APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1988, p. 1277–1279 Vol. 54, No. 5
0099-2240/88/051277-03$02.00/0

Effects of Some Alkyl Phenols on Methanogenic Degradation of Phenol

YI-TIN WANG,[1]* MAKRAM T. SUIDAN,[2] JOHN T. PFEFFER,[2] AND ISSAM NAJM[2]

Department of Civil Engineering, University of Kentucky, Lexington, Kentucky 40506,[1] and Department of Civil Engineering, University of Illinois, Urbana, Illinois 61801[2]

Received 17 September 1987/Accepted 10 February 1988

The effects of six phenolic compounds (*o*-, *m*-, and *p*-cresol and 2-, 3-, and 4-ethylphenol) on the anaerobic biodegradation of phenol was examined in batch methanogenic cultures. Results showed that ethylphenols were more inhibitory of phenol degradation than were cresols. The inhibitory effects of the three isomers of cresol and ethylphenol did not vary with the isomer but rather with the substituted functional group.

Wastewaters containing phenols have traditionally been treated by using physical, chemical, and aerobic biological processes. Recently, anaerobic biological processes have been used for the treatment of phenolic wastewaters (7, 10, 13–16, 18). Some phenolic compounds, including phenol, *m*-cresol, and *p*-cresol, have been shown to be biodegradable to methane and carbon dioxide in batch anaerobic cultures (1–3, 6, 9, 19).

Evans (5) summarized probable pathways for the anaerobic biodegradation of aromatic compounds. Ferry and Wolfe (8), however, showed that the methanogenic fermentation of benzoate requires the cooperation of several groups of bacteria and that the methanogens serve only as the terminal organism of a metabolic food chain. Removal of intermediates by methanogens provides thermodynamically favorable conditions for the degradation of many aromatic compounds. Recently, Sheridan et al. (12) and Dwyer et al. (4) observed a consortium consisting of three types of bacteria that were responsible for the conversion of phenol to CH_4 and CO_2.

In this study, the biodegradabilities of phenol, three cresols (*o*-, *m*-, and *p*-cresol), and three ethylphenols (2-, 3-, and 4-ethylphenol) in batch phenol-enriched methanogenic cultures were examined. The concentration effects of three cresols and three ethylphenols on the rate of phenol biodegradation were also investigated.

A phenol-enriched methanogenic culture was cultivated with a continuous-flow 14-liter fermentor (model MF-114; New Brunswick Scientific Co., Inc., Edison, N.J.) operated at a 30-day hydraulic retention time. The substrate for the fermentor contained 4,000 mg of phenol per liter and 5 mg of each of the following phenolic compounds per liter: *o*-cresol, *m*-cresol, *p*-cresol, 2-ethylphenol, 3-ethylphenol, 4-ethylphenol, catechol, resorcinol, hydroquinone, salicylic acid, *m*-hydroxybenzoic acid, and *p*-hydroxybenzoic acid. This solution was supplemented with a salt and vitamin nutrient solution of the actual feed composition described previously by Wang et al. (18). The fermentor was operated at a temperature of 35°C, and a pH of 7 was maintained with sodium bicarbonate. Culture transfer was performed only when the fermentor was operating at steady-state conditions.

A serum bottle modification of the Hungate technique was used for culture medium transfer, as described elsewhere (17). A 100-ml volume of culture was transferred from the fermentor to each serum bottle (125 ml) for all the batch studies. For the study of the effects of cresols and ethylphenols on anaerobic phenol degradation, each test compound was added to a series of bottles at nominal concentrations of 50, 100, 150, 250, 300, 400, 500, and 700 mg/liter. Phenol, a known biodegradable compound, was also added to each bottle at a concentration of 200 mg/liter. After equilibration for 30 min at the incubation temperature (35°C), gas volumes were zeroed to ambient pressure with a syringe; the bottles were then ready for incubation and sampling. Duplicates were run for all samples, including the blanks, which contained only the fermentor cultures and the reducing agents (sodium sulfide and L-cystine hydrochloride), and the controls, which received only 200 mg of phenol per liter.

