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ORIGINAL PAPER

C.U. Ugwu · J.C. Ogbonna · H. Tanaka

Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers

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Abstract The feasibility of improving mass transfer characteristics of inclined tubular photobioreactors by installation of static mixers was investigated. The mass transfer characteristics of the tubular photobioreactor varied depending on the type (shape) and the number of static mixers. The volumetric oxygen transfer coefficient (k_La) and gas hold up of the photobioreactor with internal static mixers were significantly higher than those of the photobioreactor without static mixers. The k_La and gas hold up increased with the number of static mixers but the mixing time became longer due to restricted liquid flow through the static mixers. By installing the static mixers, the liquid flow changed from plug flow to turbulent mixing so that cells were moved between the surface and bottom of the photobioreactor. In outdoor culture of *Chlorella sorokiniana*, the photobioreactor with static mixers gave higher biomass productivities irrespective of the standing biomass concentration and solar radiation. The effectiveness of the static mixers (average percentage increase in the productivities of the photobioreactor with static mixers over the productivities obtained without static mixers) was higher at higher standing biomass concentrations and on cloudy days (solar radiation below 6 MJ m^{-2} day^{-1}).

Introduction

Currently, open ponds are used for most algal cultivation but due to various problems, such as difficulties in controlling the culture conditions and contamination, productivities obtained in such systems are very low. In order to solve some of these problems, various closed photobioreactors have been proposed. These include airlift tubular loop reactors (Pirt et al. 1983), vertical tubular photobioreactors (Miyamoto et al. 1988), horizontal/near horizontal tubular photobioreactors (Chaumont et al. 1988; Torzillo et al. 1991; Tredici and Chini Zittelli 1998), flat panel reactors (Ramos de Ortega and Roux 1986; Tredici et al. 1991; Hu et al. 1996) and internally illuminated stirred tank photobioreactors (Ogbonna et al. 1996, 1998). Among the proposed closed systems, tubular photobioreactors are promising because they are cheap, simple and easy to construct. However, a major limitation of tubular photobioreactors is their poor mass transfer characteristics. Although carbon dioxide can easily be supplied in excess, efficient removal of photosynthetically generated oxygen still remains a major problem. These aspects of mass transfer and hydrodynamics in tubular photobioreactors have been reported (Camacho Rubio et al. 1999). Accumulation of dissolved oxygen (DO) has been recognized as a constraint in the scale-up of horizontal tubular photobioreactors (Camacho Rubio et al. 1999; Molina Grima et al. 1999). Thus, an efficient method of removing DO in tubular photobioreactors is required. Although some tubular photobioreactors are equipped with airlift systems for better gas dispersion (Lee and Low 1991; Lee et al. 1995; Molina Grima et al. 1995; Acién Fernández et al. 1997), mixing is still a problem because the gases tend to float at the upper part of the tubes. Efficient mixing systems in tubular photobioreactors require elements that can move both the culture and the gases between the upper and lower parts of the tubes. Circulation of broth between the upper and lower part of the tubes will help to induce a flashing light effect and thus more efficient light utilization in dense cultures of photosynthetic microorganisms.

Static mixers, which are motionless baffles installed inside bioreactors to break down large bubbles into tiny ones, improve mixing with a resultant increase in the air-liquid mass transfer inside the bioreactors. The use of static mixers in bioreactors has been reported (Hsu et al. 1975; Lin et al. 1976; Wang and Fan 1978; Stejskal and Potucek 1985; Chisti et al. 1990; Zhou et al. 1993). Although an attempt has been made to use static mixers in loop tubular photobioreactors, they were not effective in improving gas dispersion because they tend to trap algal

C.U. Ugwu · J.C. Ogbonna (✉) · H. Tanaka
Institute of Applied Biochemistry, University of Tsukuba, Ibaraki 305–8572, Japan
e-mail: jogbonna@sakura.cc.tsukuba.ac.jp
Tel.: +81-298-536646, Fax: +81-298-534605

