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Use of Nitrifying Activity Measurements for Describing the Effect of Salinity on Nitrification in the Scheldt Estuary

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Nitrifying activity measurements, carried out on freshly collected samples from an estuarine environment, show that the composition of the nitrifier population undergoes a progressive modification during the mixing of freshwater masses in seawater, with increasing tolerance to salt. As a result, the overall effect of increasing salinity on nitrification is much less severe than the direct effect of salt on the freshwater nitrifying population.

The Scheldt estuary is heavily polluted in its upstream part by industrial and domestic sewage, resulting in completely anoxic conditions. Downstream, mixing with well-aerated seawater promotes the microbial reoxidation of the reduced mineral substances (sulfides, reduced iron and manganese, ammonium, etc.) produced in the anaerobic zone (see Billen et al. [1] for a general discussion of the redox balance in the Scheldt estuary).

Among these microbial reoxidation processes, nitrification plays an important role because of the very high ammonium concentrations (500 to 1,200 μM) resulting from organic matter decomposition in the upstream part. It is the ultimate self-purification process before the reestablishment of oxygen saturation of the water.

Figure 1 shows a typical profile of planktonic nitrifying activity measured in the Scheldt estuary by the method of Somville (9) (measurement of N-Serve [Dow Chemical Co., Norfolk, Va.]-sensitive bicarbonate incorporation during short-term incubation under in situ conditions; the ratio of HCO_3^- incorporated to N oxidized is 0.12). These measurements show the very sudden start of nitrification and the corresponding sharp increase of nitrate concentration at salinities ranging from 1 to 5 g of Cl^- per liter. At higher salinities, the nitrifying activity decreases although ammonium is still present, and nitrate concentration is chiefly governed by the dilution of freshwater into nitrate-poor seawater, as indicated by the straight-line relationship between nitrate and salinity.

The comparison of nitrate flux from sediments and of nitrate production by planktonic nitrification (M. Somville, Ph.D. thesis, Université de Bruxelles-Faculté des Sciences, Bruxelles, Belgium, 1980) has shown that the latter process, accounting for more than 90% of the nitrate budget, is by far the most important. This is confirmed by the good agreement of measured planktonic nitrifying activities and activities computed by a model of nitrification in the Scheldt estuary (10).

To understand this peculiar distribution of nitrifying activity in the Scheldt estuary, the effect of several varying environmental factors on the dynamics of nitrifiers must be investigated. Among these factors, salinity might be important because nitrification occurs in the sharpest part of the salinity gradient.

The work described in this note was intended to investigate the effect of salinity on the rate of nitrification in the Scheldt estuary.

Samples were collected in September 1977 and January 1978 in the navigable fairway of the Scheldt estuary at five locations in the salinity gradient, covering a salinity range from 0.4 to 15 g of Cl^- per liter. For each of these samples, the effect of salinity on the potential nitrifying activity was measured in the following way.

The samples were brought to the laboratory within 1 to 2 h, and six subsamples of each sample were mixed with 2 volumes of the standard Winogradski NH_4^+ enrichment medium (7) containing 10 mM NH_4^+. In each subsample, the NaCl concentration was adjusted to give a range of final salinities from 0.5 to 20 g of Cl^- per liter. The rate of N-Serve-sensitive $H^{14}CO_3^-$ incorporation (15 to 20% of the total HCO_3^- incorporation) was then measured after 2 h of incubation at 21°C with sufficient shaking to maintain air saturation.

This procedure ensures minimum modification of the size and composition of the nitrifier populations and allows measurement of their potential activity under optimal growth conditions regarding oxygen, ammonium concentration, pH, and temperature. N-Serve is a specific inhibitor of autotrophic nitrification (2, 8). Although Laurent (6) suggested that heterotrophic nitrifiers might be optimally active at low Eh levels, autotrophic nitrification appears to be the only quantitatively important mechanism in natural waters (11). Our measurements at different salinities therefore reflect the effect of salinity on the natural nitrifier populations under optimal growth conditions, all other parameters being constant.

