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Methanogenic degradation kinetics of phenolic compounds in aquifer-derived microcosms

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Abstract

In this segment of a larger multidisciplinary study of the movement and fate of creosote derived compounds in a sand-and-gravel aquifer, we present evidence that the methanogenic degradation of the major biodegradable phenolic compounds and concomitant microbial growth in batch microcosms derived from contaminated aquifer material can be described using Monod kinetics. Substrate depletion and bacterial growth curves were fitted to the Monod equations using nonlinear regression analysis. The method of Marquardt was used for the determination of parameter values that best fit the experimental data by minimizing the residual sum of squares. The Monod kinetic constants (μ_{max}, K_s, Y, and k_d) that describe phenol, 2-, 3-, and 4-methylphenol degradation and concomitant microbial growth were determined under conditions that were substantially different from those previously reported for microcosms cultured from sewage sludge. The K_s values obtained in this study are approximately two orders of magnitude lower than values obtained for the anaerobic degradation of phenol in digesting sewage sludge, indicating that the aquifer microorganisms have developed enzyme systems that are adapted to low nutrient conditions. The values for k_d are much less than μ_{max}, and can be neglected in the microcosms. The extremely low Y values, approximately 3 orders of magnitude lower than for the sewage sludge derived cultures, and the very low numbers of microorganisms in the aquifer derived microcosms suggest that these organisms use some unique strategies to survive in the subsurface environment.

Abbreviations: GC – gas chromatography, HPLC – high performance liquid chromatography, LBSSB – likelihood based sum of squares boundaries, MPN – most probable number, NLR – nonlinear regression analysis, OFAG – oxygen free Argon gas, PCP – pentachlorophenol, RSS – residual sum of squares, SRB – sulfate reducing bacteria

Introduction

Environmental scientists have realized for some time the need for quantitative data in their research on the movement and fate of organic pollutants in the environment. With the increased ability of computer simulations to model comprehensively the physical and chemical factors affecting the fate of pollutants, microbiologists now can describe not only the types of organisms that inhabit a particular environment but also the rates at which they perform metabolic functions that affect pollutants. This quantitative approach involves the determination of parameters in equations chosen to represent

the process under study: in this instance, organic substrate utilization and concomitant bacterial growth. These parameters can then be incorporated into computer models resulting in improved simulations.

Equations chosen for describing organic substrate utilization and concomitant bacterial growth are those proposed by Monod (1949):

$$-\frac{dS}{dt} = \frac{\mu_{max} X_a S}{Y(K_s + S)} \quad (1)$$

$$\frac{dX}{dt} = \frac{\mu_{max} X_a S}{K_s + S} - k_d X_a \quad (2)$$

with initial conditions defined as:
$X_a(0) = X_{ao}$
$S(0) = S_o$
where:

μ_{max} = maximum specific growth rate, day^{-1}
K_s = half-saturation constant, mg substrate·L^{-1}
Y = yield coefficient, mg cells·mg substrate utilized^{-1}
S = substrate concentration at time t, mg·L^{-1}
X_a = active biomass at time t, mg·L^{-1}
k_d = specific bacterial decay rate, day^{-1}

The above relationships were developed by Monod from experiments using suspensions of pure cultures of bacteria utilizing single organic compounds. A major factor affecting microbial growth and decay in many environments (e.g., aquifer sediments) is the presence of solid surfaces. Surfaces may affect the bioavailability of organic chemicals, change the concentration of various organic and inorganic nutrients, or immobilize microbial enzymes or microorganisms. Bacterial cells attached to subsurface materials may have physiological activities quite different from those of cells in suspension. It remains to be determined if these equations, with or without bacterial decay, can describe the degradation of single compounds or complex mixtures of compounds in the subsurface environment by a complex mixed microbial community that is predominantly attached to the aquifer material. Furthermore, can the kinetic constants generated under as close to natural conditions as possible be used to model the movement and fate of organic compounds in the environment?

In this part of the study, we present evidence that the Monod equations adequately describe the utilization of phenol, 2-, 3-, and 4-methylphenol, major components of the water soluble fraction of creosote, at low environmental concentrations, and to a lesser degree, the concomitant bacterial growth in microcosms that simulate the subsurface environment.

