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ORIGINAL ARTICLE

Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in a Middle and Southern European climate

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Abstract Two variants of open photobioreactors were operated at surface-to-volume ratios up to 170 m^{-1}. The mean values for July and September obtained for photobioreactor PB-1 of 224 m^2 culture area (length 28 m, inclination 1.7%, thickness of algal culture layer 6 mm), operated in Třeboň (49°N), Czech Republic, were: net areal productivity, P_{net} = 23.5 and 11.1 g dry weight (DW) $m^{-2}\,d^{-1}$; net photosynthetic efficiency (based on PAR – Photosynthetic Active Radiation), η = 6.48 and 5.98%. For photobioreactor PB-2 of 100 m^2 culture area (length 100 m, inclination 1.6%, thickness of algal culture layer 8 mm) operated in Southern Greece (Kalamata, 37°N) the mean values for July and October were: P_{net} = 32.2 and 18.1 g DW $m^{-2}\,d^{-1}$, η = 5.42 and 6.07%. The growth rate of the alga was practically linear during the fed-batch cultivation regime up to high biomass densities of about 40 g DW L^{-1}, corresponding to an areal density of 240 g DW m^{-2} in PB-1 and 320 g DW m^{-2} in PB-2. Night biomass loss (% of the daylight productivity, P_L) caused by respiration of algal cells were: 9–14% in PB-1; 6.6–10.8% in PB-2. About 70% of supplied CO_2 was utilized by the algae for photosynthesis. The concentration of dissolved oxygen (DO) increased from about 12 mg L^{-1} at the beginning to about 35 mg L^{-1} at the end of the 100 m long path of suspension flow in PB-2 at noon on clear summer days. Dissipation of hydraulic energy and some parameters of turbulence in algal suspension on culture area were estimated quantitatively.

Keywords Performance · Photobioreactor · Thin layer

Abbreviations

A	size of the culture area (m^2)
C_{O_2}	concentration (DO) of dissolved oxygen (g O_2 m^{-3})
$C_{O_2,0}$	DO concentration at the beginning of culture area (g O_2 m^{-3})
$C_{O_2,L}$	DO concentration at the end of culture area (g O_2 m^{-3})
$C_{O_2}{}^*$	DO concentration in equilibrium with oxygen content in ambient atmosphere (g O_2 m^{-3})
$C_{O_2,mean}$	mean DO concentration on culture area (g O_2 m^{-3})
D_{CO_2}	carbon dioxide diffusion coefficient in the algal suspension (m^2 h^{-1})
D_{O_2}	oxygen diffusion coefficient in the algal suspension (m^2 h^{-1})
G	rate of carbon dioxide supply into the bioreactor (g CO_2 h^{-1})

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h	thickness of the suspension layer on culture area (m)
I	inclination of the culture area (−)
I_0	PAR (Photosynthetically Active Radiation) irradiance ($W\,m^{-2}$)
K_H	Henry's constant for carbon dioxide ($g\,CO_2\,m^{-3}\,kPa^{-1}$)
K_I	light saturation constant ($W\,m^{-2}$);
K_{L,CO_2},	mass transfer coefficient for carbon dioxide ($m\,h^{-1}$)
K_{L,O_2}	mass transfer coefficient for oxygen ($m\,h^{-1}$)
L	length of the culture area (m)
n	roughness of the culture area ($m^{1/3}\,s^{-1/2}$)
$pCO_2{}^*$	partial pressure of carbon dioxide in ambient atmosphere (kPa)
$pCO_{2,mean}$	mean partial pressure of carbon dioxide in algal culture (kPa)
P_L	daylight productivity ($g\,DW\,m^{-2}\,d^{-1}$)
P_{net}	net algae productivity calculated from the differences between each morning algal mass in the bioreactor ($g\,DW\,m^{-2}\,d^{-1}$)
Q	volumetric flow rate of the suspension ($m^3\,h^{-1}$)
Q_{CO_2}	volumetric rate of carbon dioxide supply ($m^3\,h^{-1}$)
$R_{CO_2,mean}$	mean rate of carbon dioxide consumption by algae referred to 1 m^2 of culture area ($g\,CO_2\,m^{-2}\,h^{-1}$)
R_{O_2}	local (at the distance x) rate of oxygen evolution by algae referred to 1 m^2 of culture area ($g\,O_2\,m^{-2}\,h^{-1}$)
$R_{O_2,mean}$	mean rate of oxygen evolution by algae referred to 1 m^2 of culture area ($g\,O_2\,m^{-2}\,h^{-1}$)
u	velocity of suspension flow on culture area ($m\,h^{-1}$)
u_{mean}	mean velocity of suspension flow on culture area ($m\,h^{-1}$)
V	volume of the suspension in the bioreactor (L)
x	coordinate considered in the direction of flow of the suspension on culture area (m)
X	biomass concentration ($g\,DW\,L^{-1}$)
Y	mass of carbon dioxide consumed by algae, related to mass of oxygen evolved by algae ($g\,CO_2\,g\,O_2{}^{-1}$)
Z	loss of supplied CO_2 not absorbed in the suspension ($g\,h^{-1}$)

Greek symbols

φ_0	quantity of CO_2 absorbed in suspension related to quantity of supplied CO_2 (−)
κ	Karman's constant (−)
$\|v\|$	velocity of turbulent eddies ($m\,h^{-1}$)
ρ_{CO_2}	density of gaseous carbon dioxide ($g\,m^{-3}$)

Introduction

There is still a noticeable discrepancy between the extent of commercially operated algal cultures and the potential of algae (Richmond, 1988, 2000). Relatively low yields and high production costs are the main reasons (Borowitzka, 1999a). Since the first experiments with large-scale algal cultures in the 1950s (Burlew, 1953), various types of culture equipment have been developed and tested on a semi-pilot plant scale (Stengel, 1970; Goldman, 1979a; Richmond & Becker, 1986; Soeder, 1986; Tredici, 2004). For most products of microalgal mass cultivation, outdoor open horizontal circular or "raceway" ponds with a 15–30 cm layer of algal suspension and flow velocity of 15–30 $cm\,s^{-1}$ are the most commonly used technology for the growth of algae (Borowitzka, 1999b). The commercial production of *Chlorella and Arthrospira* (*Spirulina*) biomass is carried out exclusively in these open systems. Closed reactors have been employed for research in small field installations (e.g. Torzillo, 1997; Tredici & Zittelli, 1997, 1998; Pulz & Scheibenbogen, 1998; Tredici, 2004). The only exception is a large-scale tubular bioreactor which started production of *Chlorella* biomass in Central Germany in 2000 (Pulz, 2001) and tubular reactors used to produce *Haematococcus* in Israel.

Horizontal ponds are characterized by simple construction and relatively low building costs. On the other hand, there are many serious drawbacks of this system: due to utilization of the light, the concentration of algae should be no higher than 500 mg algal DW per 1 liter of nutrient solution. Low algal densities increase the danger of the contamination by undesirable algal

species. The laminar and low velocity flow of poorly mixed algal cells may result in photoinhibition of the upper layer of light-oversaturated algae and lead to accumulation of oxygen dissolved in the suspension. The circulation of the suspension is a continuous (day and night) and energy-demanding process. Highly energy-demanding is also the separation of algae from the nutrient solution at their harvest, which must be performed mostly in three or more steps of centrifugation in order to concentrate sufficiently the algal biomass for further processing.

The key for reduction of cultivation and harvesting costs, and for better control of the cultivation process and, consequently, for higher yields, rests in a low areal volume of algal suspension. This can be achieved by decreasing algal layer exposed to the light to as low values as technologically possible. Several millimeters thick and well-mixed dense algal culture makes it possible to increase dramatically the frequency of light/dark periods of single cells, thus increasing the efficiency of light utilization and decreasing the photoinhibitory effect up to very high solar light intensities.

