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AMMONIA OXIDATION IN NITROSOMONAS AT NH_3 CONCENTRATIONS NEAR K_m: EFFECTS OF pH AND TEMPERATURE

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Abstract—*Nitrosomonas europaea* from continuous pure cultures was incubated with 26.4 μM NH_3 (= 0.37 mg NH_3-N l^{-1}) at various NH_4^+ concentrations, pH values and temperatures. Measured rates of nitrite formation were significantly influenced by pH. Likewise unexpectedly, the maximum ammonia oxidation rate occurred between pH 6.7 and 7.0. Temperature had an even stronger effect on the rate of ammonia oxidation than the availability of NH_3. It is concluded that the assumption of a strict dependence of the rate of ammonia oxidation on substrate concentration is an unjustified oversimplification. Among the mechanisms which could explain ammonium uptake and oxidation near or below pH 7.0, the formation of NO from HNO_2 is considered.

Key words—nitrification, NH_3, pH, temperature, inhibition, continuous cultures

INTRODUCTION

Because of the slow growth of nitrifying bacteria, nitrification is the principal bottleneck in modern activated sludge processes which are tuned for efficient removal of nitrogen compounds, in particular of NH_x-N(= NH_3–N + NH_4^+-N). Being a function of the rates of ammonia oxidation and of nitrite oxidation, the growth rates especially of the ammonia oxidizers actually determine the volumes required for the aerated tanks of an activated sludge plant. Up to now, however, the quantification of this important design criterion is in fact more hypothetical than based on exact knowledge of reaction constants under the inconstant working conditions to be met in practice (Dohmann, 1993).

The current assumption that free ammonia rather than that of ammonium is the substrate for ammonia oxidation in *Nitrosomonas* has been based on the numerous publications reviewed by Painter (1986) and Dombrowski (1991). The kinetic coefficients for either the total process of nitrification or for its two steps individually were derived from experiments with nitrifying activated sludge, enrichment cultures and with pure cultures of ammonia oxidizing or nitrite oxidizing bacteria. In preceding studies on the influences of pH, temperature or oxygen concentration on the rate of ammonia oxidation (e.g. Painter and Loveless, 1983; Antoniou *et al.*, 1990; Helder and de Vries, 1985) at least one of the following kinetic parameters was determined: μ_{max} (maximum specific growth rate), K_s (half saturation constant for ammonia or oxygen), or K_{si} (substrate inhibition constant).

The long and confusing debate about the correct value of K_s or K_m for ammonium seemed to have come to an end by the conclusion that free ammonia is the energy source of ammonia oxidizers like *N. europaea* (Wood, 1986). Suzuki *et al.* (1974) found the K_m value to be fairly constant (0.25–0.34 mg NH_3-N per liter) between pH 6.5 and 8.5 in cell-free extracts of *N. europaea*. The results of similar experiments with whole cells were, however, not as uniform.

For a nitrifying enrichment culture Neufeld *et al.* (1980) obtained a constant K_m for free NH_3 of 0.152 mg NH_3 l^{-1} over a pH range from 7.0 to 9.0. On the other hand, Drozd (1976) reported a decrease of K_m for free NH_3 from 0.22 mg l^{-1} at pH 9 to 0.07 mg l^{-1} at pH 6.0 for a batch culture of *N. europaea*. The same tendency was found by Laudelout *et al.* (1976) with a K_m for NH_3 of 1.15 mg NH_3-N l^{-1} at pH 8.0 and 0.04 mg NH_3-N l^{-1} at pH 6.0. Jones and Morita (1985) grew a marine *Nitrosomonas* sp. at 5°C and incubated samples at various pH values. In this case the K_m for free NH_3 decreased only from 0.014 mg NH_3-N l^{-1} at pH 7.8 to 0.011 mg NH_3-N l^{-1} at pH 6.8.

Another interesting finding of Jones and Morita (1985) was a pronounced effect of temperature during chemostatic precultivation on the rates of ammonia oxidation under standard conditions. To check whether this temperature effect might be detectable in *N. europaea*, too, pure cultures were grown at 20 and 30°C in chemostats. However, we failed to keep a continuous culture of the organism stable at 10°C.

