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Methanogenesis of Phenolic Compounds by a Bacterial Consortium from a Contaminated Aquifer in St. Louis Park, Minnesota

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Anaerobic degradation of phenolic compounds to CH_4 and CO_2 in sewage sludge digestion is well documented. Little is known of the phenomenon in other environments, but it is thought to be a common occurrence (MAH et al. 1977). EHRLICH et al. (1982) found that methanogenesis is occurring in contaminated ground water at St. Louis Park, Minnesota. The ground water had been contaminated during the operation of a coal tar distillation and wood treatment facility. Phenols, a major fraction of coal tar, were observed to degrade in both the ground water and laboratory digestors with a concomitant production of CH_4. The purpose of this paper is to present the details of the laboratory studies.

MATERIALS AND METHODS

Water samples for this study were collected from the near-surface ground water in an area contaminated with plant process wastes resulting from operation of a coal tar distilling and wood treating plant. Well construction details, well locations and water sample collection methods have been described by EHRLICH et al. (1982). Water samples were collected from three wells. Well W13 is located near the centroid of the contaminated zone directly under the area where the plant process liquids penetrated the subsurface. Fluid from Well W13 is a two-phase mixture of a dense, water insoluble hydrocarbon phase of polynuclear aromatic compounds and a less dense phenolic compound containing aqueous phase. Water from Well P14, located 143 m downgradient in the direction of ground water flow from Well W13, does not have a hydrocarbon phase but does have a distinct creosote odor. This well penetrates the area of active microbiological activity. Water from Well P119, located 430 m downgradient from Well W13, does not have a distinct creosote odor and is beyond the area of active microbiological activity (EHRLICH et al. 1982).

The aqueous phase of the two-phase fluid from Well W13 was passed through a glass wool-packed chromatography column by gravity and then through a glass fiber filter (Gelman type A/E) under vacuum to remove particulates and traces of the hydrocarbon phase. Treated water was stored unsterilized in an anaerobic glove box (ARANKI et al. 1969) under an atmosphere of 85% N_2 - 10% H_2 - 5% CO_2 for at least 5 days before use to allow for gas equilibration.

The laboratory anaerobic digestors were prepared by the technique of HEALY & YOUNG (1979). The digestors, AD1 and AD2, were prepared by adding 180 mL of treated W13 water and 2 mL of ferrous sulfide suspension (BROCK & O'DEA 1977) to 250-mL serum bottles. Well P14 water, 20 mL, was added to each as inoculum. The bottles were sealed with rubber serum stoppers and purged with O_2 - free N_2. The purged gas was bubbled through the liquid by use of a long hypodermic needle penetrating to near the bottom of the bottle and the gas was vented with a short hypodermic needle through the septum. All operations were performed in the glove box.

Digestor AD1 was operated in a semi-continuous addition mode for culture enrichment. At approximately 84-h intervals, 20 mL of fluid was removed from the reactor with a disposable plastic syringe and needle and replaced with 20 mL of treated W13 water. The second digestor, AD2, was maintained for the 8-week period of the experiment in the glove box with no volume additions or subtractions. The volumes of evolved gas in the digestors were measured by displacement of the water-wetted plunger in a 10-mL glass syringe.

Methane in the head space was determined by gas chromatography (GC). A Baseline model 1010A (Baseline Industries, Inc., Lyons, CO) equipped with a flame ionization detector was used. Gas samples (0.1 mL) were injected into a stainless-steel column (0.8 mm inside diameter by 2.5 m long) packed with 150-200 mesh Porapak Q (Waters Associates, Inc., Millford, MA). Operating conditions were as follows: column temperature, 70°C; detector temperature, 70°C; detector air flow rate, 250 mL/min; detector H_2 flow rate, 35 mL/min; and N_2 carrier gas flow rate, 35 mL/min.

The phenolic compounds were isolated from the aqueous samples by dichloromethane extraction and analyzed using programmed temperature gas chromatography. Subsamples of 5.0 mL each contained in graduated, glass stoppered centrifuge tubes were made alkaline (pH 11, indicator paper) by dropwise addition of 7N NaOH. Neutral and basic interferences were extracted with 1.0 mL CH_2Cl_2. The solvent layer was removed and the phenolics in the aqueous layer were extracted with 1.0 mL CH_2Cl_2, after acidification (pH 2, indicator paper) using concentrated H_2SO_4. The water layer was aspirated from the heavier extract. Remaining water was removed by addition of 0.1 g anhydrous Na_2SO_4 to the extract. For gas chromatography, 0.5 µL volumes of the CH_2Cl_2 extract were injected. A Vista 44 GC system equipped with a split-ratio injector, flame ionization detector (Varian Group, Palo Alto, CA) and a fused silica capillary column (Cat. No. DB-5-3W, J & W Scientific Inc., Rancho Cordova, CA) was used. The operating conditions were as follows: column temperature, initial 75°C, final 200°C, program rate 16°C/min; injection temperature, 275°C; detector temperature, 300°C; He carrier gas pressure 1.05 kg/cm^2; detector H_2 flow rate, 30 mL/min; detector air flow rate, 300 mL/min.