In Třeboň's laboratory the construction principle of a large-scale thin-layer culture unit was developed and the unit has been operated since 1963 (Šetlík et al., 1970). In the first arrangement of the bioreactor, a 50 mm layer of algal suspension flowed at a velocity of 8 cm s^{-1} down an inclined surface of 3% slope. Four centimeters high slanted baffles were placed at a distance of 15 cm perpendicularly to the flow, with slots between the cultivation surface and the baffles. The suspension flowing over and under the baffles was intensively mixed and the baffles also helped maintain the required thickness of algal layer over the whole culture area. The suspension flowing down the one-sided inclined 30 m long and 30 m wide area was returned by a collection pipe into a pump and continuously delivered to the upper edge of the inclined area. This circulation took place during the day and at night the algal culture was kept in an aerated tank. The main disadvantage of the system was the relatively high energy consumption for the operation of the pump and for aeration of the large suspension volume kept in the tank between the cultivation periods. As well as this, the installation of the baffles and everyday cleaning of the culture surface was a laborious and time consuming process.

Based on the experience with this inclined baffled bioreactor, a modified version was built and started operation at Třeboň in 1991 (Doucha et al., 1993; Doucha & Lívanský, 1995). The modifications consist in removing of the baffles, reducing the culture area inclination from 3 to 1.6–1.7% and arranging the culture area into two parts inclined in the opposite direction. Lower inclination enables the microalgae to be grown in a 6–8 mm thick layer only and to reach a very high algal density at harvest. High algal density significantly decreases the danger of culture contamination by undesirable algal species. Intensive turbulence of the thin algal layer increases the frequency of light/dark periods of individual cells and, due to low suspension volume, permits better control of cultivation parameters, resulting in higher efficiency of light utilization and higher algal productivity.

The aim of this work is to present the data collected in the above described open high density microalgal photobioreactors and to compare the results attained in the reactors operated in two climatic zones. Based on the obtained data, a modular photobioreactor for production-scale cultivation is proposed.

Materials and methods

Organism and culture medium

A thermophilic strain of *Chlorella* sp., selected in our laboratory, was used for cultivation. The medium composition was designed to reflect the mean content of basic chemical elements (P, N, K, Mg, S) in the algal biomass. Amounts of added nutrients per 1 kg (DW) of produced biomass were: macronutrients (technical grade) – urea = 182 g; KH_2PO_4 = 39.5 g; $MgSO_4 \cdot 7H_2O$ = 29 g; $FeSO_4 \cdot 7H_2O$ = 5 g; micronutrients (analytical grade) – H_3BO_3 = 137 mg; $CuSO_4 \cdot 5H_2O$ = 158 mg; $CoSO_4 \cdot 7H_2O$ = 100 mg; $MnSO_4 \cdot 4H_2O$ = 608; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ = 29 mg; $ZnSO_4 \cdot 7H_2O$ = 440 mg; NH_4VO_3 = 2.3 mg. Tap water was used for preparation of the medium. pH of this medium is stabilized and keeps in the range of pH 6.8–8.0.

Outdoor culture system

The outdoor open photobioreactor used for cultivation of microalgae in the Třeboň laboratory consisted of two inclined cultivation lanes (inclination 1.7%) (Fig. 1). The lower end of the upper lane and the beginning of the next (lower) lane, inclined in an opposite direction,

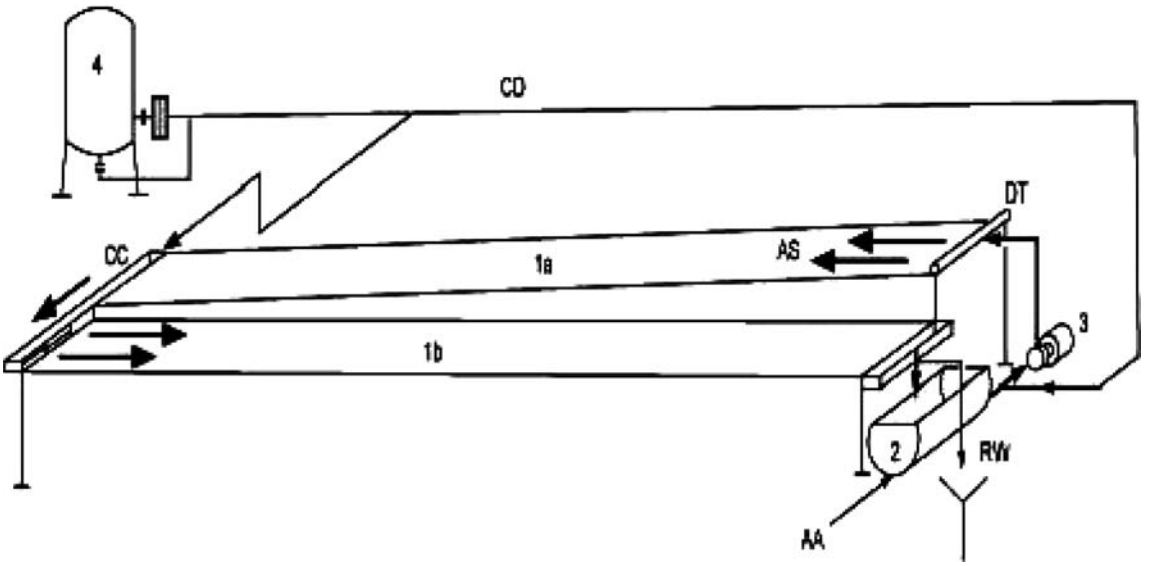

Fig. 1 Scheme of the photobioreactors. (1a, 1b) culture area; (2) retention tank; (3) circulation pump; (4) CO_2 storage tank; (M) aeration air; (AS) algal suspension flow; (CC) connecting channel; (CD) carbon dioxide; (DT) distribution tube; (RW) rain water

were connected by a channel through which the suspension flowed from the upper to the lower area. Below the end of the lower lane a retention tank was placed, which was connected through a pump to the upper edge of the culture area, where a polypropylene tube fitted with outlets serves for suspension distribution. The culture area was made from 2 × 1 m glass sheets built on a steel supporting frame. Mean velocity of the suspension flowing down the inclined area was 60 cm s^{-1}, thickness of the suspension layer was 6 mm. Two bioreactors (each 28 m long and 4 m wide) were, for technical reasons, assembled symmetrically together, giving thus 224 m^2 of bioreactor total culture area (designated PB-1). The suspension volume in the bioreactor was 2000 L.

Photobioreactor PB-2 used for comparative cultivation of *Chlorella* in Southern Greece (Kalamata) consisted of two connected lanes 50 m long and 1 m wide, arranged as in bioreactor PB-1. Inclination of the culture area was 1.6%, flow velocity of the algal suspension was 66 cm s^{-1}, thickness of the algal layer was 8 mm. The volume of the suspension in the reactor was 1000 L.

Operating mode

The algae were operated in a fed-batch mode with a starting inoculation density of 1–2 g DW L^{-1}. Nutrients were supplied twice a day (at the beginning of daily cultivation and at noon) in quantities based on the laboratory determination of urea nitrogen and P-phosphate in the medium. When the harvesting density was reached, the algae were harvested using self-desludging plate separators. A day before the harvest, no nutrients were added to the culture so that only very low amount were found in the supernatant at harvest.

Supply of carbon dioxide

To minimize loss of in algal suspension dissolved CO_2, pCO_2 at the end of culture area was maintained at 0.1–0.2 kPa, which is the minimum value for non-limited algal growth (Lívanský & Doucha, 1998). Gaseous CO_2 from a storage tank, where liquid food grade CO_2 was kept under pressure, was supplied into the suction pipe of the suspension circulating pump of the PB-1. CO_2 supply was controlled by means of an infrared analyzer as follows: A small portion of suspension from the end of culture area was fed continuously into a glass vessel where it was dispersed, creating an area for the CO_2 mass transfer from suspension to gas phase. The CO_2 concentration in gas phase was measured by infrared analyzer (model SM–95IR, International Sensor Technology, USA). The IR electric output was fed into a control unit (OMRON, model K3MA-J, Kyoto, Japan), displaying the CO_2 values in the range of 0–0.5 vol% CO_2. The set point of the control unit was adjusted to 0.15 vol% CO_2. When the CO_2 concentration dropped

below the set value, the unit activated a magnetic valve on the CO_2 pipe and the CO_2 gas was fed under pressure via a flow meter into suction pipe of the circulation pump of the bioreactor. When the CO_2 content exceeded the set point value, the magnetic valve for CO_2 delivery was switched off. In this manner the CO_2 content oscillated about the set value in the range of 0.10–0.20 vol% CO_2, corresponding to $pCO_2 = 0.10$–0.20 kPa in suspension at the end of culture area.