All five populations displayed a curve of potential activity with respect to salinity with a maximum (Fig. 2). Cooper (3) suggested the existence of such a maximum of potential nitrifying activity under various physicochemical conditions. The level of this maximum can be taken as an index of the general density of the population, whereas the salinity at which this maximum occurs reflects the adaptation of the populations towards salinity. These two aspects will be discussed in turn.

The maximum potential activity severely decreases in the upstream anaerobic reach of the Scheldt estuary when the chlorinity increases from 0 to 5 g of Cl^- per liter (Fig. 2a, b, and c). When the chlorinity increases from 5 to 15 g of Cl^- per liter (Fig. 2d and e), a certain growth occurs, but the maximum level of potential activity never reaches such high values as those measured in the freshwater.

Comparison of these potential activity measurements (under optimal conditions) with the in situ activities (Fig. 1) shows that the largest nitrifier populations are not found where the highest in situ activities are measured. The Scheldt river carries an important population of nitrifying bacteria which is completely inactive owing to the anaerobic conditions existing in the upstream part of the estuary. Once

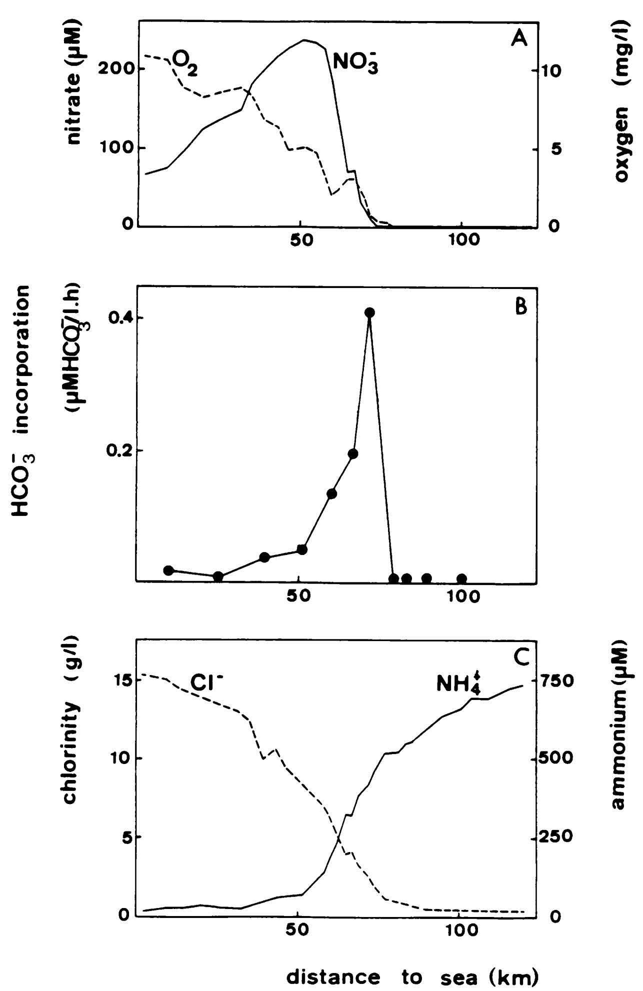

FIG. 1. Longitudinal profiles of nitrate and oxygen concentration (A), nitrifying activity (bicarbonate incorporation inhibited by N-Serve; C/N = 0.12) and redox potential (measured by a platinum electrode) (B), and ammonium and chloride concentration (C) measured at low tide in the Scheldt estuary in September 1977.

