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Rate of Demethylation of Methylmercuric Chloride by *Enterobacter aerogenes* and *Serratia marcescens*

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Since the initial report of mercury poisonings in the Minamata Bay Area, there has been considerable interest generated in both the nature of mercury found in aquatic bodies and the biological processes which affect speciation. Most of the effort to date, however, has dealt with the methylation of inorganic mercury by microorganisms with only a few studies addressing the process of demethylation.

In eight years since JENSEN and JERNOV (1969) reported biological methylation of inorganic mercury, the number of organisms identified with the methylation process has been expanded to include aerobes (BISHOP and KIRSCH 1972, WOOD et al. 1968, and JERNLOV (1971). In fact, it is generally accepted that inorganic mercury is readily converted into methylmercury by pure cultures of Methanobacterium omelianskii and, to a lesser degree, by the bacterial species Pseudomonas fluorescens, Mycobacterium phlei, Escherichia coli, Enterbacter aerogenes, Bacillus megaterium and the fungi Aspergillus niger, and Saccharomyces cervesiae (EYL et al. 1970, SIJPESTEIJN 1973).

Until recently, however, there has been less definitive information on the degradation of organic (and inorganic) mercurials. SPANGLER et al. (1973a and 1973b) isolated species of Pseudomonas from environmental samples in which demethylation of alkylmercury compounds was observed. BRUNKER and BOTT (1974) reported the formation of volatile Hg^0 by action of the yeast Cryptococcus on mercuric chloride and SHARIAT et al. (1979) identified seventeen common bacteria which were capable of demethylating aqueous solutions of methylmercuric chloride. The rates at which the demethylating reactions occur, however, are seldom reported. The purpose of this study was to investigate the kinetics of methylmercury chloride degradation by the bacterial species Enterobacter aerogenes and Serratia marcescens.

MATERIALS AND METHODS

Pure cultures of Enterobacter aerogenes and Serratia marcescens which showed significant demethylation in screening tests (SHARIAT et al. 1979) were used in a series of laboratory studies to determine the rate of demethylation of aqueous methylmercuric chloride. Demethylation rate studies were made using 18-hr pure cultures of

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the forementioned strains (approximately 10^6 cells/ml) at concentrations of 1 mg/L (4 μM/L), 2 mg/L (8 μM/L), and 5 mg/L (20 μM/L) methylmercuric chloride and pH 6, 7, and 8, respectively, at each concentration for each microbe. Test apparatuses were prepared as described by SHARIAT et al. (1979). Both controls (sterile systems) and experimental studies were performed in duplicate using a clay type soil as the primary substrate as previously described by SHARIAT et al. (1979).

Each test flask was fitted with a trap containing $HgBr_2$ and KBr solution to retain volatilized mercury (SPANGLER et al. 1973a). Flasks were flushed continuously with sterile humidified compressed air and sampled at intervals of 0, 4, 8, 12, and 16 days for methylmercury analysis by the procedure of WESTOO (1970) and SHARIAT et al. (1979). Traps were examined simultaneously for the presence of organic mercurials to ascertain if losses occurred by stripping or the formation of volatile organic products.

RESULTS AND DISCUSSION

Analysis of the data in Tables 1 and 2 suggests that the initial rate of demethylation is governed by mercury concentration and pH in the form characteristic of enzymatic reactions. Plots of mercury (substrate) concentration versus the initial velocity of the demethylation reaction for Enterobacter aerogenes and Serratia marcescens (see Figures 1 and 2) show that the initial rate of demethylation follows a first order relationship at each pH to a methylmercury concentration of at least 2 mg/L (8 μM/L). At substrate concentrations greater than 2 mg/L the effect of pH is observed on the reaction velocity at pH 6, 7, and 8 in cultures of E. aerogenes and pH 6 and 7 in S. marcescens.

Enterobacter aerogenes appeared to follow first order (or pseudo first order) kinetics in the demethylation of CH_3HgCl at concentrations less than approximately 4 mg/L (16 μM/L) at pH 6, 7, and 8. Analysis of the data illustrated in Figure 1, showed the

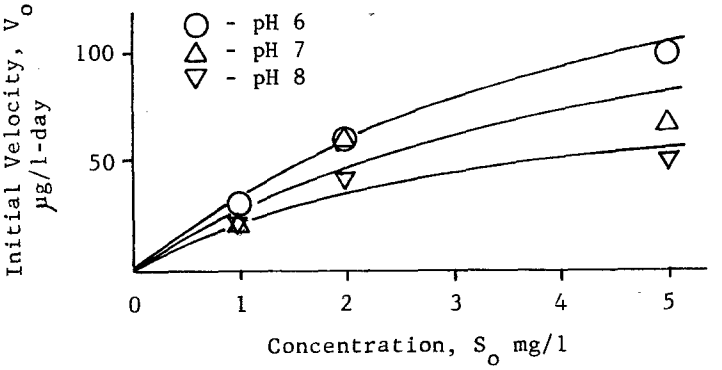

Figure 1. Rate of demethylation versus substrate concentration for Enterobacter aerogenes at pH 6, 7, and 8. Means of Duplicate Studies.

