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# Degradation of xenobiotic compounds in situ: Capabilities and limits

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**Abstract:** Exploiting microorganisms for remediation of waste sites is a promising alternative to groundwater pumping and above ground treatment. The objective of in situ bioremediation is to stimulate the growth of indigenous or introduced microorganisms in regions of subsurface contamination, and thus to provide direct contact between microorganisms and the dissolved and sorbed contaminants for biotransformation. Subsurface microorganisms detected at a former manufactured gas plant site contaminated with coal tars mineralized significant amounts of naphthalene (8–43%) and phenanthrene (3–31%) in sediment–water microcosms incubated for 4 weeks under aerobic conditions. Evidence was obtained for naphthalene mineralization (8–13%) in the absence of oxygen in field samples. These data suggest that biodegradation of these compounds is occurring at the site, and the prospects are good for enhancing this biodegradation. Additional batch studies demonstrated that sorption of naphthalene onto aquifer materials reduced the extent and rate of biodegradation, indicating that desorption rate was controlling the biodegradation performance.

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**Key words:** In situ bioremediation; Organic contaminants; Performance; Limitations; Biodegradation; Sorption/desorption

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## Introduction

The contamination of groundwater and soils with organic compounds is widespread [1]. The extensive production and use of organic compounds makes them among the most prevalent in groundwater at waste disposal sites. Over 270 million metric tons of the 50 most widely used chemicals were produced in 1988 [2]. Synthetic organic compounds accounted for approximately one-third of the chemical production. Many of these synthetic compounds are known or poten-

tial threats to public health and the environment, so there is an urgent need to understand their fate in the environment and develop effective control methods. A desire for zero contamination and zero risk is frequently voiced by the public.

A large number of contaminated sites exist in the United States. Nearly 1300 sites are on the Superfund priority list [3]. A pool of money generated by special taxes is available for clean-up efforts at Superfund sites. These sites present a high risk to public health and the environment from hazardous substances in groundwater, surface water, air, and soil. About 31 000 waste sites have been catalogued by the US Environmental Protection Agency as candidates for Superfund clean-up. There are over 300 000 landfills and

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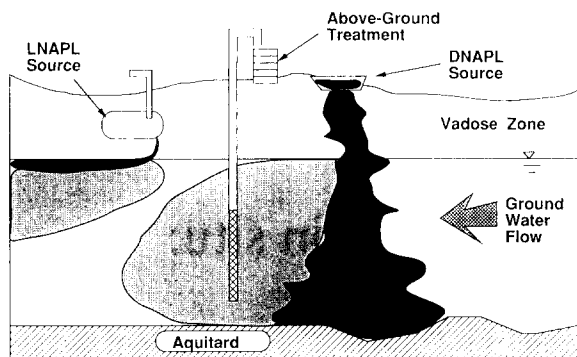


Fig. 1. Contamination of the subsurface by a light non-aqueous phase liquid (LNAPL) and a dense non-aqueous phase liquid (DNAPL) and possible location for groundwater pumping and above-ground treatment.

surface impoundments in the US which receive both hazardous and non-hazardous wastes from industries and municipalities [4]. Many of these are existing or potential sources of soil and groundwater contamination. There are about 10 million underground storage tanks in the US; approximately 2 million of them are used to store gasoline. Many of these tanks are subject to corrosion and fractures that result in leakage.

When organic compounds are spilled or leak from an underground storage tank or a surface impoundment, the subsurface environment becomes contaminated as illustrated in Fig. 1. As the compounds migrate downward by gravity force, the entrained residuals contaminate the surface soil, the unsaturated (vadose) zone between the surface soil and the groundwater table, and eventually the groundwater in the saturated zone. Organic contaminants that are lighter than water (often termed LNAPL: light non-aqueous phase liquid), such as gasoline, tend to mound and spread on the surface of groundwater and slowly dissolve to form a contaminant plume in the groundwater. Organic contaminants that are denser than water (often termed DNAPL: dense non-aqueous phase liquid), such as chlorinated solvents and coal tars, tend to sink to the bottom of the aquifer.

Remediation of groundwater and soil at contaminated sites is often attempted using the pump-and-treat method. A basic pump-and-treat

system consists of one or more wells that pump contaminated water to the surface, a surface treatment unit to remove the contaminants, and some method for disposing of the treated water (Fig. 1). The objective is to have contaminants dissolve in the water so they can be pumped to the surface for treatment. However, it is difficult to extract hydrophobic organic contaminants from the subsurface because they are generally sorbed strongly to the soils. This partitioning to soil means that pump and treat systems are generally inefficient and slow. There is typically a long tail in the contaminant levels in the extracted water which leads to exceedingly long pumping times (can be in excess of 10–100 years) [5,6]. Physical/chemical processes, such as air stripping or carbon adsorption, are frequently employed to treat the extracted water at the surface. These processes do not destroy the contaminants. Rather they sequester them or transfer them from one environmental medium to another. These limitations of pump-and-treat have caused many to consider in situ biological treatment as an alternative approach for site remediation.

