Biodegradation **8:** 167–175, 1997.

Biodegradation of benzene, toluene and naphthalene in soil-water slurry microcosms

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Accepted 2 July 1997

Key words: benzene, bioavailability, biodegradation, naphthalene, sorption, toluene

Abstract

Aerobic biodegradation of benzene, toluene and naphthalene was studied in pre-equilibrated soil-water slurry microcosms. The experiments were designed to simulate biodegradation at waste sites where sorption reaches equilibrium before biodegradation becomes important. Rates of biodegradation were reduced by the presence of soil. For example, nearly complete naphthalene biodegradation (1.28 mg/L) by indigenous soil bacteria occurred within 60 hours in aqueous solution (soil-free) while it took two weeks to degrade the same amount in the presence of 0.47 kg soil/L of water. The rate of biodegradation was observed to decrease with increasing organic compound hydrophobicity, soil/water ratio, soil particle size, and soil organic carbon content. These results clearly indicate that the rate of biodegradation is affected by both the extent and rate of sorption. Further analysis suggests that mass transfer could control the performance of in situ bioremediation for highly hydrophobic organic contaminants which exhibit a large extent of sorption and slow rate of desorption.

Introduction

Successful bioremediation depends on the close association between microorganisms and contaminants. However, in soils, sediments and aquifers, hydrophobic organic compounds, such as polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), tend to distribute among the solid, liquid and gas phases such that only a small fraction of the contaminant mass may be present in the bulk water phase and available for biodegradation (Mackay, 1992; Schwarzenbach et al., 1992; Thibodeaux, 1995).

Many studies have confirmed that biodegradation can be limited by slow desorption of organic compounds. For example, Robinson et al. (1990) observed that although most of the toluene in soil-water slurries was degraded rapidly, a small fraction was biodegraded much more slowly and at a rate limited by desorption. Al-Bashir et al. (1994) reported similar behavior for biodegradation of several nitrogen-substituted naphthalenes: an initial fast phase followed by a slow decline believed to be controlled by desorption. Pignatello and co-workers (1989) reported the fate of 1,2-dibromoethane (EDB) in soils from agricultural sites where this chemical was used as a soil fumigant. Residues of EDB were found to persist in the top few centimeters of soil for as long as 19 years after application. EDB was shown to be biodegraded readily by soil microbes. However, desorption of EDB was found to be very slow at ambient temperature. It was therefore concluded that the EDB residue was sorbed into soil micropores and unavailable for biodegradation. Rijnaarts et al. (1990) demonstrated that biodegradation of α-hexachlorocyclohexane (α-HCH) in soil slurries was totally controlled by desorption, not by the activity of the degrading microorganisms.

The objective of this research was to evaluate the significance of various factors affecting extent and rate of sorption in aerobic biodegradation studies with benzene, toluene, and naphthalene. These factors included the organic compound hydrophobicity, soil organic carbon content, soil/water ratio, soil particle size,

Table 1. Characteristics of the soil materials

Label	Soil depth (cm)	Clay (g/kg)	Silt (g/kg)	Sand (g/kg)	Organic C (g/kg)	pH (1:1)
A	0–30	231	606	163	10.6	6.9
B	30–60	300	610	90	8.1	7.4
C	60–90	280	650	70	4.7	8.0

Table 2. Weight fractions and organic carbon contents of different size fractions of soil A

Sieve No.	10–20	20–40	40–60	60–80	80–100	100–200	<200
Size range (mm)	2.0–0.85	0.85–0.42	0.42–0.25	0.25–0.18	0.18–0.149	0.149–0.075	<0.075
Weight fraction (%)	50	24.1	9.7	5.2	2.8	6.6	1.6
f_{oc} (%)	1.04	1.01	1.06	1.05	1.07	1.09	1.00

temperature, and mixing condition. Experiments were designed to measure the rate of biodegradation in pre-equilibrated soil-water slurries. Under this circumstance, sorption/desorption processes were at equilibrium prior to introduction of bacterial inocula. This likely represents the situation at many waste sites where contamination has occurred years ago before natural biodegradation becomes important and/or engineered systems are implemented to stimulate biodegradation.

Materials and methods

Soils

Soil materials were collected from a site in Bozeman, Montana. Samples were taken from 0–30 cm (soil A), 30–60 cm (soil B), and 60–120 cm (soil C) depths. The soils were air-dried, homogenized, and characterized for clay, silt, sand, and organic carbon contents, and pH (Table 1). Soil particles larger than 2 mm were removed by passing through a No. 10 standard sieve. Soil organic carbon contents (f_{oc}), measured with a simplified colorimetric method (Sims and Haby, 1970), decreased with increasing depth (Table 1). pH was measured using a 1:1 soil/water ratio to ensure that the soil pH was favorable for biodegradation. pH values ranged from 6.8 for soil A to 8.0 for soil C and increased with depth (Table 1). Soil A was also separated into seven size fractions by a standard dry sieve techniques (ASTM, 1985). Organic carbon contents of the different size fractions of soil A were quite similar to the level in the bulk material (Table 2).

