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DEVELOPMENT OF A MULTI-LEVEL PROTOCOL FOR DETERMINATION OF BIODEGRADATION KINETICS AND BIOAVAILABILITY OF ORGANIC COMPOUNDS TO ENHANCE IN-SITU BIOREMEDIATION

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INTRODUCTION

Knowledge of biodegradation kinetics in soil is needed to understand the efficacy of *in-situ* and *ex-situ* bioremediation technologies. Laboratory studies to determine biodegradation rates can be used as screening tests to determine the rate and extent of bioremediation that might be attained during remediation, and to provide design criteria. (Graves et al, 1991; (1) Tabak et al, 1992; (2) Govind et al, 1993; (3) Tabak et al, 1994, 1995) (4,5) Traditionally, *in-situ* biodegradation kinetics have been determined using soil microcosms (6,7) which are difficult to model mathematically. Laboratory studies involving soil slurry reactors have been reported by Bachmann et al. (8) Kaplan and Kaplan (9), Milhelcic and Luthy (10) and Brunner et al. (11). Currently there is no systematic methodology to quantitatively determine biodegradation kinetics of contaminants in compacted soil systems.

Biodegradation in soil is a fairly complex process which involves diffusion of contaminants in the porous soil matrix, adsorption to the soil surface, biodegradation in the biofilms existing on the soil particle surface and in the large pores as well as in the bound and free water phase after desorption from the soil surface. In soil slurry reactors, biodegradation of contaminant occurs both in the liquid phase by soil microorganisms desorbed from the soil matrix and by the biofilms immobilized on the soil particle surface. In compacted soil systems, biodegradation occurs in the free and bound water phase primarily by the soil immobilized microorganisms and the contribution of water suspended microbiota is small due to low water content. In this paper a systematic protocol based on three types of bioreactors and one representative contaminant, phenol, has been developed to determine the biokinetic parameters of the suspended and immobilized microbiota and the transport parameters of contaminant and oxygen in the soil matrix.

EXPERIMENTAL PROCEDURES AND MATERIALS

In this study, three types of bioreactors, shown in Figure 1, to determine the biokinetic parameters of the suspended and immobilized microbiota were: (1) slurry bioreactor, where soil at 5% slurry concentration is vigorously mixed with the contaminant, dissolved in water with nutrients; (2) wafer reactor, where a thin wafer of soil is spiked with contaminant and nutrients dissolved in water, to obtain a 50% total soil moisture content; and (3) porous glass tube reactor, where sieved soil with contaminant is packed in a porous glass tube with moisture content identical to the wafer reactor.

In the soil slurry reactor there are no limitations of oxygen, which freely diffuses into the well-stirred slurry, nutrients, which are initially dissolved in the aqueous phase and water. Hence, the biodegradation rate in soil slurry reactors depends on the intrinsic biokinetic rate, microorganism concentration in the soil matrix and inherent diffusivity of the contaminant. In the soil wafer reactor, oxygen diffuses freely through the thin soil matrix, and hence biodegradation rate is controlled by the water content in addition to the other intrinsic parameters, as in

the case of the soil slurry reactor. In the porous tube reactor, biodegradation rate is controlled by the water content and oxygen diffusivity and other intrinsic biokinetic parameters. The porous tube reactor provides a better estimate of biodegradation rates for *in-situ* bioremediation than the soil wafer and soil slurry reactors.

Biodegradation rates of phenol in the three soil reactor systems were determined by electrolytic respirometry (Voith Respirometer) used to continuously measure the cumulative oxygen uptake and to simultaneously monitor the carbon dioxide evolution. Appropriate concentrations of phenol stock solution in distilled deionized (DD) water were added to the reactor system containing Organization for Economic Co-operation and Development (OECD) nutrient solution composed of inorganic salts, trace salts, vitamin solution and/or yeast extract solution and different soil concentrations. Stock solution containing OECD nutrients with no phenol were used for control reactor flasks. In addition, radiolabelled contaminant was used to confirm the oxygen uptake from the contaminant rather than due to normal soil respiration.

