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Investigations on nitrification in the water and the sediment of the Kiel Bight (Baltic Sea)

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Abstract

The results of this study show that chemoautrophic nitrification is the most important source of nitrate in the brackish water of the Kiel Bight. The main nitrification potential is localized in the sediment. Nitrification activities were measured from January to March 1978 in a sand sediment from a beach station in the Kiel Fjord and in March and May 1979 in the sand sediment of the central Kiel Bight. The nitrification rates measured at the beach station lay between 0.9×10^{-3} µgat N/cm³ x h and 4.5×10^{-3} µgat N/cm³ x h and in the Kiel Bight between 1.9×10^{-2} µgat N/cm³ x h and 4.7×10^{-2} µgat N/cm³ x h.

Introduction

In the Baltic Sea nitrate plays a key role in the production of biomass (SEN GUPTA 1972). According to DUGDALE and GOERING (1967) productivity in oceanic waters is limited to the supply of nitrate from the decomposition of organic matter in the sediment and of nitrogen from N_2-fixation.

Several phytoplankton species are described as preferring nitrate to ammonia (LUND 1965, VOLLENWEIDER 1968, MULLIGAN and BARANOWSKY 1969, cited according to CHEN et. al. 1972). So the availability of nitrogen compounds might influence species succession and distribution of the plankton in the oceans (EPPLEY et. al. 1969).

In the Kiel Bight nitrate concentrations normally are rather low during the summer. After phytoplankton blooming has stopped nitrate concentration first starts to increase slowly during autumn and increases quite rapidly during the winter, reaching its maximum just before phytoplankton starts to grow.

It was the aim of this study to find out to what extent microbiological processes such as nitrification are responsible for the nitrate formation in the Kiel Bight. Nitrification has been defined as . . . ''the biological transformation of nitrogenous compounds from a reduced to a more oxidized state'' (ALEXANDER et. al. 1961). The oxidation of ammonia to nitrate in aquatic systems is carried out mainly by autotrophic nitrifying bacteria (CHEN et. al. 1972; CAVARI 1977; WEBB and WIEBE 1975). Ammonia is oxidized to nitrate in two steps (WALLACE and NICHOLS 1969):

$$NH_4 + 1.5\ O_2 \dashrightarrow NO_2 + H_2O + 2H^+$$

ΔF – 84 Kcal (ammonia-oxidizing bacteria)

$$NO_2 + 0.5\ O_2 \longrightarrow NO_3$$

ΔF – 17.8 Kcal (nitrite-oxidizing bacteria)

According to THOMSEN (1910) nitrification in the Kiel Bight is carried out by autotrophic nitrifying bacteria originating from land by wash-out and is therefore

restricted to coastal sediments. During this investigation studies were undertaken to determine if there is an autochthoneous nitrifying population in the Kiel Bight.

Materials and methods

Sampling

Water and sediment samples were taken from one station in the Kiel Fjord (Station 1, Fig. 1) and two stations in the Kiel Bight (Stations 2, 3). Station 2 is described in detail by RHEINHEIMER (1977).

Station 1, the "Falckenstein Beach", is influenced by eutrophic water from the Kiel Fjord and oligotrophic water from the Kiel Bight. It is a sand beach with sediment grain sizes between 0.2 mm to 0.6 mm (mean diameter).

Station 3 is situated outside the Eckernförde Bight and in regard to nutrient concentration and sediment quality similar to Station 2 in the Kiel Bight.