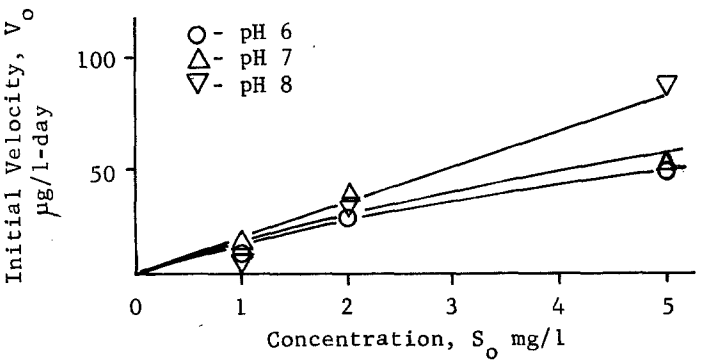

Figure 2. Rate of demethylation versus substrate concentration for Serratia marcescens at pH 6, 7, and 8. Means of Duplicate Studies.

observed reaction rate to reach one-half the maximum rate ($V_{max}/2$) at pH 6, 7, and 8 (see Table 3) but that the maximum rate of demethylation by E. aerogenes would be on the order 182 µg/L-day (0.73 µM/L-d). Values of K_m and V_{max} obtained from the data on E. aerogenes indicate uniform kinetics over the pH range followed with somewhat more rapid demethylation observed at pH 6 and 7. Serratia marcescens on the other hand, showed the highest rate of demethylation at pH 8 as compared to pH 7 and 6 (see Figure 2). Data in Table 2 and Figure 2 further suggest that while demethylation by S. marcescens is much more affected by pH than is Enterobacter aerogenes, its performance at pH 6 and 7 is not substantially different. Serratia marcescens thus appeared to be markedly better at demethylating CH_3HgCl only under alkaline soil conditions.

Methylmercury losses were noted in the sterile control systems (see Table 1) at levels up to 16% of the initial concentration after 20 days of incubation, with losses of 2-12% during the initial reaction phase. Microbial demethylation was also followed in two additional soils characterized as sand and loam by sieve analyses with no significant differences in the rate of demethylation at the 0.95 level of confidence. However, slightly higher losses of methylmercury occurred in the controls using clay soils (over sand or loam) partially as a result of increased adsorption to the finer particles and lower recoveries on extraction. Most of the methylmercury lost from the control system occurred during the first 12 days of experimentation and was in direct proportion to the initial concentration of methylmercury in the system. Highest methylmercury losses were at pH 8 in the sequence 8> 7> 6.

TABLE 1

Demethylation of CH_3HgCl at 1 mg/L (4 μM/L), 2 mg/L (8 μM/L), and 5 mg/L (20 μM/L) Initial Concentrations by Enterobacter aerogenes

pH	CH_3HgCl Conc.	1 mg/L[1] (4 μM/L)			2 mg/L (8 μM/L)			5 mg/L (20 μM/L)		
	Time Days	Average Loss %		Net Demethylation %	Average Loss %		Net Demethylation %	Average Loss %		Net Demethylation %
		Tests	Blanks		Tests	Blanks		Tests	Blanks	
6	4	29.2	2.45	26.8	30.6	1.99	28.6	14.9	3.38	11.5
	8	47.3	3.96	43.4	47.1	3.03	44.1	25.2	7.23	18.0
	12	55.1	4.90	50.2	56.8	3.83	52.9	36.4	8.11	28.3
	16	59.8	5.56	54.2	60.8	4.47	56.4	53.3	8.52	44.8
	20	64.1	6.03	58.1	63.2	5.02	58.2	60.2	8.57	51.6
7	4	21.6	3.87	17.7	30.1	2.45	27.6	16.2	3.96	12.2
	8	26.8	7.0	19.8	37.5	4.54	33.0	27.6	5.97	21.6
	12	38.2	9.18	29.0	43.0	6.52	36.4	38.7	7.63	32.0
	16	45.6	10.1	35.6	51.7	7.70	43.5	46.3	8.44	37.9
	20	56.9	10.5	46.4	56.2	8.61	47.6	50.5	8.92	41.5
8	4	26.4	3.54	22.8	19.1	3.44	15.7	6.84	6.44	0.40
	8	39.7	6.05	33.6	34.0	4.73	29.2	15.9	11.2	4.71
	12	46.5	8.21	38.2	40.3	6.60	33.7	23.7	13.6	10.0
	16	50.9	9.16	41.8	51.4	9.52	41.9	29.0	15.4	13.6
	20	53.0	10.0	43.0	54.3	11.7	42.6	37.0	16.2	20.7

[1] All studies performed in duplicate.

