

COMPLETION REPORT FY-1992 PROGRAM

**TOXIC ORGANIC CHEMICALS IN
RHODE ISLAND GROUNDWATER:
CONTAMINANT FATE & TRANSPORT**

Amy P. Gamerdinger

quality issues such as: estimating the travel time of potential groundwater pollutants from the point of release to drinking water supply wells, the amount or concentration of the contaminant that will ultimately be present in the wells, and contaminant removal rates from soil and groundwater when cleanup and remediation strategies are employed.

Most of our current understanding of organic contaminant behavior in soil and groundwater is based on studies conducted for a single chemical in a completely aqueous system. In the environment, we often have complex mixtures of chemical contaminants in aqueous systems where organic solvents are also likely to be present. We know that the fate and transport of an organic chemical will be influenced by the presence of other organic chemicals and solvents. In order to properly assess organic pollutant fate and transport from waste sites or leaking fuel storage tanks, we need to consider contaminant behavior in the presence of other chemicals, such as organic solvents, which are known to influence fate and transport.

We consider the fate and transport of hydrophobic organic chemicals (HOCs) in the presence of organic solvents and natural organic material that is present in the aqueous phase. We examine the effects on processes which control distribution between phases, retardation and transport, and biodegradation. Retardation slows the movement of the pollutant through the sediment and results from contaminant-sediment interactions (sorption).

Literature Review:

The need for understanding the fate and transport of toxic organic chemicals in order to maintain groundwater quality is apparent; the need to determine the potential for exposure to these chemicals (via surface and groundwater) in evaluating environmental health risks is obvious [Kimbrough, 1990]. Environmental fate models provide an efficient means for predicting

exposure, however, the execution of such models requires mechanistic descriptions of the relevant processes (e.g., retardation and attenuation) which govern chemical fate and transport. These mechanistic process descriptions require specific input parameters.

The descriptions and parameters which are available for describing contaminant fate and transport have generally been determined for a single chemical in an aqueous (100% water) system. However, the situations which pose the greatest threat to groundwater commonly involve complex chemical mixtures which contain organic solvents in addition to other chemicals and natural organic chemicals.

Our project considers the fate and transport of HOCs in the presence of synthetic organic solvents and natural organic material, which are known to modify pollutant behavior. We consider HOC distribution among three phases: soil, water, solvent; the effect of selected organic solvents on distribution and biodegradation; and the effect of soil organic matter (OM) on biodegradation potential. Sorption, or the partitioning of an organic contaminant between the solution and solid phase, acts to slow or retard HOC movement through soil or groundwater. Partitioning between the aqueous phase and an organic solvent will have the same effect. It is well-documented that the equilibrium partitioning of HOCs in soil or groundwater systems can be described by the following equation:

$$S_T = K_p C \quad [1]$$

where S_T is the concentration which is sorbed to sediment (sorber), K_p is the equilibrium sorption constant, and C is the equilibrium concentration of the sorbate in solution [Chiou et al., 1979; Rao 1990]. Similarly, HOC distribution between the solvent-water phase is described by a solvent-water partition coefficient (analogous to the oft-used octanol-water partition

coefficient:

$$K = C_{i, \text{ solvent}} / C_{i, \text{ water}} \quad [2]$$

The value of K_p can be determined from the retardation factor, R ,

$$R = 1 + \rho K_p / \theta \quad [3]$$

where ρ and θ are the bulk density and volumetric water content of the porous media, respectively. R is calculated from the breakthrough curve (BTC) which is generated with miscible displacement techniques (below).

Greater microbial activity, and therefore biodegradation potential, has traditionally been associated with higher OM soils. Recently, several researchers have reported that sorption in high OM soils decreases bioavailability of 2,4-D and therefore the biodegradation potential [Ogram et al., 1985; Greer and Shelton, 1992]. Another factor that has received less attention is the effect of natural organic matter (present in the aqueous phase as dissolved or colloidal material) on biodegradation.

There are few studies to date on HOC biodegradation in the presence of organic solvents. Recent work by Efroymsen and Alexander [1991] considers the biodegradation of hydrocarbons which are partitioned into an organic solvent; this is one of the few studies in an environmentally relevant system. As mentioned above, bio- and chemical process engineers have found that biologically mediated enzymatic reactions are catalyzed in bi-phasic (organic and aqueous) systems [Lilly et al., 1987; Carrea 1987; Halling 1990]. As more research on the behavior of nonaqueous phase liquids in the environment is conducted, our understanding of how organic solvents affect pollutant attenuation will become apparent.

Methodology:

General.

¹⁴C naphthalene (Sigma Chemical Co.) was used in combination with non-

labeled naphthalene in mineralization studies to obtain the desired activity and concentration of naphthalene. Liquid scintillation counting (Packard Tri-Carb, Packard Instrument Co., Downers Grove, IL) and HPLC with UV detection (HP 1050) were used to determine naphthalene activity and concentration, respectively.

Optical density (Klett-Summerson Photoelectric colorimeter, Klett MFG. Co., Inc., NY) was used as an indicator of bacterial number and growth. Standard techniques were used to plate bacterial cultures and inocula for verification of microbial number and culture purity.

Soil and aquifer material were obtained locally (Kingston, RI): aquifer material from the C-horizon, Peckham Farm; Merrimac from the B-horizon, Great Swamp Management area; and a muck soil from the A-horizon, URI Northwoods. For "washed soil" experiments, the A and B horizon soils were treated with a water-methanol solution to remove water soluble and/or leachable organic matter from the soil. Experiments were conducted with "washed" and untreated soils to determine the effect of natural organic matter (present in the aqueous phase) on biodegradation.

Naphthalene sorption and partitioning.

