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OXYGEN MICROPROFILES OF TRICKLING FILTER BIOFILMS

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Abstract—Oxygen microprofiles of photosynthetic and non-photosynthetic biofilms of trickling filters from a sewage treatment plant were studied with Clark type oxygen microelectrodes. The oxygen profiles in photosynthetic biofilms exhibited pronounced changes with changing light conditions, and the profiles of both types of biofilm were affected by addition of nutrients. The existence of a 100–500 μm thick diffusive boundary layer in the water just above the biofilm was evident from all recordings. The O_2 gradient in this boundary layer was used to calculate diffusive fluxes of oxygen exchanged between the biofilms and the overlying water phase. The calculated fluxes were compared to the overall oxygen consumption rates measured experimentally and to the photosynthetic activity as measured with the microelectrodes. Approximately 60–70% of the oxygen produced in the algal films during photosynthesis was consumed within the films. At least one third of this consumption may be due to photorespiration. Dark/light and light/dark shifts demonstrated a very dynamic nature of the O_2 status of the algal films, showing an increase from anaerobic conditions to 500% air saturation and vice versa within 25 min. In the same regime, pH profiles showed a similar dynamic change, whereby pH varied between 8.1 and 9.7 in the same period.

The data obtained with the non-photosynthetic biofilms show that the oxygen respiration and oxygen penetration are mostly limited by diffusive oxygen transport through the boundary layer. The overall oxygen consumption of the heterotrophic biofilms equalled within $\pm 10\%$ the estimates made from flux calculations.

Key words—oxygen microprofiles, photosynthesis, trickling filters, diffusion, biofilms, algal films, oxygen microelectrode, respiration, diffusive boundary layer, pH-microprofiles

INTRODUCTION

The recent technical improvement of oxygen microelectrodes has allowed a wide application of this tool to a variety of fields of research (Revsbech and Ward, 1983, 1984; Revsbech and Jørgensen, 1986). Among these, the measurement of microgradients in biofilms such as algal films (Bungay and Chen, 1981; Revsbech *et al.*, 1983; Jørgensen *et al.*, 1983) and non-photosynthetic microbial films (Bungay *et al.*, 1969; Huang and Bungay, 1973; Chen and Bungay, 1981) have been particularly rewarding. The power of resolution of our methods has now reached a dimension relevant to the size of the microorganisms which are involved in the creation of the gradients measured. The microprofiles of oxygen can be compared with theoretical profiles generated with mathematical models from data of overall rate measurements. In this way our understanding of biological processes on the microscale can be improved while at the same time our insight into the contribution of physical processes, such as diffusion and convection, to the establishment of these microgradients can be improved.

Bungay and coworkers first applied microelectrode techniques to study O_2 distribution in natural and artificial microbial films. (Bungay *et al.*, 1969; Huang and Bungay, 1973). Estimates of oxygen diffusivity and of respiratory activity of the biofilms were based on a combination of mathematical modelling and experimental data. The possibility to measure directly the diffusive boundary layer was, however, not investigated and the data generated were not checked against independent measurements of overall activities of O_2 uptake and/or production. The microelectrode measurements were also applied to study the effect of nutrient supply, pH and temperature on the overall activity of photosynthetic and non-photosynthetic biofilms of trickling filters. These initial studies clearly showed the potential of microelectrode studies to analyse in detail the dynamics of oxygen exchange in these systems (Chen and Bungay, 1981; Bungay and Chen, 1981). As pointed out by Harremoes (1977, 1978) an important field of application of such analysis will be wastewater treatment, where a better understanding of limiting factors in trickling filter systems and rotating drum systems may lead to improvements in this field of application.

The usefulness of the improved microelectrodes for the quantative measurement of gradients of oxygen,

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fluxes of oxygen and rates of photosynthesis, was recently demonstrated (Jørgensen *et al.*, 1983; Revsbech *et al.*, 1983). It is particularly important that this recent experimental work has revealed the presence of diffusive boundary layers, also referred to as the diffusive sublayers (Boudreau and Guinasso, 1982). Hence fluxes of oxygen out of, or into the biofilms, can be calculated from observed oxygen gradients by use of Fick's first law of diffusion, thereby obviating the need for indirect estimation of this flux. (Williamson and McCarty, 1976; Atkinson *et al.*, 1968; Boudreau and Guinasso, 1982).

In this study oxygen microelectrodes were used to measure gradients in photosynthetic and non-photosynthetic biofilms of trickling filters. We have thereby focussed on the effect of light and dark shifts on the dynamics of oxygen production and consumption in these films, and also on the dynamics of response of the films to organic and inorganic nutrients.

METHODS

Photosynthetic and non-photosynthetic biofilms growing on rocks were sampled from a rotating arm trickling filter of the municipal wastewater treatment plant in Viby, Denmark. This filter receives raw sewage directly from the settling tanks. The COD of the raw sewage was 100–200 mg O_2 l^{-1}. Only the rocks exposed to sunlight had algal biofilms. The sides and the bottom of these rocks as well as all rocks of lower location in the filter possessed non-photosynthetic slimy layers of microorganisms with abundant populations of sludgeworms, *Tubifex* sp., and larvae of sewage flies, *Psycoda* sp. The algal layers consisted of filamentous green algae and cyanobacteria. Immediately after sampling, the sides and bottom of the rocks were brushed clean from bacterial slimes in order to remove the very motile population of *Tubifex*. Very few *Tubifex* were present in the algal films as long as the algal films were in the bright daylight. The very moment, however, that the rocks were placed in the shade or dark, the worms moved from the non-photosynthetic slimes into the algal films. Upon arrival in the laboratory the remaining *Tubifex* were removed from the biofilm with forceps. The majority of the fly larvae were removed from the bacterial slimes in a similar way. The algal biofilms were kept for no longer than one week in the laboratory and stored under a constant stream of tapwater. Most measurements were performed within 5 days. The (heterotrophic) activity of these algal films was very little affected by the storage in the lab (in daylight). The heterotrophic films were used within 2 days to avoid a drop in activity. During experiments the rock was immersed 1 cm deep into 20°C tapwater that was circulated over the biofilm at a controlled rate (usually 4 cm s^{-1}). Illumination for studies of photosynthesis was provided by a 150 W halogen lamp from a slide projector. The light intensity was 600 E $m^{-2}s^{-1}$.

