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# Artificial cyanobacterial mats: cycling of C, O, and S

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ABSTRACT: Cycling of carbon, oxygen and sulphur was studied in cyanobacterial mats growing on defaunated sediments. Like in natural stromatolitic mats, fluxes of $O_2$ in the dark and in the light and estimates of gross photosynthesis suggest that respiration is much higher in the light than in the dark. It is shown here that the rate of organic C mineralisation is only moderately higher in the light. Mineralisation is entirely anaerobic in the dark and the reoxidation of reduced end-products (notably reduced sulphur) is incomplete due to temporary sulphide immobilisation. The sediments therefore develop an $O_2$ debt during darkness and the enhanced $O_2$ uptake in the light to a large extent reflects the reoxidation of reduced end-products accumulated during darkness. This also explains the mismatch between fluxes of inorganic C and $O_2$. Oxygen fluxes between the mat and the overlying water are therefore not a measure of net photosynthesis or mineralisation rates, but cause underestimations of these processes by a factor of about 2. About 11% of the net influx of inorganic C during illumination (or about 5% of gross photosynthesis) is permanently accumulated as organic C and about 0.5% accumulates as carbonate minerals.

KEY WORDS: Cyanobacterial mats · Stromatolitic mats · Carbon cycling · Sulphur cycling · Photosynthesis · Mineralisation

## INTRODUCTION

Element cycling of benthic cyanobacterial mats has drawn attention during the last 2 decades. Reasons for this include the relative ease by which concentration gradients (especially $O_2$) can be measured with microelectrodes and used for rate estimates since the few mm thick surface film constitutes the biologically active part and transport of substances is by way of 1-dimensional diffusion. The intensity of reaction rates (on a volume basis) is reflected by several hundred percent supersaturation with $O_2$ in the light and by anoxia in the dark within the upper 2 to 3 mm of the mats. Aspects of their biogeochemistry, and the hypothesis that cyanobacterial mats represent a modern analogue of Precambrian stromatolites, have also added to their attractiveness as study objects.

A pecularity of these mats is that there appears to be a dramatic increase in respiration in the light (by a factor of 5 or more). This is usually attributed in part to a tight coupling between production and mineralisation (brought about by excretion of dissolved organics by the phototrophs in the light and a close physical association with heterotrophs) and in part to photorespiration (Jørgensen et al. 1983, Revsbech et al. 1983, Glud et al. 1992, Canfield & Des Marais 1993, Kühl et al. 1996). It has also been suggested that the effect occurs because the oxic zone is more voluminous during illumination (Epping & Jørgensen 1996), but this seems to be an incomplete explanation. Another apparent paradox is that the upwards flux of oxygen (net production of $O_2$) is substantially higher than net uptake of $O_2$ in the dark although the actual net accumulation of organic C or reduced S is low; also it has been found that there is a 'mismatch' between fluxes of inorganic C and $O_2$ (the former being higher both in the dark and in the light). Canfield & Des Marais (1993), after having examined different possible explanations, suggested that these findings reflected the accumulation of C compounds with a higher (>0) oxidation level of C than found in ordinary organic matter.

Using laboratory grown, 1 to 12 mo old mats it is shown here that the imbalance between $O_2$ uptake in the dark and $O_2$ release in the light is caused by an

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oxygen debt which builds up in the mat during darkness. During darkness, C mineralisation is exclusively anaerobic, but only marginally lower than in the light. In the light, $O_2$ consumption is in part due to the reoxidation of reduced end-products of anaerobic mineralisation (bound and free sulphides, $Fe^{2+}$, etc.) and some photosynthate is temporarily stored to become mineralised in the dark. Net $O_2$ fluxes are not, as previously suggested (Revsbech 1994), a measure of net phototrophic C fixation or organic C mineralisation, but cause underestimations of these processes by a factor of about 2 because the redox balance is buffered by sulphate production or consumption and exchange with the overlying water column. The permanent accumulation of organic C and of $CO_3^{2-}$ minerals is also quantified.

