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Nitrification in the Scheldt Estuary (Belgium and the Netherlands)[a]

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Nitrification and repartition of nitrifying bacteria were investigated in the autoepuration zone of the Scheldt estuary.

Measurements of vertical profiles of nitrate and nitrite concentration in the interstitial water of sediments show that nitrification in sediments is very low, implying that most of the nitrate and nitrite production occurs in the water of the river itself.

Nitrifying bacteria, probably of terresterial origin, are present throughout the water along a longitudinal profile of the estuary, with a regular decrease in numbers downstream. However, nitrification occurs only in a zone of favourable oxidation–reduction conditions, which coincides with the thermodynamic stability fields of nitrate and nitrite with respect to ammonium.

Introduction

The process of nitrification is important in polluted rivers because it is the ultimate step in the autoepuration of the organic load through the regeneration of oxidized forms of nitrogen from ammonium produced by decomposition of organic matter. In some cases, it can significantly affect the oxygen balance in streams (Courchaine, 1968). Therefore, an understanding of the conditions under which nitrification occurs and its kinetics in river systems is an essential factor in the modelling and management of these systems.

The oxidation of ammonium to nitrate is almost exclusively the result of the activity of bacteria and fungi. Heterotrophic nitrification has been demonstrated to occur in some natural environments (Verstraete & Alexander, 1973). However, the production of nitrate by this process is generally small and autotrophic nitrifiers (mainly *Nitrosomonas* and *Nitrobacter*) are considered to be the most important agents of nitrification in the majority of ecosystems (Painter, 1970). Thus, nitrification is a very specific process, performed by a homogeneous group of micro-organisms. Consequently, the problem of nitrification in rivers is a particularly 'pure' ecological problem and provides a good example for evaluation of several different approaches to studying the activity of micro-organisms in natural ecosystems.

A purely thermodynamical model, similar to that developed theoretically by Stumm (1966), could be applied to nitrification. Such thermodynamical models are based on the

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assumption that an internal chemical equilibrium is achieved among dissolved species. Bacteria are viewed as simple catalysts maintaining solution equilibrium. Although these models are very valuable in situations where reactions occur on a long-term time scale, as in sediments or anoxic waters with large residence times (Thorstenson, 1970), the validity of the basic assumption of internal equilibrium is questionable in stream systems. In fact, because thermodynamic modelling does not take into account the kinetics of bacterial action, the approach may appear too simplistic to biologists. The latter are accustomed to representing bacterial activity as a function of bacterial number and substrate concentration. Determination of pertinent parameters such as growth rate constants and Michaelis constants, has been the object of laboratory experiments with nitrifying bacteria (Painter, 1970; Wild *et al.*, 1971). Mathematical models based on a constant growth rate for nitrifying organisms have been applied successfully to isolated portions of rivers (Wezernak & Gannon, 1968). However, in whole stream systems, particularly when the polluting load is large, autoepuration commonly proceeds in two successive stages, corresponding, respectively, to organic carbon oxidation and ammonium nitrogen oxidation (Klein, 1957; Downing, 1962), implying that nitrifying bacteria become active only after a period of latency. The cause of this latency and the factors initiating nitrification remain obscure. The rise of dissolved oxygen above some critical level (Wheatland *et al.*, 1959) or the extent of organic degradation have been proposed as empirical parameters (Eckenfelder, 1967; Edeline, 1973), but are difficult to interpret theoretically.

This paper presents a study of nitrification in the epuration zone of the heavily polluted Scheldt estuary (Belgium and the Netherlands). Observations performed are interpreted in terms of both the thermodynamic and 'biological' approaches. One the one hand, the zone of activity of nitrifying bacteria is shown to be related to redox conditions as would be predicted by a thermodynamic model; on the other hand, the distribution of nitrifying populations in the estuary is qualitatively explained by differences in the growth rates of the bacteria in the different physico-chemical conditions present in the estuary.