Biomass samples from the effluents of the two chemostat cultures were incubated in a batch reactor at various ammonium concentrations, pH values and temperatures. Contrary to previous approaches, we measured the rates of ammonia oxidation at substrate concentrations near the K_m value (0.37 mg NH_3-N l^{-1}) which was adjusted specifically for every pH and temperature. Around K_m the oxidation rates should follow first order kinetics and any influence of pH or temperature or substrate availability should produce a clear response.

MATERIAL AND METHODS

N. europaea (Strain Nm35, obtained from H.-P. Koops, University of Hamburg) was continuously cultivated at 20 ± 0.1 and 30 ± 0.1°C in fermenters (Bioengineering) with 2 l net volume. To prevent photoinhibition (Alleman *et al.*, 1987) the fermenters were totally covered with black polyethylene sheets. The inorganic medium contained per liter: 1500 mg NH_4Cl, 80 mg KCl, 50 mg $MgSO_4 \cdot 7\ H_2O$, 15 mg $CaCl_2 \cdot 2H_2O$, 600 mg NaCl, 100 mg $Na_2HPO_4 \cdot 12H_2O$, 0.71 mg $FeCl_2 \cdot 4H_2O$, 1.35 mg Na_2 EDTA $\cdot 2H_2O$, 1130 mg $NaHCO_3$ and 1 ml of trace element solution as described by Payer and Trültzsch (1972). The medium was supplied continuously (Abimed pump) to give a dilution rate of $0.02\ h^{-1}$. The fermenters were aerated with air ($100\ l\ h^{-1}$) enriched with CO_2 (1% v/v). pH values were kept constant at 8.00 ± 0.02 by titration with a 1 mol NaOH solution (WTW pH meter: Ingold autoclavable electrode, control unit: Chessell). The cultures were regularly checked for heterotrophic contamination by incubating samples in tryptic soy broth (Merck) for 2 weeks at 30°C. Ammonia-N and nitrite-N were measured with an autoanalyzer (Skalar).

Biomass concentration was measured as optical density (OD) at 436 nm (Kontron photometer, type Uvikon 820). In previous work (Ramsch, 1991, unpublished) a fairly good linear correlation ($r = 0.97$) was found between dry matter concentration and optical density. We used that relation to convert OD into biomass concentration.

The fermenter effluent was collected and centrifuged for 10 min at 8600 *g* at 20 or 30°C, respectively (Dupont, Sorvall RC-5B). The biomass was resuspended in ammonia-free medium and stored up to two days in the dark at 20 or 30°C before being used in the batch experiments.

The aerated batch reactors had a volume of 1 l, were pH-controlled, and the desired temperature was kept constant by pumping heated or cooled water through the glass jackets of the reactors. Each batch test lasted 2 h. Samples were taken every 10 min, and analyzed for nitrite. The plots of nitrite concentration versus time gave linear correlations ($r = 0.99$).

We calculated the amounts of initial $NH_x (= NH_3 + NH_4^+)$ concentrations to obtain 0.37 mg of NH_3 per liter at different pH values and temperatures using (Emerson *et al.*, 1975):

$$F = \frac{1}{1 + 10^{pk_s\text{-pH-}S}}$$

and

$$pk_s = \frac{2792.92}{TC + 273.16} + 0.09018$$

where F is the free-ammonia fraction of NH_x, TC = temperature in °C. The salinity factor (S) was neglected.

RESULTS AND DISCUSSION

Continuous cultures

N. europaea was continuously cultivated at a dilution rate of $0.02\ h^{-1}$ over 200 days without any detectable contamination. At an influent concentration of 392 mg NH_x-N per liter, energy-limited growth was never obtained. The effluent NH_x-N concentration showed irregular oscillations between 20 and 300 mg l^{-1}. The difference between these amounts and the bound-nitrogen input equaled the amount of NO_2^--N produced (up to 370 mg l^{-1}). The biomass concentrations varied between 6 and 25 mg dry matter per liter.