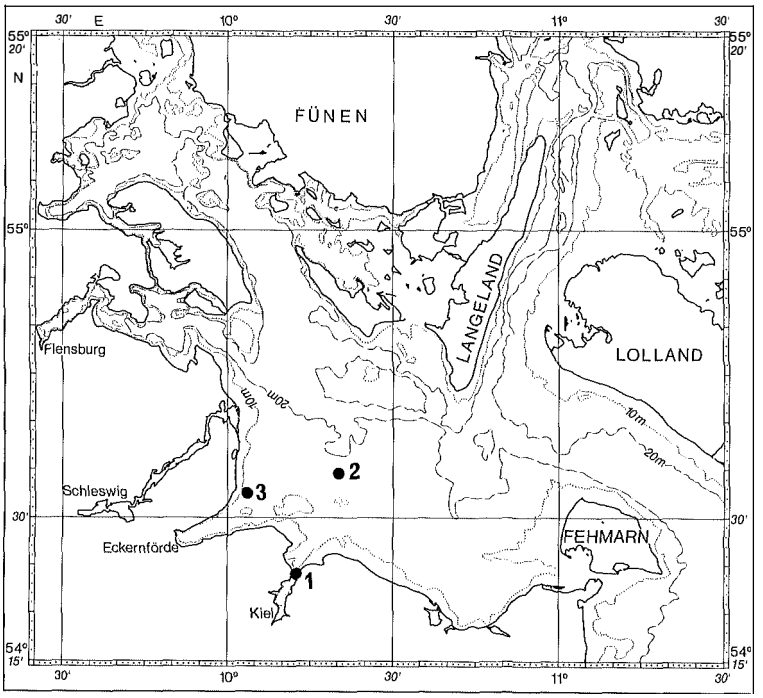

Figure 1
The position of the sampling stations (Nos. 1–3) in the Western Baltic Sea

Water samples were taken with sterile 5 l glass bottles in a modified ZoBell sampler. Sediment sampling at Station 2 was performed with a Van Veen grab. To take off the upper one-centimeter thick layer of the beach sediment a sterile household dustpan was used. All sediment samples were collected in sterile glass vessels.

Methods

To isolate autotrophic ammonia-oxidizing bacteria from the water of the Baltic Sea a 500 ml water sample from Station 3, depth 5 m, was enriched with minerals (see

nitrification medium below) and incubated in the dark. As soon as 250 mg $(NH_4)_2SO_4$ were oxidized to nitrite 1 ml of the enrichment-culture, diluted to 10^{-2}, 10^{-3}, 10^{-4} of the original concentration, was inoculated on a sterile agar medium of the following composition: nitrification medium (simplified acc. to CARLUCCI and STRICKLAND 1968) 1 g $(NH_4)_2SO_4$, 40 mg K_2HPO_4, 600 mg $CaCO_3$, 10 g agar-agar (Baker, No. 1892), traces of phenol red indicator, 1000 ml aged seawater (diluted with distilled water, salinity $16^0/_{00}$).

Using a microscope (magnification 16 x) microcolonies of 1 to 2 mm diameter were picked up from the agar surface after an incubation time of 4 weeks. Single colonies were inoculated into 10 ml of liquid nitrification medium. Nitrite-positive cultures were checked for heterotrophic contaminants by inoculating 1 ml of nitrifier culture into 10 ml of 1/10 concentrated medium acc. to OPPENHEIMER and ZOBELL (1952), salinity $16^0/_{00}$.

Taxonomic tests with *Nitrosomonas* spec. were performed with logarithmically growing cultures. The pH value was controlled automatically by a pH-chemostat (Eschweiler and Co, Kiel). The culture was aerated with sterilized air.

For transmission electron microscopy *Nitrosomonas* spec. was prepared acc. to RUDORF (1978). The cells were observed under a Philips Electron Microscope 201 at 39500-fold magnification.

The cytochrome-spectrum was measured acc. to KOOPS et. al. (1976). To determine the nitrification and growth rates of *Nitrosomonas* spec. nitrite formation and cell number (ZIMMERMANN 1977) of a fresh culture were determined just after incubation and then every 24 hours until the stationary growth phase was reached.

To determine the temperature demand of *Nitrosomonas* spec. nitrite formation of a logarithmically growing culture was measured in a temperature range from 5° to 40°C after an incubation time of 2 hours. The apparatus used is described by HOPPE (1972).

The sodium demand of the nitrifier was measured with artificial seawater (Mc LEOD et. al. 1954) with 1 g $(NH_4)_2SO_4$/l, $16^0/_{00}$ salinity. All sodium salts were replaced by equimolar potassium salts. The medium was enriched with NaCl in $2^0/_{00}$ increments from 0 up to $30^0/_{00}$. 10 ml of each NaCL-concentration was inoculated with 100 µl of a cell suspension washed with NaCl-free artificial seawater. Nitrite formation was measured after 1 hour incubation time.