A Clark type oxygen microelectrode constructed according to Revsbech and Ward (1983) was used to measure the oxygen microprofiles in the various biofilms. One advantage of this electrode is that it can be used across the air–water interface since it does not need external liquid contact for its electrical circuit. The current was measured with a picoamperemeter connected to a recorder. Calibration of the electrode was done in air saturated tap water of 20°C before each measurement. The zero current was recorded in anaerobic layers of the biofilms. The electrodes were very stable and kept their calibration for at least 15–30 min. Drift was often < 1% per hour. The response time of the electrode and the attached equipment was 0.2 s for 90% scale deflection. The electrode was attached to a micromanipulator. The position of the electrode tip could be followed through a dissecting microscope. The precision of its positioning was better than 10 μm. Details of the technique for analysis of the surface topography of microbial films have been published elsewhere (Jørgensen *et al.*, 1983).

Rates of photosynthesis were measured by determing the rate of decrease in oxygen concentration within the biofilm just after a light–dark shift. (Revsbech and Jørgensen, 1983). As discussed in detail by Revsbech *et al.* (1981) and Revsbech and Jørgensen (1983) the steady state oxygen concentration in the film is the result of three important processes, namely oxygen production, oxygen consumption and oxygen diffusion. During the first few seconds in the dark the latter two parameters do not change significantly. Hence the initial change after a L–D shift represents the rate of oxygen production due to photosynthesis: The change in oxygen concentration (c) with time (t) can be written as

$$\frac{dc}{dt} = D \cdot \frac{\partial^2 c}{\partial x^2} + f(I) - g(c) \quad (1)$$

in which D is the diffusion coefficient, x the depth in the biofilm, $f(I)$ photosynthesis as a function of light intensity (I) and $g(c)$ the respiration as a function of the oxygen concentration.

In steady state in the light, at zero time the $dc/dt = 0$ and hence

$$D\frac{\partial^2 c}{\partial x^2} = g(c) - f(I). \quad (2)$$

Immediately after darkening [$f(I) \rightarrow 0$] equation (1) becomes

$$\frac{dc}{dc} = D\frac{\partial^2 c}{\partial x^2} - g(c). \quad (3)$$

Combinations of (2) and (3) gives

$$\frac{dc}{dt} = -f(I), \text{ immediately after darkening.}$$

As the oxygen gradients change rapidly in the dark, the rate of decrease should be read within the first 1–2 s. The spatial resolution of the measurement will then be approx. 0.1 mm (Revsbech and Jørgensen, 1983).

Measurements of the oxygen uptake by the biofilms were made as follows. A perspex cylinder of 5 cm dia was firmly pressed down over a selected part of the biofilm so that a closed chamber was formed. The cylinder was filled with sewage water and closed free from air bubbles with a tightly fitting stopper. The stopper was equipped with a normal Clark-type polarographic oxygen electrode and a stirrer to allow mixing of the liquid in the chamber. After recording the oxygen uptake activity of the biofilm + sewage, a separate measurement was made to correct for the uptake of the sewage alone. Following this experiment, while keeping the rate of stirring constant, the large oxygen electrode was removed and an oxygen microelectrode was lowered through the hole in the stopper into the biofilm to record oxygen microprofiles.

pH-measurements were carried out with a pH-microelectrode manufactured in our laboratory according to Revsbech and Jørgensen (1986). It had a sensitive tip of 40 × 100 microns and a 90% response time of < 5 s. A calibration curve was made using standard buffers. The response of the electrode to changes in pH was linear. The electrode was mounted in a micromanipulator as described above for the oxygen electrode.

Calculations

In order to calculate the time required for a diffusible substrate to penetrate into a biofilm the penetration theory

(Crank, 1975; Beek and Muttzal, 1975) was used:

$$c(x, t)/c_0 = 1 - \mathrm{erf}\left\{\frac{x}{2\sqrt{Dt}}\right\}$$

where

$c(x, t)$ = the concentration of the diffusible substrate at distance x and time t
c_0 = the concentration of the substrate at the interface for $t > 0$
x = the distance from the interface into the mat
t = time
erf = the error function
D = the diffusion coefficient.

When a certain nutrient concentration is established above the biofilm with no substrate consumption, and with a diffusive boundary layer of 0.1 mm, the time required to establish 10% of the initial concentration would be < 10 s at 0.05 mm depth in the film, and take in the order of 20 min at 2 mm. Therefore, whenever a steady state was observed to have established on the basis of subsequent measurements, the time required to reach this steady state was checked against the time for 10% penetration as indicated by the penetration theory.

RESULTS

Rocks with photosynthetic biofilms (algal films) from a trickling filter were immersed in a waterbath and tapwater was circulated over the films. Oxygen profiles were measured at 50–100 μm depth intervals with an O_2-microelectrode both in the light (600 μE m^{-2} s^{-1}) and in the dark. Figure 1 shows a typical example of steady state oxygen profiles in light and dark. The linear change of the O_2 concentration just above the film revealed the diffusive boundary layer, which under these conditions of flow was approx. 300 μm thick. The curves obtained were very smooth due to the rapid diffusion of oxygen at these dimensions.

In order to obtain information on the dynamics of photosynthesis, of oxygen exchange, and of oxygen consumption of these films, the oxygen profiles were measured during a light–dark (L–D) shift and dark–light (D–L) shift. The recording of an oxygen microprofile took about 20 s, and consequently the oxygen concentration at each particular depth was measured about 3 times per min. An example of the oxygen concentration at constant depth is plotted as a function of time in Fig. 2. From the time course of each depth Fig. 3a, b were constructed. These results show that the oxygen concentration in the photosynthetic biofilms may change from zero to 5 times air saturation in < 10 min.