Methods

Sampling

Measurements were performed during nine cruises undertaken in the Scheldt estuary on February, April–July, September–November, 1973, and February 1974. The zone studied extends about 100 km from the sea; sampling stations were separated by 2 to 5 km (Figure 1). On each cruise, each station was visited at the same state of the tide, either at low water or high water. A complete longitudinal profile was established during three successive days.

All measurements were made on surface water, collected from the boat with a bucket. No important vertical stratification exists in the Scheldt estuary (Wayc *et al.*, in preparation).

Electrochemical measurements

Determinations of pH and *E*h were made on shipboard immediately after collection of the water. The pH was measured using a Beckman combination electrode, standardized against a pH 6·5 buffer. The *E*h was measured by means of a platinum electrode relative to a calomel electrode. Calibration was made against a solution of $0{\cdot}33 \times 10^{-2}$ M-ferrocyanide, $0{\cdot}33 \times 10^{-2}$ M-ferricyanide and 1×10^{-1} M-KCl (Zobell, 1946). The fact that potentials measured at an inert metal electrode in natural aquatic environments do not represent thermodynamic potentials and are not amenable to direct quantitative interpretation has been pointed out

by Stumm (1966) and Morris & Stumm (1967). However, *E*h measurements can be used as empirical environmental parameters and related to biochemical activity, as done successfully by Baas-Becking *et al.* (1960), Borchardt (1966), Whitfield (1969) and others.

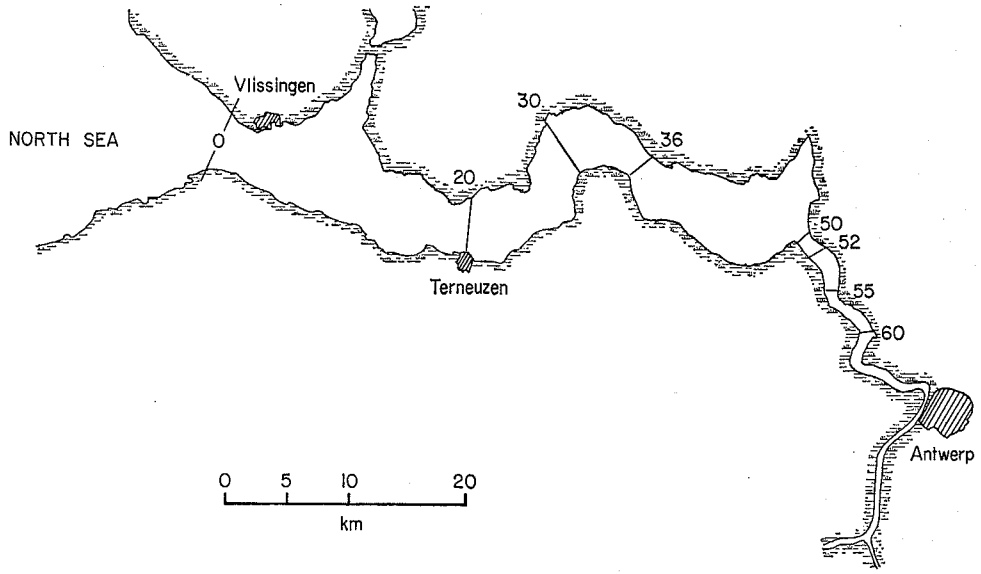

Figure 1. The Scheldt Estuary illustrating the situation of the sampling stations. Distances from the river mouth are given in kilometers.

Chemical analysis

Samples for chemical analysis were immediately treated with chloroform. After returning to the laboratory, they were filtered through 0·2 μ Millipore filters and frozen until analysis. Nitrate, nitrite and ammonium were determined, respectively, according to the procedures described in Armstrong *et al.* (1967) and Slawyc & McIsaac (1972). The salinity of the surface water was determined by measurement of the electrical conductivity. In pore waters extracted from sediments, salinity was estimated by $AgNO_3$ titration following the procedure of Strickland & Parsons (1968).

