High Density Outdoor Microalgal Culture

Jiří Doucha and Karel Lívanský

Abstract Despite the common use of open raceway pond technology for algae production, the system has many serious drawbacks resulting in low productivities and relatively high production costs. The key to higher yields and a cheaper product rests with the lowering of culture volume by decreasing the thickness of the algal layer exposed to the light. The higher the culture surface-to-volume ratio (S/V), the higher the culture density and the lower the cost of handling and harvesting. Basic parameters (light, temperature, mixing, carbon dioxide, oxygen, nutrition) affecting algal productivity in thin-layer (TL) photobioreactor have been assessed. In a low volume of vigorously mixed culture, utilization of light energy and algal yields are increased. Production costs are reduced to about one fifth (20%) compared to raceways ponds.

Keywords Microalgae · *Chlorella* · Culture parameters · High density culture · Open ponds · Thin layer photobioreactor · Productivity · Photosynthetic efficiency · Economic consideration

List of Acronyms

DO	dissolved oxygen
dw	dry weight
EPDM	ethyl propylene dimer
PAR	photosynthetic active radiation
PE	photosynthetic efficiency
PUFA	polyunsaturated fatty acid
S/V	surface to volume ratio
TL	thin layer

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1 Introduction

Microalgae have high potential for use in many fields of human activities. Due to their biological quality, (high protein content, nearly all essential vitamins, pigments, carotenoids, unsaturated fatty acids, organically bound minerals) most of algal products have been used in human and animal nutrition, pharmacy and cosmetics.

Lately, increasing attention has been focused on microalgae as a feedstock for the production of biofuels, currently produced from crop plants containing starch (corn, wheat) or sucrose (sugarcane, sugar beet), for the production of bioethanol, and lipids (rapeseed, soya, palm oil) for the production of biodiesel (Chisti 2007, 2006; Chen et al. 2011; Lam et al. 2012).

One of the factors limiting the extent of algal utilization is their production economics. While the market price of dried *Chlorella* biomass used for the production of health food supplements ranges from 40 to 60 USD per 1 kg, which makes it feasible to grow the algae even under extensive culture conditions, the price of the biomass for economically viable biofuel production should be about two orders of magnitude lower. Thus, cheap and high yielding culture technology is essential.

2 Open Ponds

In the 1960s, large-scale cultivation of unicellular *Chlorella* was established in Japan and Taiwan, and some years later, *Spirulina* and *Dunaliella*, using open circular or oblong ("raceway") ponds (Fig. 1). Nowadays, these ponds are also located in the USA, China, India, Thailand, Indonesia, and other countries (Belay 1997; Lee 1997; Borowitzka 1999; Tredici 2004; Spolaore et al. 2006).With the exception of a large tubular photobioreactor producing *Chlorella* biomass in Klötze, Germany (Pulz 2001), and *Haematococcus* for production of astaxanthin in Israel (Algatechnologies, Ltd.), this open pond technology is the only system used commercially for the production of algae. Global annual biomass production is estimated to be up to 6,000 t of *Spirulina* and about 5,000 t of *Chlorella*.

2.1 Circular Ponds

Circular ponds are still used for production of *Chlorella* biomass in Japan and Taiwan. The 20–40 cm thick algal layer is mixed by means of a centrally anchored rotating arm. The diameter of the pond, made of concrete, can be up to 45 m. The main disadvantages of the system are too high thickness of the algal layer requiring very low culture densities, low turbulence in the poor mixed culture especially in the middle of the pond, insufficient supply of CO_2 by nozzles located on the arm, high energy consumption for mixing and downstream processing of biomass and low productivity.

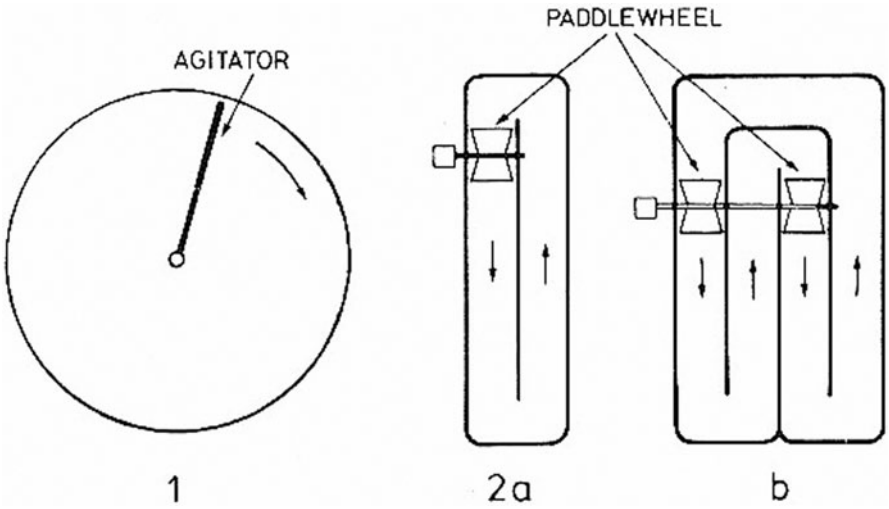

Fig. 1 Scheme of a circular and raceway pond (courtesy E. W. Becker). *1* algal layer is mixed by means of a centrally anchored rotating arm; *2* **a**, **b** oblong ponds with a central dividing wall, around which the algal suspension is circulated by means of paddle wheels

2.2 Raceway Ponds

Raceway ponds are mostly oblong basins equipped with a central dividing wall, around which an algal suspension circulates by means of paddle wheels. They are characterized by simple construction and relatively low building costs. The bottom is often sand upon which a high quality UV-resistant plastic lining is laid. The walls of the pond are constructed from bricks or concrete blocks covered with plastic sheets. The lining must be carefully laid to prevent wrinkling at the bottom or water accumulation beneath the lining.

For higher productivity of *Chlorella*, mixotrophic culture technology has been used by Taiwanese producers, utilizing acetic acid or glucose as an organic carbon source. To prevent excessive bacterial growth, addition of organic carbon ceases during the night. Despite this, the use of these substrates increases contamination of algal cultures by heterotrophic microorganisms. Removal of bacterial cells by size segregation or by heat and radiation treatments decreases the quality of the algal product and increases production costs.

Despite the common use of raceway technology, the system has many serious drawbacks resulting in low productivities (Pulz and Scheibenbogen 1998; Tredici 2004) and relatively high production costs:

- due to high thickness of the culture layer the concentration of algae should not be higher than 500 mg algal dry weight (dw) per 1 l. With increasing densities the productivity sharply decreases (Richmond 1988; Grobbelaar et al. 1990; Vonshak 1997). Low algal densities increases the danger of

contamination by bacteria and undesirable algal species (Apt and Behrens 1999; Doucha and Lívanský 2006).

- low velocity flow (10–30 cm s^{-1}) of poorly mixed algal suspension may result in photoinhibition of the upper overlighted algae and in the accumulation of oxygen, thus increasing photorespiration and decreasing photosynthetic efficiency and productivity (Demirbas 2010; Park et al. 2011).
- circulation of a large volume of algal culture is a continuous (day and night) and thus costly process. Separation of algae at harvest is also energy demanding and requires high capital expenditure. To obtain the desired concentration of algae for further processing, the culture requires at least two stages of thickening using expensive plate separators.
- a large volume algal culture accumulates heat energy from the sun during the course of cultivation, thus increasing the night respiration loss of biomass.
- supply of the algal culture with CO_2 is performed by its bubbling through the perforated tubes laying on the bottom of the pond. CO_2 utilization is only 13–20 % (Richmond and Becker 1986; Becker 1994).

2.3 Thin-Layer Culture Technology

The key to higher yields and lower production costs rests with a low volume culture by decreasing algal layer exposed to light to as low value as is technologically possible. The higher the surface-to-volume ratio (S/V), the higher the culture density and the lower the cost of handling and harvesting. In a well-mixed culture, several millimeters thick, it is possible to optimize the frequency of light/dark periods of single cells, thus increasing the efficiency of light utilization (photosynthetic efficiency, PE) and decreasing the photoinhibitory effect up to very high solar light intensities.

