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CO_2 and O_2 gas exchange in outdoor thin-layer high density microalgal cultures

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Abstract

Two thin layer culture units operated as batch cultures with the alga *Chlorella kessleri* were used in gas exchange experiments. The mass transfer coefficient K_g [g m^{-2} h^{-1} kPa^{-1}] of O_2 and CO_2 desorption from culture surface decreased with increasing culture temperature. Between 60–70% of supplied CO_2 was used for algal growth. It was estimated that the length of growth surface may be extended to about 50 m, without additional saturation by CO_2. On average 1.35 g CO_2 was consumed by the alga per 1 g of produced O_2. Net CO_2 consumption (R_{CO2}) and O_2 production (R_{O2}) were not inhibited by irradiance. R_{O2} did not decrease (in some cases it even increased) along the culture surface, despite increased accumulation of O_2. Measurement of pO_2 where the culture leaves the reactor before being pumped back onto the illuminated surface, correlated with O_2 production and CO_2 consumption and may be used to monitor the reactors growth performance.

Introduction

In outdoor algal ponds during peak hours of photosynthesis, oxygen supersaturation of more than 400% is quite common – Weissman et al. (1988). High O_2 concentration in culture results in inhibition of photosynthesis due to photorespiration and photooxidation (Richmond & Becker, 1986; Becker (1994). Suppression of growth in laboratory culture of *Chlorella pyrenoidosa* occured at an O_2 partial pressure of 30% and higher – Amman and Lynch (1966). Specific growth rate of *Chlorella vulgaris* in a turbidostatic culture deteriorated appreciably with the increase of pO_2 = 0–100 kPa. The inhibition due to oxygen was from 22–38%.

Large scale algal cultures are subjected to cycles of CO_2 and O_2 regardless of the design of the culture system. Careful process analysis and system design can limit the frequency and amplitude of these cycles. To this purpose gas exchange (CO_2 and O_2) experiments were done in thin-layer open outdoor bioreactors with the aims of elucidating the following:

– dependence of the mass transfer coefficient K_g, for desorption of CO_2 and O_2 from algal culture into atmosphere, on culture temperature

– what are the maximu values of pO_2 (partial pressure of dissolved oxygen) reached in the culture suspension in both types of growth surface used,

– what is the time course of pCO_2 in the culture,

– variation in the rates of photosynthesis along the reactor length,

– dependence of the rates of O_2 evolution and CO_2 consumption on irradiance, and - what is the maximum allowable reactor length before CO_2 becomes limiting.

Materials and methods

In our laboratory large scale thin – layer culture units have been designed and operated since 1960 (Šetlík et al., 1970). The growth area of 3% inclination is constructed from transparent material and can be used as a roof of a greenhouse. In this cultivation system the 40–50 mm layer of algal suspension flows at 8 cm s^{-1}.

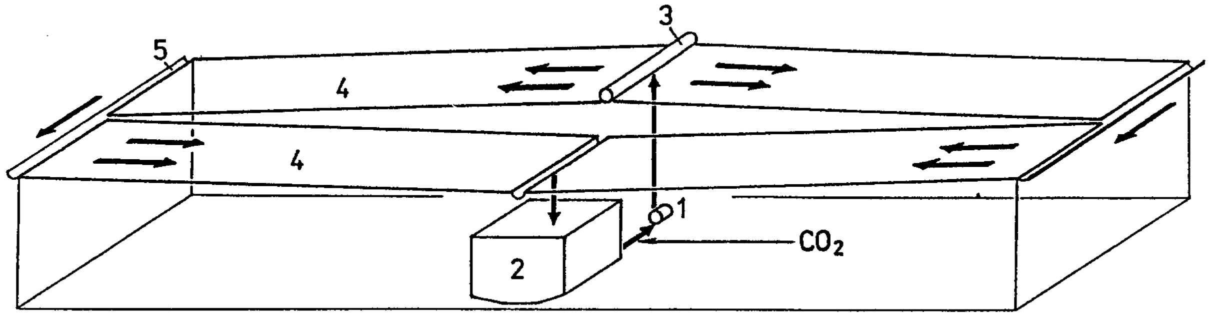

Figure 1. Schematic view of a thin – layer culture unit; 1, pump; 2, tank; 3, distribution tube; 4, growth surface; 5, connecting channel.

