Bacterial nitrification and denitrification rates in the Rhône River plume (northwestern Mediterranean Sea)

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ABSTRACT: Water samples were collected in May 1992 from turbid plume water along several transects of increasing salinity from the Rhône River mouth to the sea. Nitrogen salt concentrations (NH4+, NO2-, NO3-) and nitrification, denitrification and nitrate reduction were determined. NH4+ values, measured in the plume water, were lower than those corresponding to a conservative dilution, demonstrating a loss of 2 μmol l⁻¹ of NH4+. In situ concentrations of NO2- differed by 10 to 30 μmol l⁻¹ from theoretical values (conservative dilution), showing a net consumption of this compound. NO3- concentrations stayed close to the conservative dilution curve plots. Along the salinity gradient, ammonium and nitrite oxidation rates decreased from 2 to 0.2 and 1 to 0.1 μmol l⁻¹ d⁻¹ respectively. These 2 rates correlated well with in situ NH4+ concentrations. 15% of the allochthonous NH4+ was nitrified. Dissimilative nitrate and nitrite reduction rates displayed similar values, decreasing from 380 to 7 μmol l⁻¹ d⁻¹. Denitrification ranged from 0 to 7 μmol l⁻¹ d⁻¹, independently of the salinity value. 3.5% of the allochthonous NO3- was denitrified. In the plume, denitrification rates were 30 to 100 times lower than nitrate reduction, while at a salinity > 20 psu, these 2 processes occurred at similar rates. A significant correlation was demonstrated between the nitrate reduction rate and the difference between theoretical and in situ NO3- concentrations. The deficit in nitrate in the plume could arise from the nitrate reduction process.

KEY WORDS: Nitrification · Denitrification · Nitrogen budget · Rhône River plume · Mediterranean Sea

INTRODUCTION

In contrast to most of the Mediterranean Sea, where evaporation processes dominate the freshwater inputs by rivers and rain, the northwestern Mediterranean basin is diluted by the Rhône River outflow. This river constitutes an important source of fresh water (average of 2000 m³ s⁻¹). A layer of about 1 m vertical thickness and 1 to 6 km length, with a high load of dissolved and particulate matter, overlays the particulate- and nutrient-poor seawater. As the Mediterranean Sea is oligotrophic, the river runoff contribution to the nitrogen budgets and fluxes is of great importance. An increase in primary production has already been demonstrated near the edge of the plume (Coste et al. 1977, Minas et al. 1988, Videau & Leveau 1990), but the role of these nutrient enrichments in the global productivity of the northwestern Mediterranean Sea remains in question.

Nitrogen inputs from the Rhône River are mainly composed of nitrate (50 to 160 μM), the concentration of which displays annual fluctuations 1 to 3 times the average concentration (Coste 1974). Ammonium contributes about 10 to 15 μM, while nitrite inputs stay low (≤3 μM) and neither of them shows annual variations (Coste 1974). Chemical and physical processes such as flocculation, adsorption and desorption do not influence the concentrations of other mineral compounds, e.g. phosphorus (Coste 1974).

Ammonium and nitrate concentrations in estuaries are greatly influenced by anthropogenic inputs. In estuaries with high nutrient content (reviewed by Sharp 1983), conservative or nonconservative dilution can be demonstrated on the basis of nitrogen salt concentrations. Microbial processes, e.g. nitrification and denitrification, are able to influence the turnover of inorganic nitrogen inputs. In particular, ammonium could
be oxidized by nitrification processes, as was shown for estuaries of the Tamar River (Owens 1986), the Delaware River (Lipschultz et al. 1986) and Narragansett Bay (Berounsky & Nixon 1990). On the other hand, in the oxygenated water column, the particulate material provides micrones allowing anaerobic processes (Bianchi et al. 1992). In highly turbid estuarine waters which are nepheloid layers the abundance of particles, combined with high nitrate concentration, favours nitrate reduction and denitrification, which has to be taken into account for the nitrogen budget.

In the superficial water of the Rhône River plume, corresponding to a nepheloid layer (Aloisi et al. 1979), nitrification, denitrification and nitrate reduction rates were measured along the gradient of increasing salinity. The influence of these bacterial processes on the behaviour of the nitrogen inputs in the marine area is discussed.