The sodium demand of natural nitrifying populations of the Kiel Bight attached on sand grains (Station 2) was determined in a NaCl-concentration range from 0 to $32^0/_{00}$. Each 3 cm^3 sample of the sediment was washed with 100 ml of artificial seawater (500 mg $(NH_4)_2SO_4$/l), adjusted to the special NaCl-concentration and incubated in a 300 ml Erlenmeyer flask with 100 ml of the NaCl-solution at 20°C under mechanical shaking. After 3 days the concentrations of ammonia, nitrite and nitrate were measured.

Heterotrophic nitrite formation from ammonia was tested in ''Fischer-medium'' (WITZEL 1973). Instead of destilled water artificial seawater, $16^0/_{00}$ salinity with traces of phenol red indicator was used. Each 50 ml of test-medium was inoculated with 1 drop of saprophyte culture grown in ,,Fischer-medium''. Acid formation was neutralized with sterile 0.5 % Na_2Co_3-solution. After an incubation time of 14 days at 20°C in the dark nitrite formation was determined.

The autotrophic nitrifying activity in sand sediments was measured by $^{14}CO_2$ incorporation in the dark at *in situ* temperatures (acc. to BILLEN 1976). Using N-Serve

(2-chloro-6-trichloromethyl-pyridine, Dow Chemical Co., Michigan) as a specific inhibitor for autotrophic nitrification the CO_2-fixation rate of autotrophic nitrifying bacteria was calculated by the difference from total CO_2 incorporation and related to the nitrification rate.

In deviation from the method of BILLEN (1976) the incorporated carbon of the sediment from Station 1 was combusted by the method of VAN SLYKE et. al. (1951) as modified by MEYER-REIL (1978). The samples from Station 2 (Kiel Bight) were combusted automatically by a Packard sample oxidizer, "TriCarb", under oxygen atmosphere. Each 3 cm^3 of sediment sample was weighed, mixed with 200 mg of cellulose-powder p.a. (Merck 2330), poured into percussion-cups (Packard) and formed to pills by a press at 1.5 tons. The pills were burned for 3 minutes in the TriCarb analyser. CO_2 was trapped with a Carbosorb-Permafluor V-mixture (9 + 15, Packard) at a yield efficiency of 83 %. The fixed ^{14}C-radioactivity was measured in a scintillation-spektrometer (Betacint 5000, Berthold & Frieseke). The ammonia concentration was determined acc. to KOROLEFF (1976), nitrite and nitrate acc. to GRASSHOFF (1976).

The alkalinity measurement was carried out as described by BALZER (1978).

The organic substances of the sediment were determined acc. to „Deutsche Einheitsverfahren" (1960).

The grain-size analysis was carried out acc. to WEISE and RHEINHEIMER (1978).

Salinity and temperature were measured by a TS-sonde (salinity temperature bridge MCS, Electronic Switchgear Ltd., London).

Results and Dicussion

Taxonomy of an autotrophic ammonia oxidizing bacterium isolated from the water of the Kiel Bight

An autotrophic ammonia oxidizing bacterium was isolated from the brackish water of the Kiel Bight at Station 3 (Fig. 1). This strain was tested for its morphological and physiological characteristics. They are listed in Table 1. As shown by the electron-micrograph (Fig. 2) the ammonia-oxidizer isolated from the Kiel Bight has cytomembranes arranged in flattened lamellae in the peripheral region of the cell. According to Bergey's Manual (BUCHANAN and GIBBONS 1974) organisms that oxidize ammonia to nitrite with endomembranes like this belong to the genus *Nitrosomonas*. The cells have rounded ends similar to those of a marine *Nitrosomonas* strain described by WATSON (1971). This is different from the terrestrial *Nitrosomonas europaea* (WATSON 1971) which has pointed ends. The isolated *Nitrosomonas* strain grows autotrophically and does not use organic substances. In the logarithmic growth phase at 20 °C the generation time of this organism is 13.2 hours, the oxidation rate 4.5 x 10^{-9} μgat NH_3-N/h. cell. The temperature optimum of the strain lies in the range of 32°C (Fig. 3). At 5°C only 2 % nitrification activity is measured in comparison to the optimum.

