Journal of Applied Phycology **7**: 47–51, 1995.

Design and performance of an α-type tubular photobioreactor for mass cultivation of microalgae

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Received 25 April 1994; revised 28 September 1994; accepted 2 October 1994

Key words: photobioreactor, microalgae, design performance

Abstract

An α-shape tubular photobioreactor was designed and constructed based on knowledge of algal growth physiology using sunlight. The algal culture is lifted 5 m by air to a receiver tank. From the receiver tank, the culture flows down parallel polyvinyl-chloride tubes of 25 m length and 2.5 cm internal diameter, placed at an angle of 25 ° with the horizontal to reach another set of air riser tubes. Again the culture is lifted 5 m to another receiver tank, then flows down parallel tubes connected to the base of the first set of riser tubes. Thus, the bioreactor system looks like the symbol α. As there is no change in the direction of the liquid flow, high liquid flow rate and Reynolds Number can be achieved at relatively low air flow rate in the riser tubes. Due to the high area-volume ratio of the bioreactor, and equable photosynthetically available radiance and culture temperature, biomass density of exceeding 10 g dry weight L^{-1} and daily output rate of 72 g dry weight m^{-2} land d^{-1} were achieved.

Introduction

Expression of the biological potential of photosynthetic microalgae in using sunlight for growth and production of valuable biomolecules is ultimately determined by growth parameters. The growth kinetic responses of these photosynthetic cells to culture parameters (such as temperature and pH) and nutritional status in culture medium are no different from other microbial cells (Cunningham & Mass, 1982; Rhee *et al.*, 1981). The uniqueness of photosynthetic cultures is largely due to the form and frequency of energy supply. It is the aim of this work to develop a practical enclosed photobioreactor which would allow the photosynthetic culture to maximize their efficiency in utilizing sunlight energy for growth and product formation.

Principles of photobioreactor design

The amount of light energy received by a photosynthetic culture over a finite interval is a function of the photon flux density measured at the surface of the culture and the illuminated surface area. In the light limited linear growth phase of an algal culture where all photosynthetically available photons are absorbed, the biomass output rate is determined by the area to volume ration (Pirt *et al.*, 1980). Thus, in order to achieve high cell density to minimise the cell harvesting and processing costs, it is desirable to use a photobioreactor of high area-volume ratio, *i.e.* a shallow culture. High cell density is also desirable to avoid the effect of photoinhibition of photosynthesis at high sunlight intensity (Vonshak & Guy, 1988; Lee & Vonshak, 1988).

In high density algal cultures, the effect of 'mutual shading' which results from the absorbance of the incident light by cells nearer to the illuminated surface, is however pronounced. As a consequence, cells circu-

lating in the culture receive an intermittent supply of energy. In such an intermittent light regime, the maintenance energy requirement was found to increase with increasing dark period (Lee & Pirt, 1981), possibly due to light stimulation of the CN-sensitive portion of mitochondrial respiration (Falkowski *et al.*, 1985) and dark excretion of carbonaceous compounds (Law & Bannister, 1980). It was suggested that when exposed to saturated photon flux density, algal cells could store some energy for subsequent growth in the dark (Lee & Pirt, 1981). Thus, a high turbulent culture which prevents cells from staying in the dark portion of the culture for longer than the dark growth period will minimize the dark maintenance energy requirement. Rapid mixing is also desirable, as it prevents cells from over staying in the light saturated portion of the culture resulting in dissipation of unprocessed energy or even photoinhibition as discussed earlier. Vigorous mixing also increases exchange rates of nutrients and metabolites between cells and their growth medium (Grobbelaar, 1994). The effect of mixing is therefore most pronounced in high density culture as demonstrated in laboratory studies (Markl, 1980; Pirt *et al.*, 1983) as well as outdoor cultures (Richmond & Vonshak, 1978).

In outdoor cultures, photosynthetic cells are subjected to diurnal illumination of varying intensity. The photon flux density varies by more than 300 μmol photon m^{-2} s^{-1} in an hour in most part of the day. It was consistently observed in outdoor algal cultures that the photosynthetic conversion efficiency of the cultures was high in the very early morning (Lee & Low, 1991) but dropped precipitously before mid-morning to about a fourth of the earlier value. Low overall productivity in outdoor photosynthetic cultures was attributed to their inability to respond rapidly to rapid increase in photon flux density (Lee & Low, 1992; Chanawongse *et al.*, 1994). A study on the transitional productivity of *Chlorella* cultures in unstable weather conditions (Lee & Low, 1993) further suggested that algal cultures were able to achieve a higher energy conversion efficiency and productivity after having exposed to a higher morning photo flux density, perhaps through light activation of certain key enzymes, e.g. ribulos bisphosphate carboxylase (Bassham *et al.*, 1978). Indeed, by positioning a tubular photobioreactor at an angle with the horizontal, thus exposing the culture to higher photon flux density in the early morning and relatively constant photon flux density throughout the day (Lee & Low, 1991), resulted in higher bioenergetic growth yield. The basic physiological studies discussed above suggest that an effective large scale photobioreactor should possess the following characteristics:

1. Shallow culture, 2. high cell density, 3. high flow turbulence, 4. incline at an angle with the horizontal.

Materials and methods

The photobioreactor

The α-type tubular photobioreactor is diagrammatically depicted in Fig. 1. It consisted of two sets of 10 parallel polyvinyl chloride tubes (25 m length and 2.5 cm internal diameter), placed at an angle of 25 ° with the horizontal. The upper end of each tube was connected to a receiver tank, while the lower end connected to the base of a vertical air-riser tube (5 m tall, 3.2 cm internal diameter). The culture volume was about 300 L. The bioreactor covered a land area of about 12 m^2. The algal culture in the bioreactor was lifted up in the air riser tubes to a receiver tank and then flowed down the tubular photobioreactor to reach the opposite set of air riser tubes. Again, the culture was lifted up 5 m to another receiver tank, then flow down the parallel photobioreactor tubes connected to the base of the first set of riser tubes. Thus, the bioreactor system looked like the symbol α. The photobioreactor was placed in a East-Westernly direction (in Singapore, where sun rises in the East, passing overhead and sets in the West).

Pure industrial grade carbon dioxide gas was injected into the photobioreactor tubes just after the receiver tanks. The gas flow rate was regulated by electronic control valves (Brooks 5835A) at values which gave a dissolved CO_2 partial pressure measured in the photobioreactor tubes before the air-risers, at about 1 KPa. The dissolved CO_2 partial pressure was measured by Ingold CO_2 electrodes (Urdorf, Switzerland).

Compressed air (200 KPa) was injected into the air-riser tubes through sintered glass spargers. The air flow rate was regulated by either electronic control valves (Brooks 5835A) or manual needle valves.

The temperature of the culture was allowed to equilibrate with the ambient temperature. Because urea was the N-source, the pH of the culture did not vary beyond 6.6±0.1.

Cell adhesion on the photobioreactor tubes could happen in old cultures or under unfavourable weather conditions for growth. Wall growth could be easily removed by blowing air through the tubes from the upper ends.

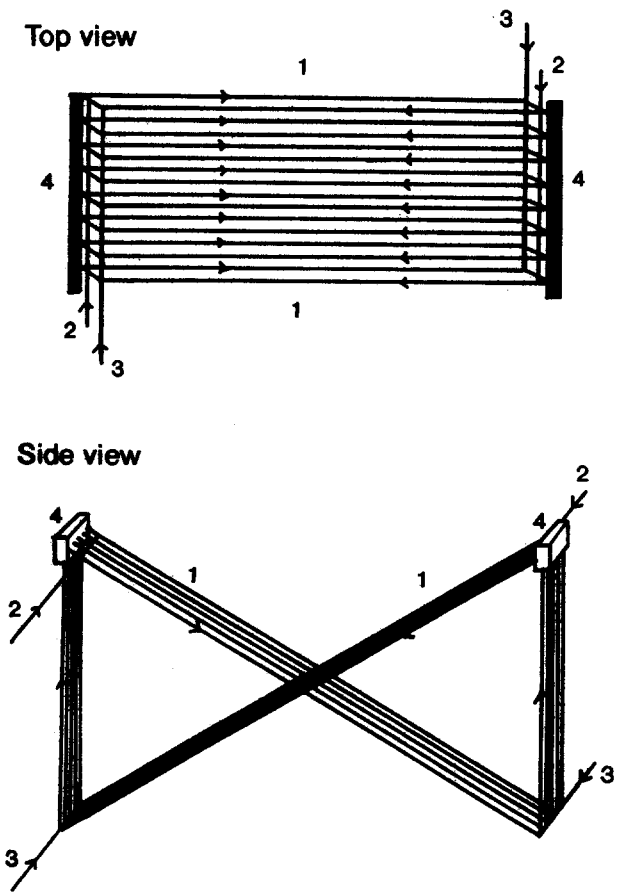

Fig. 1. Diagrammatic representation of the α-photobioreactor system: (1) tubular bioreactor; (2) CO_2 inlet; (3) air inlet; (4) receiver tank; (5) air riser tubes.

