu_{\text{max}}\) and \(K_l\) were obtained from a plot of \(1/\mu\) vs. \(1/I_c\).

The specific rate of decrease in cell concentration when cells were subjected to the dark condition was determined in 100 ml Roux bottles. Cells were cultivated autotrophically to the stationary phase, subjected to total darkness (by wrapping the Roux bottles containing the culture broth with aluminium foil) and the rate of decrease in the concentration of cells inside the Roux bottles was monitored. During cultivation, the media, the incident light intensities and the aeration rates were as described above.

FIG. 1. Schematic diagrams of (A) unseparated photobioreactor where cells could randomly move between the surface and bottom zones and (B) separated photobioreactor where cell movement was restricted to each half of the reactor. BC, Bottom compartment; E, gas outlet; FT, fluorescent tubes; GBF, glass ball filter; L, reactor lid; MB, magnetic bar; MS, magnetic stirrer; SC, surface compartment and TAS, transparent acrylic sheet.
tions were the same for all compartments. The symbols are O: first (surface) compartment; 1: second compartment; (2): third compartment; 4: fourth (bottom) compartment and №: total productivity.

Analytical methods

The dry cell concentration was determined after washing (by repeated centrifugation and suspension in distilled water) and drying at 105°C for 12 h or by measuring the optical density at 680 nm (Spectronic 20A, Shimadzu Scientific Instruments, Japan). In the latter, the OD readings were converted to dry cell concentrations using predetermined calibration curves for *Chlorella* and *Spirulina* cells. In the case of separated reactors, the total cell concentrations (Fig. 3) were calculated as the average of the cell concentrations in the two compartments.

RESULTS

In homogeneously mixed nutrient-saturated cultures, light can be considered to be the only growth-limiting factor. In that case, movement of cells between the surface and bottom of the reactor would affect productivity only when there are differences in the productivities at different depths of the reactor (due to light intensity gradient within the reactor). *Chlorella* productivity at different depths of a deep reactor (depth = 16 cm, \(I_0 = 325 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\)) was investigated by dividing the reactor with transparent acryl sheets into 4 equal zones. They were inoculated with an equal initial cell concentration and then the reactor was illuminated from one surface. The time course of cell growth in each zone was taken and the relative productivity (the ratio of cell productivity in one zone to the total productivity) was calculated at different time intervals (corresponding to various cell concentrations in the surface compartment) as summarized in Fig. 2. At a low cell concentration of about \(0.1 \text{ g} \cdot \text{l}^{-1}\), about 50% of the total productivity was obtained at the surface compartment. This means that even at low cell concentrations, about half of the total cells are produced only in the surface zone corresponding to 25% of the total reactor volume. At such a low cell concentration, cell growth was also observed in the other zones including the bottom compartment of the reactor. However, with increasing cell concentration, productivity at the zones remote from the illumination surface decreased sharply. At a cell concentration of about \(1 \text{ g} \cdot \text{l}^{-1}\), the bottom 75% of the reactor (the last 3 zones) was not productive at all (Fig. 2). When the cell concentration increased above \(2 \text{ g} \cdot \text{l}^{-1}\), light intensity at the last three compartments was almost zero and slight decreases in cell concentrations were observed in those compartments. This led to the question of whether the productivity of the entire reactor would be affected if the cells were allowed to move freely (by random mixing) between the surface (illuminated) and bottom (dark) zones of the reactor.

Separated reactors (Fig. 1B) were used to investigate the effects of random cell movement between the surface and bottom of the reactors on the reactor productivities. During the early growth phase, when the total cell concentration (the average of the cell concentrations in the surface and bottom compartments) was less than \(2.2 \text{ g} \cdot \text{l}^{-1}\), movement of cells between the two zones of the shallow reactor had no significant effect on *Chlorella* cell growth (Fig. 3A). During this period, productivity in the lower zone was still relatively high (Fig. 4A). However, as the total cell concentration increased above \(2.2 \text{ g} \cdot \text{l}^{-1}\), movement of cells between the surface and bottom of the shallow reactor was found to be beneficial for cell growth. At such high cell concentration (after about 100 h of cultivation), volumetric productivity at the surface zone of the separated reactor was almost the same as the total volumetric productivity of the unseparated reactor (Fig. 4A). However, at the same time, productivity in the bottom zone of the separated reactor (black circles) was very low so that its total productivity (the average of the productivity in the surface and bottom compartments) was lower than that of the unseparated one.