In contrary to freshwater and terrestrial bacteria marine and brackish water bacteria require sodium to live, whereas most bacteria from freshwater, wastewater and soil are normally killed within a short time by the bactericidal action of the brackish water (RHEINHEIMER 1966). The nitrification rate of *Nitrosomonas* spec. as a function of the sodium chloride concentration is illustrated in Fig. 4. This strain is able to oxidize ammonia within a wide NaCl concentration range. 50 % nitrification was measured between 1‰ and 20‰. The optimum lies at 6‰ NaCl. According to MEYER-REIL (1973) this strain can be classified as being a facultative halophilic brackish water organism.

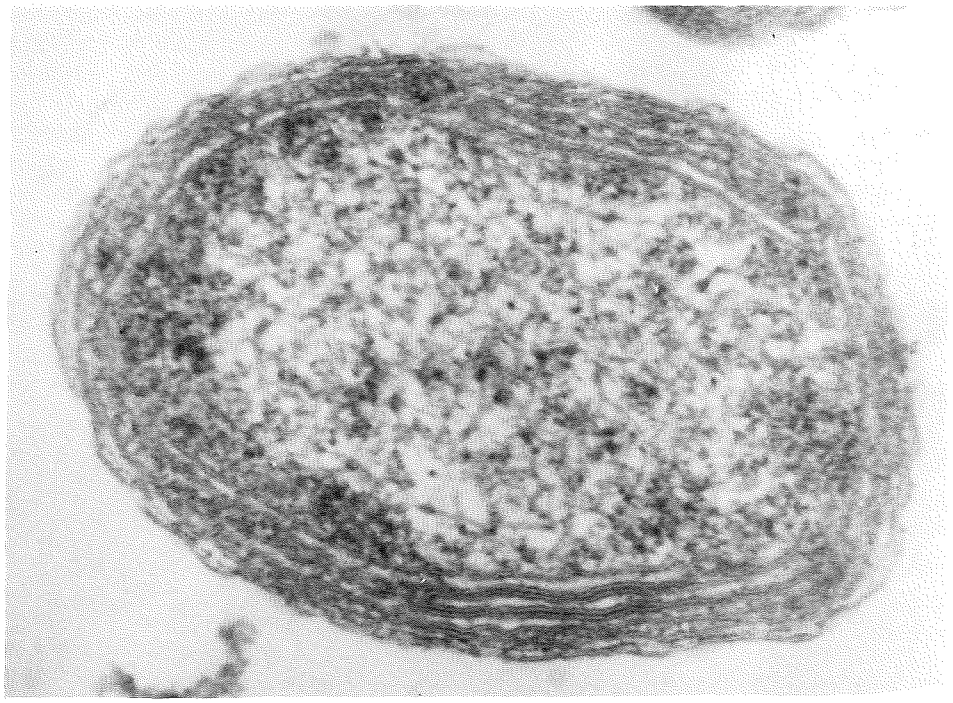

Figure 2
Electron-micrograph of *Nitrosomonas* spec. isolated from the brackish water of the Kiel Bight (*magnification* 114.000 x)

With this strain it could be proved that there are autochthoneous ammonia oxidizing bacteria in the brackish water of the Kiel Bight.

Salinity demands of natural populations of nitrifying bacteria in the Kiel Bight

In order to obtain information about the origin of the nitrifying population in a brackish water habitat, nitrifying bacteria attached on the sand sediment from Station 2 were tested for their salinity demands.

Figure 5 illustrates the nitrification potential of a sediment sample from the Kiel Bight (Station 2) as a function of the NaCl concentration. Nitrification is expressed by the increase of nitrate within 3 days. Depending on the wind, fast salinity fluctuations occur in the Kiel Bight (LENZ 1974). This area is dominated by bacteria with a high fresh and sea water tolerance (MEYER-REIL 1973). Maximum nitrate formation was measured at 12‰ NaCl with a small shoulder at 28‰ NaCl. The actual NaCl concentration at the station was at about 11‰ (16‰ salinity). So the highest nitrification activity corresponds to the natural NaCl concentration. Obviously the NaCl concentration has an important influence on the composition of the nitrifying community. Besides this it

