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Urea and DON uptake by a *Lyngbya gracialis* dominated microbial mat: a controlled laboratory experiment

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ABSTRACT: The uptake of urea and dissolved organic nitrogen by a *Lyngbya gracialis* dominated microbial mat was studied in a double chambered continuous flow-through system in a 12:12 h light:dark cycle experiment and during a prolonged dark incubation. In addition, the fluxes of total inorganic carbon, oxygen, dissolved free amino acids, nitrate and ammonium across the microbial mat were studied. In the 12:12 h light:dark cycle, the microbial mats grew exponentially (0.5 d^{-1}) with dissolved organic nitrogen as a nitrogen source. Dark urea uptakes were 50 to 85% of the urea uptakes in light. The uptake of urea continued during the 2 wk of prolonged dark incubation. Natural microbial mats may be important regulators of urea dynamics in shallow marine environments, as the microbial mat efficiently took up urea at concentrations somewhat higher than *in situ* urea concentrations in surface sediments.

KEY WORDS: Cyanobacteria · Fluxes · Flow-through system · Microbial mats · Nitrogen retention

INTRODUCTION

The release of nitrogen from coastal marine sediments can supply 10 to 200% of the nitrogen requirements of pelagic primary producers (Blackburn & Henriksen 1983, Klump & Martens 1983). Measurements of the nitrogen efflux from the sediment to the water column have traditionally included inorganic nitrogen compounds (NH_4^+, NO_2^- and NO_3^-), whereas studies on dissolved organic nitrogen (DON) fluxes from the sediment to the water column are rare (Enoksson & Rüdén-Berg 1983, Hopkinson 1987, Boucher & Boucher-Rodoni 1988, Hansen & Blackburn 1991, 1992, Burdige & Zheng 1998, Lomstein et al. 1998). Urea can be a quantitatively important component of the nitrogen efflux from the sediment to the water column (Boucher & Boucher-Rodoni 1988, Lomstein et al. 1989), as the efflux of urea can account for 10 to 100% of the total urea + inorganic nitrogen flux (Lomstein et al. 1989, Lomstein unpubl.)

Microbial mats situated on the sediment surface may be important regulators of the exchange of DON between the sediment and the water column in shallow marine environments. Microbial mats are complex communities of photoautotrophic, photoheterotrophic, chemoautotrophic and heterotrophic microorganisms (Jørgensen et al. 1983, Stal et al. 1985). Cyanobacteria are, in many cases, the most important mat-building organisms (Jørgensen et al. 1983). Microbial mats are found in physically and chemically versatile environments such as marine intertidal flats, hypersaline waters and hot springs (Jørgensen et al. 1983, Bauld 1984, Castenholz 1984, Gerdes & Krumbein 1984, Stal et al. 1985). Numerous field and laboratory investigations have studied the influence of microbial mats on inorganic nitrogen exchange between the sediment and the water column (Henriksen et al. 1980, Andersen & Kristensen 1988, Sundbäck & Granéli 1988, Sundbäck et al. 1991, Bebout et al. 1994). However, the impact of microbial mats on benthic urea and DON fluxes has to our knowledge not been studied previously.

The aim of the present study was to evaluate (1) the ability of microbial mats to take up urea supplied from

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below the mat, (2) the diel variation in urea uptake and (3) the effect of a prolonged dark incubation on the uptake of urea. The experiments were conducted in a double chambered continuous flow-through system. The advantage of this system was that physical and chemical parameters such as light intensity, pH, temperature, salinity and nutrient concentrations could be controlled and sediment processes were excluded.

MATERIALS AND METHODS

Sampling (microbial mat enrichment). Seawater, mud, sand, eelgrass, and mussels were collected from Gammel Løgten Beach (Aarhus Bay, Denmark) and transferred to 3 aquaria. The eelgrass and mussels were placed on the bottom of the aquaria and covered with a layer of sand. The mud was placed above the sand and finally seawater was added. The 3 aquaria were incubated at 16°C in a 12 h:12 h light:dark cycle. Light was supplied from a fluorescent lamp (Osram L30W/77 fluora) and the light intensity was 90 to 250 µE m^{-2} s^{-1}. Dense cyanobacterial mats developed after 3 wk of incubation. The mats were dominated by *Lyngbya gracialis*, but they also contained *L. aestuarii*, *Oscillatoria* spp., *Beggiatoa* spp., diatoms, coccoid cyanobacteria, flagellates such as *Exuviaella* sp., and unidentified bacteria.