The first pilot-scale open photobioreactor for microalgae cultivation in a thin-layer (TL) was built at Třeboň's division of the Institute of Microbiology (Czech Republic) and started operation in 1963 (Šetlík et al. 1970). A 50 mm layer of algal suspension flowed down an inclined surface of 3 % slope, with 4 cm high slanted baffles placed at 15 cm distance perpendicularly to the flow. The baffles maintained the required thickness of algal layer over the whole culture area. The vigorously mixed suspension flowing down the 30 m × 30 m inclined area was returned by a collection channel to a tank, from which it was continuously pumped to the upper edge of the incline. A culture of *Scenedesmus quadricauda* started at a density of about 0.5 g (dw) per liter and was harvested at a density of about 3 g (dw) per liter. Productivity was relatively low (10–14 g algal dw per m^2 per day for 150 days of cultivation season). The main disadvantage of this system was the high energy consumption for circulating 50,000 l of algal suspension during the day and for night aeration of the suspension in the tank (Doucha and Lívanský 2006). Nevertheless, for 15 years this technology was used successfully for the commercial production

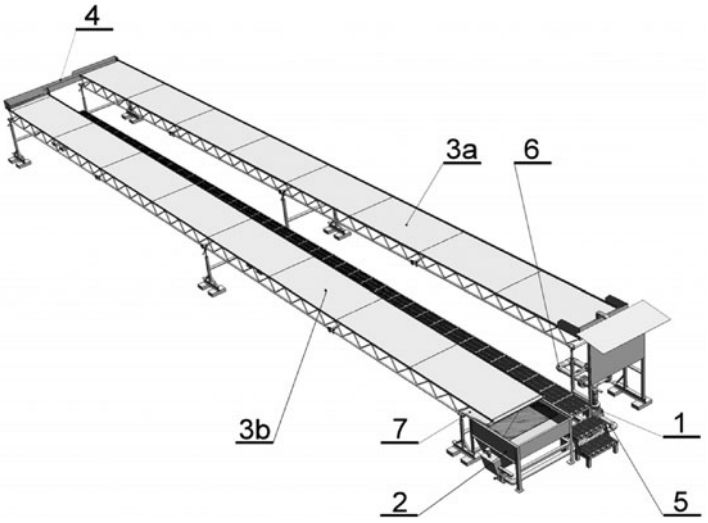

Fig. 2 Scheme of an open solar pilot plant photobioreactor for growth of microalgae in thin layer. The culture area consists of two lanes, inclined in the opposite directions and connected by a channel, *4*. The algal culture is supplied by means of a centrifugal pump, *1*, equipped with a frequency converter and flows from the retention tank, *2*, onto the upper edge of the culture area and along the inclined glass lanes, *3a* and *3b*. An optimal flow velocity is 50–60 cm s^{-1} at an inclination of 1.6–1.7%, and a culture layer thickness of 6–10 mm; the inclination can be changed. At the end of the lower lane, *3b*, the culture falls through the sieve to the tank, *2*. Scattering of the culture due to flow through the sieve decreases concentration of the DO and prevents impurities falling into the open culture. At night, the culture is kept in the tank and aerated by means of a pump, *1*, set on a night circulation regime. Supply of carbon dioxide: if pure CO_2 is used, it is supplied to the pipe, *5*, that enters the pump, *1*. If flue gas (containing 10–15% vol CO_2) is used, it is supplied to the porous ethylpropylendimer (EPDM) tubes placed in the pipe loop, *6*. A sliding plate, *7*, covers the suspension in the tank at night or on rainy days. The tank is equipped with a sensor that controls the addition of water lost by evaporation during the course of cultivation and keeps the darkened culture flowing through the tank at the required low level. The optimal CO_2 concentration is monitored by a pCO_2 electrode and maintained by means of a pH-stat or infraanalyzer (Doucha and Lívanský, 2006). Oxygen concentration in the culture which indicates the rate of photosynthesis during algal growth is measured by means of the Clark electrodes located at the beginning and end of the culture lane

of algal biomass in Rupite, Southern Bulgaria, where a photobioreactor of 3,000m^2 culture area was built (Fournadzieva and Pilarski 1993).

A modified version of an inclined-area photobioreactor whose culture parameters were in course of years in details analyzed and further optimized (Lívanský and Doucha 1996, 1997, 1998, 1999; Doucha 1998; Doucha and Lívanský 1995a, b, 1998, 1999, 2006, 2009; Doucha et al. 1993, 2005) started operation at Třeboň in 1991. Modifications consist of removing the baffles, reducing the culture layer to 6–8 mm, changing the inclination of the culture area to 1.6–1.7% and arranging the culture area into two lanes inclined in opposing direction (Figs. 2, 3). At night the algal culture is maintained in an aerated tank.

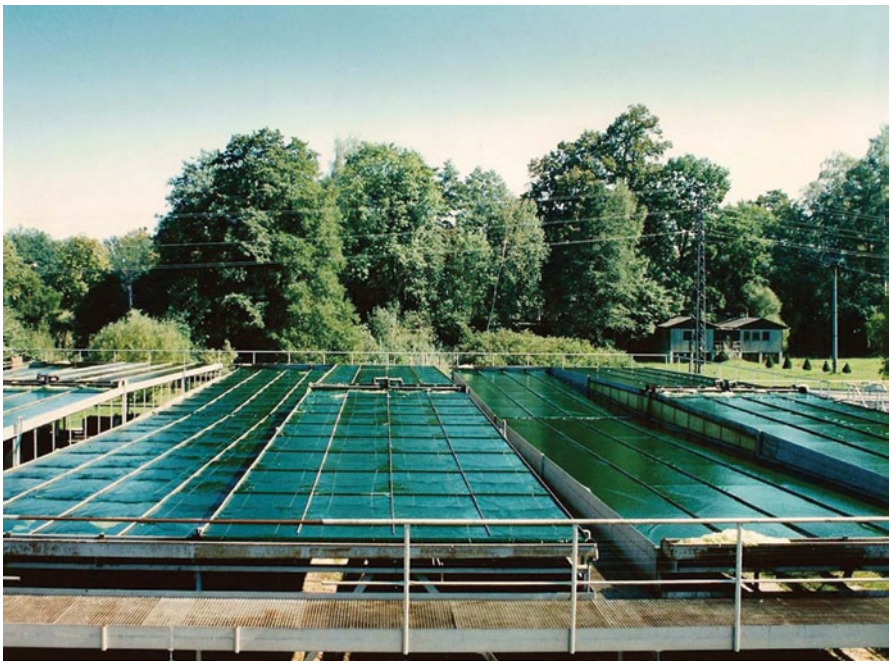

Fig. 3 Open thin-layer photobioreactor of 700 m^2 culture area in Třeboň's Institute. The photobioreactor serves for both growth experiments and production of high-quality biomass used in human and animal nutrition. (Photograph by J. Doucha)

Basic Characteristics of Thin-Layer Culture Technology

- high turbulence of the 6–8 mm layer of algal suspension flowing at a velocity of 50–60 cm s^{-1} along the inclined surface results in a high frequency of light/dark periods for single cells thus increasing light utilization and reducing the photoinhibition effect even at high sunlight intensities. This results in elevated PE and productivity.
- the culture volume per unit of cultivation area is 50x lower and the algal density at harvest is 100x higher than in raceways significantly reducing the cultivation and downstream processing costs; low culture volume permits better control of growth parameters. High algal density minimizes culture contamination.
- growth rate of the algal culture does not decrease with culture density up to high harvesting densities thus making the downstream processing cheaper.
- supply of the culture with CO_2 is achieved by (1) its supply into the suction pipe of the culture circulating pump (in case of pure CO_2) or (2) in case of flue gas, by CO_2 delivery to the EPDM tubes placed in the pipe loop between the circulation pump and the upper edge of the cultivation lane. In both cases, the utilization of CO_2 by algal cultures is about 70 % and reaches values achieved with closed culture systems.

- due to high cooling effect by evaporation of water from the thin algal layer, growth is possible even under high air temperatures and high solar light intensities (Doucha and Lívanský 2006, 2009).

