

Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture

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Abstract

Relatively large (0.19 m column diameter, 2 m tall, 0.06 m³ working volume) outdoor bubble column and airlift bioreactors (a split-cylinder and a draft-tube airlift device) were compared for monoseptic fed-batch culture of the microalga *Phaeodactylum tricorutum*. The three photobioreactors produced similar biomass versus time profiles and final biomass concentration (~4 kg m⁻³). The maximum specific growth rate observed within a daily illuminated period in the exponential growth phase, had a value of 0.08 h⁻¹ on the third day of culture. Because of night-time losses of biomass, the specific growth rate averaged over the 4-days of exponential phase was 0.021 h⁻¹ for the three reactors.

The biomass in the vertical column reactors did not experience photoinhibition under conditions (photosynthetically active daily averaged irradiance value of 1150 ± 52 μE m⁻² s⁻¹) that are known to cause photoinhibition in conventional thin-tube horizontal loop reactors. Because of good gas-liquid mass transfer, the dissolved oxygen concentration in the reactors at peak photosynthesis remained <120% of air saturation; thus, oxygen inhibition of photosynthesis and photo-oxidation of the biomass did not occur. Carbohydrate accumulation (up to ~13% w/w) by the biomass was favored during light-limited linear growth. A declining light intensity caused a more than five-fold increase in cellular carotenoids but the chlorophylls increased only by about 2.5-fold during the course of the culture. In the stationary phase, up to 2% of the biomass was chlorophylls and carotenoids constituted up to 0.5% of the biomass dry weight.

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1. Introduction

Microalgae are a source or potential source of high-value products such as polyunsaturated fatty acids [1], natural colorants [2], biopolymers, and therapeutics [3]. In addition, a significant quantity of microalgal biomass is produced as essential aquaculture feed for shellfish and fish juveniles. Commercial monoculture of large quantities of microalgal biomass is usually carried out outdoors in closed continuous-run tubular loop bioreactors with tubes that are typically less than 0.08 m in diameter [4–6]. These photobioreactors occupy a large land surface and are expensive to build and operate.

An alternative to conventional tubular reactors, is to use relatively large-diameter (diameter > 0.1 m) vertical column reactors such as bubble columns and airlift bioreactors [7,8]. Vertical column reactors are compact, low-cost, and easy to operate monoseptically [6,7]. In addition, vertical column reactors are a realistic option for producing large quantities of microalgal biomass [6]. Little information exists on characterization of microalgal culture in large outdoor bubble columns and airlift vessels. This work compares biomass production in three compact, large-diameter vertical reactors (a bubble column, a split-cylinder airlift device, and a draft-tube airlift bioreactor) of the same overall configuration. The biochemical composition of the biomass produced is discussed, as this is important for determining the suitability of the biomass as a source of chemicals (e.g. pigments). The composition of the biomass can also influence its value as an aquaculture feed.

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2. Materials and methods

2.1. The microalga

Phaeodactylum tricornutum UTEX 640 was the microalga used. This alga is a good producer of eicosapentaenoic acid (an essential fatty acid) and is, therefore, of potential economic significance [1]. The culture was obtained from the collection of the University of Texas, Austin, USA. The inoculum for the photobioreactors was grown indoors under artificial light ($230 \mu\text{E m}^{-2} \text{s}^{-1}$ light flux at the vessel's surface) in a 201 bubble column (0.15 m diameter) aerated at 1 vvm. The preculture medium was identical to that used in the final reactor cultivation (see later).

2.2. The photobioreactors and culture medium

Three kinds of photobioreactors were used: a bubble column, a split-cylinder airlift device and a concentric draft-tube

airlift vessel sparged in the draft-tube (Fig. 1). All vessels were made of 3.3 mm thick, transparent poly(methyl methacrylate), except for the lower 0.25 m sections that were made of stainless steel (Fig. 1). The vessels were 0.193 m in internal diameter. The riser-to-downcomer cross sectional area ratio was unity for the split-cylinder and 1.24 for the draft-tube airlift vessel. The internal diameter of the draft-tube was 0.144 m. The draft-tube and the baffle were located 0.091 and 0.096 m from the bottoms of the reactors, respectively. The gas-free liquid height was about 2 m in all cases, to provide a working volume of 0.06 m^3 . The fluid was mixed by sparging with air through perforated pipe spargers (13–17 holes of 1 mm diameter) (Fig. 1). The superficial aeration velocity was 0.01 m s^{-1} , based on the total cross section of the reactors. This aeration rate corresponded to a pneumatic energy input of 98 W m^{-3} , in all reactors. The complete geometric details of the culture vessels appear in Fig. 1 [9].