Experimental set-up. Two 25 l water reservoirs supplied media to the top and bottom half-chambers, respectively of the experimental continuous flow-through system (Fig. 1). Two Erlenmeyer flasks (500 ml) were inserted between the reservoirs and the chambers, to bubble the medium with sterilised atmospheric air. The Erlenmeyer flasks were submerged into a thermostated water bath (20°C). A combined bubble trap and a drip system between the pump and each growth chamber captured air bubbles and prevented bacterial contamination of the reservoir media. The complete chamber set-up consisted of a top and a bottom half-chamber, which were connected by a permeable filter membrane overlaid with solidified gelrite (Fig. 1). A detailed description of the filter is given below. The aerated media was pumped through the 50 ml top and bottom half-chambers with a Watson-Marlow pump at a flow rate of 1 ml min^{-1}. The resultant renewal time of water in the top and bottom half-chambers was 50 min. All connections within the system were made of Viton VA 70 (Verneret) tubes. The seawater flowing through the top and bottom half-chambers of the continuous flow-through system was a modified Waterbury (1992) SNAX medium. The medium supplied to the top half-chamber was phosphate-free and contained vitamin B_{12} (50 pg l^{-1}). The medium supplied to the bottom half-chamber contained urea (1 mmol urea-N l^{-1}). The SNAX medium was made of seawater from the sampling site and the final pH and salinity of the medium was 8.2 and 11.6‰, respectively. The use of natural seawater as part of the SNAX medium resulted in minor changes in the concentrations of specific compounds between batches.

Inoculation and establishment of the microbial mat. The mats were rinsed with GF/C filtered seawater under dim light in order to remove eelgrass and sand particles. An autoclaved gelrite solution (0.002 w/v) was pipetted onto the top of an anodisc filter, which was fixed to the bottom of a polycarbonate cylinder (inner diameter 20 mm, height 2 mm; Fig. 1). The rinsed mats were inoculated on top of the gelrite solution immediately after the gelrite solution reached a temperature of 30°C. Two top half-chambers were inoculated with mat material. One chamber set-up was used in a light:dark experiment and the other chamber set-up was used in the long-term dark experiment. A third chamber set-up was not inoculated and served as a control. The chambers were placed in a thermostated water bath at 17.0 ± 0.5°C.

The growth of the microbial mats was restricted to the filters as phosphate was only supplied to the bottom half-chamber. The water in the top half-chamber was stagnant during the first 2 d to allow the mat to establish; hereafter the top half-chamber was connected to the flow. The water bodies of the top and bottom half-chambers were stirred with a magnet (60 rpm). The microbial mats were preincubated for an additional 2 d in a 12:12 h light:dark cycle. Light was supplied from an Osram (41860 SP, 12 V, and 20 W) halogen lamp and the light intensity was 35 µE m^{-2} s^{-1}. The tube system was rinsed with 1 N HCl followed by a thorough wash with Milli-Q water the day before the flux measurements were initiated. Day 1 refers to the first day of the flux measurement.

Incubation and sampling. The appropriate sampling time in the light and the dark periods, where steady state was established, was determined in a pilot experiment. In the pilot experiment samples for urea, O_2 and CO_2 measurements were taken 4 times in the light period and 4 times in the dark period. Urea uptake rates and O_2-production and -consumption rates were constant after ~2 h in the light (urea: 0.516 ± 0.006 mmol N m^{-2} h^{-1}, n = 4) and in the dark (0.455 ± 0.006 mmol N m^{-2} h^{-1}, n = 4; O_2-production: 4.26 ± 0.34 mmol m^{-2} h^{-1}, n = 4; O_2-consumption: 3.12 ± 0.74 mmol m^{-2} h^{-1}, n = 4). CO_2-production and -consumption rates were constant after ~4 h in the dark or in the light (CO_2 production: 7.12 ± 0.32 mmol m^{-2} h^{-1}, n = 3; CO_2 consumption: 5.64 ± 1.03 mmol m^{-2} h^{-1}, n = 3). The results obtained at the first sampling in the light or in the dark period were not included in the calculation of the average CO_2 fluxes as steady state was not reached at that time. On the basis of the results ob-