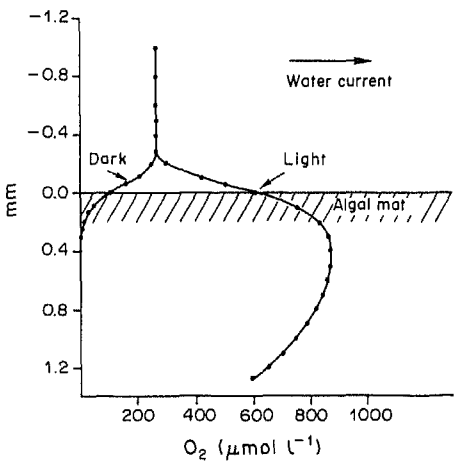

Fig. 1. Oxygen microprofile in algal film growing on trickling filter rock with overlying water in the light and in the dark. Zero depth is at the algal film/water interface.

The initial rate of oxygen disappearance in the dark (Fig. 2) allowed an accurate estimate of the rate of photosynthesis at different depths (see "Materials and Methods"; Revsbech *et al.*, 1981; Revsbech and Jørgensen, 1983). Figure 4 shows the rates of photosynthesis obtained with this method. The decrease of the rate of photosynthesis in the deeper regions of the layer was due to light limitation, since an increase of the light intensity resulted in an increase of the rate of photosynthesis in these layers (results not shown). In the top 0–0.2 mm the algae were light saturated at 600 μE m^{-2} s^{-1}.

As the process of photosynthetic oxygen evolution is stoichiometrically related to the fixation of carbon dioxide the observed production of oxygen should also be reflected in a change in the bicarbonate concentration, which could be followed indirectly by pH-measurements as shown in Fig. 5a, b. When comparing these results with those of Fig. 3 it should be realized that the sensitive tip of our pH-microelectrode is 100 micron long, and that the spatial resolution of this measurement was therefore one order of magnitude less than the one obtained with the oxygen electrode. Nevertheless there is a very good correlation between the data shown in Fig. 3 and Fig. 5. A steady state in the bicarbonate concentration was clearly not reached at the lower depths even after 25 min in the light. This was probably due to a high buffering capacity created by the relatively high bicarbonate concentration in the algal films.

When observed with the naked eye the algal film seemed very smooth, but when inspected under the dissection microscope the surface appeared to undulate and to be heterogenous. Figure 6 shows a schematic cross section of the surface of the film together with 4 isopleths of oxygen concentrations. The heterogeneity in oxygen conditions within the film is evident. From Fig. 6 it can also be inferred that the diffusive boundary layer follows the topography of the film surface. An impression of the relative heterogeneity of the film can also be obtained from Fig. 7 which gives oxygen profiles and rates of photosynthesis at points B, C, D and E positioned at the 4 corners of a 1 cm square around point A, which was the location of the measurements in Fig. 4. The results show that, while profiles and also the diffusive boundary layers are completely analogous, the absolute values for rate of photosynthesis may differ by a factor of 2 over such short distance.

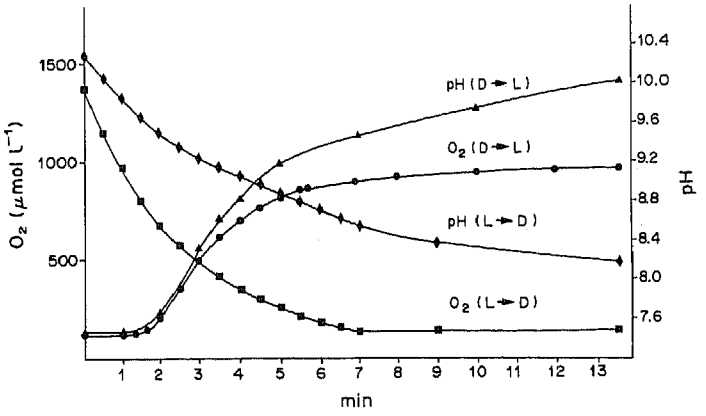

Fig. 2. Oxygen concentration and pH changes as a function of time during a light–dark shift (L–D) and a dark–light shift (D–L) at 0.5 mm depth in the algal film shown in Fig. 1. At zero time the light was switched on or off. The time lag after switching on the light is unexplained.

The thickness of the diffuse boundary layer is dependent on the rate of water flow over the mat. This is demonstrated in Fig. 8 which shows light and dark profiles of oxygen at different flow rates. The boundary layer was reduced to approx. 100 μm at a flow rate of 10 cm s^{-1} while in stagnant water it extended more than 1 mm above the film.

In a subsequent series of experiments in a very similar algal film the effect of nutrients on the oxygen respiration activity of the algal film was tested by rapidly mixing broth and/or nitrate with the bulk liquid flowing over the biofilm both in the light and in the dark. The results are summarized in Table 1, which gives the (slopes of) O_2 gradients above this algal film in the diffusive boundary layer under the different experimental conditions. In the light the addition of 1:1 nutrient broth plus yeast extract (final concn 500 ppm in the overlying water) resulted in a gradual, slow, decrease in oxygen concentration at a depth of 0.5 mm in the film of about 500 μmol oxygen l^{-1} h^{-1}, demonstrating that the films possessed some heterotrophic potential. Nitrate had no