The reactors were located outdoors in Almería ($36^{\circ}50' \text{N}$, $2^{\circ}27' \text{W}$), Spain. The photosynthetically active irradiance on

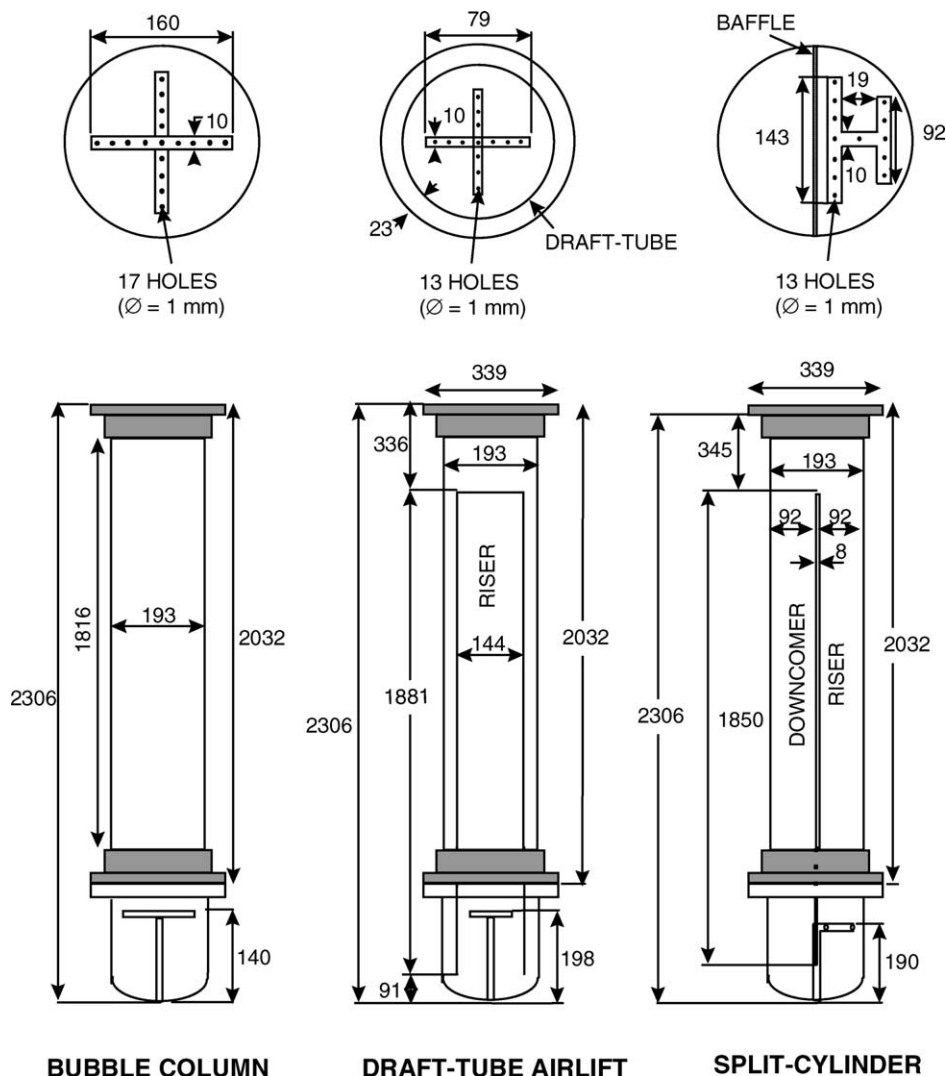


Fig. 1. The configurations of photobioreactors and air spargers, all dimensions in mm.

a horizontal plane at the location of the reactors was measured using a quantum scalar irradiance meter (QSL-100 Biospherical Instruments Inc., San Diego, CA, USA). The dissolved oxygen concentration was monitored continuously during each run, as an indicator of the photosynthetic activity. The monitoring electrodes were calibrated before each run. The culture temperature was controlled at $22 \pm 1^\circ\text{C}$ by circulating thermostated water through a jacket that surrounded the lower steel portion of the reactors (Fig. 1). The pH was controlled at pH 7.6 by automatically injecting carbon dioxide, as needed. The microporous sparger used for injecting carbon dioxide was different than the main gas sparger. This prevented mixing of CO_2 with the main air supply and enhanced mass transfer of CO_2 .

Prior to each run, the reactors were cleaned of salt deposits. The reactor was filled with tap water, concentrated hydrochloric acid (40 ml, 35% w/w HCl) was added, and the water was aerated for at least 2 h. The reactor was then emptied and rinsed with tap water. For sterilization, the reactors and associated pipework were filled with filtered seawater (60 l), 30 ml of sodium hypochlorite (10% solution) was added, and the fluid was allowed to circulate for 2 h. The reactors were then rinsed with filter sterilized seawater until the pH of the rinse was pH 7.6. The reactors were now filled with the medium.

