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Effect of Molybdenum, Vanadium and Tungsten on the Activity of *Nitrobacter agilis*

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Nutritional studies have indicated that the growth requirements of *Nitrobacter* include calcium,¹⁾ iron, phosphorus and magnesium.²⁾ Zavarzin³⁾ was the first to note that Mo enhances the development of *Nitrobacter* in freshly inoculated cultures as measured by nitrite oxidation. Later on Finstein and Delwiche⁴⁾ found that Mo is essential for *Nitrobacter* and maximum growth takes place when it is present in the concentration of 10^{-9} g moles/litre. They estimated that a minimum of 2000 atoms of Mo is required for the synthesis of one *Nitrobacter* cell. Mo has proved to be essential for every reaction in the nitrogen cycle. It has been observed that for some organisms (viz-*Azotobacter*), vanadium can serve the same purpose as Mo, but for others (such as *Beijerinckia*), it can not.

EXPERIMENTAL

The following solutions were prepared:

(i) Culture medium—The composition of the culture medium employed was as follows:

Na₂CO₃ 1.0 g, K₂HPO₄ 0.5 g, NaCl 0.5 g, CaCl₂·6H₂O 0.3 g, MgSO₄·7H₂O 0.3 g and distilled water 1000 ml.

To avoid the deficiency of some other micro-

nutrients, the following salts were added at approx. 10^{-5} g moles per litre concentration:

ZnSO₄, CuCl₂, Ni(NO₃)₂, Co(NO₃)₂ and FeSO₄·7H₂O

(ii) Sodium nitrite solution containing 1 mg. nitrogen per ml.

(iii) Ten solutions of each of Na₂MoO₄·2H₂O, NaVO₃ and Na₂WO₄ were prepared in such a way that when 5 ml of each were added to 45 ml medium, their concentration in the medium was 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10} g mole Mo or V or W per litre respectively.

To study the effect of Mo on the oxidation of nitrite by *Nitrobacter agilis*, ten 250 ml conical flasks were taken; 40 ml culture medium and 0.2 ml sodium nitrite solution were added to each flask. Then 5 ml of the respective solution of sodium molybdate were added separately to obtain a concentration of Mo at 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10} respectively in these flasks. One flask was left as such with no molybdate solution. The volume of the solution of each flask was made up to 49 ml by adding the requisite amount of distilled water. All the flasks were sterilized at 15 lbs. pressure for 30 min. After cooling 1 ml of inoculum of pure culture of *Nitrobacter agilis* was added to each flask and all the flasks were then kept in an incubator at 32°C. For the preparation of pure culture the medium used was the same as has been described above [(i) Culture medium]. The amount of nitrite nitrogen (Griess-Ilosovay method)⁵⁾ and the total of nitrite and nitrate nitrogen (Brucine method)⁶⁾

1) T. Y. Kingma-Boltjes, *Arch. Microbiol.*, **6**, 79 (1935).

2) M. I. H. Aleem, and M. Alexander, *Appl. Microbiol.*, **8**, 80 (1960).

3) G. A. Zavarzin, *Dokl. Akad. Nauk. S.S.S.R.*, **113**, 1361 (1957).

4) M. S. Finstein and C. C. Delwiche, *J. Bacteriol.*, **89**, 123 (1965).

5) J. Meiklejohn, *J. Gen. Microbiol.*, **4**, 185 (1950).

6) F. L. Fisher, E. R. Ibert and H. F. Beckman, *Anal. Chem.*, **30**, 1972 (1958).

in 1 ml of the inoculum were estimated at the beginning of the experiment after introducing it into a flask containing 40 ml medium and 10 ml distilled water. The inoculum contained 0.003 mg nitrogen as nitrite nitrogen and 0.06 mg as the total of nitrite and nitrate nitrogen per ml.

Similar experiments were set up to study the effect of V and W.

To study the effect of V and W in presence of Mo, similar experiments were set up, but here in every flask 5 ml of sodium molybdate solution was added so as to obtain a concentration of Mo in each flask at 10^{-8} g moles/litre.

TABLE I. EFFECT OF Mo ON THE OXIDATION OF NITRITE BY *Nitrobacter agilis*

Conc. of Mo (g moles/litre)	Amount of nitrite nitrogen left at different intervals of time (in mg)			
	Time in hours			
	24	72	168	240
Control	0.176	0.074	0.007	—
10^{-2}	0.184	0.097	0.021	—
10^{-3}	0.180	0.068	—	—
10^{-4}	0.176	0.058	—	—
10^{-5}	0.169	0.054	—	—
10^{-6}	0.167	0.049	—	—
10^{-7}	0.167	0.047	—	—
10^{-8}	0.167	0.045	—	—
10^{-9}	0.173	0.066	—	—
10^{-10}	0.173	0.072	—	—

Control—containing no Mo

TABLE II. EFFECT OF V ON THE OXIDATION OF NITRITE BY *Nitrobacter agilis*

Conc. of V (g moles/litre)	Amount of nitrite nitrogen left at different intervals of time (in mg)			
	Time in hours			
	24	72	168	240
Control	0.176	0.074	0.007	—
10^{-2}	0.184	0.119	0.053	—
10^{-3}	0.180	0.085	—	—
10^{-4}	0.180	0.073	—	—
10^{-5}	0.180	0.065	—	—
10^{-6}	0.176	0.056	—	—
10^{-7}	0.169	0.056	—	—
10^{-8}	0.173	0.059	—	—
10^{-9}	0.176	0.071	—	—
10^{-10}	0.180	0.072	—	—

Control—containing no V

The nitrite left was estimated in 10 ml fraction of each flask after 24, 72, 168 and 240 hr.