The most important principle of in situ bioremediation is that microorganisms can be used to destroy dissolved and sorbed contaminants or convert them to less harmful products [7]. In some cases, existing conditions at the contaminated site are sufficient to permit natural bioremediation. More often, bioremediation requires the installation of engineered systems to stimulate the growth of indigenous or introduced microorganisms in regions of subsurface contamination, and thus provide direct contact between microorganisms and the dissolved and sorbed contaminants for biotransformation [8,9]. The process typically entails perfusion of nutrients and one or more electron acceptors through the contaminated subsurface to optimize the environment for biodegradation of the contaminants. The reduction in contaminant mass which provides long-term protection of public health is a benefit of in situ bioremediation.

This paper addresses a few issues of importance to in situ bioremediation of organic contaminants. A case study is presented on how to evaluate the feasibility of using in situ bioremediation.

ation to remove benzene and polyaromatic hydrocarbons (PAHs) present in the groundwater at the site of a former manufactured gas plant in Baltimore, MD. The biodegradation in laboratory cultures is compared with field samples to illustrate two complicating factors. These include the need for proper growth-stimulating chemicals and the influence of mass transfer rate (desorption rate) on the biodegradation performance.

## Materials and Methods

### *Organic contaminant biodegradation in the field*

#### *Sampling of aquifer material*

Aquifer materials were collected from the Baltimore Gas & Electric Company (BG&E) Spring Gardens Facility located in Baltimore, MD. A significant amount of benzene and PAHs entered the subsurface as a result of coal tar wastes generated by former coal gasification operations at the site. Slow to dissolve, denser than water, and highly concentrated, these coal tars are persistent in the soil and groundwater beneath the site. The site is underlain by an approx. 90 foot heterogeneous sequence of unconsolidated quaternary, pleistocene, and cretaceous sediments. Fifty-seven samples of these sediments were aseptically obtained from five boreholes across the site using techniques described by Madsen et al. [10]. Rhodamine-WT was added to the drilling mud to ensure the absence of drilling fluids in sediment samples. Borehole site selection was geared toward sampling at the edge of the PAH plume under the premise that the most active PAH degraders would be found where PAH concentrations were below inhibitory levels and dissolved oxygen was available.

Analyses of ground-water samples obtained from monitoring wells located within 50 feet of each borehole and screened at depths corresponding with aquifer sample depth provided an indirect assessment of the quality and aeration state of the in situ aqueous environment. The groundwater data indicated a prevalence of microaerophilic (< 2 mg O<sub>2</sub>/l) conditions at most locations, with anaerobic conditions occurring in

some of the more contaminated areas. Consequently, laboratory analysis of most of the subsurface sediments was conducted under aerobic conditions. Sampling and analysis of sediments from anaerobic areas was conducted in a portable anaerobic glove-bag and laboratory glove box, respectively.

#### *Enumeration of sediment bacteria*

Within 24–48 h after sampling, viable populations (colony forming units, cfu) adhered to the subsurface sediments were enumerated on serial dilution plates of 5% PTYG agar (recipe from Balkwill and Ghiorse [11]). Total counts on these populations were measured using a modification of the Acridine orange epifluorescence microscopy technique described by Wilson et al. [12]. The modification entailed the use of filtering Acridine orange-stained sediment bacteria extracts through a 0.2- $\mu$ m Nucleopore filter based on the work of Hobbie et al. [13].

#### *Mineralization assay*

Sediment–water batch microcosms were used to assay the ability of the sediment bacteria to mineralize <sup>14</sup>C-labeled forms of primary coal tar constituents (benzene, naphthalene, and phenanthrene). Microcosms were prepared in 14-ml serum vials containing 5 g sediment and 3 ml ground water from a well within 50 feet of the sediment sample borehole and screened at depths corresponding to sediment sample depth. The general intent was to give the microcosms an aqueous environment that closely approximated the in situ conditions, thus enabling an evaluation of biodegradation under conditions analogous to those in the site subsurface. Microcosms were Teflon<sup>TM</sup>-capped, aluminum-crimped, and silicone-sealed in order to limit volatilization of substrates and entry of oxygen or other electron acceptors into the microcosms.