In the case of the moderately deep reactor, movement of *Chlorella* cells between the surface and bottom of the reactor had no significant effect on the reactor productivity when the total cell concentration was less than \(1.3 \text{ g} \cdot \text{l}^{-1}\). However, in contrast to the shallow reactor, as
the total cell concentration increased beyond 1.4 g l⁻¹, cell movement between the surface and the bottom of the reactor resulted in a decrease in cell growth (Fig. 3B).

As shown in Fig. 4B, for the moderately deep separated reactor, productivity at the bottom zone was zero when cell concentration was high, however, cells continued to grow in the surface zone even when the total cell concentration was 1.7 g l⁻¹ (when cell concentration in the surface zone was higher than 3 g l⁻¹). On the other hand, productivity in the unseparated reactor decreased to almost zero at a cell concentration of 1.2 g l⁻¹.

Even at low cell concentrations, circulation of *Chlorella* cells between the surface and bottom of deep reactor had an adverse effect on cell growth (Fig. 3C). When cells were allowed to randomly move between the two zones (unseparated reactor), productivity at the bottom zone was zero when cell concentration was high (0.075 g l⁻¹ d⁻¹) and decreased to almost zero when the cell concentration increased to just 0.4 g l⁻¹. In contrast, as shown in Fig. 4C, although productivity at the bottom zone of the separated reactor decreased to almost zero at a total cell concentration of 0.2 g l⁻¹, productivity at the surface zone was comparatively high up to a cell concentration of about 0.7 g l⁻¹.

The productivity of *Chlorella* increased when cell concentration was 1.4 g l⁻¹. However, in the unseparated reactor, productivity at the bottom zone was zero when cell concentration was high, however, cells continued to grow in the surface zone even when the total cell concentration was 1.7 g l⁻¹. The net result is that higher reactor productivity was obtained by allowing the cells to freely circulate between the surface and bottom of the reactor.
In view of the difference between the results obtained with Chlorella and Spirulina cells, some of their kinetic parameters were compared. As shown in Fig. 5 and Table 1, compared with Chlorella, Spirulina's light saturation constant (K_t), and saturation light intensity (light intensity at which \( \nu = \nu_{\text{max}} \)) are lower. Furthermore, when the cells were autotrophically grown to the stationary phase and then subjected to total darkness, the specific rate of decrease in the concentration of Chlorella cells was higher than that of Spirulina (Fig. 6).

**DISCUSSION**

Photosynthetic growth involves reactions which require light (light reactions) and those which do not (dark reactions). Under light condition, cells can store energy and produce intermediate products (e.g., reducing power and ATP) which are used for carbon dioxide fixation and synthesis of biomass whether the cells are in light or dark condition. It has been shown that when algal cells are transferred from light to dark, photosynthesis continues for a certain period of time in the dark (2), implying that the dark reaction can be the rate-limiting step in the overall process. Therefore enhancement of light utilization efficiency (flashing light effect) can be achieved if the cells are subjected to a condition whereby at certain time intervals, the accumulated intermediate products are processed in the dark. The extent of the flashing light effect depends on the length of time cells can continue to grow under dark condition (on the amount of energy/intermediate products which the cells are able to accumulate while under light condition). For a given strain of algae, this depends, at least to a certain extent, on the light intensity and the length of time the cells are exposed to light.

When the cells move to the dark zone, they continue to grow until all the energy/intermediate products stored during their stay in the light zone are used up. After this, growth stops and endogenous respiration (11) may take place, leading to the decrease in cell concentration. The maximum flashing light effect (total light integration) will be observed if the cells return to the light zone immediately after the stored energy/ATP are exhausted.

Movement of Chlorella cells between the surface and bottom of the reactor resulted in increased productivity. Since mixing conditions as well as the incident light intensities were the same in both separated and unseparated reactors (Fig. 1), higher volumetric productivity obtained in the unseparated reactor can be attributed to cell circulation between the surface illuminated and bottom dark zones.