The total of Nitrite and nitrate nitrogen was also determined at the end of each experiment to know, if any loss of nitrogen had taken place.

DISCUSSION*

In agreement with the observations of Finstein and Delwiche, we also found that Mo enhances the rate of oxidation of nitrite to nitrate by *Nitrobacter agilis*. Table I clearly shows that the rate of nitrite oxidation is less when the concentration of Mo is 10^{-2} g moles/litre. But when the concentration is 10^{-3} to 10^{-10} g moles/litre, the rate of oxidation increases. Maximum nitrite oxidation takes place when the concentration of Mo is kept at 10^{-8} moles/litre. This indicates that the activity of *Nitrobacter agilis* is maximum at 10^{-8} M concentration of Mo. Finstein and Delwiche found this concentration to be 10^{-9} M.

Table II shows that V behaves very much like Mo but it is slightly less effective in increasing the rate of nitrite oxidation and

TABLE III. EFFECT OF V ON THE OXIDATION OF NITRITE BY *Nitrobacter agilis* WHEN Mo IS ALSO PRESENT AT THE CONC. OF 10^{-8} M

Conc. of V (g moles/litre)	Amount of nitrite nitrogen left at different intervals of time (in mg)			
	Time in hours			
	24	72	168	240
Control	0.167	0.045	—	—
10^{-2}	0.184	0.121	0.038	—
10^{-3}	0.173	0.078	—	—
10^{-4}	0.171	0.053	—	—
10^{-5}	0.169	0.047	—	—
10^{-6}	0.167	0.047	—	—
10^{-7}	0.167	0.043	—	—
10^{-8}	0.168	0.045	—	—
10^{-9}	0.167	0.046	—	—
10^{-10}	0.168	0.043	—	—

Control—containing no V

* The total of nitrate and nitrite nitrogen remained the same at the beginning and at the end of each experiment, which clearly showed that no loss of nitrogen took place and that all the nitrite which disappeared had been changed to nitrate only.

TABLE IV. EFFECT OF W ON THE OXIDATION OF NITRITE BY *Nitrobacter agilis*

Control of W (g moles/litre)	Amount of nitrite nitrogen left at different intervals of time (in mg)			
	Time in hours			
	24	72	168	240
Control	0.176	0.074	0.007	—
10 ⁻²	0.191	0.160	0.117	0.052
10 ⁻³	0.187	0.131	0.066	—
10 ⁻⁴	0.187	0.117	0.027	—
10 ⁻⁵	0.187	0.108	0.017	—
10 ⁻⁶	0.184	0.095	0.009	—
10 ⁻⁷	0.176	0.089	—	—
10 ⁻⁸	0.179	0.087	—	—
10 ⁻⁹	0.176	0.080	—	—
10 ⁻¹⁰	0.178	0.071	—	—

Control—containing no W

TABLE V. EFFECT OF W ON THE OXIDATION OF NITRITE BY *Nitrobacter agilis* WHEN Mo IS ALSO PRESENT AT THE CONC. OF 10⁻⁸M

Conc. of W (g molef/litre)	Amount of nitrite nitrogen left at different intervals of time (in mg)			
	Time in hours			
	24	72	168	240
Control	0.167	0.045	—	—
10 ⁻²	0.184	0.113	0.023	—
10 ⁻³	0.184	0.109	0.018	—
10 ⁻⁴	0.180	0.097	0.008	—
10 ⁻⁵	0.180	0.080	—	—
10 ⁻⁶	0.180	0.076	—	—
10 ⁻⁸	0.176	0.074	—	—
10 ⁻⁷	0.173	0.072	—	—
10 ⁻⁹	0.173	0.069	—	—
10 ⁻¹⁰	0.169	0.067	—	—

Control—containing no W

the maximum nitrite oxidation takes place when it is present in the concentration of 10⁻⁷M. When Mo is added along with V, it is found that there is no additional effect.

TABLE VI. EFFECT OF W ON THE OXIDATION OF NITRATE BY *Nitrobacter agilis* WHEN V IS ALSO PRESENT AT THE CONC. OF 10⁻⁷M

Conc. of W (g moles/litre)	Amount of nitrite nitrogen left at different intervals of time (in mg)			
	Time in hours			
	24	72	168	240
Control	0.171	0.058	—	—
10 ⁻²	0.187	0.149	0.102	0.034
10 ⁻³	0.187	1.131	0.078	—
10 ⁻⁴	0.187	0.117	0.057	—
10 ⁻⁵	0.182	0.113	0.053	—
10 ⁻⁶	0.180	0.111	0.047	—
10 ⁻⁷	0.182	0.095	0.024	—
10 ⁻⁸	0.173	0.082	—	—
10 ⁻⁹	0.173	0.066	—	—
10 ⁻¹⁰	0.169	0.061	—	—

Control—containing no W

The rate of nitrite oxidation is equal to the rate which obtains in the presence of optimum concentration of Mo alone. This clearly shows that Mo is required for the activity of *Nitrobacter agilis* only upto a certain amount. In the absence of Mo, V can serve the purpose of Mo, though not as efficiently as the latter.

It is found that the presence of W alone in the medium decreases the rate of nitrite oxidation. The decrease is proportional to the concentration of W in the medium. At such a high dilution of W as that of 10⁻¹⁰M concentration, the rate is approximately the same which obtains in the medium without any W. It is further observed that when Mo is added along with W, the depressing effect of W is very much reduced. This indicates that Mo substitutes W and so the latter becomes less active. But when V is added along with W, there is no change in the rate of nitrite oxidation.