METHODS

In May 1992, water samples were collected from the surface water using polycarbonate bottles along several transects from the Rhône River to the sea. Salinity and temperature were measured with a YSI probe (Yellow Spring Instrument Co.). Subsamples were used for measurements of NH$_4^+$ (Solorzano 1969), NO$_3^-$ (Bendschneider & Robinson 1952) and NO$_2^-$ by Technicon Analyzer (method of Wood et al. 1967, modified according to Tréguer & Le Corre 1975). To counterbalance the salt effect, a first measure was carried out with sulfanilamide only, and the optical density was subtracted in each case from the value obtained with all the reagents together.

Total bacterial counts were performed by an image-analysis system on cells stained with DAPI (Porter & Feig 1980).

Nitrification rates were obtained by measurement of the increase or decrease of nitrate concentrations in subsamples containing allylthiourea (ATU) or NaClO$_3$ to inhibit the oxidation of ammonium and nitrite respectively. The sample was divided into 3 × 1 l subsamples. One (control) was unaltered, one received 10 mg l$^{-1}$ of ATU (final concentration) and the third received 10 mM of NaClO$_3$. No substrate was added, because of the high natural concentrations of ammonium and nitrite. The subsamples were incubated in the dark in a 200 l culture chamber (Facis S.A., France) at the in situ temperature. The nitrite concentration in the subsamples was measured every 4 h from time zero to 24 h, using 10 cm path-length cells. Nitrification rates were calculated from the exponential phase of increase or decrease of nitrite, as already described in Bianchi et al. (1992).

Denitrification rates were measured using 100 ml subsamples kept in 130 ml serum flasks. 100 µl of a NH$_4$Cl solution, giving a final concentration of about 1 mmol ammonium l$^{-1}$ seawater, was added to repress the nitrate assimilation pathway (Tiedje 1988). Flasks were sealed with rubber stoppers and anaerobic conditions were obtained by flushing N$_2$ through the flask for 5 min. Acetylene (15 kPa), which inhibits the reaction from NO$_3^-$ to N$_2$, was added to the flask (Balderston et al. 1976). NO$_3^-$, NO$_2^-$ and N$_2$O analyses were performed after 0.5, 1, 2 and 3 h of incubation at in situ temperature in the dark.

The rate of nitrite reduction can be determined from the difference between the rate of nitrate consumption and the net rate of nitrite production (Bonin & Raymond 1990).

The initial linear rate of N$_2$O accumulation is considered as the in situ denitrification activity. After incubation, 3 ml of the gas phase was sampled using a pre-evacuated venoject tube. Extraction of N$_2$O from the liquid phase was carried out by the procedure of Chan & Knowles (1979) modified by the multiple equilibrium technique (McAullife 1971). Nitrous oxide was determined using a Girdel series 30 gas chromatograph equipped with an electron capture detector as previously described (Bonin et al. 1987).

RESULTS AND DISCUSSION

The distributions of ammonium, nitrite and nitrate concentrations versus salinity are reported in Fig. 1. The difference between theoretical and measured values demonstrates the net production (or loss) for the considered nitrogen compound. The measured in situ values of NH$_4^+$, NO$_2^-$ and NO$_3^-$ were in the same range as those reported by Coste (1974) for the Rhône River plume.

The maximum NH$_4^+$ concentration was 10 µmol l$^{-1}$, a low value compared to those from the Scheldt estuary in Belgium (Somville 1978), the Tamar estuary in England (Owens 1986) and Narragansett Bay, USA (Berounsky & Nixon 1990), where the NH$_4^+$ concentrations were 100 to 1000 µmol l$^{-1}$, 0 to 45 µmol l$^{-1}$ and 10 to 20 µmol l$^{-1}$ respectively. The NH$_4^+$ concentration decreased by a factor of 10 from the river mouth (10 µmol l$^{-1}$) to the sea (1 µmol l$^{-1}$) (Fig. 1a). Below a salinity of 30 psu (practical salinity units), most of the in situ values stayed below the value corresponding to a conservative dilution, demonstrating a loss of ammonium. This loss was around 2 µmol l$^{-1}$. Above 30 psu, a low production of ammonium (1 to 2 µmol l$^{-1}$) was noted (Fig 1a).

