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Protocol for determining bioavailability and biokinetics of organic pollutants in dispersed, compacted and intact soil systems to enhance *in situ* bioremediation

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The development of effective *in situ* and on-site bioremediation technologies can facilitate the cleanup of chemically-contaminated soil sites. Knowledge of biodegradation kinetics and the bioavailability of organic pollutants can facilitate decisions on the efficacy of *in situ* and on-site bioremediation of contaminated soils and determine the attainable treatment end-points. Two kinds of compounds have been studied: (1) phenol and alkyl phenols, which represent hydrophilic compounds, exhibiting high water solubility and moderate to low soil partitioning; and (2) polycyclic aromatic hydrocarbons which are hydrophobic compounds with low water solubility and exhibit significant partitioning in soil organic carbon. Representative data are given for phenol and naphthalene. The results provide support for a systematic multi-level protocol using soil slurry, wafer and porous tube or column reactors to determine the biokinetic parameters for toxic organic pollutants. Insights into bioremediation rates of soil contaminants in compact soil systems can be attained using the protocol.

Keywords: biodegradation kinetics; bioavailability; slurry, wafer, tube and column bioreactors; respirometry; bioremediation; soil

Introduction

Bioremediation of Superfund soil and sediment sites using treatment technologies, such as bioremediation, requires a fundamental understanding of biodegradation kinetics and physicochemical factors that control the rate of biodegradation [2,4,5]. Knowledge of the kinetics of biodegradation is essential for evaluation of the persistence of most organic pollutants in soil and can provide useful insights into the favorable range of important environmental parameters for improvement of the microbiological activity, enhancement of the biodegradation rates of the contaminants in soil, sediments and aquifers and consequently for enhancing the bioremediation of these environments [9,12].

This paper highlights biodegradation studies on phenol, several alkyl phenols (*p*-cresol, 2,4-dimethyl phenol, catechol, hydroquinone, and resorcinol) and selected polycyclic aromatic hydrocarbons (PAHs): naphthalene, phenanthrene, acenaphthene, and acenaphthylene. A multilevel experimental protocol is presented which incorporates the use of soil microcosms for acclimation of soil microbiota, measurement of respirometric oxygen uptake in soil slurry, wafer and porous tube or column reactors, and determination of adsorption/desorption equilibria and kinetics. Mathematical models for soil slurry, wafer, column or porous tube reactors [6] are used to determine the contaminant diffusivities and biodegradation kinetics in soil slurry and compacted soil systems.

Two kinds of compounds were selected for this study: phenol and alkyl phenols, and polycyclic aromatic hydrocarbons (PAHs). Phenolic compounds constitute a significant fraction of water-soluble organic compounds present in wastes from many industrial processes. Polycyclic aromatic hydrocarbons (PAHs) exhibit low aqueous solubility and high octanol-water partition coefficients. Hence, sorption of PAHs to soil organic matter is more significant than for hydrophilic compounds such as phenol and alkyl phenols.

Scow *et al* [19] reviewed biodegradation kinetics in soil and discussed the effects of diffusion and adsorption. Kinetically, sorption is a two-phase process, with an initial fast stage ($<$1 h) followed by a slower long phase (days), controlled by diffusion to internal adsorption sites [15,17]. It has been postulated that time-dependent sequestration reactions render contaminants in soil unavailable to microbial attack [14,23]. However, the complex interactions between the contaminant and various soil components is currently not well understood, and results on contaminant sequestration are strongly dependent on types of soil and contaminant used, methods for soil sterilization and even methods used for extraction of the soil [18]. Recently, studies were conducted on the reduced biodegradability of desorption-resistant fractions of polycyclic aromatic hydrocarbons in soil and aquifer solids [7]. These studies suggested that the fraction of compound resistant to desorption has to be evaluated before the compound's environmental fate can be esimated. It was further found that the soil organic matter content influences the availability of the desorption-resistant fraction [21].

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Materials and methods

Soil characterization

Uncontaminated silt loam soil was obtained from a farm in Florence, Kentucky. It had the following characteristics [6]: soil moisture 17%, organic matter 0.415%, classification silt loam, cation exchange capacity 6.5, soil pH 6.1, bulk density 1.06, nutrients in soil (ppm): phosphorus 17, potassium 90, magnesium 80, calcium 1100 and sodium 17. The Brunauer, Emmett and Teller (BET) specific surface area was 20.27 $m^2\ g^{-1}$, BET void volume was 0.029 $cm^3\ g^{-1}$ and the BET average pore diameter was 58 Å [1,3]. The soil was air dried and sieved to pass a group of sieves (2, 10, 20, 75, 150, 300, 600 and 1000 mm). The average soil particle size obtained was 0.0334 cm. Soil porosity, pore size distribution, pore volume and surface area were determined by nitrogen adsorption using Micrometrics ASAP 200 [3].

Preparation of contaminated soil

In the case of phenols, the phenol stock solution was added directly to soil while preparing the soil slurry, soil wafer, porous tube or soil column reactors. In the case of PAHs, which exhibited low water solubilities, the PAH compound was dissolved in acetone and the acetone solution was used to contaminate the soil. Specifically, 700 mg of naphthalene was dissolved in 0.5 L acetone. The acetone was mixed well to ensure complete dissolution of added naphthalene. One kilogram of the uncontaminated sieved soil was spiked with 500 ml of acetone solution. The soil was mixed thoroughly as the solution was added. The soil was then spread on an inert surface as a thin layer and left open in the fume hood for 24 h to allow the acetone to evaporate. Periodically the soil was turned to expose fresh surface during the 24-h period. Four samples were taken from the soil before and after the contaminant solution was added and the soil concentration of naphthalene was determined using standard EPA methods [10,22].

Nutrient solution composition

The nutrient solution used in the respirometer was an Organization for Economic Cooperation and Development (OECD) synthetic medium [16] containing mineral salts and vitamin solution [6]. The soil served as a source of inoculum.

Measurement of soil-bacterial adsorption isotherm

The adsorption isotherm for the bacterial cells was determined by incubating soil microbiota with radiolabeled phenol in a respirometric reactor until an oxygen uptake plateau was obtained, indicating that phenol had biodegraded into $^{14}CO_2$, which was absorbed in the KOH solution, and into ^{14}C biomass. The soil suspension was allowed to settle for about 30 min. One milliliter of the supernatant phase was sampled and the ^{14}C activity was measured by liquid scintillation counting. Equilibrium amounts of the ^{14}C biomass adsorbed to the soil were determined by subtracting the ^{14}C present in the biomass in suspension and the ^{14}C present as carbon dioxide absorbed in the KOH solution from the total ^{14}C added initially. The ratio of the biomass adsorbed to the soil and the biomass present in the suspension gave the biomass/soil adsorption isotherm, Kb.