Materials and methods

Sample site

The sample site is located at Pensacola, Florida, U.S.A., adjacent to the site of an abandoned wood preserving plant (Godsy et al. 1992). The wood preserving process consisted of steam pressure treatment of pine poles with creosote and/or PCP (pentachlorophenol). For more than 80 years, large but unknown quantities of wastewaters, consisting of extracted moisture from the poles, cellular debris, creosote, PCP, and diesel fuel, from the treatment processes were discharged to nearby surface impoundments. The surface impoundments were unlined and in direct hydraulic contact with the sand-and-gravel aquifer. Contamination of the ground water resulted from the accretion of wastes from these impoundments. After the waste mixed with the anoxic ground water, two distinct phases resulted: a dense (1.17 g·cm^{-3}) insoluble hydrocarbon phase that moved vertically downward somewhat perpendicular to the ground water flow, and an organic rich aqueous phase. The aqueous phase is enriched in organic acids (35%), phenolic compounds (36%), single and double ring polynuclear aromatic hydrocarbon compounds (4%), and single and double ring nitrogen, sulfur, and oxygen containing heterocyclic compounds (25%). The four phenolic compounds tested account for 67% of the total phenolic compounds in the water soluble fraction of creosote.

Laboratory microcosms

Microcosms used for this study were prepared in 4 L glass sample bottles and contained approximately 3 kg of aquifer material anaerobically collected from the approximate centroid of the active methanogenic zone at a depth between 5 and 6 m at a site approximately 50 m down gradient from the source (Godsy et al. 1992). The microcosms were equipped with both liquid and gas sampling ports to permit periodic sampling (Godsy et al. 1992). The phenolic compounds were added to 2.5 L of mineral salts solution at concentrations similar to those found in the aquifer: 20 to 40 $mg \cdot L^{-1}$. The mineral salts solution was composed of the following (L^{-1}): KH_2PO_4, 0.75 g; K_2HPO_4, 0.89 g; $MgCl_2 \cdot 6\ H_2O$, 0.36 g; NH_4Cl, 0.9 g; trace metal solution (Zeikus 1977), 9.0 mL; and vitamin solution (Wolin et al. 1963), 5.0 mL. The pH was adjusted to 5.9, and the medium was then boiled, cooled and dispensed under a stream of OFAG (O_2-free Ar gas). The medium was then sterilized at 121° C (1.05 $kg \cdot cm^{-2}$) for 15 min. Amorphous FeS was used as a reducing agent (Brock & O'Dea 1977) to insure methanogenic conditions. The microcosms were prepared, incubated, and sampled in an anaerobic glove box containing an OFAG atmosphere at 22° C. A killed cell control (autoclaved) for each compound was prepared as above. A single viable-cell organic-free control was prepared to account for CH_4 and CO_2 production from any biodegradable organics that might be present on the aquifer material.

Chemical analysis of microcosms

Two mL liquid subsamples for substrate utilization analysis were removed from the microcosms at approximately 3-day intervals. Analyses were done by reverse-phase gradient-elution HPLC (High Performance Liquid Chromatography) after centrifuging the sample at $2{,}000 \times g$ for 10 min. The apparatus consisted of two Isco Model 2350 pumps equipped with an Isco Chemresearch System Controller, an Isco Model V[4] UV variable wavelength detector set at a wavelength of 280 nm, and an Isco C^{18} chromatography column.[1] A linear gradient from a 5% solution of acetonitrile in water to 100% acetonitrile was accomplished in 12 min and then held for 2 min at 100% acetonitrile. The flow rate was maintained at 2.0 $mL \cdot min^{-1}$.

Analyses for CH_4 and CO_2 were determined by the GC (Gas Chromatography) head space method described by Godsy et al. (1992). Corrections were made for gas production from the substrate that was removed during analyses. Dissolved gas concentrations in the microcosms were calculated from the head space concentrations (Furutani et al. 1984).

Microbial characterization of microcosms

The total biomass concentration in the microcosms at the onset and at the end of incubation was determined by total protein on 10.0 g subsamples of the sediment added to the total protein present in 5.0 mL subsamples of the liquid in suspension. Total protein was determined by the method described by Gälli (1987). Total aerobic, denitrifying and methane producing bacteria attached onto the sediment and in suspension were determined using a 5-tube MPN (Most Probable Number) procedure described by Godsy et al. (1992). SRB (Sulfate Reducing Bacteria) were enumerated using a 5-tube MPN procedure using the basal medium previously described with the addition of 3.0 g/L of Na_2SO_4, 3.0 g/L sodium acetate$\cdot 3\ H_2O$, and a head space atmosphere of 70% H_2 – 30% CO_2.