Naphthalene sorption onto aquifer material, glacial outwash, and muck soils was measured using the miscible displacement technique in a laboratory soil column [Zhong et al., 1986; Lee et al., 1988; Gamerdinger et al., 1990, 1991]. The soil-water partition coefficient (K_p) was calculated from the retardation factor ($R = 1 + \rho K_p / \theta$) which was determined through mathematical moment analysis [Valocchi, 1985].

Naphthalene partitioning between each of the solvents was measured in two-phase solvent-water, and three-phase soil-solvent-water systems (in progress). A known concentration and activity of ^{14}C naphthalene in acetone

was added to 1 ml of each solvent followed by 7 mls of D.I. water. The vials were tightly sealed with teflon lined caps, equilibrated for 6 days, and centrifuged prior to sampling and analysis of each phase.

Bacterial isolation and growth.

A bacterium that was able to use naphthalene as a carbon source was isolated from soil collected in the vicinity of an underground oil storage tank; characterization is in progress. Standard isolation techniques were used; glycerol stocks were prepared and stored at -20°C.

Pure culture inocula for use in mineralization studies were prepared as follows. A flask containing M-9 mineral media and excess crystalline naphthalene was inoculated with bacteria from a frozen glycerol stock and placed on an incubator/shaker until the late log-phase of growth was reached (3-5 days). The culture was then centrifuged at 5000 rpm for 15 minutes, the supernatant was discarded, and the pellet was resuspended in M-9 media. This procedure was repeated twice. The final resuspension was diluted to a predetermined optical density and used as the inoculum in mineralization studies.

Mineralization experiments.

Sterilized soil, M-9, the solvent treatment, naphthalene in acetone, ¹⁴C-labeled naphthalene in acetone, and the inoculum were added to each 250 ml flask, depending upon the treatment. The total acetone in each flask did not exceed 0.2%, a concentration which was shown to have no effect on bacterial growth [Bauer and Capone, 1985].

An inlet to each flask was connected to an air pump; the outlet was connected to 3 CO₂ traps in series, which contained a 4:1 methanol:ethanolamine solution [Zhou and Traxler, 1992]. The flasks were closed except during sampling and were placed on a shaking water bath at a

temperature of 27 +/- 1 °C throughout the experiment.

The CO₂ traps were changed at discrete time intervals following a ten minute aeration period; a 0.3 ml sample was taken and ¹⁴CO₂ was measured by liquid scintillation counting. Microbial number of the inoculum was estimated from optical density and subsequently verified by plating of a dilution series using standard techniques.

Results and Discussion:

Naphthalene sorption and mineralization in 3 soils.

Results of miscible displacement studies of naphthalene transport in each of the 3 soils (aquifer material, outwash, and muck) are presented in Figures 1-3. As anticipated, greater retardation and sorption to soil was observed with increasing organic carbon content.

Results of the mineralization studies (complete conversion of naphthalene to CO₂) in each of the three soils and in "washed" soils are shown in figures 4-6. In untreated soil, mineralization increased with increasing organic carbon content of the soil (Figure 4). Because sorption is thought to decrease bioavailability, we were surprised with this result. To explore the controlling mechanism, the experiments were repeated on "washed" soil with water soluble organic matter removed. Mineralization decreased in these treatments (Figures 5 and 6), but the results are not clear because biological activity (as measured in the control treatment) was different for the two experiments. These experiments will be repeated.

Naphthalene partitioning and mineralization in solvent-water systems.

Solvent-water partition coefficients were similar and on the order of 500-600 for naphthalene distribution between a solvent (hexane, octane, decane, dodecane, and hexadecane) and aqueous phase. Experiments in 3-phase (aquifer material, solvent, water) are not yet complete.

Mineralization of naphthalene was enhanced in some solvent-water systems (Figures 7-8). Activity is correlated with solvent hydrophobicity as reported by others [Laane et al., 1987]. The effect of organic solvents on biodegradation was systematically evaluated by comparing mineralization of naphthalene in the presence of hexane, octane, decane, dodecane, and hexadecane in 2-phase solvent water and 3-phase soil-solvent-water systems. Analysis of these experiments is not yet complete, but it appears that activity is higher in the longer chain, more hydrophobic solvents.

Principle Findings and Conclusions:

Complete degradation of naphthalene in the presence of 3 soils (A horizon muck, B horizon outwash, and aquifer material) was measured. The extent of degradation was positively correlated with the organic carbon content of the soil, suggesting that organic matter from the soil enhanced degradation. Degradation was reduced when water-soluble organic matter was removed from the two surface soils. These results suggest that enhanced degradation is positively correlated with the amount of organic carbon available via the aqueous phase. This could result from increased substrate availability due to the cosolvency effect of the water soluble fraction of the soil organic matter, or, due to growth of the bacteria on this water-soluble fraction of organic matter. Further experimentation and evaluation is required to understand the mechanism and under what conditions enhancement will be observed.

Complete degradation of naphthalene in the presence of 5 organic nonaqueous phase liquids (hexane, octane, decane, dodecane, hexadecane) was evaluated. The extent of degradation was enhanced in the presence of the more hydrophobic organic solvents. This could be due to a direct effect on the microorganism, or due to indirect effects such as increasing substrate

availability to the bacteria. We suspect that degradation will be diminished in the presence of more hydrophilic solvents. Further experimentation is required to establish the relationship between solvent properties and their effect on biodegradation.

Bacteria which degrade PAHs were present in hydrocarbon contaminated soil. The bacterial population was viable in the presence of hydrophobic organic solvents (alkanes with chain length of 8 or greater). These solvents are also classified as L-NAPLs (nonaqueous phase liquids which are less dense than water). The overall extent of naphthalene degradation to CO₂ was enhanced in the presence of selected organic solvents, although the initial rate was slower. This suggests that contaminant degradation is a function of the mixture of contaminants that may be present on-site. Understanding the controlling mechanisms is important for predicting contaminant fate and for optimizing bioremediation strategies.

