Effects of Chromate and Cadmium on Most Probable Number Estimates of Nitrifying Bacteria in Activated Sludge

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Recent concern over pollution of the environment with heavy metals extends to natural waterways and water treatment systems. Extensive use of heavy metals by industry has resulted in the presence of significant concentrations of these potential intoxicants in sewage treatment influents, effluents and receiving water resources (NYTELKA et al., 1973). Evaluation of the effects of such metals on natural and artificial water systems should include the study of biological phenomena such as nitrification. This study was an effort to determine the effects of chromate and cadmium on the number of nitrifying bacteria obtained from activated sludge samples. Most probable number (MPN) estimates of these bacteria were made in media containing several concentrations of K_2CrO_4 and $CdCl_2$.

MATERIALS AND METHODS

Activated Sludge Samples - Activated sludge samples were obtained from two local sewage treatment facilities (San Jose Creek and Whittier Narrows). Samples were taken from the effluent immediately following completion of treatment in the aeration tank.

Chemicals - All chemicals used were reagent grade. Sulfanilamide and Marshall's reagent (WEGNER, 1972) were obtained from Van Waters and Rogers, Los Angeles, California.

Media - The media used for MPN estimates followed formulas previously described (MATULEWICH et al., 1975). The trace metals mixture no. 44 was modified to include $CoSO_4$ instead of $Co(NO_3)_2$. Cadmium, as $CdCl_2$, was added to yield concentrations of 0.1, 1.0, 10.0 and 100.0 ppm. Chromate, as K_2CrO_4, was added at concentrations of 0.01, 0.1, 1.0 and 10.0 ppm. Addition of intoxicants was both to ammonia medium used for the growth of Nitrosomonas and nitrite medium used for the growth of Nitrobacter. The pH of the media ranged from 7.2 to 7.8.

Inoculation - Activated sludge samples were serially diluted ten-fold in phosphate buffer (pH 7.2) to give final dilutions of 10^{-9}. One ml of each dilution was used as the inoculum. Uninoculated controls were incubated and tested with inoculated tubes.

Tests for Ammonia, Nitrite and Nitrate - Spot tests for ammonia used Nessler's reagent. Nitrite was determined with sulfanilamide and Marshall's reagent (N-(1-naphthyl)-ethylene diamine dihydrochloride, WEGNER, 1972). Nitrate was evaluated in tubes negative for nitrite by addition of zinc powder to reduce any nitrate to nitrite which would then react with the nitrite reagents already present. Tubes were sampled by aseptically transferring a few drops of medium to each of three wells of a spot plate. The first well was tested for ammonia, the second for nitrite and nitrate, and the third was reserved for confirmation of any test already completed.

MPN Estimates - Numbers of nitrifying bacteria were determined by a most probable number method previously described (ALEXANDER, 1965). Five replicate tubes were inoculated from each serial dilution for each concentration of chromate and cadmium. Such series were prepared with both the ammonia and nitrite media. Incubation was with stationary conditions at room temperature for a maximum of 10 weeks. At intervals of 1 week, each series of tubes was subjected to tests for ammonia, nitrite and nitrate to determine the state of oxidation. Tubes positive for oxidation were considered positive for growth. Tests were run throughout the series of increasing dilutions until 2 consecutive dilutions showed negative results. The MPN was determined from an MPN table for ten-fold dilutions with 5 replicate tubes per dilution. The 95% confidence limits were determined using a standard factor of 3.3 (ALEXANDER, 1965).

RESULTS AND DISCUSSION

Tests for the number of nitrifiers continued for a maximum of 10 weeks. The MPN estimates for Nitrosomonas increased until the fourth or fifth week of incubation. Nitrobacter was not present in significant numbers until after 4 weeks of incubation, with an increase apparent until 7 or 8 weeks. Accordingly, the MPN values from the eighth week were used for comparative purposes in the following discussion.

Table 1 shows severe inhibition of growth of Nitrosomonas by 1.0 and 10.0 ppm chromate. The MPN for these concentrations was well below the

95% confidence limits for 1.0 ppm ($4.6 - 0.42 \times 10^5$) and show almost no growth at 10.0 ppm. As shown in table 2, cadmium did not appear to significantly inhibit Nitrosomonas, although one sample indicated inhibition at 0.1, 10.0 and 100.0 ppm. Such results suggest that chromate could be restrictive to the overall nitrification process by inhibiting the initial oxidation of ammonia to nitrite. Correlation of an MPN with the percent of ammonia oxidized in natural or artificial water systems such as activated sludge could permit a prediction of the toxicity of this ion in nature.

TABLE 1

K_2CrO_4 (ppm)	MPN Nitrosomonas/ml San Jose Creek	MPN Nitrosomonas/ml Whittier Narrows
NONE	1.40×10^5	7.90×10^5
0.01	2.20×10^5	7.90×10^5
0.10	2.30×10^5	4.90×10^5
1.00	0.01×10^5	0.01×10^5
10.00	1.00×10^0	3.00×10^0

TABLE 2

$CdCl_2$ (ppm)	MPN Nitrosomonas/ml San Jose Creek	MPN Nitrosomonas/ml Whittier Narrows
NONE	1.40×10^5	7.90×10^5
0.1	1.30×10^5	2.20×10^5
1.0	0.79×10^5	3.30×10^5
10.0	0.79×10^5	2.20×10^5
100.0	0.49×10^5	1.10×10^5

As shown in tables 3 and 4, Nitrobacter was not significantly inhibited by chromate and only slightly inhibited by exposure to cadmium at 100.0 ppm.

TABLE 3

K_2CrO_4 (ppm)	MPN Nitrobacter/ml San Jose Creek	MPN Nitrobacter/ml Whittier Narrows
NONE	3.30×10^4	7.00×10^4
0.01	7.00×10^4	2.30×10^4
0.10	3.10×10^4	1.30×10^5
1.00	1.30×10^5	4.60×10^4
10.00	3.30×10^4	1.10×10^5

TABLE 4

$CdCl_2$ (ppm)	MPN Nitrobacter/ml San Jose Creek	MPN Nitrobacter/ml Whittier Narrows
NONE	3.30×10^4	7.00×10^4
0.1	4.90×10^4	1.10×10^5
1.0	2.30×10^4	4.60×10^4
10.0	4.90×10^4	1.30×10^5
100.0	0.46×10^4	1.30×10^4

This study indicates that chromate severely inhibits growth of Nitrosomonas and that cadmium in concentrations below 100.0 has little effect on either Nitrosomonas or Nitrobacter. The lack of an effect on Nitrobacter may be due to differences in intracytoplasmic membranes in the cell envelope. These membranes encircle the entire cell of Nitrosomonas but are restricted to lesser areas of the Nitrobacter cell (MURRAY and WATSON, 1965). Differences in respiratory enzymes and cytochromes could also account for the sensitivity of Nitrosomonas to chromate. Further study is necessary to elucidate the exact effect of such ions on nitrifying bacteria. However, it is clear that such materials have a potential for significantly

affecting nitrification processes during activated sludge treatment of industrial wastes.

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