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Translated by Lorna Heller

In a lake the nitrogen compounds are liable to regular cycling in which nitrate is reduced and ammonium oxidised. As a nitrate maximum is regularly established in the upper part of the hypolimnion of a stratified summer lake, we have dealt in particular with the oxidising side of the nitrogen cycle. In the study described here partial results in the nitrification in PluBsee are presented. The PluBsee was chosen, since it is almost entirely without inflows, and, lying in a wooded basin, is well protected from the wind, and therefore stably stratified. The epi- and hypolimnion are well-formed, over a surface area of only 13.5 ha and at the relatively great depth of 25m. The lake can therefore be considered as typical of the eutrophic stratified Baltic lakes (OHLE 1962, OVERBECK 1968).

Ammonium is constantly oxidised in the lake; although algae and bacteria continuously take up nitrate, these compounds do not disappear completely, apart from periods of heavy nitrogen consumption. Nitrate is also permanently supplemented.

As, under natural circumstances a purely chemical oxidation of the ammonium is to be eliminated (according to RAKESTRAW and HOLLAENDER 1936), it must be assumed that this is brought about by bacteria.

Two groups of bacteria are involved in the nitrification.

1. The autotrophic forms Nitrosomonas and Nitrobacter discovered by WINOGRADSKY (1904), as well as several equally autotrophic spores (WATSON 1965). These bacteria use the free chemical energy of the ammonium - oxydation for the autotrophic CO_2 - fixation and for the purpose of their other energy requirements.
2. Heterotrophic bacteria and fungi, which oxidise ammonium to nitrite and nitrate in a still unknown reaction.

On the subject of the micro-organisms of the second group, the following is well-known (ALEXANDER et al.1961, HIRSCH et al.1961, RUSCHKE 1967, DOXTADER and KOBIRA 1968):

The NH_4^+ oxidation does not add to the energy yield.

The organisms live heterotrophically and cannot be induced to adopt autotrophic habits.

The organisms do not belong to a particular taxonomic group. There are to be found amongst them, as well as fungi, which can form large quantities of nitrites and nitrates, also bacteria and actinomycetes, which only form small amounts of nitrites and nitrates. Moreover, the capacity of bacteria and fungi to NH_4^+ - oxidation seems to be widely distributed.

The organisms can not only oxidise NH_4^+ ions, but also act on amino acids, amines and other organic compounds like oxime and hydroxylamine. Where nitrate is formed, nitrite appears simultaneously. The formation of nitrate was until now only known in fungi, but not in heterotrophic bacteria.

NH_4^+ - oxidation does not take place parallel to growth but preferably after the conclusion of the log. growth phase.

While BOLTJES (1935), IMSENECKI(1946) and BOMEKE (1948) still strictly rejected the existence of heterotrophic nitrificants, and in doing so justly referred to the fact that the publications relating to this were faulty or insufficiently detailed, there can now be no doubt as to the existence of heterotrophic bacteria which can oxidise NH_4^+.

Materials and Methods

The determination of microbial activities in situ, is fundamentally problematic. To record nitrification RHEINHEIMER (1959) developed a method for determination of strength, which was taken over by BRAUN and UHLEMANN (1968). In accordance with RHEINHEIMER, a small quantity of tenfold concentrated nutrient solution is filled up with a tenfold quantity of test water. The nutrient solution contains NH_4^+ for Nitrosomonas and NO_2 for Nitrobacter. After 14 to 19 days incubation the quantity of nitrite produced (Nitrosomonas)resp. used up (Nitrobacter) is determined, and serves as a measure for the nitrification strength.

This was the first method used. However, the results obtained were difficult to interpret, eg. the greatest strength was found in mud containing H_2S, and it has already been shown that the addition of only 10mg/l of organically bound carbon severely altered the values (GODE 1970). Because, in the Plußsee content of dissolved organic compounds in the water can exceed this value and the quantities vary greatly at different depths of the lake, the wider uses of this method were put aside. Otherwise, the differences in the dissolved carbon compounds of the water could have simulated differences in the nitrification power.

The determination of the bacterial count remained as another method. It resulted in the tubule-dilution test in triplicate, according to the MPN method of PRESCOTT et al.(1950) from tables of the "Standard Methods" 1959.