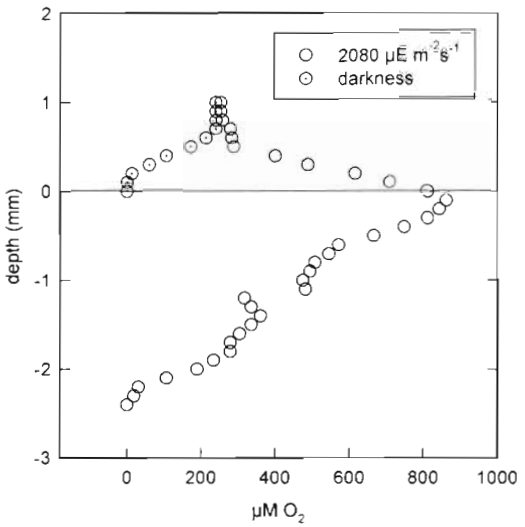


Fig. 1. Oxygen concentration gradients of an approximately 5 mo old mat in the dark and in the light

## MATERIAL AND METHODS

Cyanobacterial mats, based on defaunated inoculates and grown on natural sediments or on foam rubber, were prepared and maintained as described in Fenchel (1998a); their physical structure and biota are described in more detail in Fenchel (1998b). In the present study mats between 1 and 13 mo old were used. Simultaneous measurements of inorganic C and $O_2$ fluxes were based on a set of mats approximately 8 mo old. At the time these were about 7 mm thick (including the purple zone) and extended 1.5 to 2 mm above the surface of the sediment/foam rubber. Extended measurements (not shown) indicated that light and dark fluxes of $O_2$ remained almost constant for 1.5 to 2 mo after the establishment of the mats.

Oxygen was measured with $O_2$-microelectrodes prepared according to Revsbech & Jørgensen (1986). Net oxygen fluxes were measured in 2 ways. One method was based on oxygen concentration gradients determined by measurements at depth intervals of 100 µm, see Fig. 1. Measurements were made while bubbling the supernatant water with air. Oxygen tensions were converted to concentrations according to temperature and salinity. The slope of the linear part of the gradient immediately above the sediment surface multiplied by the diffusion coefficient for dissolved $O_2$ (corrected for temperature and salinity) is then a measure of the (negative or positive) $O_2$ flux (Revsbech 1994). Alternatively, the cores were covered (avoiding air bubbles) with a perspex lid fitted with a mounted magnetic stirrer bar and a 1 mm hole for inserting an $O_2$-electrode; the amplifier for the electrode was connected to a strip chart recorder and the decrease/increase in $O_2$ tension was followed for 0.5 or 1 h. The 2 methods ($O_2$ gradients, total $O_2$ uptake/release of 78.5 $cm^2$ mat surface) yielded identical results as far as dark $O_2$ uptake is concerned (not shown). This was not tested critically in the case of $O_2$ production in the light (that is, as simultaneous measurements by the 2 methods in a particular mat). It is possible that the very steep gradients are deformed by the presence of the microelectrode which again yields an overestimate of the flux (Glud et al. 1994). However, quite comparable flux estimates resulted from the 2 methods for a range of mats of different ages (cf. Table 1, Fig. 3). Estimates of $O_2$ production of the mats were usually made after 2 to 3 h light exposure.

Gross $O_2$ production was measured at different depths in the mat following the method of Revsbech (Revsbech & Jørgensen 1986, Revsbech 1994). The $O_2$-electrode was positioned at a given depth in the light exposed mat. The light was thereafter turned off and the initial (linear) decrease in $O_2$ concentration is then a measure of the previous gross $O_2$ production. This assumes that steady state was attained in the previous light period and that sudden darkness initially affects only $O_2$ production, but not the various sinks for $O_2$. The mats were exposed to light for 10 min after each measurement and before the electrode was repositioned and a new measurement took place.

Fluxes of inorganic C were measured simultaneously with $O_2$ exchange using the above-mentioned lid with a magnetic stirrer and taking 1 ml water samples before the experiment and after 1.5 or 2 h incubation. The samples were injected into airtight vials containing 1 ml 1 N HCl. The vials were shaken for 2 h and $CO_2$ contents of the headspace were quantified with a gas chromatograph fitted with a thermal conductivity detector.

Carbonate deposition was measured on a mat growing on foam rubber. Squares (1 $cm^2$) of the foam rubber

+ mat were cut with a scalpel, briefly rinsed in distilled water and dried at 100°C. The pieces were then placed in vials which were capped with an airtight seal and 1 ml 1 N HCl was injected. After 2 h on a shaking table, gas samples were analysed for $CO_2$. Organic C contents were measured as decoloration of dichromate-sulphuric acid solutions (Strickland & Parsons 1968). Subsamples of the mat (0.1 $cm^2$) were taken. The upper 4 to 5 mm thick coherent mat was gently blotted and placed in 4 ml dichromate-sulphuric acid for 1 h at 100°C. The liquid was diluted to 50 ml and extinction read at 440 nm. A glucose solution was used as standard.