The culture medium was a modified Ukeles [10,11] medium made in filter sterilized Mediterranean sea water at twice the components concentrations. The medium contained the following components (kg m^{-3}): $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.016; Na_2EDTA , 0.01; Fe(III) citrate , 4.9×10^{-3} ; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 9.9×10^{-4} ; ZnCl_2 , 1.36×10^{-4} ; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 2.4×10^{-5} ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 2.5×10^{-5} ; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 2.42×10^{-4} ; and sodium nitrate, 1. The composition of seawater has been published [6]. The medium (50 l) was inoculated using 10 l of inoculum in the late exponential growth phase. The biomass concentration in the freshly inoculated reactors was about 0.07 kg m^{-3} .

2.3. Biomass recovery

The biomass was recovered by passing the broth through a continuous centrifuge (Westfalia LG205-1, Germany) running at $6300 \times g$. The recovered biomass paste (approximately 80% water content) was washed with saline (NaCl , 9% w/w). The washed paste was freeze dried (Edwards freeze drier, model-4K).

2.4. Analytical methods

2.4.1. Cell viability

Cell viability was determined by the Trypan blue dye exclusion method. Microalgal suspension (0.2 ml) was diluted with 0.3 ml of sterile saline and mixed with 0.5 ml of Trypan blue solution (0.4% w/v). The mixture was held for 5–15 min. Nonviable cells took up the blue dye. The live and dead cells were counted in a Neubauer chamber under an

optical microscope, to calculate the percentage of the viable cells.

2.4.2. Biomass concentration

The biomass concentration was determined spectrophotometrically by reading the culture absorbance at a wavelength of 625 nm. A calibration curve established the following dependence between the optical density (OD_{625}) and the dry biomass concentration (C_b , kg m^{-3}):

$$C_b = 0.38 \times \text{OD}_{625}. \quad (1)$$

The measurements of the biomass concentration were made during the daylight hours only.

2.4.3. Total carbohydrates

The anthrone–sulfuric acid method suitably adapted for microalgal biomass was used [12]. Thus, 8 ml of perchloric acid (20% w/w) was added to algal biomass (100 mg) in a round-bottomed flask (100 ml). The mixture was ultrasonicated for 10 min and left to hydrolyze for 12 h. The mixture was filtered and diluted to 250 ml using distilled water. Freshly prepared anthrone reagent (5 ml) was added to 1 ml of the filtrate. The resulting mixture was brought to 100°C over a period of 12 min. A green color developed because of the formation of a glucose–anthrone complex. The mixture was cooled (ice bath) and the optical density was read at a wavelength of 630 nm against a blank of the anthrone reagent (5 ml) mixed with distilled water (1 ml). The anthrone reagent had been prepared by dissolving anthrone (10 mg) in 100 ml of sulfuric acid (72% w/w). A calibration curve was prepared for each experiment, using D+ glucose dissolved in distilled water. The glucose concentration (C_g , mg ml^{-1}) and the optical density had the following relationship:

$$C_g = 0.536 \times \text{OD}_{630} + 0.0028. \quad (2)$$

2.4.4. Chlorophylls

The spectrophotometric method of Hansmann [13] was used for estimating the chlorophylls content in the cells. A culture volume corresponding to 5 mg dry biomass was centrifuged ($800 \times g$, 2 min) to recover the cells. The biomass was resuspended in an acetone–water mixture (8 ml, 90% v/v acetone) to extract the pigments. A mark on the glass tube indicated the exact volume of the slurry. The tube was shaken vigorously, ultrasonicated for 10 min, and allowed to stand in the dark at 4°C for 48 h. Further processing occurred at room temperature. Acetone was added to compensate for any evaporation and the sample was centrifuged ($800 \times g$, 5 min). The supernatant was recovered and its optical density was read at 665, 645 and 630 nm in a spectrophotometer. The following equations [13] were used to calculate the concentrations of chlorophylls:

$$\text{Ch}_a = 11.6 \times \text{OD}_{665} - 1.31 \times \text{OD}_{645} - 0.14 \times \text{OD}_{630} \quad (3)$$

$$Ch_b = 20.7 \times OD_{645} - 4.34 \times OD_{665} - 4.42 \times OD_{630} \quad (4)$$

$$Ch_c = 55.0 \times OD_{630} - 4.64 \times OD_{665} - 16.3 \times OD_{645} \quad (5)$$

where Ch_a , Ch_b and Ch_c are the concentrations (mg l^{-1}) of chlorophyll a, b and c, respectively, and OD_{xxx} is the optical density measured at the specified wavelength. The optical density measurements were made in quartz cuvettes of 1 cm light path. If the absorbance value exceeded 0.8 units, the sample was diluted with the solvent (acetone–water) to bring the measurement within range.