Bacteriological methods

Numbers of nitrifying bacteria were evaluated by the most probable number method. Five samples of 0·5 ml and five of 0·05 ml were diluted under sterile conditions in about 5 ml of the following enrichment media. For nitrous bacteria, the medium used was essentially that of Winogradsky (Rodina, 1972):

$(NH_4)_2SO_4$	1·32 g l^{-1}
K_2HPO_4	1
$MgSO_4 \cdot 7H_2O$	0·5
$FeSO_4 \cdot 7H_2O$	0·3
$CaCO_3$	precipitated
NaCl	2 (fresh water medium)
	33 (marine medium)
distilled water	1000 ml.

One ml of a solution of chelated metals (Carlucci & Strickland, 1968) was added to the above medium, and the pH adjusted to 7·5 with NaOH. Production of nitrites was qualitatively detected with nitrite-sensitive paper ('Merckoquant Nitrite Test'). For nitric bacteria, the same medium was used, but ammonium sulphate was replaced by $NaNO_2$ (0·0345 g l^{-1}). Disappearance of nitrites was considered as evidence of nitrification only when nitrates were produced at the same time.

Coring

A core was collected from shipboard in an area of the Scheldt (Figure 1) where the water depth was about 6 m. The coring apparatus was a simple plastic tube of 5 cm diameter and 30 cm length, equipped with a rubber piston. The core was frozen after collection and then sawed in 1-cm thick sections. Interstitial water was extracted by pressure filtration.

Results and discussion

Longitudinal profiles of nitrate, nitrite and ammonium

Typical profiles of nitrate, nitrite and ammonium concentrations in the Scheldt estuary are shown in Figure 2(a) as a function of distance to the sea. It can be seen by comparison of these profiles with the profile of chlorinity [Figure 2(b)] that the rise in nitrate and nitrite concentration beginning at about 60 km cannot be explained by an import of nitrate and nitrite from sea water but is the result of the production of these nitrogen compounds in the river.

For each cruise profile, it is possible to discern two zones, as shown in Figure 2(a): an upstream zone where ammonium is the highly dominant form of dissolved mineral nitrogen, nitrate and nitrite being present in concentrations below 2 µmol/l, and a downstream zone where oxidized forms of nitrogen are produced; i.e. a zone where nitrification does not take place and a zone where it does. The beginning of nitrification is very abrupt; the location of the nitrification zone can be assessed within 3 km from the data collected.

Dependence of nitrification on Eh

After leaving the region of Antwerp (75 km from the sea), the very polluted water of the Scheldt undergoes rapid autoepuration which is accelerated by sedimentation of particulate organic matter and mixing with aerated sea water. The redox potential as measured by platinum electrode (Eh_{Pt}), rises progressively from values as low as about +200 mV in winter and −100 mV in summer near Antwerp, to about +350 mV downstream, for both seasons.

The pH and *E*h values corresponding, respectively, to the zone of presence and absence of nitrate and nitrite were plotted on a *E*h–pH diagram [Figure 3(a)]. It is apparent that the separation between the two zones coincides with a particular oxidation–reduction potential. An empirical boundary can be obtained from the data having the form

$$Eh_{Pt} = 0{\cdot}820 - 0{\cdot}08\ \mathrm{pH}.$$

To compare these empirical data with predictions of a thermodynamical equilibrium model, an *E*h–pH equilibrium diagram for the system NH_4^+, NO_2^-, NO_3^- was constructed