Transverse baffles at distances of 15 cm apart ensure thorough mixing of the cell suspension. At the lower end of the growth surface, the suspension is collected in a trough and returned by a pump to the upper part of the culture surface. The suspension is circulated during the day while at the night it is kept in the aerated tank. The concentration of algae in this system was 2–3 g (d wt) l^{-1}, several times higher than in commonly used raceway ponds.

Based on the experience with the above described baffled unit, a modified version of the thin-layer system was built (Doucha & Lívanský, 1995). The modifications consisted of reducing the surface inclination from 3 to 1.7%, in a meandering growth surface and in the reduced thickness of the suspension layer to 6–10 mm, depending on the roughness of the growth surface.

A schematic view of the modified culture unit is given in Figure 1. The growth surface consists of four meandering sections each 14 m long and 4 m wide. The total culture area is 224 m^2.

In the gas exchange studies, two growth surfaces were used, a thin-layer smooth surface (TLSS) consisting of glass sheets with a culture layer thickness of 6 mm, and a thin-layer surface with baffles (TLBS). The baffles were 1 m long, 13 mm in diameter PVC rods 1.5 m distant from each other. The rods were placed 3 mm above the growth surface perpendicular to the flow. High turbulence is a characteristic of the TLBS and the average culture thickness is 10 mm. Characteristics of the TLSS and TLBS units are given in Table 1.

Chlorella kessleri strain P12 (from collection in our laboratory) was used and operated in a batch mode. Nutrients were added daily based on uptake by the alga during cultivation (Doucha & Lívanský, 1995). Gaseous CO_2 was supplied from a storage tank into the suction part of the circulation pump (Figure 1), in order to ensure that some was present at the end of growth surface (after travelling 28 m the pCO_2 was about 0.2 kPa).

Table 1. Characteristics of the TLSS and TLBS culture units. (TLSS - thin-layer smooth surface; TLBS - thin-layer surface with baffles).

Parameter	TLSS	TLBS
Culture area (m^2)	224	224
Growth surface	smooth	baffles
Inclination of surface (%)	1.7	1.7
Thickness of culture (mm)	6	10
Mean velocity of suspension (cm s^{-1})	50	30
Volume of suspension in unit (l)	2200	3200

Table 2. Overall CO_2 and O_2 mass balance in TLSS and TLBS culture units.

CO_2	Supplied	100 %
	Loss by incomplete absorption in pump	20
	Loss by desorption from growth surface	10–20
	Utilization of supplied CO_2 by alga	60–70
	Theoretical requirement of CO_2 (g) per g of grown alga	1.83
	Actual supply of CO_2 (g) per g grown alga	2.6–3.0
	CO_2 (g) consumed by alga per 1 g of produced O_2	1.35
O_2	Loss by desorption from growth surface (% produced O_2)	40
	Loss by desorption in the tank (% produced O_2)	60

Irradiance was measured with an integration solarimeter (Kipp and Zonen, type CC1, Delft, Holland). Dissolved oxygen (DO) was measured with a portable dissolved oxygen meter using a Clark mem-

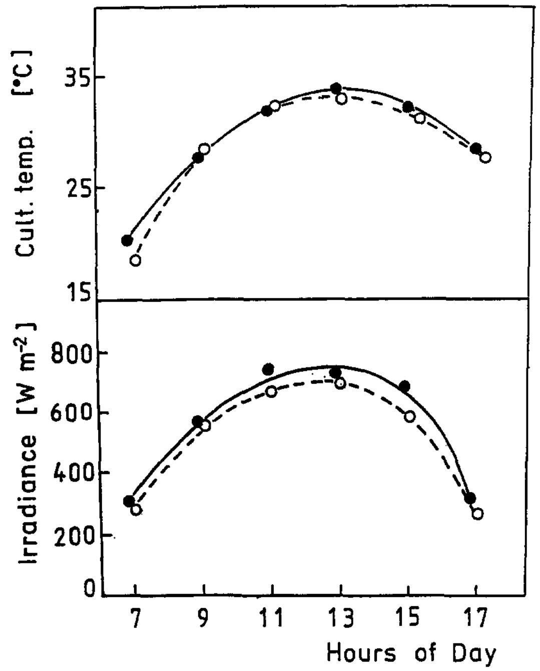

Figure 2. Course of irradiance and culture temperature. (○ – TLSS and ● – TLBS unit).