Gas production was measured by the syringe method described by Owen et al. (11). Gas composition was determined with a gas partitioner (model 1200; Fisher Scientific Co., Pittsburgh, Pa.) with certified calibration standards. The volumetric methane production data were corrected for moisture content and converted to standard temperature and pressure (0°C and 1 atm [101.29 kPa]). These data were also corrected for the methane content of the liquid as determined from Henry's law and the partial pressure of the methane in the gas phase. Aqueous samples (1.5 ml each) were withdrawn from test bottles with a syringe at proper intervals and were immediately acidified to a pH of 2. A portion of each sample (0.5 ml) was used for volatile acid analysis, while the remaining 1.0 ml was extracted with ethyl ether for determination of the concentrations of the phenolic compounds. Concentrations of phenolic compounds and volatile organic acids were analyzed by gas chromatography by the procedure described previously (18).

Since the presence of phenol at a concentration of 200 mg/liter did not inhibit methane production or affect the rate of phenol degradation (data not shown), this concentration of phenol was selected as a background level in tests designed to determine the effects of other phenolic compounds on phenol fermentation. Six model phenolic compounds were evaluated: *o*-cresol, *m*-cresol, *p*-cresol, 2-ethylphenol, 3-ethylphenol, and 4-ethylphenol. At the same time, the biodegradabilities of these compounds when present at an initial concentration of 100 mg/liter were also examined (Table 1).

o-Cresol and the three isomers of ethylphenol were not significantly degraded to methane during a 5-month incubation period. Complete disappearance of *p*-cresol was noted within 192 h, with an observed methane yield which was

* Corresponding author.

TABLE 1. Summary of screening experiment

Substrate[a]	Methane production (ml)	
	Measured[b]	Theoretical
o-Cresol	N	8.8
m-Cresol	7.5	8.8
p-Cresol	7.9	8.8
2-Ethylphenol	N	9.2
3-Ethylphenol	N	9.2
4-Ethylphenol	N	9.2

[a] At a concentration of 100 mg/liter.
[b] Corrected for methane production from blank test bottles. N, Not significantly different (10%) from results with blanks.

approximately 90% of the theoretical yield. A lag period of approximately 70 h was observed, however. The degradation of *m*-cresol required a much longer time. Methane production did not exceed that observed for the blanks during the first 1,010 h of incubation, but complete disappearance of *m*-cresol was noted after incubation for 1,400 h. Net CH_4 production from *m*-cresol accounted for 85% of the theoretical yield. Previous work has shown that *m*-cresol is resistant to degradation under anaerobic conditions (3, 6). However, Boyd et al. (2) reported the complete disappearance of *m*-cresol in 10% anaerobic sewage sludge within 7 weeks of incubation. The difference could be due either to variability in culture inoculum or to length of incubation period.

The data in Fig. 1 show the effects of the *o*-, *m*-, and *p*-cresol concentrations on the rate of degradation of phenol. Concentrations of the three cresols lower than 150 mg/liter did not seem to affect the rate of phenol degradation appreciably. However, higher concentrations of these compounds were observed to affect the rate of phenol degradation and methane production. The production of volatile organic acids was monitored throughout the study, and no significant acid accumulation, compared with that in the controls, was observed, even when the rate of methane production was severely reduced. Acetic acid was the only low-molecular-weight volatile acid detected, and the measured concentrations in all test bottles were generally in the 10- to 40-mg/liter range. This observation indicated that phenol-degraders were more susceptible to inhibition by cresols than were the methanogens.

The inhibition of the biodegradation of phenol by cresols seemed to increase with increasing concentrations of cresol

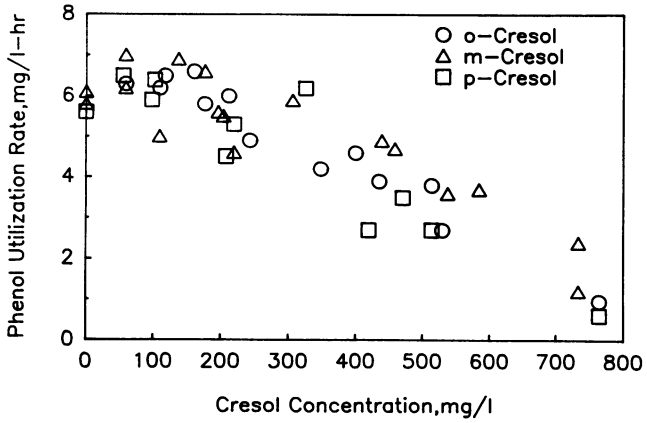

FIG. 1. Effect of cresol concentration on phenol utilization rate.