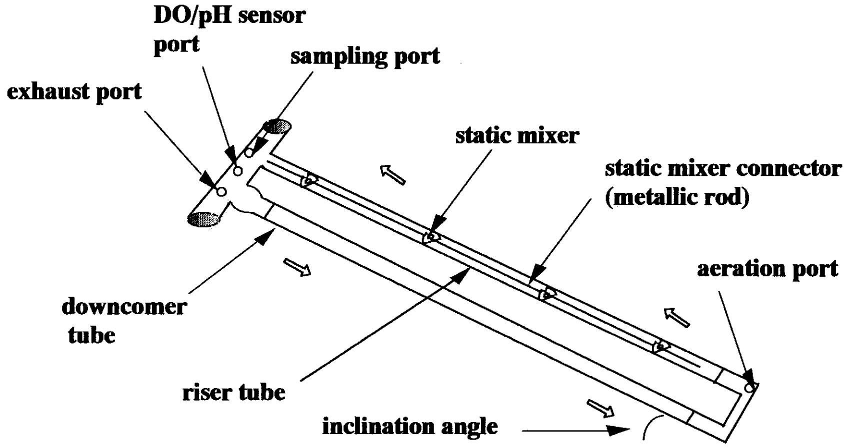


Fig. 1 Schematic diagram of the inclined tubular photobioreactor with internal static mixers

cells and consequently block the tubes (Pirt et al. 1983). Possible reasons for the ineffectiveness of static mixers in the latter case may be due to the small diameter of the tubes and the type of static mixers used. Static mixers have good potential in horizontal tubular photobioreactors but their efficiency depends on their design being appropriate for easy installation and gas bubble dispersion with minimal decrease in liquid circulation. Static mixers should be designed so that the cells will not stagnate in the tubes, thus ensuring free circulation of culture broth along the tubes.

In this report, the design and construction of static mixers and their installation in tubular photobioreactors were investigated. The effects of such static mixers on the mass transfer characteristics of tubular photobioreactors – mixing time, gas hold up and volumetric oxygen transfer coefficient (k_La) – were studied. The effectiveness of the static mixers in outdoor cultures of *Chlorella sorokiniana* under various standing biomass concentrations was also investigated.

Materials and methods

The photobioreactors

A schematic diagram of the tubular photobioreactor is shown in Fig. 1. The photobioreactor consists of a riser and a downcomer tube. The tubes are joined together at the bottom by a gassing chamber and on the top by a degasser, and are equipped with ports for pH, temperature and DO measurements. The internal diameter of each tube is 38 mm while the total length of the riser and the downcomer tubes is 4 m. The photobioreactor was operated at a working volume of 6 l. Aeration with 5% CO_2 in air is done only in the riser tube, resulting in liquid circulation between the riser and the downcomer section. After cultivation, the sampling point was opened and the culture broth flowed out freely from the photobioreactor. The tubular photobioreactor was installed on a 2-m stage located at the Agricultural and Forestry Research Center of the University of Tsukuba, Japan. The surface area covered by the photobioreactor was 0.18 m^2.

The static mixers

Schematic diagrams of four different types of static mixers designed and constructed in this study are shown in Fig. 2 (A–D). Transparent acrylic panels (thickness =2 mm) were used for the construction of A-type, B-type and D-type static mixers. The A-type static mixer was constructed by making a v-cut on top of a circular transparent acrylic material. The culture broth and air bubbles circulate through the v-cut. The B-type static mixers were constructed by reducing the lower part of the A-type static mixers in order to ensure free flow of the culture broth along the tubes. The C-type static mixers were made from perforated stainless steel plates (thickness of the plate =1 mm, pore diameter =3 mm). The D-type static mixers were Y-shaped transparent acrylic panels. The static mixers were connected by a metallic rod (diameter =8 mm). In each of the static mixers, there is a small hole at the center for attachment to the connecting rod.

Effects of operational parameters on mass transfer characteristics

The effects of operational parameters such as aeration rate, angle of inclination, types and spacing distances of the static mixers on mixing time, gas hold up and k_La were investigated. Aeration rates were varied from 0.125 vvm to 1.250 vvm while the inclination angle was varied from 8° to 45°. The number of static mixers used varied from one to eight, corresponding to a decrease in the spacing distance from 1 m to 0.25 m. Mixing time in the photobioreactors was measured by introducing an alkali tracer into the aeration point and measuring the length of time taken to achieve a constant pH at the degasser chamber of the photobioreactor. The gas hold up was calculated as the ratio of gas volume to the total volume of liquid inside the photobioreactor. This was measured by estimating the volume of the liquid displaced by the gas (expansion of liquid volume due to gas). A calibrated glass cylinder was connected to an outlet pipe at the degasser port of the photobioreactor. The reactor was filled with water to the brim before aeration. As the aeration was started, the gas bubbles displaced the liquid into the cylinder and the volume of the displaced liquid was measured after achieving equilibrium. Measurement of k_La in the photobioreactor was carried out using a static gassing out method. The photobioreactor was filled with tap water and aerated with nitrogen gas to remove oxygen before aerating with air. The DO sensor was placed in the degasser port to monitor the increase in DO concentration at time intervals. The k_La was calculated from the slope of a plot of DO concentration vs time.