3 Closed Photobioreactors

In the last decade, increasing attention has been focused on closed culture systems. Basic principles have been reviewed by many authors (Grima et al. 1999; Janssen et al. 2003; Carvalho et al. 2006; Chisti 2007; Xu et al. 2009; Posten 2009). The following parameters are considered to advantageous in closed bioreactors in comparison with open culture technology: prevention of, or minimizing contamination, growth temperature can be maintained higher and better controlled, prolonged cultivation season in climate with lower ambient temperature, better control of basic culture parameters (pH, pO_2, pCO_2), lower water and CO_2 losses, smaller area requirements in the case of vertically arranged bioreactors (tubes, plates).

However, there are some serious drawbacks to closed culture technology, which arise from its large-scale use: high building costs, diminished PE due to age-related decreasing transparency of the material from which walls of the reactor are made and due to loss of light incident under angle on the culture surface, and excessive accumulation of oxygen which inhibits photosynthesis. Removal of oxygen must be carried out in non-illuminated degassing columns (Chisti 2007). Attachment of algae to the inside surface of the culture area and fouling of the culture at higher algal densities decreases the absorbance of light energy and reduces PE. High energy demand: the culture must be forced through the tubes or in meandering way arranged channels of the flat-plates continuously (day and night), the density of growing algae is low (mostly less than 1.5 g dw l^{-1}) increasing thus further energy demands for algal separation at the harvest.

Many types of closed bioreactors have been developed (Tredici 2004; Carvalho et al. 2006; Posten 2009): tubular systems of various length, volume and diameter constructed from glass or transparent plastic tubes arranged either vertically, horizontally or in the form of coils; flat plates in which the suspension is exposed directly to the sunlight and mixed by air bubbling; panels arranged vertically where the algal suspension moves through horizontal channels in a meandering way by means of a pump.

The closed cultivation units often equipped with artificial lights were widely used in experimental work in laboratories and in the preparation of the high valuable products requiring special culture technology (e.g. production of labeled compounds, microalgae in aquacultures, preparation of PUFA's for pharmaceutical use). Only very few bioreactors have been practically operated on a large scale. The largest plant for *Chlorella* production is running in Klötze, Northern Germany (Pulz 2001). This plant, installed in a greenhouse, consists of 500 km of 50 mm diameter glass tubes arranged horizontally in 20 modules that form 3 m high vertical walls.

Nevertheless, the volumetric productivity is relatively low. A considerable increase of algal yield is achieved by use of mixotrophic culture technology (light and an organic carbon source). The product, spray-dried algal biomass is mostly used in the food and cosmetic industries.

4 Parameters Affecting Algal Productivity in Dense Open Cultures

4.1 Light

Solar radiation covers a broad wavelengths spectrum, of which only a part, photosynthetically active radiation, (PAR, wavelengths 400–700 nm) is useful for photosynthesis. For most applications, efficient capture of light energy represents one of the most important factors controlling culture productivity. Microalgal growth is mostly saturated at a PAR of about 200 μmol m^{-2} s^{-1}, which is about 1/10 of the maximum light intensity outdoors in summer (Torzillo et al. 2003). For solar light a conversion factor of 1 W m^{-2} = 4.94 μmol m^{-2} s^{-1} (μE m^{-2} s^{-1}) was found for PAR (Doucha and Lívanský 2006). Chlorophyll fluorescence measurements in high-density mass cultures exposed to strong light showed a decrease in photoinhibition with increasing biomass concentration (Richmond 2000). A high column of water in the pond absorbs much higher portion of light which could not be used for the cells, compared to a thin-layer culture. This factor alone justifies the use of low areal densities in ponds.

The occurrence of the "light saturation effect" is one of the most serious limitations of high solar irradiance utilization. Three types of approaches have been proposed to alleviate the saturation effect: (1) increase culture density and the mixing rate; (2) use of special designs of photobioreactors to dilute light intensity incident upon the culture (Mori 1986; Carlozzi 2003); (3) search for strains having small antenna size (Nakajima and Ueda 1997; Melis et al. 1999).

However, in certain cases, saturating light is a prerequisite for stimulating the synthesis of valuable products such as secondary carotenoids (e.g. by the alga *Haematococcus*).

Algal productivity is influenced mainly by light intensity available to cells inside the thin culture layer. The PAR intensity I (W m^{-2}) at a distance z (m) from the algal culture surface can be expressed, in accordance with the Lambert-Beer law, as: $I = I_0 \exp(-\varepsilon X z)$, where: I_0 (W m^{-2}) is PAR irradiance of the algal culture, ε(m^2 g dw^{-1}) is the extinction coefficient, X (g dw m^{-3}) is the cell concentration. At an algal cell concentration higher than ca 5 g dw l^{-1}, almost all incident light is absorbed in the layer (Doucha and Lívanský 2009).

The mean light intensity in the layer of thickness h is:

$$I_{mean} = \frac{1}{h}\int_0^h I\, dz = \frac{I_0 - I_h}{\varepsilon_{mean} X\, h} \tag{1}$$

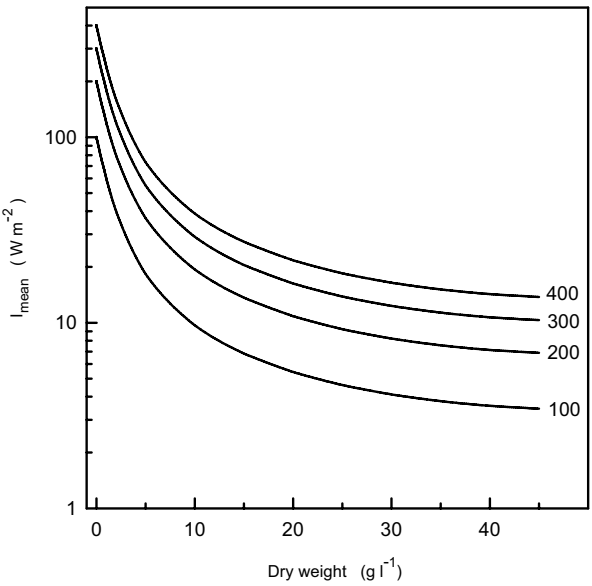

Fig. 4 Dependence of the mean light intensity inside an 8 mm thick culture layer on *Chlorella* sp. dry weight. Numbers at curves: PAR irradiance (Wm^{-2})

where $I_h = I_0 \exp(-\varepsilon_{mean} X h)$ is the unabsorbed light intensity in the layer, and ε_{mean} is the mean extinction coefficient. The following correlation was found for *Chlorella* culture: $\varepsilon_{mean} = \varepsilon_0 (1 - a_1 h/2)(1 - a_2 X)$, with values of empirical coefficients: ε_0 (m^2 g dw^{-1}) = 0.175; $a_1 = 46.165$; $a_2 = 9.664.10^{-6}$ (Doucha and Lívanský 2009). The mean light intensity inside the layer decreases with increasing cell concentration (Fig. 4). At high cell concentrations, this decrease is slow. This may partly explain why algal productivity in high density thin-layer cultures is influenced only slightly by cell concentration (Doucha and Lívanský 2006, 2009).

4.2 Temperature

Temperature significantly affects biomass yield. Due to a very thin algal layer and a low culture volume in the TL photobioreactor, the optimum growth temperature is reached very quickly after beginning of the cultivation day. The rapid rise in temperature may prevent photoinhibition of algal growth, which occurs in raceway ponds, where the temperature rises more slowly due to the large volume of suspension that must be heated. On the other hand, even on very hot and sunny days, cooling of the culture in the TL photobioreactor due to water evaporation is very effective: the maximum temperature of a 6 mm thick algal layer does not exceed 38.5 °C at noon hours. The evaporated water is replaced by means of a controller (Fig. 2). Loss of the evaporated water (5–6 l m^{-2} d^{-1} in clear summer days) is comparable, at the same solar energy input, to the consumption of water for cooling of closed bioreactors by spraying from the outside (Doucha and Lívanský 2006, 2009).

In addition, the relatively low evening culture temperature in TL bioreactor causes smaller loss of algal biomass by its night respiration in the tank.

4.3 Mixing

Mixing of algal culture in photobioreactors avoids thermal stratification, distributes the nutrients and improves gas (CO_2, O_2) transfer and mass transfer between the cells and the liquid. Adequate mixing can counter the negative effect of a light gradient. Turbulence exerts the following major effects: it prevents culture stratification which could result in photoinhibition of cells exposed to high irradiance, decreases the cell boundary layer and prevents sedimentation of the cells.