Studies using soil microcosms

The soil microcosm reactor, shown in Figure 1, consists of an airtight rectangular container (50 cm × 30 cm × 30 cm) constructed of glass and supported by stainless steel panels. The nutrients and appropriate contaminants are sprayed from the top using liquid atomizing sprays. The bottom of the reactor is equipped with ports to allow the drainage of leachates. A controlled flowrate of CO_2-free air is passed through the reactor and the exit gas is bubbled through potassium hydroxide solution to quantify the average evolution rate of CO_2 in the reactor.

Each microcosm reactor represents a controlled site, which eventually selects out the acclimated indigenous microbial population in the soil for the contaminating organics. Samples of soil are then taken from the microcosm reactors and used as the source of acclimated microbial inoculum for subsequent studies.

Some microcosm reactors were contaminated with a mixture of phenolic compounds dissolved in deionized distilled water so that the total chemical oxygen demand per kg of soil in the microcosm reactor was 300 mg. Equal concentrations of phenol, resorcinol, catechol, 2,4-dimethyl phenol, cresol and hydroquinone were used in the mixture. The other microcosm reactors were contaminated with 25 ppm each of the following polycyclic aromatic hydrocarbons: dibenzothiophene, naphthalene, anthracene, phenanthrene, acenaphthene, acenaphthylene, chloronaphthalene, fluorene, fluoranthene, chrysene, pyrene, and bezoanthracene, dissolved in a mixture of deionized-distilled water containing 0.5% (v/v) solution of a surfactant, Triton X-100. Control microcosm reactors contained uncontaminated soil which was sprayed with an equal volume of deionized-distilled water and soil contaminated with 0.5% Triton X-100. At appropriate time intervals during the microcosm runs, soil core sampling was undertaken and the samples were subjected to solvent extraction and GC/MS analysis to determine the residual levels of the parent phenolic and PAH compounds and their metabolites [10,22].

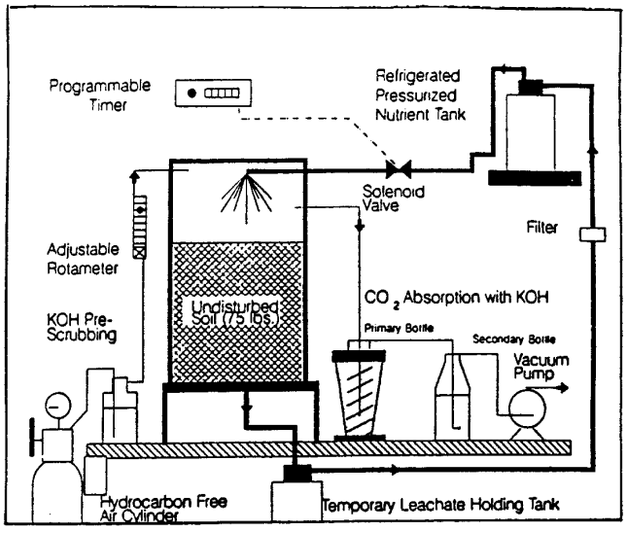

Figure 1 Schematic of soil microcosm reactor.

Abiotic soil adsorption studies

Soil adsorption kinetics and equilibria were measured using well-stirred bottles. The soil was initially air dried and then sieved to pass a 2-mm mesh size sieve. Ten grams of soil sample were placed in each bottle and mixed with 100 ml of distilled deionized water containing a known amount of the compound and mercuric chloride to minimize biodegradation. The soil : solution ratio was expressed as the oven-dry equivalent mass of adsorbant in grams per volume of solution.

The liquid was sampled at predefined time intervals. Before the liquid sample was taken, the bottle contents were centrifuged and the liquid sample was withdrawn using a syringe connected to a 0.45-μm pore size porous silver membrane filter (Poretics, CA, USA) to prevent soil particles from entering the sample. The liquid samples were analyzed using GC/MS analysis and liquid scintillation analysis for radiolabeled compounds using standard EPA methods [10,11,13,22].

From the initial amount of compound and analysis of the liquid phase, the amount of compound absorbed in the soil was obtained by difference. Equilibrium is defined when the liquid concentration reaches a stationary value, which is usually attained in 24 h. Data taken at equilibrium are used to obtain Freundlich isotherm parameters.

Desorption studies

Desorption studies were conducted by first adsorbing the chemical in the soil until equilibrium was achieved. One hundred milliliters of deionized distilled water were mixed with 20 g of soil and a specified concentration of chemical for adsorption. After adsorption equilibrium was attained, the sample was diluted with an equal volume of deionized distilled water and with 20 g L^{-1} of mercuric chloride to inhibit biodegradation. Twenty-milliliter samples were withdrawn at 4, 8, 16, 24, 48, 72, 96 and 120 h. Each sample was withdrawn from a separate adsorption bottle. The sample was analyzed using extraction with methylene chloride followed by GC/MS analysis [10,22].

Respirometric studies

The concentration of selected soil in the reactor flask varied from 2 to 10% by weight, using dry weight of soil as the basis. The total volume of the slurry in the flask was 250 ml. Three types of bioreactors were used to determine the biokinetic parameters of the suspended and immobilized microbiota and the transport parameters of contaminant and oxygen in the soil matrix. These three types of reactors, shown in Figure 2, were [6]: (1) slurry bioreactor, where soil at 5% slurry concentration was vigorously mixed with the contaminant, dissolved in water with nutrients; (2) wafer reactor, where a thin wafer of soil was spiked with contaminant and nutrients dissolved in water, to obtain a 50% total soil moisture content; and (3) porous tube or column reactor, where sieved soil with contaminent was packed in a porous glass tube or column with moisture content identical to the wafer reactor. In the soil slurry reactor, there were no limitations of oxygen, which freely diffused into the well-stirred slurry and nutrients, which were initially dissolved in the aqueous phase. Hence, the biodegradation rate in soil slurry reactors depended on the intrinsic