Microorganism and culture conditions

Chlorella sorokiniana IAM-212, obtained from the Institute of Applied Microbiology, University of Tokyo (Culture Collection Center), was used in this study. The culture medium was composed of (g l^{-1}); KNO_3, 5.0; KH_2PO_4, 1.25; K_2HPO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 2.5; NaCl, 1.8; A_5 solution (consists of H_3BO_3, 2.85 g; $MnCl_2 \cdot 4H_2O$, 0.222 g; $CuSO_4 \cdot 5H_2O$, 0.08 g; $NaMoO_4$, 0.021 g; in

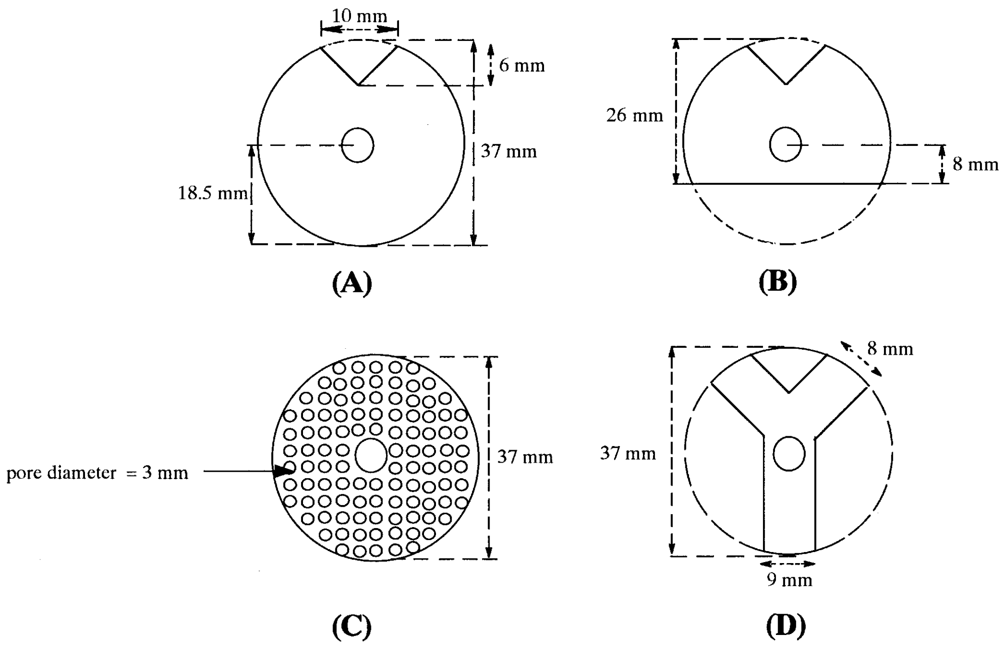


Fig. 2A–D Schematic diagrams of the static mixers developed for tubular photobioreactors

1 l of distilled water) 1 ml l^{-l}; iron solution (consists of $FeSO_4 \cdot 7H_2O$, 25 g; EDTA, 33.5 g in 1 l of distilled water) 1 ml l^{-l}. The pH was adjusted to 6.0.

Pre-culture

Pre-culture of *C. sorokiniana* was performed by inoculating a loop of the slant culture into 1.2 l of the medium in a 1.5 l Roux flask under continuous illumination at 30°C for 48 h. Seven daylight fluorescent lamps for plant growth experiments (8FL-40-s-PG, National Electric, Tokyo) arranged in parallel on a vertical plane, were used as the light source. The light intensity at the surface of the Roux bottle was 300 µmol m^{-2} s^{-1}. Aeration and mixing were achieved by sparging air enriched with 5% CO_2 through a glass-ball filter, which was inserted to the bottom of the Roux flask, at 0.3 vvm.