2.4.5. Carotenoids

Carotenoids were determined by a suitably modified procedure of Whyte [14]. The culture volume corresponding to 5 mg dry biomass was immediately treated with aqueous potassium hydroxide (1 ml, 60% w/w KOH) to hydrolyze the lipids. The mixture was subjected to ultrasound for 10 min, homogenized, warmed to 40 °C for 40 min, and left on a shaker in the dark for 24 h at 4 °C. The carotenoids were extracted using 1 ml aliquots of ethyl ether until the organic extract was clear. The solvent in the combined extracts was evaporated with nitrogen and the residue resuspended in acetone (5 ml). The optical density of the resulting solution was read at 444 nm wavelength in a spectrophotometer. The total carotenoids were calculated as follows:

$$C_t = 4.32 \times OD_{444} - 0.0439 \quad (5)$$

where C_t is the carotenoids content in mg l^{-1} .

3. Results and discussion

3.1. Biomass production

The biomass concentration versus time profiles in the three photobioreactors are shown in Fig. 2. The data shown were obtained in parallel runs during August. The photosynthetically active daily averaged irradiance (PAR) value and its standard deviation during the culture period were $1150 \pm 52 \mu\text{E m}^{-2} \text{s}^{-1}$. The three culture profiles are virtually identical, suggesting that the cells experienced identical values of average internal irradiance in the three cases. In all cases, the culture profiles followed the expected pattern of a lag phase, exponential growth, linear growth, and a stationary phase (Fig. 2).

To prevent damage to culture by intense sunlight during the optically dilute lag phase, the south face (i.e. the face towards the Sun) of the reactors was covered by a dark sheet. The sheeting was removed on Day 2 of culture when the cells had become established and exponential growth had commenced. Generally, onset of linear growth (Fig. 2) indicates the limitation of some nutrient. In our case, the inorganic nutrients were fed every 48 h (as a concentrate) and carbon dioxide was injected according to demand. Thus, the

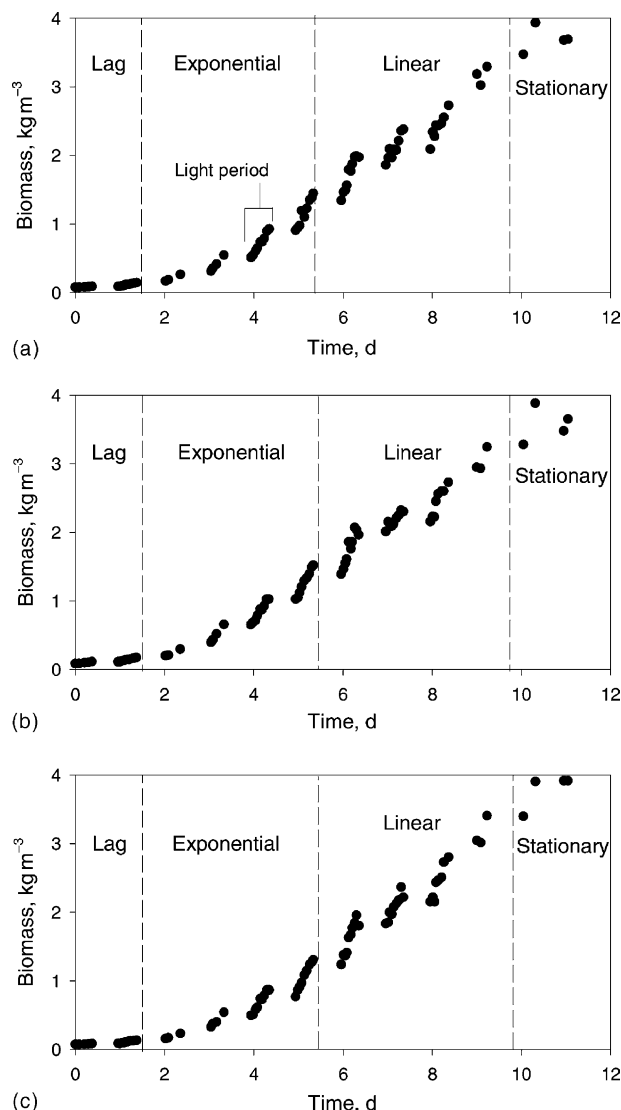


Fig. 2. Biomass concentration vs. time in fed-batch culture: (a) split-cylinder airlift reactor; (b) draft-tube airlift reactor; (c) bubble column; the data were measured during the light period only.

only limiting nutrient was light. Light limitation arose because of attenuation of light by the growing cell population. Eventually, light became so limiting that the cultures entered the stationary phase.

