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Ammonia or Ammonium Ion as Substrate for Oxidation by *Nitrosomonas europaea* Cells and Extracts

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The effect of pH on the K_m values for ammonia was studied in its oxidation by *Nitrosomonas* cells and cell-free extracts. The K_m values decreased markedly with increasing pH, suggesting (NH_3) rather than (NH_4^+) as the actual substrate for oxidation.

Nitrosomonas europaea and other nitrifying bacteria are normally considered to use ammonium ion (NH_4^+) as substrate for oxidation to nitrite (NO_2^-), because ammonia exists largely in its cationic form at pH values optimal for its oxidation (around pH 8 for *N. europaea*).

We investigated the effect of pH on the K_m values for ammonia in its oxidation by whole cells as well as by cell-free extracts of *N. europaea*. It was concluded from the results that the undissociated form of ammonia rather than NH_4^+ may possibly be the actual form of substrate for oxidation.

N. europaea cells and cell-free extracts were

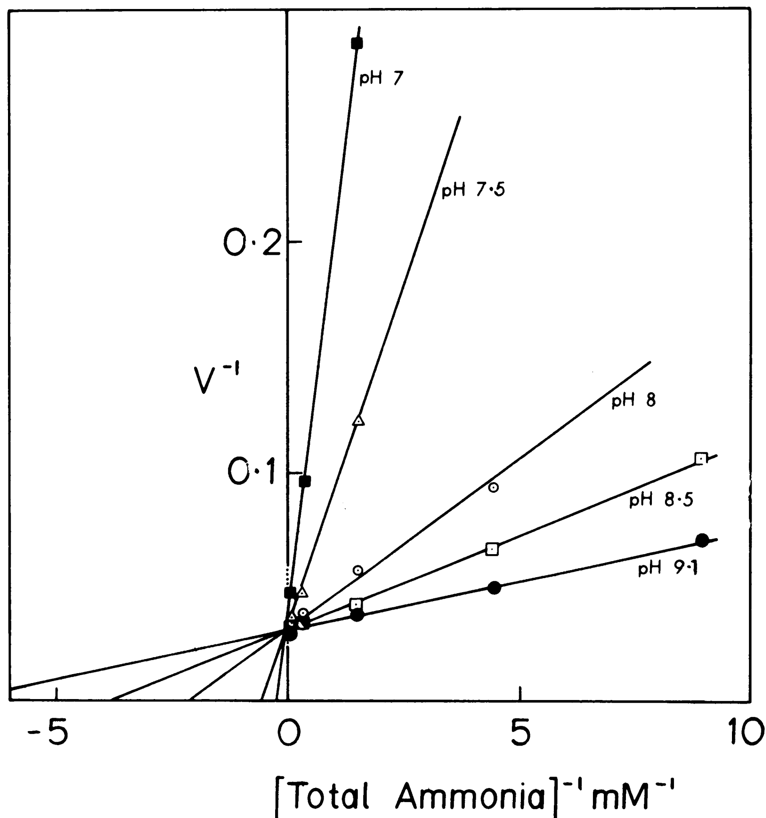

FIG. 1. *Effect of pH and ammonia concentration on the oxidation of ammonia by Nitrosomonas cells. The reaction mixture contained (in a total volume of 1.5 ml) 0.6 mg of wet cells, 0.1 M potassium phosphate buffer, and ammonia as indicated. The initial rate of oxidation (v) was expressed as nanomoles of O_2 consumed per minute.*

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prepared as described previously (3), except that extracts were prepared in the presence of 20 mg of bovine serum albumin per ml from a cell suspension of 30 mg of wet cells per ml in 0.1 M potassium phosphate buffer (pH 7.5).

Oxidation of ammonia or hydroxylamine was followed in a Gilson Oxygraph with a Clark Oxygen Electrode at 25 C. The reaction was started with the addition of ammonia as ammonium sulfate.

As shown in Fig. 1 and 2, the rate of ammonia oxidation by *Nitrosomonas* cells or extracts was markedly influenced by the pH and the concentration of ammonia. The effect of increasing pH was to reduce the slope of the double reciprocal rate-concentration plots without affecting the maximal velocity. The K_m values decreased with increasing pH (Table 1).