Sulphide was measured with calibrated sulphide electrodes according to Revsbech & Jørgensen (1986) and total $S^{2-}$ calculated from the measured potential and pH. A commercial needle electrode (Microelectrodes, Inc., USA) was used for pH measurements. Sulphate (using 50 µl samples taken with a syringe at 1 mm depth intervals, diluted with distilled $H_2O$ and filtered through a membrane filter) was measured turbidometrically as a $BaSO_4$ suspension in gelatine (Standard methods 1975).

The mats were in most cases illuminated with a cold light lamp for measurements of photosynthetic activity; alternatively a 100 W microscope lamp was used. For infrared illumination the microscope lamp was fitted with a 3 mm RG9 filter which absorbs light at wavelengths <700 nm. For visible light, intensity was calibrated with a flat underwater quantum sensor (Li-Cor Li 1000 radiation sensor); the reported values represent incident light on the mat surface. The simultaneous measurements of $O_2$ and $CO_2$ fluxes were made in darkness and with an incident radiation of 2000 µE $m^{-2}$ $s^{-1}$. All experiments were performed at room temperature (20 to 25°C).

## RESULTS AND DISCUSSION

An example of gross $O_2$ production at different light intensities and depths is seen in Fig. 2. Photosynthetic activity was highest at a depth of 100 to 300 µm and was detectable down to a depth of about 1.8 mm. Integration of the curves with depth yields an estimate of the gross $O_2$ production. These are shown in Fig. 3 together with the net $O_2$ fluxes. Extensive measurements of different mats at various ages showed that for mats more than 1.5 to 2 mo old, net dark $O_2$ uptake was always 350 to 400 nmol $cm^{-2}$ $h^{-1}$ and net production of $O_2$ satiated at about 600 nmol $cm^{-2}$ $h^{-1}$ at an incident radiation of >250 µE $m^{-2}$ $s^{-1}$. Gross $O_2$ production satiated at about 3000 nmol $cm^{-2}$ $h^{-1}$. The respiration rate of the mat is given by the difference between net and gross $O_2$ production, implying a 6-fold higher uptake

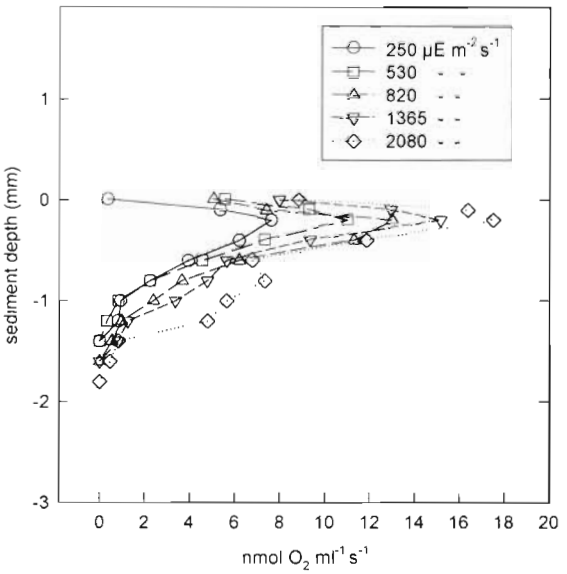


Fig. 2. Gross $O_2$ production as a function of depth and of incident light in an approximately 2.5 mo old mat

of $O_2$ in the light than in the dark. To the extent that these fluxes are measures of net phototrophic C fixation and net C mineralisation, respectively, then the difference between dark uptake and light production would imply that about 30% of the production is accumulated in the sediment in the form of organic C or reduced S, a figure which far exceeds actual accumulation rates (see below).