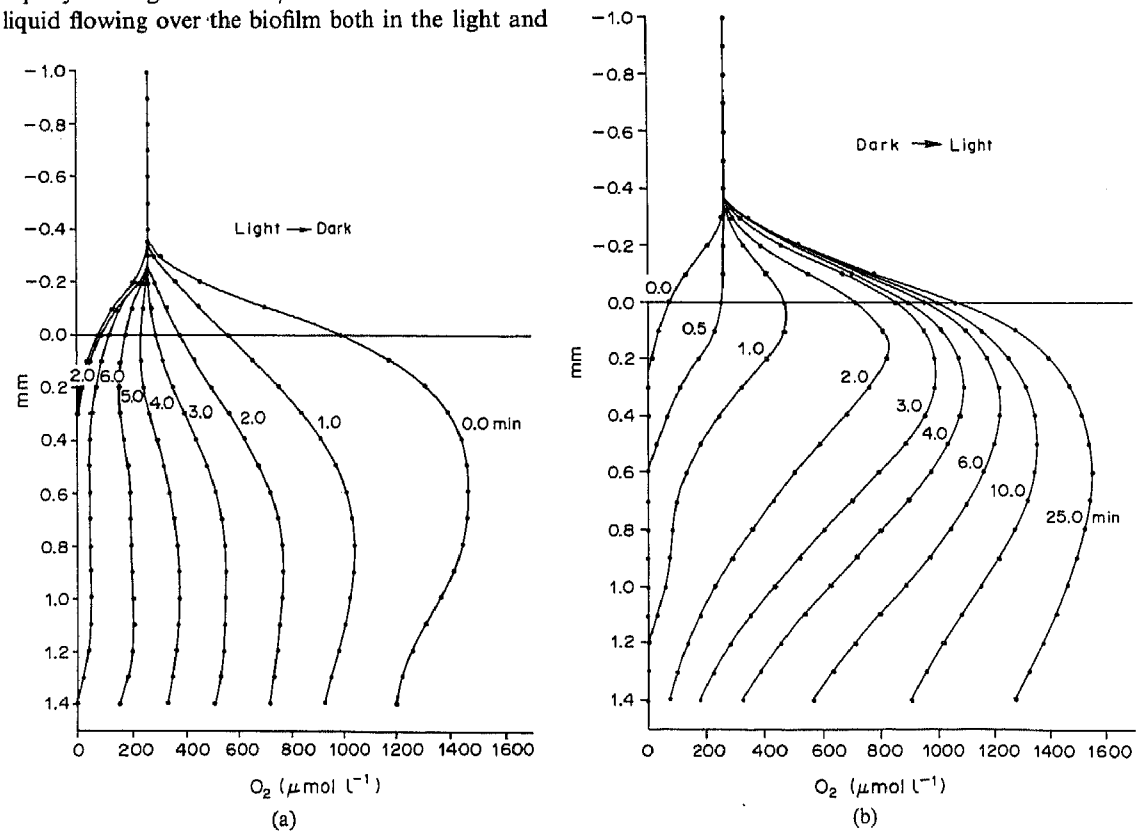

Fig. 3. Oxygen microprofiles in algal film taken at different time intervals after a L–D shift (a) or a D–L shift (b). Zero time shows the profile at steady state. The profiles in A and B were calculated from time-course measurements at different depths similar to those in Fig. 2.

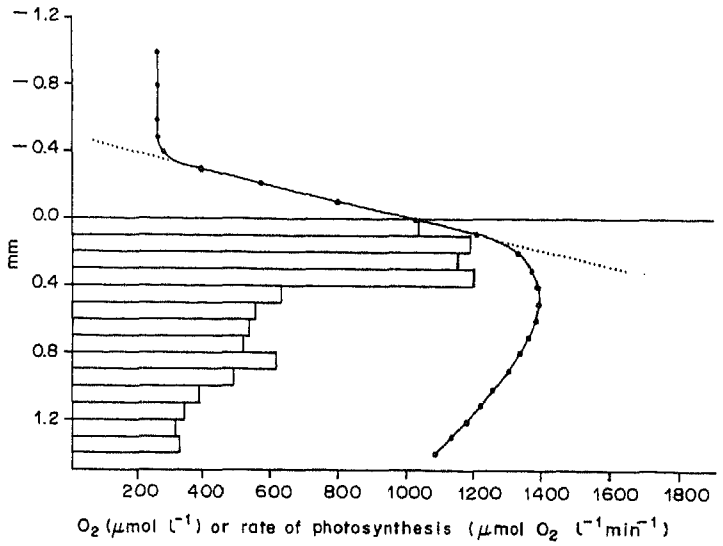

Fig. 4. Oxygen microprofile in algal film at position "A" in the light, and rates of photosynthesis at various depths. Rates of photosynthesis were calculated from the initial (2–3 s) rate of decrease in oxygen concentration after darkening the film.

effect on the rate of respiration of the film (data not shown). The heterotrophic potential of the algal film was also tested with sugars (glucose and fructose) and organic acid salts (acetate, propionate and lactate), including a well-known algal excretion product, glycollate, at concentrations of 1 mmol l^{-1}. The effect of glycollate was not detectable at all, while the positive effect of the other compounds was always less than that of 500 ppm nutrient broth and/or yeast extract. The fluxes of oxygen leaving the film were calculated from the slope of the oxygen profile in the diffusive boundary layer with and without nutrients with Fick's first law for diffusion. The flux is $F = D \cdot dc/dx$, in which, D = the diffusion coefficient for a given chemical and dc/dx the slope of the profile of that given chemical. The O_2-diffusion coefficient was taken as 2.35×10^{-5} cm^2 s^{-1} at 20°C (Broecker and Peng, 1974). In the absence of nutrients the O_2 gradient was 2.1 mmol O_2 l^{-1} mm^{-1} (or 21 μmol O_2 cm^{-4}) which gives a flux of 30 nmol O_2 cm^2 min^{-1} leaving the film. In the presence of nutrient broth and yeast extract the respiration was increased and thus resulted in less oxygen leaving the film.