For each daylight period in Fig. 2, the biomass concentration increased rapidly from dawn up to 2 h before the solar midday. Towards solar noon, there was a decline in the rate of biomass production because vertical column photobioreactors receive least light when the Sun is directly overhead (i.e. at 14:00 h local time in August), as shown in Fig. 3. The vertical photobioreactors did not experience photoinhibition that is commonly seen in the narrow tubes of horizontal tubular reactors [5,15]. From mid-day up to about 17:00 h, the external irradiance level rose again (see Fig. 3) and so did the biomass concentration (Fig. 2). Because the

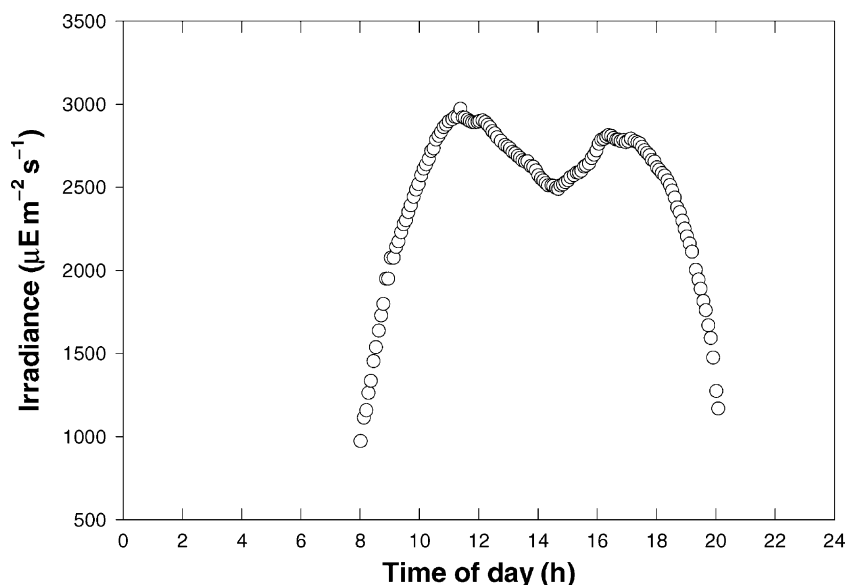


Fig. 3. Daily variation in internal irradiance at the axis of the bubble column (tap water, no aeration, July).

flow patterns in the three reactors are obviously different, an identical culture behavior (Fig. 2) can seem surprising. However, fairly different hydrodynamic regimes can lead to cells receiving the same cumulative average irradiance over a given period.

The maximum specific growth rate observed for the illuminated period in the exponential growth phase, had a value of 0.08 h^{-1} on the 3rd day of culture in the split-cylinder reactor. This value is comparable to specific growth rates of 0.06 to 0.09 h^{-1} reported for the same alga under constant indoor lighting in a horizontal tubular photobioreactor with thin (0.03 m diameter) tubes [16]. In an outdoor horizontal tubular photobioreactor with 0.03 m diameter tubes, Urda Cardona [17] observed a maximum specific growth rate of only 0.017 h^{-1} and attained a final biomass (*P. tricorutum*) concentration (exponential growth phase) of 4.21 kg m^{-3} . The latter value is comparable to the concentrations attained in Fig. 2 in much deeper reactors. The reactor used by Urda Cardona [17] was in the same geographic location as our reactors and was operated during the same time of the year as our cultures. The exceptionally low specific growth rate of Urda Cardona [17] is explained by intense photoinhibition experienced in the small diameter tubes.

Clearly, large-diameter vertical photobioreactors such as the ones used in our work can generate high specific growth rates and final biomass yields that are comparable to the values attained in more traditional horizontal tubular reactors. Although the average irradiance in a 0.2 m diameter bubble column or airlift reactor is unavoidably lower than in a 0.02 m diameter horizontal tube, the vertical reactors experience little or no photoinhibition. Also, because of good gas–liquid mass transfer [9], the oxygen generated by photosynthesis is readily removed from bubble columns and airlift bioreactors. Consequently, the peak oxygen concentration in

these reactors does not exceed about 115% of air saturation (Fig. 4). In comparison, the dissolved oxygen concentration commonly approaches 400% of air saturation in horizontal tubular photobioreactors [6,15,18]. High concentrations of dissolved oxygen are known to suppress photosynthesis [18] and a combination of an elevated dissolved oxygen level and intense sunlight causes photo-oxidative damage to biomass. Photo-oxidation and oxygen inhibition of photosynthesis occur in conventional tubular bioreactors but not in vertical column reactors. These factors may explain why the culture in a relatively large diameter vertical bioreactor can perform at least as well as in a narrow-bore horizontal tubular reactor.

Because of nighttime losses of biomass, the specific growth rate averaged over the 4-days of exponential phase was 0.021 h^{-1} for the three reactors. This value was of course much lower than the specific growth rate for only the illuminated period on any day of exponential growth (Fig. 2). However, the averaged specific growth rate value was comparable to 0.023 h^{-1} that has been reported in a draft-tube airlift vessel with a 0.09 m diameter outer tube, under continuous artificial illumination of $230 \mu\text{E m}^{-2} \text{ s}^{-1}$ at the surface of the reactor [19].