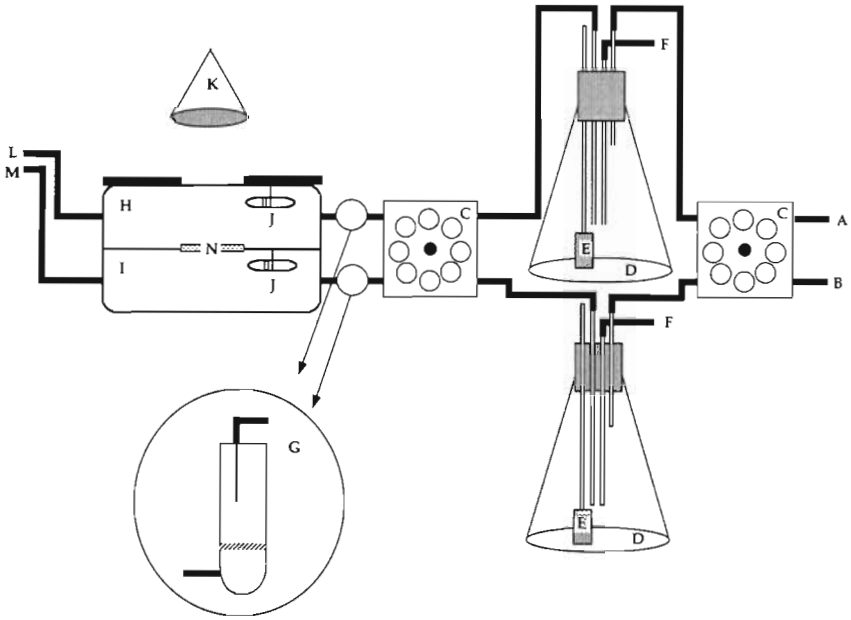

Fig. 1. The double-chambered, continuous flow-through system. (A) medium (25 l reservoir) for the top half-chamber, (B) medium (25 l reservoir) for the bottom half-chamber, (C) Watson-Marlow pump, (D) 500 ml Erlenmeyer flasks submerged in a thermostated water bath, (E) inlet of sterilized atmospheric air through a bubble stone, (F) sampling of inflowing water to the top and bottom half-chambers, (G) a combined bubble trap and drip system, (H) top half-chamber, (I) bottom half-chamber, (J) Teflon-coated magnet driven by a large rotating magnet (not shown), (K) Osram (41860 SP, 12V, 20W) halogen lamp, (L) sampling of outflowing water from the top half-chamber, (M) sampling of outflowing water from the bottom half-chamber and (N) polycarbonate cylinder on which the anodisc filter was mounted

tained in the pilot experiment it was decided that sampling was to be carried out after 10 h in the light or in the dark in order to ensure that steady-state conditions were reached.

Two experiments were carried out: (1) a long-term diel study where the microbial mat was exposed to a 12:12 h light:dark cycle (Expt 1) and (2) a study on the effect of a prolonged dark period (Expt 2). The total incubation time in the long-term diel study was 11 d and sampling of inflow and outflow water took place on Days 1, 2, 5, 7, 8, 10 and 11. In Expt 2 (the prolonged dark incubation), the microbial mat was exposed to a 12:12 h light:dark cycle for 7 d followed by a 16 d dark incubation. Sampling was performed as described in Expt 1. On Days 12 and 23 (i.e. after 5 and 16 d in the dark) the inflow and outflow water was sampled twice a day.

Inflow water was collected from the Erlenmeyer flasks except for samples for the determination of O_2. These samples were taken from the O_2-collection chambers mounted before and after the growth chamber set-up (Fig. 1). Outflow water was collected from the outflow of the top and bottom half-chambers.