Each microcosm received  $6.76 \times 10^{-2}$   $\mu$ Ci of <sup>14</sup>C radiolabeled and 1000  $\mu$ g/l unlabeled benzene, naphthalene, or phenanthrene (separate set of vials for each compound). Abiotic loss was measured in a control microcosm amended with 0.5 N HgCl<sub>2</sub> (biocide). A sterile, 2-ml glass vial containing 0.5 ml of 1 N KOH was placed in each

microcosm to trap  $^{14}\text{CO}_2$  generated from the mineralization of the  $^{14}\text{C}$ -labeled substrate. The extent of mineralization of the test compounds was determined by monitoring (using liquid scintillation counting) the average amount of  $^{14}\text{CO}_2$  generated in the trial microcosms less the amount generated in the  $\text{HgCl}_2$  control.

*Batch studies to test the influence of sorption / desorption on biodegradation of naphthalene*

*Soil materials*

Batch studies were conducted to evaluate the effects of soil/water ratio, organic carbon content, and particle size on aerobic naphthalene biodegradation. Soil samples were obtained from 0–30 cm (designated Soil A), 30–60 cm (designated Soil B), and 60–120 cm (designated Soil C) depths corresponding to the A, B and C horizons of the soil from a site at Bozeman, MT. The soils were air-dried and homogenized. Soil particles larger than 2 mm were removed by passing through a Number 10 standard sieve. Soil A was separated in four size classes by standard dry sieve techniques [14] to test the influence of particle size on biodegradation. Soil A, B, and C and the four size classes of Soil A were analyzed for total organic carbon using the methods reported by Sim and Haby [15] and Page et al. [16]. The pH was measured using a 1:1 soil/water ratio to ensure that pH was favorable for biodegradation.

*Bacterial culture*

The bacteria used in this study were enriched from the Bozeman soil obtained from the A horizon (0–30 cm depth). The enrichment procedure involved incubation for 30 days with 50 g of the soil in 500 ml of nutrient solution ( $\text{KH}_2\text{PO}_4$ , 8.5 mg/l;  $\text{K}_2\text{HPO}_4$ , 21.8 mg/l;  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 33.4 mg/l;  $\text{NH}_4\text{Cl}$ , 29.8 mg/l;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 22.5 mg/l;  $\text{Na}_2\text{SO}_4$ , 1.85 mg/l;  $\text{NaNO}_3$ , 13.8 mg/l;  $\text{CaCl}_2$ , 27.5 mg/l;  $\text{NaHCO}_3$ , 20 mg/l;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.25 mg/l;  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ , 1.0 mg/l). The nutrient media was also spiked with 1 mg/l naphthalene. 10 ml of the supernatant from this culture was transferred to a 500-ml batch of fresh nutrient media every 10 days in order to enrich for naphthalene-degrading microorgan-

isms. Three sequential transfers were performed. The enrichment culture was kept at  $4^\circ\text{C}$  prior to its use in the biodegradation studies.

*Biodegradation assay*

Three sets of batch biodegradation studies were conducted. The first set was designed to test the influence of the soil/water ratio on rate and extent of naphthalene biodegradation. Soil A was sterilized by autoclaving for 1 h. Either 1, 10, or 15 g of soil were placed into 26-ml borosilicate serum bottles. Then the bottles were spiked with 0.01 mM (1.28 mg/l with 1000–2000 dpm/ml of  $\text{U-}^{14}\text{C}$ -naphthalene obtained from Sigma Chemical Co.) naphthalene and filled with the sterilized nutrient media to eliminate headspace. This resulted in test soil/water ratios of 40 g/l, 470 g/l, and 790 g/l. The bottles were immediately sealed with Teflon<sup>TM</sup>-faced septa and aluminum seals. The bottles were incubated for 30 days to attain sorption equilibrium. Then 0.2 ml of the enriched bacterial culture solution ( $5 \times 10^7$  cfu/ml) was introduced into the bottles to initiate naphthalene biodegradation. The temperature during the incubation was maintained at  $22 \pm 3^\circ\text{C}$ .

A second batch study with the Bozeman soil was designed to test the influence of soil organic carbon content on rate and extent of naphthalene biodegradation. Sterilized Soil A, Soil B, and Soil C were added to separate 26-ml bottles at a soil/water ratio of 470 g/l. The rest of the procedure is identical to the first batch experiment described above.

The third batch study with the Bozeman soil was designed to test the influence of soil particle size on rate and extent of naphthalene biodegradation. Soil A was sieved into four different size fractions: 1500–850  $\mu\text{m}$ , 420–250  $\mu\text{m}$ , 140–75  $\mu\text{m}$ , and  $< 75 \mu\text{m}$ . Each size fraction was added to a separate 26-ml bottle at a soil/water ratio of 80 g/l. The rest of the procedure is identical to the first batch experiment described above.