The soil was characterized to determine soil moisture content, clay, sand and silt content, percent organic matter, soil pH, cation exchange capacity, bulk density, and nutrients in soil. The soil selected for the study was uncontaminated silt loam soil with the following characteristics: soil moisture 17%, organic matter 2.9%, 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. Table 1 summarizes the soil characterization data. The Brunauer, Emmett and Teller (BET) specific surface area was 20.27 m^2/g, BET void volume was 01029 cm^3/g and the BET average pore diameter was 58Å. The soil porosity was 6.53%. Soil particle size distribution was determined using outline by Day (1965) (12). Soil porosity, pore size distribution, pore volume and surface area was determined by nitrogen adsorption using Micrometrics ASAP 200.

Abiotic adsorption and desorption kinetics of the contaminant into the soil matrix and oxygen uptake data obtained for the soil slurry, soil wafer and porous tube reactors are used in conjunction with detailed mathematical models to derive the intrinsic biokinetic and transport parameters. To determine the adsorption and desorption characteristics, the soil was air dried and passed through 2 mm sieve. Abiotic adsorption and desorption equilibria were quantified using the Freundlich isotherm equation. A systematic protocol for conducting the abiotic adsorption and desorption kinetics was initially developed using soil slurry systems.

Soil Slurry Experiments

To simulate biodegradation of substrate in soil slurry bioreactors (1) 20 g of soil was mixed with 250 mL of DD water containing OECD nutrients and phenol at different concentration levels; (2) 10 mL of KOH solution (pH 9.5) was injected in carbon dioxide trap; (3) Reactor vessel was closed and cumulative continuous oxygen uptake measurements were made using respirometer. Reactor vessels were stirred continuously with teflon coated stirring bars. (4) KOH solution was withdrawn from CO_2 trap and the amount of CO_2 evolved, was quantified by measuring the change in pH of KOH solutions. (5) In radiorespirometric studies both gaseous and aqueous 14 CO_2 and radiolabelled intermediates were analyzed by Packard scintillation counting apparatus.

Soil Wafer Experiments

To more closely simulate the actual biodegradation of phenol in intact soils, soil wafer studies were developed for respirometer experimentation. The wafer system consisted of a thin layer (or wafer) of soil with a known moisture content. Procedures for the wafer experiments were as follows: (1) 20.0 g of soil and measured amount of water, approximately 20 to 30 mL in volume were placed in reaction flask, well mixed to give uniform initial biomass concentration, and the entire flask was weighed; (2) the flask(s) were placed in fume hood overnight and re-weighed. The weighing procedure was repeated until weight of flask indicated that the soil contained the desired moisture content; (3) the soil wafer was contaminated with 2.5 to 10.0 mL of experimental stock solution, depending on the desired concentration, using a syringe. Steps 3 thru 5 for the slurry experiments were followed without stirring the soil wafer.

Microporous Tube Experiments

Microporous tube reactors were developed for use in electrolytic respirometer to study the effects of oxygen diffusivity. The porous glass tubes used were purchased from Corning (Corning, NY) and were made from vycor glass with pores averaging 40 Angstroms in diameter. This pore size was selected because it was found to be optimum for containing the soil and water within the tube and allow free flow of oxygen into the soil. The porous glass tube pore sizes exceeding 40 A° were found not to effect the oxygen uptake results. The experimental procedure was as follows: (1) the microporous glass tubes were placed in the laboratory oven set at 200°C and left overnight to evaporate any water or contaminant in the pores or on the surface of the tubes; (2) the tubes were removed from oven and allowed to cool for several hours to room temperature. The tubes were placed in a beaker containing DD water to initially saturate the pores in the glass; (3) the tubes were removed from the water and filled with 20.0 g of soil, plugging both ends with glass wool; (4) the soil was contaminated evenly in the tube with 1.25 to 5.0 mL of experimental stock solution by inserting a syringe through the plugs at either end and mixing the soil around with the syringe as much as possible while injecting the solution; (5) two tubes were placed into each flask and procedures according to steps 3 through 5 for the slurry experiments were followed.

Adsorption and Desorption Experiments

For adsorption experiments, 50 g soil sample were placed in a 250 mL glass bottle. Various concentrations of each compound, namely 10, 25, 50, 100 and 150 mg/L were used. The volumes of the total solution added to each bottle were 250 mL in order to maintain a minimum head space. The head space was important, as it not only affected the degree of mixing during stirring but also controlled loss of phenol due to volatilization. Blanks containing only the phenol solution allowed the measurement of volatilization losses. In order to prevent phenol degradation in solution, 1 mL $HgCl_2$ saturated solution was added to each bottle. All adsorption experiments were conducted in a fume hood, so that the temperature of adsorption could be controlled at 24°C.