Periods of 20–30 days with variations in optical density of less than 10% were considered as steady states. At 30°C, the "steady state" biomass concentration was 18.3 ± 1.8 mg l^{-1} and the residual NH_x-N concentration in the effluent of the chemostat was 88 ± 15 mg N l^{-1}. From these data, the calculated true growth yield was 0.06 g biomass · g N^{-1} which agrees roughly with the few published data on chemostatic cultures of *Nitrosomonas* (Keen and Prosser, 1986) whereas growth yields reported for enrichment cultures are about twice as high (Dombrowski, 1991).

At 20°C the variations in the concentration of biomass and residual NH_x were much stronger. At an average concentration of 10 ± 4 mg biomass l^{-1} the NH_x concentration was 200 ± 100 mg N l^{-1}. This means that the substrate utilization rate was significantly lower than at 30°C.

Substrate inhibition

The likely reason for the observed oscillations of biomass and especially of NHx-N concentration in the effluent is a permanent fluctuation between substrate inhibition (influent concentration 392 mg NH_x-N l^{-1}) and product inhibition (up to 350 mg NO_2^- l^{-1}). Evidence for this assumption came from two series of batch tests. *Nitrosomonas* cells were harvested, washed and exposed to different concentrations of NH_x-N at pH 8.0. The oxidation rates obtained followed a Haldane kinetics (see e.g. Rozick and Castens 1986) with inhibition at concentrations above 100 mg NH_x-N l^{-1}. From this amount, approximately 8 mg was free NH_3 at pH 8.0 which agrees well with to the minimum inhibition concentration of 8.2 mg l^{-1} of free ammonia reported by Anthonisen *et al.* (1976) and the incipient inhibition found by Neufeld *et al.* at 10 mg l^{-1} (1980).

Product inhibition

On a molar basis the difference between ammonia concentrations in inflow and effluent was always identical with the amount of nitrite produced. Hence, the amount of ammonia incorporated into biomass was negligible.

Nitrite is, of course, in a pH dependent equilibrium with nitrous acid which can inhibit growth and activity of *Nitrosomonas* (Anthonisen *et al.*, 1976). For comparison we examined the influence of nitrite (up to 3000 mg NO_2^--N l^{-1}) on the ammonia oxidation rate in the presence of 10 mg NH_x-N l^{-1}.

At pH 8.0 and 30°C, an inhibition of the oxidation rate was detectable above 80 mg NO_2^--N l^{-1}. This effect was reversible. *Nitrosomonas* cells exposed to 3000 mg NO_2^--N l^{-1} (the oxidation rate was 20% of the control with minor amounts of NO_2^--N) immediately reached the same oxidation rate after washing with NO_2^--free medium as the controls not exposed to inhibitory concentrations of nitrite.

From the results of the batch experiment we interpret the variable performance of the chemostatic cultures of *N. europaea* as follows. The influent concentration of NH_x was so high that it inhibited the bacteria significantly but not completely (low biomass concentration, high effluent NH_x). After reaching slowly a sufficient biomass concentration the ammonia oxidation reduced NH_x below the inhibition threshold. This must have induced faster bacterial growth, until the concentration of nitrite became so inhibitory that the biomass concentration and the rate of ammonia oxidation per unit volume declined. Thus, the observed fluctuations appear as changes from substrate (NH_3) inhibition to product (NO_2^-) inhibition and vice versa. At concentrations of NH_x below the amount leading to substrate inhibition, Keen and Prosser (1986) and Verhagen and Laanbroek (1991) were able to obtain true energy limited steady states with 100 mg NH_x-N l^{-1} (pH 7.0 ± 0.2) and 70 mg NH_x-N l^{-1} (pH 7.8), respectively.

Influence of pH

From the data fitted to the Haldane equation we obtained a V_{max} for ammonia oxidation of 0.7 mg NH_x-N mg biomass^{-1} h^{-1} and a K_m of 7 mg NH_x-N l^{-1}. Based on NH_3, the K_m value would be 0.52 mg N l^{-1}. To study the influence of pH and temperature on the rate of ammonia-oxidation, an initial NH_3-N concentration of 0.37 mg N l^{-1} (variable NH_x-N) was chosen for one set of batch experiments. Another series of tests was conducted with a constant initial concentration of 5 mg NH_x-N l^{-1}.