Bacterial culture

An aerobic bacterial culture was enriched from soil A to provide a consistent mixed inoculum for several sets of aerobic experiments described in this study. The enrichment procedures involved incubation for 30 days with 50 grams of soil A in 500 mL of nutrient solution containing 1 mg/L of naphthalene. Ten mL of supernatant from this culture was then transferred to a 500 mL batch of fresh nutrient media containing 5 mg/L naphthalene. Three sequential transfers were performed. This bacterial culture was stored at 4 °C prior to its use in biodegradation studies.

Chemicals

Benzene and toluene were purchased from Fisher Scientific. Naphthalene was obtained from Aldrich Chemicals. The purity of chemicals were re-measured using a HP 5890 gas chromatography, confirming the purity of 98+% for the model compounds that were used throughout the experiments. All radiolabeled chemicals were from Sigma (benzene-UL-^{14}C, 10 mCi/mmol; toluene-ring-UL-^{14}C, 10 mCi/mmol; naphthalene-UL-^{14}C, 20 mCi/mmol).

Biodegradation in aqueous (soil-free) solution

Biodegradation experiments with benzene, toluene and naphthalene were conducted under aerobic conditions in batch microcosms. Air was bubbled through a sterile growth medium for 12 hours to achieve oxygen saturation (8–10 mg/L). The growth medium contained the following ingredients: KH_2PO_4, 8.5 mg/L; K_2HPO_4, 21.8 mg/L; $Na_2HPO_4{\cdot}7H_2O$, 33.4 mg/L; NH_4Cl,

Table 3. ^{14}C-radiotracer analysis for evaluating biodegradation in water sample

Sample no.	1#	2#	3#	4#
pH	>12	>12	<1	<1
Air Stripping	-	$\surd$	$\surd$	$\surd$
Filtration	-	-	-	$\surd$
^{14}C Counted	^{14}VOC			
	$^{14}CO_2$	$^{14}CO_2$		
	^{14}Cell	^{14}Cell	^{14}Cell	
	^{14}C-metabolites	^{14}C-metabolites	^{14}C-metabolites	^{14}C-metabolites
Results	^{14}Organic compound remianing = 1#–2#			
	$^{14}CO_2$ produced = 2#–3#			
	^{14}Cell synthesized = 3#–4#			

* VOC – volatile organic compound.

29.8 mg/L; $MgSO_4{\cdot}7H_2O$, 22.5 mg/L; Na_2SO_4, 1.85 mg/L; $NaNO_3$, 13.8 mg/L; $CaCl_2$, 27.5 mg/L; $NaHCO_3$, 20 mg/L; $FeCl_3{\cdot}6H_2O$, 0.25 mg/L; $FeCl_2{\cdot}4H_2O$, 1.0 mg/L; $MnCl_2{\cdot}4H_2O$, 0.125 mg/L; $Na_2MoO_4{\cdot}2H_2O$, 0.025 mg/L; $CuCl_2$, 0.0075 mg/L; Na_2SeO_3, 0.005 mg/L; H_3BO_3, 0.0125 mg/L; $ZnCl_2$, 0.0125 mg/L; $AlCl_3$, 0.0125 mg/L; $CoCl_2{\cdot}6H_2O$, 0.0125 mg/L; $Ni(NO_3)_2$, 0.0125 mg/L. Benzene, toluene, or naphthalene stock solutions were prepared by dissolution in the sterile medium at 50°C (benzene, 500 mg/L; toluene, 200 mg/L; naphthalene 31.5 mg/L). Planned concentrations ($1–5x10^{-2}$ mM) for sorption and/or biodegradation experiments were achieved by diluting the concentrated stock solutions with the air-sparged growth media. The bacterial culture was introduced at an initial cell density of 10^6 CFU/mL. The solution was maintained in 20 mL serum bottles (20 mL, Wheaton 400 brand clear borosilicate glass, Wheaton, NJ) filled without headspace and sealed with Teflon septa (Teflon-faced butyl septa, 20 mm, Wheaton, NJ) and aluminum caps (standard aluminum, Wheaton 224178, 20 mm). The serum bottles were incubated in the dark at 22 ± 1 °C.

Periodically, a serum bottle from the batch samples was sacrificed to obtain a time course for the following analyses: concentration of the model organic compound, accumulation of CO_2, production of biomass, and production of intermediates. ^{14}C-radiotracers were used for the biodegradation measurements. During biodegradation, a portion of the ^{14}C in the parent organic molecules is oxidized to $^{14}CO_2$ to generate energy and reducing power (electrons). A portion of the ^{14}C is also incorporated into new bacterial cells and appears in intermediate products. The rate of $^{14}CO_2$ production was assumed to indicate the biodegradation rate of the parent compound. The procedures used to quantify the four fractions of ^{14}C (^{14}C-parent compound, $^{14}CO_2$, ^{14}C-cells, ^{14}C-intermediates) are presented in Table 3.