Outdoor culture

The pre-culture was used to inoculate the 4-m tubular photobioreactor (working volume =6 l). The culture was aerated at 0.25 vvm with air fortified with 5% CO_2. The photobioreactor was naturally illuminated by solar light energy. Cooling was carried out occasionally by sprinkling the surface of the photobioreactor with tap water. The effects of standing biomass concentrations (1.5 g l^{-l}, 2.3 g l^{-l}, 3.4 g l^{-l} and 5.0 g l^{-l}) on biomass productivity were investigated by daily dilution of the culture with fresh medium. Every morning, the optical density was measured to estimate the cell concentrations from a predetermined calibration curve. The culture was then diluted to the desired standing biomass concentration with fresh medium. The cultivation period was between 6 a.m. and 6 p.m. (12-h daylight period). The outdoor culture experiments were carried out between May and September of the years 2000 and 2001.

Analytical methods

Cell dry weight determination was made using duplicate samples of the culture. The cells were washed with 0.5 M HCl to remove the precipitated salts and other non-organic substances, rinsed with distilled water, dried at 105°C for 24 h, cooled over silica gel in a desiccator, and weighed (Ogbonna and Tanaka 1996; Ogbonna et al. 1997). The optical density was measured at 680 nm using a spectrophotometer (Spectronic 20A; Shimadzu, Tokyo, Japan). The solar light intensity on the surface of the photobioreactor was measured using a photorecorder (PHR-51; T&D, Japan). DO concentrations in the photobioreactors were measured using a DO controller (Mk-250 DO, Marubishi, Japan). The increase in the biomass concentration between 6 a.m. and 6 p.m. was calculated as the daily productivity (g l^{-1} day^{-1}). The effectiveness of the static mixers (E_s) was calculated as:

$$E_s = \frac{P_w - P_0}{P_0} \times 100 \qquad (1)$$

Here, P_w and P_0 are the biomass productivities in the photobioreactor with and without static mixers, respectively. Experiments on mass transfer were done in 4 replicates. The standard error of the mean (SE) and confidence limits were calculated and the mean values were plotted with 95% confidence limits ($\bar{X} \pm SE$) (Parker 1980). Analysis of variance (ANOVA) followed by least significant difference tests was used to compare the results.

Results

Effect of various types and spacing distances of static mixers on mass transfer characteristics of the photobioreactors

The effects of the various types and numbers (spacing distances) of static mixers on k_La, gas hold up and mixing time (the inclination angle was 45° while the aeration rate was 0.3 vvm) are shown in Figs. 3 and 4. The k_La and gas hold up increased as the number of static mixers was increased from one to eight (corresponding to a decrease in spacing distance from 1 m to 0.25 m) (Fig. 3). On the other hand, the mixing time became longer as the number of static mixers was increased (Fig. 4). All four types of static mixers were effective in increasing the mass transfer capacity of the photobioreactor. Regardless of the type of static mixer installed, the k_La and gas hold

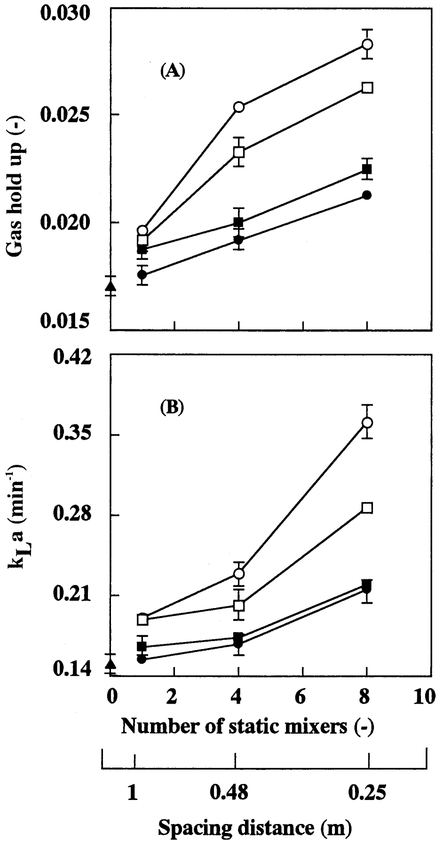


Fig. 3 Effect of different types and number of static mixers on **A** gas hold up, and **B** volumetric oxygen transfer coefficient (k_La) in a 4 m tubular photobioreactor. The angle of inclination was 45° and the aeration rate was 0.3 vvm. *Open squares* A-type static mixers, *open circles* B-type static mixers, *closed squares* C-type static mixers, *closed circles* D-type static mixers, *closed triangles* without static mixers