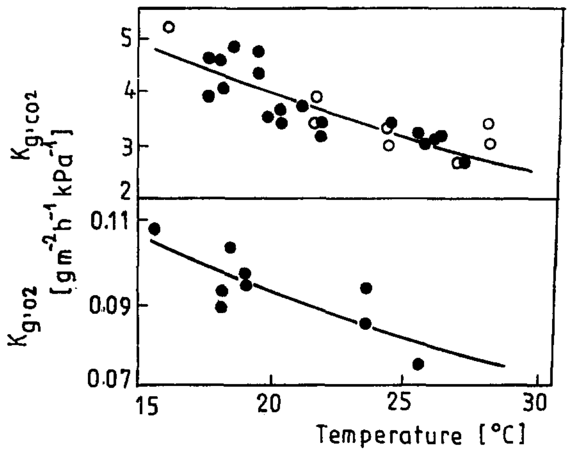

Figure 3. Temperature dependence of the mass transfer coefficient K_{g,CO_2} for the desorption of CO_2 and K_{g,O_2} for the desorption of O_2 from the nutrient solution into the atmosphere. (○ – TLSS unit and ● – TLBS unit).

brane covered electrode with automatic temperature compensation.

pCO_2 was measured with a pCO_2 membrane electrode. Mass transfer coefficients K_g for O_2 and CO_2 were determined from pO_2 and pCO_2 profiles in the nutrient solution on the growth surface saturated with either oxygen or carbon dioxide. The gas exchange experiments were done from 21–26 July 1995 in the TLBS unit and 31 July–4 August 1995 in the TLSS unit. pCO_2 and pO_2 were measured at 2-h intervals between 07.00 and 17.00 under cloudless days at Trebon.

Results

An example of irradiance and culture temperature in TLSS and TLBS units is shown in Figure 2, showing a maximum at 13.00. From Figure 3 it can be seen that the mass transfer coefficient K_g decreased with culture temperature. There is almost no difference between values of K_g for the two types of growth surface used.

Examples of the pCO_2 and pO_2 changes are given in Figures 4 and 5, respectively. Both gases were also desorbed from the culture in the channel (5 in Figure 1), causing thus a drop of pCO_2 and pO_2 at the length 14 m of reactor. Partial pressure of dissolved oxygen reached a maximum at noon of more than 60 kPa and did not increased further. Little difference was seen between the TLBS and TLSS units.

The net rate of O_2 production, R_{O_2} [g O_2 m^{-2} h^{-1}] in the culture was estimated from the differential O_2 mass balance given by the following equation:

$$R_{O_2} = Q_1 K_H \cdot dpO_2/dx + Kg(pO_2 - pO_2^*) \quad (1)$$

where Q_1 = flow rate of algae suspension per 1 m width of growth surface (m^3 m^{-1} h^{-1}); K_H = Henry's constant for oxygen (g O_2 m^{-3} kPa^{-1}) for the desorption of O_2 into atmosphere; pO_2 = partial pressure (kPa) of oxygen in algal culture; pO_2^* = partial pressure (kPa) of oxygen in the ambient atmosphere. In equation (1) the following values were used: $Q_1 = 10.5$ m^3 m^{-1} h^{-1} and $PO_2^* = 21$ kPa. The temperature dependence of Henry's constant KH was estimated using the correlation of Buhr & Miller (1983):

$$K_H(gO_2m^{-3}kPa^{-1}) = 173.26/(25131 + 709.2 \cdot t) \quad (2)$$

where t = temperature (°C).

Rates of R_{O2} (net oxygen production as measured in the TLSS and TLBS units are shown in Figures 6 and 7. Contrary to our expectation, the O_2 production did not decrease along the flow length of the culture as a result of growth inhibition by increased O_2 concentrations (Figure 5). A sharp decrease in O_2 production