Periodic measurements of dissolved oxygen (DO) concentration were made to assure that oxygen was not a limiting factor for biodegradation. DO concentrations were initially between 8 to 10 mg/L. The final DO concentrations after completion of biodegradation were above 1.5 to 2 mg/L. Mass balances of the organic compounds studied were monitored by measuring organic concentrations in sterile samples that did not receive the bacterial inoculum (control). At the end of a four-week incubation period, 92.2% of benzene, 94% of toluene and 94.4% of naphthalene remained in the control samples. These results indicated that a good mass balance was maintained in the absence of the bacterial inoculum. Mass balances were also established by monitoring the total ^{14}C activities during biodegradation. On the average, greater than 90% of the initial ^{14}C could be recovered during the entire experimental period.

Sorption experiments

Preparation of samples for batch sorption experiments involved the following procedure: (1) add known quantities of aquifer solids to glass serum bottles (Tubular serum bottle, amber, 5 mL), (2) autoclave the soils and bottles for two 60 minute sessions, (3) spike the resultant samples with a known quantity of contaminant (mixed with ^{14}C radiolabeled chemical), (4) fill the serum bottles with sterilized water containing 10^{-2} M NaCl, (5) seal the bottles with Teflon septa, (6) incu-

bate the samples for a given period of time at 22±1 °C, (7) samples were then periodically centrifuged (6000 rpm for 30 minutes), and 1 mL of the supernatant was collected in liquid scintillation cocktail for assay of ^{14}C activity to measure organic concentration in the aqueous phase (C). The solid phase concentrations (S) were calculated from the mass balance of organic compound in the batch system.

Biodegradation in pre-equilibrated soil-water slurry microcosms

Procedures used to prepare batch soil-water slurry microcosms included: (1) add known quantities of dried aquifer solids to glass serum bottles (Tubular serum bottle, amber, 5 mL, Wheaton, Millville, NJ), (2) autoclave the soils and bottles for two 60 minute sessions, (3) spike the resultant samples with a known quantity of contaminant (mixed with ^{14}C radiolabeled tracer), (4) fill the serum bottles with growth medium without headspace, (5) seal them with Teflon septa, (6) incubate the samples for a period of 30 days to reach sorption equilibrium (for the three compounds studied, separate sorption experiments confirmed that sorption equilibrium was reached within 10 days), (7) after the 30 day equilibrium period, the serum bottles were uncapped and 100 μL aliquot of the bacterial culture was introduced into each serum bottle to obtain an initial bacterial concentration at 10^6 CFU/mL, and (8) the serum bottles were then resealed and incubated in the dark at 22 ± 1 °C.

Biodegradation in the soil-water slurry microcosms was determined by monitoring production of $^{14}CO_2$ from the parent organic compounds overtime. Periodically the contents of microcosms were centrifuged (6000 rpm for 30 minutes), and a portion of the supernatant was collected in liquid scintillation cocktail for assay of $^{14}CO_2$ and organic concentration in the water (Table 3).

Experimental results

Biodegradation of benzene, toluene and naphthalene in aqueous (soil-free) solution

Several series of batch biodegradation experiments were conducted using either benzene, toluene or naphthalene as the sole organic substrate (single organic compound per series). A concentrated aqueous solution of each aromatic compound was added to the growth medium to attain an initial organic concentration of 3.9 mg/L ($5x10^{-2}$ mM) for benzene, 4.6 mg/L ($5x10^{-2}$ mM) for toluene, and 1.28 mg/L (10^{-2} mM) for naphthalene. The aqueous solution also contained trace amount of either benzene(-UL-^{14}C), toluene(-ring-UL-^{14}C), or naphthalene(-UL-^{14}C). The bacterial inoculum enriched from soil A was added to achieve an initial cell concentration of 10^6 CFU/mL.

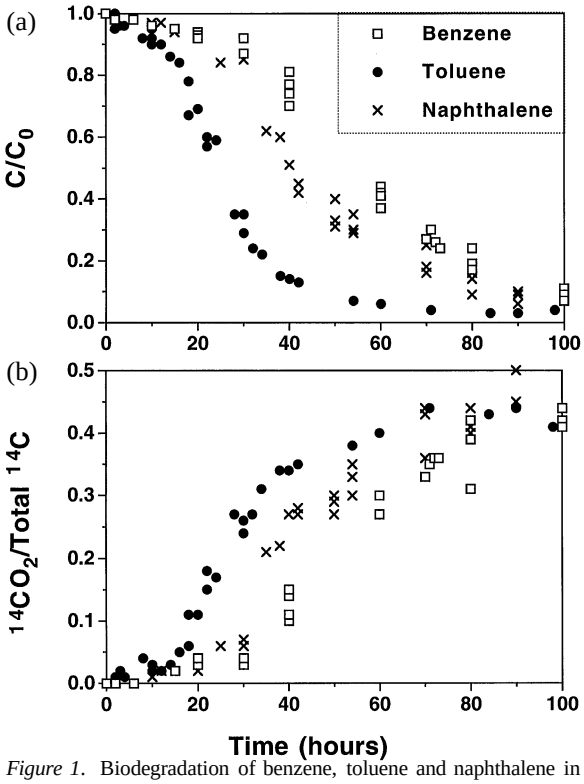

Figure 1. Biodegradation of benzene, toluene and naphthalene in water: (a) concentrations of parent organic compounds, and (b) carbon dioxide production.

