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Impact of microsensor-caused changes in diffusive boundary layer thickness on O_2 profiles and photosynthetic rates in benthic communities of microorganisms

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ABSTRACT: Although O_2 microsensors used for study of benthic photosynthesis have tip diameters of less than 10 µm and stem diameters of about 150 µm at a distance of 0.5 cm from the tip, they do change the local flow pattern in the overlying water and thereby reduce the thickness of the diffusive boundary layer (DBL). The reduced DBL resulted in a lower diffusional resistance towards export of O_2 from communities with a net production of O_2, and insertion of a microsensor in such a community therefore resulted in lowered O_2 concentrations. The total diffusion flux out of the photosynthetic layer was not significantly affected by the presence of the microsensor. The higher upward flux into the water phase was counterbalanced by a reduced downward flux into the sediment. The O_2 profiles and diffusion fluxes were only affected when the surface was smooth, while the effect of microsensor insertion was undetectable when the surface was made irregular by small tufts, bubbles, etc. In the investigated cyanobacteria-dominated mats, the gross photosynthetic rates as measured by the light/dark shift technique were unaffected by the presence of a microsensor.

KEY WORDS: Microelectrodes · Oxygen · Diffusive boundary layer · Benthic photosynthesis

INTRODUCTION

During the last decade, O_2 microsensors have been used extensively in the study of microbenthic photosynthesis. By microsensor analysis it is possible to obtain detailed microprofiles of O_2 concentration and rate of gross photosynthesis (Revsbech & Jørgensen 1983), and, by calculation of diffusive O_2 efflux from the benthic community, rates of net photosynthesis can be obtained (Revsbech et al. 1981, Revsbech & Jørgensen 1986, Glud et al. 1992). Microsensor analysis has also been used to study the distribution and dynamics of the diffusive boundary layer (DBL) (Jørgensen & Revsbech 1985, Gundersen & Jørgensen 1990). The DBL is a thin layer of water that covers all submersed surfaces and through which diffusion is the main transport mechanism for dissolved material. The diffusive exchange through the DBL can be a limiting factor for transport of solutes between sediment and overlying water (Santschi et al. 1983, Archer et al. 1989). It was demonstrated by Jørgensen & Revsbech (1985) that a few particles protruding from the general sediment surface had no detectable effect on the DBL if the size of these particles was less than 50% of the diameter of the DBL. It was therefore anticipated that O_2 microsensors with tips of <10 µm and shafts of about 150 µm at a distance of 0.5 cm from the tip had no effect on the generally 200 to 1000 µm thick DBL (Gundersen & Jørgensen 1990, Jørgensen & Des Marais 1990). However, it has recently been shown by Glud et al. (1994) that microsensors coming from above do significantly affect the DBL structure. The reduction in DBL thickness below the microsensor was sufficient to cause an increase in the rate of O_2 uptake of 59% in very active sediments where all O_2 consumption

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occurred within the uppermost 100 to 200 µm of the sediment. The effect on O_2 uptake and overall O_2 profile in less extreme sediments with O_2 penetrations of more than 2 mm was below 10%. It was, however, also demonstrated that microsensor tips protruding into the DBL from below had no effect on the DBL structure (Glud et al. 1994).

Photosynthetically highly active microbial communities are characterized by very steep O_2 gradients near the substrate-water interface (e.g. Revsbech & Jørgensen 1986, Revsbech 1990), and the finding of very significantly altered O_2 profiles due to insertion of microsensors in non-photosynthetic communities did suggest that serious artifacts could be associated with microsensor-based determinations of photosynthetic rate. The determinations of net photosynthetic rates especially could be affected, as the net rates are calculated directly from the O_2 gradients. An effect on gross photosynthetic rates, which are calculated from rates of change in O_2 concentration during light/dark shifts, is less likely. This paper investigates possible effects of microelectrode insertion on O_2 profiles and net and gross photosynthetic rates in benthic communities of microalgae.