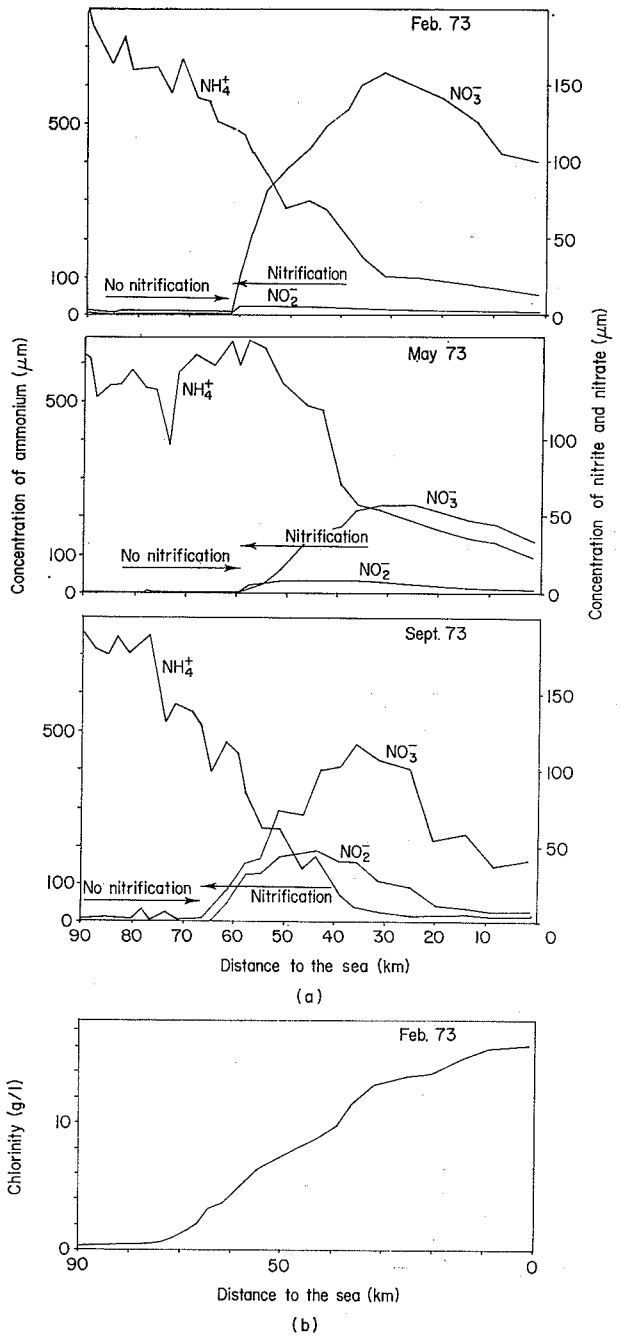

Figure 2. (a) Longitudinal profiles of NH_4^+, NO_2^-, NO_3^- concentration in the water of the Scheldt as a function of the distance to the mouth in February, May and September 1973. (b) Longitudinal profile of chlorinity in the water of the Scheldt in February 1973.

[Figure 3(b)], using free-energy data cited in Garrels & Christ (1965). The following reactions were considered:

$$NH_4^+ + 2\ H_2O \longrightarrow NO_2^- + 8\ H^+ + 6\ e^- \quad E^\circ = 0{\cdot}897 \qquad (1)$$

$$NH_4^+ + 3\ H_2O \longrightarrow NO_3^- + 10\ H^+ + 8\ e^- \quad E^\circ = 0{\cdot}882 \qquad (2)$$

$$NH_4^+ 2\ H_2O \longrightarrow HNO_2 + 7\ H^+ + 6\ e^- \quad E^\circ = 0{\cdot}864 \qquad (3)$$

$$NH_3 + 2\ H_2O \longrightarrow NO_2^- + 7\ H^+ + 6\ e^- \quad E^\circ = 0{\cdot}806 \qquad (4)$$

$$NO_2^- + H_2O \longrightarrow NO_3^- + 2\ H^+ + 2\ e^- \quad E^\circ = 0{\cdot}835 \qquad (5)$$

$$HNO_2 + H_2O \longrightarrow NO_3 + 3\ H^+ + 2\ e^- \quad E^\circ = 0{\cdot}935 \qquad (6)$$

$$HNO_2 \longrightarrow NO_2^- + H^+ \quad pK\text{a} = 3{\cdot}37 \qquad (7)$$

$$NH_4^+ \longrightarrow NH_3 + H^+ \quad pK\text{a} = 9{\cdot}24 \qquad (8)$$

In the theoretical equilibrium diagram, the field of stability of oxidized nitrogen (defined as including both the field of stability of nitrate and that of nitrite) is separated from the ammonium field by the equilibrium line corresponding to reaction (1)