Benzene biodegradation as evidenced by appearance of $^{14}CO_2$ commenced after 12 hours of incubation (Figure 1). 50% of the benzene was degraded after 60 hours and nearly complete biodegradation (>99%) was achieved within 90 hours. For toluene, biodegradation commenced immediately after incubation with no apparent lag phase (Figure 1). 50% of the toluene was degraded at 7 hours, and biodegradation was nearly complete within 14 hours. For naphthalene, biodegradation began after 6 hours of incubation. After 28 hours, 50% of the naphthalene was degraded and nearly complete biodegradation was accomplished within 60 hours. Following nearly complete biodegradation of the compounds, the portion of the benzene,

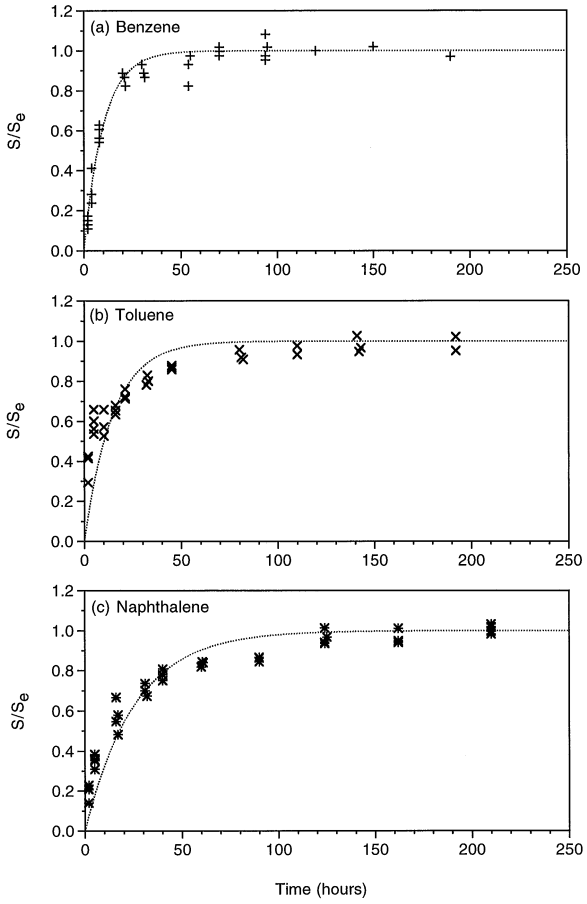

Figure 2. sorption of benzene (a), toluene (b), and naphthalene (c) to Soil A. S is the organic concentration in soil (mg/kg), and S_e is the equilibrium concentration of the organic compound in soil. The solid curves are best-fit results using first-order kinetics.

toluene, and naphthalene carbon converted into carbon dioxide was 56.7%, 58.4%, and 53.6%, respectively.

Rate of sorption

Sorption rate experiments were conducted in batch systems with soil water ratio $R_{s/w}$=0.12 kg/L (Figure 2). S in Figure 2 is the organic concentration in soil (mg/kg) and S_e is the equilibrium concentration of the organic compound in soil. The aqueous concentrations (C) were from direct measurements and the solid phase concentrations (S) were calculated from mass balances. Initial organic concentrations in the aqueous phase were 3.9 mg/L ($5x10^{-2}$ mM) for benzene, 4.6 mg/L ($5x10^{-2}$ mM) for toluene, and 1.28 mg/L (10^{-2} mM) for naphthalene. These aqueous solutions also contained 1,500 dpm/mL of (UL-^{14}C)benzene,

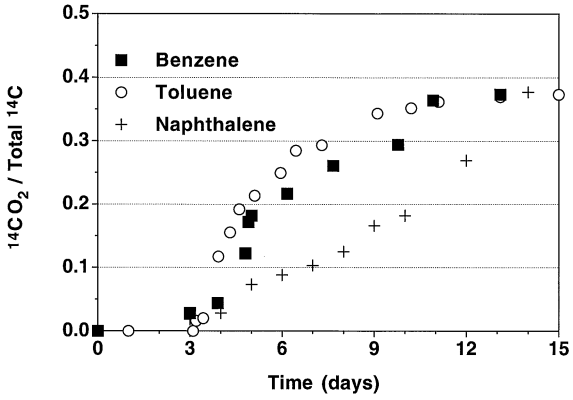

Figure 3. Evolution of $^{14}CO_2$ from benzene, toluene and naphthalene biodegradation in pre-equilibrated soil-water slurry microcosms ($R_{s/w}$ = 0.47 kg/L).

4,400 dpm/mL of (UL-^{14}C)toluene, and 2,500 dpm/mL of (UL-^{14}C)naphthalene.

The equilibration rate experiments indicated that benzene sorption equilibrium was closely approached within 72 hours ($\frac{S}{S_e} \geq 0.99$). Toluene sorption equilibrium was achieved within 150 hours and naphthalene sorption equilibrium within 240 hours (Figure 2). These time scales were generally much higher than those for biodegradation (10–90 hours).