Photobioreactor design should ensure an optimum light-dark cycling of the cells to enhance PE (Chisti 2006). Algal growth can be influenced by three types of intermittent illumination (light/dark periods): high frequency fluctuations of 100 ms (10 Hz) or less ("flashing light effect") (Kok 1953; Nedbal et al. 1996), medium frequency fluctuations of seconds to minutes and low frequency cycles of hours to days (Grobbelaar 1991). Open raceway ponds, mixed mostly by paddle wheels at flow rates of 10–30 cm s^{-1}, operate with random light/dark frequencies (seconds to minutes). No photosynthetic or growth rate enhancement occurred at these frequencies (Weissman et al. 1988; Grobbelaar 1989). Slow light/dark changes, in the range of seconds, diminishes the specific growth rate below the value expected for the same net irradiance applied as continuous light (Morweiser et al. 2010). On the other hand, Ogbonna et al. (1995) reported an increase in productivity caused by random mixing of a dense culture of *Chlorella pyrenoidosa*. This effect was most pronounced in a photobioreactor with a shallow suspension layer. From these findings we may expect that even random mixing (microeddies) caused by turbulence in an optically dense culture, in outdoor sloped photobioreactors, could lead to better light utilization. Nonrandom mixing designs, using foils (Laws et al. 1983) or baffles (Doucha and Lívanský 1995a, b) immersed in a dense shallow algal culture, producing vortices with rotation rates of ca. 0.5–1.0 Hz, taking advantage of the flashing light effect on productivity.

Mixing rate and layer thickness determine frequencies of light/dark periods. Quantitative estimation of turbulence parameters were derived for algae flowing in a 6–8 mm layer down a inclined culture area. The flow is turbulent (Reynolds number 3,600–6,000). A mean transfer time for a turbulent vortice from the irradiated culture surface to the layer bottom is in the range of $T = 0.38–0.45$ s, and the transverse motion frequency of the algal cells is $1/T = 2.2–2.6$ Hz. Thus, a layer of well mixed dense algal culture several millimeters thick increases the frequency of dark/light periods of the single cells thus increasing PE and decreasing photoinhibition up to very high solar light intensities.(Doucha and Lívanský 2006).

The Manning equation was used (Oswald 1988; Weissman et al. 1988) to calculate algal culture velocity u (m s^{-1}) in deep-layer ponds where the culture is mixed by paddle-wheels: $u = (1/n)\ h^{2/3}\ I^{1/2}$, where n ($m^{1/3}\ s^{-1/2}$) is the roughness coeffi-

cient, h (m) is the layer thickness, and I (−) is the inclination of the culture area. This equation was verified for a small (24 m^2) outdoor TL photobioreactor made of glass sheets with $n = 7.945 \cdot 10^{-3}$ m $^{1/3}$ $s^{-1/2}$ (Doucha and Lívanský 2006). Oswald (1988) reported $n = 10 \cdot 10^{-3}$ $m^{1/3}$ $s^{-1/2}$ for smooth concrete. Turbulent flow, efficient transverse mixing of the culture layer on an inclined surface and a very low surface roughness coefficient prevent sedimentation and sticking of the cells.

Hydraulic power E required for pumping an algal suspension onto a culture area is the product of volumetric flow rate Q, specific weight $\gamma = \rho g$ and head loss (Weissman et al. 1988). In the case of an inclined area of length L, and inclination I, the hydraulic power needed for lifting the suspension from the end to the beginning of the culture area is: $E = \rho g L Q I$. Volumetric flow rate Q is proportional to the product of suspension velocity u, layer thickness h and culture area width b: $Q = u h b$. Thus, hydraulic power for 1 m^2 of culture area will be: $E_A = E/(bL) = \rho g u h I$. For a layer thickness $h = 0.006$ m, it follows from the Manning equation that $u = 0.54$ m s^{-1}. For $\rho = 1{,}000$ kg m^{-3}, $g = 9.81$ m s^{-2}, $I = 0.017$ we obtain $E_A = 0.54$ kg $s^{-3} = 0.54$ W m^{-2}. Similarly, for a layer thickness of 0.008 m, we obtain $u = 0.66$ m s^{-1} and $E_A = 0.88$ kg $s^{-3} = 0.88$ W m^{-2}. The total hydraulic power needed for pumping the suspension onto a culture area will however, be higher than the above values due to pressure drop within the tubes and less than 100 % efficiency of the circulation pump.

Substituting for velocity u from the Manning equation into the formula for E_A, the following proportionality can be found: $E_A \approx h^{5/3} I^{1.5}$. Hence, to save pumping energy, the suspension layer thickness and culture area inclination should be as low as possible. On the other hand, a thicker layer may have some beneficial effects in summer cultivation in regions with high solar energy inputs: a greater capacity of the culture layer for dissolved CO_2 and O_2, thus diminishing concentration and pH gradients along the flow path of the suspension. A greater culture volume on the area diminishes overheating. The optimum thickness of the algal layer must be a compromise between energy cost for pumping and the influence of the above factors on cultivation conditions in the TL bioreactor. From practical reasons, a 6–8 mm thick algal layer in a sloped culture system seems to be optimal (Doucha and Lívanský 1995b, 2006).

4.4 Carbon dioxide

Carbon comprises 45–50 % of algal dry weight (dw), so that 1.65–1.83 g CO_2 is theoretically required for the biosynthesis of 1 g dw. Carbon is stored in nutrient solution as a free carbon dioxide, bicarbonate or carbonate. The relative amount of each species is pH-dependent. In autotrophic microalgal mass cultures, such as *Chlorella*, CO_2 is used as the main carbon source. Carbon dioxide is responsible for the physiological processes and influences the buffering capacity and electrolyte balance of the nutrient solution. Although HCO_3^- is easily absorbed by the chlorococcal cells it is a poor source of carbon compared to CO_2 (Goldman et al. 1981). Alkaline bicarbonate alone cannot provide sufficient carbon to optimize biomass

yields due the formation of chemical precipitates and, therefore, CO_2 must be added to the cultures. *Arthrospira* (*Spirulina*) is the only alga produced in large scale that can use carbonate or bicarbonate. In *Chlorella* sp. grown outdoors, the maximum rate of CO_2 consumption per 1 m^2 of TL bioreactor area was about 10 g CO_2 m^{-2} h^{-1} at noon hours on clear summer days (Doucha and Lívanský 2006).

The cost of pure CO_2 for large-scale algal cultures is of prime importance to total production economics. In our experience, the cost of CO_2 represents 60–65 % of the total cost of nutrients needed for algal growth. This proportion can be lowered considerably by use of waste CO_2.

Large-scale algal cultures are subjected to change in the CO_2 concentration regardless of the culture system design. The CO_2 supply depends on: (1) the efficiency of mass transfer; (2) loss by escape into the atmosphere; (3) consumption by algal cells. To minimize CO_2 loss, the partial pressure (pCO_2) of dissolved CO_2 must be as low as possible. The minimum value for non-limiting *Chlorella* growth was found to be $pCO_2 = 0.1–0.2$ kPa (Weissman et al. 1988; Lívanský and Doucha 1996, 1998).

The question of CO_2 supply to algal cultures is a key engineering problem in autotrophic production of algae (Xu et al. 2009). Several techniques have been developed for raceway ponds to distribute CO_2 in the culture medium ranging from plastic dome exchangers, air stones and commonly used perforated pipes placed at the bottom of the pond. However, only 13–20 % of the CO_2 supplied is absorbed (Richmond and Becker 1986; Becker 1994). In the open TL bioreactor, pure CO_2 is supplied into the suction pipe of the suspension circulating pump (Lívanský and Doucha 2005). Alternatively, CO_2 containing flue gas is supplied via porous ethylpropylene dimer (EPDM) membrane tubes inserted between the circulation pump and the culture area. 80 % of the CO_2 is absorbed and about 70 % is utilized for photosynthesis. This compares well with the utilization of CO_2 in closed systems (Sobczuk et al. 2000).

