

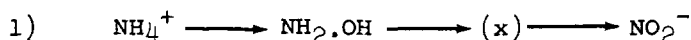
NITROHYDROXYLAMINE: THE UNKNOWN INTERMEDIATE IN NITRIFICATION?*

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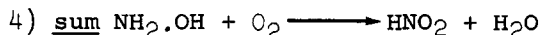
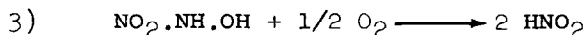
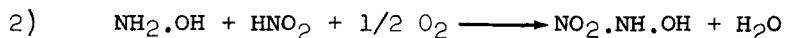
Received February 2, 1962

It is known that the chemosynthetic autotroph Nitrosomonas oxidizes ammonium to nitrite in the following way:-



but, up to now, all attempts to identify (x) have failed.

We recently began to speculate on whether the last two oxidation steps in the reaction sequence given above might not be semi-cyclic, involving an initial oxidative condensation between hydroxylamine and nitrite. These steps might formally be written:-



involving nitrohydroxylamine as the "unknown" intermediate.

Nitrohydroxylamine (Na salt) was therefore prepared according to the method of Angeli (1896) and incubated with washed Nitrosomonas suspensions in 10^{-1} M phosphate buffer, pH 8.0 in standard Warburg flasks for 30 minutes and the O_2 uptakes measured. The nitrite concentrations of the suspensions

*This work was supported by grants from the National Research Council of Canada.

were measured by the normal Griess Ilosva method at the beginning and end of the incubation. Experimental figures typical of several obtained are given below. Each result cited is the mean of two observations. The calculated O_2 uptakes are based on nitrite formation according to Equation 3 above.

Nitrohydroxylamine concentration ($\mu M/ml$)	Nitrite formed ($\mu M/ml$)	O_2 uptake (μ atoms)	
		Observed	Calculated
12	9.5	5.5	4.7
15	12.5	6.5	6.2

There was no O_2 uptake or nitrite formation when nitrohydroxylamine was incubated with boiled Nitrosomonas cells or with buffer alone in the absence of cells. These results, together with our observations that the velocity of nitrohydroxylamine oxidation is of the same order as the velocity of ammonium or hydroxylamine oxidation, suggest that nitrohydroxylamine may indeed be the hitherto unknown, or unidentified, intermediate in ammonium oxidation by Nitrosomonas.

REFERENCE

Angeli, A. (1896) Gazzetta Chimica Italiana 27 (II), 17.