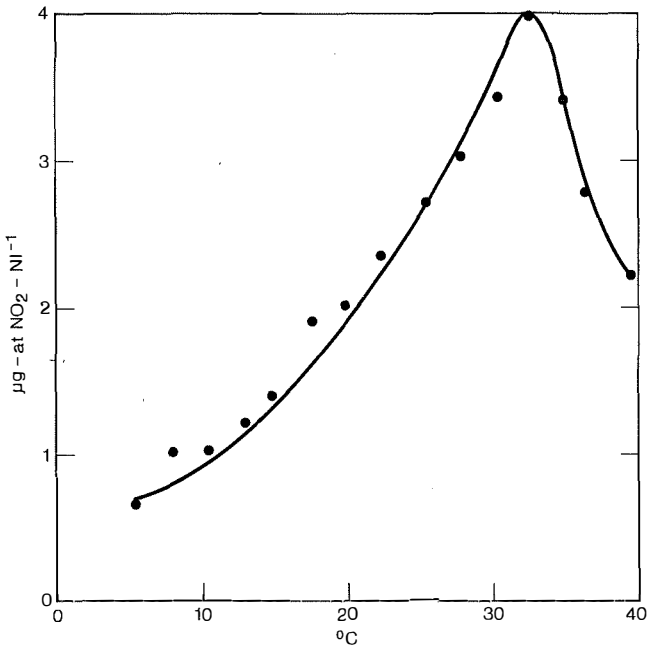

Figure 3
Nitrite formation of the autotrophic ammonia oxidizer as a function of the temperature

Table 1
Characteristics determined for the *Nitrosomonas* spec. isolated from water, depth 5 m, at Station A

Characteristics	*Nitrosomonas* spec.
Size	0.5 – 1 µ x 1 – 2.5 µ
Shape	rods with rounded ends, single in pairs
Flagellation	none observed
Gram reaction	negative
Origin	brackish water of the Kiel Bight
Endomembranes	flattened lamellae in the peripheral region of the cell
Cytochrome spectrum	434 (Cyt. c) 462 (Cyt. a) 524 (Cyt. c) 553 (Cyt. c) 602 (Cyt. a)
Generation Time	13.2 hours at 20°C
Nitrification rate	4.5 x 10^{-9} µgat N/h · cell (during the log-phase; 1g) $(NH_4)_2SO_4$/l; pH-controlled, 20°C)
NaCl optimum	6‰
Temperature optimum	32°C

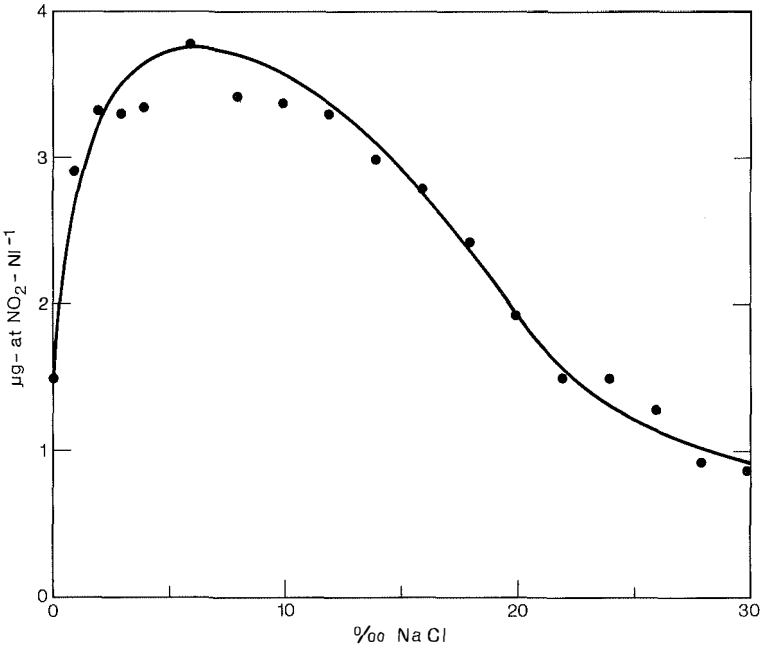

Figure 4
Nitrite formation of the autotrophic ammonia oxidizer at NaCl concentrations from 9 to 30‰