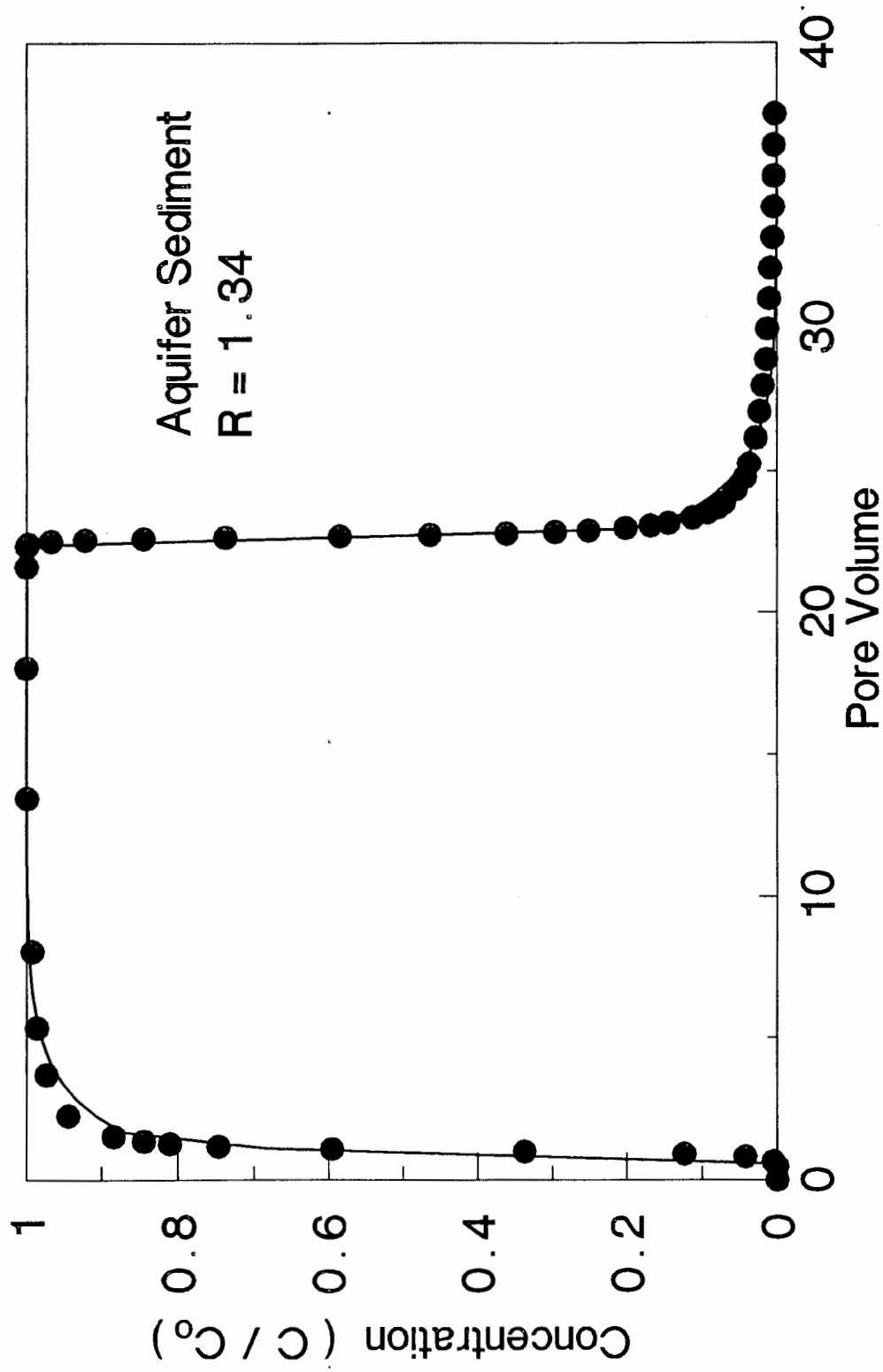


Figure 1. Naphthalene Sorption/Retardation in Aquifer Material.