The time course of photosynthetic activity, as reflected by oxygen evolution, during a 24-h cycle is shown in Fig. 4 for the three reactors. In all cases, the rate of photosynthesis rose rapidly from about 7:30 h to 10:00 h, as the irradiance increased (see Fig. 3). During 10:00 and 16:00 h, the rate of photosynthesis leveled off or declined (Fig. 4) because of a low irradiance level (Fig. 3). The latter was caused by a close alignment of the Sun with the vertical axes of the column reactors, as discussed in the past [6]. The general pattern of daily oxygen generation by photosynthesis (Fig. 4) was consistent with the pattern of the irradiance–time curve

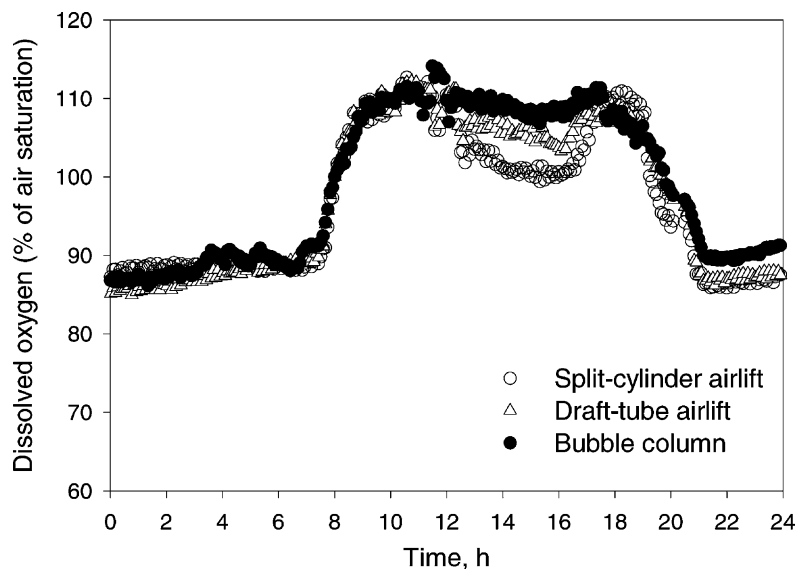


Fig. 4. Dissolved oxygen concentration vs. time during a daily cycle (10 August 1999) in the three photobioreactors.

(Fig. 3) although the peaks and troughs in the two figures did not match exactly. This was because the data in Figs. 3 and 4 were measured about a month apart.

Of the three reactors, the culture in the bubble column was the least sensitive to the mid-day decline in irradiance (Fig. 4). The differences in culture sensitivity to mid-day loss of irradiance in the three reactors can be explained by the differences in the nature of the fluid flow in the reactors. Because of radial mixing of fluid in the entire cross section of the bubble column, the cells from the central darker core of the reactor continually moved into the well-lit periphery of the column. This radial light–dark movement occurred with a higher frequency in the bubble column than in the airlift photobioreactors because in the bubble column the light and dark zones did not have an intervening partition such as a draft-tube or a baffle. Thus, in the bubble column, a cell did not face an extended period of darkness. In contrast, in the split-cylinder and the draft-tube airlift vessels, the vertical baffle and the draft-tube prevented radial motion of fluid.

Because of differences in geometry, the internal irradiance profiles in the three reactors were necessarily slightly different, although the external irradiance profiles were identical for the three cases. Although the average internal irradiance levels were evidently different, the similar levels of biomass productivity (Fig. 2) in the reactors occurred because of the different magnitudes of effects such as the light–dark cycle frequency [5,20].

3.2. Cellular carbohydrates

Carbohydrates are the primary product of photosynthesis. The time profile of the carbohydrates in the biomass is shown in Fig. 5, for the three bioreactors. In every case, during any daylight period, there was an accumulation of carbo-

hydrates in the cells because of photosynthesis. During the night, some of the accumulated carbohydrate was used up to support metabolism. Thus, the carbohydrate content of the biomass at the start of each day were substantially less than at the end of the previous daylight period (Fig. 5). However, the nightly consumption of carbohydrate was a little less than the carbohydrate reserve accumulated in the previous daylight period. Consequently, during the course of the culture, there was a small net accumulation of carbohydrates in the cells (Fig. 5).

Within a given daylight period, the rate of accumulation of the carbohydrates was low when light was abundantly available (i.e. Days 2–5 in Fig. 5) in an optically dilute culture. This was because in a dilute culture the specific biomass growth rate was high and the cells consumed carbohydrates to produce structural components (mainly proteins). In contrast, under limiting irradiance of linear growth (Days 6–9 in Fig. 5), the cells prepared for the dark phase by accumulating energy storage compounds (carbohydrates and lipids). Under low indoor irradiance ($72 \mu\text{E m}^{-2} \text{s}^{-1}$), *P. tricornutum* has been observed to accumulate more than 40% of its cell mass as carbohydrates [16].

A plot of the carbohydrate contents in the biomass versus the biomass concentration in the reactor (Fig. 6) clearly shows that in the absence of light limitation, the carbohydrate contents were independent of the biomass concentration. In the light limited region (Fig. 6), the carbohydrate contents increased as the cell concentration increased (i.e. as the culture became darker).