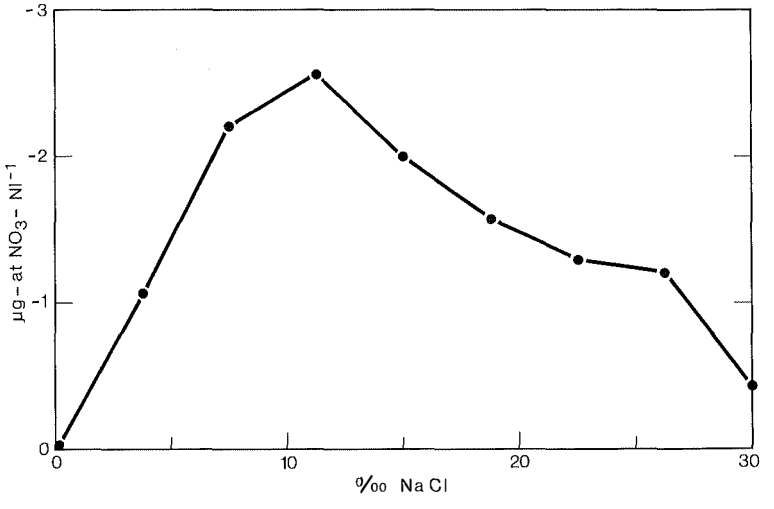

Figure 5
The NaCl demand of a natural nitrifying population in sand sediment from the Kiel Bight, measured by the nitrate formation

could be shown once more that nitrification in the Kiel Bight is carried out by autochthoneous, halophilic or halotolerant brackish water bacteria.

Heterotrophic nitrificaton in the Kiel Bight

Besides the autotrophic nitrifying bacteria of the family Nitrobacteraceae several heterotrophic bacteria and fungi are known to be able to form nitrite and nitrate from ammonia and organic nitrogen compounds (EYLAR and SCHMIDT 1959; HIRSCH et. al. 1961, OBATON et. al. 1968, VERSTRAETE 1970, WITZEL 1973).

Therefore 563 strains of heterotrophic bacteria isolated monthly by BÖLTER (1976) from water and sediment of the Kiel Fjord and the Kiel Bight during the period from January 1974 to March 1975 were tested for their ability to oxidize ammonia to nitrite. Heterotrophic nitrate formation has been reported of from fungi (HIRSCH et. al. 1961) and from the genus *Arthrobacter*. None of the isolated saprophytes belonged to either of these species (BÖLTER 1976).

Only 4.3 % of the tested saprophytes were able to form nitrite from ammonia. During an incubation time of 14 days dense cell suspensions of the heterotrophic nitrifiers formed 0.7 to 3 µgat NO_2-N/l. According to BÖLTER (1976) the heterotrophic ammonia oxidizers belong to the following genera:

Genus	Number of Strains
Bacillus	1
Corynebacterium	10
Enterobacterium	3
Pseudomonas	2
Vibrio	1
Flavobacterium	6
Achromobacter	1

During the investigation period the number of heterotrophic ammonia-oxidizing bacteria lay between 0 and 10 cells/cm^3 of water as well as sediment. In contrast to the report on the potential nitrification rate as measured during the same time in the investigation area (SZWERINSKI 1977), no significant seasonal cycle of the numbers of heterotrophic nitrifiers was detected.

These results indicate that although heterotrophic nitrite-forming bacteria can be found, heterotrophic nitrification should be unimportant in the investigated area. With about 20 µg C/l of dissolved free carbohydrates in the water (DAWSON, personal comm.) and 50 to 170 µg glucose/l (MEYER-REIL et. al. 1978) in the sand sediment at Station 2 the concentration of soluble free carbohydrates seems too low for this process (VERSTRAETE and ALEXANDER 1973).

The autotrophic in situ nitrification rate in the Kiel Bight sediment

Previous investigations indicated that the main nitrifying potential is localized in the sediment (SZWERINSKI 1977). Data about the in situ nitrification rate are needed in order to get an idea of the significance of nitrification for the turnover of substances in the Kiel Bight. By measuring the CO_2-fixation rate of the autotrophic nitrifying bacteria BILLEN (1976) developed a method to determine in situ nitrification rates in sediment samples. The nitrification rate can be calculated from the CO_2-fixation rate by the C/N ratio for nitrifying bacteria (BILLEN 1976).

In short term experiments nitrification rates were determined in the sand beach sediment of the Kiel Fjord (Station 1) at *in situ* substrate and temperature conditions from January to March 1978 and in the Kiel Bight (Station 2) during March and May 1979 (Table 2).