Nonlinear regression analysis

Only the substrate depletion curves were fitted to the Monod equations using NLR (Nonlinear Regression Analysis). Biomass increase was not included in the model fit because of the lack of data points and the uncertainty associated with the biomass analyses. The method of Marquardt (Bard

[1] The use of brand or product names in this article is for identification purposes only and does not constitute an endorsement by the U.S. Geological Survey.

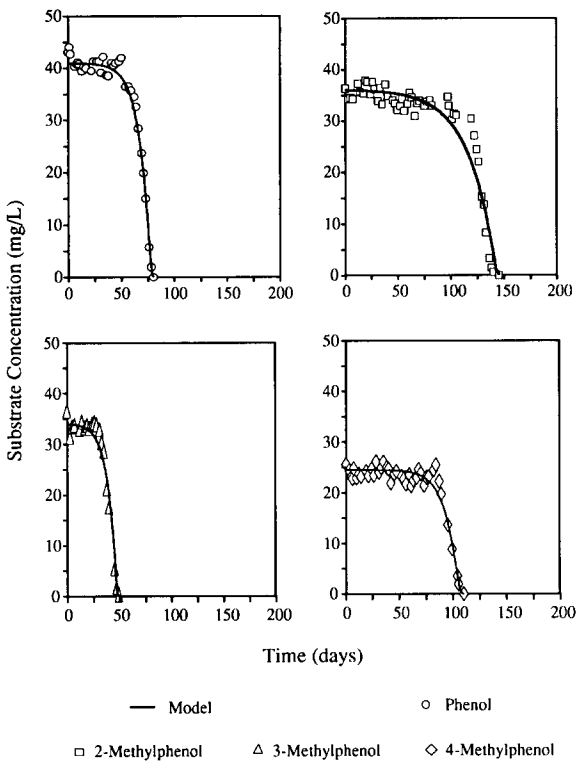

Fig. 1. Phenolic compound utilization compared to the Monod model prediction for each of the compounds tested. All values are averages of two determinations.

1974) was used for the determination of parameter values that best fit the experimental substrate depletion data by minimizing the RSS (Residual Sum of Squares). Because the Monod equations do not have explicit analytical solutions for substrate and biomass concentrations as a function of time, a simultaneous solution of both equations was accomplished using a fourth-order Runge-Kutta numerical-procedure (Constantinides 1987). The statistical basis for these analyses is presented by Robinson (1985), and requires that the sensitivity of the dependent variable to changes in each of the parameters be calculable. The partial derivatives of S with respect to μ_{max}, K_s, and Y satisfy this requirement. These expressions are derived from the integrated Monod substrate utilization equation by implicit differentiation. Unique determination of the parameters can best be obtained when S_o (initial substrate concentration) is in the mixed-order region (0.5 K_s to 2 K_s) and then letting S proceed through the first-order region ($< 0.5\ K_s$) during the course of the experiment.

Results

Monod parameter determinations for phenol, 2-, 3-, and 4-methylphenol degradation with the 95% confidence intervals are given in Table 1. The substrate disappearance curves with the model predictions are shown in Fig. 1. The time interval before the onset of rapid methanogenesis varied from 28 days for 3-methylphenol to 119 days for 2-methylphenol, even though the inoculum history suggests that the microbial population in the microcosms had been exposed to all of the phenolic compounds for considerable length of time (~ 80 years). Degradation of organic compounds was not observed in the killed cell (autoclaved) controls and, consequently, are not shown in Fig. 1. The viable-cell organic-free control did not produce any detectable amounts of CH_4 and/or CO_2, and is also not shown.

Fig. 2 shows the effect of X_{ao}, the fitted starting biomass concentration, on the substrate utilization curve for the phenol microcosm. Increasing or de-

Table 1. Kinetic constants determined for each of the compounds tested ± 95% confidence intervals.