The fluxes and process rates presented above agree closely with what has previously been measured for a variety of natural cyanobacterial mats (e.g. Revsbech et al. 1983, Glud et al. 1992, Canfield & Des Marais 1993, Revsbech 1994, Kühl et al. 1996). This suggests that the rates are controlled by some basic physical constraints such as light absorption and diffusion rates in these 1-dimensional microbial communities. However, $O_2$ production stabilised at somewhat lower light

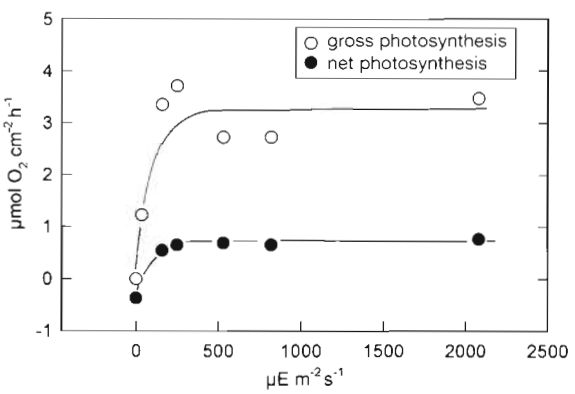


Fig. 3. Gross and net production of $O_2$ as a function of incident light in an approximately 5 mo old mat

Table 1. Fluxes of inorganic C and $O_2$ through the surface of an 8 mo old mat in nmol $cm^{-2}$ $h^{-1}$ based on concentration changes in the overlying water; numbers in parentheses are standard deviations

| | Light | Dark |
|---|---|---|
| $CO_2$ | −1244 (305) | +1235 ( 236) |
| $O_2$ | +644 (58) | −364 (16.5) |

intensities relative to what has previously been recorded from natural mats (e.g. Revsbech et al. 1983).

Simultaneous measurements of light and dark fluxes of inorganic C and of $O_2$ are shown for an 8 mo old mat in Table 1. It can be seen that net uptake and net production of inorganic C approximately balance, indicating a very modest accumulation of organic C. It can also be seen that fluxes of inorganic C in the light and in the dark are about 2 and 3.5 times higher, respectively, than are the corresponding $O_2$ fluxes.

In the light, free sulphide appears in appreciable amounts only beneath the oxic zone (the upper 2.5 mm; Figs. 1 & 4), although free sulphide was detectable (but not quantifiable) throughout most of the oxic zone. When irradiated only with infrared light, the sediment remains anoxic almost to the surface (not shown); since the sulphide gradient is very similar to that in visible light (Fig. 4), it is likely that the bulk of the sulphide oxidation is phototrophic at depths beneath 2 to 2.5 mm. In the dark, sulphide is found above the mat surface, overlapping with $O_2$ somewhere between 0 and 1 mm above the surface and it accumulates at concentrations of several hundred µM in the uppermost part of the mat. If light is turned off after a longer period of illumination, sulphide first starts to accumulate 100 to 300 µm beneath the mat surface (Fig. 5). Whether this reflects a continuous sulphide production which is immediately reoxidised under oxic conditions (cf. Fründ & Cohen 1992) or whether sulphate reduction first is initiated after conditions have become anoxic cannot be answered on the basis of the present observations. However, the data show that the peak in sulphate reduction coincides with the photosynthetically most active level of the mat.

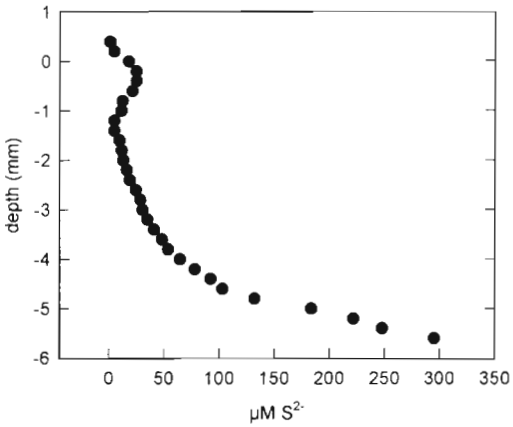


Fig. 5. Concentration gradient of total free sulphide approximately 0.5 h after an illuminated mat was placed in darkness; the mat was approximately 5 mo old

The distribution of $SO_4^{2-}$ in the light and in the dark is shown in Fig. 6. In terms of spatial resolution the employed method is much too crude to use for the estimation of fluxes. However, the data suggest that there is a net flow into the mat in the dark and a net outflow during the light.