MATERIAL AND METHODS

Two different microbial mats were used in this study. One mat dominated by cyanobacteria was collected from the tidal flats just west of Aggersund, Limfjorden, Denmark. The second mat was grown in the lab on the surface of a nutrient-enriched marine sediment using material from the Aggersund mats as inoculum. The microsensor analyses were carried out in a rectangular flow chamber (9 cm wide, 24 cm long, and 5 cm tall) (Fig. 1). A sieve plate made of a 1 cm thick Plexiglas plate with 11 times 21 holes of 2 mm diameter was placed 3 cm from the inflow in the chamber and helped to establish a near-linear flow. Water was siphoned out at the outflow of the chamber. Care was taken to adjust the height of the outflow from the siphon so that the rate of water removal from the chamber corresponded to the rate of inflow Flow velocities were measured as the rate of outflow divided by the water-filled cross area of the chamber. The water depth was about 1.5 cm. The chamber had 5 mm holes in the bottom through which microelectrodes could be inserted from below. The holes and the bottom of the chamber were covered with a 1 cm thick layer of 1.5% agar. Rectangular slices (about 25 × 25 × 5 mm) of microbial mat were fastened in the surface of the agar by gentle insertion just before the agar hardened at 40°C. O_2 concentrations and rates of gross photosynthesis were determined by use of Clark-type O_2 microelectrodes with guard cathodes (Revsbech 1989) which had tip diameters <10 µm, shaft diameters of about 150 µm at a distance of 0.5 cm from the tip, and 90% response times of less than 0.3 s. The signals from the microelectrode circuits were collected in a computer equipped with an A/D converter. The computer also contained a motor control board by which the motor-equipped micromanipulator could position the microelectrode tips with <1 µm accuracy. Electrical circuits for microsensors and computerized data acquisition were described by Revsbech & Jørgensen (1986).

RESULTS

In order to determine if the microsensor had a detectable effect on the O_2 profiles in photosynthetic sediment, we conducted a simple experiment using 2 microsensors on a laboratory-grown mat dominated by cyanobacteria while a flow rate of 0.7 cm s^{-1} was applied. One microsensor was inserted from below through the agar-filled holes in the bottom of the flow chamber (lower sensor, LS); another sensor (upper sensor, US) was placed vertically aligned above the LS with the aid of a dissecting microscope (Fig. 1). We now continuously monitored the O_2 concentration at a certain depth in the sediment with the LS for 2 min. At the beginning of this continuous recording the US was situated 5.0 mm above the sediment surface. After 30 to 50 s we moved the US to a position 0.2 mm above the sediment surface, i.e. well inside the DBL. This resulted in a decrease in O_2 concentration as recorded by the LS (Fig. 2). Then we moved the LS into another depth in the sediment and started again to record the O_2 concentration continuously. This time the US was situated at a distance of 0.2 mm above the sediment surface at the beginning of the recording and, after 30 to 50 s, was then moved away from the sediment to a distance of 5.0 mm. This resulted in an increase in O_2 concentration as recorded by the LS. We repeated this

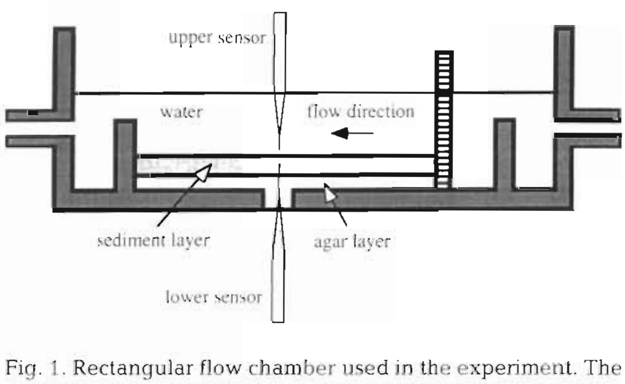

Fig. 1. Rectangular flow chamber used in the experiment. The agar-filled hole in the bottom of the chamber made it possible to measure with 2 microelectrodes along the same vertical axis

experiment with the LS placed in depths ranging from 1.5 mm below the surface to 0.15 mm above the surface. Typical changes in O_2 concentrations as recorded with the LS caused by either placing the US close to the surface (0.2 mm) or further away (5.0 mm) are shown in Fig. 2. The drop in O_2 concentration following the positioning of the US in the DBL was most pronounced in the DBL itself and in the uppermost part of the cyanobacterial mat.

These results show that an O_2 profile might be significantly altered by the insertion of an electrode from above. O_2 concentrations are lowered and the O_2 gradients used to calculate net photosynthetic rates could be altered — the upward gradient becoming larger and the downward gradient becoming smaller than in the undisturbed case, i.e. when the US was placed 5 mm above the sediment surface. When the surface of the mat was made irregular by, for example, small tufts or bubbles, no changes in O_2 concentration could by detected by the LS when the US was moved in or out of the DBL (data not shown).