Compound	μ_{max} (day^{-1})	K_s ($mg \cdot L^{-1}$)	Y ($mg \cdot mg^{-1}$)	k_d (day^{-1})
Phenol	0.111 ± 0.005	1.33 ± 0.07	0.004 ± 0.003	0.001 ± 0.012
2-Methylphenol	0.044 ± 0.001	0.25 ± 0.82	0.003 ± 0.003	0.002 ± 0.008
3-Methylphenol	0.103 ± 0.078	0.55 ± 6.67	0.002 ± 0.003	0.000 ± 0.019
4-Methylphenol	0.099 ± 0.110	3.34 ± 11.1	0.042 ± 0.012	0.000 ± 0.032

creasing the value of X_{ao} only displaces the curve to the left or right without changing the shape of the curve. Fitting this parameter alleviates the problem of arbitrarily picking the point at which a lag or adaptation period ends and degradation starts. This is warranted for this study because the microbial population used for the inoculum for all of the microcosms had been exposed to these compounds in the aquifer for approximately 80 years.

The values for S_o, X_a, X_t (total measured biomass), and MPN of methane producing bacteria for each of the microcosms are given in Table 2. The value of X_t in the microcosms approximately doubled during the course of the experiment and the methanogenic bacteria increased by approximately 1.5 orders of magnitude; however, the methanogenic bacteria only account for approximately 0.1% of the total bacterial population. Total facultative aerobes, denitrifying, and SRB bacterial numbers are given in Table 3. The total aerobic population increased by approximately by an order of magnitude, while the SRB and denitrifying bacteria approximately doubled in numbers.

Fig. 3 compares the biomass increase predicted by the Monod equations to the measured biomass values given in Table 2. In all cases, the fitted value of X_{ao} required to account for the long onset times was much less than the measured biomass at the outset, and this value presumably is a measure of that population that initiates the attack on the compounds tested. The final measured biomass was approximately one third of the predicted value in all cases, with the exception of 4-methylphenol. In this case, the measured value was about one order of magnitude less than the predicted value. This large predicted biomass value results from the rather large value for Y for this compound.

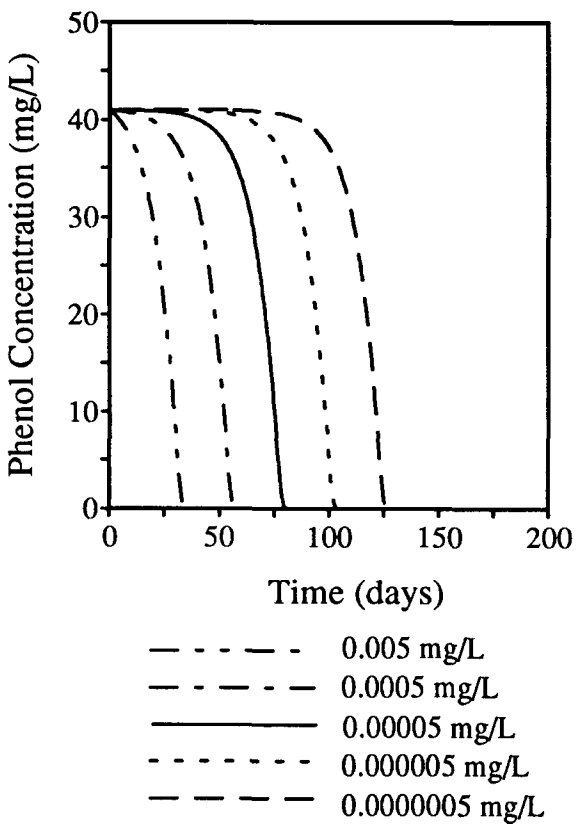

Fig. 2. Effect of the starting biomass concentration on the displacement of the phenol utilization curve based on model predictions.

Table 2. Initial substrate and biomass concentration and changes in measured biomass during substrate utilization in the microcosms.