$$E\text{h} = 0{\cdot}897 - 0{\cdot}079\ \text{pH}.$$

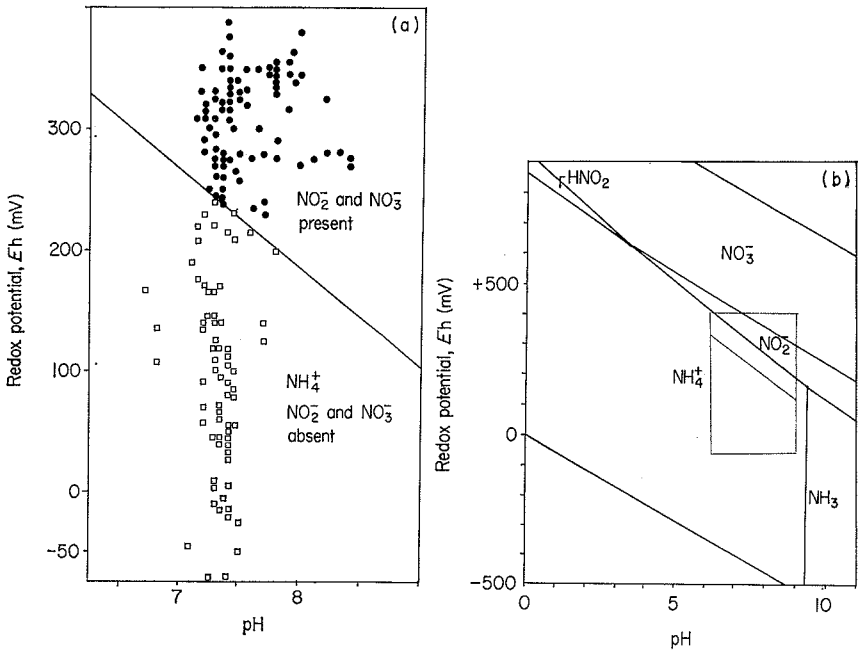

Figure 3. (a) Relationship between redox potential (Eh), pH and nitrification. Each point is plotted according to its Eh (measured with a platinum electrode) and pH value, as a white square if it corresponds to the zone where ammonium is the only form of mineral nitrogen present in concentration higher than 2 µmol/l, or as a black point if it corresponds to a zone of presence of oxidized forms of nitrogen. (Data from February, May, June, July, November 1973 and February 1974.) (b) Thermodynamical stability diagram of the system NH_4^+–NO_2^-–NO_3^-. Calculated from free energy data cited in Garrels & Christ (1965).

The parallelism between the empirical limit of the nitrification zone and the thermodynamic boundary between oxidized nitrogen and NH_4^+ is striking. The difference of about 77 mV between the two boundaries can be explained by technical problems associated with *E*h measurements; because of the irreversibility of reactions at the platinum electrode, measured potentials may not give true thermodynamical values, as pointed out above.

This accordance, however, does not imply that a thermodynamic equilibrium is established in the water of the Scheldt. If so, ammonium would be entirely oxidized in the nitrification zone, as predicted from substitution of observed *E*h values in the potential equation for reaction (2).

$$Eh = 0{\cdot}882 - 0{\cdot}074\ pH + 0{\cdot}007 \log (NO_3^-/NH_4^+).$$

In fact, ammonium is still the dominant nitrogen species in a large part of the nitrification zone and the NO_3^-/NH_4^+ ratio never rises above a value of 3. The coincidence in terms of pH and *E*h of the nitrification zone with the thermodynamical stability fields of NO_2^- and NO_3^- merely shows that nitrification proceeds only in redox conditions where oxidized forms of nitrogen are thermodynamically favoured; in other words, nitrifying bacteria are only active in physico-chemical conditions where oxidation of ammonia is exoenergetic. This fact is easy to understand if it is remembered that nitrifying bacteria use the oxidation of nitrogen as their energy source.