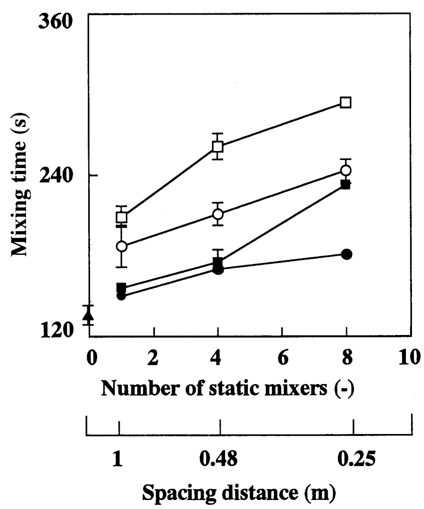


Fig. 4 Effect of different types and number of static mixers on mixing time in a 4 m tubular photobioreactor. The angle of inclination was 45° and the aeration rate was 0.3 vvm. *Open squares* A-type static mixers, *open circles* B-type static mixers, *closed squares* C-type static mixers, *closed circles* D-type static mixers, *closed triangles* without static mixers

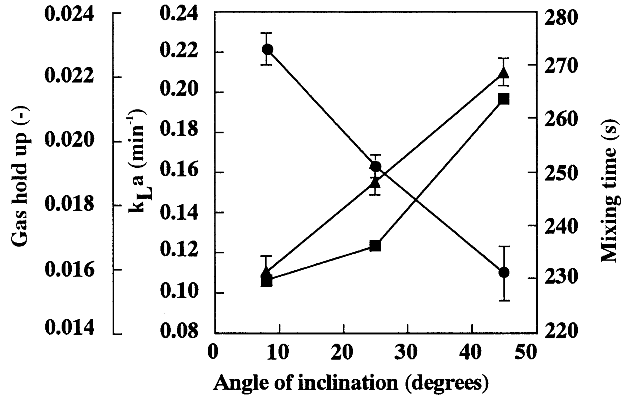


Fig. 5 Effect of angle of inclination on mass transfer characteristics of a 4 m tubular photobioreactor with four B-type static mixers. The aeration rate was 0.25 vvm. *Closed triangles* Gas hold up, *closed squares* k_La, *closed circles* mixing time

up values obtained were significantly higher than those obtained without static mixers. There was no significant difference between the gas hold up and k_La values obtained with the C-type and D–type static mixers ($P<0.05$). The effectiveness of the static mixers in increasing the k_La and gas hold up can be ranked in the following order: B>A>C, D>without static mixers. Although the mixing times in C-type and D-type static mixers were shorter than those obtained with A-type and B-type static mixers (without static mixers<C, D<B<A), the k_La and gas hold up were lower. Of all the types of static mixers, B-type was considered the best as reflected by the high gas hold up, volumetric mass transfer coefficient (k_La) and moderate mixing time. By installing eight B-type static mixers, there was over 140% increase in the k_La compared to the value obtained without static mixers. Also, the gas hold up increased by 65%, but the mixing time became longer by about 70%. Although the k_La and gas hold up increased as the number of static mixers was increased from one to eight, a compromise between the increase in k_La and gas hold up and the increase in mixing time was made. Thus, four B-type static mixers were selected for further studies.

Effect of inclination angle of the photobioreactor on mass transfer characteristics

The effects of photobioreactor inclination angles on mass transfer characteristics of the photobioreactor equipped with four B-type static mixers are shown in Fig. 5. At an aeration rate of 0.25 vvm, both the k_La and gas hold up increased (8°<25°<45°) while the mixing time decreased (8°>25°>45°) as the angle of inclination was increased from 8° to 45°. Although increasing the angle of inclination above 45° would result in better mass transfer characteristics, installing and supporting photobioreactors at high inclination angles would be technically difficult and expensive. Furthermore, a

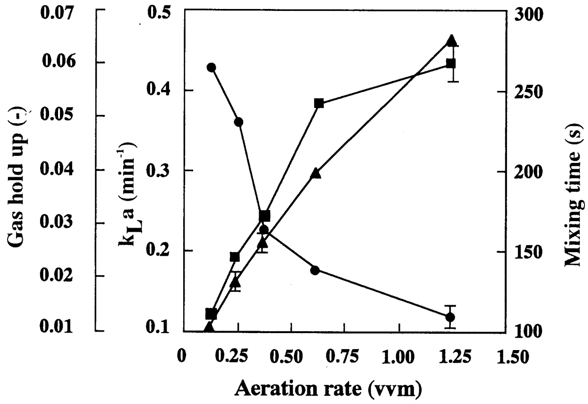


Fig. 6 Effect of aeration rate on mass transfer characteristics of a 4 m tubular photobioreactor with four B-type static mixers. The angle of inclination was 45°. *Closed triangles* Gas hold up, *closed squares* k_La, *closed circles* mixing time

higher inclination angle would mean lower light intensity on the photobioreactor surface. Thus, 45° was used in all subsequent experiments.