*Measurement of naphthalene biodegradation*

A subset of batch bottles was periodically removed from incubation and analyzed to monitor the time response of naphthalene biodegradation. Biodegradation was established by measuring the

production of  $^{14}\text{CO}_2$  from  $^{14}\text{C}$ -naphthalene. Sample  $^{14}\text{CO}_2$  was quantified with a liquid scintillation counter according to the procedure of Bouwer and McCarty [17]. 1-ml aqueous samples were mixed with 10 ml of counting cocktail (Fisher Scientific Scintiverse II). Samples were assayed at high pH (pH 12) with the addition of KOH (2 drops of 6 N KOH/ml of sample) and low pH (pH 1) with the addition of HCl (2 drops of 6 N HCl/ml of sample). Both samples were purged with 50 ml air per min for 5 min. Naphthalene is semi-volatile and is stripped from solution at both low and high pH.  $^{14}\text{CO}_2$  is only stripped under low pH. The difference in radioactivity between the high pH and low pH samples after stripping reflects the  $^{14}\text{CO}_2$  activity. The fraction of naph-

thalene mineralized was computed with the following expression:

$$\text{Mineralization} = {}^{14}\text{CO}_2 \text{ activity} / Y_{\text{CO}_2}$$

where  $Y_{\text{CO}_2}$  is the relationship between  $^{14}\text{CO}_2$  evolved and naphthalene  $^{14}\text{C}$  consumed. The value of  $Y_{\text{CO}_2}$  was obtained from batch cultures in the absence of soil. The average from 18 measurements was  $0.57 \pm 0.045$ .

## Results and Discussion

### Coal tar biodegradation in field samples

At the former manufactured gas plant site, viable populations ranging from  $10^2$  to  $10^4$  cfu/g

Table 1

Summary of enumeration and 4-week mineralization assay results for sediment samples from three boreholes at the former manufactured gas plant site

Location	Sample depth (feet)	Texture	Viable count <sup>a</sup> (cfu g dry weight)	Total count (cells/g dry weight)	Percent biotic mineralization <sup>b</sup>			In situ aqueous naphthalene ( $\mu\text{g/l}$ )	In situ dissolved $\text{O}_2$ (mg/l)	
					Benzene	Naphthalene	Phenanthrene			
A	8-10	Clay	$3.2 \times 10^3$	$1.4 \times 10^5$	NM <sup>c</sup>	$29 (\pm 5)$ <sup>d</sup>	$2 (\pm 1)$	BDL <sup>e</sup>	1.6	
	28-30	Gravel	$4.3 \times 10^2$	$7.3 \times 10^2$	NM	$30 (\pm 3)$	$7 (\pm 11)$	BDL	~ 0.5	
	42-44	Gravel	$5.7 \times 10^2$	$3.1 \times 10^4$	NM	$25 (\pm 7)$	$5 (\pm 5)$	BDL	~ 0.5	
						$8 (\pm 1)$ AN	0 AN	BDL	~ 0.5	
	54-56	Sand	$1.8 \times 10^3$	$5.2 \times 10^4$	NM	$29 (\pm 1)$	$9 (\pm 10)$	BDL	~ 0.5	
	56-58	Silt	NG <sup>f</sup>	BDL	NM	$1 (\pm 1)$	NM	BDL	~ 0.5	
	72-74	Gravel	$8.4 \times 10^4$	$1.4 \times 10^4$	NM	$8 (\pm 8)$	NM	4200	~ 0.5	
$13 (\pm 2)$ AN										
B	10-12	Sand	$2.4 \times 10^3$	$8.6 \times 10^3$	$24 (\pm 1)$	$43 (\pm 7)$	$31 (\pm 3)$	< 20	4.5	
	14-16	Silt	NG	$9.5 \times 10^3$	$15 (\pm 4)$	$13 (\pm 6)$	$12 (\pm 11)$	< 20	4.5	
	54-56	Silt	NG	BDL	3	0	0	< 20	2.5	
	76-78	Sand	$6.0 \times 10^2$ AN	NM	0 AN	0 AN	0 AN	0 AN	2300	BDL
					0 AN	0 AN	0 AN	0 AN	2300	BDL
93-94	Gravel	$1.4 \times 10^4$	$1.4 \times 10^5$	NM	$4 (\pm 3)$	0	2300	BDL		
C	20-22	Silt	NG	BDL	NM <sup>f</sup>	NM	NM	BDL	~ 0.5	
	36-38	Gravel	NG	$5 \times 10^6$	NM	$8 (\pm 2)$	$10 (\pm 3)$	BDL	~ 0.5	
	48-50	Sand	$8.5 \times 10^3$	$4 \times 10^6$	NM	$8 (\pm 6)$	$11 (\pm 11)$	BDL	~ 0.5	
	58-60	Gravel	NG	$3.6 \times 10^6$	NM	$13 (\pm 3)$	$6 (\pm 5)$	BDL	~ 0.5	
	68-70	Gravel	NG	$3.6 \times 10^5$	NM	$20 (\pm 4)$	NM	4200	~ 0.5	

<sup>a</sup> Counts shown are aerobic except for anaerobic counts denoted by AN.