TABLE 2

Demethylation of CH_3HgCl at 1 mg/L (4 μM/L), 2 mg/L (8 μM/L), and 5 mg/L (20 μM/L) Initial Concentrations by *Serratia marcescens*

	CH_3HgCl Conc.	1 mg/L[1] (4 μm/L)			2 mg/L (8 μM/L)			5 mg/L (20 μM/L)		
pH	Time days	Average Loss % Tests	Average Loss % Blanks	Net Demethylation %	Average Loss % Tests	Average Loss % Blanks	Net Demethylation %	Average Loss % Tests	Average Loss % Blanks	Net Demethylation %
6	4	11.7	2 72	9.0	19.1	3.16	15.9	11.7	4.41	7.24
	8	15.5	3.81	11.7	24.4	5.36	19.0	23.6	8.36	15.3
	12	18.4	4.45	13.7	28.6	6.60	22.0	27.5	9.84	17.7
	16	22.1	5.17	17.0	34.4	7.46	27.0	31.1	10.5	20.6
7	4	12.1	2.80	9.28	11.2	5.31	5.86	13.2	5.43	7.73
	8	18.5	5.51	13.0	25.1	7.61	17.5	20.5	7.91	12.5
	12	26.0	6.82	19.2	32.6	9.19	23.4	33.4	9.53	20.9
	16	36.4	7.75	28.7	39.2	10.2	29.0	37.9	10.5	27.4
8	4	12.5	6.00	6.55	17.2	6.09	11.1	22.3	6.07	16.2
	8	21.7	9.60	12.1	34.9	10.0	24.8	35.9	9.90	26.0
	12	26.5	11.2	15.3	48.0	12.2	33.8	39.1	10.7	28.4
	16	33.1	12.5	20.6	50.4	13.3	37.1	40.8	11.7	29.0

[1] All studies performed in duplicate.

TABLE 3

Michaelis Constants (K_m)* and Maximum Velocities (V_{max}) for Enterobacter aerogenes and Serratia marcescens at pH 6, 7 and 8

	Enterobacter aerogenes			Serratia marcescens		
pH	6	7	8	6	7	8
K_m	16.4 μM/L (4.09 mg/L)	16.8 μM/L (4.2 mg/L)	15.6 μM/L (3.9 mg/L)	33.6 μM/L (8.4 mg/L)	34.0 μM/L (8.5 mg/L)	153 μM/L (38.3 mg/L)
V_{max}	0.73 μM/L-d (192 μg/L-d)	0.57 μM/L-d (143 μg/L-d)	0.37 μM/L-d (92 μg/L-d)	0.49 μM/L-d (122 μg/L-d)	0.56 μM/L-d (139 μg/L-d)	2.64 μM/L-d (661 μg/L-d)

* $\frac{1}{V_o} = \frac{1}{V_{max}} \cdot \frac{K_m}{V_{max}} \frac{1}{[S_o]}$

Note: $\frac{1}{V_o} = \frac{2}{V_{max}}$, $\frac{1}{K_m} = \frac{1}{[S_o]}$

$\mu M/L = \frac{\mu g/L}{250}$

The fact that suitable conditions for rapid demethylation exist at pH 6 and 8 with the two common organisms evaluated, suggests that pH is not likely to be a controlling factor in the demethylation of CH_3HgCl under normal soil or sediment condition.

REFERENCES

BISHOP, P.L. and E.J. KIRSCH: Proc. 27th Ind. Waste Conf. 628 (1972).

BRUNKER, R.L. and T.L. BOTT: Appl. Micro 27, 870 (1974).

EYL, T.B., K.R. WILCOX, JR., and M.S. REIZEN: Mich. Med. 69, 873 (1970).

JENSEN, S. and A. JERNLOV: Nature 223, 753 (1969).

JERNLOV, A.: Environmental Mercury Contamination, Hartung and Dennan (Ed.) Ann Arbor, Michigan, 167, (1972).

SHARIAT, M., A.C. ANDERSON, and J.W. MASON: Bull. Environ. Contam. and Toxicol. In press (1979).

SPANGLER, W.J., J.L. SPIGARELLI, J.M. ROSE, R.S. FLIPPIN, and H.H. MILLER: Appl. Micro. 26, 488 (1973a).

SPANGLER, W.J., J.L. SPIGARELLI, J.M. ROSE, and H.H. MILLER: Science, 180, 192 (1973b).

VONK, J.W. and A.K. SIJPESTEIJN: Antorni Van Leeuwenhoek 39, 505 (1973).

WOOD, J.M., K.F. SCOTT, and C.G. ROSEN: Nature 220, 1973 (1968).