In PB-2, carbon dioxide for the 50 m long first lane of the culture area was supplied into the suction pipe of the circulation pump. The suspension on the second lane was sparged via two porous ethylpropylene dimer (EPDM) membrane tubes (each 1 m long, diameter 26 mm, FORTEX, Czech Republic) placed at the bottom of the channel, which connected the two lanes of the bioreactor.

Analytical methods

The concentrations of anions (PO_4^{3-}, SO_4^{2-}) in nutrient solution were determined by ion exchange chromatography (DIONEX ICS-90, USA), using an IONPAC AS9-HC column (4 × 250 mm), flow rate 1 mL min^{-1}. The concentration of urea was determined by the Biolachema test 450 (LACHEMA, Czech Republic), based on the reaction of urea with diacetylmonoxime, yielding a coloured complex. Determination of the other basic cultivation parameters (DW estimation, pCO_2, dissolved oxygen, PAR values, pH) has been described elsewhere (Doucha et al., 2005). Daylight algal productivity, P_L per culture area, was estimated from the difference between morning and evening dry weight. Net algal productivity, P_{net}, including night biomass loss, was estimated from the difference between successive morning algal dry weights. Net efficiency of solar PAR energy utilization (%) was estimated from the P_{net} (g DW m^{-2} d^{-1}) times a conversion factor 6.4 Wh PAR g DW^{-1} (Morita et al., 2001) × 100, divided by the integrated PAR (Wh PAR m^{-2} d^{-1}). For solar light a conversion factor 1 W m^{-2} = 4.94 mol m^{-2} s^{-1} (μE m^{-2} s^{-1}) was found for PAR (400–700 nm).

Results

Growth, productivity and photosynthetic efficiency of algae

We analyzed and compared fed-batch growth cycles performed in July and September (PB-1) and in July and October (PB-2) in 1997–2001. The time course of algal biomass concentration during fed-batch growth cycle in both PB-1 and PB-2 bioreactors is shown in Fig. 2. The growth rate of the alga was almost linear up to very high cell concentrations. Mean net productivity P_{net} (g DW m^{-2} d^{-1}) for cultivation cycle lasting n days

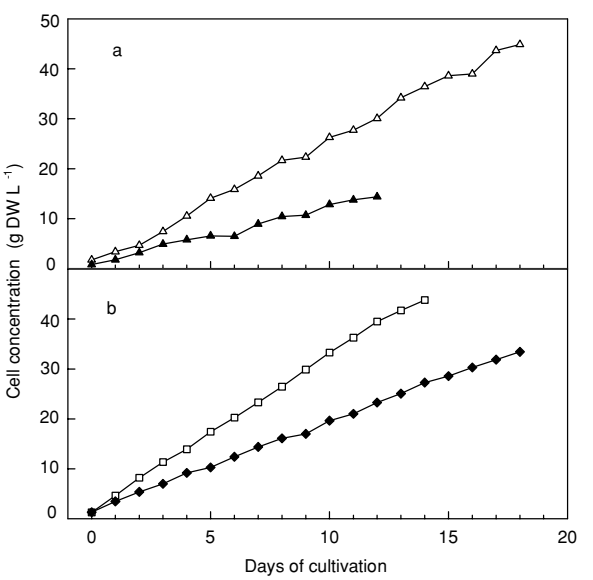

Fig. 2 Time course of algal cell concentration in the PB-1 and PB-2 bioreactors. (Morning algal dry weights are plotted). (a) Bioreactor PB-1, 224 m^2 culture area with 6 mm layer of suspension, Třeboň. Culture period: (△) July 7–26; (▲) September 16–29. (b) Bioreactor PB-2, 100 m^2 culture area with 8 mm layer of suspension, Kalamata. Culture period (□) June 22–July 6; (■) October 12–31

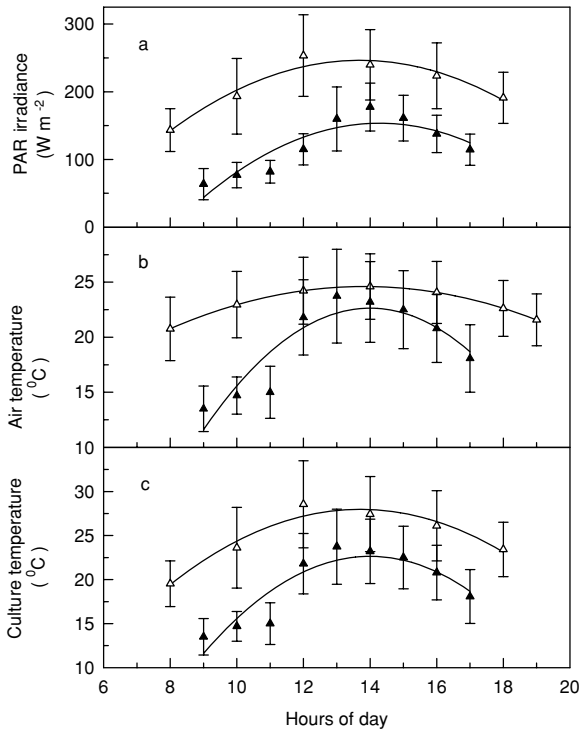

Fig. 3 Daily course of: PAR irradiance, (a), air temperature, (b) algal culture temperature, (c), in Třeboň (bioreactor PB-1). Culture period (△) July 7–26; (▲) September 16–29

was estimated as:

$$P_{\text{net}} = \frac{(X_n - X_0)}{n}\frac{V}{A} \tag{1}$$

where X_0 is biomass concentration at the beginning of the growth cycle, which started with about 5% of washed algal biomass obtained at the harvest of the previous cycle, X_n is the morning concentration of algae after n days of cultivation, V is the volume of the suspension in the bioreactor, A is the area of the culture area.

The daily changes in PAR, air and culture temperature for both bioreactors are shown in Figs. 3 and 4. The mean daily PAR energy inputs and mean air temperatures over the year in Třeboň and Kalamata are shown in Fig. 5. Whereas the cultivation season in Třeboň lasts usually 140–150 days (May–September), the cultivation period in Kalamata is longer (230–240 days, March–October).

The productivity in both bioreactors increased linearly with daily solar energy input (Figs. 6a and b) except at the highest energy input observed, where a deviation from linearity occurred. Net photosynthetic efficiency decreased with energy input (Fig. 6c). This may be due to a general decrease of light utilization with increasing irradiance observed in photosynthetic systems.

Carbon dioxide utilization

The amount of CO_2 to be supplied to the algal culture depends on: (i) efficiency of gas sparging; (ii) CO_2 loss from algal culture into ambient atmosphere; (iii) CO_2 consumption by algal cells. The overall CO_2 mass balance for an open algal bioreactor can be expressed as:

$$G = Z + K_{\text{L,CO}_2} K_{\text{H}} A(p\text{CO}_{2,\text{mean}} - p\text{CO}_2{}^{*}) + R_{\text{CO}_2,\text{mean}} A \tag{2}$$

Equation (2) takes into account the loss of CO_2 as a result of its incomplete absorption in algal suspension, its loss from the suspension on culture area due to escape into the atmosphere caused by $p\text{CO}_2$ gradients and consumption of CO_2 by algae. Assuming that the rate of CO_2 intake by the algae was proportional to the rate of oxygen evolution (coefficient of proportionality Y), we assessed by means of Equation (2) the CO_2 mass balance in both photobioreactors (Table 1). In the calculations, the mass transfer coefficient $K_{\text{L,CO}_2}$