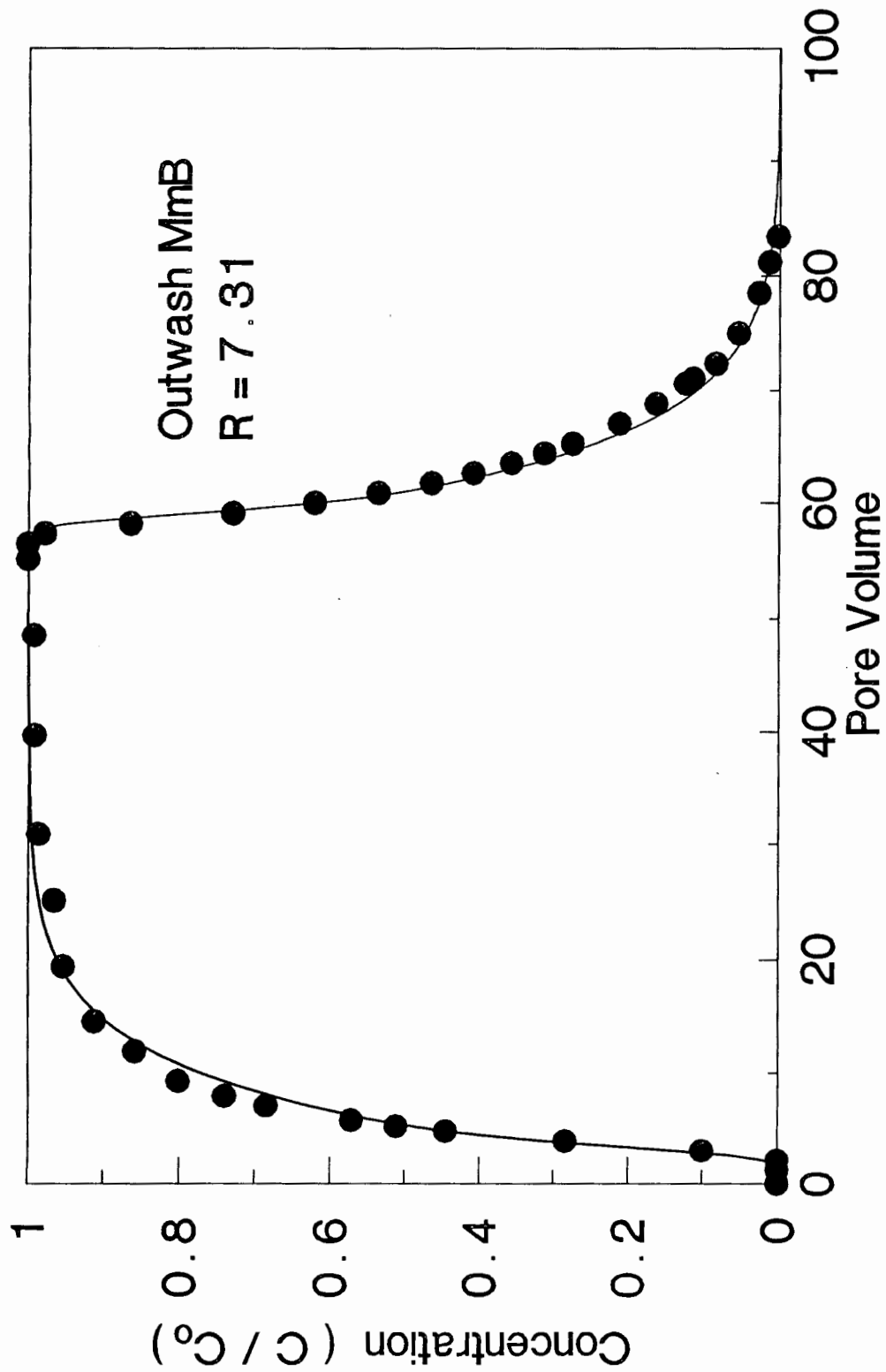


Figure 2. Naphthalene Sorption/Retardation in Outwash.

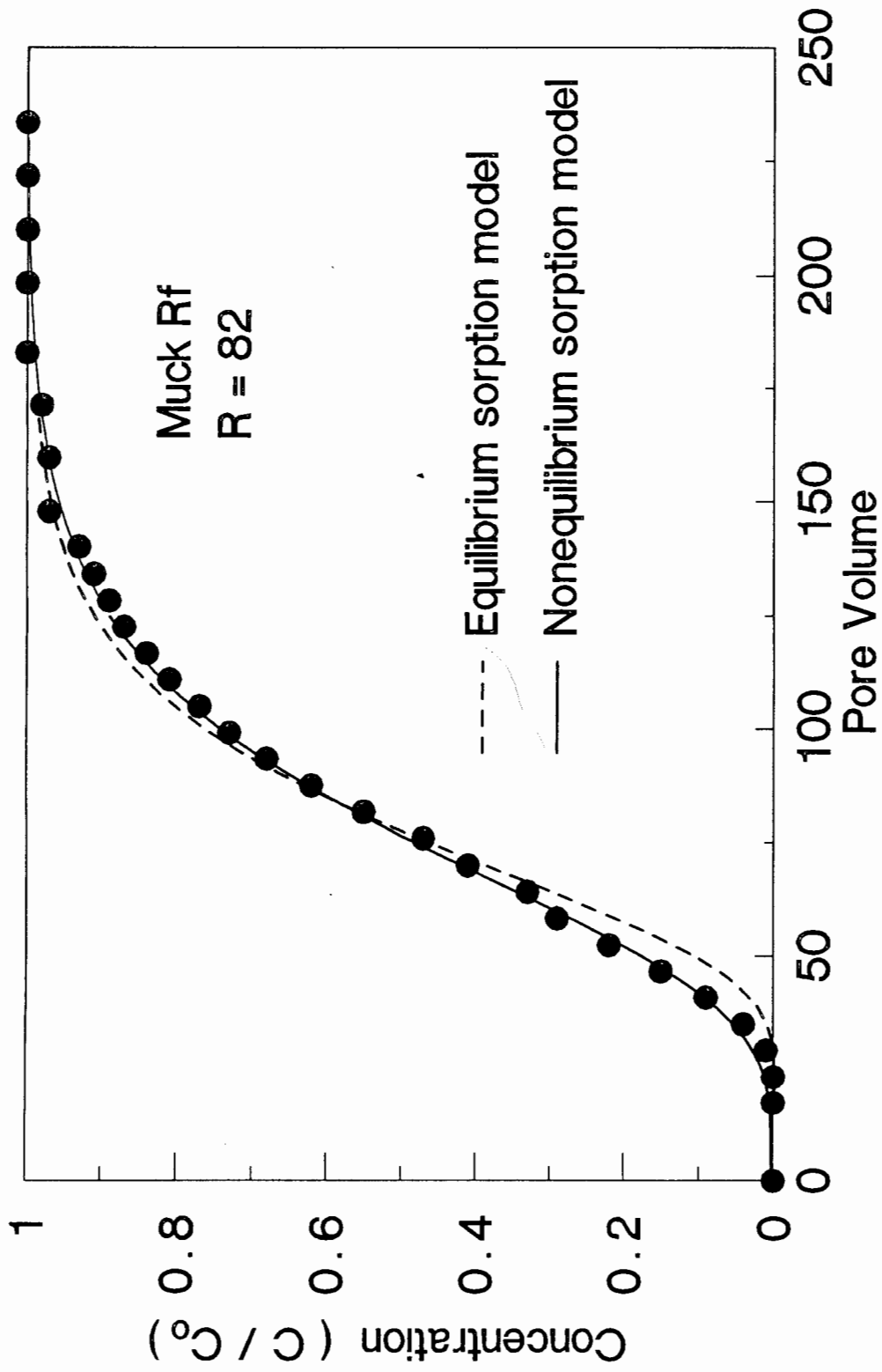


Figure 3. Naphthalene Sorption/Retardation in Outwash.