Water samples for the analysis of DON, dissolved free amino acids (DFAA), urea-N, NH_4^+ and NO_3^- were filtered through a 0.2 µm Sartorius filter and frozen for later analysis. Samples for the determination of total inorganic carbon ($C_T = HCO_3^- + CO_3^{2-} + CO_2$) were collected in 6.5 ml gas-tight vials (Labco, Exetainers), conserved with 40 µl 5% (w/v) $HgCl_2$ solution and stored in the dark at 5°C for later analysis. The samples were never stored for more than 2 wk. O_2 samples were pumped with a peristaltic pump (LKB BROMMA, BRD) from the O_2-collection chamber to a chamber equipped with an O_2 microelectrode for direct measurement of the O_2 concentration (Revsbech 1989)

Analytical procedure. The concentrations of urea-N and NH_4^+ were measured on a Technicon Autoanalyzer by the diacetylmonoxime method described in Price & Harrison (1987) and the salicylate-hypochlorite method (Bower & Holm-Hansen 1980), respectively. The concentration of NO_3^- was analysed by HPLC (Sykam, Gilching, Germany) equipped with an UV detector (Spectro-Monitor 3200) at a wavelength of 220 nm. The eluent was 20 mM NaCl and the flow rate was 1.5 ml min^{-1}. Total dissolved nitrogen (dissolved organic + inorganic nitrogen) was analysed on a modified Antek 7000 system as described in Lomstein et al. 1998). The concentration of C_T was measured on a flow injection system as described by Hall & Aller (1992). DFAA concentrations were determined by high performance liquid chromatography (HPLC; Waters Chromatographic System) according to Lindroth & Mopper (1979).

Calculations. The fluxes (F) of gasses and dissolved compounds across the microbial mat-water interface were, with the exception of the urea flux, calculated by use of the Nishio et al. (1983) formulation:

$$F = V(C_o - C_i)/A$$

where V is the flow rate, C_i is the concentration in the inflowing water, C_o is the concentration in the outflowing water and A is the surface area of the mat (A = 3.1 cm^2). The net urea fluxes were calculated from the concentration difference in the top half-chambers of the inoculated set-up and the control set-up. The net uptake rates of DON, urea-N, DFAA, NH_4^+, NO_3^- and C_T and the net production rate of O_2

by the microbial mat were calculated as the difference between the net fluxes in the control chamber set-up and the microbial mat chamber set-up. Gross primary production was calculated as C_T uptake in light plus dark C_T release. It was assumed that respiration in the light equalled the measured dark respiration.

The C:N uptake ratio by the microbial mat was determined as the gross primary production divided by the DON uptake. The average C:N uptake ratio given in the discussion is the mean of the C:N ratios obtained on Days 8, 10 and 11 in Expt 1.

RESULTS

The diffusion of urea-N from the bottom half-chamber to the top half-chamber of the control chamber set-up resulted in a urea-N concentration in the top half-chamber of 5.8 to 6.8 µM N. Differences in the urea concentration in the SNAX medium resulted in slightly variable urea-N concentrations in the control top half-chambers. The urea concentration in the top half-chamber in the long-term diel experiment varied between 1.9 and 3.28 µM in the light and 3.09 and 4.32 µM in the dark. In the prolonged dark experiment the concentration varied between 1.0 and 1.4 µM in the light and 2.0 and 3.5 µM in the dark. The variation in the urea concentration was due to differences in the urea concentration in the batch of natural seawater, which was used for the modified SNAX medium. The difference in the O_2 concentration in the inflow and outflow water of the top half-chamber of the control chamber set-up was 1 to 2 µM. However, the O_2 concentration in the inflow water changed from 258 µM in the beginning of the experiment to 224 µM on Day 10. After Day 10 the O_2 concentration remained constant. In the long-term diel experiment the O_2 concentration in the outflow water differed, with ~70 µM between light and dark conditions. The lowest out flow O_2 concentration obtained in the dark was 210 µM and the highest concentration in the light was 282 µM. A similar difference in the outflow O_2 concentration between light and dark was obtained during the light:dark cycles (first 7 d) of the prolonged dark experiment. The DON concentration in the inflow water fell in the range of 5.6 to 11.6 µM dependent on the batch of seawater that was used for the modified SNAX medium. The C_T concentration in the inflow water varied between 1750 and 1900 µM, which was also dependent on the seawater batch used for the modified SNAX medium.