With the NH_x-N of 5 mg l^{-1} at all pH values and gross differences in NH_3-concentration, the maximum rate of oxidation was obtained between pH 7.5 and 8.0 (Fig. 1). At pH 8.0, the ammonia oxidation rate was 0.27 mg N mg biomass^{-1} h^{-1}, slightly below the calculated half V_{max} value of 0.35 mg N mg biomass^{-1} h^{-1}. Between pH 6.7 and 9.0, the curve relating *Nitrosomonas* activity to pH was comparatively flat-topped and therefore reminiscent of results obtained with nitrifying activated sludge (Nolting, 1991). Between pH 6.7 and 6.0 the activity dropped sharply. There was still some activity at pH 11.0 which is in accordance with the findings of Prakasam and Loehr (1972) cited in Focht and Verstraete (1977) who showed that ammonia oxidation is possible up to pH 11.2.

Since these results did not indicate a simple dependence of the rate of ammonia oxidation on the concentration of free ammonia (calculated values see

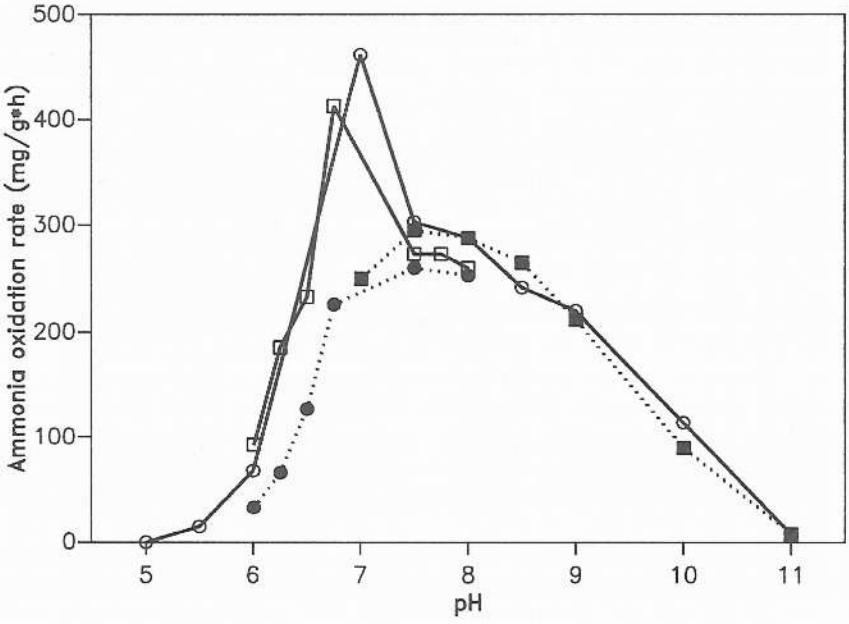

Fig. 1. Plots of ammonia oxidation rates versus pH at 30°C. Open squares and circles: data from two different batches of *Nitrosomonas* biomass incubated with 0.37 mg NH_3-N l^{-1}. Solid symbols: data from incubations with 5 mg NH_x-N l^{-1}.

Table 1. (a) The amount of NH_x-N mg/l necessary to obtain 0.368 mg/l NH_3-N in dependence of pH at 30°C. (b) Equilibrium concentration of NH_3-N in μg/l at different pH values and temperatures with 5 mg NH_x-N/l

	Temperature (°C)	pH value 5	5.5	6	6.5	7	7.5	8	8.5	9	10	11
(a)	30	4632	1465	463.56	146.84	46.69	15.02	5.00	1.83	0.83	0.41	0.37
(b)	0	0.041	0.129	0.407	1.29	4.07	12.82	40.33	125.36	376.02	2242	4452
	5	0.062	0.195	0.615	1.94	6.15	19.38	60.76	187.24	547.75	2758	4624
	10	0.092	0.290	0.917	2.90	9.15	28.84	90.07	274.04	775.04	3236	4752
	15	0.135	0.426	1.35	4.26	13.45	42.28	131.30	392.94	1062	3646	4821
	20	0.195	0.617	1.95	6.16	19.45	60.92	187.77	548.98	1403	3980	4875
	25	0.281	0.887	2.80	8.86	27.90	87.16	265.60	773.42	1797	4244	4912
	30	0.397	1.26	3.97	12.53	39.42	122.57	308.08	1004	2210	4410	4938

Table 1), we decided to determine this relationship under more stringent conditions, i.e. at a fixed NH_3 concentration. Therefore, two sets of experiments were run with an initial concentration of 0.37 mg NH_3-N l^{-1} at pH values between 5.0 and 11.0 which implied variable NH_x-concentrations (Table 1).