Production of nitrate by bottom sediments

To estimate the role of sediments in the production of nitrates, a core was obtained at low water in the zone of increase of nitrate concentration in January 1974. The concentrations of nitrate and nitrite in the extracted interstitial water as a function of depth in the core are given in Figure 4(a). The fact that nitrite and nitrate disappear below 3 cm depth indicates that denitrification dominates at that level. The observation that nitrate and nitrite concentrations are higher in the upper two centimeters of the sediments than in the overlying water is the result of (i) nitrification in the most shallow depth of the sediment, or (ii) a tide effect, the maximum of nitrate concentration in the sediments corresponding to the remembrance of the composition of the overlying water at high tide, which has a higher nitrate concentration than at low water (Vanderborght, J. P. & Billen, G., in preparation). The observed vertical profile of chlorinity in the pore water [Figure 4(b)] supports the latter interpretation because the maximum in nitrate concentration in the first two centimetres coincides with a maximum of chlorinity. This observation, however, does not exclude the possibility of some nitrification in the sediments.

Because the core was sampled at low water and the nitrate concentration in the overlying water is higher at high water the value of the concentration gradient at the interface, estimated from the data of Figure 4(a), can be used to calculate a maximum value of the flux of nitrate from sediments to the water in the nitrification zone (Berner, 1971). The calculation gives a maximum value of 0·01 μmol m^{-2} s^{-1}. Using the mean residence time of the water of the Scheldt calculated by Wollast (1973), it can be shown that the nitrate flux from the sediments can increase the concentration in the water by at most 1·5 μmol l^{-1} during the time the water flows 5 km downstream. This estimate is one order of magnitude lower than the concentration increase observed, implying that most of the nitrification takes place in the overlying water.

Numeration of nitrifying bacteria in the water

The numbers of autotrophic ammonia-oxidizing bacteria were counted both in saline and fresh water enrichment media at some stations along the longitudinal profile. Results

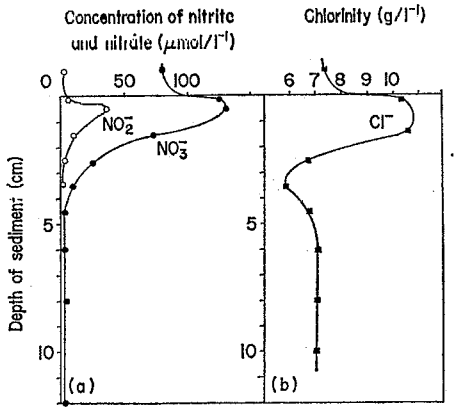

Figure 4. (a) Vertical profile of nitrate and nitrite concentration in the interstitial water of bottom sediments and in overlying water. (b) Vertical profile of chlorinity in the interstitial water of bottom sediments at low water.

Core collected in January 1974 at low water near Saaftinge (55 km from the sea).

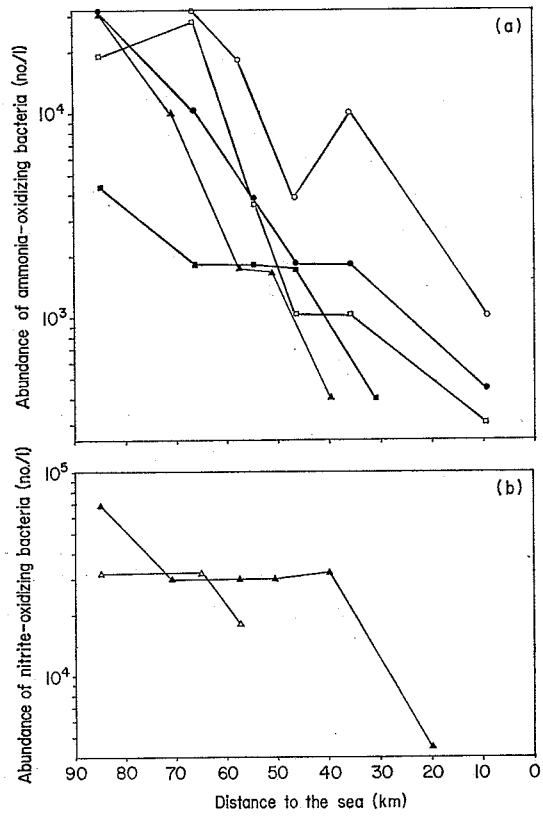

Figure 5. (a) Longitudinal profiles of the numbers of ammonia-oxidizing bacteria as a function of the distance to the sea. (b) Longitudinal profiles of the numbers of nitrite-oxidizing bacteria as a function of the distance to the sea.