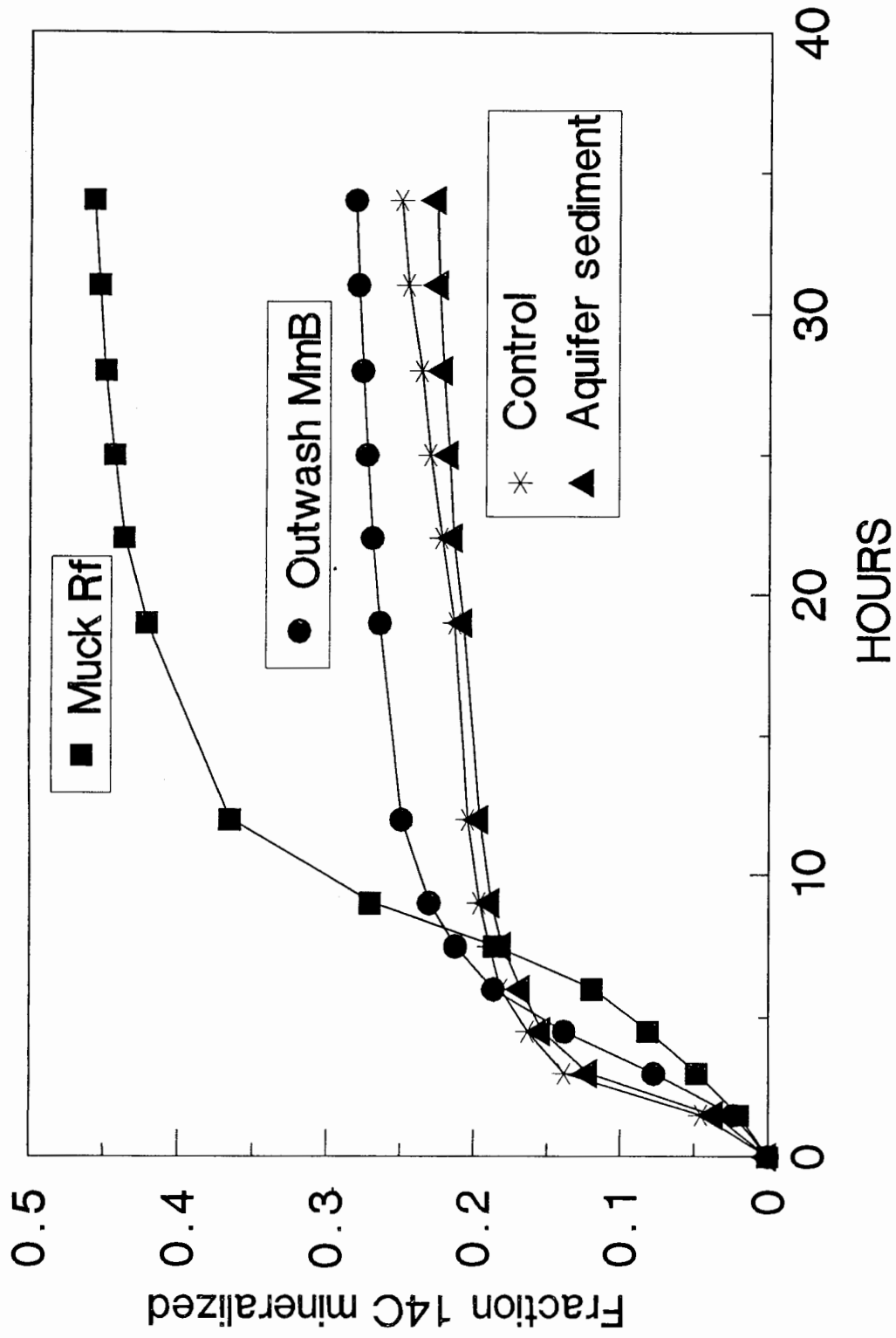


Figure 4. Naphthalene Biodegradation in 3 Soils.