Compound	S_o (mg·L^{-1})	X_{ao} [1] (mg·L^{-1})	Measured initial biomass X_{to} [2] (mg·L^{-1})	Measured final biomass X_{tf} (mg·L^{-1})	Initial methane bacteria (MPN·L^{-1})	Final methane bacteria (MPN·L^{-1})
Phenol	41.0	0.0001	0.017 ± 0.0019	0.037 ± 0.0032	3.1 x 10^3	1.6 x 10^5
2-Methylphenol	36.0	0.0004	0.021 ± 0.0019	0.043 ± 0.0036	3.6 x 10^3	5.6 x 10^4
3-Methylphenol	34.0	0.0003	0.017 ± 0.0016	0.044 ± 0.0037	3.1 x 10^3	2.7 x 10^5
4-Methylphenol	24.5	0.0001	0.018 ± 0.0016	0.030 ± 0.0034	3.1 x 10^3	2.4 x 10^5

[1] Fitted active biomass to account for the delay before the onset of rapid methanogenesis.
[2] Biomass ± 95% confidence interval.

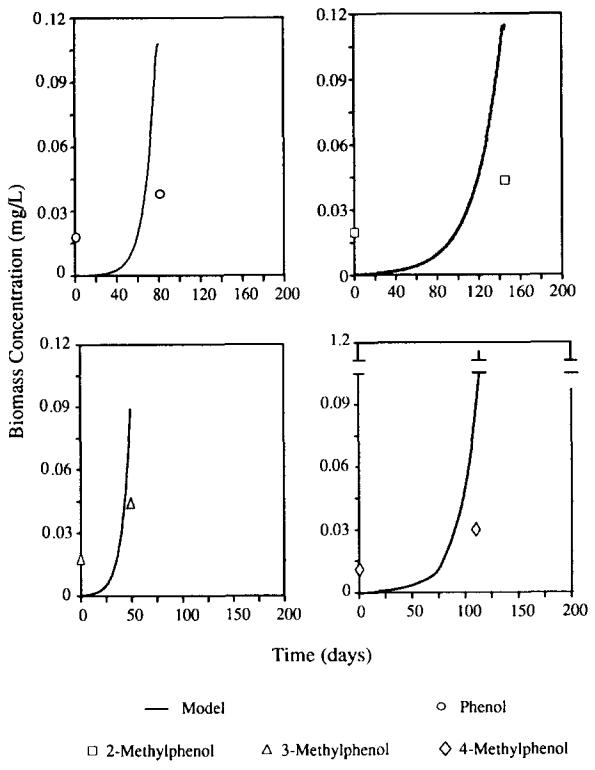

Fig. 3. Measured biomass compared to the Monod model predictions for each of the compounds tested. All values are averages of three determinations.

Mass balances of phenolic compound utilization with CH_4 and CO_2 production were determined and based on the following equation proposed by Tarvin & Buswell (1934):

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \quad (3)$$

The measured production of CH_4 and CO_2 for each of the compounds were compared to the theoretical gas yields calculated from the above equation (Fig. 4). The mass balances on the degradation of the phenolic compounds were corrected for substrate removed for HPLC analyses. Total gas values were computed from the CH_4 and CO_2 values and yielded 95.3% of theoretical total gas production for phenol, 85.4% for 2-methylphenol, 90.9% for 3-methylphenol, and 92.2% for 4-methylphenol. These values are well within acceptable and expected ranges.

Given in Table 4 are the RSS and the LBSSB (Likelihood Based Sum of Squares Boundaries) values for each determination (Bates & Watts 1988). In NLR, the assumption is made that the model being fitted is the correct one, and that the observations deviate from the model in a random fashion. These values may be used internally to give an estimate of how well the data fit the model prediction. The high RSS value for 2-methylphenol reflects the inability of the model to account for the shoulder at the onset of rapid methanogenesis (~ day 105). That family of substrate utilization curves generated from parameter values within the 95% confidence interval parameter that yields a RSS $\leq$ LBSSB will form boundaries around the predicted model curve. This is roughly equivalent to a 95% confidence interval for the substrate utilization curves and is based on the F-statistic at the 0.05 level of significance (Bates & Watts 1988). The RSS value determined as the boundary for phenol, 102.8, would displace the model curve by ±3–4 days along the time axis. The boundaries for 2-

Table 3. Initial and final concentrations of selected bacterial types for the compounds tested. All values given as MPN·L^{-1}.