The effect of microsensor insertion on the O_2 profile was further investigated in an experiment where O_2 concentration profiles were measured using alternately the US and the LS along the same vertical axis. The used flow rate was 2.3 cm s^{-1}. The US was placed 5 mm above the surface for 5 min before a profile with the LS was measured. Concentration profiles at each location were analyzed twice with both US and LS, and as 4 locations were investigated, this resulted in a total of 8 profiles for each sensor. These 8 profiles were averaged and standard deviations were calculated for each depth (Fig. 3). It is evident that there is a marked difference in the O_2 profiles obtained by the 2 electrodes; the use of the US resulted in lower O_2 concentrations throughout the sediment as compared to the LS profile, as well as in a reduction of the O_2 penetration depth.

Having analyzed the O_2 profile it is possible to calculate fluxes (J) by use of Fick's first law of diffusion (Crank 1983):

$$J = D_s \times \Phi \times \delta C / \delta x$$

where J is the net flux, D_s is the apparent sediment diffusion coefficient, Φ is the porosity and C is the O_2 concentration at depth x. We found the upward flux into the water phase to be larger and the downward flux into the sediment to be smaller when the US was used as compared to the LS (Table 1). A larger part of the photosynthetically produced O_2 diffuses upwards into the water when the thickness of the DBL is reduced by insertion of a microsensor coming from above as compared to the situation where the DBL is undisturbed, i.e. when the sensor is coming from below. No significant difference (standard deviation overlap) could be detected in the total O_2 efflux from the photic zone between the 2 treatments (Table 1). But upon insertion of the US, the balance between O_2 export into the water phase and O_2 consumption in the sediment is changed towards a larger export. The standard deviations of the net fluxes being rather large (11%) suggest that spatial heterogeneity might be more important in producing uncertainty about an estimate of net photosynthesis rate than the disturbance of the DBL caused by the insertion of a microsensor from above.

The influence of DBL disturbance by microsensors on gross photosynthetic rates as determined by the light/dark shift technique (Revsbech & Jørgensen 1986, Glud et al. 1992) was also investigated. The method is based on the fact that in the light there is an equilibrium between O_2 production and import on one side and O_2 consumption and export on the other side. During the first 4.6 s after darkening, O_2 consumption, export and import have been shown to be unaltered (Glud et al. 1992), while O_2 production is zero, and the O_2 concentration therefore initially decreases with the former photosynthetic rate. It is important, though, to stress that a