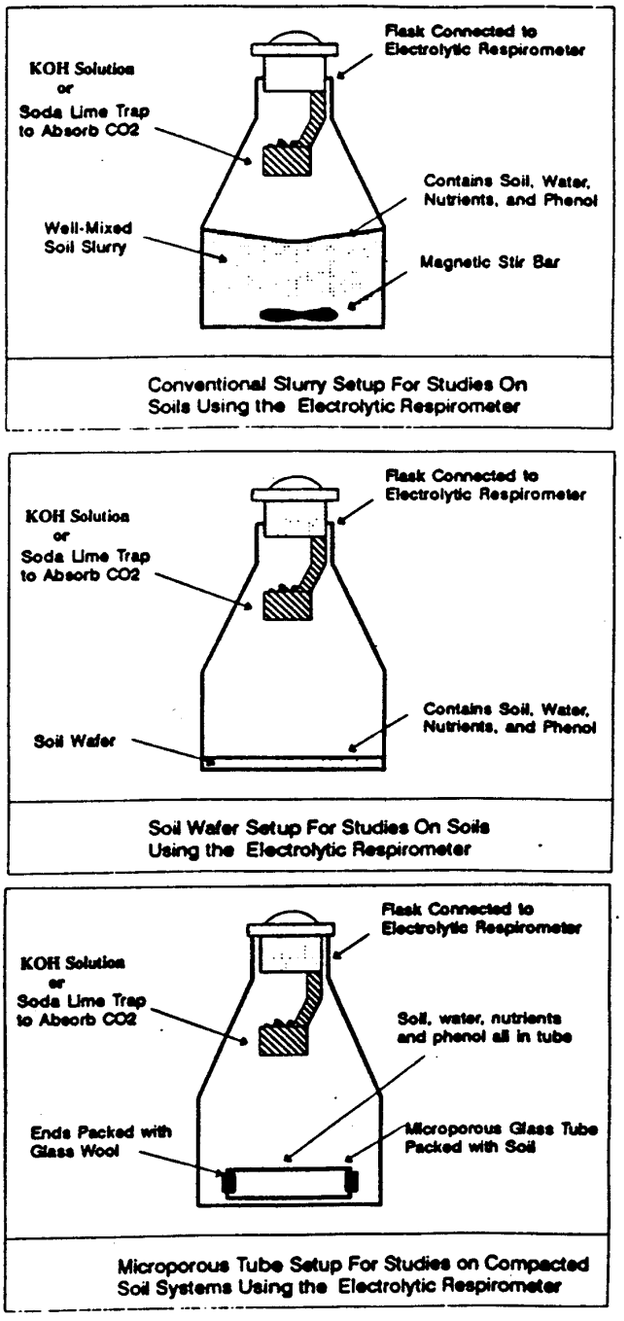

Figure 2 Schematic of soil slurry, wafer, porous tube and column bioreactors.

biokinetic rate, microorganism concentration in the soil matrix and inherent diffusivity of the contaminant. In the soil wafer reactor, oxygen diffused freely through the thin soil matrix, and hence the biodegradation rate was controlled by the water content in addition to the other intrinsic parameters, as in the case of the soil slurry reactor. In the soil column or porous tube reactor, the biodegradation rate was controlled by the water content and oxygen diffusivity and other intrinsic biokinetic parameters. The soil column or porous tube reactor provided a better estimate of biodeg-

radation rates for *in situ* bioremediation than the soil wafer and soil slurry reactors.

Ten shaker flasks, each with 100 g of soil from the acclimated microcosm reactor mixed with 1 L of double-distilled water containing 4 ml of secondary activated sludge and OECD nutrients [16], were set up to serve as the inoculum source for all soil reactor experiments. Inoculum was obtained by simply withdrawing a fixed volume of the slurry mixture from the shaker flasks.

Slurry reactor experiments were conducted to obtain better insight into the slurry biotreatment process. The specific procedures for the soil slurry experiments were: (1) 25 g of spiked soil was mixed with 250 ml of double-distilled water and OECD nutrients [16] and 7 ml of inoculum from the shaker flask using a Teflon-coated stir bar (duplicate experiments were conducted); (2) duplicate control reactors were set up containing 25 g of uncontaminated soil with 250 ml of double-distilled water and OECD nutrients [16] and 7 ml of inoculum from the shaker flasks; (3) the flasks were connected to an aerobic respirometer (N-CON Systems, Crawford, GA, USA). In the case of phenols, 20 g of uncontaminated soil was mixed with 250 ml of double-distilled water, and 2.5–10.0 ml of experimental stock solution to produce the desired phenol concentrations in each flask.

Specific procedures for the soil wafer reactor experiments were: (1) 25 g of spiked soil was mixed with OECD nutrients and 7 ml of inoculum from the shaker flasks and placed in each flask (experiments were conducted in duplicate); (2) duplicate control experiments, each containing 25 g of uncontaminated soil mixed with OECD nutrients [16] and 7 ml of inoculum from the shaker flasks were also conducted; (3) each flask was connected to an aerobic respirometer (N-CON Systems). In the case of phenols, 20 g of uncontaminated soil mixed with 20–30 ml of double-distilled water were placed in the reaction flask and mixed well to give uniform biomass concentration in the soil matrix. Water from the reaction flask was evaporated at room temperature until the soil had attained the desired water content. The soil wafer was contaminated with 2.5–10.0 ml of experimental stock solution, depending on the desired concentration, and the soil wafer was mixed with the syringe needle while the stock solution was injected.

Unlike the soil slurry system, the water present in the wafer reactor was significantly smaller and stationary, which increased the contaminant concentration and decreased mass transfer within the liquid phase. The oxygen uptake and CO_2 evolution were greatly affected by these differences.

Specific procedures for the soil column experiments were: (1) 100 g of spiked soil mixed with OECD nutrients and 28 ml of inoculum from the shaker flasks were placed in each column reactor (experiments were conducted in duplicate); (2) duplicate control reactors were set-up; each contained 100 g of uncontaminated soil with OECD nutrients and 28 ml of inoculum from the shaker flasks; (3) all flasks were connected to an aerobic respirometer (N-CON Systems). In the case of phenols, porous glass tubes made of vycor glass were used, with an average pore diameter of 40 nm. The pore size was chosen because it was found to be best at holding all the soil and water within the porous tube while allowing free flow of oxygen from the surrounding air. In this way, the glass tube did not affect the results of the oxygen uptake experiments and served only to support the soil during contamination and biodegradation.

The soil column reactor is similar to the porous tube reactor since it allows determination of the effect of oxygen profile on biodegradation rate in compacted soil systems. Soil columns were used in studies of PAHs because porous tube systems can be used when small amounts of soil, typically less than 30 g, have to be used. When compounds exhibiting low water solubility, such as PAHs, are studied, spiking with water saturated with the contaminant results in low contaminant concentrations in the soil. Further, low contaminant concentrations may have to be used to prevent inhibition effects. When the contaminant concentration in the soil is low, more soil has to be used in the reactor to achieve significantly higher cumulative oxygen uptake than in uncontaminated-soil controls. Hence, the soil column reactor was developed, in which significantly greater amounts of soil (exceeding 30 g) can be tested compared to the porous tube reactor.

Abiotic adsorption and desorption kinetics of the contaminant into the soil matrix and oxygen uptake data obtained for the soil slurry, soil wafer and column reactors were used in conjunction with detailed mathematical models to derive the intrinsic biokinetic and transport parameters. The mathematical models used for analyzing the experimental oxygen uptake data from soil slurry, wafer, porous tube and column models were presented earlier [6].