Compound	Initial facultative aerobic bacteria	Final facultative aerobic bacteria	Initial denitrifying bacteria	Final denitrifying bacteria	Initial SRB bacteria	Final SRB bacteria
Phenol	3.6×10^6	1.2×10^7	5.5×10^3	6.3×10^3	3.5×10^5	1.2×10^6
2-Methylphenol	4.4×10^6	9.3×10^6	6.4×10^3	8.7×10^3	4.0×10^5	7.3×10^5
3-Methylphenol	3.6×10^6	9.4×10^6	5.5×10^3	6.9×10^3	3.4×10^5	9.4×10^5
4-Methylphenol	3.85×10^6	1.1×10^7	5.6×10^3	9.0×10^3	3.5×10^5	1.1×10^6

methylphenol would likewise be displaced by ±6–7 days.

Discussion and conclusions

Laboratory microcosms containing aquifer material used in this study attempt to simulate, with as little change as possible, the biotic and abiotic interactions that occur in the subsurface at the Pensacola study site. Most other kinetic studies utilize microbial cultures that have been adapted to a particular substrate by enrichment or continuous culture techniques. Often times the microbial populations undergo significant changes during these procedures (Mackey 1987). Populations that are not required for the degradation of complex organics, but affect the rate at which the organics are degraded, may be selectively removed from the consortium. Dills et al. (1980) found evidence for the presence of multiple uptake systems for glucose in marine microorganisms. Presumably, over several generations of growth at different substrate concentrations, progeny cells could be enriched for a transport system not in use under natural conditions, again altering the kinetic constants. If the Monod equations are accepted as viable models, the task is really one of estimating μ_{max} and K_s, as Y and k_d are relatively easy to evaluate. Templeton & Grady (1988) demonstrated that μ_{max} is often overestimated and K_s is often underestimated when bacterial populations are enriched by continuous culture or fed-batch techniques. The primary significance of this phenomenon to environmental engineers concerned with the fate of organic compounds in the environment is the recognition that

Table 4. Residual Sum of Squares (RSS) and Likelihood Based Sum of Squares Boundaries (LBSSB) for the Monod equations without decay for each of the phenolic compounds tested.

Compound	RSS	LBSSB
Phenol	62.5	102.8
2-Methylphenol	334.9	459.5
3-Methylphenol	130.9	229.4
4-Methylphenol	109.9	140.2

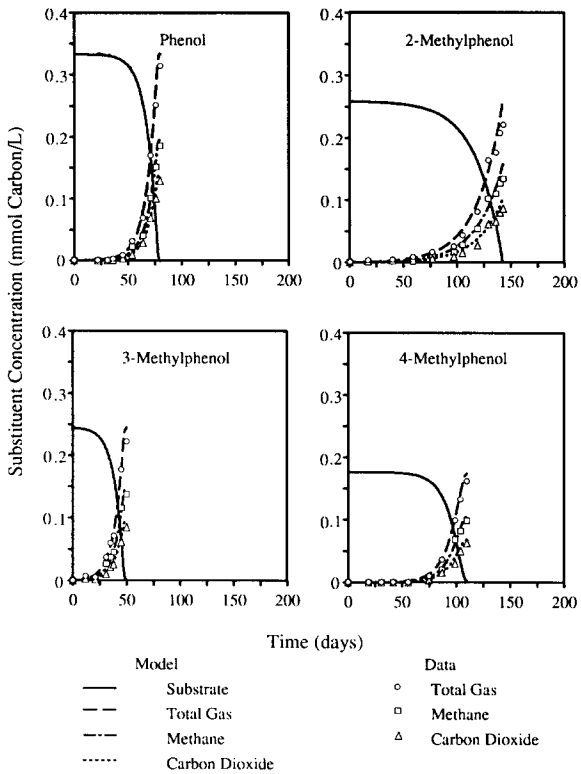

Fig. 4. Concentrations of methane, carbon dioxide, and total gas compared to theoretical yields as predicted by equation 3.

kinetic analyses will be influenced by the history of the culture used, and it is for these reasons, that no attempts were made to enrich or adapt the ground water cultures to the specific compounds tested.

The sorption of substrate and biomass to the aquifer sediment are important considerations in determining the ultimate environmental fate of contaminants. Studies in the laboratory and at the research site have shown that substrate adsorption to aquifer sediments of the four phenolic compounds tested was insignificant and that greater than 95% of the biomass was associated with the aquifer sediment (Godsy et al. 1992). However, for modeling purposes in this study, the biomass attached to the sediment can be treated as if it were uniformly distributed throughout the liquid volume. The experimental design allows the determination of the growth kinetics on the phenolic compounds at substrate concentrations similar to the

study site and under conditions as close as possible to natural conditions.