Biodegradation of benzene, toluene and naphthalene in pre-equilibrated soil-water slurry microcosms

The influence of organic compound hydrophobicity on biodegradation was demonstrated by comparing $^{14}CO_2$ evolution during biodegradation of benzene, toluene and naphthalene in pre-equilibrated soil-water slurry microcosms (soil A) with a soil/water ratio at 0.47 kg/L (Figure 3). A concentrated aqueous solution of each aromatic compound was added to the growth medium to attain an initial concentration of 3.9 mg/L for benzene, 4.6 mg/L for toluene, and 1.28 mg/L for naphthalene (separate microcosms were prepared for each compound). The bacterial inoculum enriched from soil A was added to achieve an initial cell concentration of 10^6 CFU/mL. These conditions were maintained for all experiments described hereafter for comparison of experimental results.

Biodegradation in soil-water slurry microcosms shown in Figure 3 differed from the results in the aqueous solution (Figure 1) in three aspects: (1) biodegradation in the presence of the soil took place with longer lag phases. Significant biodegradation in soil-water

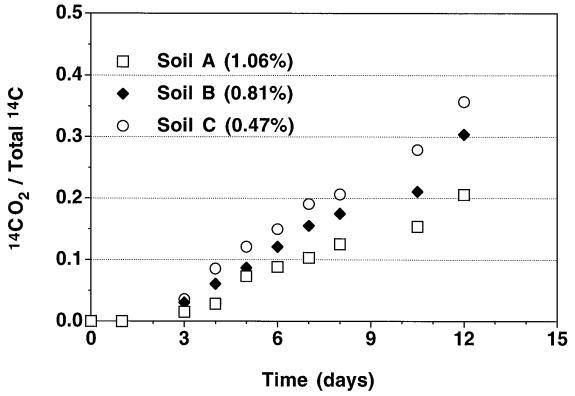

Figure 4. Effect of soil organic carbon content (f_{oc}) on $^{14}CO_2$ evolution from naphthalene biodegradation ($R_{s/w}$ = 0.47 kg/L).

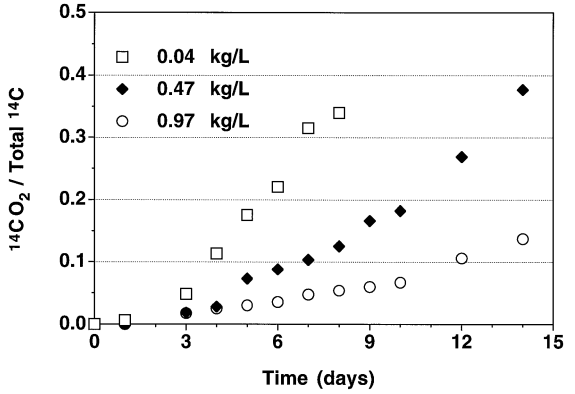

Figure 5. Effect of soil/water ratio ($R_{s/w}$) on $^{14}CO_2$ evolution from naphthalene biodegradation.

microcosms was not detected until three days while biodegradation in water started between 0 and 24 hours, (2) rates of biodegradation were significantly slower in the presence of soil. It took two weeks to degrade nearly all the three aromatic compounds while nearly complete biodegradation in water required only between 12 to 72 hours; and (3) the relative rate (sequence) of biodegradation shifted from toluene > naphthalene > benzene in water (Figure 1) to toluene > benzene > naphthalene in the soil-water slurry microcosms (Figure 3).

Effect of soil organic carbon content

A batch experiment was conducted to test the influence of soil organic carbon content (f_{oc}) on biodegradation of naphthalene (Figure 4). Pre-equilibrated soil-water slurry microcosms ($R_{s/w}$= 0.47 kg/L) were prepared with three soil samples: soil A (f_{oc}=1.06%), soil B (f_{oc}=0.81%), and soil C (f_{oc}= 0.47%). $^{14}CO_2$ evolution from benzene, toluene and naphthalene biodegradation began three days after the introduction of the bacterial inoculum (Figure 4). Throughout the 12-day experimental period, the rate of $^{14}CO_2$ evolution in the microcosm with soil A was consistently lower than the rate in the microcosm with soil B, which in turn was consistently lower than the one with soil C (Figure 4).

Effect of soil/water ratio

The influence of increasing the amount of soil relative to the volume of water on $^{14}CO_2$ evolution from naphthalene biodegradation was examined by batch experiments in three soil-water slurries with $R_{s/w}$ values of 0.04, 0.47 and 0.97 kg/L, respectively (Figure 5). For example, within nine days, 60% of the carbon from naphthalene was transformed into carbon dioxide for $R_{s/w}$ = 0.04 kg/L (nearly complete degradation), about 16% was mineralized for $R_{s/w}$ = 0.47 kg/L, and only 5% for $R_{s/w}$ = 0.97 kg/L (Figure 5). Within 14 days, 38% of the naphthalene was mineralized for $R_{s/w}$ = 0.47 kg/L, and only 14% for $R_{s/w}$ = 0.97 kg/L.