The nitrite concentration fluctuated very little, between 3 and 0.5 µmol l$^{-1}$ (Fig. 1b). Low concentrations
of nitrite are generally found in natural waters. The in situ values stayed close (± 0.5 μmol l⁻¹) to the theoretical ones.

Nitrate constituted the most important reservoir of inorganic nitrogen, ranging from 110 μmol of the river to 2 (seawater) μmol l⁻¹. Near the river mouth, when the salinity was less than 20 psu, the in situ concentrations of NO₃ were 10 to 30 μmol l⁻¹ lower than the conservative dilution, indicating a net consumption of this nitrogen salt (Fig. 1c).

From the river mouth to the sea, ammonium and nitrite oxidation rates decreased 10-fold, from 2 to 0.2 and 1 to 0.1 μmol l⁻¹ d⁻¹ respectively. Although the ammonium oxidation rates stayed 2 times higher than nitrite oxidation rates, these 2 rates were also well correlated (r = 0.871, n = 19, p < 0.01) (Table 1). In the Tamar estuary, Owens (1986) also measured higher oxidation rates for ammonium than for nitrite. This author suggested that in these estuarine areas the heavy loads of particulate material favour ammonium oxidizer activity because these bacteria exhibit a greater affinity for attachment to particles than do nitrite oxidizers. The ammonium oxidation rates stayed around 2 μmol l⁻¹ d⁻¹ in the plume, and correlated well with in situ ammonium concentrations (r = 0.888, n = 19, p < 0.01) (Table 1), which exhibited a 10-fold decrease in the salinity gradient. This significant correlation between the rate of ammonium oxidation and the environmental concentration of ammonium is also corroborated when comparing rates reported from different geographical sites. In the Scheldt estuary, rates of 0 to 24 μmol l⁻¹ d⁻¹ were reported for NH₄⁺ concentrations of 100 to 1000 μmol l⁻¹ (Somville 1978); rates of 0.98 to 11.1 μmol l⁻¹ d⁻¹ for 45 μmol l⁻¹ were observed in Narragansett Bay (Berounsky & Nixon 1990) and rates of 1 to 2 μmol l⁻¹ d⁻¹ were reported in the Rhône River plume at NH₄⁺ concentrations close to 10 μmol l⁻¹.

The amount of nitrified ammonium corresponded to the difference (2 μmol l⁻¹) between the NH₄⁺ theoretical dilution curve and the in situ values. In the plume water, where the high load of particulate material drastically limits the light for photosynthesis (Videau & Leveau 1990), the lack of ammonium could be due to its removal by nitrification processes. Furthermore, the shadowing effect of particles removes the inhibition of nitrification by light (Ward 1985). The production of NO₃⁻ by nitrification was low (0.1 to

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Table 1. Spearman rank correlation coefficients for variables: NH₄⁺ oxidation vs NH₄⁺ concentration; NH₄⁺ oxidation vs NO₃⁻ oxidation; nitrate reduction (NAR) vs nitrite reduction (NIR); nitrate reduction (NAR) vs difference between theoretical and in situ NO₃⁻ concentration [NO₃⁻(T - isi)]

<table>
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<tr>
<th>Parameters</th>
<th>r</th>
<th>Slope</th>
<th>Intercept</th>
<th>p</th>
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1 μmol l⁻¹ d⁻¹). On account of the very high load of nitrate (20 to 60 μmol l⁻¹) in the plume water, the impact of nitrate oxidation was difficult to assess.

Bacterial density did not display great changes along the salinity gradient (Table 2).

Dissimilative nitrate and nitrite reduction rates displayed similar values, and demonstrated a strong decrease from plume water to seawater (>380 μmol l⁻¹ d⁻¹ to 7 μmol l⁻¹ d⁻¹). Denitrification rates ranged from 0 to 7 μmol l⁻¹ d⁻¹, regardless of the salinity values (Table 2). In the plume water, denitrifying activity was about 10-fold lower than that generally measured in marine sediments from the western Mediterranean coast (Raymond et al. 1992). Below a salinity of 20 psu, denitrification rates were 30- to 100-fold lower than nitrite reduction, while above this value, denitrification and nitrite reduction occurred at similar rates.