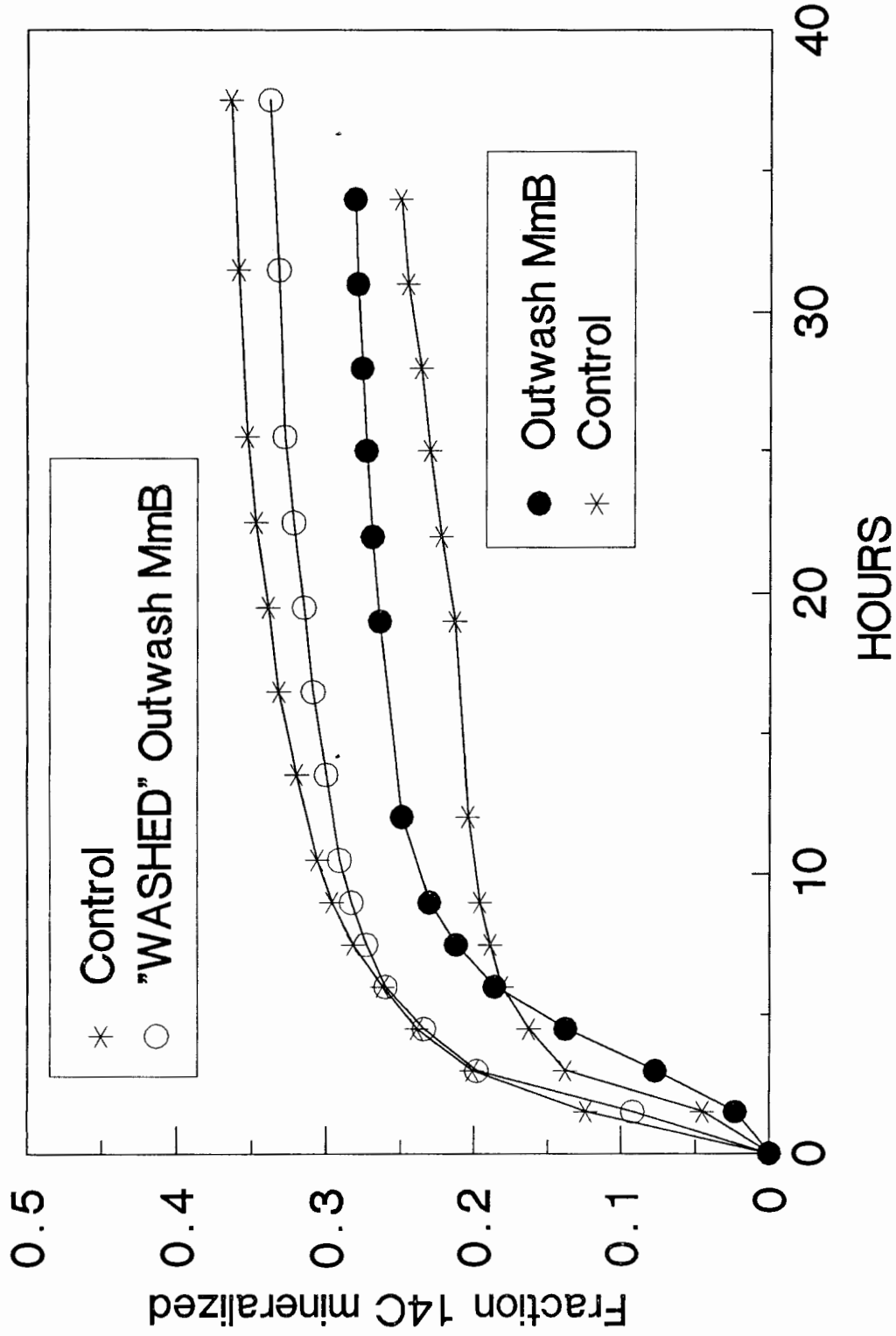


Figure 5. Decrease in Biodegradation in "Washed" Soil.

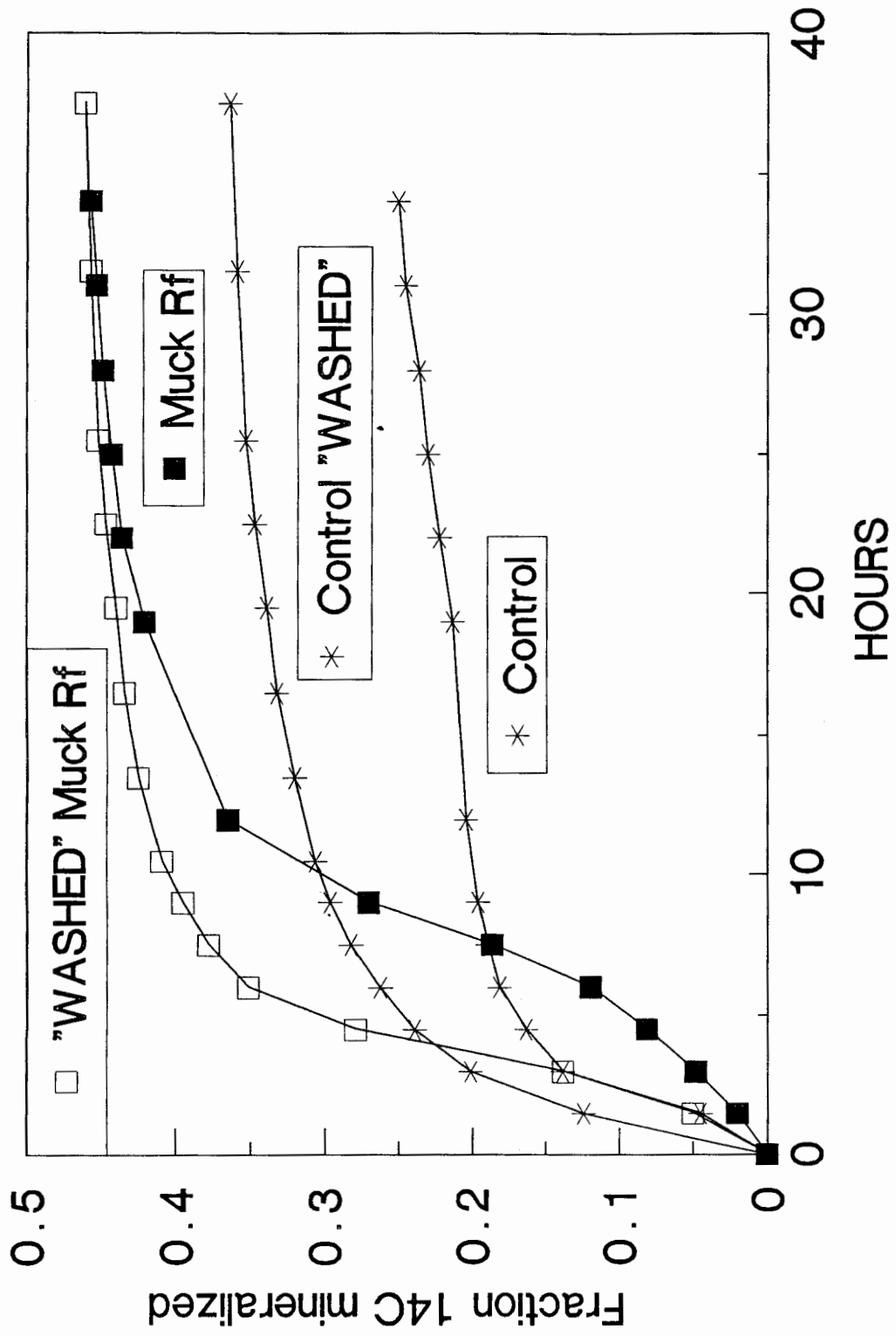


Figure 6. Decrease in Biodegradation in "Washed" Soil.