Results and discussion

The multi-level experimental protocol developed for analyzing biodegradability of soil contaminants is shown in Figure 3. It incorporates measurement of abiotic adsorption

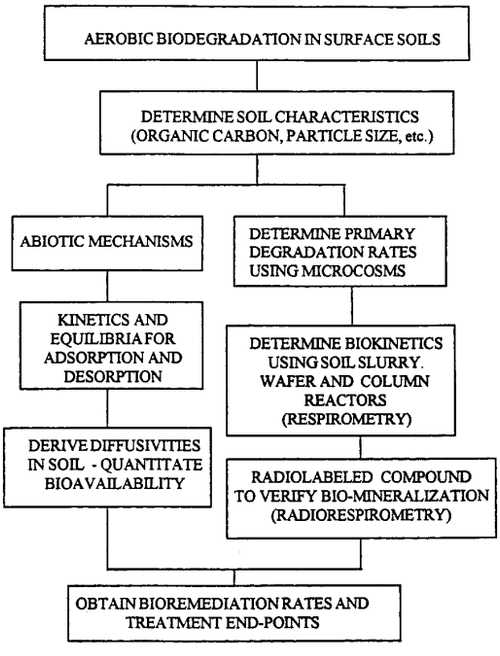

Figure 3 Overall protocol for determining biodegradation kinetics and attainable end-points in soil slurry and compacted soil biotreatment systems.

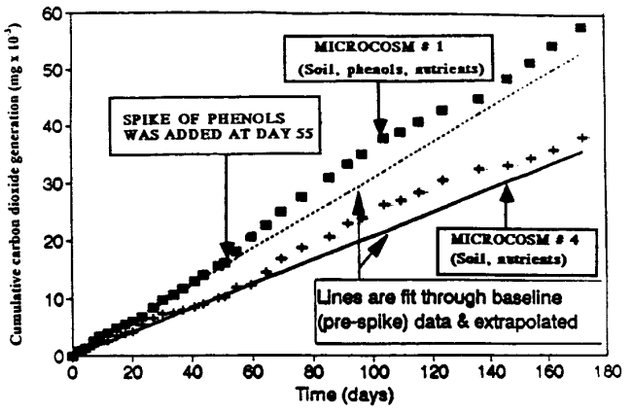

Figure 4 Cumulative carbon dioxide generation from the soil microcosm reactor spiked with a mixture of phenols and the control reactor.

and desorption rates and equilibria and quantitation of cumulative oxygen uptake using soil slurry, wafer, porous tube and column reactors. The adsorption and desorption Freundlich isotherm parameters and the biokinetic parameters, determined from the soil slurry, wafer, porous tube and column bioreactor experiments, can be used to stimulate bioremediation rates in bio-slurry and *in situ* treatment of contaminated soil. It must be noted that these parameters are intrinsic for a specific soil and contaminant. Experimental studies with aged soils are currently in progress, to determine the applicability of biokinetic parameters, determined with freshly spiked soils.

Studies using soil microcosms

Using the specially designed microcosm reactors, it was possible to acclimate the indigenous microbiota to each class of compounds. Figure 4 shows the cumulative CO_2 generated as a function of time for two microcosm reactors, before and after spiking microcosm 1 with a solution containing six phenolic compounds and microcosm 4 with OECD nutrients. The cumulative CO_2 production increased after one microcosm was spiked with nutrients and six phenolic compounds. Acclimation of the soil microbiota to the six phenolic contaminants was achieved quickly, and biodegradation of the contaminants resulted in increased carbon dioxide evolution.

In the case of the PAHs, Figure 5 shows the net cumulative CO_2 evolution, ie actual CO_2 evolution from microcosm minus the CO_2 evolved from the control reactor spiked only with OECD nutrients. The figure shows that after spiking, the net CO_2 evolution increased and approached the theoretical CO_2 limit of 7400 mg. It was concluded that after 250 days of microcosm operation, a reasonable degree of PAH acclimation was achieved in the microcosm soil reactor.

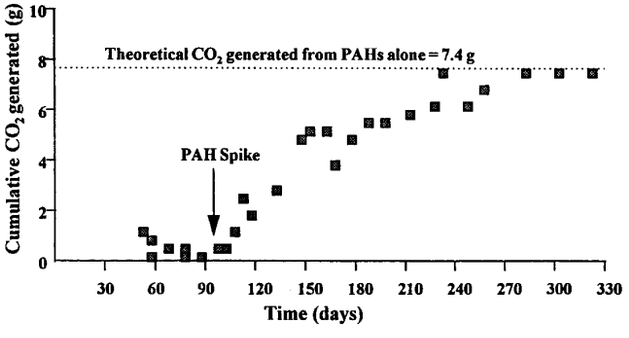

Figure 5 Cumulative carbon dioxide generation from the soil microcosm reactor spiked with a mixture of PAHs.

Adsorption and desorption of phenols

The Freundlich isotherm adsorption parameters (Ka and 1/n) and desorption parameters (Kd and 1/n) are listed in Table 1. The phenol adsorption equilibrium isotherm was highly non-linear compared to the desorption equilibrium isotherm and the extent of adsorption was significantly higher than the desorption extent. This suggested that significant amounts of phenol remained irreversibly bound to the soil matrix. Further, there was no hysteresis effect, ie, the adsorption and desorption isotherms are nearly identical. This demonstrates that there was no degradation of phenol during the adsorption/desorption study.

The diffusion coefficients for all five compounds, calculated from the adsorption data are also listed in Table 1. Diffusivities in water, calculated from correlations, are included for comparison. Diffusivities in soil are much lower (three orders of magnitude) than diffusivities in water, indicating that diffusion in soil pores is retarded due to interaction with soil organic carbon. However, compounds which have high diffusivity in water also exhibit high diffusivity in soil. Similar results were found for desorption, as shown in Table 1. However, soil diffusivities were lower for desorption when compared to adsorption.

The liquid-phase mass transfer coefficient was calculated using the standard empirical correlation equation [6]. The mass transfer coefficient was also derived from the experimental adsorption and desorption data, using non-linear regression techniques. The experimentally determined values are close to the theoretical values, as shown in Table 1. This shows that the theoretical mass transfer coefficient equation can be used to estimate the liquid-phase mass transfer coefficient in soil slurry systems.

The kinetics of adsorption/desorption strongly depend on the isotherm parameters and soil diffusivity. The soil diffusivities reported in this paper can be used to estimate the adsorption/desorption rates in soil slurry systems.

Adsorption and desorption of PAHs

The extent of partitioning of PAHs tested depends on the octanol-water partitioning coefficient for the compound and its diffusivity in water. Table 2 shows the diffusion adsorption parameters for several PAHs. Diffusivities in water, estimated for each compound, are also listed for comparison. Diffusivities in soil are three orders of magnitude lower than diffusivity in water, indicating that diffusion in soil pores is retarded due to interaction with soil organic carbon. Desorption rates are much slower than adsorption rates, and equilibrium was attained in about 60 h. Table 2 also lists the diffusion desorption parameters estimated from experimental data. Soil diffusivities for desorption are lower than for adsorption. The experimentally determined best-fit value of the mass transfer coefficient is close to