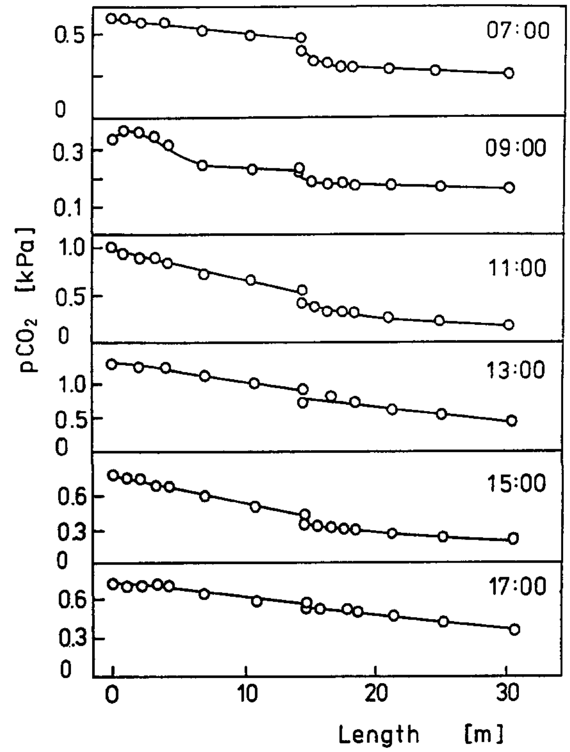

Figure 4. Hourly values of pCO_2 in the TLSS culture, as measured on 31 July 1995 along the horizontal length of growth surface.

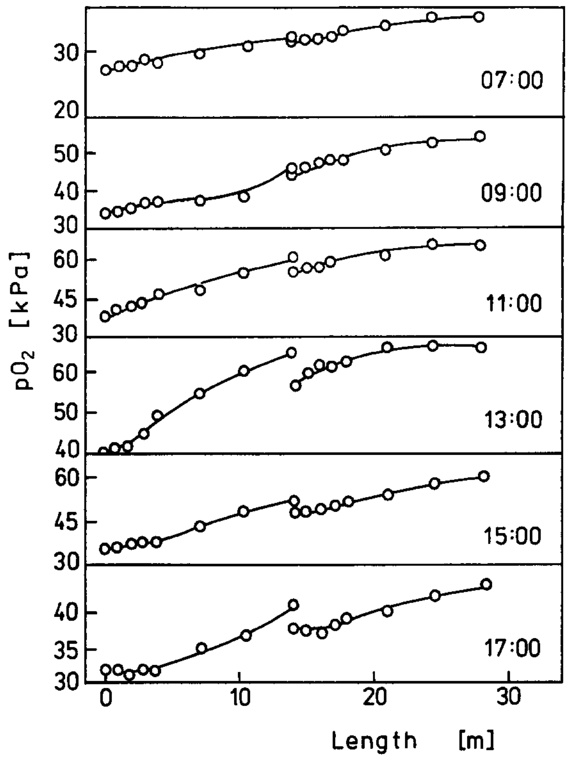

Figure 5. Hourly values of pO_2 in the TLSS culture, as measured on 31 July 1995 along the length of growth surface.

was seen after the pump (distribution tube 3 in Figure 1) and after the connecting channel (5 in Figure 1). The residence time of culture in the tank and in the distributing tube was about 30 s. In both of these the algae were essentially in the dark.

Of interest is to note the relatively slow increase in the rates of oxygen production (Figures 6 and 7), following a period in the dark. This may indicate a stress that is imposed on the culture.

Calculation of the net rate R_{CO2} of CO_2 consumption by the alga from measured pCO_2 values along the culture flow was difficult, because of evolution of CO_2 by dehydration chemical reactions from the carbonates. Using some assumptions we were able to estimate R_{CO2} rates. In most cases R_{CO2} increased with distance from the distribution tube, for both TLSS and TLBS cultivation units. This trend is in accordance with the course of R_{O2} measurements.

It is apparent from Figures 6 and 7 that the rate of oxygen production dropped when the culture passed through collecting channel and pump. For increased productivity it would be better to keep the alga exposed to light.

A further requirement is that sufficient carbon dioxide must be present to prevent limitation. From Figure 4 it is seen that pCO_2 decreased exponentially along the length of the algal culture flow. Our results indicated that sufficient CO_2 may be maintained in cultures of 50 m in length if sufficient CO_2 quantity was supplied, with a good economy of CO_2 use.

Net rates of CO_2 consumption and O_2 production were approximately linearly dependent on the irradiance of culture surface (Figure 8). No significant differences could be found between the TLSS and TLBS units. These results are supported by measurements of dry weight increases over 24 hours and gas exchange experiments.

The pO_2 of the cultures entering the storage tank correlated with the rates of CO_2 consumption and O_2 production (Figure 9). This may be used for an on line estimation of algae productivity (monitoring pO_2 data at the end of growth surface).