3.3. Pigments content

The time course of chlorophylls and carotenoids contents of the biomass is shown in Fig. 7 for the cells cultured in

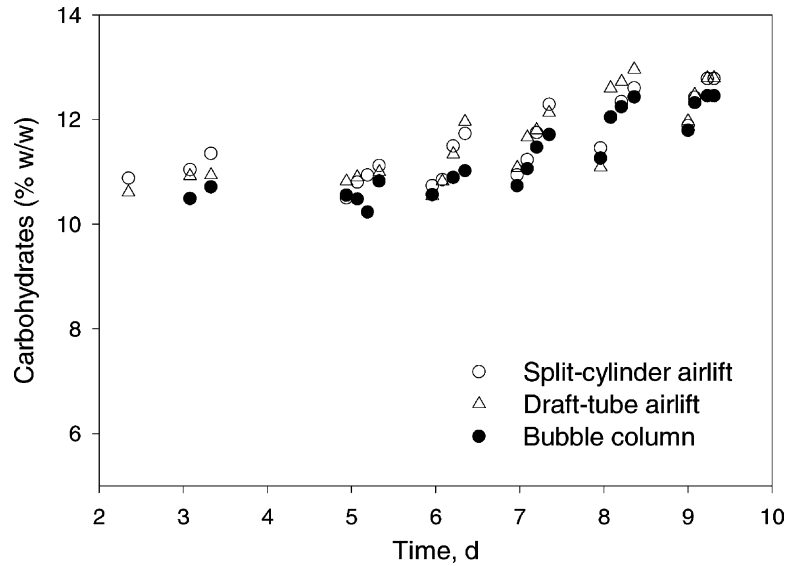


Fig. 5. Carbohydrate content of algal biomass during fed-batch culture in the three photobioreactors; data correspond to the biomass profiles shown in Fig. 2.

the split-cylinder reactor. Similar profiles were observed in the other reactors. The increasing concentration of pigments in the biomass (Fig. 7) is attributable to the light harvesting role of these pigments. As the concentration of the cells in the reactor increased, the average light intensity in the vessel declined because of self-shading of cells. Consequently, the cells responded by increasing the concentration of the photopigments in attempts to harvest more of the available light. This behavior is fairly typical of microalgae. In all reactors, a rapid rise in the rate of pigment accumulation was seen with the onset of the linear growth (i.e. Day 6 in Fig. 7).

The increase in the biomass pigments content was not a linear function of the biomass concentration, as revealed

clearly in Fig. 8 where the pigments contents are plotted as a function of the biomass concentration in the culture. This nonlinear relationship was expected because light attenuation by the biomass is known to follow a nonlinear pattern, the Beer–Lambert relationship [5]. Significantly, in Figs. 7 and 8, the increasing cell population (i.e. a declining intensity of available light in the bioreactor) caused a more than five-fold increase in carotenoids but the chlorophylls increased only by about 2.5-fold during the course of the culture. This was consistent with the light harvesting role of both these pigments. This behavior was seen in all reactors.

A relatively small increase in the chlorophylls content (Figs. 7 and 8) is partly explained by the fact that the

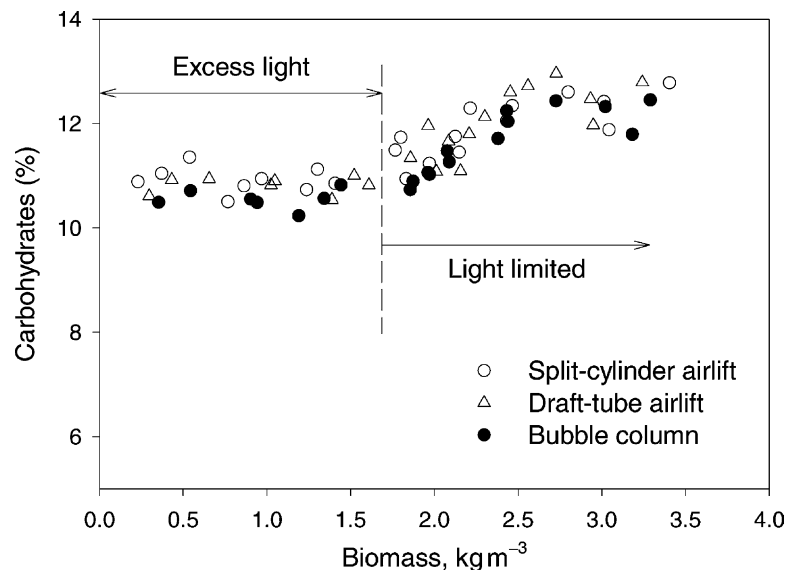


Fig. 6. Carbohydrate content of algal biomass vs. biomass concentration in the photobioreactors; data correspond to the biomass profiles shown in Fig. 2.