The following solutions served as media:
For Nitrosomonas: 0.5g K_2HPO_4, 0.05g $FeSO_4$, 0.1g $MgSO_4$, 1g $CaCO_3$, 0.26g $(NH_4)_2\ SO_4$, 1 litre distilled water.

For Nitrobacter: The same, but 1mg/l $NaNO_2$ instead of 0.26g/l $(NH_4)_2\ SO_4$.

Such a low nitrite concentration was chosen to check that a weak growing bacteria could also show a positive result. After 4 weeks' incubation period a test was made with α Naphthylamine and sulfanilic acid for the production (Nitrosomonas) or consumption (Nitrobacter) of nitrite.

According to HOFLICH (1969) the bacterial counts fluctuate according to the method used. After individual studies the counts of Nitrosomonas and Nitrobacter are maximal ie. the actual counts are not larger than those already found (GODE 1970). No useful methods for the recording of the heterotrophic nitrifying bacteria were known. A process, therefore, was developed, which allows one to determine their number.

The following nutrient solution (modified according to FISCHER 1956, amounts in mg) was divided, each 15ml in a 100ml Erlenmeyer flask: Natriumacetate 50, NH_4Cl 30, $NaHCO_3$ 10, $MgSO_4$ 60, $CaCl_2$ 1. To this were added 2ml sterile phosphate buffer pH7.2[1] and 0.1ml AZ- trace-element solution according to HOAGLAND, to which a further 0.5mg/l Na-Molybdate were added. The whole was made up to 100ml. In several cases we added a further 0.1ml of a solution of 0.2% tryptose and 0.2% extract of yeast to every 100ml nutrient solution.

For the determination of the bacterial count of heterotrophic nitrificants 1ml of water was added for every 15 ml of nutrient

1) A 200 ml mixture of a solution of 0.2745g $NaHPO_4$ and 0.4213g of KH_2PO_4 in distilled water.

solution. Every dilution was applied in triplicate and incubated for 3 weeks at 27°C in 100 ml Erlenmeyer flasks with cellulose stoppers. A test was then made for nitrite and nitrate (according to WALTHER 1966) with Diphenylamine - H_2SO_4. The accuracy of the method is limited, as the actual bacterial count can be higher than that estimated, but it can never be lower. The reason for this is: nitrite or nitrate being formed from other bacteria could be assimilated. Further, it must be born in mind, that nitrifying microorganisms in a natural biocoenose can be arrested. The evaluation, consequently did not take place according to the MPN method, but through estimation of the tenfold strength as the minimum value of the still positive dilution.

The experiment with pure cultures was conducted partly with growing cultures, partly with washed cells from a pre-culture with acetate, aeration and 27°C. Throughout the duration of the experiment of 8-10 days, sterile specimens were withdrawn and tested for nitrite, biomass, contamination, Nitrosomonas, and Nitrobacter.

Results

Spore counts of nitrifying bacteria.

Between 27.4.1967 and 5.6.1969, samples were taken at approx. 6-week intervals from various depths of the Plußsee, to determine the number of autotrophic nitrificants.

The distribution of the Nitrosomonas spores in the lake corresponded to expectations: In the aerobic epilimnion and also frequently in the limiting zone of the hypolimnion their numbers were relatively high, in the anaerobic hypolimnion,