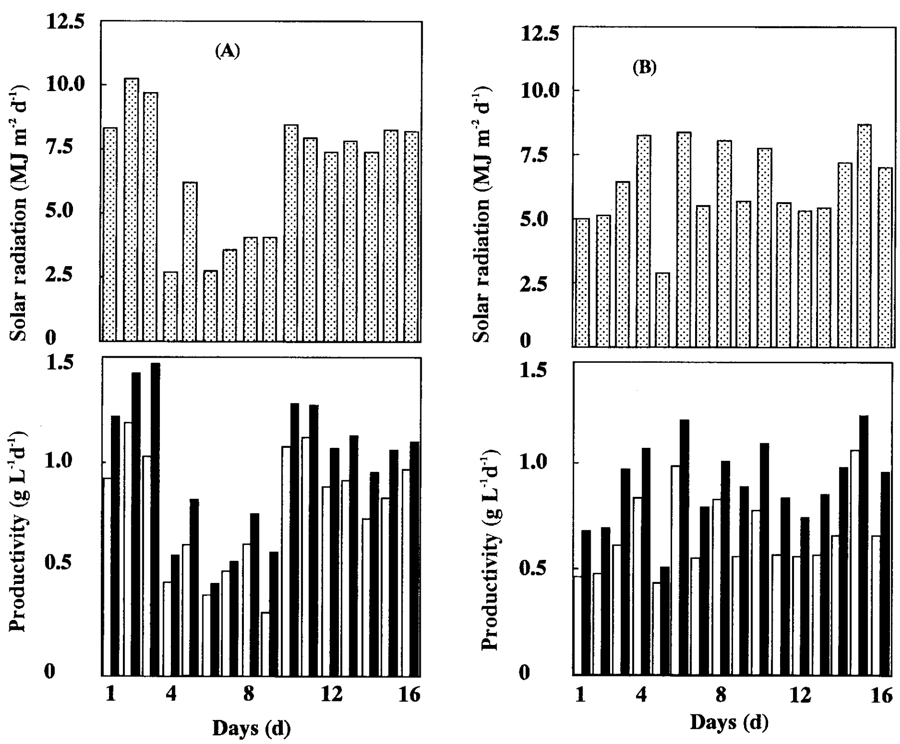
Effect of aeration rate on mass transfer characteristics of the photobioreactor

The effect of aeration rates on mass transfer characteristics of the photobioreactor with four B-type static mixers is shown in Fig. 6. By varying the aeration rate from 0.125 vvm to 1.250 vvm (inclination angle =45°), the gas hold up and k_La increased while the mixing time decreased. Although increase in aeration rates resulted in higher mass transfer, a lower aeration rate (0.250 vvm) was used during the outdoor cultures in order to avoid foaming and also to reduce the cost of operation.

Outdoor cultures of *C. sorokiniana*

Daily variations in biomass productivities of *C. sorokiniana* at standing biomass concentrations of 1.5 g l^{-1} and 5.0 g l^{-1} are shown in Fig. 7A–B. Productivities varied depending on the prevailing solar light radiation. At a standing biomass concentration of 1.5 g l^{-1}, productivity varied between 0.30 g l^{-1} day^{-1} and 1.19 g l^{-1} day^{-1} in the photobioreactor without static mixers; with static mixers, it varied from 0.43 g $l^{-1}day^{-1}$ to 1.47 g l^{-1} day^{-1}. At a higher biomass concentration (5.0 g l^{-1}), productivity varied between 0.50 g l^{-1} day^{-1} and 1.22 g l^{-1} day^{-1} in the photobioreactor with static mixers, i.e., 15–70% higher than those without static mixers. It is apparent that, regardless of the weather conditions and the standing biomass concentrations, the photobioreactor with static mixers gave higher biomass productivities than the one without static mixers. The effect of standing biomass concentration on the productivity of the photobioreactors with static mixers under bright weather conditions (solar radiation above 6 MJ m^{-2} day^{-1}) is shown in Fig. 8. Although within the range tested (1.5 g l^{-1} to 5.0 g l^{-1}), standing biomass concentrations had no significant effect on the average productivity ($P<0.05$), slightly higher productivities were obtained at lower standing biomass concentrations. As shown in Fig. 9, the effectiveness of the static mixers was higher at a standing biomass concentration of 5.0 g l^{-1}. Furthermore, the static mixers