From pH 7.5 to pH 11.0 the rates of ammonia oxidation were essentially the same as obtained with 5 mg NH_x-N (Fig. 1). At pH 8.0, they should have been the same anyway, because 5 mg NH_x-N gives 0.37 mg NH_3-N at 30°C. Below pH 7.5 there was a striking difference to the 5 mg NH_x-N series. A peak of activity with oxidation rates of 0.49 and 0.41 mg NH_3-N mg biomass^{-1} h^{-1} was found at pH 7.0 and 6.7, respectively. At lower pH values the oxidation rates dropped almost as sharply as in the 5 mg NH_x-N series (Fig. 1), but activities were higher. At pH 5.5, the amount of NH_x-N required to obtain 0.37 mg NH_3-N was 1465 mg l^{-1} and still an oxidation rate of 0.015 mg NH_3-N mg biomass^{-1} h^{-1} was measured.

Influence of temperature

Besides pH, temperature has a pronounced influence on the equilibrium between NH_4^+ and NH_3 (Table 1). At rather low concentrations of ammonia and at low temperatures the availability of NH_3 will become limiting. In that situation it could be advantageous for the operation of nitrifying activated sludge plants to increase the pH value somewhat. For instance, the concentration of NH_3 at pH 8.0 and 30°C is 368 μg l^{-1} and only 131 μg l^{-1} at 15°C (Table 1). A slight increase in pH to 8.5 at 15°C would give again 392 μg NH_3 l^{-1}.

In order to find out whether the influence of temperature on the rate of ammonia oxidation can be (partly) compensated by increasing substrate availability, *Nitrosomonas* cells from continuous cultures grown at 20 and 30°C were incubated with either 5 mg of NH_x-N l^{-1} (variable NH_3) or 0.37 mg NH_3-N l^{-1} (variable NH_4^+-N concentration). The rates of oxidation were measured between 0 and 30°C

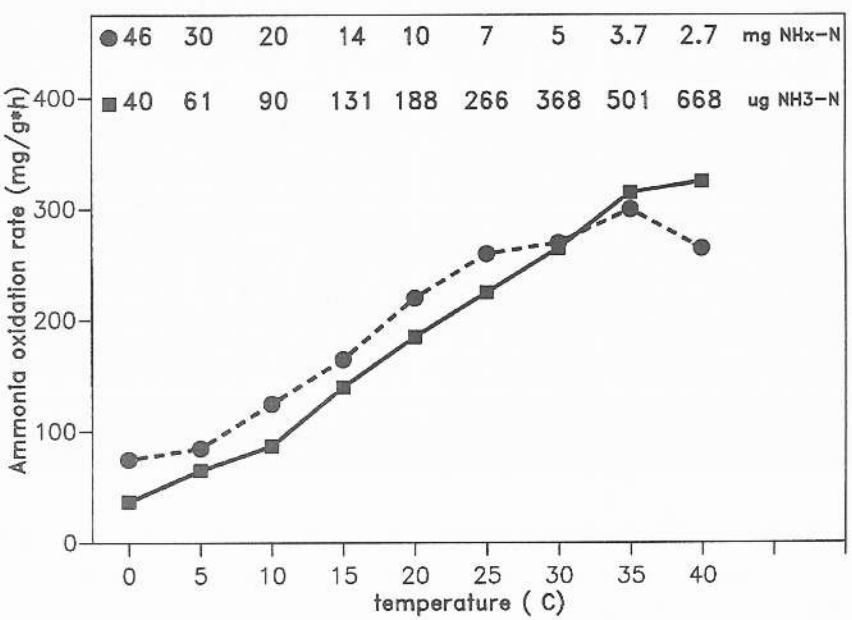

Fig. 2. Temperature dependence of ammonia oxidation rates at pH 8.0 in the presence of 5 mg NH_x-N l^{-1}. Solid line: varied concentration of NH_3-N. Broken line: 0.37 mg NH_3-N l^{-1} at varied NH_x-N concentrations.