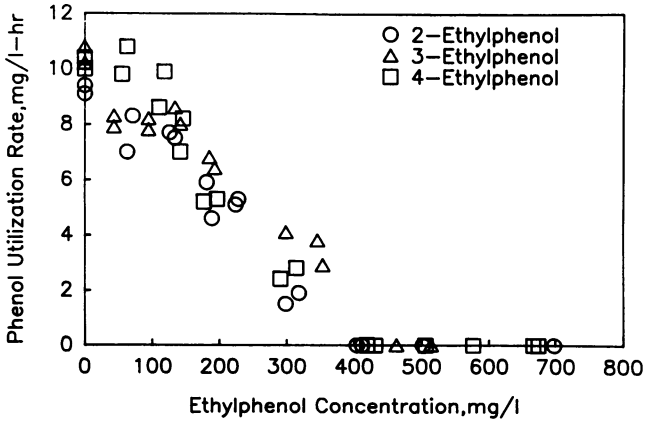

FIG. 2. Effect of ethylphenol concentration on phenol utilization rate.

(Fig. 1). These data, however, do not indicate a difference between the nature of the inhibition caused by the three cresol isomers, thus implying that the position of substitution of the methyl group does not play a major role in the inhibitory effects of the compound. This observation is very interesting, since the position of substitution of the methyl group appeared to have a significant effect on the biodegradability of the cresols. *p*-Cresol was observed to degrade in all the test cultures; however, no degradation of *p*-cresol occurred until phenol was completely utilized (data not shown). When present at 500 and 700 mg/liter, *p*-cresol was degraded at a very slow rate, despite the complete disappearance of phenol. The slower rate of *p*-cresol degradation compared with that of phenol may be attributed to the culture inoculum, grown primarily on phenol, used in this study. However, other researchers have reported that *p*-cresol degraded at rates slower than those observed for phenol. Young and Rivera (19) observed that the rate of phenol degradation was five times faster than that of *p*-cresol degradation and that acclimation did not result in an increase in the rate of *p*-cresol utilization in batch methanogenic cultures. Using bromoethanesulfonate to inhibit methanogenesis, Young and Rivera (19) detected phenol during the initial metabolic phase of *p*-cresol. They suggest that demethylation was the rate-limiting step during anaerobic *p*-cresol degradation.

The concentration effects of three ethylphenols on the anaerobic metabolism of phenol were also investigated. Similar to the effects of cresols, the effects of the three isomers of ethylphenol (2-, 3-, and 4-ethylphenol) did not differ significantly.

The data in Fig. 2 summarize the concentration effects of three ethylphenols on the rate of phenol degradation. The 50% rate reduction in phenol degradation occurred at an ethylphenol concentration of about 250 mg/liter. At about 400 mg/liter, ethylphenols appeared to be toxic to phenol degradation; phenol degradation virtually ceased in the presence of 400 mg of 2-, 3-, or 4-ethylphenol per liter (Fig. 2). Ethylphenols were not degraded in all cultures even at the very low concentrations (50, 100, 150, and 200 mg/liter) at which complete disappearance of phenol was observed. Therefore, ethylphenols can be regarded as more refractory and more inhibitive than methylphenols (cresols) under anaerobic conditions. In addition, as was the case with cresols, acetic acid was the only volatile organic acid detected, and the measured concentrations in all test bottles

were generally lower than 40 mg/liter. Thus, phenol-degrading organisms seemed to be more susceptible than the methanogens to inhibition by ethylphenols. Furthermore, the effects on phenol fermentation did not differ significantly among the cresols or among the ethylphenols. Therefore, the substitution group, rather than the substitution position, may be the important factor affecting toxicity in anaerobic cultures.