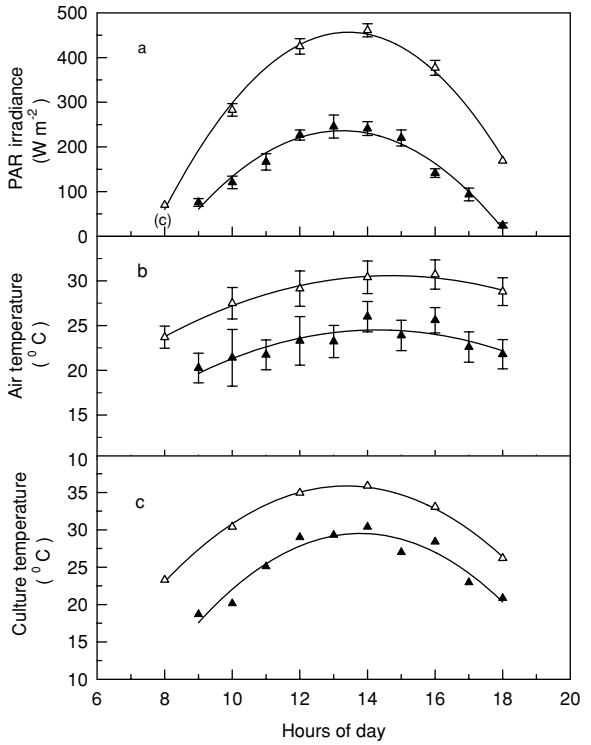

Fig. 4 Daily course of: PAR irradiance (a), air temperature (b), algal culture temperature (c) Kalamata (bioreactor PB-2). Culture period (△) June 22–July 6; (▲) October 12–31

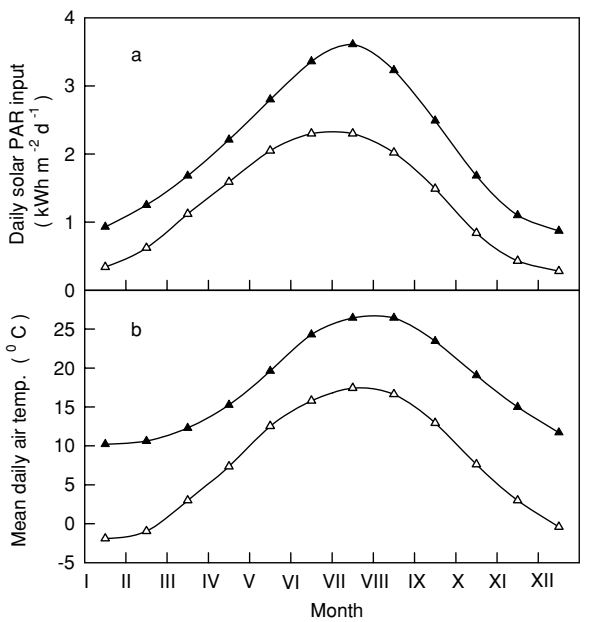

Fig. 5 The yearly course of: mean daily PAR energy input (a), mean air temperature (b), in Třeboň (△) and Kalamata (▲)

was estimated from the determined mass transfer coefficients for oxygen mass transfer from algal suspension into the surrounding atmosphere using the formula (Talbot et al., 1991): $K_{L,CO_2} = K_{L,O_2}(D_{CO_2}/D_{O_2})^{1/2}$. Values of Henry's constant K_H were computed from the formula given by Buhr and Miller (1983). Based on the measurements of pCO_2 and DO concentration profiles in the suspension on the culture area, we estimated the value of parameter $Y = 1.115 \pm 0.238\ g\,CO_2\,g\,O_2^{-1}$. The percentage of absorbed CO_2

Table 1 CO_2 mass balance for PB-1 and PB-2 photobioreactors operated on sunny days (Mean ± Std. Dev.)

Photobioreactor	Length of the culture lane (m)	Date of evaluation	Culture temperature (°C)	Mean rate of CO_2 supply per 1 m^2 of culture area ($L\,h^{-1}$)	Related to flow rate of CO_2 supplied: CO_2 absorbed in suspension (%)	Related to flow rate of CO_2 supplied: CO_2 utilized by algae (%)	Note
PB-1	0–28	31.VII.–3.VIII.	29.5 ± 6.0	4.07 ± 1.20	81.2 ± 8.44	69.0 ± 13.3	(a)
PB-2	0–50	5. VII.	33.5 ± 2.0	5.88 ± 1.38	84.2 ± 10.4	75.2 ± 8.5	(a)
	50–100		33.8 ± 2.5	6.78 ± 1.34	77.5 ± 3.4	70.7 ± 3.0	(b)
	0–50	25. X.	27.1 ± 4.1	3.86 ± 1.18	89.6 ± 1.7	76.7 ± 5.1	(a)
	50–100		27.4 ± 7.7	4.72 ± 1.24	83.7 ± 1.6	70.3 ± 5.2	(b)

(a) CO_2 supplied into suction pipe of the circulation pump; (b) CO_2 supplied into porous tubes

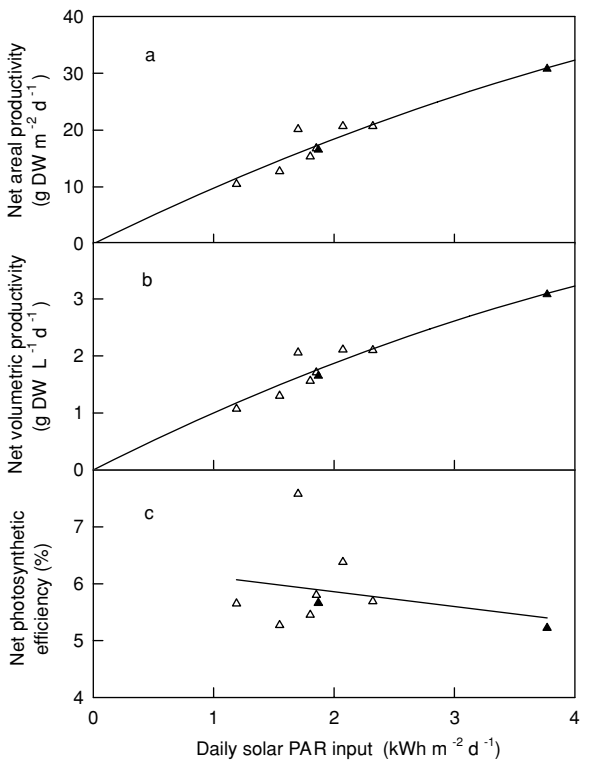

Fig. 6 Dependence of: net areal productivity (a), net volumetric productivity (b), net photosynthetic efficiency (c), on daily solar PAR input. (△) bioreactor PB-1; (▲) bioreactor PB-2

in algal suspension was influenced by the rate of CO_2 supply related to a unit volume of algal suspension which passed through the sparging site of the bioreactor (Fig. 7). The decrease in Figs. 7b and c was caused probably by coalescence of bubbles at high CO_2 gas flow rates. The circulation pump of the PB-1 had higher revolutions ($n = 2835\,min^{-1}$) than that of PB-2 ($n = 1400\,min^{-1}$). pCO_2 decreased on culture area in both bioreactors approximately exponentially, accompanied by an almost linear pH rise of the culture (Fig. 8).