The overall CO_2 and O_2 mass balances in the culture units are given in Table 2. It was found that 1.35 g CO_2 was consumed by the algae per 1 g of O_2 produced. This is in accordance with Buhr & Miller (1983), who

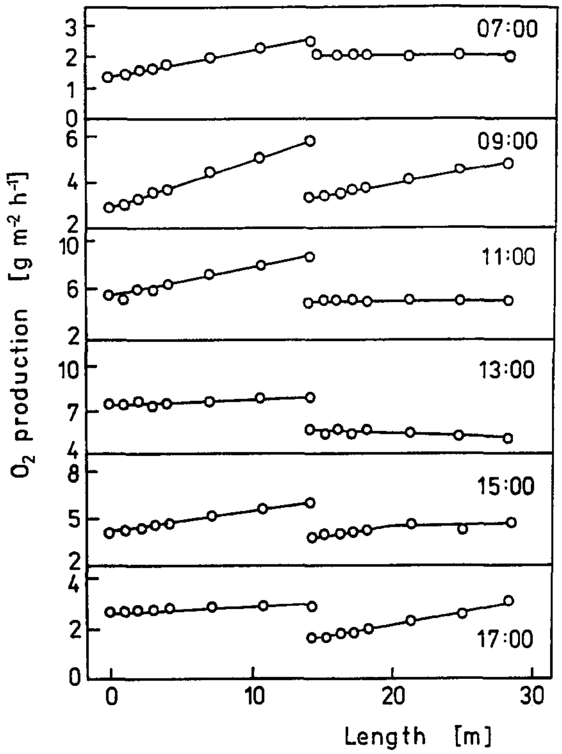

Figure 6. Net hourly rates of O_2 production in the TLSS culture as measured along the length of growth surface.

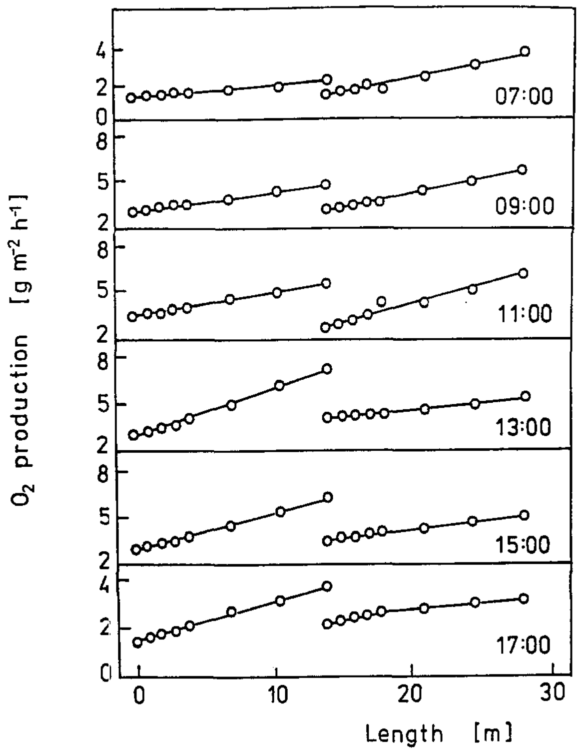

Figure 7. Net hourly rates of O_2 production in the TLBS culture as measured along the length of growth surface.

found 1.37 g CO_2 per 1 g O_2 (approximately 1 mol CO_2 per 1 mol O_2).

Discussion

In this work, values of mass transfer coefficient K_{g,O_2} were found to be more than order of magnitude higher than K_{g,CO_2}. This may be caused by much lower solubilities of O_2 than that of CO_2 in medium. In thin-layer cultures K_{g,O_2} K_{g,CO_2} were twice higher than these reported by Weissman et al. (1988) for outdoor ponds. Thus, mixing of thin-layer culture in the reactors was good.

Weissman et al. (1988) reported that at least pCO_2 about 0.2 kPa was sufficient for optimal productivity of *Chlorella* in outdoor ponds. Such is also our experience with outdoor and laboratory cultures of *Chlorella* and *Scenedesmus*. Thus, growth of alga in thin-layer reactor was not limited by CO_2 shortage (Figure 4).

pO_2 in thin-layer culture reached a maximum at noon about 70 kPa. This is lower figure than pO_2 = 80–120 kPa found in ponds (Weissman et al., 1988). Better mixing of thin-layer culture lowered pO_2.