The work described in this paper was funded wholly by the U.S. Environmental Protection Agency under assistance agreement EPA Cooperative Agreement CR 806819 to the Advanced Environmental Control Technology Research Center, University of Illinois at Urbana-Champaign.

LITERATURE CITED

1. **Boyd, S. A., and D. R. Shelton.** 1984. Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Appl. Environ. Microbiol. **47**:272–277.
2. **Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje.** 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. **46**:50–54.
3. **Chmielowski, J., A. Grossman, and S. Labazek.** 1965. Biochemical degradation of some phenols during the methane fermentation. Zesz. Nauk. Politech. Slask. Inz. Sanit. **8**:97–122.
4. **Dwyer, D. F., M. L. Krumme, S. A. Boyd, and J. M. Tiedje.** 1986. Kinetics of phenol biodegradation by an immobilized methanogenic consortium. Appl. Environ. Microbiol. **52**: 345–351.
5. **Evans, W. C.** 1977. Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. Nature (London) **270**:17–22.
6. **Fedorak, P. M., and S. E. Hrudey.** 1984. The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis. Water Res. **18**:361–367.
7. **Fedorak, P. M., and S. E. Hrudey.** 1986. Anaerobic treatment of phenolic coal conversion wastewater in semicontinuous cultures. Water Res. **20**:113–122.
8. **Ferry, J. G., and R. S. Wolfe.** 1976. Anaerobic degradation of benzoate to methane by a microbial consortium. Arch. Microbiol. **107**:33–40.
9. **Healy, J. B., and L. Y. Young.** 1978. Catechol and phenol degradation by a methanogenic population of bacteria. Appl. Environ. Microbiol. **35**:216–218.
10. **Khan, K. A., M. T. Suidan, and W. H. Cross.** 1981. Anaerobic activated carbon filter for the treatment of phenol-bearing wastewater. J. Water Pollut. Control Fed. **53**:1519–1532.
11. **Owen, W. F., D. C. Stuckey, J. B. Healy, Jr., L. Y. Young, and P. L. McCarty.** 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res. **13**: 485–492.
12. **Sheridan, W. G., W. J. Jones, R. S. Wolfe, and M. T. Suidan.** 1985. Fundamentals associated with biodegradation of phenols and polycyclic N-aromatic compounds. Report to U.S. Environmental Protection Agency, Cooperative Agreement CR806819. U.S. Environmental Protection Agency, Washington, D.C.
13. **Suidan, M. T., W. H. Cross, and M. Fong.** 1980. Continuous bioregeneration of granular activated carbon during the anaerobic degradation of catechol. Prog. Water Technol. **12**:203–204.
14. **Suidan, M. T., W. H. Cross, M. Fong, and J. W. Calvert.** 1981. Anaerobic carbon filter for degradation of phenols. J. Environ. Eng. Div. Am. Soc. Civ. Eng. **107**:563–579.
15. **Suidan, M. T., G. L. Siekerka, S. W. Kao, and J. T. Pfeffer.** 1983. Anaerobic filters for the treatment of coal gasification wastewater. Biotechnol. Bioeng. **25**:1518–1596.
16. **Suidan, M. T., C. E. Strubler, S. W. Kao, and J. T. Pfeffer.** 1981. Treatment of coal gasification wastewater with anaerobic filter technology. J. Water Pollut. Control Fed. **55**:1263–1270.
17. **Wang, Y.-T., M. T. Suidan, and J. T. Pfeffer.** 1984. Anaerobic biodegradation of indole to methane. Appl. Environ. Microbiol. **48**:1058–1060.
18. **Wang, Y.-T., M. T. Suidan, and B. E. Rittmann.** 1986. Anaerobic treatment of phenol by an expanded-bed reactor. J. Water Pollut. Control Fed. **58**:227–233.
19. **Young, L. Y., and M. D. Rivera.** 1985. Methanogenic degradation of four phenolic compounds. Water Res. **19**:1325–1332.