Along the algal flow path, the pCO_2, which is buffered by formation of CO_2 from bicarbonate ions, decreases approximately exponentially along length of the flow while the culture pH correspondingly rises (Lívanský and Doucha 2005, Fig. 5). The decrease in pCO_2 is less at a more alkaline pH and higher at high irradiance of the culture. The log pCO_2 decreased linearly with increasing pH of the culture (Lívanský and Kajan 1994; Lívanský and Doucha 1998). For a prediction of pCO_2 changes in outdoor open TL photobioreactors, models that can be applied to rational design and scale-up of the photobioreactors were developed (Lívanský et al. 1993, 2006).

Experiments with laboratory *Chlorella vulgaris* culture showed that use of KNO_3 as a nitrogen source for growth caused a considerable shift in the log pCO_2-pH relationship to higher pH values. This behavior can be attributed mainly to the accumulation of K^+ ions during cultivation. Buffering of culture pH by addition of bicarbonate (1 g $KHCO_3$ l^{-1}) was not much efficient. With urea as the nitrogen source, the log pCO_2-pH shift was much less (Lívanský and Kajan 1994). An example of the pCO_2-pH relationship in a *Chlorella* culture in a TL photobioreactor is shown in Fig. 6.

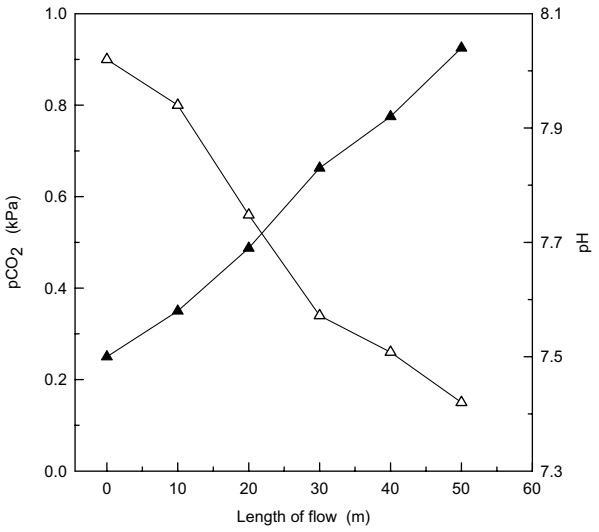

Fig. 5 The course of pCO_2 (Δ) and pH (▲) along the flow path of a *Chlorella* culture (layer thickness 8 mm). Sunny day, July, 14:00 h

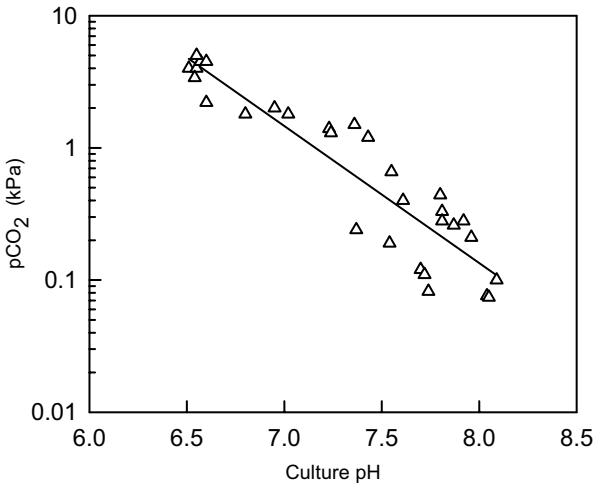

Fig. 6 Relationship between pCO_2 and culture pH in the TL photobioreactor. (Doucha et al. 2005)

To minimize CO_2 loss and ensure sufficient CO_2 for growth, the rate of CO_2 supply must be controlled. One way is to use a pH-stat system: CO_2 is supplied to balance the production of OH^- ions and pH control is by means of an on-off valve and standard controllers, without taking plant dynamics into account. As an example, in the pilot TL photobioreactor containing 290 l of *Chlorella* suspension, the response of pCO_2 (and hence culture pH) to changes in light intensity lasted several minutes to reach a new quasi steady-state (unpublished results). The pH sensor, connected to a regulation pH-meter, was placed in the pipe that enters to circulation pump of the bioreactor. The pH meter was set at values in the range pH = 7.9–8.0. In this

Fig. 7 Pilot-scale photobioreactor for thin-layer cultivation of *Chlorella* algae in a dairy farm (1,200 pcs of dairy cattle) at Dublovice, Czech Republic. After combustion of anaerobically generated CH_4, flue gas is used as a source of CO_2 for algal photosynthesis. A modified liquid fraction of anaerobically digested manure can serve as an additional source of nutrients. (Photograph by J. Langová, BCS Engineering)

way, the pCO_2 at the end of the culture flow is held approximately constant during cultivation. Another method using an infraanalyzer was described by Doucha and Lívanský (2006) for pCO_2 control in an outdoor open thin-layer photobioreactor. About 70 % of supplied CO_2 is utilized by the algae and, for the production of 1 kg (dw) of *Chlorella,* about 2.6 kg of CO_2 is required.

In contrast, the system dynamics in raceway ponds do not permit a rapid response to disturbances in culture parameters (light intensity, culture temperature) due to the large volume of culture and the buffering capacity of CO_2-HCO_3^- in the nutrient solution.

Using a microalgal photobioreactor as the CO_2 mitigation system is a practical approach for eliminating CO_2 emission and for decreasing biomass production costs. Commercially produced pure carbon dioxide can be replaced with CO_2-containing flue gas generated by e.g. combustion of the biogas formed by anaerobic digestion of organic wastes, (Eureka project OE 09025 for 2009–2012) by combustion of municipal wastes (Doušková et al. 2009; Eureka project OE 221 for 2006–2009), and other sources (limekiln and cement industry, power stations, fermentation processes etc).

Flue gas containing 13 % vol. CO_2 after combustion of anaerobically generated CH_4 from cattle manure was used successfully for outdoor production of *Chlorella* in a pilot plant TL photobioreactor (Fig. 7, Doucha 2012).

4.5 Oxygen

Oxygen accumulating in the nutrient solution (dissolved oxygen, DO) during the course of algal growth is one of the most important factors influencing algae productivity. Its high concentration lowers the photosynthetic rate due to inhibition of ribulose-1,5 bisphosphate carboxylase (RuBisCO), which catalyses the incorporation of CO_2 to the first organic product - phosphoglycerate. Most investigations of the oxygen influence on algal growth were carried out on laboratory cultures using constant cultivation parameters: temperature, irradiance, gas mixture composition, etc. (Ogawa et al. 1980; Torzillo et al. 1984; Akyev and Tsoglin 1992; Lívanský 1996). These studies may not accurately reflect the adaptation of algae to changing culture conditions outdoors.

Due to poor mixing of a 30 cm thick culture, oxygen removal in raceways is insufficient. Its concentration does not vary much along the flow path of the culture and the cells may be constantly exposed to inhibitory oxygen concentration. This is not the case in TL bioreactor, where the culture is getting rid off oxygen by flow through the scattering sieve placed between the lowest part of the culture lane and the pump which pushes the culture up to the upper edge of the culture area. Therefore the concentration of DO is lowest at the beginning of algal flow along the culture lane and is highest at the end of it. Thus, growth inhibiting concentration of DO can occur mainly at the end of the culture area.

A combination of high DO, high temperature and high irradiance of a low density raceway culture may result in photooxidative damage to algal cells (Richmond 1986). In a TL photobioreactor, the photooxidative effect is reduced due to the high flow velocity (50–60 cm s^{-1}) and strong turbulence, causing a high frequency of light/dark periods for single cells (Grobbelaar et al. 1995; Tichý et al. 1995).

For non-limited growth, oxygen concentrations should be maintained below 400 % of air saturation value (Kunjapur and Eldridge 2010)which corresponds to 30 mg l^{-1} of DO, assuming that the equilibrium solubility of O_2 in air is 7.5 mg l^{-1} (21 % O_2 v/v) at 30 °C. The maximum DO found in ponds is typically 25–40 mg l^{-1} (Weissman et al. 1988). In dense *Chlorella* cultures, the DO at the end of the inclined culture area should not be greater than 30–35 mg l^{-1}. The typical course of DO concentration in *Chlorella* culture grown in an open TL photobioreactor in Southern Greece is shown in Fig. 8.