Long-term diel experiment

The diel variation in the net fluxes of O_2, C_T and DON and in the uptake of urea during the 11 d of incubation is shown in Fig. 2. There was a net production of O_2 and a net uptake of C_T by the microbial mat in the light, except during the first 2 d of incubation, when there was a net uptake of O_2 and a net production of C_T (Fig. 2a,b). The net production of O_2 and the C_T uptake in the light varied between 2.8 and 11.3 mmol O_2 m^{-2} h^{-1} and 4.1 and 12.1 mmol C_T m^{-2} h^{-1}, respectively, during the remaining part of the experiment. Net dark O_2 uptake and C_T production rates varied between 1.7 and 6.6 mmol O_2 m^{-2} h^{-1} and 0.6 and 5.3 mmol C_T m^{-2} h^{-1}, respectively, from Day 5 to Day 11. The gross primary production rate increased from 93.4 mmol C m^{-2} d^{-1} on Day 5 to 154.1 mmol C m^{-2} d^{-1} on Day 8. After Day 8 the gross primary production remained almost constant (150.7 to 151.7 mmol C m^{-2} d^{-1}, data not shown). There was an exponential increase in the cumulated net primary production by the microbial mat during the time span of the incubation (Fig. 3) and the growth rate constant was 0.5 d^{-1}.

There was a net uptake of DON by the microbial mat in both light and dark from Day 8. The DON uptake rates varied between 0.5 and 0.8 mmol N m^{-2} h^{-1} (Fig. 2c). On Days 5 and 7, there was a release of DON in the light of 0.7 and 0.6 mmol N m^{-2} h^{-1}, respectively, whereas DON was released in the dark on Day 5 (0.3 mmol N m^{-2} h^{-1}) and taken up in the dark on Day 7 (0.5 mmol N m^{-2} h^{-1}). It was not possible to calculate a flux of DFAA, as the concentration of DFAA was always below the detection limit of the analytical system. The microbial mat took up urea-N in both light and dark, but the uptake rates were higher in the light

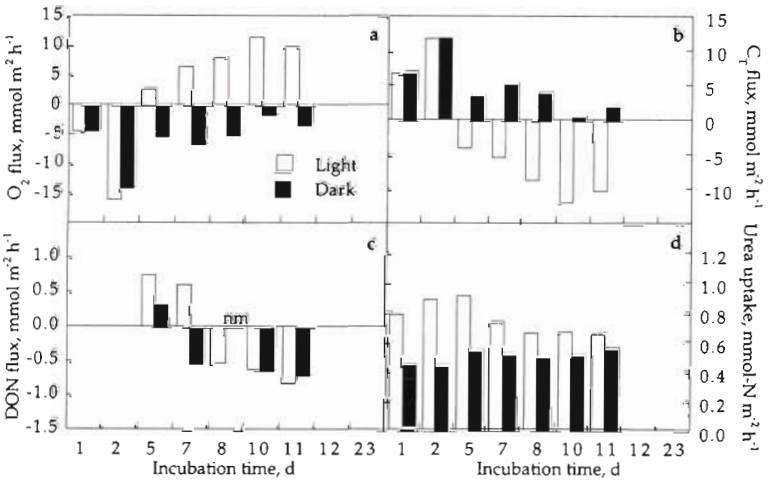

Fig. 2. Diel variation in net fluxes of (a) O_2, (b) total inorganic carbon (C_T), (c) DON and (d) net urea-N uptake during the 11 d of incubation in the long-term diel experiment. The net DON fluxes given in (c) are fluxes of DON other than urea

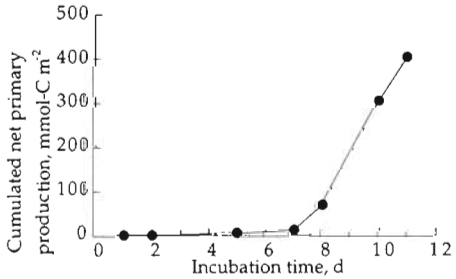

Fig. 3. Cumulated net primary production of the microbial mat in the long-term diel experiment

than in the dark (Fig. 2d). Light and dark urea-N uptake rates varied between 0.7 and 0.9 and between 0.4 and 0.6 mmol N m^{-2} h^{-1}, respectively, during the 11 d of incubation. The difference between light and dark urea-N uptake rates decreased with increased time of incubation. NH_4^+ was taken up by the microbial mat during the first 2 d of incubation, where the uptake rates were 0.8 and 1.1 mmol m^{-2} h^{-1}, respectively (data not shown). NO_3^- was not taken up by the microbial mat, despite the NO_3^- concentration in the inflowing water being 35 to 43 µM during the entire experiment.