Identification of major component peaks was done using a Hewlett-Packard 5995A GC/MS system. Authentic compounds were used to confirm identifications.

RESULTS AND DISCUSSION

Gas chromatograms of the extracts of water from near the center of contamination, Well W13, down-gradient, Well P14, and the two laboratory digestors are shown in Figure 1. No chromatogram is shown for Well P119 because the phenols in this water were below the detection limits of less than 0.03 mg/L for each, if present.

The chromatograms of the phenols from samples from Well W13 and those from the two digestors are nearly comparable although there is a slight volumetric error because of the dilution of the digestor AD2. The peak heights of the chromatogram of Well P14 are attenuated relative to W13 owing to biodegradation and hydrodynamic dispersion in the ground water.

The values given in Table 1 are adjusted for dilution and disperson. Adjustments for dispersion were made by comparing the concentration of sodium ion in samples from Well W13 to that of the other wells. Sodium hydroxide was used in the coal tar processing and the sodium ion accompanied the waste. In using sodium as a tracer, it is assumed that this cation behaves rather conservatively in the ground water

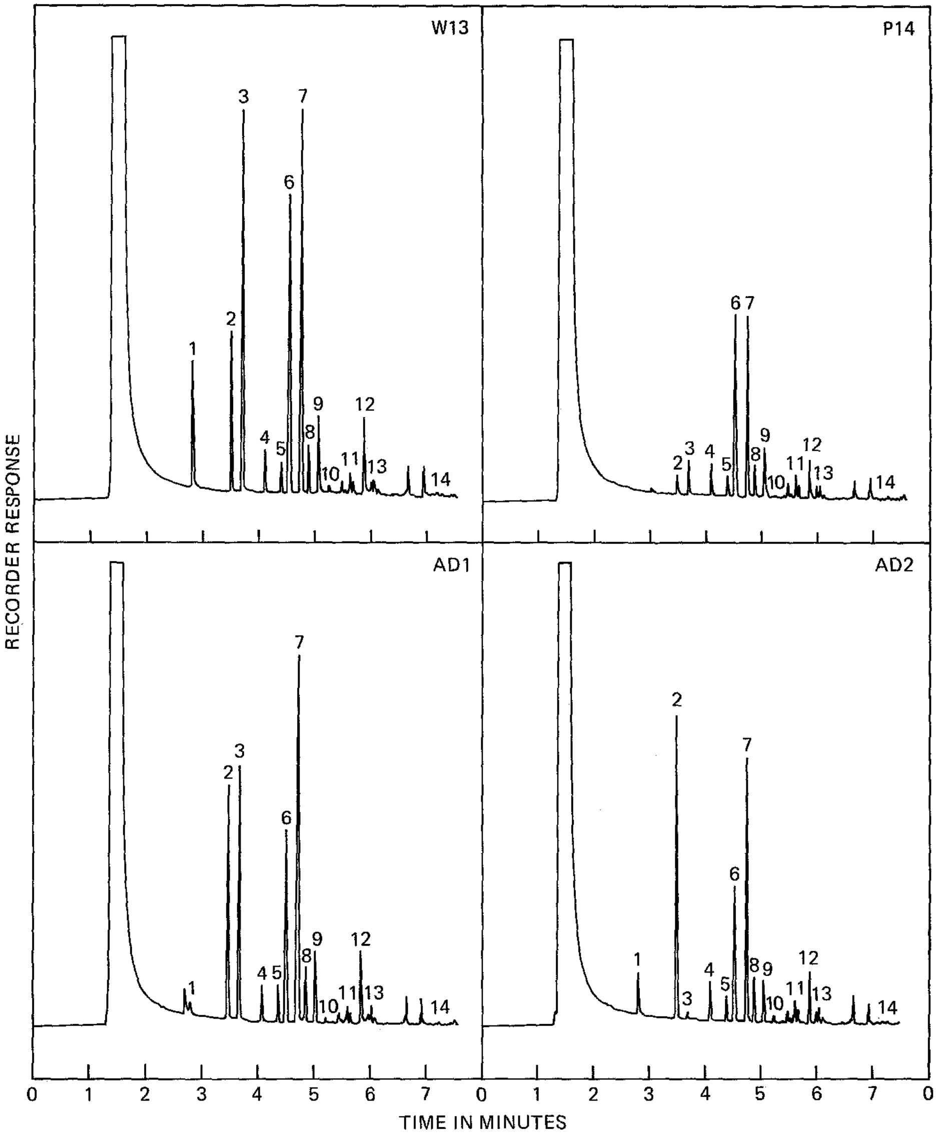

Figure 1. Gas Chromatograms of Phenolic Compounds in Water Samples and Digestors.