<sup>b</sup> Data shown for aerobic microcosms except for those denoted by AN.

<sup>c</sup> NM, not measured due to insufficient sample.

<sup>d</sup>  $\pm$  values represent one standard deviation from replicate mean.

<sup>e</sup> BDL, below detection limit.

<sup>f</sup> NG, no growth.

dry sediment were detected at most depths between ground surface and the top of bedrock at 95 feet (Table 1). The most dense viable populations were observed in deep gravel deposits (Location A, 72 feet and Location B, 93 feet) in the presence of elevated aqueous naphthalene concentrations (2300 and 4200  $\mu\text{g/l}$ ). These data suggest that subsurface bacteria at the site may proliferate in the presence of the PAH plume. Bacteria from microaerophilic (0.5 mg  $\text{O}_2/\text{l}$ ) and anaerobic zones were capable of growth on aerobic plates, indicating that some of the site microorganisms are facultative anaerobes.

Total counts usually exceeded viable counts by one to two orders of magnitude, which was expected given that the total count includes active, dormant, and dead cells (Table 1). Other researchers [12,18] have observed this same difference between viable and total counts. A strong positive correlation was observed between total counts and sample grain size, which is consistent with the research of Beloin et al. [19], Sinclair and Ghiorse [20], and Sinclair et al. [21].

A typical result for the mineralization microcosm study is illustrated for benzene in Fig. 2. A small amount of  $^{14}\text{CO}_2$  evolved in the controls, suggesting incomplete inhibition of microbial activity in the presence of 0.5 N  $\text{HgCl}_2$ . The control values were subtracted from the  $^{14}\text{CO}_2$  evolution in borehole sediment samples to provide a conservative estimate of the extent of mineralization.

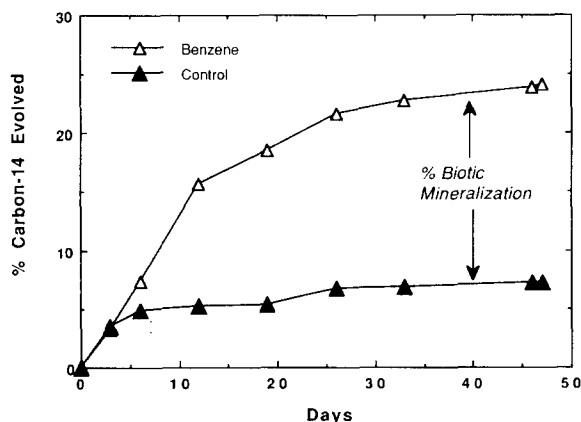


Fig. 2. Benzene mineralization in a subsurface sample from a former manufactured gas plant site.

Bacteria in many samples exhibited capacity for aerobically mineralizing significant amounts of naphthalene (Table 1). Percent mineralization ranged between  $8 \pm 2\%$  and  $43 \pm 7\%$  after 4 weeks of incubation. Anaerobic mineralization of naphthalene of between  $8 \pm 1\%$  and  $13 \pm 2\%$  was only observed in the two microcosms containing significant levels of nitrate in the groundwater. A few sediment samples from the site exhibited capacity to mineralize significant amounts of phenanthrene ( $10 \pm 3\%$  to  $31 \pm 3\%$ ) and benzene ( $24 \pm 1\%$ ) in aerobic microcosms, but neither of these compounds was degraded in anaerobic microcosms. No acclimation period was observed for any microcosm exhibiting significant biodegradation.

The positive biodegradation results for the sediment microcosms (Table 1) suggest that site bacteria have developed enzymes necessary to metabolize certain coal tar constituents, and that natural in situ biodegradation is occurring at some locations across the site. These data are consistent with other studies that have shown that PAH- and benzene-degrading bacteria are likely to occur in petroleum-contaminated subsurface environments. Aquifer bacteria from PAH-contaminated sites have been shown to degrade certain PAHs under aerobic laboratory conditions [18,22,23]. Biodegradation of PAHs has also been observed in the laboratory under denitrifying conditions by mixed bacteria from non-contaminated surface soil [24]. Benzene degradation by aquifer microorganisms has been reported for laboratory studies conducted under aerobic conditions [25,26], denitrifying conditions [27], and sulfate reducing conditions [28].

