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Diurnal behaviour of nitrogenase activity in a benthic cyanobacterial mat community in the Wadden Sea (North Sea)

M. Villbrandt*, W. E. Krumbein* and L. J. Stal**

* Institut für Chemie und Biologie des Meeres (ICBM) an der Universität Oldenburg
Postfach 2503, D-2900 Oldenburg, Germany

** Institute of Microbiology, University of Amsterdam
Nieuwe Achtergracht 127, NL-1018 WS Amsterdam, The Netherlands

Abstract

Versicoloured microbial mats (Farbstreifen-Sandwatt) in the intertidal zone of the North Sea island of Mellum were investigated with respect to oxygenic photosynthesis and nitrogen fixation. In laboratory cultures of the cyanobacterium *Oscillatoria limosa* a temporary separation of both incompatible processes occurs. Also *in situ* a negative correlation of sedimentary oxygen concentration and nitrogenase activity was found. If the sediment turned anaerobic during the night, no acetylene reduction was measurable. In systems, however, which stayed oxygenated during the night, nitrogenase activity was found at night. Peaks of nitrogenase activity were often detected at sunrise. Sometimes acetylene reduction was measured during the day when oxygenic photosynthesis was interrupted or depressed, due to light conditions (clouding effects). The diurnal pattern of nitrogenase activity as well as the acetylene reduction rate was influenced by the variation of all environmental conditions. The highest rate of nitrogenase activity (25 µmol C_2H_4/mg Chl $\underline{a}$/h) detected *in situ* agreed well with maximal nitrogenase activity found in pure cultures of *Oscillatoria limosa*.

Introduction

The fixation of molecular nitrogen counteracts losses of combined nitrogen due to denitrification. The enzyme responsible for nitrogen fixation, nitrogenase, has only been found in procaryotic organisms. Although nitrogenase is extremely sensitive to oxygen, several species of cyanobacteria are known as potent nitrogen fixers. A number of mechanisms have been shown to be instrumental in protecting nitrogenase in these oxygen evolving phototrophs. These mechanisms include the separation of oxygenic photosynthesis and nitrogen fixation. This separation can be either spatial (heterocystous species) or temporal (non-heterocystous species). In contrast with the considerable solid data that are available for cultures of cyanobacteria, only very little is known about the mechanisms of nitrogen fixation in natural populations.

The marine environment is nitrogen depleted. In spite of this it was thought for a long time that the marine environment did not inhabit nitrogen-fixing cyanobacteria. This point of view was probably promoted due to the absence of heterocystous species which were regarded for a long time as the only cyanobacteria capable of fixing nitrogen. However, in the tropics the non-heterocystous *Trichodesmium* spp. forms nitrogen-fixing blooms (CARPENTER and PRICE 1976). Likewise marine intertidal sediments have also been shown to inhabit non-heterocystous nitrogen-fixing cyanobacteria. In this paper an overview is given on the interactions of nitrogen fixation, dissolved oxygen and photosynthesis in cyanobacterial mats found in intertidal sediments of the Wadden Sea (southern North Sea).

Results and discussion

North Sea microbial mats

The Shallows of the southern North Sea (Wadden Sea) are an extended area which is influenced by the tidal movement of the sea. A chain of islands separates the Shallows from the North Sea. The littoral zone consists of sediments composed of fine sand and silt. Extended sand flats are alternately flooded or exposed to the atmosphere. The higher parts of the intertidal flats are only irregularly flooded by the sea and particularly here, cyanobacteria may colonize the sediment surface. The (mainly) filamentous species of cyanobacteria stabilize the sediment by trapping the sand grains in their entangled trichomes and glueing the sediment particles by their extracellular polymers (EPS). Eventually a fabric develops, which is generally referred to as a microbial mat. The microbial mats that are located on the North Sea island and nature reserve Mellum have been studied extensively (STAL et al. 1985). It has been established that 2 species of cyanobacteria are of spatial importance. *Microcoleus chthonoplastes* is a cosmopolitan mat-forming cyanobacterium and also presents the main component of established mats in the North Sea. *Oscillatoria limosa* is usually found in considerable numbers in microbial mats and is very often a pioneer organism in barren quartz sand, probably because of its ability to fix nitrogen. Although *M. chthonoplastes* and *O. limosa* are undoubtedly the dominant cyanobacteria, a number of other species are found. These include some different species of *Oscillatoria, Phormidium* and *Spirulina* and the unicellular *Merismopedia* sp. and *Gloeocapsa* sp.. Microscopic examinations did not reveal the presence of heterocystous cyanobacteria.