Effect of soil particle size

The significance of soil particle size on naphthalene biodegradation is presented in Figure 6. Pre-equilibrated soil-water slurry microcosms with four size fractions of soil A (<0.075 mm, 0.075–0.149 mm, 0.425–0.85 mm, and 0.85–2.00 mm) were prepared. Initial rates of biodegradation (as evidenced by $^{14}CO_2$ evolution) were nearly the same for the four size fractions (Figure 6). However, after several days of incubation, differences in biodegradation rates among the four soil-water slurries gradually became evident. The smallest size fraction (<0.075 mm) exhibited the greatest extent of biodegradation while the largest size fraction (0.85–2.0 mm) had the lowest (Figure 6). For example, 50% of the organic carbon was mineralized for soil slurries with particles less than 0.075 mm (the smallest particles) within 12 days. Within the same time period, only 37% of the organic carbon was mineralized for soil slurries with the largest particles (0.85–2.00 mm).

Effect of temperature

Biodegradation of naphthalene in pre-equilibrated soil-water slurry microcosms was evaluated under two tem-

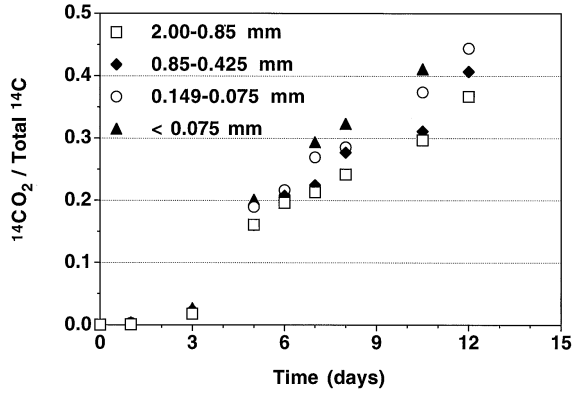

Figure 6. Effect of soil particle size (d_p) on $^{14}CO_2$ evolution from naphthalene biodegradation ($R_{s/w}$ = 0.47 kg/L).

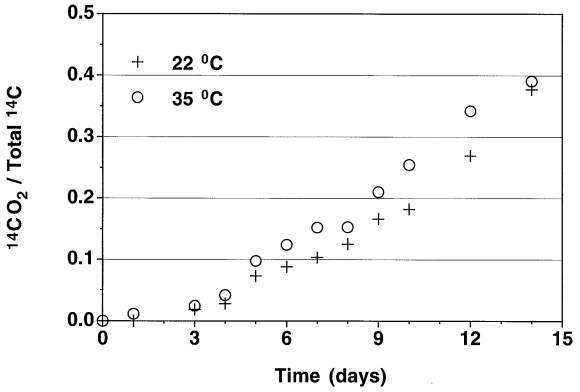

Figure 7. Effect of temperature on $^{14}CO_2$ evolution from naphthalene biodegradation ($R_{s/w}$ = 0.47 kg/L).

peratures: 22 °C and 35 °C, with all other environmental conditions maintained the same. Biodegradation rate at 35 °C was consistently higher than at 22 °C throughout the 14-day experimental period (Figure 7). Biodegradation at 35 °C also exhibited a shorter lag phase of less than 24 hours.

Effect of mixing

Effect of mixing intensity on the rate of naphthalene biodegradation was demonstrated by comparing $^{14}CO_2$ evolution rate from two batch measurements. One series of batch samples was mixed on a shaker table for only the first 48 hours after the bacterial culture was introduced. The second series was continually mixed during the entire experimental period (two weeks). Virtually identical results were recorded for the two series (Figure 8). This also suggests consistent experimental

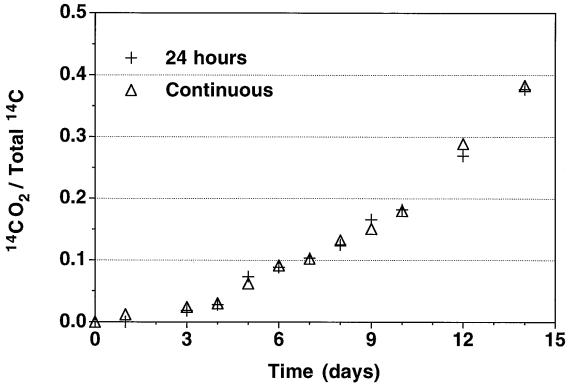

Figure 8. Effect of mixing condition on $^{14}CO_2$ evolution from naphthalene biodegradation ($R_{s/w}$ = 0.47 kg/L).

procedure. Comparisons are useful for other results as well.

Discussion

In considering the above observations, effects of sorption on biodegradation can be classified into two categories: *concentration effects* and *desorption rate effects*.