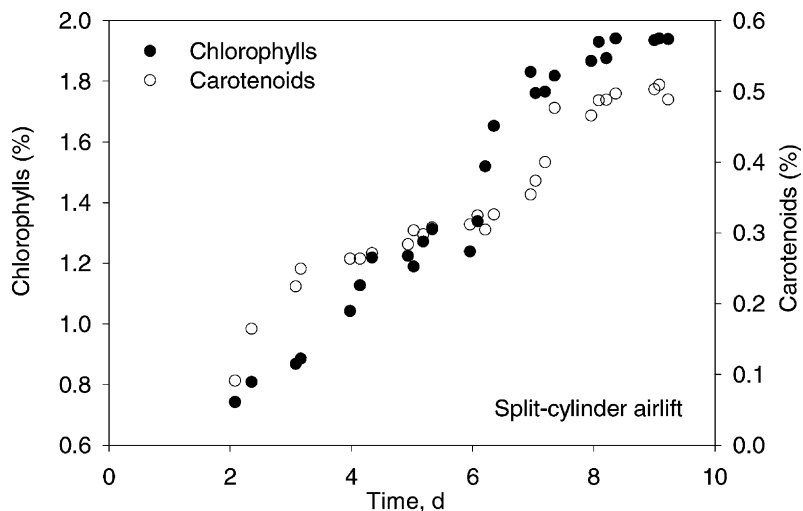


Fig. 7. Variation of chlorophylls and carotenoids contents in biomass during fed-batch culture in the split-cylinder airlift photobioreactor; data correspond to the biomass profiles shown in Fig. 2.

principle antenna pigment of *P. tricornutum* is fucoxanthine [21] and not chlorophyll. Although carotenoids have been ascribed a photoprotective role, at least in some cases, this does not explain the accumulation of carotenoids in low light. Obviously, the light capture role of carotenoids is quite important especially under low available irradiance.

The pigments contents of the biomass in the three reactors differed by less than $\pm 20\%$; however, there were consistent differences. Generally, the biomass from the draft-tube reactor was the most pigmented whereas the biomass from the bubble column had the least pigments. This suggested a higher average irradiance in the bubble column than in the draft-tube reactor. In the latter case, the cells were unavoidably subjected to a relatively long dark period while passing through the draft-tube. The pigments contents of

the split-cylinder reactor were comparable to that in the draft-tube vessel. Clearly, the cells compensated for the small differences in the light regimens of the three reactors by altering the pigments in the cells such that the overall biomass production profiles were similar in all reactors (Fig. 2).

4. Concluding remarks

Light-saturated culture in exponential growth provides algal biomass that is low in carbohydrates but rich in structural components of the cell. Pigment-rich biomass is generated in light-limited culture. The carotenoids contents in *P. tricornutum* are much more sensitive to available light

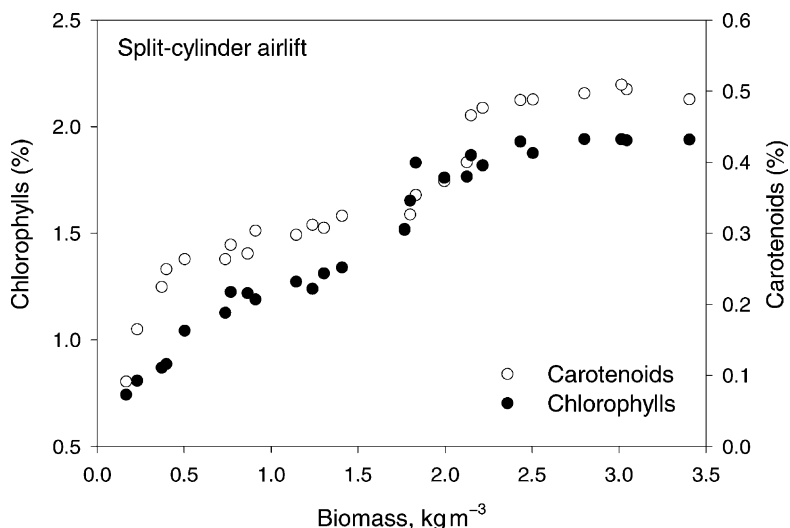


Fig. 8. Pigments contents (chlorophylls and carotenoids) in biomass vs. biomass concentration in fed-batch culture in the split-cylinder airlift photobioreactor.

in light-limited culture than are the chlorophyll contents. Bubble column and airlift photobioreactors of up to 0.19 m in diameter can attain a final biomass concentration and specific growth rate that is comparable to values typically reported for narrow tubes (e.g. 0.03 m in diameter) of conventional horizontal tube reactors. The good performance of the large-diameter vertical reactors is explained partly by an absence of severe photoinhibition and photo-oxidation of the biomass in them. Also, because of a good capacity for removing oxygen, the biomass in vertical reactors does not experience oxygen inhibition of photosynthesis.

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