When the K_m values for ammonia were expressed in the concentration of undissociated form of ammonia rather than the total concentration of ammonia (using a pK value of 9.25), the results shown on the right side of Table 1 were obtained. The effect of pH on the K_m had virtually disappeared and the values remained, within experimental error, unchanged at various hydrogen ion concentrations. At very acidic or alkaline conditions, experiments were difficult because of reduced rate of oxidation, substrate inhibition, or instability of the ammonia-oxidizing system.

Hydroxylamine, an intermediate of ammonia oxidation, was oxidized by *Nitrosomonas* cells with a K_m value of 0.2 to 0.3 mM between pH 6.5 and 9.0. The constant K_m value was possibly due to the very low level of dissociation of hydroxylamine above pH 6.5 (8% at pH 7.0, using a pKa value of 5.95). With cell-free extracts, the K_m value for hydroxylamine was too low to be measured in an Oxygraph. Hooper

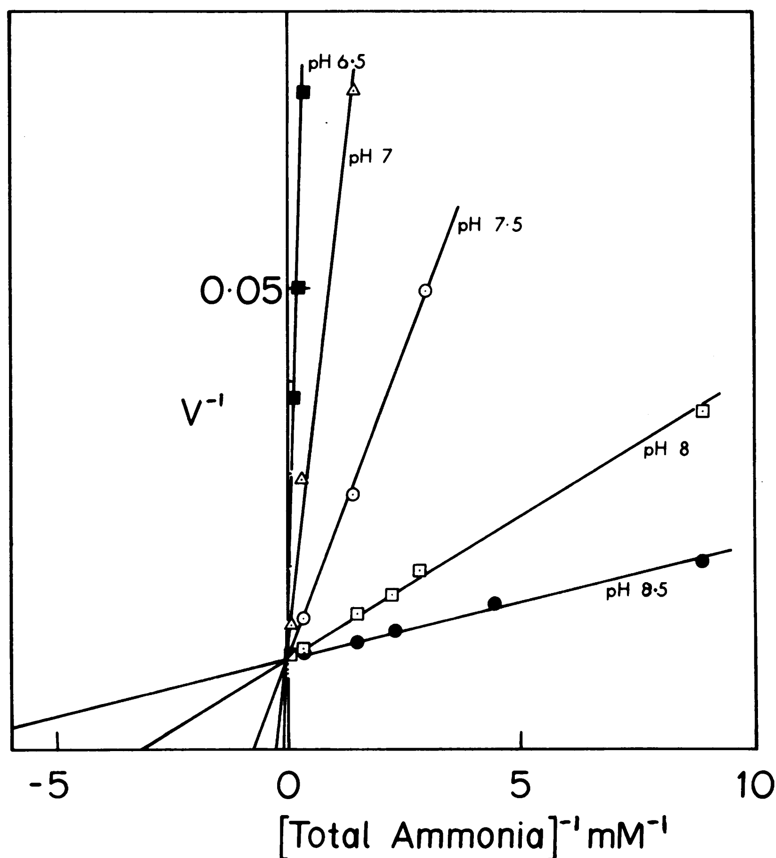

FIG. 2. *Effect of pH and ammonia concentration on the oxidation of ammonia by Nitrosomonas extracts. The reaction mixture contained (in a total volume of 1.5 ml) 0.5 ml of extract, 2 mM spermine, 0.1 M potassium phosphate buffer, and ammonia as indicated. The pH indicated was the value after mixing all the ingredients. v = nanomoles of O_2 consumed per minute.*

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TABLE 1. *Effect of pH on the K_m for ammonia in Nitrosomonas europaea*

pH	K_m			
	$NH_4^+ + NH_3$ (mM)		NH_3 (μM)	
	A[a]	B[b]	A[a]	B[b]
6.5		10.0		18
7.0	4.0	4.0	23	23
7.5	1.6	1.3	29	24
8.0	0.48	0.32	26	18
8.5	0.30	0.12	46	20
9.1	0.14		58	

[a] Whole-cell experiments (data obtained from Fig. 1).

[b] Cell-free extract experiments (data obtained from Fig. 2).

and Nason (1) reported a K_m value of 3.6 μM for hydroxylamine-cytochrome *c* reductase of the organism.

These results suggest that the actual form of substrate used for ammonia oxidation by *N. europaea* may be its undissociated form, NH_3, rather than ammonium ion, NH_4^+. A similar situation seems to exist in *Nitrobacter agilis*, and HNO_2 rather than NO_2^- was suggested as the substrate for the nitrite oxidase system (2).

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