Finally Table 2 shows the accumulation of carbonate (which was deposited as a discrete layer about 300 µm beneath the mat surface, cf. Fenchel 1998b) and the accumulation of organic C. It is seen that carbonate deposition (which probably takes place in the light

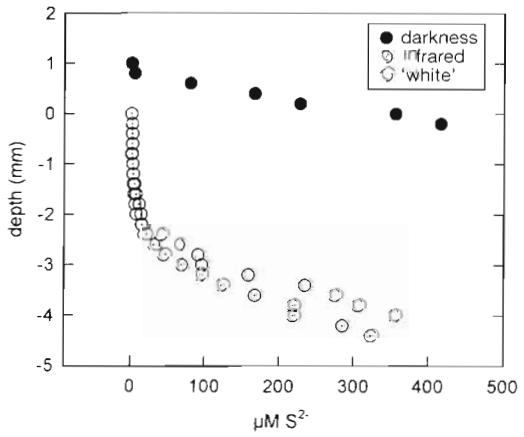


Fig. 4. Concentration gradients of total free sulphide after 16 h in the dark and after 2 h in light and in infrared light, respectively, for an approximately 5 mo old mat

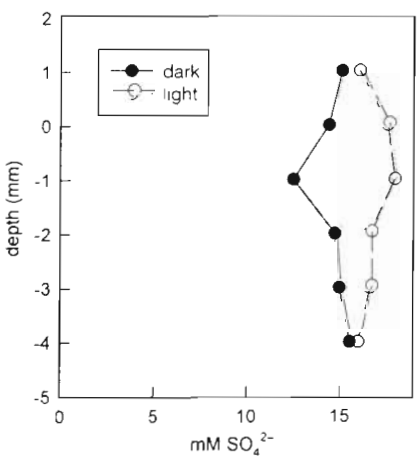


Fig. 6. Concentration gradients of $SO_4^{2-}$ after 16 h darkness and after 5 h in the light in an approximately 8 mo old mat

Table 2. Accumulation of organic C (in the upper 4 to 5 mm of the mat) and of $CO_3^{2-}$ (numbers in parenthesis are standard deviations). Rates of increment are calculated only for periods in the light (14 h $d^{-1}$)

| | Age of mat (d) | μmol $cm^{-2}$ | nmol $cm^{-2}$ $h^{-1}$ |
|---|---|---|---|
| Organic C | 238 | 899 (438) | 270 |
| | 356 | 710 (174) | 142 |
| $CO_3^{2-}$ | 147 | 30.5 (1.64) | |
| | 180 | 37.4[a] | |
| | 233 | 42.4[a] | |
| | | | 14.3[b] |

[a]Only 1 sample was analysed
[b]Calculated from regression of the 3 data points

when pH approaches 9 in the upper 0.5 mm of the mat) represents about 1% of the uptake of inorganic C. Measurements of organic C accumulation were more uncertain as results showed considerable scatter. This may in part be due to spatial variation in mat thickness, but probably first of all because the thickness of the samples for the analysis was ill defined. Nevertheless, accumulation is probably of the order of 10% of the net C fixation during light exposure (the remaining being mineralised during the subsequent dark period).

A budget which accounts for the measured fluxes of inorganic C and $O_2$ and for gross $O_2$ production in the light is shown in Figs. 7 & 8. It represents a simplification for several reasons. The model ignores the N cycle as well as methanogenesis and thus some processes which may alter the reduction-oxidation state of C al-

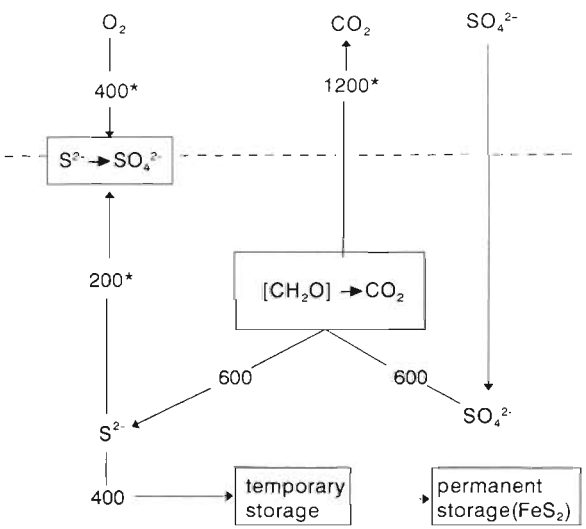


Fig. 7. Principal transformations of C, O, and S in darkness. Rates are in nmol $cm^{-2}$ $h^{-1}$; those marked * have been measured while the other values have been inferred indirectly (see text)