Fig. 7A, B Biomass productivities during outdoor cultivation of *Chlorella sorokiniana* at a standing biomass concentration of **A** 1.5 g l^{-1} and **B** 5.0 g l^{-1}. *Open bars* Without static mixers, *closed bars* with four B–type static mixers



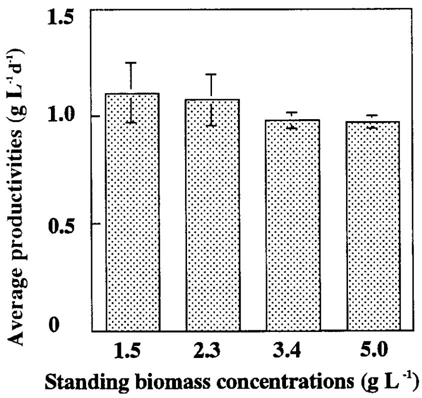


Fig. 8 Effect of standing biomass concentration on biomass productivity during bright days (solar radiation above 6 MJ m^{-2} day^{-1}). The tubular photobioreactor with four B-type static mixers was used

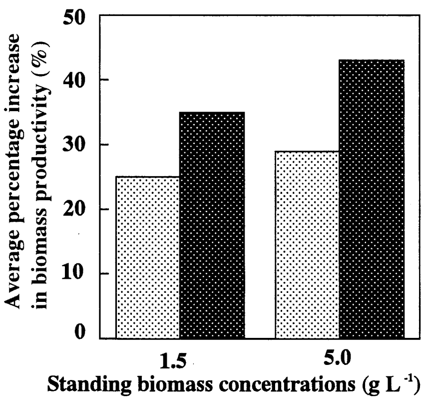


Fig. 9 Effect of weather conditions and standing biomass concentration on the effectiveness of the static mixers. *Gray bars* Bright days, *black bars* cloudy days. Bright days are defined as days with solar radiation above 6 MJ m^{-2} day^{-1}

were more effective on cloudy days (solar radiation <6 MJ m^{-2} day^{-1}) than on bright days (solar radiation >6 MJ m^{-2} day^{-1}).

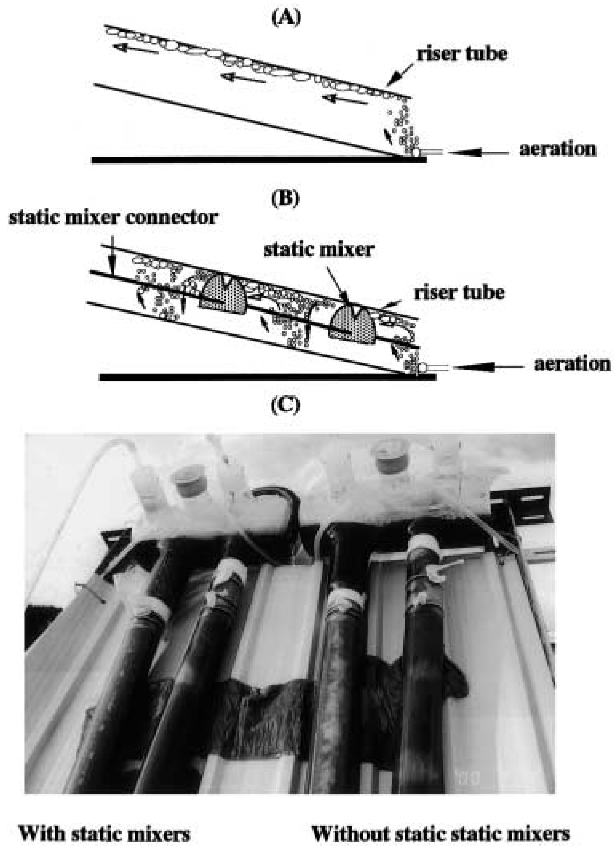


Fig. 10 Schematic diagram of a section of the riser tube, **A** without static mixers and **B** with B-type static mixers. **C** Photographs of the photobioreactors