Table 1 Transport parameters for adsorption and desorption of phenols

Transport parameter	Adsorption					Desorption				
	Phenol	2,4 Dimethyl phenol	Catechol	Resorcinol	*p*-Cresol	Phenol	2,4 Dimethyl phenol	Catechol	Resorcinol	*p*-Cresol
Average soil diffusivity $\times 10^5$ ($cm^2\ h^{-1}$)	4.75	3.01	3.66	4.17	3.52	4.14	2.07	3.26	3.04	2.84
(aqueous diffusivity)	(3329)	(2721)	(3203)	(3203)	(2980)					
Freundlich isotherm										
$K \times 10^3$	10.50	15.14	6.55	12.37	9.01	12.60	8.04	0.19	10.02	5.98
1/n	0.84	0.768	0.945	0.825	0.945	0.774	0.968	1.872	0.674	0.894
Experimental mass transfer coeff ($cm\ h^{-1}$)	10.25	7.55	10.11	9.89	9.93	11.55	6.41	10.92	10.88	7.62
(calculated value [6])	(9.06)	(7.93)	(8.83)	(8.83)	(8.42)					

Table 2 Transport parameters for adsorption and desorption of PAHs

Transport parameter	Adsorption				Desorption			
	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	Naphthalene	Acenaphthene	Fluorene	Phenanthrene
Average soil diffusivity $\times 10^5$ ($cm^2\ h^{-1}$)	6.22	2.79	2.87	1.67	5.96	2.06	1.88	1.49
(aqueous diffusivity)	(2576)	(2398)	(2289)	(2113)				
Freundlich isotherm								
$K \times 10^3$	4.6	10.1	14.1	24.9	4.6	10.2	8.9	15.4
1/n	0.90	1.01	0.92	0.97	0.84	1.01	0.82	0.67
Experimental mass transfer coeff ($cm\ h^{-1}$)	7.99	6.56	6.77	6.35	6.78	6.59	5.46	5.36
(calculated value [6])	(7.63)	(7.28)	(7.06)	(6.70)				

the calculated value [6]. This shows that the theoretically calculated mass transfer coefficient is reasonable.

The liquid concentrations for all PAHs did not vary more than 5% after 20 h, which indicated that equilibrium was achieved. Since the concentration of the compound did not change after 48 h even though the experiments were conducted for 96 h, there was no biological degradation of the compounds under the experimental conditions.

Comparing the adsorption/desorption equilibrium time (approximately 20 h) with biodegradation acclimation time (42 h for 100 mg L^{-1} initial concentration of naphthalene), it is clear that adsorption/desorption equilibrium was achieved much before the onset of biodegradation in soil slurry reactors.

Studies involving phenol respirometry

Figures 6 and 7 show the oxygen uptake data for the slurry, wafer and porous tube reactors when the actual amount of phenol was 25 mg and 12.5 mg phenol in each reactor system. Clearly, the oxygen uptake curve for the slurry reactor reaches a higher plateau than the curves for the soil wafer and porous tube reactors, indicating that more phenol was being degraded in the slurry reactor. Furthermore, the data from the slurry reactor attain a plateau value faster than the wafer and porous tube oxygen uptake data. This shows that biodegradation rates in soil slurry reactors are the highest since there are no limitations of oxygen, nutrients or water

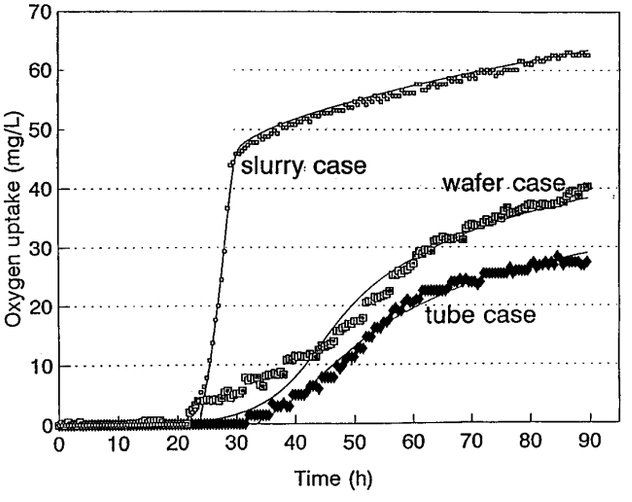

Figure 6 Cumulative oxygen uptake and model fits for slurry, wafer and porous tube reactors at 25 mg of phenol added to each reactor. □, ⊡, ◆, Experimental; ———, model.

and biodegradation occurs both in the liquid phase by the suspended microorganisms and by biofilms immobilized on soil particles. In the soil wafer reactor, there was no oxygen limitation and the biodegradation rate was governed by contaminant desorption and subsequent degradation in the

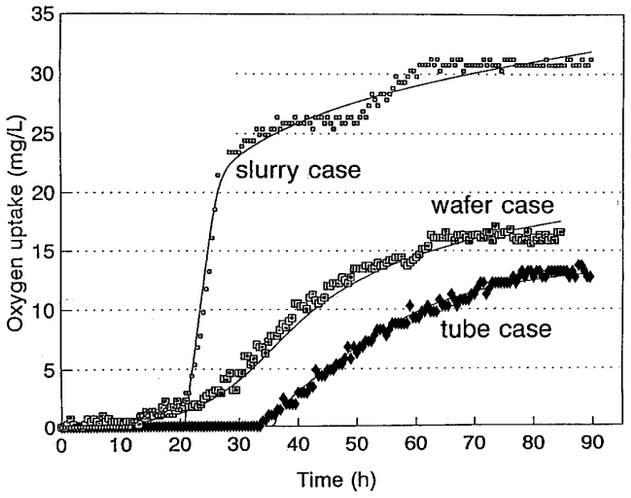

Figure 7 Cumulative oxygen uptake and model fits for slurry, wafer and porous tube reactors at 12.5 mg of phenol added to each reactor. □, ⊡, ◆, Experimental; ———, model.

free and bound water phase by microorganisms immobilized in biofilms. Since the water content of the soil in the wafer reactor was significantly less than in the slurry reactor, the biodegradation rate was also lower than in the slurry reactor. In the porous tube reactor, in addition to the limited water content in the soil, as in the case of the soil wafer reactor, oxygen diffusion in the soil matrix was also limited. Limited oxygen diffusion in the soil matrix caused little phenol biodegradation in the outer region of the tube while phenol present in the interior of the tube did not biodegrade due to unavailability of oxygen.

Data from the bioreactor demonstrate that *in situ* bioremediation rates are significantly lower than biodegradation rates achievable in soil slurry reactors due to limited water content and oxygen diffusivity. Furthermore, nutrient limitations may further limit bioremediation rates in contaminated soils. While bioventing approaches may maximize availability of oxygen, delivery of water and nutrients are still major limitations for maximizing *in situ* bioremediation rates.

Detailed mathematical models were developed for analyzing oxygen uptake data from the soil slurry, wafer and porous tube reactors [6]. In the soil slurry reactor, significant degradation of contaminant occurs in the aqueous phase by the suspended soil microorganisms rather than by the cells immobilized in biofilms. Biodegradation rates in soil wafer and porous tube reactors increase linearly with contaminant concentrations and active microbiota concentration. Eighty-one per cent of the phenol added initially was biodegraded in the soil wafer reactor and 64% was degraded in the porous tube reactor. As shown in Figures 6 and 7, the mathematical model fitted the experimental data quite well. The soil slurry reactor data were used to derive the biokinetic parameters for the suspended and immobilized microorganisms. These parameters when used with the appropriate amount of free water, were used to fit the wafer reactor data. The wafer reactor data were used to obtain additional information with no oxygen limitations and the porous tube reactor data provided quantitative estimation of oxygen diffusivity in the soil matrix. The porous tube reactor data were used to derive the oxygen diffusivity in the soil phase.