The colonization of the intertidal sediment is not always immediately noticeable because the green layer of cyanobacteria may be covered by a layer of sand. The sand serves as a filter and protects the cyanobacteria against excessive high light intensity. However, the filamentous cyanobacteria in the mat move with a gliding movement and the organisms may appear at the surface particularly during rainy days with very low light intensity.

The development of a cyanobacterial community on the barren sand is largely dependent on the supply of nutrients. Among these, combined nitrogen presents a problem since sea water is usually depleted of it, while the demand for growth is high. It is therefore not surprising that the pioneer organism is capable of fixing molecular nitrogen. It seems paradoxical that the non-heterocystous cyanobacterium *O. limosa* colonizes the sediment. In culture this species has been shown to be able to fix nitrogen very efficiently. The reason why not the apparently much better adapted heterocystous cyanobacteria become dominant is still obscure. PEARSON et al. (1979) reported the capacity of nitrogen fixation in a strain of *M. chthonoplastes* but nitrogenase activity was much lower than in *O.*

limosa. The strains of *M. chthonoplastes* that were isolated from the microbial mats of the island Mellum induced nitrogenase only under completely anoxic conditions and were not able to grow diazotrophically. At the end of the growth season *M. chthonoplastes* sometimes becomes dominant in freshly colonized sediment, apparently when sufficient input of nitrogen in the sediment has occured.

In freshly colonized sediment the cyanobacteria are loosely associated with the substrate and are distributed in the upper 1 cm. The presence of cyanobacteria at this relatively great depth was explained by the fact that the low biomass attenuated the light only little. Respiration was low because the low photosynthetic biomass allows only limited heterotrophic activity. Another important feature of freshly colonized sediment is that the loose sediment promotes gas diffusion. Oxygen diffuses well into the deeper layers of the sediment. Even during the dark the rate of respiration does not usually exceed the transport of oxygen into the sediment by diffusion from the air. This means that the freshly colonized fine sandy sediment will generally not be faced with anoxic conditions. During the day when the cyanobacteria are actively photosynthesizing, oxygen supersaturation occurs. As a result of permanent oxic conditions, sulfate reduction does not take place at a significant rate. This is also obvious from the absence of the black layer of FeS.

Established microbial mats are found as a strip along the vegetation border. The cyanobacterial layer is characterized by a very high biomass and is almost entirely organic. In this type of mat the cyanobacteria form a tough and leathery fabric with their entangled trichomes and extracellular polymers. The high organism density results in a very steep light gradient. The cyanobacteria are therefore limited to the uppermost 1-2 mm of the sediment. Established microbial mats generally consist of *M. chthonoplastes*. However, *O. limosa* frequently occurs in considerable numbers and its presence correlates with nitrogen fixation in the *Microcoleus*-mat.

The high biomass content of established mats promotes high rates of respiration which greatly exceed oxygen input by diffusion. Therefore such mats turn anoxic in the dark. Underneath the layer of cyanobacteria, oxygenic photosynthesis cannot take place and permanent anoxic conditions prevail. The established microbial mat inhabit very active populations of sulfate-reducing bacteria. The sulfide that is produced may serve as an electron donor for anoxygenic photosynthesis. Although it is likely that in North Sea microbial mats the cyanobacteria are responsible for a considerable part of the oxidation of the sulfide, the purple sulfur bacteria sometimes appear as a distinct pink layer underneath the cyanobacteria (the german designation 'Farbstreifensandwatt' applies to this particular microbial community).

