



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 $26.4 \mu\text{M}$ NH_3 ($= 0.37 \text{ mg NH}_3\text{-N l}^{-1}$) 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 $\text{NH}_3\text{-N}$ ($= \text{NH}_3\text{-N} + \text{NH}_4^+\text{-N}$). 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 ($0.25\text{--}0.34 \text{ mg NH}_3\text{-N per liter}$) 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 $0.152 \text{ mg NH}_3\text{ l}^{-1}$ 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 $1.15 \text{ mg NH}_3\text{-N l}^{-1}$ at pH 8.0 and $0.04 \text{ mg NH}_3\text{-N l}^{-1}$ 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 $0.014 \text{ mg NH}_3\text{-N l}^{-1}$ at pH 7.8 to $0.011 \text{ mg NH}_3\text{-N l}^{-1}$ 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 .