Table 3 summarizes mean values for the transport, diffusivity and biokinetic parameters determined from the cumulative oxygen uptake and CO_2 evolution data of the soil slurry, soil wafer and soil porous tube reactor systems. These parameters are intrinsic for a specific soil and contaminant, and will vary with soil type, contaminant and bioreactor conditions.

The oxygen profile in the porous tube soil using the model developed for tube reactor showed that the radial oxygen concentration decreased rapidly, attaining a zero value at a radial distance of 0.25 R from the tube center, where R is the radius of the porous tube. This confirmed that there were oxygen limitations in the porous tube reactor.

In the soil slurry reactor there were no limitations of oxygen, which freely diffused into the well-stirred slurry. Hence, the biodegradation rate in soil slurry reactors depended on the intrinsic biokinetic rate, microorganism concentration in the soil matrix and inherent diffusivity of the contaminant. In the soil wafer reactor, oxygen diffused freely through the thin soil matrix, and hence the biodegradation rate was controlled by the water content in addition to other intrinsic parameters, as in the case of the soil slurry reactor. In the porous tube reactor, the biodegradation rate was controlled by the water content in addition to other intrinsic parameters, as in the case of the soil slurry reactor. In the porous tube reactor, the biodegradation rate was controlled by the water content and oxygen diffusivity and other intrinsic biokinetic parameters. The porous tube reactor provided a better estimate of biodegradation rates for *in situ* bioremediation than the soil wafer and soil slurry reactors.

Oxygen uptake data for microporous tube experiments were run with the regular 40-Å pore diameter tubes, as well as with similar tubes with pores 3200 Å in diameter. The results indicate that no appreciable difference was seen in the data gained using the different tubes. This confirmed the earlier assumption that the 40-Å pore size tubes would not limit oxygen uptake and could be used for subsequent experimentation.

Experiments with uniformly labeled ^{14}C phenol and measurement of CO_2 evolution [11] showed that the net oxygen uptake (actual uptake minus the oxygen uptake in the control flask) was solely due to phenol degradation. This verified the initial assumption that the net cumulative oxygen uptake in each type of soil reactor could be used to derive the biokinetic and transport parameters.

Respirometry of PAHs

Figure 8 shows the cumulative oxygen uptake curve for naphthalene in the soil slurry reactor with 25 g of contaminated soil. The cumulative oxygen uptake curve attained a plateau after about 400 h. The corresponding curve, obtained using uncontaminated soil, is also shown in this figure. The acclimation time, not shown in the figure, was 52 h. The model fits, obtained using the best-fit parameter values and the model equations [6] are also shown. The cumulative oxygen uptake in the control reactor was mainly

Table 3 Summary of biokinetic parameters for phenol in soil slurry, wafer and porous tube reactors

Biokinetic parameter/type of bioreactor	Soil slurry	Soil wafer	Porous tube
Maximum specific growth rate in soil phase (L h^{-1})	0.294	same as slurry	same as slurry
Half velocity constant for soil phase (mg L^{-1})	2.12	44.8	same as wafer
Maximum specific growth rate in aqueous phase (L h^{-1})	0.228	same as slurry	same as slurry
Half velocity constant for aqueous phase (mg L^{-1})	1.99	30.9	same as wafer
Biomass yield	0.342	same as slurry	same as slurry
Maximum specific growth rate for oxygen in soil phase (mg L^{-1})	–	–	0.404
Half velocity constant for oxygen in soil phase (mg L^{-1})	–	–	0.749
Maximum specific growth rate for oxygen in aqueous phase (mg L^{-1})	–	–	0.477
Half velocity constant for oxygen in aqueous phase (mg L^{-1})	–	–	0.473
Fractional amount of phenol biodegraded	1.0	0.81	0.64

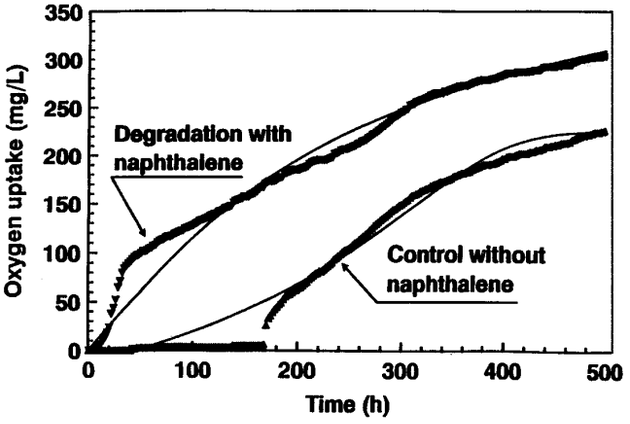

Figure 8 Cumulative oxygen uptake in naphthalene-contaminated soil slurry and control slurry reactor. ▲, ▼, Experimental data; ———, model.

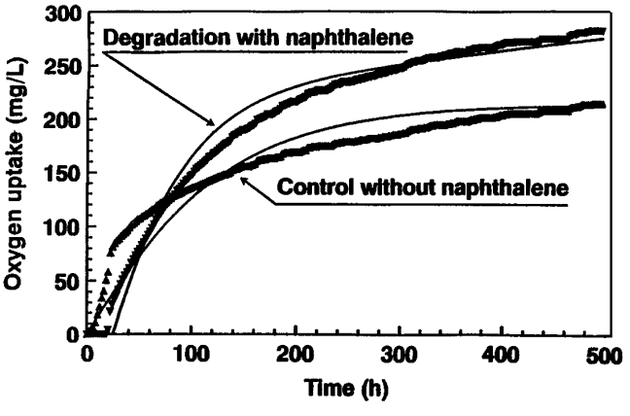

Figure 9 Cumulative oxygen uptake in naphthalene-contaminated soil wafer and control wafer reactor. ▲, ▼, Experimental data; ———, model.

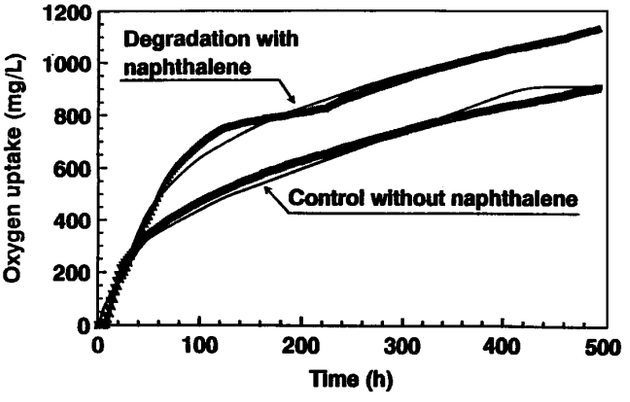

Figure 10 Cumulative oxygen uptake in napthalene-contaminated soil column and control column reactor. ▲, ▼, Experimental data; ———, model.