Table 2

Nitrification rates (N-Rate) in sand sediments from the Falckenstein Beach (Station 1) and from the central area of the Kiel Bight (Station 2) with physical and chemical parameters of the water (W) and the pore-water (PW)

Parameter	Station 1		Station 2			
	11. Jan. 78	6. Feb. 78	3. March 78	14. March 79	27. March 79	3. May 79
NH_3-N µgat/l (W)	9,6	–	5,8	3,43	1,6	–
NO_2-N µgat/l (W)	1,6	–	1,4	1,05	0,8	–
NO_3-N µgat/l (W)	30,4	–	38,2	4,09	0,49	–
NH_3-N µgat/l (PW)	9,7	4,8	4,1	–	–	–
NO_2-N µgat/l (PW)	1,4	2,6	0,8	–	–	–
NO_3-N µgat/l (PW)	69,0	73,0	(114,0)	–	–	–
Inorganic carbon (mg C/l)	28,08	27,72	27,96	20,37	18,53	22,09
Salinity ‰	17,4	18,8	13,6	16,1	16,3	15,1
Temperature °C	3,5	0,7	2,8	– 0,015	0,6	4,0
Grain size, mean (mm)	0,192	0,632	0,247	–	–	–
Organic substance (mg/g)	4,53	9,68	5,52	3,37	2,8	2,9
N-Rate, 0–1 cm µgat N/cm³ · h	$3{,}9 \cdot 10^{-3}$	$4{,}5 \cdot 10^{-3}$	$0{,}87 \cdot 10^{-3}$	$5{,}9 \cdot 10^{-2}$	$1{,}9 \cdot 10^{-2}$	$3{,}9 \cdot 10^{-2}$

The nitrification rates at the beach station ($0.87 – 4.5 \times 10^{-3}$ µgat NO_3–N/cm³ x h) were by about one order of magnitude lower than those at Station 2 in the Kiel Bight ($1.9 – 5.9 \times 10^{-2}$ µgat NO_3–N/cm³ x h), although water temperature and salinity were comparable. This was probably due to the higher ammonia concentrations in the pore-water at Station 2. Unfortunately it was not possible to obtain pore-water samples for inorganic nitrogen analysis from Station 2. The inorganic nitrogen concentration of the pore-water and the overlying water listed in Table 2 can differ strongly, as can be seen from the data of the water and the pore-water at the Falckenstein Beach.

Based on these results a rough estimation of the nitrate formation by autotrophic nitrifying bacteria during the four nonproductive months of the year was undertaken. This calculaton was done in order to determine whether or not the measured nitrification activities are sufficient to produce the nitrate amounts accumulating in the water of the Kiel Bight during wintertime. For this calculation the activities determined from January to March 1978 at Station 1 were chosen because they represented nitrification rates under winter conditons.

The following simplifications were necessary:

1. The mean nitrification rate in Kiel Bight during wintertime is about 3×10^{-3} µgat NO_3–N/cm³ x h.

2. The main part of the bed of the Kiel Bight is covered with coarse to fine sand sediment at a depth of about 18 m (DIETRICH and KÖSTER 1974).
3. Denitrification processes are suspected to be low during winter. During this time the upper layers of the sand sediment are well aerated by wave action. Besides this the concentration of organic substances is quite low because no sedimentation from phytoplankton production occurs.
4. Presumably all nitrate produced up to a depth of 1 cm is delivered quantitatively into the overlying water column.

At a mean nitrification rate of 3×10^{-3} µg at NO_3–N/cm³ x h the total nitrate production during the four winter months in the upper one-centimeter deep layer of the sand sediment then amounts to 9 µg at NO_3–N/cm³. This nitrate quantity is transferred into an overlying water column of about 18 m height and is diluted to a nitrate concentration of 5 µg at NO_3–N/l after four months. Nitrate concentrations in the Kiel Bight (Station 2) that are measured just before the spring phytoplankton bloom starts, normally range from 5 to 10 µgat NO_3-N/l.

These empirical data coincide quite well with those calculated from the nitrification rates. The results from this calculation indicate that autotrophic nitrification is a main factor in the formation of nitrate in the Kiel Bight.

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