An adaptation period can be considered the time required for the adjustment of a bacterial population to a new environment. The bacterial cells may be taking in substrate, they may be synthesizing new enzymes, and they may be undergoing enlargement prior to division, but, they have not begun the orderly and steady replication. The time required for the adaptation of the population varies widely and is generally not predictable. The organisms from the centroid of the contaminated aquifer will have seen low concentrations of the phenolic compounds tested for a considerable length of time and transfer to a microcosm that duplicates the aquifer as much as possible should not alter the environmental conditions. As a consequence, the time required for the onset of methanogenesis is presumably the time required for the populations of the organisms responsible for the initial attack on the phenols to increase to a level that is sufficient for rapid degradation of the phenols and not an adaptation period in the true sense.

Analyses of the viable bacterial populations in the microcosms revealed a relatively small but diverse population. These analyses accounted for somewhat less than 10% of the total population estimated by the total protein method. Using methods described by Godsy (1980), several distinct populations of bacteria were identified. Xenic cultures (by microscopic observation) were obtained by enrichment culture methods (Zeikus 1977) that included: short fluorescing methane producing rods by formate enrichment; large, blunt-ended, crooked, fluorescing methane producing rods resembling *Methanobacterium bryantii* by H_2–CO_2 enrichment; long, curved, slender methane producing rods resembling *Methanospirillum hungatii* by a combination of acetate and H_2–CO_2 enrichment; blunt-ended, methane producing rods in long chains resembling *Methanothrix soehngenii* by acetate enrichment; a small rod-shaped sulfate reducing bacterium, similar to *Desulfobacterium* by sulfate, acetate, and H_2–CO_2 enrichment; and numerous facultative aerobic and denitrifying rods capable of heterotrophic growth on various media. Probably many other types of facultative aerobic and anaerobic bacteria exist in the ground water. We did not attempt a comprehensive program to investigate the general heterotrophic populations that exist; however, the general description of microorganisms is similar to other methanogenic consortia degrading phenolic compounds (Ehrlich et al. 1983) and aromatic compounds in general (Healy et al. 1980).

Kinetic constants for the anaerobic degradation of phenol in sewage sludge (Table 5) have been published by Neufeld et al. (1980) and for methanogenic conditions by Suidan et al. (1989). When anaerobic domestic sewage sludge is used as the inoculum, large quantities of organic compounds are presumably available for microbial utilization. The energy available from phenol under methanogenic conditions shown below is small and must be shared by a number of bacterial populations

$$C_6H_6O_{(aq)} + 4\ H_2O \rightarrow 3.5\ CH_4 + 2.5\ CO_2$$
$$\Delta G^{o'} = -149\ kJ \cdot mol^{-1}$$

and would require a relatively high substrate concentration before a microbial population would initiate the degradation of this compound when other,

Table 5. Comparison of published kinetic constants for the anaerobic degradation of phenol in sewage sludge and the parameters obtained in this study.

	μ_{max} (day^{-1})	K_s ($mg \cdot L^{-1}$)	Y ($mg \cdot mg^{-1}$)	k_d (day^{-1})
Aquifer microcosm, this study, *Methanogenic*	0.111	1.33	0.004	0.001
Neufeld et al. 1980, *Anaerobic*	0.08	686	0.82	0.008
Suidan et al. 1989, *Methanogenic*	0.106	0.03	0.16	0.192

more energetically favorable and readily biodegradable, compounds are available. This effect may explain the high K_s value (686 mg·L^{-1}) reported by Neufeld et al. (1980). Several other researchers, studying the methanogenic degradation of phenol with sewage sludge cultures, did not determine kinetic constants (Young & Rivera 1985; Healy & Young 1978); however, the approximate K_s values can be determined from the substrate utilization progress curves and appear to be slightly less than 50 mg·L^{-1}. In our study and the study by Suidan et al. (1989), cultures were used that had been exposed only to phenol and similar compounds for long periods of time. The low K_s values obtained in these studies (1.33 and 0.03 mg·L^{-1}, respectively) demonstrate that the responsible enzyme systems have developed a high affinity for phenol.