In the case of the moderately deep reactor with cell concentrations more than 1.4 g·L⁻¹ or in the deep reactor, the proportion of the reactor volume under light condition is very small and random mixing of cells between the surface and bottom of the reactors results in the cells spending too long a time (longer than that required for the processing of the accumulated energy/intermediate products) in the dark zone. Under such condition, there would be no growth and endogenous respiration as well as cell death could take place during some part of the time the cells spend in the dark zone (3). Lower productivities observed in the moderately deep (at high cell concentrations) and deep reactors when Chlorella cells were allowed to move between the surface and bottom of the reactors can thus be attributed to the high proportion of time the cells spend in the dark. This view is supported by the results shown in Fig. 6 where there are decreases in the concentration of cells subjected to total darkness. Thus it can be concluded that while intermittent dark periods may lead to improved light utilization efficiency (due to the flashing light effect), reactor productivity would decrease if the duration of the dark period becomes too long. Also in flashing light experiments, it has been reported that maintenance energy increases in proportion to the duration of the dark period (3) while Terry (12) also noted that at low proportion of time the cells spent in the light, there was no enhancement of net photosynthetic efficiency because of respiration.

In contrast, higher cell growth and productivity were consistently observed when Spirulina cells were allowed to move between the surface and bottom of the deep reactor. Some possible explanations of the difference in the results obtained between Spirulina and Chlorella can be made from the results shown in Table 1. The lower saturation light intensity \( (\nu_{\text{max}}) \) for Spirulina implies that at low cell concentrations (when the amount of light absorbed by the cells is still low and high proportion of the light is transmitted to the bottom zone), the difference in the average light intensity between the separated and unseparated reactors would not result in much difference between the productivities of the two reactors (since both light intensities may be saturating for the cells). The lower \( K_t \) value for Spirulina means higher affinity for light which implies that Spirulina cells can utilize low light intensities more efficiently than Chlorella cells. Furthermore, the growth rate of Spirulina is very low \( (\nu_{\text{max}} = 0.083 \text{ h}^{-1}) \) which means that the rate of decrease in light intensity during the cultivation (due to cell absorption) would be lower than that of Chlorella with a higher growth rate. Also, Spirulina's lower specific decrease in cell concentration (md) when incubated in the dark would mean that when compared with Chlorella, it can spend a longer time in the dark without exhibiting a significant decrease in cell concentration.

The effects of cell movement between the surface (illuminated) and bottom (dark) zones observed in this study may not be completely attributed only to the flashing light experienced by the cells during the movement since quantitative analysis of the cycle frequencies (which under the present experimental conditions would vary with cultivation time and among the individual cells) was not made. Furthermore, cell growth and productivity were used rather than the more accurate photosynthetic rates. Aside from the flashing light experienced by the cells as they move between the surface and bottom of the reactor, other factors such as degree of mixing and cell adaptation might have contributed to the results. However, since the aeration rate and the magnetic stirring rates were kept equal (in both separated and unseparated reactors) and were high enough to maintain a homogeneous condition, contribution due to difference in the degree of mixing, if any, can be considered to be negligibly small. In view of this, the flashing light experienced by the cells might have contributed more to the results obtained in this study. The important thing, however, is that at high cell concentrations, the movement of cells between the surface and bottom of the reactor has an effect on the reactor productivity. The net result may be positive or negative depending on the inci-
dent light intensity per unit volume of the reactor (for algal ponds, the incident light intensity and the depth of the ponds) as well as the light requirements of the algal strain. These results have some practical implications.

Although the average volumetric productivity of *Chlorella* in the moderately deep reactor was about half that in the shallow one, productivity per unit time was about the same for the two reactors while productivity per unit area was about twice as high in the moderately deep reactor. On the other hand, in comparison with the moderately deep pond, the maximum productivity per unit volume, time and area were only 20%, 40% and 80%, respectively, in the deep reactor. Construction of algal cultivation ponds so deep that the incident light intensity per unit volume is less than 2,000 \( \mu \text{mol} \cdot \text{m}^{-3} \cdot \text{s}^{-1} \) should be avoided since the maximum cell concentration in such ponds would be too low and random mixing would even decrease the productivity further.

Furthermore, since except for very deep ponds, random mixing between the surface and the bottom of the reactors had no significant effect on the productivity at low cell concentrations, mixing of ponds operated at such low cell concentrations should be reduced to the level necessary to avoid cell sedimentation and mass transfer limitations. If the ponds are operated at high cell densities, mixing intensity should be regulated according to the available incident light intensities. During cloudy days, mixing should be reduced since under such low incident light intensity, movement of cells between the surface and bottom of the reactor will result in lower reactor productivity.

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