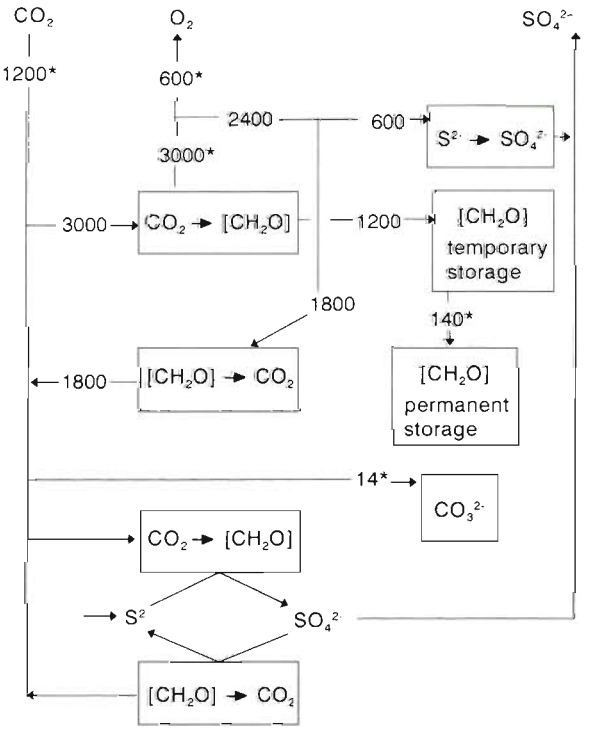


Fig. 8. Principal transformations of C, O, and S in the light. Rates are in nmol $cm^{-2}$ $h^{-1}$; those marked * have been measured directly while the other values have been inferred indirectly (see text)

though they are probably quantitatively modest when compared to the role of the sulphur cycle. It also ignores the fact that a number of sulphur compounds with oxidation states intermediate between sulphate and sulphide may accumulate (e.g. van Gemerden 1993) and that chemotrophic sulphide oxidation involves some C fixation. In Figs. 7 & 8 no attempt has been made to balance quantitatively minor processes (deposition of carbonate, permanent accumulation of organic C and reduced S).

In the dark (Fig. 7), all mineralisation of organic C must be anaerobic since the entire mat is anoxic (cf. Fig. 1). The principle terminal electron acceptor is sulphate (although $Fe^{3+}$ and $NO_3^-$ reduction and methanogenesis may contribute). The rate of organic C mineralisation must equal the flux of inorganic C (~1200 nmol $cm^{-2}$ $h^{-1}$). This again corresponds to the reduction of 600 nmol $SO_4^{2-}$ $cm^{-2}$ $h^{-1}$. The $O_2$ flux to the mat is about 400 nmol $cm^{-2}$ $h^{-1}$, corresponding to the oxidation of 200 nmol sulphide, and the dark $S^{2-}$ gradient (Fig. 4) is consistent with an upward flux of this magnitude, assuming that the diffusion coefficient of sulphide is 0.67 times that of $O_2$ (Cussler 1989). Consequently, 400 nmol of sulphide (or corresponding reduction equivalents) must be stored in the mat per hour during darkness. Accumulation of free sulphide (alto-

gether about 150 nmol during a night) can only account for a fraction of this and so most temporarily stored reduction equivalents are probably immobilised as ferrous sulphide. The pool of free sulphide builds up relatively slowly during darkness (over several hours, cf. Figs. 4 & 5) while $O_2$ disappears within minutes. This also suggests that most sulphide is immobilised, presumably mainly as FeS.

In the light (Fig. 8), net phototrophic production must equal the flux of inorganic C into the mat (approximately 1200 nmol $cm^{-2}$ $h^{-1}$), most of which is subsequently mineralised in the dark, although a smaller fraction (~11% as estimated from the accumulation of organic C; Table 2) seems to be permanently stored and represents the growth of the mat. The apparent discrepancy between inorganic C and $O_2$ fluxes (by a factor of about 2) reflects the fact that $O_2$ is consumed by the reoxidation of reducing materials accumulated during darkness (the oxygen debt). Since gross $O_2$ production amounts to about 3000 nmol $cm^{-2}$ $h^{-1}$, 1800 nmol inorganic C must be recycled. This rate of organic C mineralisation is, however, only about 50% higher than what takes place in the dark. The somewhat higher rate in the light probably reflects a higher rate of excretion of dissolved organics by the phototrophs.