Dissolved oxygen concentration and rate of oxygen evolution

The concentration of dissolved oxygen (DO) increased along the 100 m path of flowing suspension

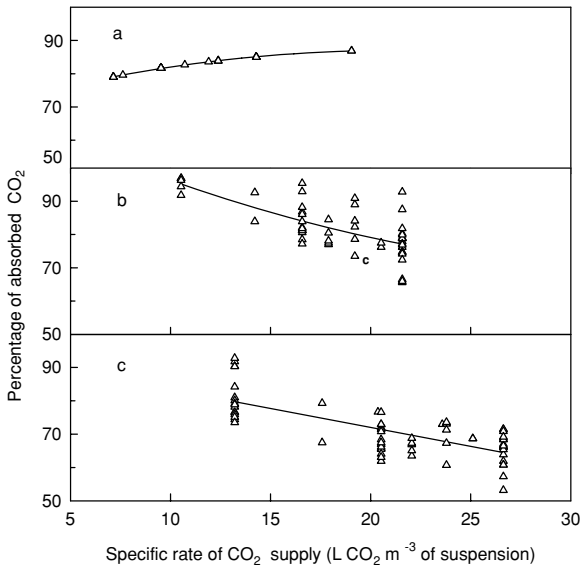

Fig. 7 Percentage of carbon dioxide absorption in photobioreactors PB-1 and PB-2 as influenced by the specific rate of CO_2 supply. (a) PB-1, CO_2 supply into circulation pump; (b) PB-2, CO_2 supply into circulation pump, sparging of the 0–50 m lane; (c) PB-2, CO_2 supply into porous tubes, sparging of the 50–100 m lane

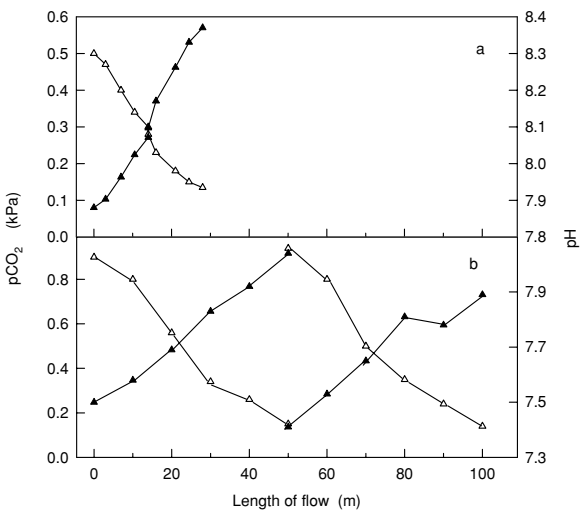

Fig. 8 The course of pCO_2 (△) and pH (▲) along the length of flow of algal culture in PB-1, (a) and PB-2, (b) photobioreactors. Sunny day, July, local time 14:00 h

in PB-2 to about 35 mg O_2 L^{-1} (Fig. 9). The DO concentration in PB-1 on sunny days at noon, ranged from 12 mg L^{-1} at the beginning, to 22 mg L^{-1} at the end of the 28 m suspension flow path. A certain decrease in oxygen concentration was found after flow of the culture through the channel connecting upper and lower lanes of the PB-2 bioreactor, where the algal cells were not sufficiently illuminated. Besides, some oxygen was removed during additional sparging of the culture in the channel by CO_2 gas.

The net rate of oxygen evolution by algae, referred to a unit of culture area of the bioreactor, was calculated from the DO data as:

$$R_{O_2} = uh(dC_{O_2}/dx) + K_{L,O_2}(C_{O_2} - C^*_{O_2}) \quad (3)$$

The first term on the right-hand side of the equation represents DO accumulation along the flow path of the suspension, the second term expresses mass transfer of oxygen from the algal culture into the atmosphere. Oxygen concentration $C^*_{O_2}$ at the algal culture-air interface was taken as that for water at the algae culture temperature being in equilibrium with ambient O_2 partial pressure ($pO_2 = 21$ kPa).

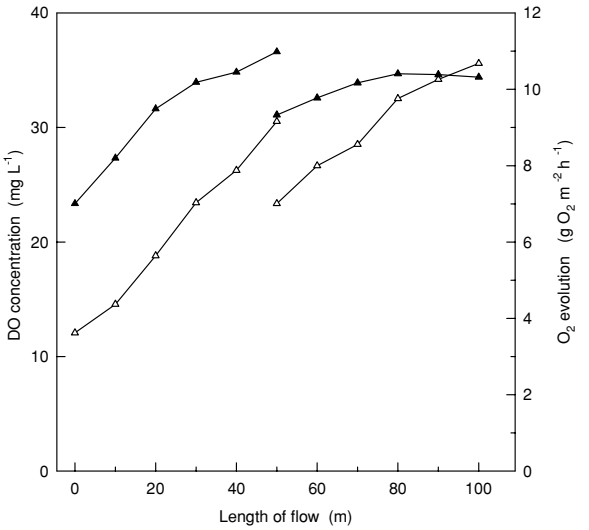

Fig. 9 The course of dissolved oxygen (DO) concentration (▲) and rate of oxygen evolution by the alga (△), referred to 1 m^2 of culture area, along the length of flow of algal culture. (Photobioreactor PB-2, sunny day, July, local time 14:00 h)

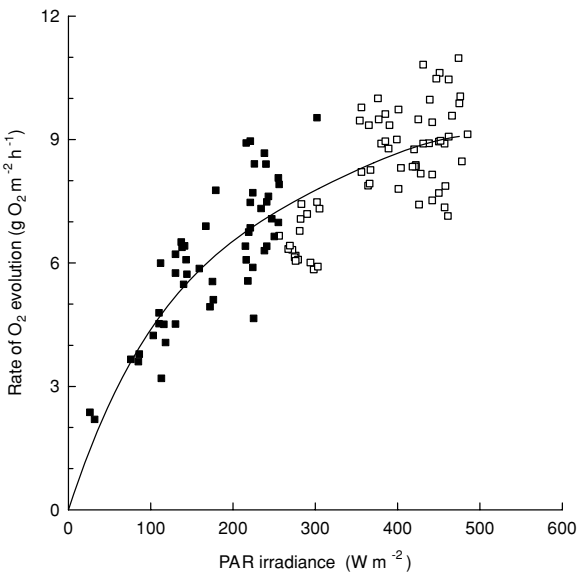

Fig. 10 Dependence of the net rate of oxygen evolution by the alga referred to 1 m^2 of culture area in PB-2 photobioreactor on PAR irradiance. Culture period: (□) June 22–July 6; (■) October 12–31

For the estimation of K_{L,O_2} we averaged Equation (3) for the flow path L of algae suspension as:

$$R_{O_2,\text{mean}} = (uh/L)(C_{O_2,L} - C_{O_2,0}) + K_{L,O_2}(C_{O_2,\text{mean}} - C_{O_2}{}^*) \quad (4)$$

Assuming that $R_{O_2,\text{mean}}$ was the same in both lanes of the culture area of the bioreactor, we estimated by means of Equation (4) and measured DO concentrations: $K_{L,O_2} = 0.24\,\text{m}\,\text{h}^{-1}$ for PB-1, $K_{L,O_2} = 0.18\,\text{m}\,\text{h}^{-1}$ for PB-2. The Reynold's number in PB-1 was lower (Re = 3600) than in PB-2 (Re = 5400) but the mass transfer coefficient K_L was higher in PB-1 than that in PB-2. Weissmann et al. (1988) reported for a 20 cm deep pond with a suspension velocity of $30\,\text{cm}\,\text{s}^{-1}$ (Reynold's number about 60 000) the value $K_L = 0.1\,\text{m}\,\text{h}^{-1}$. This value is lower than the K_L found for our thin-layer sloping bioreactors. In our opinion, the value of mass transfer coefficient is influenced mainly by surface renewal caused by turbulence near the liquid-gas interface. The distribution of turbulent eddies in the vicinity of the interface could be specified by instruments and methods which were not directly

Table 2 Verification of Manning formula in a thin-layer bioreactor with microalgae (*Chlorella* sp.) suspension[a]

Inclination of the bioreactor culture area	Layer thickness (Mean ± S.D.) (mm)	Mean velocity of suspension flow ($m\,s^{-1}$)	
		Experimental (Mean ± S.D.)	Estimated (Manning formula)
0.013	7.94 ± 1.52	0.574 ± 0.010	0.571
0.014	7.64 ± 1.35	0.566 ± 0.005	0.577
0.015	7.60 ± 1.20	0.595 ± 0.008	0.596
0.016	7.58 ±.1.23	0.618 ± 0.008	0.614
0.017	7.30 ± 0.95	0.622 ± 0.010	0.617

[a]Culture area 24 m long and 1 m wide, flow rate $Q = 270\,L\,min^{-1}$

available in this work. Future studies are needed to clarify the difference of the mass transfer coefficients found in our bioreactors.

The mean rate of oxygen evolution (calculated from the determined local R_{O_2} values along the length of flow of the suspension) increased hyperbolically (Fig. 10) with increasing PAR, similarly to the course of productivity given in Fig. 6. The fitted curve in Fig. 10 corresponds to the empirical formula: $R_{O_2,mean} = kI_0/(K_I + I_0)$, with k=12.82 g $O_2 m^{-2}\,h^{-1}$, $K_I = 193.4$ W (PAR) m^{-2}, correlation coefficient $r = 0.844$.