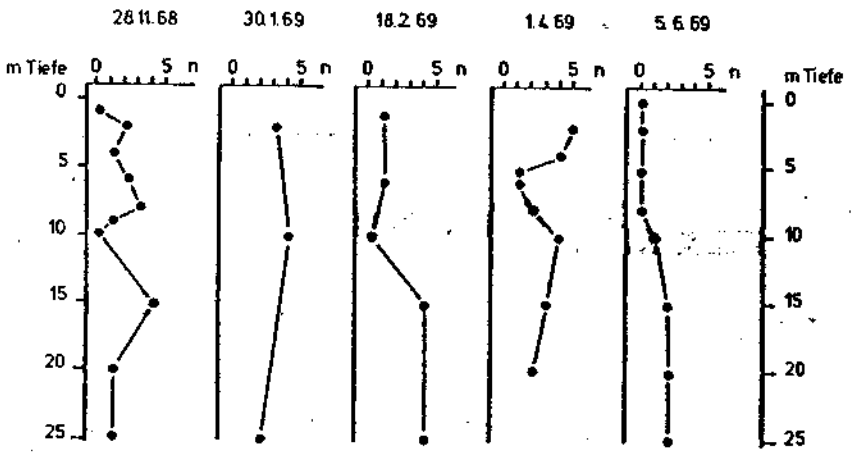
relatively low. The low level, however, was surprising: no more than one spore of Nitrosomonas per ml was found. According to ENGEL (1930) a Nitrosomonas cell can oxidise 10^{-7} µg/hour N under optimal conditions. This means that in the case of the small spore count Nitrosomonas can play no material role in the nitrogen cycle.

The spore counts of Nitrobacter attained values of 200 bacteria/ml and more. The greatest values lay, surprisingly, in the sediment and in the anaerobic part of the metalimnion. As 200 spores/ml according to ENGEL (1930) can oxydise a maximum of 14 µg/l nitrate per month, a weak efficacy of Nitrobacter (as an antagonist of nitrification only) in the lake cannot be excluded, however. For the oxidation of ammonia to nitrate, Nitrobacter is probably insignificant, as it can no longer oxidise nitrite in this oxidation chain, when it has previously been produced from Nitrosomonas.

Between the 28.11.1968 and 5.6.1969, the nitrifying heterotrophic bacteria on the other hand reached much higher counts with values up to 10,000 cells/ml. Maxima lay partly in the limiting layer between aerobic and anaerobic water, partly several metres under it. In the lower part of the O_2- containing epilimnion, the spore count was very low, while, on the other hand, in full circulation was high. (Fig. 1)

Even if nothing is known about the oxidation capacity of a single cell, these high spore contents bring out as probable that the oxidation of NH_4^+ in the lake is above all due to the heterotrophic bacteria. In order to confirm this conjecture, further studies were carried out in the laboratory.

Tiefe = depths



Grenzzone = border zone

Heterotrophic nitrificants given in 10^n spores/ ml.

Fig. 1. Depth profile of the heterotrophic nitrificants in the PluBsee. On 30.1.1969 the lake was well aerated in full circulation.

Studies on the biology of heterotrophic nitrificants

From the estimates on the determination of spore numbers of the heterotrophic nitrificants, 14 species in the pure culture were isolated and examined from morphological, biochemical and taxonomic viewpoints. The types divided themselves into the genera Flavobacterium (1), Xanthomonas (2), Achromobacter (2), Pseudomonas and Vibrio (7), Arthrobacter (1), Micrococcus (1) and reveal the wide distribution of the capacity for heterotrophic nitrification. Common features were only eatablished in the negative: none of the types belonged to the spore-forming genera, none was acid resistant, and none formed acetone.

The Arthrobacter - species, which grew well and characteristically on plates, was selected for the research on some of the ecological factors of heterotrophic nitrification.

In non-growing cell suspensions out of an acetate-preliminary culture, the nitrogen oxydation increased with rising NH_4^+ - and PO_4^{3-} content in the nutrient solution and became weaker with rising Mg^{2+} content. (Fig.2.)

With peptone as the only C-source (5 g/l), a strong cell growth sets in after a lag-phase, but no NH_4^+ is oxydised (Fig. 3). Decrease of the dry weight on the 3rd day is not due to autolysis, but to the fact that the bacteria settle on the glass wall. With Na-acetate (5 g/l) with and without additionof tryptose - yeast extract the bacteria nitrificate, after they have used up the originally existing NO_2^- and NO_3^-. The highest values occur after the termination of the log. growth phase.