Oxygen produced in the culture is removed by mass transfer to the atmosphere. The extent of transfer depends on the rate of oxygen diffusion and on intensity of turbulence, both of which influence the overall mass transfer coefficient for oxygen K_L (m h^{-1}). Additional removal of oxygen takes place outside the culture area trough the following processes: (1) flow of suspension from the culture area into the retention tank of the bioreactor through the scattering sieve; (2) contact of algae with carbon dioxide (or by CO_2 containing flue gas) where dissolved oxygen is transferred from the culture to the gas phase; (3) depletion of oxygen by dark respiration of algal cells in non-illuminated regions of the culture, i.e. in the retention tank, in the circulation pump and in pipes that supply and distribute the culture throughout the

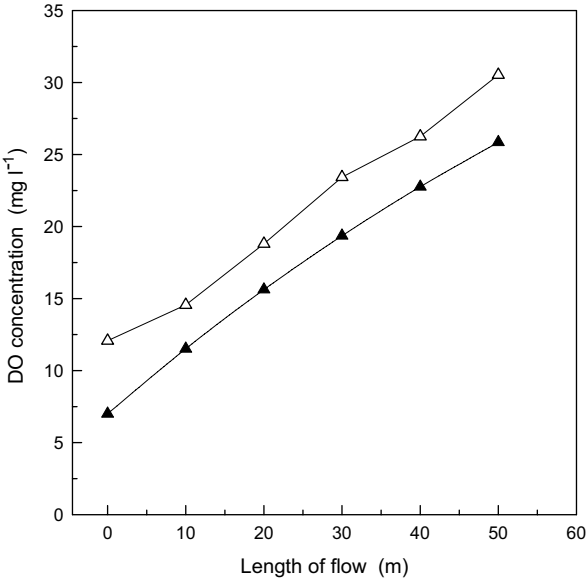

Fig. 8 DO concentration along the flow path of a *Chlorella* culture (layer thickness 8 mm). Sunny day, July, 14:00 h. Δ pure CO_2 was supplied; ▲ estimated from the model (Lívanský and Doucha 1999) for supply of flue gas after combustion of anaerobically generated CH_4

bioreactor. Generally, under a quasistationary state, the mass of oxygen produced by algae is equal to that which is removed from the culture as described above.

Knowledge of DO concentration in a photobioreactor can provide useful and rapid information about the state of the culture. DO is proportional to the rate of algal growth (Ben-Yaakov et al. 1985; Fournadzieva et al. 1993). A mathematical model concerning the hydraulics of culture flow, oxygen transfer to the atmosphere and the rate of oxygen evolution during the course of algal photosynthesis was described by Lívanský and Doucha (2003). The model was verified for a *Chlorella* culture, several millimeters thick, grown outdoors up to a density of 40 g dw l^{-1}. The model enables to estimate the net rate of oxygen evolution R (g O_2 per 1 m^2 of culture area) and the mass transfer coefficient K_L. This value for a 6–8 mm thick *Chlorella* culture was in the range of $K_L = 0.18–0.24$ m h^{-1}. The mean net rate of oxygen evolution in the culture was correlated with PAR irradiance I_0 (W m^{-2}) as: R (g O_2 m^{-2} h^{-1}) $= k\, I_0/(K_I + I_0)$, where $k = 12.82$ g O_2 m^{-2} h^{-1}, $K_I = 193.4$ W (PAR) m^{-2} (Fig. 9, Doucha and Lívanský 2006).

The efficiency of all techniques used to date to provide oxygen removal from microalgal cultures are still not at a satisfactory level. There are a few solutions open to the reactor designer for lowering oxygen concentration: increasing turbulence; O_2 stripping with air (e.g. in the airlift zone of a tubular bioreactor); increasing the velocity of the culture flow, accompanied however by higher energy consumption for pumping and higher shear stress that may damage the cells. Algal strains that can tolerate high oxygen concentrations have not yet been isolated.

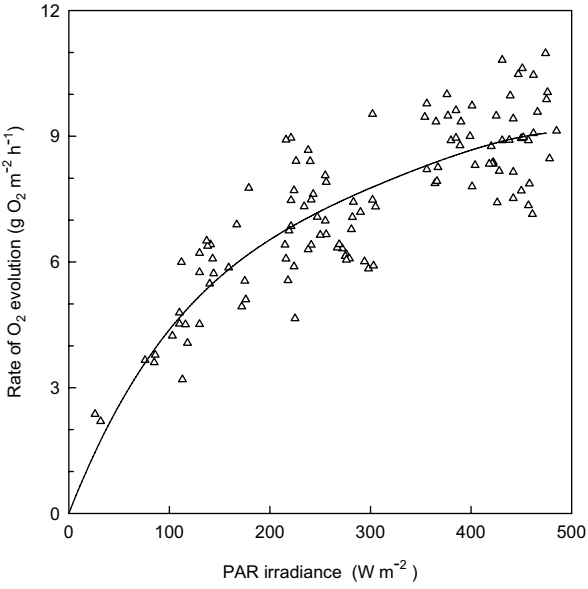

Fig. 9 Relationship between the net rate of oxygen evolution by *Chlorela* sp. per 1 m^2 of culture area and PAR irradiance

4.6 Nutrition

Carbon, nitrogen and phosphorus are the most important nutrients for algae production. Usually, macronutrients are supplied at concentrations in g l^{-1} and the micronutrients in mg l^{-1}.

The nitrogen content of algal biomass may be up to 10 % dw. Nitrogen is usually supplied as urea, nitrate (NO_3^-), ammonium salts (NH_4^+) or their combination. Ammonium nitrogen is often the preferred N source. Assimilation of nitrate N leads to an increased pH of the nutrient solution whereas assimilation of ammonium N causes a pH decrease. In an outdoor *Chlorella* culture with KNO_3 as a sole nitrogen source, an increased concentration of DO was observed, compared to when urea was used as a N source. We have found (unpublished results), for both laboratory and outdoor *Chlorella* cultures, that up to 50 % of urea nitrogen can be replaced by NH_4HCO_3 or $(NH_4)_2CO_3$ without any negative effect on algal productivity or pH of the medium. Ammonium nitrogen is utilized well by cells, with a minimum of NH_4-N remaining at the end of several days culture cycle. From a practical point of view, the best N source for growing *Chlorella* is urea - it is cheap, well soluble, well utilized by the cells and has little influence on culture pH.

The preferred form of phosphorus for algae is phosphate (PO_4^{3-}). Algal biomass usually contains less than 1 % P. Based on our results, to reduce the cost of nutrients, H_3PO_4 and K_2CO_3 can replace commonly used KH_2PO_4 for *Chlorella* growth.

The composition of nutrient solution is designed to reflect the mean content of basic chemical elements (P, N, K, Mg, S) in algal biomass. Nutrients are supplied daily at the beginning of cultivation (a variant of fed-batch culture technology) in

quantities based on a determination of urea-N and phosphate-P consumption. In this way, a balanced nutrient composition is maintained throughout the fed-batch growth cycle. To economize nutrients utilization, their addition is stopped two days before harvest.

5 Productivity and Photosynthetic Efficiency

Basic factors that influence the productivity of autotrophically grown algae are irradiance, temperature, biomass density, mixing and nutrients. These factors are in a close relationship with culture requirements and the type of culture system.