Hydraulics and power requirements for mixing of the suspension

The Manning equation (Weissman et al., 1988; Oswald, 1988) was used for calculating the suspension velocity flow u ($m\,s^{-1}$) in outdoor deep-layer ponds where algal culture was mixed by paddle-wheels: $u = (1/n)\,h^{2/3}\,I^{1/2}$, where n ($m^{1/3}\,s^{-1/2}$) is the roughness coefficient, h (m) is layer thickness, I (−) is inclination of channel. We verified this equation for a small outdoor thin-layer photobioreactor of 24 m^2 culture surface made of glass sheets (Table 2). The measurements of layer thickness were performed with an accuracy of one decimal point. Ten measurements of layer thickness were performed at each culture area inclination. The layer thickness values given in Table 2 are mean values expressed to two decimal points. It was found that $n = 7.945 \times 10^{-3}\,m^{1/3}\,s^{-1/2}$ (correlation coefficient between experimental and calculated velocity $r = 0.965$).

Hydraulic power E required for pumping the algal suspension is the product of volumetric flow rate Q, specific weight $\gamma = \rho g$ and head loss (Weissman et al., 1988). In the case of a photobioreactor with an inclined culture area of length L, hydraulic power is given as: $E = gLQI$. Volumetric flow rate Q is proportional to the product of suspension velocity u and thickness of the suspension layer h: $Q = uhb$, where b is the width of the culture area. Hydraulic energy dissipated per unit of the culture area is: $E_D = E/(bL) = guhI$. Substituting $\rho = 1000\,kg\,m^{-3}$, $g = 9.81\,m\,s^{-2}$, $u = 0.60\,m\,s^{-1}$, $h = 0.006$ m, $I = 0.017$, we get $E_D = 0.60\,W\,m^{-2}$ for PB-1. For PB-2 ($u = 0.66\,m\,s^{-1}$, $h = 0.008$ m, $I = 0.016$) we get $E_D = 0.83\,W\,m^{-2}$. However, the total mixing power needed is higher than this due to the geometric height to which the suspension is delivered onto the upper rim of the bioreactors, pressure loss in the pipes and less than 100% efficiency of the circulation pump.

Discussion

Growth, productivity and photosynthetic efficiency of algae

Large-scale commercial production of *Chlorella, Arthrospira*, or *Dunaliella* is currently carried out in open circular or raceway ponds in which a deep layer of algal culture is mixed by paddle-wheels.

The main drawbacks of open ponds are low productivity, low utilization of added CO_2, poor temperature regulation, contamination of algal culture by other algal species and heterotrophic microorganisms. Using thin-layer culture technology, most of the above critical factors are eliminated due to the efficient method of CO_2 supply, whose utilization by the algal culture is

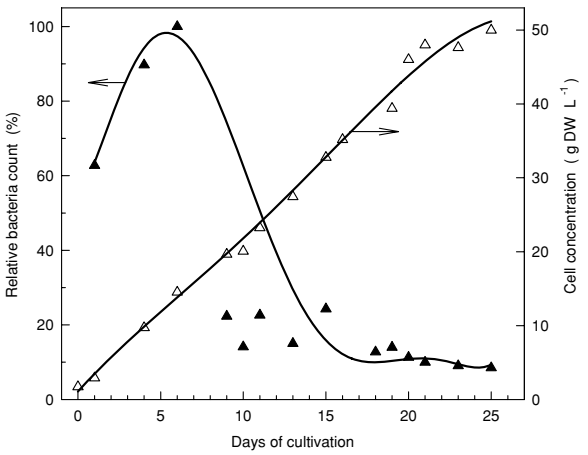

Fig. 11 Change in bacterial count and cell concentration in photobioreactor PB-1

>60%, which compares well with utilization of this gas in closed systems (Sobczuk et al., 2000). The cooling effect of water evaporating from the culture surface is very high. Even on very hot and sunny days, the maximum temperature of a 6–8 mm thick layer of dense algal suspension in July in Kalamata, Greece did not exceed 38.5 °C at noon. Due to the very thin algal layer, the optimal growth temperature is reached quickly after the beginning of the daily growth cycle and, on conversely, the evening temperature before the culture is put into the tank is relatively low; thus decreasing the night loss of algal biomass.

In detailed studies of bacterial contamination of *Chlorella* cultures grown outdoors in fed-batch cycles in a 6 mm layer, we have found that the beginning of each new growth cycle, for which the inoculum of washed algae of previous cycle is used, is characterised by very low levels of bacteria. Nevertheless, bacterial concentration increases relatively quickly to a maximum algal density of about 10–15 g L^{-1} algal DW. With further growth of the algal culture up to harvesting density (∼35 g or more algal DW L^{-1}), the amount of bacteria distinctly decreases (Fig. 11). Growth of bacteria occurs mostly in the night, when the suspension is kept and aerated in retention tank.

Net productivity of algae in a bioreactor depends on the production rate of the culture during the day and on night biomass loss caused by dark respiration of reserve materials (starch, glycogen). This process is strongly temperature-dependent. Night biomass loss of *Chlorella* in PB-1 (Třeboň) was 9–14% and in PB-2 (Kalamata) 6.6–10.8% of the daylight algae productivity. The night loss of biomass was estimated from the decrease of biomass (algae + bacteria) concentration (determined as dry weight) in the bioreactor. Because the mass of bacteria in suspension was much smaller than the mass of algae, the determined night biomass losses were caused by algal cells.

Previous fed-batch growth cycles of our pilot-plant thin-layer cultures (1991–2003) repeatedly showed that growth of *Chlorella* in the PB-1 bioreactor with a 6 mm thick layer of suspension proceeded linearly with the time of cultivation up to culture densities higher than 40 g DW L^{-1}. This was confirmed also in the PB-2 bioreactor with the suspension layer 8 mm thick (Fig. 2). These results are in sharp contrast with the frequently published data describing the algal productivity as being strongly algal density dependent. For example, the maximal productivity of *Spirulina* grown in a raceway pond is reached at a density of about 0.4 g algal DW L^{-1} and at a density of about 1 g DW L^{-1} the productivity drops to half the maximum value (Richmond, 1988; Vonshak, 1997). The sharp decrease in algal productivity in raceway ponds is probably caused by an excessively thick cultivation layer in which a severe shortage of light energy for algal photosynthesis occurs with increasing density of the poorly mixed algal culture.

The rapid rise of culture temperature with light intensity at the beginning of the cultivation day (Figs. 3 and 4) in thin-layer bioreactors may prevent strong photoinhibition of algal growth at high light intensity and low culture temperature, which occurs in raceway ponds. Chlorophyll fluorescence measurements in high-density mass cultures exposed to strong light revealed a decrease in photoinhibition with increasing

biomass concentration (Torzillo et al., 1996; Richmond, 2000).

The optimal areal density for maximal productivity of *Scenedesmus obliquus* and *Coelastrum sphaericum* in outdoor ponds with a thick suspension layer was 38–41 g DW m^{-2} (Grobbelaar et al., 1990). Similarly, Hartig et al. (1988) reported an optimal areal density of 40–45 g DW m^{-2} for *S. obliquus* grown in outdoor ponds with a culture depth of 150 to 300 mm. These values are much lower than those found in our bioreactors: we have not observed any significant decrease in productivity up to an areal density greater than 300 g DW m^{-2} (Fig. 2). Grobbelaar et al. (1996) found that in medium light path systems (such as raceway ponds) light/dark periods are in the range of seconds to minutes, photosynthetic rates are low and the compensation light/dark ratio is high. Consequently productivities would be low and attainable biomass densities would be also low. The thick column of water in the ponds absorbs certainly a much higher portion of light which cannot be used for the cells, compared to the thin-layer culture. The greater the depth of culture, the stronger is photolimitation and photoinhibition (Fernandéz, 1998).