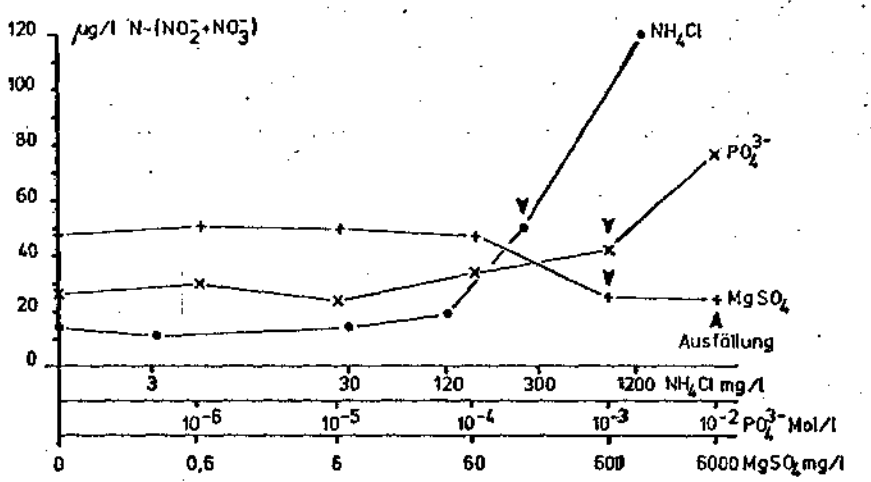


Abb. 2. Heterotrophe Nitrifikation bei verschiedenen Konzentrationen von NH_4^+, PO_4^{3-} und Mg^{++}

Fig. 2. Heterotrophic nitrification at varying concentrations of NH_4^+, PO_4^{3-} and Mg^{++}

Ausfällung = precipitation

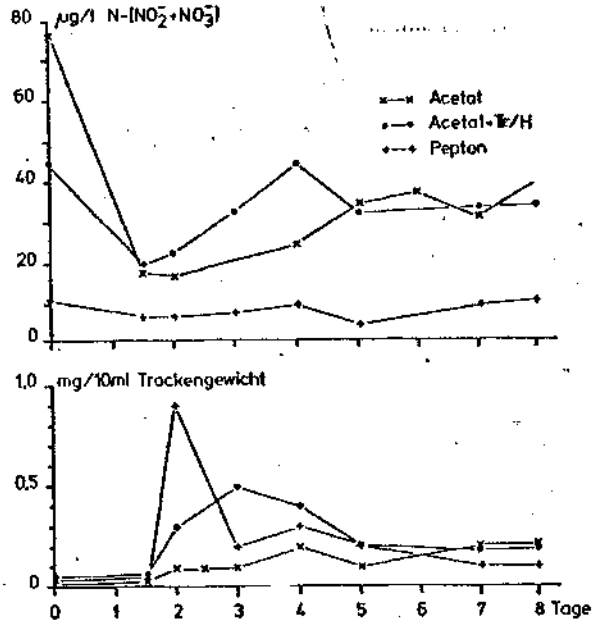


Abb. 3. Nitrifikation und Zellwachstum von *Arthrobacter spec.* mit Acetat bzw. Pepton als C-Quelle (Tr/H = Tryptose-Hefeextrakt)

Fig. 3. Nitrification and cell growth of Arthrobacter sp. with acetate resp. peptone as C-source (Tr/H = Tryptose - yeast extract.

Trockengewicht = dry weight.

In growing cultures, cell increase and ammonia-oxydation of the acetate content of the nutrient solution were interdependent in the following way (Fig.4): In the curves 2, 3 and 4 (5g, 2g resp. 0.5g acetate/l) cell increase and nitrification ran parallel. In the case of 0.2 g/l and 0.5 g/l (curves 5 and 6) growth slowed up with sustained nitrification. Also, in the case of the high concentration of 50 g/l (curve 1), which admits no growth, strong nitrification could be traced.

It follows from this, that no relationship between growth and nitrification must definitely exist, but no doubt can exist. Under favourable conditions it goes to nitrification without a corresponding increase in the mass of the bacteria. However the nitrification is definitely connected with the existence of the bacteria.