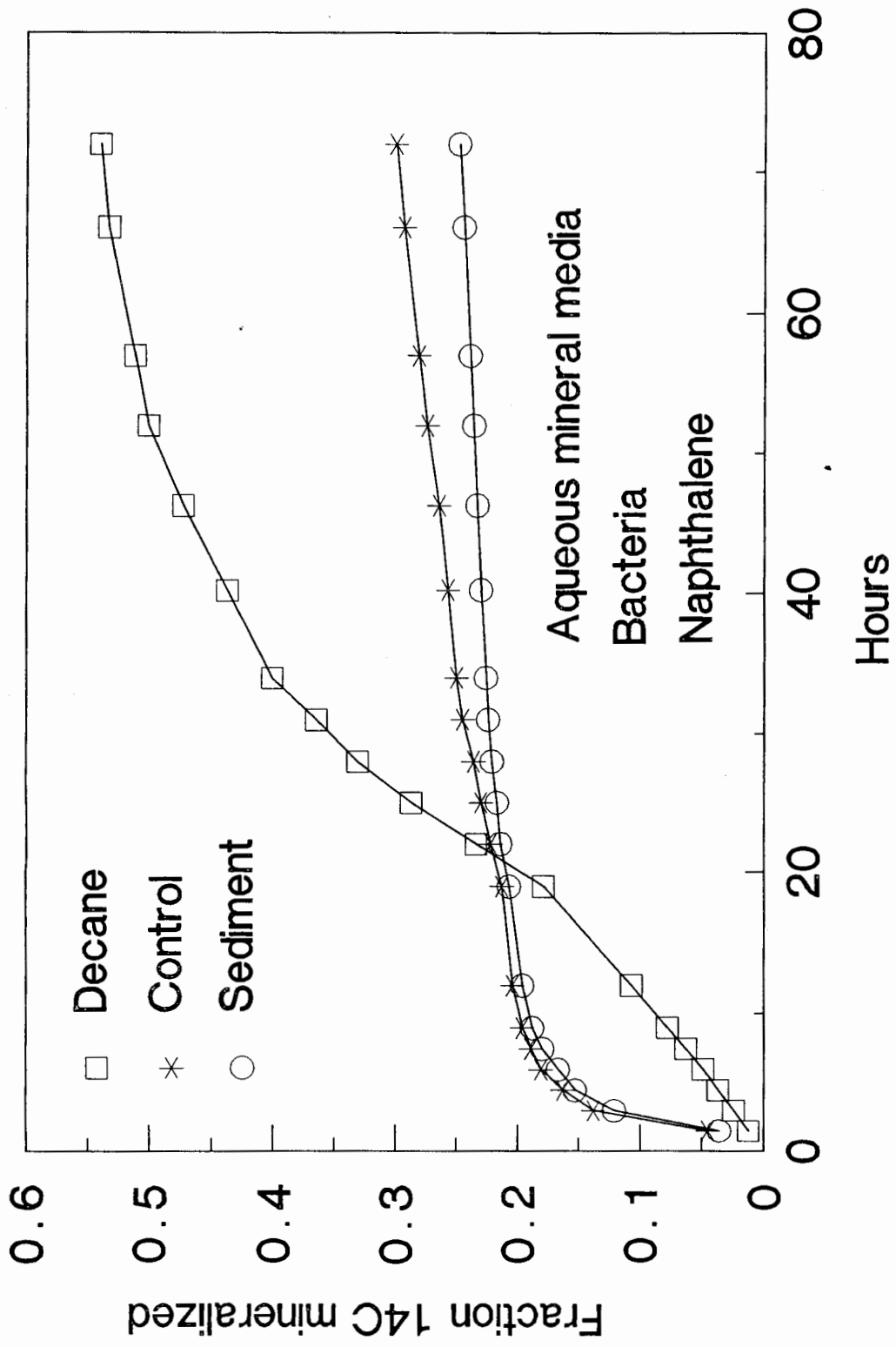


Figure 7. Biodegradation in Solvent-water and Soil-water systems.