Unfortunately, no data concerning photosynthetic efficiency of algal cultures grown in raceway ponds are available. The mean net PAR efficiency of 5.4% (this work, Fig. 6c) found for *Chlorella* cultures grown in Kalamata in July is lower than that found in summer months in Třeboň ($\eta = 6.5\%$). This may be due to several factors: higher solar energy input found in Kalamata causing an oversaturating effect of light intensity on the growth of the alga, high culture temperature at noon (about 38 °C), high dissolved oxygen concentration at noon (about 35 mg L^{-1}) in the suspension leaving the culture area of the bioreactor.

Carbon dioxide utilization

It has been shown that the cost of CO_2 in large-scale algal cultures is of prime importance to the total economics (Tapie & Bernard, 1988; Oswald, 1988; our calculations, unpublished). Carbon forms 45–50% of algal dry weight, so that 1.65–1.83 g CO_2 is needed for the biosynthesis of 1 g dried algal biomass. Mass of CO_2 consumed by algae, related to mass of oxygen evolved by algae $Y = 1.115\ g\,CO_2\ g\,O_2{}^{-1}$ found in this work is close to the published data for *Chlorella* sp. grown on urea as nitrogen source: $Y = 1.21\ g\,CO_2\ g\,O_2{}^{-1}$ (Beljanin et al., 1980).

Several techniques have been developed to distribute CO_2 into the culture medium, ranging from plastic dome exchangers to air stones and perforated PVC pipes. It has been reported (Richmond & Becker, 1986; Becker, 1994) that only 13–20% of supplied CO_2 was absorbed in raceway ponds. We found that sparging of suspension by CO_2 in our bioreactors was much more efficient (Table 1) and about 70% of the CO_2 supplied was utilized for photosynthesis. It is apparent from Figs. 7a and b that the percentage of absorbed CO_2 in the algal culture decreased with an increasing rate of CO_2 supply. The decrease may be caused by coalescence of CO_2 bubbles at higher gas flow rates, resulting in a decreased specific gas-liquid interfacial area. On the other hand, the amount of absorbed CO_2 in the algal culture, circulated by a pump with higher revolutions, increased a little with the rate of CO_2 supply (Fig. 7c), due to a positive effect of high shear rate in the pump on CO_2 dissolution. Introducing CO_2 to the circulation pump of the bioreactors had no adverse effect on the delivery of the suspension to the culture area.

We showed earlier (Lívanský & Doucha, 1999) that the pCO_2 in dense thin-layer algal culture during summer sunny days was lower than that in equilibrium with the concentration of bicarbonate ions in the culture. Fortunately, the pCO_2 gradient along the flow of suspension was lowered by the formation of CO_2 from bicarbonate ions, which caused a better utilization of supplied CO_2 in the bioreactors.

Dissolved oxygen concentration and rate of oxygen evolution

The rate of oxygen evolution by the algae in the PB-2 increased hyperbolically with increasing irradiance. This may be contributed to a general decrease of light utilization with increasing irradiance. (Goldman, 1979b) in photosynthetic systems.

Little work has been done so far to determine the influence of oxygen concentration on the productivity of algae in large-scale reactors. Most investigations have been done on laboratory cultures using constant cultivation parameters (Amman & Lynch, 1966; Torzillo et al., 1984; Akyev & Tsoglin, 1992). These studies may not adequately reflect the adaptation of algae to

changing culture conditions outdoors. R_{O_2} in PB-2 was about 2.5–20.4% higher in the second lane (50–100 m of the algal flow path) than in the first one (0–50 m), despite the higher DO concentration. The photosynthetic response of algal cells to light intensity was slower in the first lane of the reactor probably due to adaptation of algae to a sudden exposition to high irradiance occurring after delivery of algae suspension from the darkened tank to the irradiated culture area.

Hydraulics, power requirements for suspension mixing, turbulence

In accordance with the Manning equation, volumetric flow rate of the suspension delivered per 1 meter width of the culture area is proportional to $h^{5/3}$ at any inclination I of the culture area. Roughness of culture area made of glass: $n = 7.945 \times 10^{-3}\,\mathrm{m}^{1/3}\,\mathrm{s}^{-1/2}$, found in this work, was close to this as reported for smooth concrete ($n = 10 \times 10^{-3}\,\mathrm{m}^{1/3}\,\mathrm{s}^{-1/2}$ – Oswald, 1988).

The minimum suspension layer thickness on a sloped culture area was 6 mm. However, greater thickness of the layer may have some beneficial effects for summer cultivation in regions with high solar energy inputs: a greater capacity of the culture layer for dissolved CO_2 and O_2 diminishes their concentration and pH gradients along the flow-path of the suspension. A higher volume of suspension on the culture surface diminishes its overheating. A proper choice of layer thickness must be a compromise between the costs of energy for pumping and the influence of the above mentioned factors on the operation of the bioreactor.

The rate of mixing and the thickness of layer determine the frequencies of light/dark periods. In order to obtain some quantitative estimates, we applied equations derived for turbulent flow of water in channels (Gallacher & Chobbs, 1981) to the case of algae suspension flowing as a thin layer down an inclined culture area. Velocity of turbulent fluctuations can be computed from the formula:

$$|v| = l_y \frac{\partial u}{\partial y} \tag{5}$$

where l_y, mixing length; u is local velocity considered here at the distance y from the bottom of layer. Mixing length varies with the coordinate y as follows:

$$l_y = \kappa_y (1 - y/h) \tag{6}$$

The velocity u depends on the y as:

$$u = (u_*/\kappa)\ln(y/y_0) \tag{7}$$

where κ is Karman's constant ($\kappa = 0.42$); y_0 is thickness of bottom boundary layer. From Equations (5)–(7) we obtain:

$$|v| = u * (1 - y/h) \tag{8}$$

For the dynamic velocity u_* it holds:

$$u_* = \sqrt{ghI} \tag{9}$$

Average velocity of turbulent fluctuations in a layer of thickness h can be defined as:

$$|v|_{av} = (1/h)\int_0^h |v| d = u_*/2 \tag{10}$$

Average mixing length in a layer of thickness h can be defined similarly:

$$(l_y)_{av} = (1/h)\int_0^h |l_y| d = 0.067h \tag{11}$$

Taking into account the parameters of the PB-1 (or PB-2) bioreactors: $h = 0.006$ m (0.008 m), $I = 0.017$ (0.016), for $g = 9.81\,\mathrm{m\,s^{-2}}$, we obtained from the above equations: $|v|_{av} = 0.0158\,\mathrm{m\,s^{-1}}$ ($0.0177\,\mathrm{m\,s^{-1}}$), $(l_y)_{av} = 4.02 \times 10^{-4}$ m (5.36×10^{-4} m). The mean time of fluctuations will be: $\tau_{\mathrm{mean}} = (l_y)_{av}/|v|_{av} = 0.0254$ s (0.0303 s). The time needed for the transfer of a turbulent fluctuation from the irradiated surface of the suspension to the bottom of the layer is: $T = h/|v|_{av} = 0.380$ s (0.452 s). Intensity of turbulence defined by the ratio $u_\lambda/u_{\mathrm{mean}}$ (Bird et al., 1968), was equal to $0.01655/0.6 = 0.028$; i.e. 2.8% in the PB-1 and $0.01747/0.66 = 0.026$; i.e. 2.6% in the PB-2. For comparison, the reported (Bird et al., 1968) intensity of turbulence is from 1 to 10% at turbulent flow of a liquid in tubes.

Some authors (Kok, 1953; Grobbelaar et al., 1996; Nedbal et al., 1996) have found that light/dark periods of frequency >1 Hz caused an increase in photosynthetic rates and light utilization efficiencies. From these findings we may expect that even random mixing (micro-eddies) caused by turbulence in an optically

dense algal culture in the thin-layer bioreactors contributed to a better utilization of light by algal cells.

Based on the described results, a modular system is proposed for large-scale production of algal biomass: one module of 1000 m^2 of culture area consists of two 10 m wide and 50 m long meandering sloped lanes on which a 6–8 mm thick layer of flowing and well mixed algal suspension is exposed to sunlight. Extremely low cultivation volumes and high harvesting densities substantially reduce the costs of energy input for suspension flow and processing of the product (Doucha & Lívanský, 1998, 1999).