at pH 8.0. In the series with the constant NH_3 concentration, the ammonia oxidation activity up to 30°C (Fig. 2) was higher than in the series with 5 mg NH_x-N l^{-1} because of the better substrate availability.

At 15°C, the oxidation activity was 137 and 165 mg g^{-1} h^{-1} with 131 and 368 μg NH_3-N l^{-1}, respectively. At 30°C and 368 μg NH_3-N l^{-1} the oxidation rate was 260 mg g^{-1} h^{-1}, which means that temperature has a much stronger effect on oxidation activity as can be compensated for by better substrate availability.

Nitrosomonas precultivated at 30°C displayed basically the same temperature dependence. Arrhenius plots of the data from the tests run at 5 mg NH_x-N per liter gave a linear relationship between activity and temperature between 10°C and 30°C for cells precultivated at 30°C. For *N. europaea* precultivated at 20°C the corresponding plot deviated from linearity above 20°C which indicated an effect of temperature preadaptation as described by Jones and Morita (1985).

NH_3 or NH_4^+ as a substrate

When we lowered pH from 8.5 to 6.75 (NH_x-N: 5 mg l^{-1}), the concentration of NH_3-N decreased almost over two orders of magnitude from 1.0 mg to 0.023 mg N l^{-1}. At the same time the rate of ammonia oxidation remained fairly constant with values between 0.23 and 0.30 mg N mg biomass^{-1} h^{-1}. This differed markedly from the results of the experiments with constant NH_3 concentration (Fig. 1), where the 0.37 mg NH_3-N l^{-1} corresponded to the K_m value found by Suzuki *et al.* (1974) for whole cells between pH 7.0 and 8.0 (0.32–0.44 mg NH_3-N l^{-1}). As mentioned above, we found under these conditions maximum rates of NH_3 oxidation at pH values between 6.7 and 7.0 with 0.41 and 0.49 mg NH_3-N mg biomass^{-1} h^{-1}, clearly above 0.5 V_{max}. This implies that the K_m value for NH_3 was not constant over a broad range of pH values as reported by Suzuki *et al.* (1974). Instead, the apparent K_m values decreased at lower pH values also found by Drozd (1976), Laudelout *et al.* (1976), and Jones and Morita (1985). It might be that the peak activity obtained at pH 6.7–7.0 reflects the pH optimum for the ammonia monooxygenase (AMO)/hydroxylamine oxidoreductase (HAO) complex.

An approach to explain this obvious deviation from a simple relationship between NH_3 concentration and the rate of NH_3 oxidation could be the assumption of an active NH_4^+ transport system below pH 7.0, although it is unlikely that bacteria grown in the presence of high concentrations of NH_4^+ do possess a NH_4^+ pump (Kleiner 1980, 1984). On the other hand, Bhandari and Nicholas (1979) concluded from their experiments with *N. europaea* that the uptake of NH_4^+ is energy dependent and would result in the extrusion of protons from the cells.

By means of an energy-consuming export of protons near pH 7.0 many bacteria are able to maintain an internal pH exceeding that of the medium (Kleiner 1984). For endogenously respiring *N. europaea* cells, Frijlink *et al.* (1992) observed actually a slight pH gradient, inside alkaline below pH 7.0 and inside acid above pH 7.0 in the medium.

The conclusions of Bhandari and Nicholas (1979) and the measurements of Frijlink *et al.* (1992) probably reflect the kinetics of the ammonia utilization rather than of ammonia oxidation as the current model of electron transport in *Nitrosomonas* supposes Bock *et al.*, (1991). According to this model, AMO is assumed to be located at the outer side of the cytoplasmic membrane and the HAO in the periplasmic space. Under these conditions, a transport of ammonia across the cytoplasmic membrane will not be a prerequisite for ammonia oxidation.