The total uptake of DON + urea-N by the microbial mat increased from 4.5 mmol N m^{-2} d^{-1} on Day 5 to 32.6 mmol N m^{-2} d^{-1} on Day 11. This change was mainly due to the fact that the microbial mat was a source of DON other than urea in the beginning of the experiment, whereas it was a sink for both DON and urea during the remaining part of the experiment.

Effect of a prolonged dark incubation

The effects of a prolonged dark incubation on net O_2 and DON fluxes and the uptake of urea are shown in Fig. 4. Prior to the dark period (Day 7), the net fluxes of O_2 and DON and the urea-N uptake in the light and in the dark were similar to the respective light and dark rates obtained on Day 7 in the long-term diel incubation (see Fig. 2a,c,d). Due to analytical problems, C_T was not measured during the prolonged dark incubation.

There was a decrease in the O_2 uptake from Day 8 (3.9 mmol O_2 m^{-2} h^{-1}) to Day 10 (1.5 mmol O_2 m^{-2} h^{-1}); hereafter the O_2 uptake remained low (1.6 to 2.1 mmol O_2 m^{-2} h^{-1}, Fig. 4a). The net uptake of DON was higher during the first 3 d of the prolonged dark incubation (0.3 to 1.2 mmol N m^{-2} h^{-1}) than the dark uptakes of DON in the same period in the long-term diel incubation. The dark DON uptake rates varied between 0.6 and 0.7 mmol N m^{-2} h^{-1} in the long-term diel incubation (compare Fig. 4b and Fig. 2c). The uptake of urea remained unchanged in the prolonged dark incubation (Fig. 4c) compared to the dark uptake of urea in the long-term diel incubation.

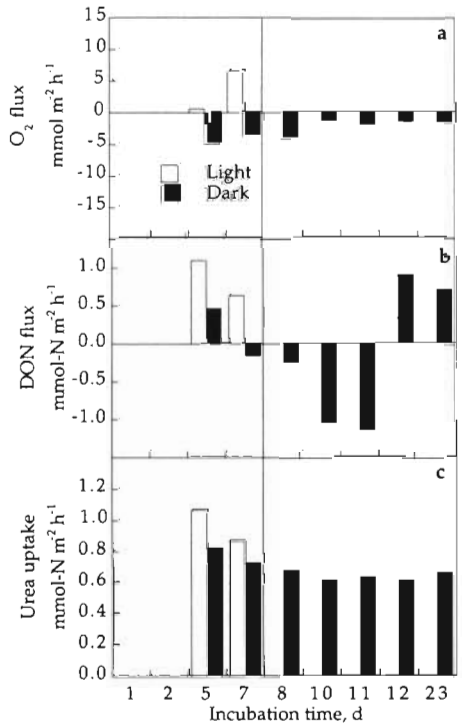

Fig. 4. Net (a) O_2 and (b) DON flux rates and (c) net urea uptake rates during the 23 d of incubation in the prolonged dark experiment. During the first 7 d the microbial mat was incubated in a 12:12 h light:dark cycle. After Day 7 the chamber was incubated in the dark (indicated by the vertical line)