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References

Akyev AY, Tsoglin LN (1992) Effect of oxygen on O_2 exchange and increase of cell biomass in the development cycle of *Chlorella*. Soviet Plant Physiol 39:312–317

Amman ECB, Lynch VH (1966) Gas exchange of algae. II. Effect of oxygen, helium, and argon on the photosynthesis of *Chlorella pyrenoidosa*. Appl Microbiol 14:178–183

Becker EW (1994) Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge, pp 293

Beljanin VN, Sidko FJ, Trenkenschu AP (1980) Energetika fotosintezirujushchei kultury Mikrovodoroslei (Energetics of photosynthetically active culture of microalgae). Nauka, Sibirskoe otdelenie, Novosibirsk

Bird RB, Stewart WE, Lightfoot EN (1968) Přenosové jevy (Transport phenomena). Academia, Praha, pp 799

Borowitzka MA (1999a) Economic evaluation of microalgal processes and products. In: Cohen Z (ed) Chemicals from Microalgae. Taylor & Francis Ltd, London, pp 387–409

Borowitzka MA (1999b) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70:313–321

Buhr HO, Miller SB (1983) A dynamic model of the high-rate algal bacterial wastewater treatment pond. Water Res 17:29–37

Burlew JS (ed.) (1953) Algal Culture from Laboratory to Pilot Plant. Carnegie Institute of Washington, DC, pp 357

Doucha J, Lívanský K, Bínová J, Kubičko P, Novotný P (1993) Thin-layer high density microalgal culture system: Productivity and energy costs. In: Masojídek J, Šetlík I (eds) Progres in Biotechnology of Photoautotrophic Microorganisms. Book of Abstracts, 6th International Conference on Applied Algology, České Budějovice 6–11

Doucha J, Lívanský K (1995) Equipment for outdoor thin-layer cultivation of algae. Czech Patent 279579

Doucha J, Lívanský K (1998, 1999) Process of outdoor thin-layer cultivation of microalgae and blue-green algae and bioreactor for performing the process. Greek Patent 1002924; USA Patent 5981271 A

Doucha J, Straka F, Lívanský K (2005) Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. J Appl Phycol 17:403–412

Fernández FGA, Camacho FG, Pérez JAS, Sevilla JMF, Grima EM (1998) Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: Effects of dilution rate, tube diameter, and solar irradiance. Biotechnol Bioeng 58:605–616

Gallacher L, Chobbs CD (1981) Rasprostranenie zagryaznenii v estuarii. [Dispersion of pollutions in estuary.]. In: James A (ed) Matematicheskie modeli kontrolya zagryaznenii vody (Mathematical Models in Water Pollution Control). Moskva, Mir. pp 229–261

Goldman JC (1979a) Outdoor algal mass cultures – I. Applications. Water Res 13:1–19

Goldman JC (1979b) Outdoor algal mass cultures – II. Photosynthetic yield limitations. Water Res 13:119–136

Grobbelaar JU, Soeder CJ, Stengel E (1990) Modeling algal productivity in large outdoor cultures and waste treatment systems. Biomass 21:297–314

Grobbelaar JU, Nedbal L, Tichý V (1996) Influence of high frequency light/dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation. J Appl Phycol 8:335–343

Hartig P, Grobbelaar JU, Soeder CJ, Groeneweg J (1988) On the mass culture of microalgae: areal density as an important factor for achieving maximal productivity. Biomass 15:211–221

Kok B (1953) Experiments on photosynthesis by *Chlorella* in flashing light. In: Burlew JS (ed) Algal Culture from Laboratory to Pilot Plant. Carnegie Institute of Washington DC, pp 63–158.

Lívanský K, Doucha J (1998) Influence of solar irradiance, culture temperature and CO_2 supply on daily course of O_2 evolution by *Chlorella* mass cultures in outdoor open thin-layer culture units. Arch Hydrobiol/Algolog Stud 89:137–149

Lívanský K, Doucha J (1999) Liquid film mass transfer coefficients K_L for O_2 and CO_2 desorption from open thin-layer microalgal cultures into atmosphere. Arch Hydrobiol Suppl 127/Algolog Stud 92:109–132

Morita M, Watanabe Y, Okawa T, Saiki H (2001) Photosynthetic productivity of conical helical tubular photobioreactors incorporating *Chlorella* sp. under various culture medium flow conditions. Biotechnol Bioeng 74:136–144

Nedbal L, Tichý V, Xiong F, Grobbelaar JU (1996) Microscopic green algae and cyanobacteria in high-frequency intermittent light. J Appl Phycol 8:325–333

Oswald WJ (1988) Micro-algae and waste-water treatment. In: Borowitzka MA, Borowitzka LJ (eds) Micro-algal Biotechnology. Cambridge University Press, Cambridge, pp 305–328

Pulz O, Scheibenbogen K (1998) Photobioreactors: Design and performance with respect to light energy input.

In: Scheper T (ed) Advances in Biochemical Engineering/Biotechnology. Springer-Verlag, Berlin. pp 123–152

Pulz O (2001) Photobioreactors: Production systems for phototrophic microorganisms. Appl Microb Biotechnol 57:287–293

Richmond A, Becker EW (1986) Technological aspects of mass cultivation–a general outline. In: Richmond A (ed) CRC Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, FL. pp 245–263

Richmond A (1988) A prerequisite for industrial microalgaculture efficient utilization of solar irradiation. In: Stadler T, Mollion J, Verdus MC, Karamanos Y, Morvan H, Christiaen D (eds) Algal Biotechnology. Amsterdam, Elsevier Science, pp 237–244

Richmond A (2000) Microalgal biotechnology at the turn of the millenium: A personal view. J Appl Phycol 12:441–451

Sobczuk TM, Camacho FG, Rubio FC, Fernández FGA, Grima EM (2000) Carbon dioxide uptake efficiency by outdoor microalgal cultures in tubular airlift photobioreactors. Biotechnol Bioeng 67:465–475

Soeder CJ (1986) An historical outlet of applied algology. In: Richmond A (ed) CRC Handbook of Microalgal Mass Culture, CRC Press, Inc., Boca Raton, Fl. pp 25–41

Stengel E (1970) Anlagentype und Verfahren der technischen Algenmassenproduktion. Ber Dt Bot Ges 83:589–606

Šetlík I, Šust V, Málek I (1970) Dual purpose open circulation unit for large scale production of algae in temperate zones. I. Basic design considerations and scheme of pilot plant. Algolog Stud (Třeboň) 1:111–164

Talbot P, Gortares MP, Lencki RW, Noue de la J (1991) Absorption of CO_2 in algal mass culture systems: A different characterization approach. Biotechnol Bioeng 37:834–842

Tapie P, Bernard A (1988) Microalgae production: Technical and economic evaluations. Biotechnol Bioeng 32:873–885

Torzillo G, Giovanetti L, Bocci F, Materassi R (1984) Effect of oxygen concentration on the protein content of Spirulina biomass. Biotechnol Bioeng 26:1134–1135

Torzillo G, Accolla P, Pinzani E, Masojidek J (1996) In situ monitoring of chlorophyll fluorescence to assess the synergistic effect of low temperature and high irradiance stresses in *Spirulina* cultures grown outdoors in photobioreactors. J Appl Phycol 8:283–291

Torzillo G (1997) Tubular bioreactors. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): Physiology, Cell-Biology and Biotechnology. Taylor & Francis, London, pp 101–115

Tredici MR, Zittelli GC (1997) In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*). Physiology, Cell-biology and Biotechnology. Taylor & Francis, London, pp 117–130

Tredici MR, Zittelli GC (1998) Efficiency of sunlight utilization: Tubular versus flat photobioreactors. Biotechnol Bioeng 57:187–197

Tredici MR (2004) Mass Production of Microalgae: Photobioreactors. In: Richmond A (ed) Handbook of Microalgal Culture. Blackwell Science Ltd, Oxford, pp 178–214

Vonshak A (1997) Outdoor mass production of *Spirulina*: the basic concept. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): Physiology, Cell-Biology and Biotechnology. Taylor & Francis, London. p 233

Weissman JD, Goebel RP, Benemann JR (1988) Photobioreactor design: Mixing, carbon utilization, and oxygen accumulation. Biotechnol Bioeng 3:336–344