Laboratory cultures in the absence of aquifer materials generally exhibit much faster biodegradation rates and greater extents of mineralization than observed in field samples. For example, a denitrifying consortium was able to nearly completely biotransform toluene within 13 days when initially present at 12 and 70 mg/l [29]. Similarly, naphthalene biotransformation was nearly complete after 4 days when tested at 1 mg/l initial concentration. Much less aromatic hydrocarbon biodegradation occurred in samples from the former manufactured gas plant site (Table 1). Two

important reasons for the differences in the laboratory and field behavior are environmental conditions and mass transfer effects as discussed in the next two sections.

### *Environmental conditions*

The presence of a microbial population in the subsurface capable of biodegrading a contaminant does not insure that bioremediation will occur. Often environmental factors, such as pH, concentration, temperature, and nutrient availability, control the rate and extent of biotransformation [30]. Relatively few microbial species can grow in acidic ( $\text{pH} < 4$ ) or basic ( $\text{pH} > 10$ ) environments. The rates of many microbial reactions typically double for each  $10^\circ\text{C}$  rise in temperature. Above a certain temperature cellular components become irreversibly inactivated. Below  $10^\circ\text{C}$ , many microbial reactions are slow which leads to long operating times for bioremediation in cold climates. Many organic contaminants become toxic to microorganisms at high concentrations. At low concentrations, often in the  $\mu\text{g}/\text{l}$  to  $\text{ng}/\text{l}$  range, insufficient energy and carbon may be available for growth and maintenance. Consequently, biodegradation of organic contaminants may be inhibited at both high and low concentrations.

In order to stimulate microbial growth in subsurface bioremediation, nutrients and an electron acceptor often must be supplied. These chemicals are usually depleted in contaminated subsurface environments, and growth is limited without them. The reaction stoichiometry helps to define the chemical needs of a microbial system. Stoichiometric relationships can be used to determine the appropriate solution of nutrients and electron acceptors to flush throughout the zone of contamination.

The chemical needs for a hypothetical spill of naphthalene (surrogate for coal tar) are illustrated in Fig. 3 as an example of the required reaction stoichiometry. For each  $\text{m}^3$  of soil, the aerobic biodegradation of the 17.3 kg naphthalene requires 16.9 kg oxygen, 3.1 kg nitrogen, and 0.52 kg phosphorus. During the aerobic oxidation of the naphthalene, 24.7 kg of biomass would be

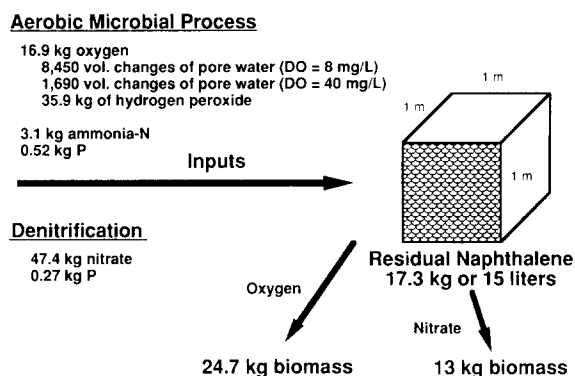


Fig. 3. Chemical requirements and products of naphthalene bioremediation for a hypothetical  $1 \text{ m}^3$  soil under aerobic and denitrifying conditions.

formed per  $\text{m}^3$  of soil. The aerobic growth forms a larger mass than that of the original naphthalene. The limited solubility of oxygen in water greatly limits the biotransformation of naphthalene in the contaminated soil. In order to deliver the amount of oxygen required using water at air saturation (approx. 8 mg oxygen/l at  $25^\circ\text{C}$ ), 8450 pore volumes of this water must be passed through the contaminated zone. Use of pure oxygen to saturate the water with about 40 mg oxygen/l reduces the pore volumes of water required to 1690. Hydrogen peroxide and nitrate are two possible sources of electron acceptor. These two chemical additives have a much higher water solubility than oxygen and are easier to deliver in the subsurface. The mass balances with hydrogen peroxide and nitrate are included in Fig. 3. The use of hydrogen peroxide to augment the oxidant capacity of aquifers for bioremediation is reviewed by Pardieck et al. [31].

The introduction into the subsurface environment of chemicals needed by microorganisms for growth is an important engineering challenge. A common method is to inject water solutions of the chemicals into the contaminated zone. Problems with this approach include the need for large quantities of injection fluid because of the limited solubility of gases (e.g. oxygen), the difficulty to achieve good mixing in the subsurface, and excessive microbial growth near the injection point. The complexities of the subsurface and

contaminant that make it difficult to extract the contaminant by pumping (hydraulic and sorption/desorption limitations) also make it difficult to introduce growth-stimulating chemicals dissolved in water to where they are needed. Use of multiple injection points and/or pulsed addition of chemicals are possible strategies to help minimize these problems. Field experience with such strategies is limited, and more effort should be directed at improving the efficiency of chemical delivery systems.