The flux out was 16 nmol O_2 cm^{-2} min^{-1}, which implies that $30 - 16 = 14$ nmol O_2 cm^{-2} min^{-1} had been respired. In order to ensure sufficiently high concentrations of nutrients in the biofilm, the oxygen profile was measured after 60 min while a new steady

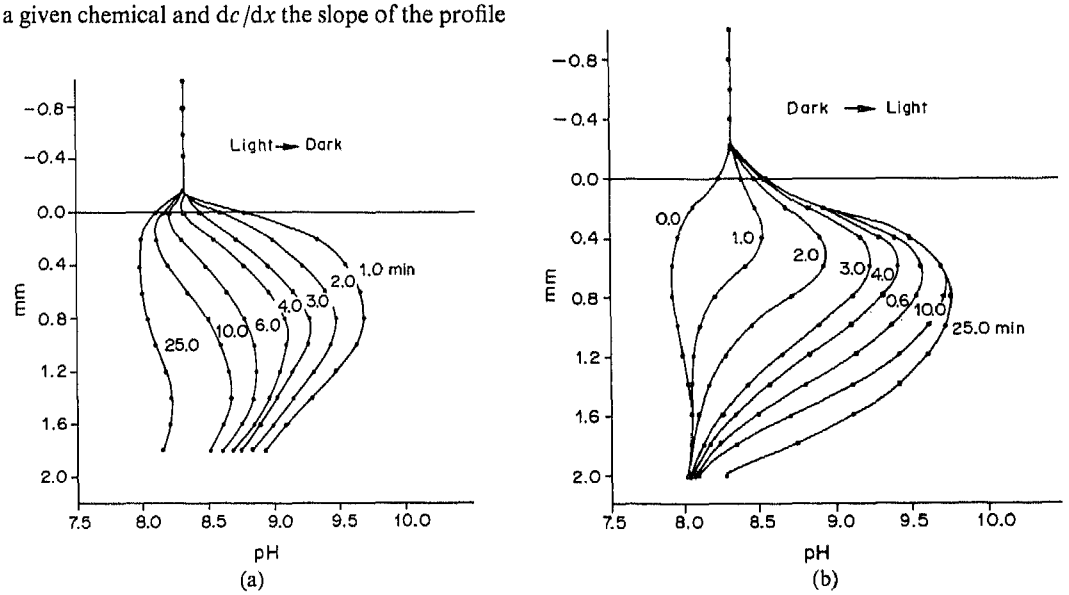

Fig. 5. pH microprofiles taken at difference time intervals after a L–D shift (a) or a D–L shift (b). The profiles were constructed from graphs of time-dependent pH changes at different depths analogous to the O_2 change exemplified in Fig. 2.

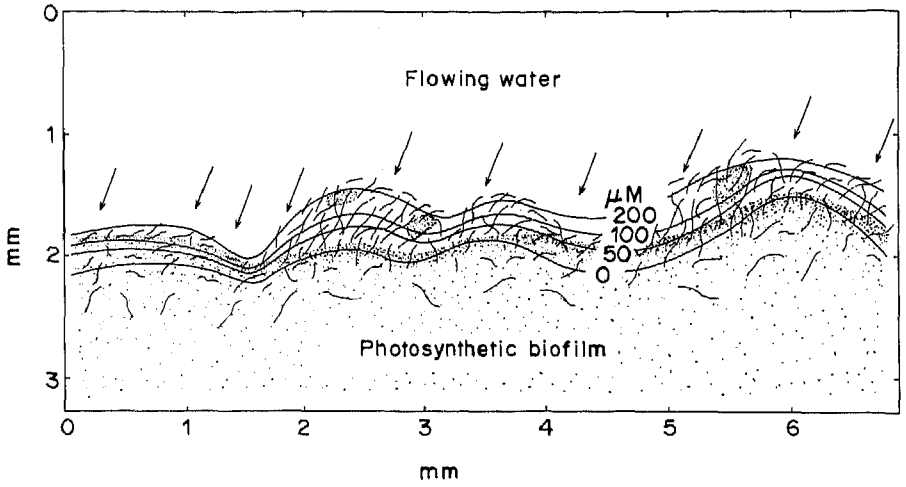

Fig. 6. Vertical section through an algal film growing on a trickling filter rock, in dim light. Isopleths of oxygen concentration, expressed in μmol l^{-1} (μM) show how the unstirred boundary layer covers the algal film as a blanket.

state oxygen profile had been reached after only 30 min (see "calculations"). The effect of nutrients was also measured in the dark. In the dark the oxygen reached down to 0.6 mm in the algal film. Addition of nutrient broth and yeast extract resulted in a small change in the oxygen gradient above the film, whereby the oxygen flux into the film changed from 10 to approx. 13 nmol cm^{-2} min^{-1}. In the light, oxygen concentrations of almost 5 × air saturation (i.e. water saturated with pure oxygen) are natur (see Fig. 1), and therefore also in the dark pu oxygen saturated water was passed over the film i order to ensure that oxygen would not becor respiration limiting to the photosynthetic layer und non-photosynthetic conditions. Under these cond tions, the oxygen reached 1.7 mm down into the fil while during photosynthesis the penetration w 2.0 mm. The change in oxygen flux after addition