Discussion

The use of static mixers in tubular photobioreactors requires carefully designed mixers. This is the first report on the design, construction and use of static mixers to improve mass transfer in tubular photobioreactors. It is evident that all the various types of static mixers developed in this study were effective in improving the mass transfer (k_La and gas hold up) of the tubular photobioreactor. However, the mixing time became longer due to restricted liquid flow through the static mixers. The mechanism by which static mixers enhance the mass transfer can be explained in terms of the turbulence generated inside the photobioreactors. In the conventional tubular photobioreactor, even when fine spargers are used to produce fine bubbles, these bubbles soon coalesce to form large bubbles, creating an interface between the liquid and the walls of the tube (Ogbonna and Tanaka 2001). Thus, the contact area between the liquid and gas is reduced resulting in poor mass transfer characteristics (Fig. 10A). However, by installing static mixers, such big bubbles are broken down into fine bubbles, thus increasing the interfacial surface area and producing turbulence with a consequent increase in mass transfer characteristics (Fig. 10B).

Chisti et al. (1990) reported that in an external airlift bioreactor, the presence of static mixers improved the k_La by 30–500% depending on the viscosity of fluid. In the present study, tap water was used for measurement of mass transfer characteristics. The effectiveness of the static mixers in increasing the k_La is expected to be higher when high viscosity fluids are used.

As shown in Fig. 7, installation of static mixers resulted in higher productivity of *C. sorokiniana* regardless of the prevailing weather conditions (solar radiation) and standing biomass concentrations. Possible reasons for higher productivity obtained with static mixers during the outdoor culture of *C. sorokiniana* are: better supply of CO_2, better removal of photosynthetically generated oxygen and/or movement of the cells between the upper and lower parts of the tubes (flashing light effect). Photographs of the upper part of the tubular photobioreactors during the outdoor experiments are shown in Fig. 10C. As shown in these photographs, the accumulation of photosynthetically generated oxygen in the downcomer section of the photobioreactor without static mixers was higher than in the photobioreactor with static mixers. In the course of the experiments, the maximum DO concentrations observed at midday at a standing biomass concentration of 3.4 g l^{-1} were 130% and 150% of air saturation in the photobioreactor with and without static mixers, respectively. Although the static mixers were effective in reducing the photosynthetically generated oxygen in the photobioreactors, this alone cannot account for the differences in productivity obtained in the two photobioreactors. Indoor culture of *C. sorokiniana* showed that a DO of about 160% air saturation did not result in a significant decrease in the productivity of *Chlorella* cells (data not shown). Nevertheless, in longer tubes, DO concentrations as high as 10 times air saturation have been reported (Weissman et al. 1988). It is expected that installation of static mixers in such long tubes will help to reduce DO below the inhibitory level and thus improve productivity. With the aeration rate used for the outdoor culture, the k_La (O_2) was 3.27× 10^{-3} s^{-1}, which corresponds to a k_La (CO_2) of 2.92× 10^{-3} s^{-1} (Talbot et al. 1991). This k_La (CO_2) can sustain a biomass productivity of 2.0 g l^{-1} day^{-1} (Mazzuca Sobczuk et al. 2000). By installing the static mixers, the liquid flow changed from plug flow to turbulent mixing so that cells were moved between the surface and bottom (flashing light effect) of the photobioreactors (Fig. 10). This may be the major reason for the increase in productivity obtained when the static mixers were installed. This view is supported by the fact that the static mixers were more effective at higher standing biomass concentrations and on cloudy days (Fig. 9). Improved productivities of photobioreactors due to the flashing light effect has been reported by many other authors (Phillips and Myers 1954; Lee and Pirt 1981; Ogbonna et al. 1995; Merchuk et al. 1998).

A maximum biomass productivity of about 1.47 g l^{-1} day^{-1} and an average productivity of about 1.11 g l^{-1} day^{-1} were obtained at a standing biomass concentration of 1.50 g l^{-1}. During the outdoor experiments, the culture temperature varied between 26°C and 41°C. It was observed that spraying of the surface of the photobioreactor with tap water was not very effective in controlling the culture temperature to within the optimum for *C. sorokinian*a (36°C). An indoor experiment showed that at a temperature above 38°C, the productivity of *C. sorokiniana* decreased by about 25% (data not shown). Thus, for higher productivity, it is necessary to develop a system for efficient temperature control.

The static mixers developed in this study are cheap, easy to install and are maintenance-free. Installation of such static mixers is therefore a promising method of improving the mass transfer, and thus the productivity, of tubular photobioreactors. The effectiveness of the static mixers is expected to be higher with longer tubes as well as for highly viscous cultures.

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