N. europaea possesses an elaborate cytoplasmic membrane system whereby the periplasmatic space extends in superimposed layers into the cytoplasm (Bock *et al.*, 1991). Due to diffusion limitation at external pH values below 7.0, ammonia oxidation could acidify the inner periplasmatic space more strongly than in its outer layer(s). If NH_3 is the substrate for AMO (Wood, 1986), free ammonia will not be available for a large part of the AMO molecules located in the inner periplasmic space. This would explain the aforementioned discrepancies between K_m values obtained for whole cells and cell homogenates (Suzuki *et al.*, 1974). A possible mechanism to increase the pH value in the periplasmic space, thereby restoring NH_3 availability, could consist in the production of nitric oxide. NO and N_2O are known to be produced anaerobically from the nitrite by *N. europaea* and other ammonia oxidizers (Remde and Conrad, 1990). Under aerobic conditions, however, NO is produced in larger amounts than N_2O.

As an explanation for the observed aerobic NO production by Nitrobacter, Bock *et al.* (1991) propose the following reaction to take place:

$$\text{cyt } c^{2+} + NO_2^- - 2H^+ \rightarrow H_2O + NO + \text{cyt } c^{3+}.$$

An energy-consuming H^+ system at pH values around and below pH 7.0 would explain the steep drop of the rate of ammonia oxidation below pH 6.5. Most of the energy toilsomely gained by ammonia oxidizers would be needed to maintain a favourable pH in the periplasmic space and almost no energy would be left for growth. This hypothesis would explain why ammonia oxidation activity can be measured in acid soils, but, on the other hand, all attempts failed to grow *Nitrosomonas* species at pH values at or below 6.0 (Frijlink *et al.*, 1992;

Painter and Loveless, 1983). Although direct evidence obtained under identical conditions is still lacking, it seems that the pH dependence of NH_3 oxidation may significantly differ from the pH dependence of *N. europaea* growth. If so, the rate of ammonia oxidation would not be a reliable indicator of the energetic gain of the cells.

CONCLUSIONS

(1) Concentrations of $NH_x(=NH_3+NH_4^+)$ such as commonly recommended for batch cultures of *N. europaea* are not suitable for the continuous cultivation of this organism because they lead either to substrate inhibition or to inhibition by the nitrite produced.

(2) Rates of ammonia oxidation at NH_3 concentrations around the apparent K_m value for NH_3 are pH dependent.

(3) At concentrations of NH_3 near apparent K_m the optimal pH value for ammonia oxidation is probably not identical with the optimal pH for growth of *Nitrosomonas* quoted in Bock *et al.*, 1991 (pH 7.8–8.0). Therefore the data reported in this paper should be regarded only as short-term (2 h) effects that are relevant for the elimination of nitrogen compounds from sewage in a fluctuating pH regime.

(4) Temperature as such had a much stronger effect on the rate of ammonia oxidation than the temperature dependent shift in the NH_3 availability.

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REFERENCES

Alleman J. E., Keramida V. and Pantea-Kiser L. (1987) Light induced *Nitrosomonas* inhibition. *Wat. Res.* **21,** 499–501.

Anthonisen A. C., Loehr R. C., Prakasam T. B. S. and Srinath E. G. (1976) Inhibition of nitrification by ammonia and nitrous acid. *J. Wat. Pollut. Control Fed.* **48,** 835–852.

Antoniou P., Hamilton J., Koopman B., Jain R., Holloway B., Lykeratos G. and Svoronos S. A. (1990) Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. *Wat. Res.* **24,** 97–101.

Bhandari B. and Nicholas D. J. D. (1971) Ammonia and O_2 uptake in relation to proton translocation in cells of *N. europaea*. *Arch. Microbiol.* **122,** 249–255.

Bock E., Koops H.-P., Harms H., Ahlers B. (1991) The biochemistry of nitrifying organism. In *Variations in Autotrophic Life* (Edited by Shively J. M. and Barton L. L.). Academic Press, New York.