Concentration effects

Both organic degradation rate and microbial growth rate decrease with reduced organic concentration in water. The most commonly assumed relationship between microbial growth and bulk water substrate concentration (C) is the Monod equation (Monod, 1949):

$$\mu = \frac{\mu_m C}{K_s + C} \tag{1}$$

where μ is the specific growth rate (1/hour), μ_m is the maximum specific growth rate, K_s is the half-velocity constant for microbial growth (mg/L), and C is the organic concentration in the bulk water phase (mg/L). In porous media, where available concentrations are usually low ($C << K_s$), Equation (1) will approximately reduce to a linear relation and the microbial growth rate (μ) is therefore proportional to the available (aqueous) concentration (C).

In porous media, it is useful to consider the fraction of an organic compound present in the bulk water phase at sorption equilibrium (f_w), which can be estimated from the following relationship:

$$f_w = \frac{1}{1 + \frac{m}{V} K_d} \quad (2)$$

where m is the mass of the solid material (kg/L), V is the volume of water (m^3) and K_d is the soil/water distribution coefficient (L/kg).

For unconsolidated granular deposits in the subsurface, $\frac{m}{V}$ values extend from 4 to 11 kg/L (Freeze and Cherry, 1979). For example, if K_d is equal to 1 L/kg, then over 80% of the organic compound resides in the solid phase. Values of K_d were determined to be 0.44 L/kg for benzene, 2.34 L/kg for toluene and 6.43 L/kg for naphthalene (Zhang, 1995). It becomes clear that, for a strongly sorbing organic compounds such as toluene and naphthalene, only a small fraction of the organic contaminant may present in the bulk water phase.

At very low substrate concentration, such as the micrograms to nanograms per liter range commonly encountered for groundwater contaminants, insufficient energy and carbon may be available for microbial growth and maintenance. That is, a threshold concentration may exist below which biomass growth cannot be sustained. Such a minimum concentration for sustainable growth, C_{min}, can be defined as the concentration at which microbial growth is just balanced by decay (Rittmann and McCarty,1980):

$$C_{min} = \frac{K_s b}{\mu_m - b} \quad (3)$$

where b is the decay coefficient (1/hour). If sorption reduces the concentration to below $C_{min,}$ a steady-state microbial population cannot be maintained and biodegradation rate will decrease and eventually stop with time due to the net loss of biomass.

At high concentrations, on the other hand, many organic compounds may be inhibitory to microbial growth or, in some cases, toxic. One model for estimating the specific microbial growth rate for an inhibitory chemical is given below (Andrews, 1968):

$$\mu = \frac{\mu_m C}{K_s + C + \frac{C^2}{K_i}} \quad (4)$$

where K_i is the inhibition constant (mg/L). In the case of an inhibitory or toxic chemical, sorption may have beneficial results by reducing dissolved organic concentration well below the K_i value, thus lessening the toxic impact.

Observations presented in Figures 3–5 indicate that the larger the extent of sorption, the slower the rate of biodegradation. The data shown in Figure 3 indicate that as the organic compound hydrophobicity increases, the rate of biodegradation decreases. As shown in Figure 4, soil A had the highest organic carbon content which resulted in the least amount of naphthalene in the bulk water and the slowest biodegradation rate. The data shown in Figure 5 illustrate that the higher the soil/water ratio, the slower the rate of biodegradation.

Desorption rate effects

The overall biodegradation process can be conceptually divided into the following major steps: desorption to the aqueous phase, mass transfer to biologically accessible regions, and biological uptake and transformation. These steps occur sequentially, such that the overall bioremediation rate can be limited and controlled by any of the steps. Practically it is often difficult to distinguish among the various steps and they are perhaps more usefully divided into just two 'apparent' processes: desorption (desorption and mass transfer) and biodegradation (uptake and biological transformation). With this nomenclature, we must recognize that 'desorption' now also includes the mass transfer of contaminant from all regions inaccessible to bacteria.

Results presented in Figures 6, 7 and 8 suggest that the slower the desorption, the lower the biodegradation rate. Observations shown in Figure 6 indicate that large particles show a slower approach to equilibrium than smaller particles, implying intraparticle-diffusion limited biodegradation. 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 biodegradation.

Temperature could potentially affect the apparent biodegradation in three ways (Figure 7). First, microbial growth rate increases with higher temperature. The rate of an enzymatically catalyzed reaction tends to double when temperature is increased by 10 °C. Second, diffusion coefficients and the rate of desorption increase with temperature. Third, the soil/water distribution coefficient (K_d) also changes with temperature. Piatt et al. (1996) reported studies on the sorption of naphthalene, phenanthrene and pyrene to low organic carbon aquifer sediments. The soil/water distribution coefficient (K_d) decreased 1.1 to 1.6 times with an increase in temperature of 22 °C. Desorption rate increased 1.2 to 2.6 times. Since temperature change

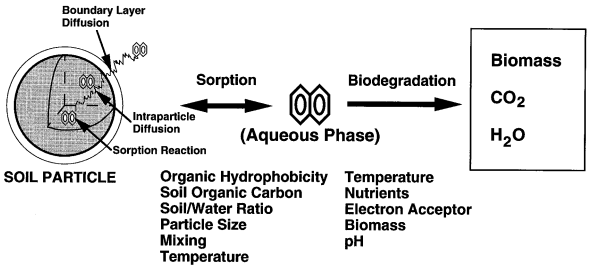

Figure 9. Summary of factors influencing sorption and biodegradation in soil-water slurries.

influences so many factors, more delicate experiments are needed to elucidate the overall impact of temperature on biodegradation in soil/water slurries.