Microalga and culture medium

The green alga *Chlorella pyrenoidosa* was a gift from Sun-chlorella, Kyoto, Japan. The urea-salt chemical defined medium A9 (Lee & Pirt, 1981) was used throughout the study. The medium A9 supports only 4 g L^{-1} cell growth. To ensure nutrient saturation, the culture was replenished by adding concentrated stock medium in the day when necessary.

Analytical methods

To determine the biomass dry weight, duplicate known volumes of the algal culture were filtered through preweighed glass microfiber filters (Whatman, UK), washed with 0.5 N HCl and distilled water to remove nonbiological materials such as mineral salt precipitates, dried at 80 °C, and then weighed.

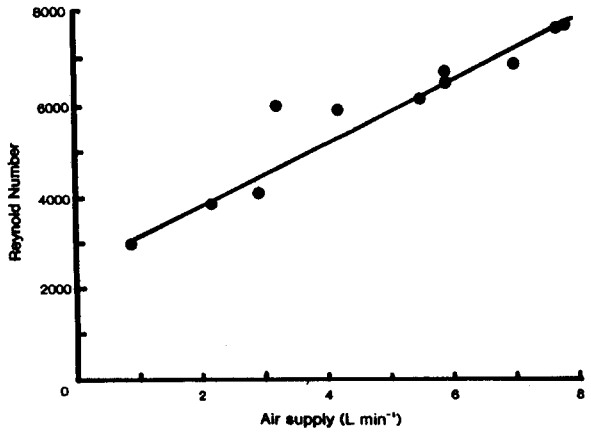

Fig. 2. Influence of air flow rate in the air lift system on Reynolds number (N_R) in the tubular photobioreactor.

The linear flow rate of liquid in the tubular photobioreactor was estimated from the retention time of small solid particles suspended in the liquid.

Photosynthetically available radiance at the surface of the bioreactor was measured by a radiometer (LI-CORLI-170).

Results

Influence of air-flow rate in air lift system on liquid recycle rate

The influence of air-flow rate in the air lift system on the liquid recycle rate was studied. The measurement (Fig. 2) show that a Reynolds number (N_R) Of 2000 could be reached with an air supply rate of lower than 1 L min^{-1}. The Reynolds number is given by $N_R = vDP/u$ where v is the liquid velocity, u is the viscosity, D is the diameter of the tube and P is the liquid density. It is assumed that turbulence is reached when $N_R = 2000$. For water flow through the photobioreactor at 37 °C, $u = 0.6915 \times 10^{-3}$ kg s^{-1} m^{-1}, $P = 0.993 \times 10^3$ kg m^{-3}.

At an air supply rate of 7.8 L min^{-1}, a liquid flow rate of 16.2 L min^{-1}, which corresponds to a N_R of>7600, was reached.

Cell growth

A day in Fig. 3 began at 0700 h in the morning and ended at 1900 h in the afternoon. For the batch of culture presents in Fig. 3, the biomass dry weight doubled twice within the first day after inoculation. The inocu-

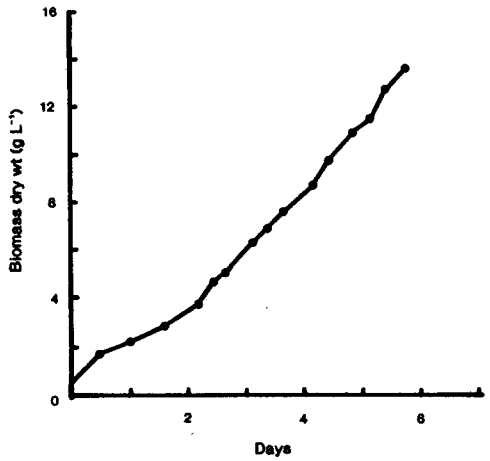

Fig. 3. Growth kinetics of a *Chlorella pyrenoidosa* culture in an α-photobioreactor. Day = 0700–1900 h, air supply rate in air lift system = 3 L min^{-1}.