#### *Influence of sorption / desorption (mass transfer) on biodegradation*

There must be a close association between a microorganism and contaminant for biodegradation to occur. The contaminant must be available for uptake and utilization by the microorganism. Microorganisms and pollutants are distributed among the solid, liquid, and gas phases within the subsurface. Many organic contaminants are hydrophobic and tend to sorb onto soil such that only a small fraction of the compound may actually be in the bulk water phase. Over long contact time, sorbing pollutants slowly diffuse into the inorganic and organic matrix and may also form bound residues. Most evidence indicates the uptake of compounds by bacteria proceeds via the liquid phase. Consequently, a process such as sorption or volatilization that reduces the solution concentration tends to reduce the biotransformation rate. Furthermore, the accumulation of contaminants in fissures and cavities within subsurface solids may render them inaccessible to microorganisms and their enzymes. These pro-

Table 2

Organic carbon content and pH for the three soils used in the batch sorption/biodegradation studies

Soil type	Soil depth (cm)	Organic carbon content (%)	pH (1:1)
A	0-30	1.06	6.9
B	30-60	0.81	7.4
C	60-120	0.47	8.0

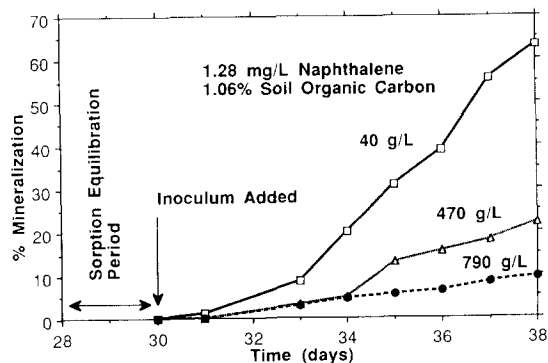


Fig. 4. Effect of soil/water ratio on the extent and rate of naphthalene biodegradation.

cesses decrease the bioavailability, and the rate of mass transfer may control the overall removal rate by biodegradation.

Batch studies were conducted to evaluate the influence of soil on the rate and extent of aerobic naphthalene biodegradation. The fraction of organic carbon and pH for the three Bozeman soils used in the batch studies are shown in Table 2. The organic carbon content decreased with depth from 1.06% to 0.47%. Consequently, sorption to the surface soil is more significant than sorption to the deeper soil. The pH increased from 6.9 to 8.0 with depth.

The influence of increasing amounts of Soil A relative to nutrient media on naphthalene mineralization is shown in Fig. 4. With 40 g soil/l, 63% of the naphthalene was mineralized within 18 days of initiating biodegradation. Less than 10% was degraded with 790 g soil/l during the same incubation period. As the soil/water ratio increases, a greater amount of the naphthalene becomes sorbed which reduces the naphthalene concentration in the aqueous media. The lower aqueous naphthalene concentration caused the biodegradation rate to decrease. In the absence of soil, the same level of naphthalene was nearly completely mineralized within 3 days [29].

The naphthalene mineralization observed in the presence of Soils A, B, and C with different fractions of organic carbon is shown in Fig. 5. As the fraction of organic carbon increased from 0.47 to 1.06%, the mineralization rate declined.



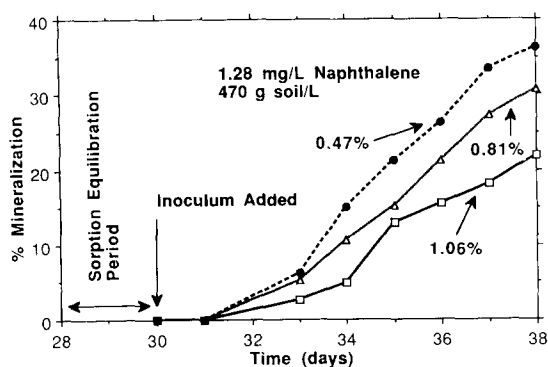


Fig. 5. Effect of soil organic carbon content on the extent and rate of naphthalene biodegradation.

Since the amount of solids was kept constant for the three soils, the amount of naphthalene sorbed was proportional to the organic carbon content. Soil A with highest organic carbon content had the least amount of naphthalene in bulk solution available for biodegradation and resulted in the lowest rate.