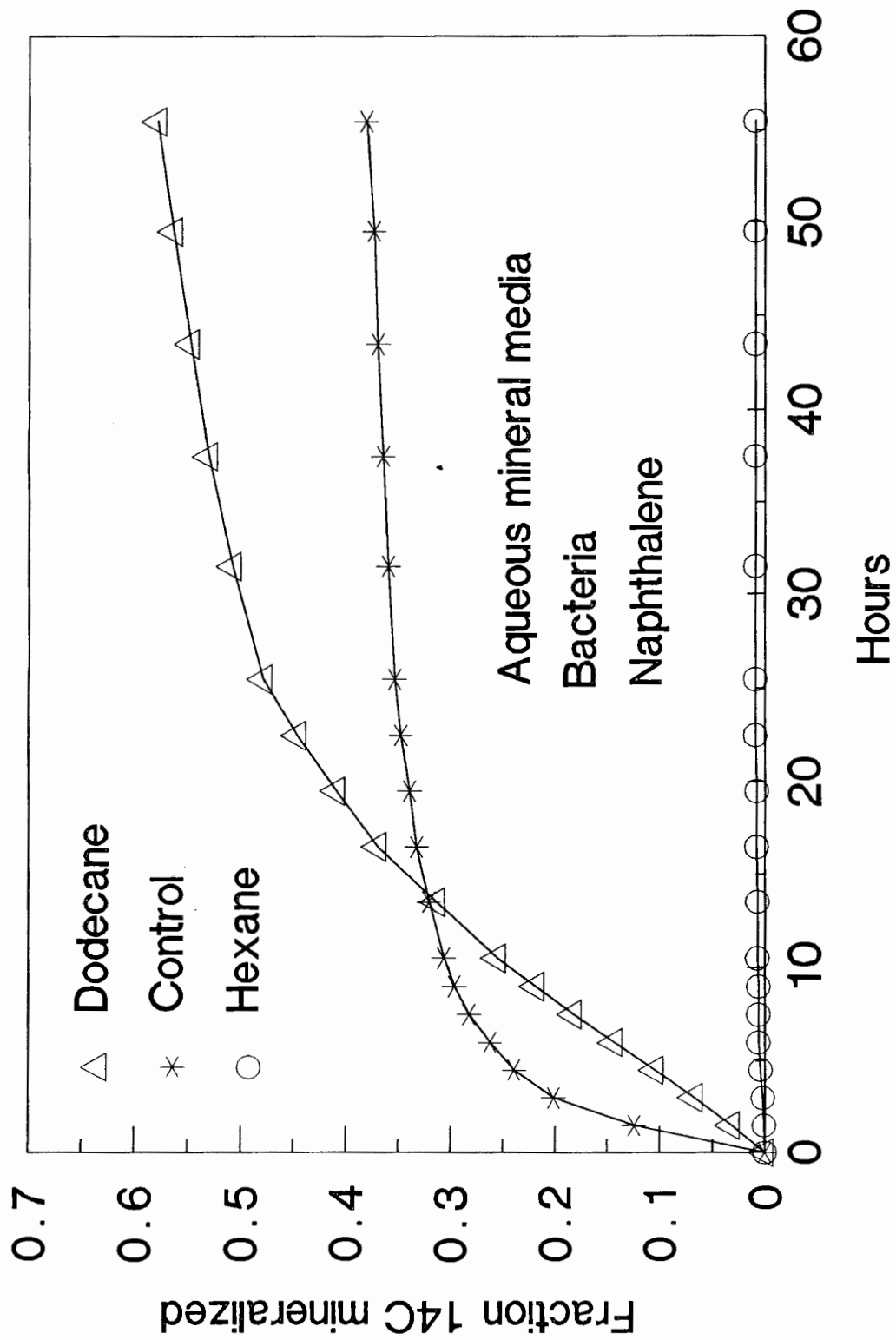


Figure 8. Biodegradation in Solvent-Water Systems.

References:

Bauer, J.E., and D.G. Capone. 1985. Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments. *Appl. Environ. Microbiol.* 50:81-90.

Carrea, G. 1987. Biocatalysis in water-organic solvent two-phase systems. In *Biocatalysis in Organic Media*, C. Laane, J. Tramper, and M.D. Lilly (eds.), Elsevier, NY, p. 157-169.

Chiou, C.T., L.J.. Peters, and V.H. Freed. 1979. A physical concept of soil-water equilibria for nonionic organic compounds. *Science* 206:831-832.

Efroymsen, R.A., and M. Alexander. 1991. Biodegradation by an *Arthrobacter* Species of hydrocarbons partitioned into an organic solvent. *Appl. Environ. Microbiol.* 57:1441-1447.

Gamerding, A.P., A.T. Lemley, and R.J. Wagenet. 1991. Sorption nonequilibrium and degradation of three 2-chloro-s-triazine herbicides in soil-water systems. *J. Environ. Qual.* 20:815-822.

Gamerding, A.P., R.J. Wagenet, and M.Th. van Genuchten. 1990. Application of two-site/two-region models for studying simultaneous nonequilibrium transport and degradation of pesticides. *Soil Sci. Soc. Amer. J.* 54:957-963.

Halling, P.J. 1990. Solvent selection for biocatalysis in mainly organic systems: Predictions of effects on equilibrium position. *Biotechnol. Bioeng.* 35:691-701.

Kimbrough, R.D. 1990. Environmental Protection: Theory and Practice. Environ. Sci. Technol. 24: 1442-1445.

Laane, C., S. Boeren, R. Hilhorst, C. Veeger. 1987. Optimization of biocatalysis in organic media. In Biocatalysis in Organic Media, C. Laane, J. Tramper, and M.D. Lilly (eds.), Elsevier, NY.

Lee, L.S., P.S.C. Rao, M.L. Brusseau, and R.A. Ogwada. 1988. Nonequilibrium sorption of organic contaminants during flow through columns of aquifer materials. Environ. Toxicol. Chem. 7:779-793.

Lilly, M.D., A.J. Brazier, M.D. Hocknull, A.C. Williams, and J.M. Woodley. 1987. Biological conversions involving water-insoluble organic compounds. In Biocatalysis in Organic Media, C. Laane, J. Tramper, and M.D. Lilly (eds.), Elsevier, NY, p. 3-17.

Ogram, A.V., R.E. Jessup, L.T. Ou, and P.S.C. Rao. 1985. Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl. Environ. Microbiol. 49:582-587.

Rao, P.S.C. 1990. Sorption of organic contaminants. Wat. Sci. Technol. 22:1-6.

Roberts, R., P.H. Abelson, R. Britten, D. Cowie, E.T. Bolton. 1955. Studies of biosynthesis in *Eschericia coli*. Carnegie Institution of Washington Publication 607, Washington, DC, p.5.

Valocchi, A.L. 1985. Validity of the local equilibrium assumption for modeling

sorbing solute transport through homogeneous soils. *Water Resour. Res.* 21:808-820.

Zhong, W., A.T. Lemley, and R.J. Wagenet. *W.Y. Garner, R.C. Honeycutt, and H. Nigg (eds.)*, Evaluation of Pesticides in Groundwater. ACS Symposium Series No. 315. Am. Chem. Soc., Washington, DC. 1986; pp.61-67.

Zhou, X., and R.W. Traxler. 1992. Comparison of carbon metabolism between cholramphemicol-treated and untreated cultures on *Clostridium acetobutylicum*. *Appl. Microbiol. Biotechnol.* 38:254-258.