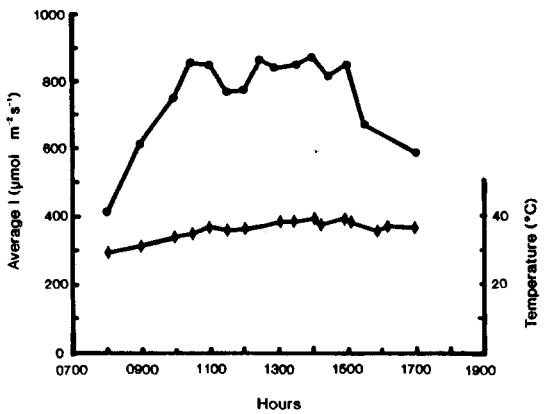

Fig. 4. Profile of sunlight in the spectral range of PAR (I) measured at the surface of the bioreactor and temperature of the *Chlorella* culture, observed on the 6th day of cultivation.

lum was cultured in a smaller tubular photobioreactor (Lee & Low, 1991) outdoors. For the subsequent days, the average doubling time for biomass decreased gradually, but the biomass output rate increased gradually from 37.5 g m^{-2} land d^{-1} on the 1st day to 45 (2nd day), 55 (3rd day), 62.5 (4th day), 70 (5th day) to reach 72.5 (6th day) g m^{-2} land d^{-1}. The biomass density reached 13.6 g L^{-2} on the 6th day.

Temperature and photon flux density during the day

The average photosynthetically available radiance (PAR) received by the culture during the day was estimated from measurements made at both the upper and lower side of the bioreactor tubes, at 1 m interval. The photobioreactor tubes facing the East received more light energy in the morning, while those facing the West received more light in the afternoon. However, the average PAR measured at the surface of the bioreactor remained relatively constant during most part of the day (Fig. 4). As a consequence, the culture heated up rapidly in the morning and then remained at around 38 °C for the rest of the day (Fig. 4).

Discussion

Tubular loop photobioreactor of the type described by Pirt *et al.* (1983), Torzillo *et al.* (1986), Chaumont *et al.*, 1988) and Tapie & Bernard (1988); Vertical tube (Cook, 1950; Miyamoto *et al.* (1988), alveolar panel (Tredici *et al.*, 1991), plate-type photobioreactor (Puiz, 1994) and tubular biocoil (Robinson, 1987) systems fulfill most of the requirements of an effective photobioreactor listed in this paper. Vertical tube and alveolar panel are not easy to scale-up. A comparative study (Chini Zittelli *et al.*, 1993) has also shown that biomass productivity in an alveolar panel is not as high as the tubular loop photobioreactor. In the report of Pirt *et al.* (1983), it was shown that 15% of the energy consumed for recycling a culture in a tubular loop photobioreactor of 1 cm bore, was to move culture around bends. Eighty fiver percent of the energy consumed was due to the shear head (resistance factor for friction between the water flow and tubing wall). The velocity head (that required to maintain the velocity of water flow) was negligible. The shear head is an inverse function of the diameter of tubing and therefore the proportion of the friction head in bends becomes larger with increasing tubing diameter. The fraction head is expected to be high in a coiled tubular photobioreactor, because the direction of the culture flow is changing all the time in a circular pattern.

The flow of culture in the α-photobioreactor did not change in direction except when it was lifted up in the air-lift system. It is therefore understandable that high liquid flow rate and Reynold number in the culture system could be achieved at relatively low air supply rate in the riser tubes (Fig. 2).

High surface area to volume ratio and turbulence, and a fairy constant culture temperature and PAR received by the culture throughout the day allowed

us to attain high biomass productivity even at high biomass density (exceeding 10 g L^{-1}). At a biomass density of about 0.5 g L^{-1} (1st day in Fig. 3), the culture biomass doubled twice in a day with a biomass out put rate of 37.5 g m^{-2} land d^{-1}. The biomass output rate gradually increased to reach 72.5 g m^{-2} land d^{-1} on the 6th day of cultivation. The culture growth was probably limited by the specific rate of uptake or metabolism of an essential substrate, including CO_2. The culture was certainly not limited by the availability of light energy, which would otherwise give a constant daily biomass output rate. The high biomass output rate of 72 g m^{-2} land d^{-1} was maintained for several months in a fed batch culture operation, where the biomass density in the early morning was diluted to about 10 g L^{-1} every day.

A technical problem associated with high cell density culture is foam formation. Foam could be controlled by addition of antifoam agent polypropylene glycol (mol. wt. 2000), however, the chemical is costly. To avoid severe foam formation, the biomass density in the α-photobioreactor was maintained at below 20 g L^{-1}. In order to maintain high biomass productivity, the culture needs to be replenished with concentrated stock medium every day. Also, the CO_2 supply needed to be stopped in the evening to prevent the dissolved CO_2 partial pressure from reaching a growth inhibiting level (Lee & Tay, 1991). These manipulations were laborious, but could be overcome by implementation of process automation.

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