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Light/dark cyclic movement of algal culture (*Synechocystis aquatilis*) in outdoor inclined tubular photobioreactor equipped with static mixers for efficient production of biomass

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Abstract

Synechocystis aquatilis SI-2 was grown outdoors in a 12.5 cm diam. tubular photobioreactor equipped with static mixers. The static mixers ensured that cells were efficiently circulated between the upper (illuminated) and lower (dark) sections of the tubes. The biomass productivity varied from 22 to 45 g m^{-2} d^{-1}, with an average of 35 g m^{-2} d^{-1}, etc which corresponded to average CO_2 fixation rate of about 57 g CO_2 m^{-2} d^{-1}. The static mixers not only helped in improving the biomass productivities but also have a high potential to lower the photoinhibitory effect of light during the outdoor cultures of algae.

Introduction

Microalgal mass cultures have been widely investigated for production of health foods, pharmaceuticals, pigments, vitamins, proteins, carbohydrates, and other fine chemicals (Borowitzka 1999). Microalgae have higher CO_2 fixation rates than plants and thus, can utilize CO_2 from flue gas to produce biomass (Negoro *et al.* 1991). However, in order to utilize solar energy for mass algae production, efficient cultivation systems with high illumination surface area are required. In this regard, closed photobioreactors such as tubular photobioreactors are promising for production of algal biomass and alga-derived products (Gudin & Chaumont 1983, Tredici 1999). Nevertheless, most of the proposed tubular photobioreactors are still very expensive for microalgal mass cultivation.

In order to utilize the potential advantages of tubular photobioreactors, some aspects of hydrodynamics and mass transfer characteristics of tubular photobioreactors have been studied (Camacho Rubio *et al.* 1999). Furthermore, the need to improve light-dark cycling (radial mixing) in tubular photobioreactors and the scale-up criterion were reported (Molina Grima *et al.* 1999, 2000). We have previously reported the feasibility of scaling up an inclined tubular photobioreactor with internal static mixers by increasing the diameter of the tubes (Ugwu *et al.* 2003). In this study, the outdoor biomass yields of *Synechocystis aquatilis* SI-2 was evaluated in inclined tubular photobioreactor with internal static mixers, which was scaled up by increasing the diameter of the tube.

Materials and methods

The tubular photobioreactor

The tubular photobioreactor used in this study consisted of two parallel tubes that were joined at the bottom by aeration port and on the top by

degasser chamber. The internal diameters of the riser and downcomer tubes were both 12.5 cm while the total length of the tube was 4 m. Detailed description of the tubular photobioreactor has been given elsewhere (Ugwu *et al*. 2002).

Microorganism and culture conditions

Synechocystis aquatilis SI-2 (obtained from Marine Biotechnology Institute, Japan) was grown on modified SOT medium which was composed of (in 1 l): $NaHCO_3$, 5 g; $NaNO_3$, 4 g; K_2HPO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; Clewat 32 microelemental mixture, 0.05 g; aged sea water, 0.1 l; and tap-water, 0.9 l.

The working volume of the tubular photobioreactor was 58 l. The photobioreactor was naturally illuminated by solar light energy. The cultivation period was from 6 am to 6 pm. The cultures were aerated at 0.25 vvm with air fortified with 5% CO_2. The effects of standing biomass concentration on biomass productivity were investigated by daily dilution of the cultures 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 culture temperature in the outdoor tubular photobioreactor varied from 28 to 40 °C. The surface of the photobioreactor was sprinkled with tap water to avoid overheating of the cultures. The outdoor culture experiments were operated during the summer of 2002 (from July to September) at the Agricultural Research Center, University of Tsukuba, Japan.