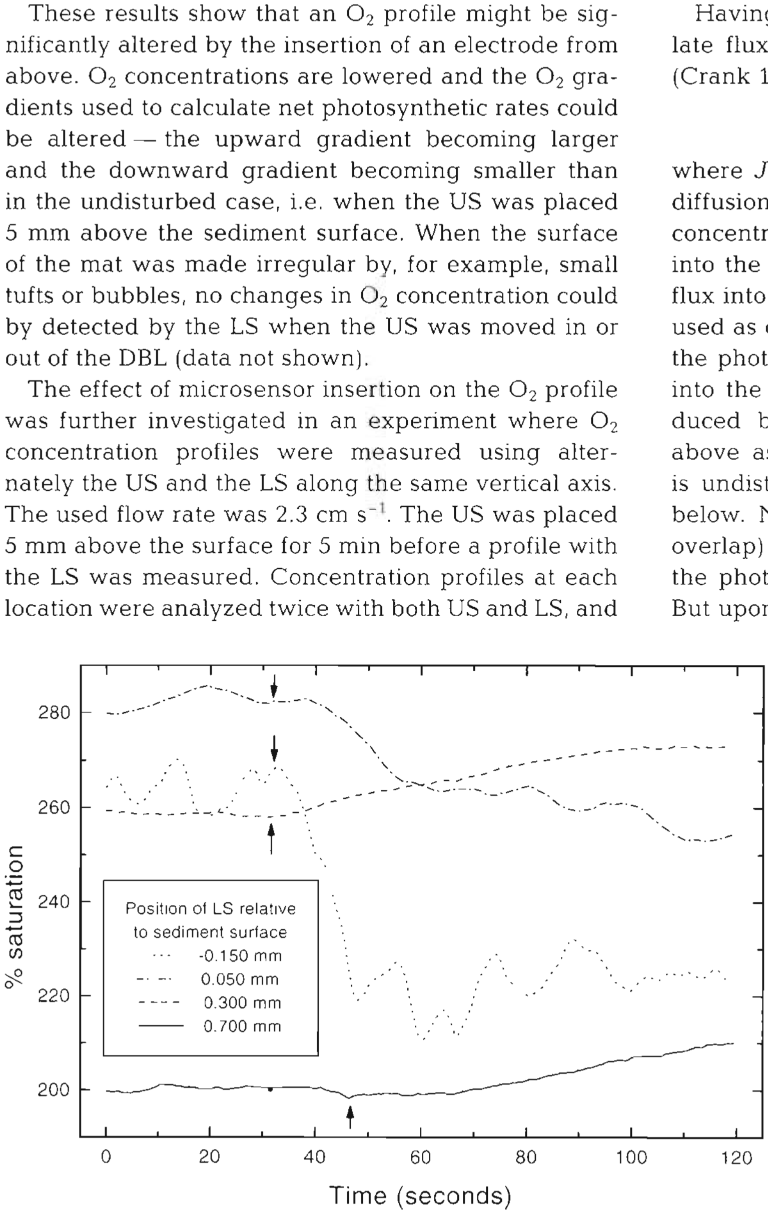

Fig. 2. O_2 concentration versus time measured with the lower microsensor (LS) at various depths in the mat while the upper sensor was moved between 5.000 mm and 0.200 mm above the sediment surface. Upward arrow indicates upper sensor leaving the diffusive boundary layer (DBL) and downward arrow indicates upper sensor entering the DBL. Applied flow rate was 0.7 cm s^{-1} Positive depths in the legend refer to positions of the tip of the LS below the sediment surface, while a negative depth refers to a position above the sediment surface

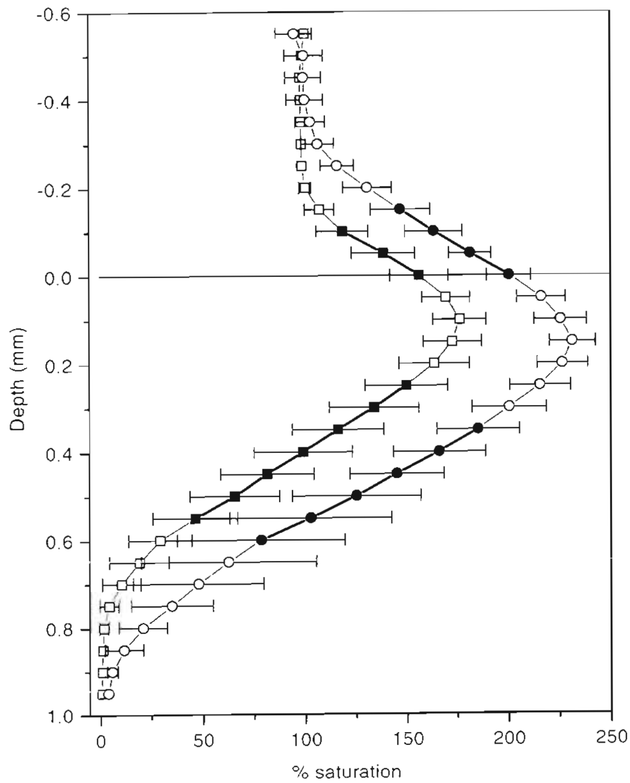

Fig. 3. O_2 profiles through a cyanobacterial mat (averages of 8 profiles ± SD). Profile obtained with the upper sensor is marked with □–□. Profile obtained with the lower sensor is marked with ○–○. The diffusion fluxes shown in Table 1 were calculated from the concentration gradients in the regions marked with solid symbols. Applied flow rate was 2.3 cm s^{-1}

steady state O_2 profile must be achieved before any reliable gross photosynthesis measurements can be obtained. The actual O_2 concentration at the measuring point is of less importance to the measurement of gross photosynthesis, but a change in the O_2 concentration could indirectly affect the photosynthetic and photorespiratory rates through a change in the ratio between CO_2 and O_2.

Table 1. Upward and downward fluxes and net photosynthetic rates calculated from the profiles shown in Fig. 3. $D_s\Phi$-values of 2.01×10^{-5} cm^{-2} s^{-1} for water (Broecker & Peng 1974) and 1.41×10^{-5} cm^{-2} s^{-1} for sediment (Glud et al. in press) were used. D_s: apparent sediment diffusion coefficient; Φ: porosity

	Upper sensor	Lower sensor
	(nmol O_2 cm^{-2} s^{-1})	
Flux up	0.191 ± 0.025	0.180 ± 0.020
Flux down	0.122 ± 0.010	0.151 ± 0.018
Net photosynthetic rate	0.313 ± 0.035	0.331 ± 0.038

We investigated a possible effect of an altered DBL on measured gross photosynthetic rates by inserting a microsensor into the O_2 maximum from below. The thickness of the DBL was switched between 2 thicknesses by alternately applying 2 different flow velocities (0.6 and 1.2 cm s^{-1}) 3 times each. A total of 19 and 21 gross photosynthetic rates and O_2 concentrations were measured with the low and high flow velocity, respectively. The duration of the dark incubations was 1.6 s.