Net productivity is defined as gross daylight productivity minus night biomass loss caused by dark respiration of energetically rich cell reserve materials (starch, glycogen). This process is tightly temperature dependent. Net areal productivity is related to volumetric productivity P_V (g dw l^{-1} d^{-1}) as: P_{24} (g dw m^{-2} d^{-1}) = P_V V/A, where V is the volume (l) of the culture in the bioreactor, A is the culture area (m^2). Volumetric productivity can be expressed as a product of mean daily specific growth rate μ_{mean} (d^{-1}) and cell dry weight X (g l^{-1}). By evaluating μ_{mean} using several mathematical models found in the literature, and applying this to the areal rate of oxygen evolution (g O_2 m^{-2} h^{-1}) in a thin-layer *Chlorella* culture, we concluded (unpublished results) that μ_{mean} yielded a better fit to local light intensity (at a distance z from the irradiated culture surface) than to the mean light intensity in the layer. Thus, specific growth rate of the cells at a distance z can be expressed for light-limited growth of the cells as: $\mu = \mu_{max}\, I/(K_I + I)$. Here μ_{max} is the maximum specific growth rate, K_I is the light saturation constant, I is the intensity of PAR at distance z. The above formula is analogous to the Monod equation for the growth of microorganisms, with light considered as a specific substrate for growth of algae. From this equation and I, expressed by the Lambert-Beer law, for the mean specific growth rate in an algal culture layer of thickness h, we have (Simmer 1979):

$$\mu_{mean} = \frac{1}{h}\int_0^h \mu \quad dz = \frac{\mu_{max}}{\varepsilon_{mean} X h} \ln\left(\frac{I_0 + K_I}{I_h + K_I}\right) \tag{2}$$

Taking into account Eq. (1), this equation can be modified:

$$\mu_{mean} = \frac{\mu_{max} I_{mean}}{I_0 - I_h} \ln\left(\frac{I_0 + K_I}{I_h + K_I}\right) \tag{3}$$

It can be seen that mean specific growth rate is proportional to mean light intensity in a layer of algal culture. This was verified for a dense *Chlorella* culture in the pilot TL bioreactor (Doucha and Lívanský 2009). The following values were found: $\mu_{max} = 2.15$ d^{-1}, $K_I = 102.7$ W m^{-2}.

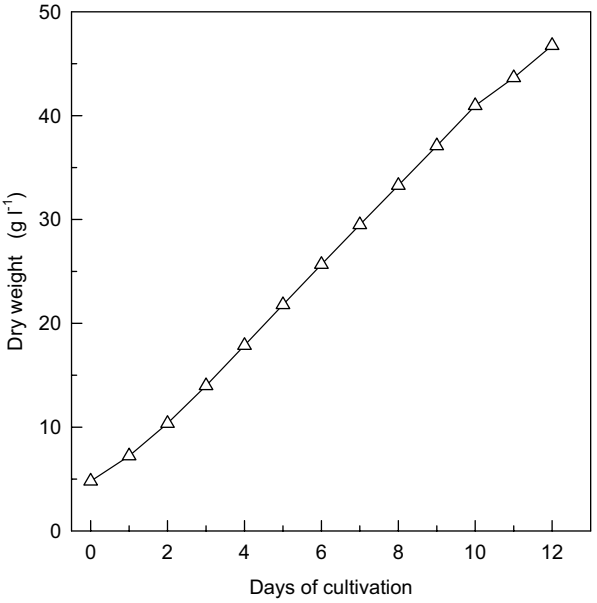

Fig. 10 Time course of *Chlorella* cell concentration in a TL photobioreactor. Daily solar PAR input is 3,400 Wh m^{-2}

Net photosynthetic efficiency, PE, expressed in % of solar PAR energy utilization, can be estimated from the net areal P_{24} productivity (g dw m^{-2} d^{-1}) and daily solar PAR energy input E_0 (Wh m^{-2} d^{-1}) incident on 1 m^2 of culture area, as: $PE = 6.4 \times 100 \times P_{24}$ divided by the energy E_0. The conversion factor 6.4 represents the energy (Wh) of chemical bonds in 1 g (dw) of algae (Kubín 1983; Morita et al. 2001). Generally, PE decreases with increasing irradiance in photosynthetic systems. This can also be derived from Eq. (2), for a dense algal culture $I_h << K_I$, $PE \approx (1/I_0) \cdot \ln [1 + (I_0/K_I]$. Thus, the PE decreases with increasing I_0.

Productivity of a dense *Chlorella* culture grown in thin layer is practically independent of algal dry weight. This is in sharp contrast with the frequently published data describing algal productivity in raceway ponds as being strongly density dependent. While an optimal areal density for maximal productivity was found to be 38–41 g (dw) m^{-2} (Grobbelaar et al. 1990) and 40–45 g (dw) m^{-2} for *Scenedesmus obliquus* (Hartig et al. 1988), we have not observed any decrease in productivity of *Chlorella* up to a cell concentration of 40–50 g (dw) l^{-1} (areal biomass densities 240–300 g (dw) m^{-2}) commonly processed for harvest (Fig. 10).

The mean seasonal net productivity of *Chlorella* cultures in TL bioreactors, depending on climatic conditions, ranges from about 22–25 g dw m^{-2} d^{-1} (Pulz and Scheibenbogen 1998; Borowitzka 1999; Doucha and Lívanský, unpublished resul ts), with a peak of about 38 g for short summer culture periods (Doucha and Lívanský 2009). This corresponds to a volumetric productivity of 4.3 g l^{-1} d^{-1} and a PE of 7.05 %. In contrast, long-term productivity in commercial raceways ranges mostly between 12 and 15 g dw m^{-2} d^{-1}(Tredici 2004) corresponding to a volumetric productivity of 0.05 g l^{-1} d^{-1}. Productivities of about 20–25 g m^{-2} d^{-1} are also reported

for short growth periods in tropical regions (Pulz and Scheibenbogen 1998; Lee 2001; Tredici 2004).

However, published algal productivities obtained using different culture systems are hard to compare, because they involve different climatic conditions, algal strains and operating systems.

6 Economic Considerations and Concluding Remarks

The main advantage of raceway ponds is their simple and, therefore, relatively cheap construction. From our unpublished calculations, the building and material costs should not be greater than 25–40 € per 1 m^2 of culture area. In contrast, based on detailed analyses provided by the stock company BCS Engineering, a.s., Brno, Czech Republic, who have designed and built up to now several thin-layer photobioreactors, building cost per 1 m^2 culture area for a 1,000 m^2 production module ranges from 200 to 230 €, i.e. 5–6 times higher than raceways. Nevertheless, the areal productivity in TL bioreactors is roughly double that of ponds (for production of an equal amount of biomass we need only half the operating staff) and the volumetric productivity, one of the deciding factors on the economics of algal production, is about 50–100 times higher. Extremely low culture volumes, higher algal yields and high harvest densities reduce the production costs to about one fifth (20%) compared with raceways. For the economics of the whole process, the deciding parameter is the cost of long-term production, not investment costs.

Raceway vs thin-layer technology - comparison of parameters that differ between systems.

Power input for circulating and mixing the culture (per 24 hrs and 100 m^2 culture area)

Raceway: paddle wheels, 600 W h^{-1}100 m^{-2} (Richmond and Becker 1986), per 24 h = 14.4 kWh. For production of 1 kg of algal dw, (mean productivity 15 g dw $m^{-2}d^{-1}$), 9.6 kWh is needed.

Thin-layer: circulation pump, 288 W h^{-1}100 m^{-2}, per 12 h of growth (light) period = 3.46 kWh; night circulation: the culture is kept in a tank, aeration requires 40% of the power input necessary for culture circulation during the course of algal growth,115 W × 12 h = 1.38 kWh; mixing and aeration: 3.46 + 1.38 = 4.84 kWh. For production of 1 kg of algal dw, (mean productivity 25 g $m^{-2}d^{-1}$), 1.94 kWh is needed.

For both culture systems, cooling of CO_2 stored in liquid form in a 10 m^3 tank requires about 1.3 kWh per 1 kg of biomass produced.

Supply of pure CO_2 **Raceway:** utilization of the pure CO_2 added to the algal suspension by means of tubes laid on the bottom of the ponds ranges from 13–20% (Richmond and Becker 1986; Becker 1994). Thus, for an average net productivity of 15 g dw m^{-2} d^{-1}, about 13.7–21.0 kg CO_2 per 100 m^2 culture area is required and for production of 1 kg dried biomass, 9–14 kg of CO_2 must be supplied.

Thin-layer: pure CO_2 is supplied via the pump (see Fig. 2). Its utilization by algae is about 70 % (Doucha and Lívanský 2006). Thus, for average net productivity 25 g dw.m^{-2}.d^{-1}, 6.5 kg CO_2 per 100 m^2 culture area is needed and for production of 1 kg of dry biomass, 2.6 kg CO_2 must be supplied.