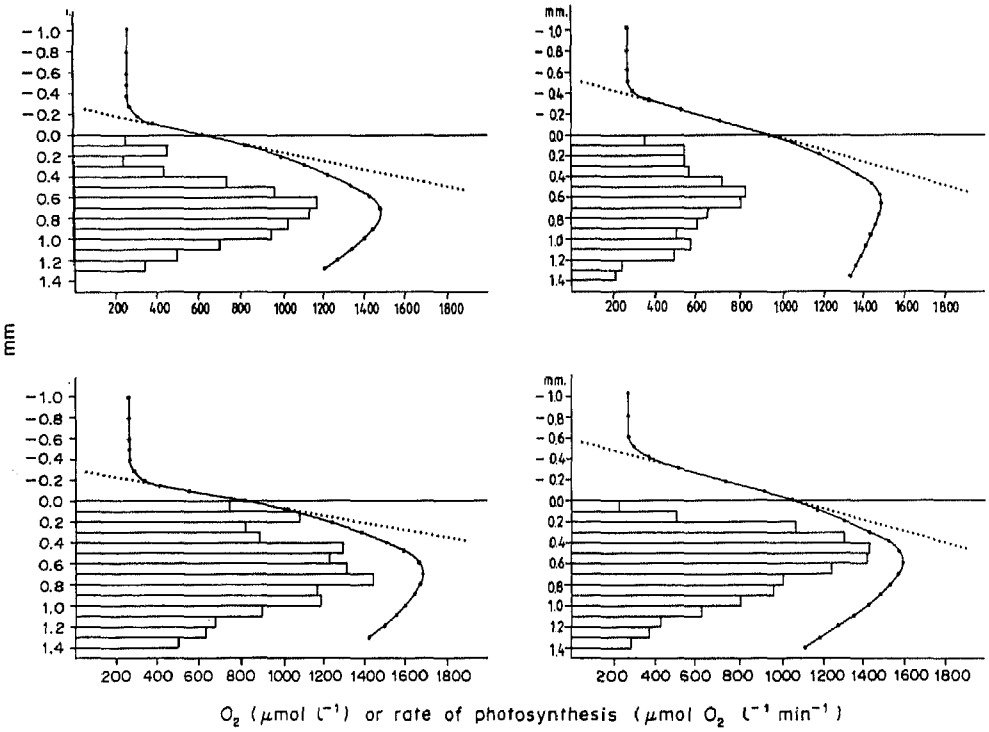

Fig. 7. Microprofiles of oxygen and photosynthesis in locations B, C, D and E, positioned at the 4 corners of a 1 cm square with position A as the centre (Fig. 4), showing the degree of heterogeneity of the algal film.

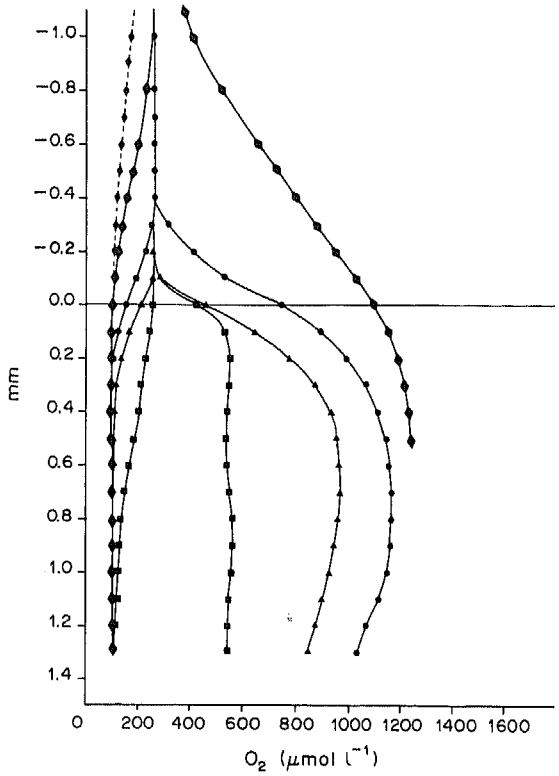

Fig. 8. Oxygen microprofiles in algal film at different flow rates of the water phase, both in the light and in the dark. Medium flow, 3–4 cm s^{-1} (●). Fast flow, 7–8 cm s^{-1} (▲). Very fast flow, 10–11 cm s^{-1} (■). No flow (◆), both dotted and drawn lines.

nutrients was about $24 - 14 = 10$ nmol cm^{-2} min^{-1} (Table 1).

The majority of the biofilms in trickling films is not photosynthetic and therefore a preliminary study was also made of this type of biolayer. The non-photosynthetic biofilm consisted of a smooth slimy material which was much more laterally homogeneous than the algal film, although it was disturbed by the presence of numerous fly larvae. A representative oxygen profile of the biofilm is shown in Fig. 9, curve A. It should be noted that the diffusive boundary layer was only half as thick as for the algal film shown in the previous figures even at the same flow rate. This was due to the much smoother surface of the non-photosynthetic biofilm (Jørgensen and Revsbech, 1983). When the flow rate of the liquid over the biofilm was reduced from 4 to 2 cm s^{-1} the diffusive boundary layer increased to 0.4 mm (data not shown). The insert of Fig. 9 shows the change in oxygen concentration which took place at a depth of 50 μm in the film upon addition of 100 ppm nutrient broth. Within 20 s a new steady state had been reached, at a rate of 9.2 mmol O_2 l^{-1} h^{-1}. The steady state profile (after 20 and 30 s respectively) in the presence of 100 and 500 ppm nutrient broth is also given in Fig. 9. The rate of change after increasing the concentration from 100 to 500 ppm nutrient broth was approx. 7 mmol O_2 l^{-1} h^{-1}. These results show the extremely rapid response of these biolayers to an increased nutrient concentration. At 100 and 500 ppm nutrient broth the flux of oxygen entering the film was also calculated using Fick's law. It was approx. 14 nmol cm^{-2} min^{-1}, while without added nutrients the consumption was about 9 nmol cm^{-2} min^{-1}. With 500 ppm nutrient broth only the outermost 0.1 mm of the biofilm was oxic, whereas without nutrients oxygen penetrated 0.4 mm into the film. As long as no nutrients had been added, the fly larvae were embedded in the film. However, the very moment that the nutrient was added they moved to the surface to achieve contact with the water phase, which emphasizes the severe O_2 limitation which this biofilm suffers upon exposure to nutrients.

In a separate experiment also the overall oxygen uptake of the biofilm in the presence of sewage was measured. This was accomplished by placing a perspex cylinder over the film (see "Methods"). The cylinder was filled with sewage and oxygen uptake was followed with time. The uptake per unit of biofilm surface was calculated after correction of uptake in the sewage itself. The net rate of uptake was 7.6 nmol of oxygen cm^{-2} min^{-1}. Without changing the rate of stirring, also the oxygen microprofile was

Table 1. Slope of the oxygen gradients (dc/dx) in the diffusive boundary layer above an algal film. From the gradients of oxygen leaving (light) or entering (dark) the film the fluxes of oxygen were calculated. Nutrients; Nutrient broth 250 ppm plus yeast extract 250 ppm (algal film) or 500 ppm nutrient broth (bacterial slime). The rate of photosynthesis was measured by the light–dark shift technique. For comparison, the fluxes of oxygen in a bacterial slime (Fig. 9) are also given (9, 10). The rates of respiration and photosynthesis are given between brackets