The extremely low Y values for the phenolic compounds obtained in this study suggest that the microbial community from an oligotrophic ground water environment has adapted to these conditions by utilizing > 99% of the available energy for maintaining cellular integrity. It seems unlikely that the other possible explanations, i.e., inefficiency at capturing the free energy available, or storing carbon as intracellular storage products, would account for the low Y values. These Y values are also consistent with the low biomass on the aquifer sediments and the high dissolved CH_4 concentrations (60–70% of saturation) throughout the contaminated field site (Godsy et al. 1992). This phenomenon is currently under further investigation.

The relatively large value for Y for growth on 4-methylphenol compared to the other phenolic compounds can not be tested statistically to determine if this value is significantly different from the other values obtained when using NLR techniques. However, Roberts et al. (1986) have observed that there is a difference in the degradative pathways for 3-methylphenol and 4-methylphenol. They determined that 92% of the methyl carbon for 4-methylphenol was oxidized to CO_2. In contrast, 87% of the methyl carbon for 3-methylphenol was converted to CH_4. Other marked differences in the behavior of 3- and 4-methylphenol degrading consortia have been observed by Fedorak & Hrudey (1986). It remains for enrichment and pure culture studies in progress to determine if these consortia are different with different growth characteristics and kinetic parameters – a distinct possibility considering the diversity of microorganisms observed in this study.

The bacterial decay term ($-k_dX_a$) in the biomass equation (2) is apparently not required to describe substrate utilization and/or biomass increase in the batch growth microcosms. The values determined by NLR are such that $\mu_{max} \gg k_d$, and k_d can be neglected. NLR analyses of substrate disappearance data using Monod equations without the decay term resulted in essentially the same kinetic constants being generated. Using the Monod equations without decay alleviates the problem of increased uncertainty associated with fitting four parameters versus three parameters. This is not meant to imply that in a continuous culture microcosm or in a field situation, the decay term is not important and/or necessary to describe the kinetics of utilization.

The biomass predicted by the Monod equation (2) was, in all cases, greater than the measured biomass at the end of the growth period. The protein content of bacteria depends on the organism and on its growth state and it is assumed that most bacteria have a protein content of 50% of their dry weight (Gälli 1987). An assumption is made that during the course of the determination, all of the biomass on the aquifer sediment or in solution is solubilized for subsequent assay. Either of these assumptions may be in error and may lead to erroneous biomass values; both assumptions are currently under investigation. It is for these reasons that only the substrate utilization curves were used for the kinetic analyses.

The bacterial substrate utilization for all of the compounds tested was modeled successfully using the Monod equations. The long delay before the onset of rapid methanogenesis of the phenolic compounds may be attributed to an extremely low initial active biomass concentration in the microcosms. The kinetic constants for all of the phenolic compounds are similar and may represent the same rate limiting bacterial population. Although there

is not a statistical test for this supposition when the parameters are generated by NLR (Bates & Watts 1988), visual examination strongly bears this supposition out. Given that the inocula were acclimated to all of the phenolic compounds, it is unclear why the range of onset times was so great. There is also a lack of correlation between the onset times and the model parameters. Although we know of no other kinetic studies conducted under similar conditions, and only two studies of the anaerobic degradation of phenol, it appears that the value of the parameters obtained are reasonable and consistent both with the values expected of organisms from oligotrophic environments and with field observations.

Independent verification of the Monod kinetic parameters generated in this study was obtained by the use of a one-dimensional solute-transport model. The model used was developed by Kindred and Celia (1989) and was modified to simulate the steady-state biomass and phenolic compound concentrations (manuscript in preparation). Boundary conditions and parameter values for the groundwater flow portion of the model were taken from Franks (1988). Model simulations were compared to actual field data obtained over a 10 year study period. The model takes into account a steady-state biomass and decreases in phenolic compound concentration during down gradient travel due to advection, dispersion, sorption onto aquifer sediments using laboratory determined coefficients, and the Monod kinetic and growth parameters determined in this study. The four phenolic compounds tested have maintained a steady-state concentration over the study period and approach zero concentration after approximately 100 m of travel in the aquifer. The steady-state concentration profiles generated by the model simulations accurately represent the actual field profiles.

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