DISCUSSION

Growth of the microbial mat

The microbial mat grew exponentially with C_T as the carbon source and DON as the nitrogen source during the long-term diel experiment. The growth rate constant was 0.5 d^{-1} (Fig. 3). We assume that *Lyngbya gracialis* was the major primary producer, as microscopic inspection of the microbial mat showed that it was the dominating phototrophic organism. The calculated growth rate constant of 0.5 d^{-1} was comparable to previously reported growth constants for pure cultures of cyanobacteria grown on urea as the only nitrogen source: *Anabaena variabilis*, 0.5 d^{-1} and *Nostoc muscorum*, 0.8 d^{-1} (Kratz & Myers 1955) and *Pseudoanabaena catenata*, 0.7 d^{-1} (Healey 1977). The average molar C:N uptake ratio by the microbial mat of 5.2 ± 0.54 (n = 3) was in good agreement with the molar C:N ratio of cyanobacteria (5.0; Flores et al. 1983) and marine bacteria (4.3 to 5.3; Fagerbakke et al. 1996). This indicated that the microbial mat was not limited by nitrogen during growth, as nitrogen limitation would have resulted in an elevated C:N ratio.

DON and urea-N uptake

The microbial mat community was able to take up urea at concentrations exceeding those normally encountered in surface sediments (<26 µM N; Lomstein et al. 1989, 1998, Lund & Blackburn 1989, Therkildsen & Lomstein 1994). It is not possible to give the exact urea concentrations experienced by the microbial mat in the present study, as the size of the diffusive boundary layer below the filter, the diffusive boundary layer above the microbial mat and the thickness of the microbial mat were not measured. However, it was possible to make a rough estimate of the urea concentrations at the lower and upper parts of the microbial mat, respectively, by using Ficks 1' law of diffusion in the different compartments (i.e. the lower diffusive boundary layer, the filter, the microbial mat and the upper diffusive boundary layer). It was assumed that the diffusive boundary layers below the filter and above the mat were both 200 µm, that the microbial mat was 200 µm and that the diffusion coefficient of urea remained constant in the different compartments. The resultant concentration of urea at the lower side of the microbial mat was 159 µM, whereas the urea concentration at the surface of the microbial mat was 83 µM. The microorganisms responsible for the uptake of urea were not identified. Pure culture studies with cyanobacteria (Healey 1977, Rai & Singh 1987), heterotrophic bacteria (Jahns 1992) and studies of mixed natural populations of cyanobacteria (Takamura et al. 1987) have shown that both heterotrophic bacteria and cyanobacteria are able to take up urea. The stimulated urea uptake in the light infers that the process was energy dependent, as previously demonstrated for the heterotrophic marine bacterium *Deleya venusta* HG1 (Jahns 1992) and several pure cultures of cyanobacteria (Rai & Singh 1987).

In addition to urea, the microbial mat took up other DON compounds, which were present in the seawater used for the basic medium. Paerl et al. (1993) showed that microbial mats were able to take up DFAA. As DFAA were not present at detectable concentrations in our medium, we can conclude that the microbial mats in the present study were able to take up additional DON constituents other than urea and DFAA.

Dark incubation

The prolonged dark incubation experiment was performed to study the initial phase of microbial mat break down. After 2 d in the dark, the O_2 uptake was reduced to 50% of the dark rate measured prior to the dark incubation. It is likely that the decreased O_2 uptake 2 d after the onset of the dark incubation was due to changed substrate availability by the heterotrophic component of the microbial mat.

Interestingly, urea was taken up throughout the entire dark period at a constant rate, while the DON uptake (exclusive urea) increased during the first 3 d of the dark period. It is not clear whether urea and DON were stored or used as nitrogen sources by the heterotrophic mat community. However, as the microbial mat maintained urea uptake during the first days of the dark incubation, the microbial mat community remained a sink for nitrogen also under heterotrophic conditions.

There was a net release of DON from the microbial mat after 4 d of dark incubation, which was indicative of a change in the metabolism of the microbial mat community. DON may have originated from the breakdown of cell material, such as dead cyanobacteria or material from intracellular stores, which were hydrolyzed during cell degradation.

Conclusions

The microbial mats served as an efficient filter for urea supplied from the bottom chamber in the continuous flow-through system. The urea-N concentrations experienced by the microbial mat in the present study (~83 to 159 µM N) were higher than urea-N concentrations in surface sediment (<26 µM N). In addition, it was shown that the microbial mat took up other DON compounds than urea at rates similar to the urea uptake. It is suggested that microbial mats in shallow marine areas are important sinks for organic as well as inorganic nitrogen.

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