Results in Figure 8 clarify that diffusion of the organic compound in the bulk water phase played an insignificant role in determining the overall mass transfer resistance during the desorption-biodegradation process. This corroborates the previous results of Ball and Roberts (1991), who studied sorption of halogenated organic compounds to aquifer materials, that sorption (beyond 3 days) was unaffected by a reduction in mixing intensity. An order of magnitude analysis suggested that the external fluid film resistance is unlikely to be dominant although it may be large enough to exert some effect on the sorption rate (Schwarzenbach et al. 1992).

In summary, major factors influencing sorption and biodegradation in soil-water slurries are illustrated in Figure 9. The experimental results described in this work are consistent with the rationale that the rate of biodegradation is strongly affected by both the extent and rate of sorption.

Acknowledgements

Insightful comments by the two anonymous reviewers are gratefully acknowledged. This work was supported in part by Cooperative Agreement ECD-8907039 between the National Science Foundation and Montana State University and in part by the Hazardous Substance Management Research Center and the New Jersey Commission on Science and Technology headquarted at the New Jersey Institute of Technology. The authors would like to thank Drs. William Inskeep and Richard Veeh for providing the soil samples.

References

Al-Bashir B, Hawari J, Samson R & Leduc R (1994) Behavior of nitrogen-substituted naphthalenes in flooded soil – part II: Effect of bioavailability on biodegradation kinetics. Wat. Res. 28: 1827–1833

Andrews, JF (1968) A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. Biotechnology and Bioengineering, 10: 707–723

ASTM (1985) Manual on Test Sieving Methods. ASTM special technical publication 447B, Philadelphia, PA

Ball WP and Roberts PV (1991) Long-term sorption of halogenated organic chemicals by aquifer material. 2. Intraparticle diffusion. Environ. Sci. Technol. 25: 1237–1249

Brusseau ML (1994) Transport of reactive contaminants in heterogeneous porous media. Reviews of Geophysics, 32: 285–313

Freeze R & Cherry J (1979) Groundwater. Prentice-Hall, Inc

Harmon TC, Semprini L & Roberts PV (1992) Simulating solute transport using laboratory-based sorption parameters. J. Environmental Engineering, 118: 666–689

Karickhoff SW, Brown DS & Scott TA (1979) Sorption of hydrophobic pollutants on natural sediments. Wat. Res. 13: 241–248

Karickhoff, SW (1984) Organic pollutant sorption in aquatic systems. J. Hydr. Eng. 110: 707–735

Mackay D, Shiu WY & Ma KC (1992) Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. Vol. 1. Monoaromatic hydrocarbons, Vol. 2. Polynuclear aromatic hydrocarbons. Lewis Publishers, Chelsea, MI

Monod, J (1949) The growth of bacterial cultures. Annu. Rev. Microbiol. 3: 371–394

Ogram, AV, Jessup RE, Ou LT & Rao PSC (1985) Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl. Environ. Microbiol. 49: 582–587

Pignatello JJ (1989) Sorption dynamics of organic compounds in soils and sediments. pp. 31–80, Chapter 3 in Reactions and Movement of Organic Chemicals in Soils, Sawhney BL, Brown K, Eds. Soil Science Society of America Inc. and American Society of Agronomy Inc

Pignatello JJ & Xing B (1996) Mechanisms of slow sorption of organic chemicals to natural particles. Environ. Sci. Technol. 30: 1–11

Rijnaarts HHM, Bachmann A, Jumelet JC & Zehnder AJB (1990) Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α-hexachlorocyclohexane in a contaminated calcareous soil. Environ. Sci. Technol. 24: 1349–1354

Monod, J (1949) The growth of bacterial cultures. Annu. Rev. Microbiol. 3: 371–394

Robinson KG, Farmer WS & Novak JT (1990) Availability of sorbed toluene in soils for biodegradation by acclimated bacteria. Wat. Res. 24: 345–350

Schwarzenbach RP and Westall J (1981) Transport of nonpolar organic compounds from surface water to groundwater. Laboratory sorption studies. Environ. Sci. Technol. 11: 1360–1367

Schwarzenbach RP, Gschwend PM, Imboden DM (1992) Environmental Organic Chemistry. John Wiley & Sons

Sims JR & Haby VA (1970) Simplified colorimetric determination of soil organic matter. Soil Sci. 112: 137–141

Thibodeaux LJ (1995) Environmental Chemodynamics: Movement of Chemicals in Air, Water, and Soil, Second Edition. Wiley

Wu SC & Gschwend PM (1986) Sorption kinetics of hydrophobic organic compounds to natural sediments and soils. Environ. Sci. Tech. 20: 717–725

Zhang, Wei-xian (1995) Ph.D. Thesis, Department of Geography and Environmental Engineering, Johns Hopkins University, Baltimore, Maryland