The O_2 concentrations in the mat followed the expected pattern, being higher when the lower flow rate was used and the DBL was therefore thicker and being lower when the higher flow rate was used and the DBL was thinner (Fig. 4). Gross photosynthesis rates varied within treatments and among treatments and no pattern relating to applied flow rate could be detected (Fig. 4). Variation caused by other factors than the change in flow rates accounted for more variation in gross photosynthetic rates than did the change in the thickness of the DBL. The DBL thickness thus had a substantial influence on O_2 concentrations but not on gross photosynthesis rates.

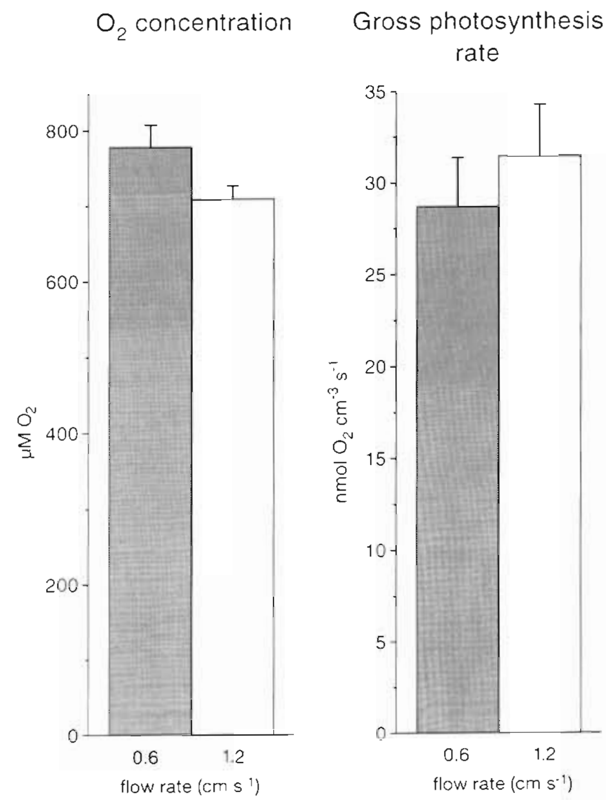

Fig. 4. O_2 concentration + SD and gross photosynthesis rates + SD in the most active layer of a cyanobacterial mat while applying 2 different flow rates and thicknesses of the DBL (n = 19 and 21 for flow rates 0.6 and 1.2 cm s^{-1}, respectively)

DISCUSSION

In general, the deformation of the DBL by the microelectrode was found only to have a significant effect on O_2 concentration in biofilms when the surface was smooth. No effect of microelectrode insertion could be detected when the surface was rough or made rough by photosynthetically produced gas bubbles (data not shown).

The presented data show that although a change in the O_2 profile may occur upon insertion of a microsensor from above, the rate of net photosynthesis calculated from the measured O_2 gradients is not significantly altered, since an increased upward flux of O_2 into the water phase is counterbalanced by a decreased downward flux into the sediment. In our cyanobacterial community, the gross photosynthetic rates are not influenced by DBL thickness. Natural variation in space and time might be a more important source of error for the estimation of net and especially gross photosynthesis rates. It is, however, possible to argue the validity of the 1-dimensional flux model used for the calculation of O_2 fluxes (Fick's first law of diffusion). The compression of the DBL around the tip of the upper sensor could result in horizontal O_2 gradients. This will cause lateral diffusion of O_2 in the DBL thus invalidating 1-dimensional calculation of fluxes. However, if lateral diffusion is of importance, the O_2 gradients in the DBL should be non-linear. Neither Glud et al. (1992) nor we detected such non-linear gradients upon insertion of a sensor from above, indicating that horizontal diffusion probably is negligible in these situations.

Photosynthetic rates of diatom communities have been shown to respond positively to an elevation of the dissolved inorganic carbon (DIC) concentration, and there seems to be a significant photorespiration at low DIC concentrations and high O_2 concentrations (Jensen & Revsbech 1989, Glud et al. 1992). The disturbance of the DBL caused by microsensor insertion from above will reduce the O_2 concentration and increase the supply of DIC. Microsensor insertion should thus lower photorespiration and increase net photosynthesis. The total effect will, however, depend on the species composition of the photosynthetic community. Instead of trying to correct for the possible error introduced by using a microsensor coming from above, it might be more helpful to think of the measurements as being obtained with a somewhat higher flow rate than applied to the general system, as both an increased flow rate and the introduction of a microsensor from above reduce the thickness of the DBL.

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