It is a complication that some of the photosynthetic activity is due to phototrophic sulphur bacteria (lower part of Fig. 8). Thus some of the inorganic C may be consumed by these organisms which are then simultaneously responsible for the reoxidation of sulphide to sulphate. However, the overall picture (in terms of mass and redox balance) will not change irrespective of the magnitude of this process. If all sulphide reoxidation beneath 2.5 mm is phototrophic, then sulphide gradients in the light (Fig. 4) indicate a flux of 130 to 140 nmol $cm^{-2}$ $h^{-1}$, corresponding to the fixation of 260 to 280 nmol inorganic C or somewhat less than 10% of the rate of oxygenic photosynthesis. Such a limit for the role of anoxygenic $CO_2$ fixation was also found by Canfield & Des Marais (1993).

The above model of element cycling of cyanobacterial mats seems to present the simplest one accounting for the measured exchanges of material between mats and the overlying water. In particular the model avoids the assumption of a dramatic difference in heterotrophic activity between the light and the dark or of an accumulation of organic C with an unusually high oxidation level. It is an implication that the mat is never in a real steady state and in particular that it slowly becomes more oxidised during light exposure. This is seen from the continuous expansion of the oxic zone during light exposure (Fig. 9). It means that $O_2$ consumption within the mat decreases with time and so net $O_2$ production must increase accordingly and

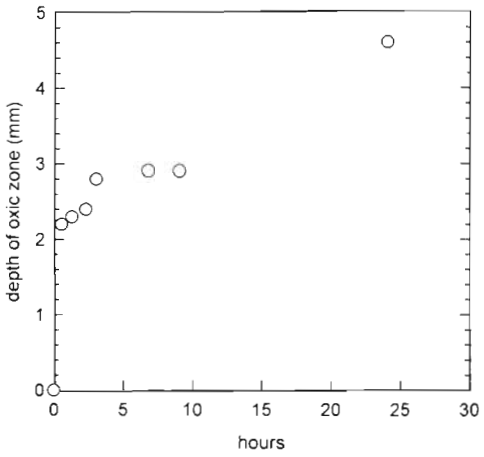


Fig. 9. Depth of the oxic zone (the deepest point where free $O_2$ was detectable with a microelectrode under a fixed point in the mat surface) as a function of time exposed to light (~2000 µE $m^{-2}$ $s^{-1}$). At time zero the mat was anoxic throughout

eventually approach the rate of $CO_2$ uptake. The net $O_2$ light production in Table 1 was that of a mat exposed to light for 2 to 4 h. The numbers in Fig. 8 therefore apply to a mat during the early part of the 14 h light period; later during the day the mismatch between inorganic C uptake and $O_2$ production will be less pronounced.

Cyanobacterial mats appear as ecosystems with a high intensity in terms of process rates. This impression, however, is caused solely by their small dimensions: the active zone is a film only a few mm thin. In terms of biological activity on an areal basis they are among the least productive ecological systems known. The net (new) production of the mat was only about 140 to 315 nmol C $cm^{-2}$ $h^{-1}$ (Table 2) or 150 to 315 g C $m^{-2}$ $yr^{-1}$ (the mats were maintained in a 14 h light:10 h dark cycle). Examples of average net production of other marine systems are: oceanic plankton, 125 g C $m^{-2}$ $yr^{-1}$; plankton in coastal and upwelling areas, 300 to 500 g C $m^{-2}$ $yr^{-1}$; and kelp forests and coral reefs, 500 to 2500 g C $m^{-2}$ $yr^{-1}$ (Valiela 1984). If the active zone of the cyanobacterial mats includes about 800 µmol organic C $cm^{-2}$ (Table 2) it means that the daily increment of the mats is only about 0.4% of the biomass. The cyanobacterial mats regenerated very slowly following disturbances; for example, scars formed by 0.5 cm core samples taken from the studied mats were still visible after a year in the form of circular depressions. The reason for this low productivity is the geometry of the mats which does not allow for the efficient use of light made possible by a canopy structure or by mixing in the upper layers of a water column. The fact that transport is restricted to molecular diffusion is also limiting.

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