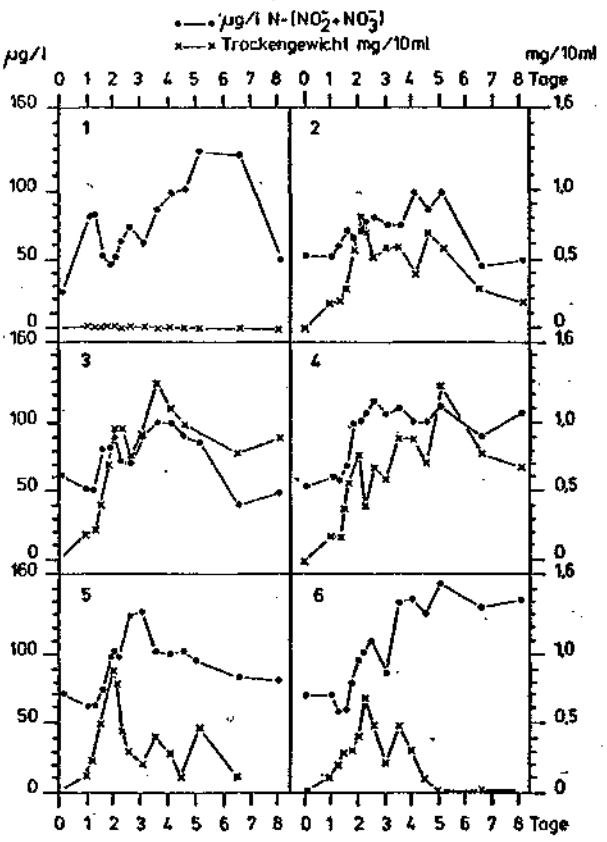


Abb. 4. Nitrifikation und Zellwachstum von *Arthrobacter spec.* bei verschiedenen Acetatkonzentrationen. Kurve 1 = 50 g Na-acetat/l, Kurve 2 = 5 g/l, Kurve 3 = 2 g/l, Kurve 4 = 0,5 g/l, Kurve 5 = 0,2 g/l, Kurve 6 = 0,05 g/l

Tage = days

Fig. 4. Nitrification and cell growth of Arthrobacter sp. at varying concentrations of acetate. Curve 1 =50g Na-acetate/l, Curve 2 = 5g/l, Curve 3 = 2g/l, Curve 4 = 0.5 g/l, Curve 5 = 0.2 g/l, Curve 6 = 0.05 g/l

Discussion

The aim of the studies was to ascertain what part of the autotrophic and heterotrophic bacteria play in the oxidation of ammonia in a lake. For the PluBsee in East Holstein the assumption that the chemolithotrophic nitrifying bacteria carry out the ammonia- oxidation, (at least for Nitrosomonas)does not prove to be correct, as the number of existing traceable cells is too low. Nitrobacter is indeed to be found more abundantly, but would be dependent upon a back-oxidation out of the denitrification, as Nitrosomonas does not yield sufficient nitrite. The numerous heterotrophic nitrificants that exist probably have a substantially greater significance. Their seasonal and local distribution

in the lake correspond with the general behaviour of heterotrophic bacteria (OVERBECK 1968). As they are not dependent on nitrification, it cannot be concluded from their number that they in fact nitrify in the lake. The laboratory experiments however, showed clearly that in the case of a very small supply of organic compounds they will nitrify well and that magnesium, ammonia and phosphate have a definite influence on the mass of the nitrogen compounds oxydised. The laboratory results do not indicate that the bacteria cannot nitrify under the conditions of the lake.

In the case of the thoroughly examined Arthrobacter species, the maximum oxidation production of a single cell was estimated at 4.10^{-7} µg/hr oxidised NH_4^+. In other experiments only about 10^{-8} µg/hr were attained. The maximum cell counts established in the lake, approx. 10^5/ml. could consequently yield NO_2^- in the milligram range. Since the method of determining spore counts produces rather low values, the production of heterotrophic ammonia-oxidation could be even higher. Since, as a rule however, greatly lower nitrite concentrations are found in the lake, and a greatly reduced capacity for nitrification by the heterotrophic bacteria in the lake against their capacity in the laboratory experiment, will not decrease their importance for the N-oxidation in the lake. Thus it is shown for the first time, that organisms other than the chemolithotrophic bacteria can play a role in the oxidative part of the nitrogen cycle of the lake, and exceed by far the significance of the case in consideration. The studies on the physiology of the heterotrophic nitrifiers will be continued.

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Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.