Table 1. Concentrations (mg/L) of Phenolic Compounds in Water Samples and Digestors

PEAK NUMBER	COMPONENT	WEIGHT PERCENT	W13	ADJUSTED1/ P14	AD1	AD2
1	Phenol	6.3	2.05	<0.03	<0.03	1.04
2	2-methylphenol	6.9	2.24	0.54	2.54	3.99
3	3-methylphenol	17.5	5.69	1.01	1.20	0.13
4	2,6-dimethylphenol	1.7	0.55	0.77	0.25	0.51
5	2-ethylphenol	1.2	0.38	0.47	0.27	0.36
6	2,5-dimethylphenol	16.8	5.44	6.73	2.00	2.45
7	3,5-dimethylphenol	19.1	6.18	5.68	3.15	3.41
8	2,3-dimethylphenol	2.2	0.73	0.97	0.49	0.62
9	2,4-dimethylphenol	3.8	1.22	2.28	0.56	0.64
10	2,4,6-trimethylphenol	0.3	0.09	0.17	<0.03	0.10
11	2,3,6-trimethylphenol	1.2	0.37	0.69	0.17	0.40
12	ethylmethylphenol 2/	5.0	1.64	1.37	1.44	0.82
13	2,3,5-trimethylphenol	0.6	0.20	0.37	0.11	0.20
14	2,3,5,6-tetramethylphenol	0.4	0.13	0.24	0.22	0.05
	Unidentified peaks (as phenol)	16.9	3.49	6.38	3.79	3.82
	Total phenols		32.46	27.73	16.84	18.39
	Sodium, mg/L		430	230		

1/ Corrected for hydrodynamic dispersion using sodium concentration ratios (see text)

2/ Authentic standard was not available

system (EHRLICH et al. 1982). Sodium in uncontaminated water was 19 mg/L and in Well P119 samples, 120 mg/L.

Concentrations of the individual phenols found in the samples from Wells W13 and P14, and in the two digestors at the end of the experiment are listed in the Table. Phenol, 2-methylphenol, and 3-methylphenol apparently degraded during transport over the 143 m distance from Well W13 to Well P14 whereas the other phenols were not utilized. Similarly, phenol and 3-methylphenol also degraded in the laboratory digestors, but 2-methylphenol did not degrade. Notably, a peak eluting at the same time as 2-methylphenol actually increased in size in the digestors. This anomaly of the digestors is not readily explainable because it did not occur in the control. The values for the phenol levels in samples from Well P14 are corrected relative to sodium. The values listed for AD2 are corrected for the inoculum volume.

When the feeding of the continuous digestor AD1 was stopped, gas evolution continued for 7 days. The incremental charge to the digestors contained 7.2 µmoles of phenolic carbon. The difference, 5.4 µmoles, was the amount of phenolic carbon utilized by the microbes from which 3.8 µmoles of CH_4 was produced for a calculated 70% conversion of phenolic carbon to CH_4. Similarly 68% of the phenolic carbon was converted to CH_4 in the batch digestor, AD2.

The stoichiometry of organic compound conversions to methane is given by the equation of TARVIN & BUSWELL (1934):

$$C_nH_aO_b+[n-a/4-b/2]\ H_2O \rightarrow [n/2-a/8+b/4]\ CO_2 + [n/2+a/8-b/4]\ CH_4$$

And, inasmuch as the substrate is composed of methyl and dimethylphenols in addition to phenol, the actual yield of methane is close to that calculated from the above equation.

The cessation of gas evolution by the digestors, even though 15-20 mg/L of phenolic compounds remained, points up the disparity between laboratory and environmental conditions. Clearly, all the phenols are utilized in the aquifer whereas only about 50% of the phenolic carbon is converted in the digestors. EHRLICH et al. (1982) confirmed that the loss of phenolic compounds was due to biodegradation and not sorption onto aquifer materials.

A significant difference between the laboratory digestors and the aquifer is the large surface area of the latter. The importance of surface to microbial growth under low nutrient concentrations has been known for many years (ZOBELL 1943). Recently, JEWELL et al. (1981) demonstrated that a plug-flow, fixed-biofilm (large surface area) anaerobic digestor is very efficient for removal of organic solutes at low temperature and low concentrations. This fits the concept of the situation occurring at the St. Louis Park, Minnesota site. The laboratory digestors did not present such a large surface area and as such did not adequately simulate the aquifer environment.

Another phenomenon which may be occurring in the aquifer but was not tested in the laboratory is the possibility of sequential degradation. CHIMILOWSKI & WASILEWSKI (1966) demonstrated the ordered degradation of resorcinol, then 3-methylphenol followed by phenol, when the mixture was added to an anaerobic digestor. Further, HEALY & YOUNG (1979) were able to show that a culture adapted to degrade a particular aromatic compound may not necessarily degrade any other aromatic compounds. In water from Well P14, phenol, 2-methylphenol and 3-methylphenol were selectively degraded, whereas the other phenols remained. At Well P119, however, all the phenols in the water samples have been utilized.

We are currently engaged in experiments to more closely simulate the aquifer environment and to study the degradation of single and mixed organic compounds in both anaerobic and aerobic conditions. The implications of this research are to study the fate of organics in aquifer systems and to point to possible solutions of problems of ground water contamination.

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