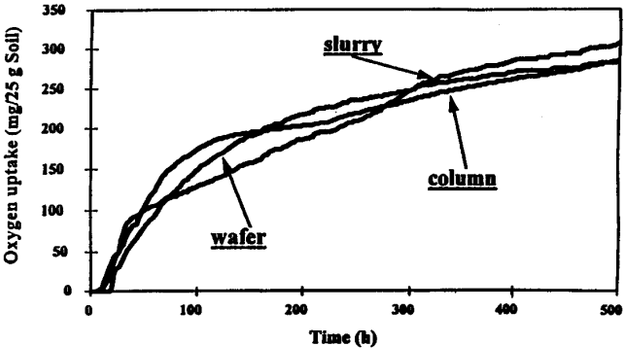

Figure 11 Net cumulative oxygen uptake for soil slurry, wafer and column reactors.

due to degradation of organic matter. Figure 9 shows the cumulative oxygen uptake data and model fit for naphthalene in the soil wafer reactor. Figure 10 shows the cumulative oxygen uptake and model fit for naphthalene in the soil column reactor. The net cumulative oxygen uptake for the soil slurry, wafer and column reactors, are shown in Figure 11.

Cumulative oxygen uptake in the control reactors occurred primarily due to biodegradation of soil organic matter. The experimental data from the control reactors provides insight on organic matter degradation, which also occurred in the actual reactors when naphthalene was present. The best-fit parameter values for naphthalene in the soil slurry, wafer and column reactors are given in Table 4. These values were used to fit the experimental oxygen uptake data. The final values are close to the initial estimates. The best-fit biokinetic parameters can be used to predict the attainable end-points in soil slurry and compacted soil treatment systems.

All three reactors produced comparable cumulative oxygen uptakes. This was mainly due to the fact that naphthalene partitioned mainly in soil organic matter, being a

Table 4 Summary of biokinetic parameters for naphthalene in soil slurry, wafer and column reactors

Biokinetic parameter/type of bioreactor	Soil slurry	Soil wafer	Column
Maximum specific growth rate in soil phase (L h^{-1})	0.298	same as slurry	same as slurry
Half velocity constant for soil phase (mg L^{-1})	12.53	13.7	same as wafer
Maximum specific growth rate in aqueous phase (L h^{-1})	0.274	same as slurry	same as slurry
Half velocity constant for aqueous phase (mg L^{-1})	23.9	155.7	same as wafer
Biomass yield	0.62	same as slurry	same as slurry
Maximum specific growth rate for oxygen in soil phase (mg L^{-1})	–	–	0.923
Half velocity constant for oxygen in soil phase (mg L^{-1})	–	–	41.1
Maximum specific growth rate for oxygen in aqueous phase (mg L^{-1})	–	–	0.746
Half velocity constant for oxygen in aqueous phase (mg L^{-1})	–	–	45.4

strongly hydrophobic compound, and biodegradation of naphthalene occurred mainly in the soil phase. The aqueous concentration of naphthalene was so small that the contribution of biodegradation in the aqueous phase was negligible. This was not true for phenolic compounds, in which case the three soil reactors exhibited varying amounts of oxygen uptake.

Analysis of the oxygen uptake data with adsorption/desorption kinetics showed that both adsorption and desorption attained equilibrium in less than 20 h, while biodegradation usually involved acclimation times exceeding 20 h. Hence, incorporating adsorption/desorption kinetics with cumulative oxygen uptake was equivalent to assuming adsorption/desorption equilibria during the biodegradation phase.

Studies with uniformly labeled ^{14}C PAHs (concentration 1 μCi per flask) showed that over 95% of the CO_2 evolved was produced due to PAH mineralization and less than 5% of total CO_2 was produced by mineralization of soil organic matter [20]. This showed that measurement of CO_2 evolution in spiked soil slurry reactors can be used to quantify the rate of contaminant mineralization.

Using the best-fit transport and kinetic parameters, the model equations were used to simulate the degradation of naphthalene in soil slurry, wafer and column reactors. The objective of this simulation was to obtain the attainable treatment endpoints. Soil slurry reactor represents *ex situ* soil treatment in a biological slurry reactor. Soil wafer represents soil treatment using land farming or bioventing, wherein oxygen diffusion in the soil matrix is sufficient and hence does not control the rate of bioremediation. Soil column reactor represents *in situ* soil treatment, wherein oxygen diffusion has a major impact on the overall bioremediation rate. The initial concentration of naphthalene in soil was assumed to be 100 mg kg^{-1}. Figure 12 shows the change in soil concentration versus time for each type of soil reactor. In the soil slurry reactor, the soil naphthalene concentration decreased rapidly and after 1000 h of treatment time, a very low naphthalene concentration remained in the soil. Hence, the final treatment endpoint attained in the case of a soil slurry reactor was very small. In the case of the soil wafer reactor, the soil naphthalene concentration decreased at a slower rate and after 1000 h of treatment time, about 80% of naphthalene had degraded. In the soil column reactor, a typical 'hockey-stick' curve was obtained, wherein there was a rapid decrease in naphthalene concentration initially, followed by a significantly lower rate of biodegradation. This is mainly attributed to slower oxygen diffusion in the column reactor. After 1000 h of treatment in the soil column reactor, about 20% of the initial naphthalene had biodegraded and the eventual endpoint was about 80 mg kg^{-1}.

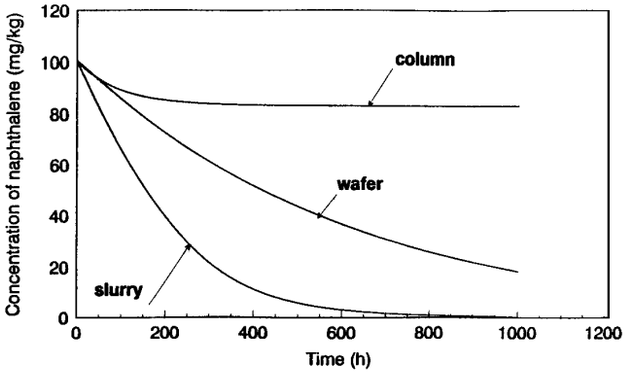

Figure 12 Change in soil concentration of naphthalene versus time during biotreatment in soil slurry, wafer and column reactors, as calculated by computer simulation.