Analytical procedures and measurements

The solar light intensity on the surface of the photobioreactor was measured using photorecorder (PHR-51, T&D Corp., Japan). The fluorescent intensity of the cultures (Fv/Fm ratio) was measured using a plant efficiency analyzer (Hansatech Instruments Ltd., Norfolk, UK). The CO_2 fixation rates were calculated as follows:

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

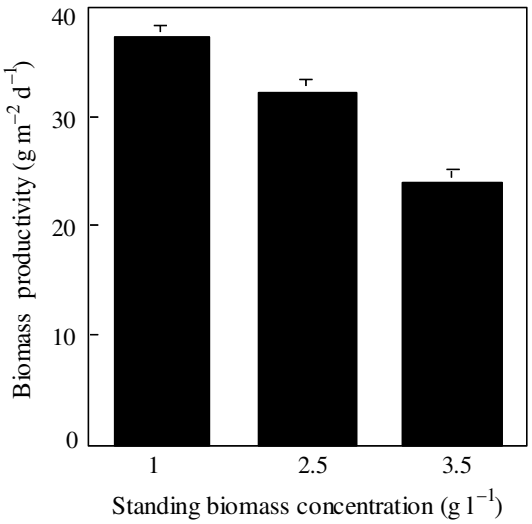

Fig. 1. Effect of standing biomass concentration on biomass productivity of *Synechocystis aquatilis* SI-2.

(where P stands for the biomass productivity while 44 and 12 are for molecular weights of carbon dioxide and carbon, respectively). The CO_2 fixation rates were calculated from the biomass productivity by using 45% as the carbon content of dried cells.

Results and discussion

Figure 1 shows the effect of standing biomass concentration on biomass productivity of *Synechocystis aquatilis*. Since the highest biomass productivity was obtained at 1 g l^{-1}, subsequent experiments were done at this standing biomass concentration (1 g l^{-1}). Figure 2 shows the effect of static mixers on biomass productivity of *Synechocystis aquatilis*. The highest biomass productivities were obtained at solar radiation of 10–12 MJ m^{-2} d^{-1}.

Table 1 shows the CO_2 fixation rates of *Synechocystis aquatilis* in the tubular photobioreactor with static mixers at various weather conditions. The CO_2 fixation rate varied from 38 g CO_2 m^{-2} d^{-1} to 76 g CO_2 m^{-2} d^{-1} (depending on the daily solar radiation). The effectiveness of static mixers (percentage increase in productivity in the tubular photobioreactor with static mixers over the one without static mixers) was higher at lower radiation than at the higher solar radiation. Possibly the response of the cells does not only

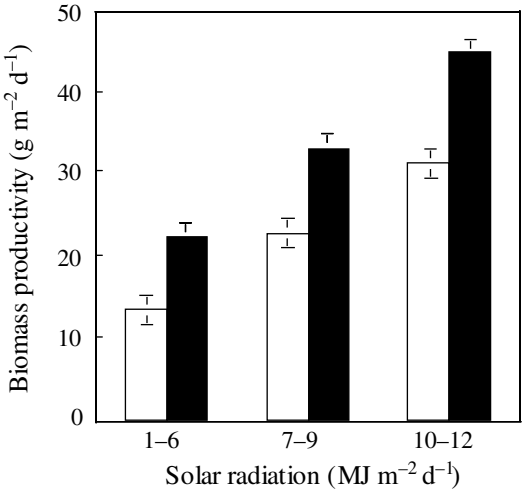

Fig. 2. Effect of static mixers on biomass productivity of *Synechocystis aquatilis* SI-2 at various solar radiations. Symbols: (□) without static mixers, (■) with static mixers. Error bars indicate the mean values obtained from 4 individual experiments.

Table 1. CO_2 fixation rates of *Synechocystis aquatilis* SI-2 at various solar radiations in the photobioreactor with static mixers.

Solar radiation (MJ m^{-2} d^{-1})	CO_2 fixation rate (g CO_2 m^{-2} d^{-1})
1–6	38
7–9	56
10–12	76

depend on the total light energy supplied but also on the light energy received by each cell as well as the light conversion efficiency by the cells. At high light intensities, the cells absorb more light than they can process and the unprocessed light is lost in form of heat or fluorescence.