Numeration by the MPN method in 2‰ NaCl medium. 1973: —■—■—, May; —□—□—, June; —●—●—, September; —○—○—, October; —△—△—, November; 1974: —▲—▲—, February.

obtained with a fresh water medium are shown in Figure 5(a). All profiles show a regular decrease downstream. The distribution of nitrite-oxidizing bacteria displays the same relationship [Figure 5(b)]. It is evident that the nitrification zone [see Figure 2(a)] does not correspond with a maximum in the number of nitrifying bacteria. Estimations made in saline medium always gave lower results and the bacteria grew more slowly, as shown by the examples given in Figure 6.

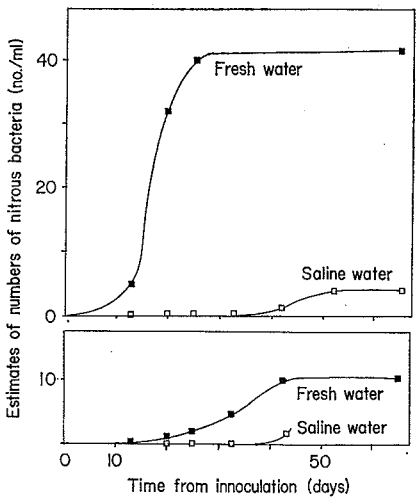

Figure 6. Estimates of growth of nitrous bacteria in fresh water and saline medium. Ten tubes with 2‰ NaCl and ten with 35‰ NaCl were inoculated at time zero as described in the Methods section. By checking periodically for the appearance of nitrite in the tubes, an estimation of the most probable number of bacteria is deduced. [Samples collected in September 1973 at stations located at 110 km (top curves) and 65 km (bottom curves) from the sea.]

To understand the distribution of nitrifying bacteria in the estuary, a distinction between fresh water and sea water nitrifying bacteria must be made. Watson (1963) found that the generation time of *Nitrosomonas europaea* and *Nitrobacter agilis* was prolonged from one day in NaCl-free media to eight days in 25‰ NaCl. Vargues & Brisou (1963) showed that marine nitrifying bacteria were more active than terrestrial ones in saline media. In the case of the Scheldt, the fact that at all stations higher numbers of bacteria were counted in 2‰ NaCl medium than in 35‰, together with the fact that growth was faster in fresh water than in saline medium, suggest that the nitrifying population of the estuary is mostly made up of fresh water bacteria. The observed longitudinal distribution of their numbers probably reflects the fact that these bacteria are of a terrigenous origin and that their population decreases in the estuary by dilution in sea water and mortality.

Conclusions

The following model can be used to explain the various observations reported above. The river carries nitrifying organisms of terrigenous origin to the estuary. No growth of the bacteria occur before the nitrification zone because physicochemical conditions do not allow metabolic activity. Therefore, the population decreases in numbers by dilution and mortality. When the bacteria enter the nitrification zone, the activity immediately begins (as suggested

by the very sudden rise of the nitrate concentration), but the rate of growth of these fresh-water organisms is strongly reduced because of increased salinity. Consequently, the population of nitrifying bacteria is diluted in sea water more rapidly than it can increase by division. This relation provides an explanation for the observations that the number of bacteria is still decreasing in the nitrification zone and that nitrate production decreases in the downstream part of the estuary, although ammonia is still present in appreciable concentration.

The observations reported provide a good example of a situation where no evident link appears between population activity and density of a group of bacteria. Such a situation is made possible owing to the ability of micro-organisms to maintain themselves for long times without displaying any significant metabolic activity. Because of this fact, simple bacterial counts cannot be used to deduce *in situ* bacterial activities.

Furthermore, the present study suggests that thermodynamic models of natural water systems can be very useful, not to describe the *state* of a system, but to predict qualitatively the *direction of change* of a system under biological influence.

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