Supply of a flue-gas CO_2 **Raceway:** in the case of flue gas containing about 13 % vol. CO_2, the amount required, is 7–8 times greater than pure CO_2. Thus, for an average net productivity of 15 g dw m^{-2} d^{-1}, 57–88 Nm^3 must be supplied and for production of 1 kg algal dry mass, the amount of flue gas required is 38–59 Nm^3 (1 Nm^3 is considered as flue gas volume at normal pressure and at temperature 20 °C. Thus, 1 Nm^3 CO_2 = 1.842 kg CO_2). To supply this gas at 0.5 bar to the algal culture, an additional 0.23–0.36 kW of power per 100 m^2 is required for a compressor, i.e. 2.76–4.32 kWh per 100 m^2 for a 12 h growth period. Therefore, the total energy consumption (mixing and flue gas supply) is 17.2–18.7 kWh per 100 m^2 d^{-1} and for the production of 1 kg algal dw, 11.5–12.5 kWh is required.

Thin-layer: flue-gas CO_2 is supplied to the EPDM tubes in the pipe loop between the circulation pump and the upper edge of the cultivation lane. At a net productivity 25 g dw m^{-2} d^{-1}, 10.9 Nm^3 must be supplied for the production of 1 kg dw algal biomass. To supply this gas at 0.5 bar to the algal culture, an additional 0.044 kW kg^{-1}dw of power is required for a compressor, i.e. 0.53 kWh kg^{-1} dw for a 12 h growth period. Therefore, the total energy consumption (mixing and flue gas supply) is 6.15 kWh per 100 m^2 d^{-1} and for the production of 1 kg algal dw, 2.46 kWh is needed.

Consumption of water According to Becker and Venkataraman (1980), water consumption for a 20 cm layer large-scale raceway pond, where *Scenedesmus* was grown at a net productivity of 15 g dw m^{-2} d^{-1}, was estimated to be about 0.8 m^3 per 1 kg algal dw (evaporation was not included). For a similar culture located in Peru, Castillo et al. (1980) calculated the liquid discharge at about 22,000 m^3 per hectare yearly (not considering evaporation). At a mean productivity of 15 g dw m^{-2} d^{-1}, the calculated specific water consumption is 0.4 m^3 of water per 1 kg dw of algal dw.

In the Třeboň laboratory, 2,000 l of *Chlorella* culture in each of three 224 m^2 TL photobioreactors are grown and successively harvested. At a net productivity of 25 g dw m^{-2} d^{-1}, after 14 days fed-batch growth, a harvest density of about 40 g dw l^{-1} and 80 kg dw of biomass is produced in each of the bioreactors. After harvest, 2,000 l of fresh water is supplied to prepare a nutrient solution for a next cultivation cycle. The corresponding specific water consumption, including water for evening washing of the culture area, (not considering evaporation, that is similar in both systems) is about 0.025 m^3 of water per 1 kg of algal dw. This is about one order of magnitude less than for raceways.

Downstream processing costs After harvesting *Chlorella* cultures, the common steps of downstream processing technology are as follows: (1) thickening of the biomass, mostly by means of plate-separators, to the density of about 150 g dw l^{-1}, (2) disintegration of algal cells by bead-mills (Doucha and Lívanský, 2008), (3) drying of the biomass using spray driers.

Table 1 Raceway vs thin-layer technology - parameters that differ between both systems

Items	Raceway	Thin-layer	Thin layer, % of raceway
Circulation and mixing (kWh per1kg dw)	9.6	1.9	19.8
Supply of CO_2			
Pure CO_2 (kg CO_2 kg dw)	9–14	2.6	18.6–28.9
Flue gas CO_2 (Nm^3 per 1 kg dw)	37.5–58.5	10.9	18.6–29.1
flue gas compression (kWh per 1 kg dw)	1.8–2.9	0.53	18.3–29.4
Water consumption (without evaporation) (m^3 per 1 kg dw)	0.4	0.025	6.3
Biomass thickening at the harvest (kWh per 1 kg dw)	2–4	0.05	1.3–2.5

Because the steps of disintegration and drying are similar in both raceway and TL technology, the calculations of downstream processing costs are only focused on the thickening the biomass after harvest. While the harvest density of algae in raceway ranges from 0.5 to 1 g dw l^{-1}, and for processing, must be increased 150–300 times, the density of algae at harvest in the TL system is about 40–50 g dw l^{-1} and, for processing, needs to be increased only 3–4 times.

For thickening of a 10 m^3 h^{-1} algal culture to a density of 150–200 g dw l^{-1} (nozzle separator, materials of GEA Westfalia Separator Group), about 2 kWh m^{-3} (including the energy input for the feed pump), i.e. 2–4 kWh per 1 kg of dry algal biomass, is needed. For TL technology, only 0.05 kWh per 1 kg of dry algal biomass is needed.

The data from these analyses are summarized in Table 1.

Labour Becker and Venkataraman (1980) provided model cost calculations for a 5 ha production plant (50 raceway ponds, each pond consisting of two 100 × 5 m joined units, yielding a 1,000 m^2 size pond). About 50 workers were needed for operation of the plant, i.e. 10 workers for 1 ha culture area.

TL production photobioreactors are equipped with automatic refilling of evaporated water and with automatic maintenance of the optimal CO_2 concentration in the growing culture. The formation of oxygen in a growing culture, as a result of algal photosynthesis, is continuously monitored by means of a Clark electrode.

Manpower requirements are focused on simple activities covering start and closing of the culture day, analysis and addition of nutrients, determination of algal productivity, control of the biological state of the culture and, work connected with processing of the harvested biomass (thickening, disintegration of the cells, drying and packing).

Based on the above data, we estimate that about 4–5 workers are needed for operation of a 1 ha plant.

Scale up Cultivation Raceway *Chlorella* or *Spirulina* production plants consist mainly of 1,000–5,000 m^2 single modules forming a production area of up to several hectares (e.g. Belay 1997).

On the contrary, for commercial production of fast growing microalgae, thin-layer culture modules of 1,000 m^2 area are proposed (Fig. 11).

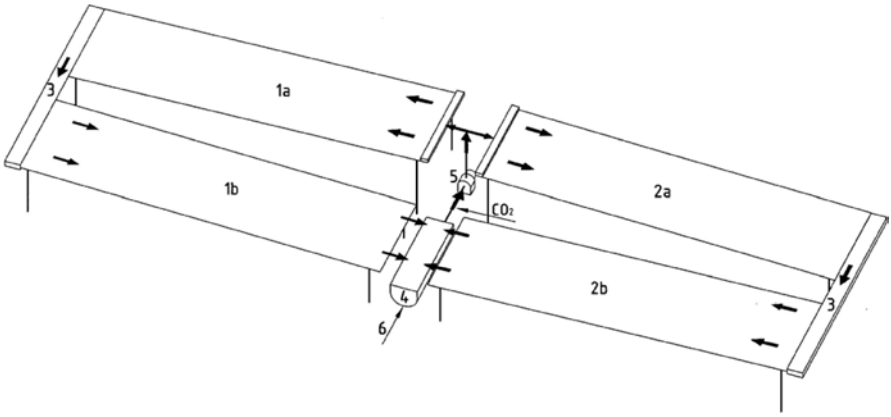

Fig. 11 Scheme of a 1,000 m^2 area production module: *1a–2b*, culture areas; *3*, connecting channels; *4*, retention tank; *5*, circulation pump; *6*, night aeration air. Flow of algae is depicted by *arrows*

The length of the inclined cultivation area is an important parameter for efficiency and economy of algal biomass production: the longer the inclined culture lane the larger the proportion of total algal volume that is exposed to sunlight. The length of the culture area is limited by DO concentration and by carbon dioxide dissolved in the algal suspension at the end of the inclined culture lane. Whilst the oxygen concentration should not exceed 30–35 mg l^{-1} (higher concentration can inhibit photosynthesis) the pCO_2 value at the end of the culture lane should be at least 0.1–0.2 kPa, and lower values limit algal growth. (Lívanský and Doucha 1998; Doucha and Lívanský, 2006).

At a volumetric productivity of 3–4 g algal dw per liter per day, a fed-batch culture cycle that starts in an algal density of about 5 g dw l^{-1}, lasts 12–14 days; 15 modules form a culture area of 1.5 ha.

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