There was no correlation between the rates of reductive processes (nitrate and nitrite reduction, denitrification) and NO₃⁻ concentrations. This suggests that nitrate did not limit either denitrification or nitrate and nitrite reduction. However, a significant correlation (r = 0.65, n = 10, p < 0.01) (Table 1) was observed between nitrate reduction rates and the difference between theoretical and measured NO₃⁻ values. From this correlation, it can be suggested that the deficit in nitrate could arise from high rates of nitrate reduction. Nitrate and nitrite reduction rates were also well correlated (r = 0.78, n = 10, p < 0.01) (Table 1). There was no nitrite accumulation after nitrate reduction, as demonstrated by the similarity between theoretical and measured values of nitrite concentrations.

At the river mouth, data indicated significant nitrite consumption rates which could not be accounted for by denitrification, but which could be due to dissimilatory reduction of nitrite to ammonium. In fact, the rate of dissimilatory nitrate reduction to ammonium may be calculated as the difference between the overall nitrate reduction and denitrification (Jørgensen & Sørensen 1985) in samples amended with acetylene (to block nitrous oxide reduction) and ammonium which may act as an inhibitor of assimilatory reduction of nitrate via nitrite (Payne 1973). However, the activity of dissimilatory nitrite reduction to ammonium could be overestimated because of (1) the failure of the acetylene block (Tam & Knowles 1979) or (2) nitrate assimilatory uptake in spite of incubation conditions usually described as unfavourable to this process (increased ammonium salt concentrations and darkness). Indeed, Anderson & Kristensen (1988) have reported that phytoplankton (diatoms) may continue to assimilate nitrate up to 2 d after their last illumination period.

Near the river mouth, denitrification consumed only 1% of the reduced nitrate, while 99% was presumably recycled to ammonium through a dissimilatory reduction. This dissimilatory reduction process can overcome denitrification in some environments (Nishio et al. 1982). Near the edge of the plume, when salinity reached 30 psu, all the reduced nitrate was due to denitrification. The occurrence of denitrification was unexpected in a superficial water layer. But, recent reports have shown that this may be due to the occurrence of aerobic denitrification (Roberston & Kuenen 1984, Bonin et al. 1989, Bonin & Raymond 1990) and/or

<table>
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<th>Salinity (psu)</th>
<th>NH₄⁺ oxidation</th>
<th>NO₂⁻ oxidation</th>
<th>NO₃⁻ reduction (μmol N l⁻¹ d⁻¹)</th>
<th>NO₂⁻ reduction</th>
<th>Denitrification</th>
<th>Bacteria (x 10⁸ ml⁻¹)</th>
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the presence of anoxic microniches which may support denitrification in oxic environments (Jannasch 1960). Denitrification rates in seawater have been estimated in only one area, the oxygen-deficient water column of the Baltic Sea, and were reported to be from about 3.6 μmol N l⁻¹ d⁻¹ (Shaffer & Ronner 1984) to < 1 μmol N l⁻¹ d⁻¹ (Ronner & Sörenson 1985, Brettar & Rheinheimer 1992).

In the Rhône River plume, the suspended matter load is very high (> 20 mg l⁻¹) allowing the presence of suboxic microzones in the oxygenated water column. Under such conditions, nitrification and denitrification processes could lead to the production of the greenhouse gas N₂O. At low oxygenation levels (ca 0.25 mg O₂ l⁻¹), the last step of denitrification can be inhibited, and the N₂O produced is accumulated (Bonin et al. 1989). Furthermore, at low oxygen concentrations and/or high NH₄⁺ concentrations, nitrification may also enhance N₂O production. So, at reduced oxygen concentrations (ca 0.18 mg O₂ l⁻¹), about 10% of the flux of ammonium through nitrification yields N₂O (Goreau et al. 1980). In addition to the N₂O produced by nitrification, Fuhrman & Capone (1991) have reported that 5% of the denitrified NO₃⁻ could yield N₂O in suboxic water, where the nitrogen cycle is accelerated by the addition of fertilizer. According to these percentages and the rates of nitrification and denitrification we measured, and referring to the annual Rhône River inputs [about 55,000 metric tons (t) of nitrate and 5500 t of ammonium; Coste 1974], 82.5 and 96.2 t of N₂O yr⁻¹ could be produced in the Rhône River plume area by nitrification and denitrification processes respectively. For these reasons, such bacterial processes affecting estuarine areas would have to be taken into account in future calculations of N₂O production.

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