These results clearly show that the attainable treatment endpoints depend strongly on the type of soil, contaminant and type of treatment. Treatment reactors which incorporate efficient transport of contaminant and oxygen achieve lower treatment endpoints than reactors wherein oxygen diffusion is rate controlling. Further it should be emphasized that in our model simulations of soil slurry reactor, there was efficient mixing and hence high transport rates of oxygen and contaminant were obtained. In actual soil slurry reactors, depending on the rate of mixing and design of impellers, mass transfer from the soil particles to the aqueous phase may become the rate controlling step. In the case of the soil wafer reactor, it was assumed that oxygen diffuses completely through the thin soil layer. However, in land farming, soil particles form larger aggregates, and oxygen diffusion through these larger aggregates may control the rate of bioremediation. Frequent turning of the soil as in land farming, may not break the soil aggregates into thin soil layers, as used in the soil wafer reactor. Hence, there is no universal treatment endpoint, and the endpoint depends on the treatment reactor design.

Conclusions

A three-step experimental protocol for determining important kinetics parameters for the *in situ* biodegradation of toxic chemicals in soils was developed using phenol and naphthalene as the test compounds. The protocol was developed so that the experimental schemes used grew in complexity toward the actual *in situ* case, but remained simple enough to allow them to be adequately modeled. The data gained for each of the schemes agreed with expectations. In the case of phenol, both the rate and extent of biodegradation decreased with the increase in the complexity of the soil systems in the experimental schemes. The amount of phenol degraded in the wafer reaction was less than in the slurry reactor primarily due to lower water content and mass transfer rates, since phenol did not partition significantly into the soil phase. In the case of the porous tube reactor, the amount of phenol degraded was even lower primarily due to oxygen diffusion limitations. In the case of naphthalene, all three reactors produced comparable cumulative oxygen uptakes. This was mainly due to the fact that naphthalene partitioned mainly into soil organic matter, being a strongly hydrophobic compound, and biodegradation of napthalene occurred mainly in the soil phase. The aqueous concentration of naphthalene was so small that the contribution of biodegradation in the aqueous phase was negligible. Modeling procedures applied to the three experimental schemes proved useful for determining biokinetic parameters for degradation of phenol. Model predictions agreed very well with experimental data. Further, the model parameters were useful in simulating treatment endpoints for these types of soil reactors. The application of this protocol to other chemicals is feasible with only minor alterations in methodology.

References

1 Black CA (ed). 1961. Methods of Soil Analysis. American Society of Agronomy, Madison.
2 Bollag J-M. 1974. Microbial transformation of pesticides. Adv Appl Microbiol 18: 75–130.
3 Brunauer S, RS Mikhail and EE Bodor. 1967. Pore structure analysis without a pore shape model. J Colloid Interface Sci 24: 451–463.
4 Dec J and J-M Bollag. 1990. Detoxification of substituted phenols by oxidoreductive enzymes through polymerization reactions. Arch Environ Contam Toxicol 19: 543–550.
5 Dhawan S, LT Fan, LE Erickson and P Tuitemwong. 1991. Modeling, analysis and simulation of bioremediation of soil aggregates. Environ Prog 10: 251–260.
6 Fu C, S Pfanstiel, C Gao, X Yan, R Govind and HH Tabak. 1996. Studies on contaminant biodegradation in slurry, wafer and compacted soil tube reactors. Environ Sci Technol 30: 743–751.
7 Ghoshal S and R Luthy. Bioavailability of hydrophobic organic compounds from nonaqueous-phase liquids: the biodegradation of naphthalene from coal tar. Environ Tox Chem 15: 1894–1900.
8 Govind R, C Gao L Lai, X Yan, S Pfanstiel and HH Tabak. 1993. Development of methodology for the determination of bioavailability and biodegradation kinetics of toxic organic pollutant compounds in soil. Paper presented at the In-Situ and On-Site Bioreclamation, 2nd International Symposium, San Diego, CA, April 5–8, 1993.
9 Graves DA, CA Lang and ME Leavitt. 1991. Respirometric analysis of the biodegradation of organic contaminants in soil and water. Appl Biochem Biotechnol 28/29: 813–826.
10 Huang T, Y Shan, M Kupferle, Q Zhao, H Zhu, GD Sayles and CM Acheson. 1996. Extracting PAHs from soil using a simple, effective, low cost shaking method. Draft paper obtained from CM Acheson, NRMRL, US EPA Cincinnati, Cincinnati, OH 45268.
11 Kessler MJ. 1989. Liquid Scintillation Analysis. Science and Technology. pp 3.25–3.33, Packard Instrument Company, Meridan, CT.
12 Khan KA, R Krishnan, TF O'Gara, C Missilian, GD Runnells and PE Flathman. 1990. Soil bioremediation treatability studies. Proceedings of 83rd Annual Air and Waste Management Association Meeting and Exhibition, p 14, Pittsburgh, PA, June 24–29, 1990.
13 Leenheer JA, TI Noyes and HA Stuber. 1982. Determination of polar organic solutes in oil-shale water. Environ Sci Technol 16: 714–723.
14 Madsen EL, CL Mann and SE Bilotta. 1996. Oxygen limitations and aging as explanations for the field-persistence of naphthalene in coal tar-contaminated surface sediments. Environ Tox Chem 15: 1876–1882.
15 McDonald JP, C Baldwin and LE Erickson. 1991. Rate limiting factors for *in situ* bioremediation of soils contaminated with hydrocarbons. Paper presented at the Fourth International IGT Symposium on Gas, Oil, and Environmental Biotechnology, Colorado Springs, CO.
16 OECD. 1981. OECD Guidelines for Testing of Chemicals. Section 3, Degradation and Accumulation, Method 301C, Ready Biodegradability: Modified MITI Test (I) adopted May 12, 1981 and Method 302C Inherent Biodegradability: Modified MITI Test (II) adopted May 12, 1981, Director of Information, Organization for Economic Cooperation and Development, Paris, France.
17 Pignatello JT. 1989. Sorption dynamics of organic compounds in soils and sediments. In: Reactions and Movement of Organic Chemicals in Soils (RL Sawhney and K Brown, eds), pp 45–80, Spec Publ No 22, Soil Sci Soc Am, Madison, Wisconsin.
18 Qiu X and MJ McFarland. 1991. Bound residue formation in PAH contaminated soil composting using *Phanerochaete chrysosporium*. Hazard Waste and Hazard Materials 8: 115–126.
19 Scow KM, S Simpkins and M Alexander. 1986. Kinetics of mineralization of organic compounds at low concentration in soil. Appl Environ Microbiol 51: 1028.
20 Speitel GE and FA Digiano. 1971. Determination of microbial kinetic coefficients through measurement of initial rates by radiochemical techniques. Water Res 22: 829–835.
21 Stuemer DH, DJ Ng and CJ Morris. 1982. Organic constituents in ground water near an underground coal gasification site in northeastern Wyoming. Environ Sci Technol 16: 582–587.
22 Test Methods for Evaluating Solid Waste. SW-846: Method 8100, 3rd Edition. 1990. USEPA Office of Solid Waste and Emergency Response, Washington, DC.
23 White J and M Alexander. 1996. Reduced biodegradability of desorption-resistant fractions of polycyclic aromatic hydrocarbons in soil and aquifer solids. Environ Tox Chem 15: 1973–1978.