Production of oxygen did not decrease (in some cases it even increased) along the flow length of culture despite the O_2 accumulation in the medium. In similar reactors Coglin et al. (1980) found an increase in the net rate of oxygen production along the flow distance of a culture of the microalga *Scenedesmus* sp. Their results are in essence similar to ours, but the decline of O_2 production after 15 m of flow of culture may have been due to CO_2 limitation. Algal cells in our reactor had been subjected to regular light-dark cycles (1–1.5 min light, 30 s dark). We may speculate that the cells had not enough time during one such cycle to be negatively influenced by varying O_2 concentrations.

Akiev & Tsoglin (1994) observed in synchronous laboratory culture of *Chlorella* sp. K, that O_2 sensitivity was different in the course of cell cycle. The young cells seemed more sensitive. From 3.5 to 6.5 h of the cycle the specific rate of O_2 evolution at pO_2 = 60–73 kPa was about the same or even a little higher than at pO_2 = 21 kPa. This is supported by our results (Figures 6 and 7). Thus, the generally accepted view on the

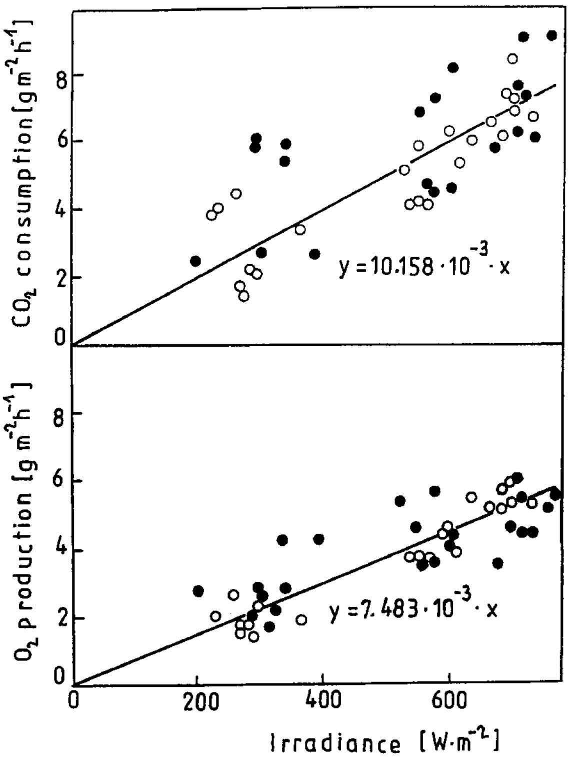

Figure 8. Relationship between net O_2 production and CO_2 consumption by alga and irradiance. (○ – TLSS unit and ● – TLBS unit).

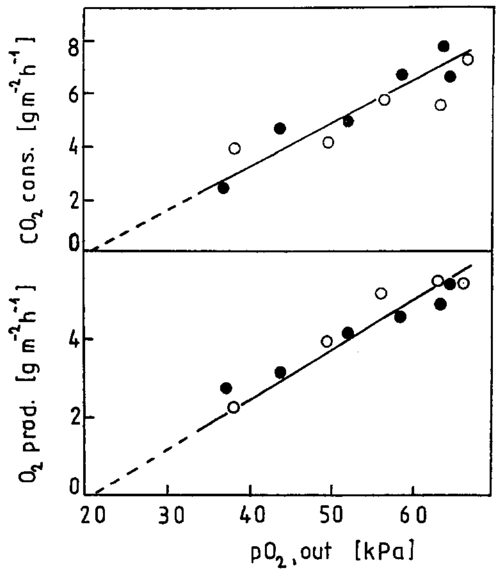

Figure 9. Relationship between net O_2 production and CO_2 consumption and pO_2 (measured before the storage tank (as the culture leaves the reactor; ○ – TLSS unit and ● – TLBS unit).

inhibitory effect of high O_2 concentrations on photosynthetic O_2 evolution holds true only for certain steps of the cell cycle or algal cultures exposed to a continuous constant concentration of O_2 for a long time.

Conclusion

1) Mass transfer coefficients of O_2 and CO_2 desorption from the cultures decreased with culture temperature.

2) No inhibition was seen in net CO_2 consumption and O_2 production in the two reactors even at an irradiance, $Io = 800$ W m^{-2}. No significant differences in CO_2 consumption and O_2 production were seen between the 6 and 10 mm thick cultures (TLSS and TLBS).

3) O_2 production was not inhibited by increased accumulation of oxygen in the culture media.

4) Dissolved oxygen concentrations reached a maximum ($pO_2 = 60$–70 kPa) at noon.

5) From measurements of pCO_2 it was concluded that sufficient CO_2 is available to extend the growth surface to 50 m.

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