Dohmann M. (1993) Bemessung der Belebungsbecken nach dem Ansatz der Hochschulgruppe (HSG-Ansatz). *Korr. Abwasser* **40,** 1240.

Dombrowski T. (1991) Kinetik der Nitrifikation und Reaktionstechnik der Stickstoffeliminierung aus hochbelasteten Abwässern. Fortschr.-Ber. VDI Reihe 15 Nr. 87. VDI-Verlag Düsseldorf.

Drozd J. W. (1976) Energy coupling and respiration in *N. europaea*. *Arch. Microbiol.* **110,** 257–262.

Emerson K., Russo R. C., Lund R. E. and Thurston E. V. (1975) Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *J. Fish. Res. Bd Can.* **32,** 2379–2383.

Focht D. D. and Verstraete W. (1977) Biochemical ecology of nitrification and denitrification. *Adv. Microbial Ecol.* **1,** 135–214.

Frijlink M. J., Abee T., Laanbroek H. J., de Boer W. and Konings W. N. (1992) The bioenergetics of ammonia and hydroxylamine oxidation in *N. europaea* at acid and alkaline pH. *Arch. Microbiol.* **157,** 194–199.

Helder W. and de Vries R. T. P. (1983) Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard Estuary). *Neth. J. Sea Res.* **17,** 1–18.

Jones R. D. and Morita R. Y. (1985) Low-temperature growth and whole-cell kinetics of a marine ammonium oxidizer. *Mar. Ecol. Prog. Ser.* **21,** 239–243.

Keen G. A. and Prosser J. I. (1987) Steady-state and transient growth of autotrophic nitrifying bacteria. *Arch. Microbiol.* **147,** 73–79.

Kleiner D. (1981) The transport of NH_3 and NH_4^+ across biological membranes. *Biochim. biophys. Acta* **639,** 41–52.

Kleiner D. (1984) Bakterien und Ammonium. *Forum mikrobiol.* **7,** 13–19.

Laudelout H., Lambert R. and Pham M. L. (1976) Influence du pH et de la pression partielle d'oxygène sur la nitrification. *Ann. Microbiol. (Inst. Pasteur)* **127A,** 367–382.

Neufeld R. D., Hill A. J. and Adekoya D. O. (1980) Phenol and free ammonia inhibition to *Nitrosomonas* activity. *Wat. Res.* **14,** 1695–1703.

Nolting B. (1989) Simultane Stickstoff-und Phosphatelimination in einstufigen Belebungsanlagen-Einflußparameter, Randbedingungen, Wechselwirkungen-. Schriftenreihe Siedlungswasserwirtschaft Bochum Nr. 16. Ruhr Universität Bochum.

Painter H. A. (1986) Nitrification in the treatment of sewage and wastewaters. In *Nitrification* (Edited by Prosser J. I.). IRL Press, Oxford.

Painter H. A. and Loveless J. E. (1983) Effect of temperature and pH value on the growth rate constants of nitrifying bacteria in the activated sludge process. *Wat. Res.* **17,** 237–248.

Payer H. D. and Trültzsch U. (1972) Ein Beitrag zur Versorgung dichter Kulturen von Grünalgen mit Mangan, Vanadium und anderen Spurenelementen. *Arch. Microbiol.* **84,** 43–53.

Ramsch B. (1991) Optimierung der Anzuchtbedingungen von Nitrifikanten in kontinuierlicher Kultur unter besonderer Berücksichtigung der Sauerstoffkonzentration. Diplomarbeit, Ruhr-Universität Bochum.

Remde A. and Conrad R. (1990) Production of nitric oxide in *N. europaea* by reduction of nitrite. *Arch. Microbiol.* **154,** 187–191.

Suzuki I., Dular U. and Kwok S. C. (1974) Ammonia or ammonium ion as substrate for oxidation by *N. europaea* cells and extracts. *J. Bacteriol.* **120,** 556–558.

Verhagen F. J. M. and Laanbroek H. J. (1991) Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. *Appl. envir. Microbiol.* **57,** 3255–3263.

Wood P. M. (1986) Nitrification as a bacterial energy source. In *Nitrification* (Edited by Prosser J. I.). IRL Press, Oxford.