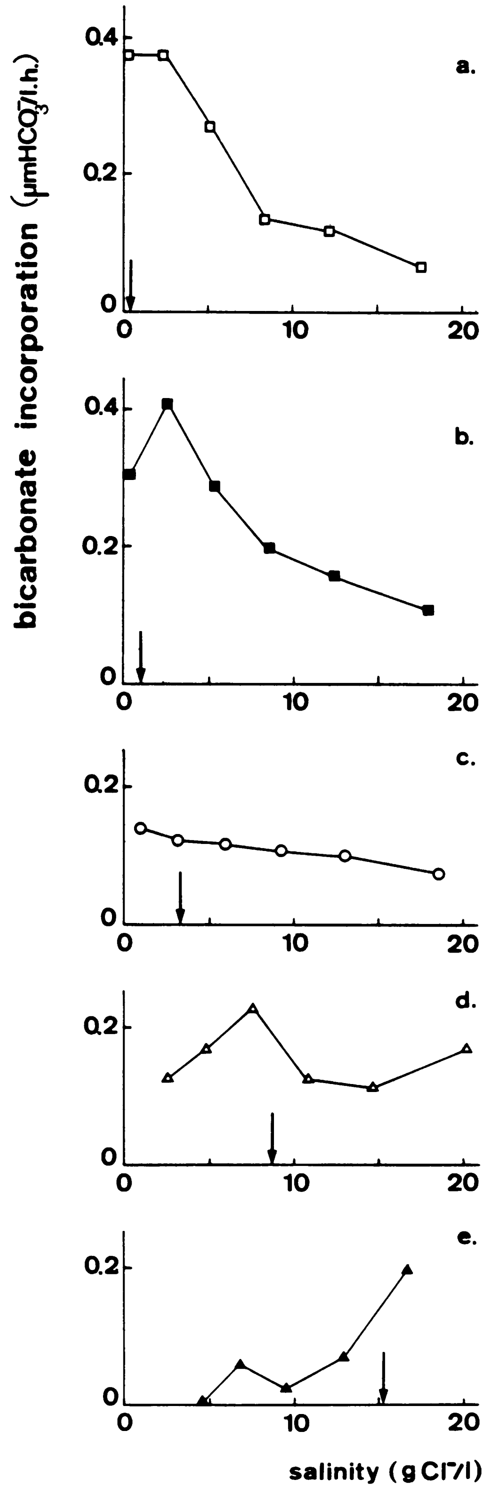

FIG. 2. Potential nitrifying activity (potential bicarbonate incorporation inhibited by N-Serve; C/N = 0.12) as a function of salinity of five natural populations of the Scheldt estuary. In each case, the arrow represents the salinity of the water mass from which the microorganisms originated.

the redox levels increase and conditions favorable to nitrification are reestablished, this population suddenly reactivates and is responsible for the very sharp peak of in situ nitrifying activity shown in Fig. 1.

Some growth then occurs, but the size of the population tends to be reduced by dilution in seawater, explaining the fact that no such high potential activities are found further downstream.

The results shown in Fig. 2 also show that the optimal chlorinity for potential activity increases regularly from river to sea. The nitrifier populations from freshwater display an optimal activity at a very low chlorinity, and their activity rapidly decreases with increasing chlorinity (Fig. 2a and b). Nitrifier populations from brackish water samples have optimum activity at 7 g of Cl^- per liter, i.e., at a salt content slightly lower than that in the water where they live. At seawater salinity, potential activity never falls below 50% of optimum (Fig. 2d). Nitrifying bacteria taken at the mouth of the Scheldt estuary have a maximum activity at 17 g of Cl^- per liter. With lower chlorinities, their activity rapidly decreases.

These results show that adaptation of the nitrifier populations to increasing salinity occurs during the travel of water masses from fresh to saline conditions. Such adaptation has already been reported in the literature. Finstein and Britzski (4) have shown that the nitrifiers adapt to salinity during long-term incubations, although different degrees in salt tolerance were noted among the selected populations. On the other hand, Koops et al. (5) have shown the existence in brackish water of an obligate moderately halophilic strain of *Nitrosococcus mobilis* growing optimally at 6 g of Cl^- per liter.

In the Scheldt estuary, the residence time of the water masses in the estuarine zone is about 2 months under mean discharge conditions (12). Adaptation of the planktonic nitrifier populations is therefore possible. It cannot be excluded, however, that migration of preadapted bacteria from the sediments to the overlying water plays some role in the observed change of the composition of the nitrifier populations.

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