	O_2 gradient (mmol O_2 l^{-1} mm^{-1})	Flux of O_2 [nmol cm^{-2} min^{-1} out (+) or in (−)]
1. Flux out of film (light)	+2.1	+30
2. Flux out of film (light) plus nutrients	+1.1	+16*
3. Flux into film (dark)	−0.7	−10
4. Flux into film (dark) plus nutrients	−0.9	−13
5. Flux into film (dark) 100% O_2	−1.0	−14
6. Flux into film (dark) 100% O_2 plus nutrients	−1.7	−24
7. Rate of photosynthesis	—	(70)
8. Respired in film in light (70–30)	—	(40)
9. Flux into bacterial slime	−0.6	−9
10. Flux into bacterial slime plus nutrients	−1.0	−14

*Extrapolated from nearly identical duplicate experiment.

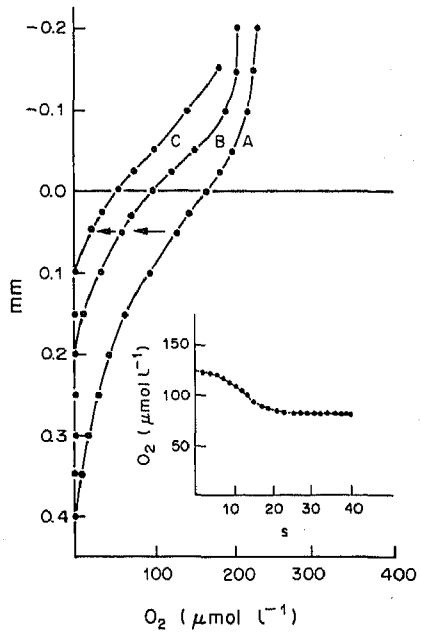

Fig. 9. Oxygen microprofiles in a non-photosynthetic biofilm with overlying water, in steady state (zero time, A) and 30 s (B) after addition of nutrient broth, at a concentration of 100 ppm. Curve C shows the profile after 20 s, when the concentration of nutrient broth had been increased to 500 ppm. The insert shows the time dependent change in ozygen concentration at 0.05 mm depth in the biofilm, after addition of 100 ppm nutrient broth to the water phase.

measured through the film. The diffuse boundary layer was 0.5 mm thick and the gradient 5.3 μmol oxygen cm^{-4}. Given a diffusion coefficient of 2.35×10^{-5} cm^{-2} s^{-1}, the flux into the film would be 7.5 nmol cm^{-2} min^{-1}, which agrees well with the rate of disappearance from the bulk phase above the film.

During operation of the trickling filter the fresh sewage is splashed over the rocks every 8–10 s through arms rotating over the trickling filter, thus providing a discontinuous supply of nutrients to the biofilms. At the same time the films are exposed to a continuous cycle of wetting and draining. This effect was simulated by draining the water from the water-bath and dropping onto the biofilm one drop of water or of nutrient broth (500 ppm) every 20 s. The result is graphically represented in Fig. 10. The dropwise addition of water to the biofilm caused a repetitive swelling and draining of the film. As the microelectrode was held in a fixed position the observed cyclic changes in oxygen were due to the varying depth (*ca* ± 0.05 mm) at which oxygen was recorded in the expanding and shrinking film. When 100 ppm nutrient broth was added to the film, however, a shift in the cyclic variation on O_2 concentration to a lower level was observed after a lag period of about 20 s. Nearly anaerobic conditions were created even at this shallow level in the biofilm as the mean O_2 level had dropped from 100 to 20 μmol l^{-1}. The supply of nitrate in addition to nutrient broth had no effect on the oxygen consumption in the biofilm.

DISCUSSION

The result of our oxygen microelectrode measurement on biofilms of trickling filters demonstrate that the oxygen profiles are very dependent on the availability of nutrients and light. Photosynthetic biofilms showed dramatic changes in oxygen concentrations with changing light conditions while the heterotrophic slimes displayed a strong dependence on nutrient supplies. The latter biofilms became even more oxygen limited as soon as nutrients were supplied above a certain level. The oxygen profiles in the overlying water just above the biofilms showed a nearly linear gradient within the diffusive boundary layer above the surface of the biofilm. It is evident that exchange of nutrients and other chemicals, and consequently life and activity of these biofilm, is ruled by the existence of this diffusion barrier, which slows down exchange of solutes between the biofilm and its environment. It should be stressed that the existence of these boundary layers cannot always be ignored in

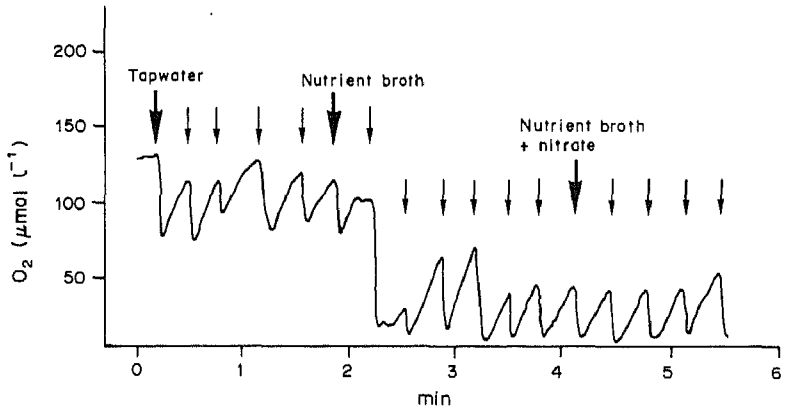

Fig. 10. Time dependent changes in oxygen concentration at 0.1 mm depth in a drained biofilm during a 20 s cycle dropwise (small arrows) addition of liquid to the biofilm. As the oxygen electrode was in a fixed position, the recorded regular fluctuations are due to repeated swelling and draining of the biofilm. The large arrows indicate the change from tapwater to nutrient broth or to nutrient broth plus nitrate.