The effect of light on chlorophyll fluorescence during outdoor cultures has been extensively studied (Torzillo *et al.* 1996, Vonshak & Torzillo 2004). The feasibility of minimizing the photoinhibitory damage of the cells in outdoor cultures by using static mixers was also investigated in terms of the Fv/Fm chlorophyll fluorescence (Figure 3). The Fv/Fm ratio varied (ranging from 0.60 to 0.68) depending on the solar intensities (during the day) and standing biomass concentrations. The cells were less affected during the midday at higher standing biomass concentration than at lower standing biomass concentration. Furthermore, the

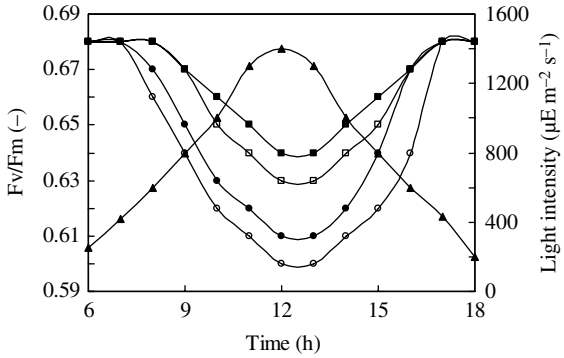

Fig. 3. Time course of the fluorescence intensity (Fv/Fm ratio) of *Synechocystis aquatilis* SI-2 during the day at various solar light intensities. Standing biomass concentrations: (○) 1 g l^{-1} without static mixers, (●) 1 g l^{-1} with static mixers; (□) 3.5 g l^{-1} without static mixers; (■) 3.5 g l^{-1} with static mixers, (▲) light intensity.

presence of static mixers ensured that cells were moved between the upper and lower sections of the photobioreactor and, thus, would reduce the photoinhibitory damage of the cells.

During the active growth of microalgae, the dissolved O_2 concentrations (which may be several fold above air saturation) may be sufficient to inhibit photosynthetic growth (Aiba 1982, Camacho Rubio *et al.* 1999). The O_2 concentration in this experiment was about 200% of air saturation (data not shown). We have previously reported that by installing static mixers in the tubular photobioreactor, the dissolved O_2 could be lowered to minimal levels (Ugwu *et al.* 2002). Generally, accumulation of dissolved O_2 is more problematic in long horizontal tubular photobioreactors as gases move from the aeration port to the degasser port. In such tubular photobioreactors, there are usually concentration gradients of nutrients, dissolved O_2, CO_2 and pH along the tube which, consequently, would result to poor mass transfer characteristics. The dissolved O_2 removal capability of a photobioreactor depends on its mass transfer rates. In other words, a tubular photobioreactor with high mass transfer rates would have high dissolved O_2 removal capability for improved algal biomass productivity and CO_2 fixation rates. However, by using tubular photobioreactor equipped with static mixers, the concentration gradients of dissolved O_2, CO_2 and pH along the tubes would diminish since the cells are circulated uniformly between the upper and lower sections of the photobioreactor.

The biomass productivities obtained in this study compare well with those reported by other authors. With vertical flat-plate photobioreactor, Zhang *et al*. (1999) reported the average biomass productivity of 32.2 g m^{-2} d^{-1} (which corresponded to CO_2 fixation rate of about 53 g CO_2 m^{-2} d^{-1}) using the culture of *Synechocystis aquatilis* SI-2. In this present study, the average biomass productivity of *Synechocystis aquatilis* SI-2 was about 35 g m^{-2} d^{-1} (based on biomass productivity per ground area). This value corresponded to CO_2 fixation rate of about 57 g CO_2 m^{-2} d^{-1}.

In conclusion, relatively high areal productivity and high CO_2 fixation rates were achieved by using large-diameter tubular photobioreactor equipped with static mixers. The static mixers generated turbulent mixing which moved the cells between the upper and lower parts of the tubes and thus, improved the mass transfer rates and light utilization in the tubular photobioreactor.

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