The batch results for the four size fractions of Soil A are shown in Fig. 6. The initial rates of biodegradation were nearly the same for the different size fractions because the initial naphthalene concentration was the same and the four size classes of soil had approximately the same organic carbon content (Table 3). However, after several days of biodegradation, the smallest size soil fraction exhibited the greatest extent of mineralization. These results are consistent with mass

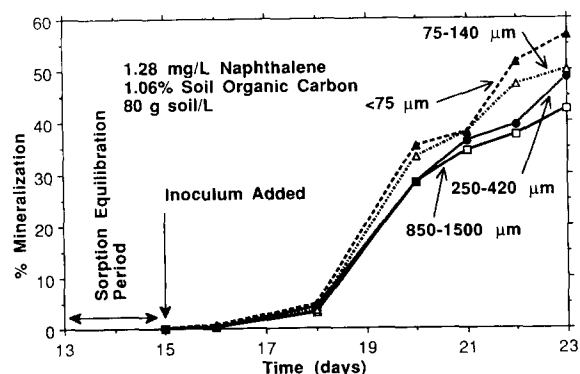


Fig. 6. Effect of soil particle size on the extent and rate of naphthalene biodegradation.

Table 3

Organic carbon contents in the four size fractions of Soil A.

Particle size range ( $\mu\text{m}$ )	Organic carbon content (%)
850-1500	1.04
250-420	1.06
75-140	1.09
< 75	1.00

transfer controlled sorption and biodegradation. Separate batch experiments (unpublished data) demonstrated that between 10 and 15 days were needed to reach sorption equilibrium with naphthalene for the four fractions of soil. Consequently, sorption/desorption rates were slow compared to the intrinsic biodegradation rate (absence of soil). The smallest size fraction has the shortest diffusion length which means faster desorption and subsequent biodegradation. Conversely, the largest size fraction has the largest diffusion length which means slower desorption and subsequent biodegradation. This is in agreement with other studies which have suggested that the kinetics of sorption to and from soil particles can be described as a radial diffusive penetration of organic contaminants into porous soil particles [32,33].

The effect of sorption on naphthalene degradation can be explained as follows: (i) sorption of naphthalene to soil decreases the aqueous concentration of naphthalene and thus reduces the amount of naphthalene available for microbial degradation; and (ii) the rate of desorption from soil to water is slower than the rate of intrinsic biodegradation. The behavior shown in Figs. 4-6 is consistent with these explanations and suggests that biodegradation of naphthalene in soil/water slurries was primarily limited by the rate of desorption.

Other researchers have observed similar decreases in the extents and rates of biodegradation with soil slurries. Rijnaarts et al. [34] demonstrated that aerobic biodegradation of  $\alpha$ -hexachlorocyclohexane in soil slurries was limited by mass transport and desorption from soil aggregates. Robinson et al. [35] found that the rate of toluene desorption controlled its overall biodegradation rate in soil slurries. The impor-

tant conclusion from the above work on bioavailability is that the overall reaction rate is controlled by the desorption rate and not by the activity of the degrading microorganisms.

### Concluding remarks

Subsurface microorganisms are capable of transforming many different organic contaminants. This has led to a great interest in exploiting biological processes for in situ treatment. The approach presented herein at the former manufactured gas plant site represents the first step in evaluating the feasibility of in situ bioremediation. Field and laboratory studies focused on characterizing the occurrence and distribution of subsurface bacteria, and assessing the extent and rates at which these bacteria degrade PAHs and benzene. The prospects for using in situ bioremediation to attenuate the coal tar compounds in the subsurface at the site appear promising. Two complicating factors are proper environmental conditions and the reduced bioavailability that results from sorption to the aquifer materials. Growth-sustaining chemicals, such as electron acceptors and nutrients, must be supplied in the proper ratio in order to stimulate growth. Their delivery to the subsurface, however, is difficult to engineer, and is an area that requires more research.

Batch experiments demonstrated that sorbing pollutants, like naphthalene, exhibit reduced extents and rates of biodegradation in the presence of soil. Consequently, the mass transfer rate becomes an important control over the biodegradation rate. The practical effect of such slow diffusion from within soil aggregates and other kinetic limitations to desorption is a decrease of the rate of removal of the contaminant, thereby increasing the time required to achieve clean-up and the amount of chemicals that must be added to sustain microbial activity.

Research in the past decade has made significant progress toward reducing the number of problems and improving our understanding of organic contaminant biotransformation. Both basic laboratory studies and well-controlled field

experiments are needed to solve the remaining complicating factors associated with in situ bioremediation. Research should be especially directed toward developing methods to improve the bioavailability of hydrophobic organic contaminants and to improve the efficiency of growth-sustaining chemical delivery systems to subsurface microorganisms.

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