natical modelling (see for example, Har-
, 1978).

possible to calculate the flux of oxygen enter-
leaving the biofilm from the slope of the
microprofiles using Fick's first law for
on. The oxygen gradient in Fig. 4 (spot A)
$\times 10^{-6}$ mol O_2 cm^{-4} (i.e. 2.1 mmol l^{-1} mm^{-1}),
gives a flux of 30 nmol cm^{-2} min^{-1}. This flux
gen leaving the film was the net result of
ynthesis and respiration within the biofilm.
oss photosynthesis taking place in the film can
en as the sum of the rates measured at 0.1 mm
intervals as shown in Fig. 4. The total oxygen
tion at point A would be 93 nmol cm^{-2} min^{-1}.
ads to the surprising conclusion that most
e oxygen produced, namely $93 - 30 =$
l cm^{-2} min^{-1}, or 68%, has been respired
the algal film. The turnover time of oxygen at
depth in the film was, in spite of its high
tration, only 60 s. Recent experiments in our
tory (Revsbech, in preparation) indicate that
fusivity constant used in our calculations may
% too high but this would not influence the
sion to any major extent.

same calculations were made for spots B, C,
E (Fig. 7), where the percentages respired were
71 and 76% respectively. A number of control
nents were run to show whether this high
tion might, at least in part, be due to the
espiration of intracellular compounds and is
artifact of the method. First of all it was found
film solidified with 0.4% agar showed the same
menon (details not given). This indicates that a
le microturbulence within the algal film was
portant relative to the diffusive transport cal-
l here. Secondly, in an independent series of
nents (Table 1) it could also be shown that the
trophic potential of the film was relatively low.
otential was calculated from fluxes of oxygen
or entering the film in the presence or absence
rients using Fick's law as in the calculation
in the previous paragraph. The results of a
r of flux calculations are shown in Table 1. In
ht, the addition of a variety of exogenous
ates such as nutrient broth, yeast extract,
or organic acids (including glycollate), at
)0 mg l^{-1} or 1 mmol l^{-1} concentrations pro-
an increase in respiration of maximally
16 =)14 nmol cm^{-2} min^{-1}. The addition of nu-
in the dark changed the flux by 3 nmol cm^{-2}
en when the concentration of oxygen in the
as artificially increased to a level similar to that
light the respiration of exogenous substrates
ed very similar to that in the dark
14 = 10). This indicates that of the 40 nmol
d in the film, in the absence of added nutrients,
order of 10–14 nmol cm^{-2} min^{-1}, at the most,
have been due to respiration of excreted prod-
he remaining $40 - 14 = 26$ (41%) must be due
iration of endogenous substrates. During these
experiments the pH increased to 9.7 which is undoubtedly due to CO_2 consumption. This would lead to a high O_2/CO_2 ratio which is known to stimulate the process known as photorespiration (Tolbert, 1979). In this process glycollate is formed due to the oxygenase activity of ribulose 1,5 biophosphate carboxylase. The intracellular glycollate is subsequently oxidized. Therefore, it may well be that the observed high endogenous respiration in the light is, at least in part, due to photorespiration.

In the presence of nutrients the non-photosynthetic biofilm became anaerobic up to 0.1 mm depth in the layer (Fig. 9). In contrast, the dark algal films became anaerobic only below 0.5–0.6 mm in the presence of nutrients. This shows that the specific activity of the biofilm, calculated per volume, is significantly higher in the bacterial slimes as compared to the algal films. However, when calculated per surface area the differences in activity are not very significant (compare in Table 1, lines 3 and 4 with lines 9 and 10 respectively). It is very interesting to note that in the light the increase of oxygen consumption as a result of nutrient addition, is much higher in the algal film than in the bacterial film which has the higher respiration potential, but suffers severe oxygen limitation. These direct measurements of oxygen profiles confirm and quantify earlier conclusions obtained from biofilm studies that the efficiency of nutrient utilisation/oxidation by mixed culture biofilms is strongly limited by their thickness which in turn is caused by limitation by oxygen diffusion (Hoehn and Ray, 1973; Harris and Hansford, 1976). Given this fact, it is obvious that the thickness of the diffusive boundary layer, which is in the order of 0.1–0.5 mm, determines the rate of oxygen transfer into the mats. Hence, in contrast to earlier conclusions (Chen and Bungay, 1981), it appears that at high respiration rates the flow rate of the medium over the slime (compare Fig. 8) is essential for efficient oxygen transfer into the film and thus will lead to a significantly higher rate of oxygen uptake at higher flow rates.

In the non-photosynthetic heterotrophic microbial film the respiration of the film was determined also by bulk uptake from the overlying medium and from the flux calculation. As mentioned in the results section, the flux of oxygen into the film during respiration of the sewage would be 6.5–7.5 nmol cm^{-2} min^{-1}. As the overall respiration measured from the disappearance of oxygen in the bulk phase above the film was 7.6 nmol cm^{-2} min^{-1} these data fit very well. This implies that microelectrode data not only are useful for comparative purposes as shown above, but can also directly be used for an estimate of the activity of the biofilm of the trickling filter.

CONCLUSIONS

(1) The oxygen microprofiles of trickling filter biofilms show a very dynamic response to nutrients and/or light.

(2) A diffusive boundary layer is governing the exchange of nutrients and products of the biofilm with its environment.

(3) In our experimental set up microturbulences within the (algal) biofilms do not significantly contribute to transport of solutes relative to that by diffusion.

(4) In photosynthetic biofilms up to about 70% of the oxygen produced may be respired within the mat, but in spite of high respiration rates in the light the heterotrophic potential for exogenous substrates was very low.

(5) The specific heterotrophic activity (calculated per volume) of non-photosynthetic biofilms is substantially higher than that of photosynthetic films.

(6) However the efficiency of nutrient utilisation/oxidation by the non-photosynthetic biofilms is strongly limited by oxygen diffusion.

(7) Oxygen microelectrode measurements can be used for direct measurements of activity of biofilms.

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