Interactions of nitrogenase and oxygen

Plain sea water only contains minimal amounts of combined nitrogen and the sand flats are particularly devoid of this essential nutrient. Such conditions are selected specifically for nitrogen-fixing cyanobacteria. This thesis was confirmed by our investigations. Nitrogen fixation was found to be particularly important during colonization of barren sand which does not contain combined nitrogen or in spring when the growth of the cyanobacterial community starts and the demand for nitrogen is high. In the proceeding section it is emphasized that heterocystous cyanobacteria are usually absent and that nitrogen fixation is carried out by the non-heterocystous cyanobacterium *O. limosa*. Culture experiments with this organism showed that nitrogen fixation and oxygenic photosynthesis are separated temporally (STAL and KRUMBEIN 1987). When O. *limosa* was grown under alternating light-dark cycles, nitrogenase activity was confined predominantly to the dark period. Also under continuous light oxygenic photosynthesis was separated temporally from nitrogen fixation. During the dark the energy demand for nitrogen fixation was covered by aerobic respiration. However, if O. *limosa* was grown under alternating light and dark periods and the latter was kept anoxic, a totally different pattern of nitrogenase activity was found. Under anoxic conditions in the dark the generation of energy is very limited, presenting a serious problem for the maintenance of nitrogenase activity. In such cultures nitrogenase activity is observed with maxima at the transition from light to dark and vice versa (STAL and HEYER 1987). There is a good deal of evidence from culture experiments with cyanobacteria that oxygenic photosynthesis and nitrogen fixation are incompatible. Our work has shown that this is also true for natural communities of cyanobacteria.

In freshly colonized sediment nitrogenase activity was usually confined to the night. During the day the sediment was often supersaturated with oxygen whereas during the night the sediment remained oxygenated. Established mats turned anoxic during the night. On several occasions the same pattern of nitrogenase activity was seen as in cultures of O. limosa that were grown under light-dark cycles with anoxic conditions during the dark: peaks at sunset and sunrise. The nitrogenase activity peak at sunrise appeared to be especially significant. It was seen on virtually every occasion and in both mat types (VILLBRANDT et al. 1990).

It must be emphasized that very distinct patterns of nitrogenase activity in the microbial mat greatly depend on the light conditions. During a bright, cloudless sunny day light intensity will follow a sinus. Oxygenic photosynthesis and the concentration of dissolved oxygen will roughly follow the variations in light intensity. However, in a temperate climate, light intensity may be very low throughout the day or may fluctuate as a result of a clouded sky. A strongly fluctuating light intensity results for instance in enormous shifts in the oxygen gradient in the microbial mat. Such shifts have been shown to have a great impact on the nitrogenase activity. A sudden increase in light intensity results in an immediate increase of dissolved oxygen and vice versa. Unfortunately nitrogenase assays in the field cannot be carried out at the same short time scale but nevertheless it was shown that when light intensity fluctuates, nitrogenase activity also showed an irregular pattern. These results suggest that nitrogenase activity may be tuned to oxygen via a switch-off/on mechanism.

Although the presently existing data indicate that oxygenic photosynthesis and nitrogen fixation are temporally separated in North Sea microbial mats, a spatial separation is also possible. STAL et al. (1984) showed that the specific nitrogenase activity (expressed as chlorophyll a) increased with depth, reaching a maximum at between 2-3 mm. At this depth oxygenic photosynthesis is not possible but due to the much greater penetration of far-red light, the anoxygenic photosystem may still be able to provide energy. Therefore it is possible that cyanobacteria move up- and downwards through the mat, carrying out oxygenic photosynthesis and nitrogen fixation in the upper and lower mat horizonts, respectively.

Conclusions

Cyanobacterial mats are found in nutrient-depleted (marine) environments. The photoautotrophic cyanobacteria are responsible for the input of organic material (primary production) which is the basis for the development of a community of microorganisms (microbial ecosystem). The standing crop biomass and photosynthetic rates of a well-developed microbial mat are high. Because the concentrations of combined nitrogen are low, nitrogen-fixing organisms are of particular importance. Obviously nitrogen-fixing cyanobacteria have an ecological advantage. Nevertheless, heterocystous cyanobacteria do not occur in significant numbers, notwithstanding the fact that these organisms are highly specialized for nitrogen fixation under oxic conditions. The reasons for the exclusion of heterocystous species from the mat are not known but it may have to do with the extremely fluctuating environmental parameters (i.e. anoxic conditions, sulfide). The non-heterocystous *Oscillatoria limosa* has been shown to be a metabolically versatile organism and is responsible for the bulk of the nitrogen fixation. Nitrogen fixation in *O. limosa* is temporally separated from oxygenic photosynthesis. The same mechanism was instrumental in the microbial mat. In addition to the temporal separation a spatial separation of oxygenic photosynthesis and nitrogen fixation at different locations of the mat may occur. A minor part of nitrogenase activity in established microbial mats may be attributed to other microorganisms than cyanobacteria (e.g. sulfate-reducing bacteria).

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