The mixture in the bottle was stirred using a magnetic stirrer for 96 hours. The samples at 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours from the beginning of the adsorption experiment were filtered using 0.45 μm silver membrane and placed in a 50 mL sample vial for extraction. The extraction method was based on U.S. EPA Method 604 & 610.

The GC used was a HP-5890A model equipped with a flame ionization detector. The following conditions were used throughout this study; initial oven temperature 60°C, initial hold time 5 minutes oven temperature rate 8°C/min., final oven temperature 280°C, final hold time 5 minutes, injector temperature 225°C, detector temperature 310°C, makeup gas (nitrogen) flowrate, 35 mL/min, detector gas flowrate 32 mL/min. hydrogen and 435 mL/min. air, carrier gas (helium) 2 mL/min., column HP-5 methyl silicon gum and 5m x 1.53mm x 2,54mm film thickness, and software: HP Chemstation.

After adsorption equilibrium was attained, the solution in a 250 mL glass bottle was mixed with 250 mL distilled deionized water in a large 500 mL glass bottle to start the desorption experiment. All the conditions for the desorption experiment were the same as for adsorption experiment. Samples for quantifying desorption were treated the same way as for adsorption studies, although the time taken to achieve desorption equilibrium was significantly longer.

RESULTS AND DISCUSSION

The Freundlich isotherm adsorption parameters (Ka and 1/n) and desorption parameters (Kd and 1/n) as well as the values for porosity of adsorbent (ϵ) and density of adsorbent (ρ) are listed in Table 2. 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.

Figure 2 shows the oxygen uptake data for the slurry, wafer and porous tube reactors for 100mg/L phenol concentration. 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 is being degraded in the slurry reactor. The soil wafer reactor degrades more phenol than the porous tube reactor. Furthermore, the slurry reactor data 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 and water and biodegradation occurs both in the liquid phase by the suspended microorganisms and by the soil immobilized biofilms. In the soil wafer reactor, there is no oxygen limitation and the biodegradation rate is governed by the contaminant desorption and subsequent degradation in the free and bound water phase by the soil immobilized biofilms. Since the water content of the soil in the wafer reactor is significantly less than in the slurry reactor, the biodegradation rate is 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 is also limited. This limits phenol degradation only in the outer region of the tube and phenol present in the interior of the tube does not biodegrade due to unavailability of oxygen.

This demonstrates, 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 soil sites. 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 the oxygen uptake data from the soil slurry, wafer and porous tube reactors. It was found that in the soil slurry reactor, significant degradation of contaminant occurs in the aqueous phase by the suspended soil microorganisms rather than by the soil immobilized biofilms. Biodegradation rates in soil wafer and porous tube reactors increase linearly with contaminant concentration and active microbiota concentration. 81% of the phenol added initially was biodegraded in the soil wafer reactor and 64% degraded in the porous tube reactor. As shown in Figure 2, 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 was used to derive the oxygen diffusivity in the soil phase.

Determining the oxygen profile in the porous tube soil using the model showed that the radial oxygen concentration decreases rapidly attaining a zero value at a radial distance of 0.25R from the tube center, where R is the radius of the porous tube. This again confirmed that there were oxygen limitations in the porous tube reactor due to limited oxygen diffusion in the soil matrix.

In the soil slurry reactor there are no limitations of oxygen, which freely diffuses into the well-stirred slurry. Hence, the biodegradation rate in soil slurry reactors depends on the intrinsic biokinetic rate, microorganism concentration in the soil matrix and inherent diffusivity of the contaminant. In the soil wafer reactor, oxygen diffuses freely through the thin soil matrix, and hence biodegradation rate is 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, biodegradation rate is 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, biodegradation rate is controlled by the water content and oxygen diffusivity and other intrinsic biokinetic parameters. The porous tube reactor provides a better estimate of biodegradation rates for *in-situ* bioremediation than the soil wafer and soil slurry reactors.

Experiments with uniformly labeled C^{14} phenol and measurement of carbon dioxide evolution by absorbing the KOH solution and scintillation counting showed that the net oxygen uptake (actual uptake minus the oxygen uptake in the control flask) was solely due to phenol degradation. This verified our initial assumption that the net

cumulative oxygen uptake in each type of soil reactor could be used to derive the biokinetic and transport parameters.

DESCRIPTION OF PROTOCOL

The overall protocol for evaluating soil biodegradation kinetics involves the following steps: (l) Measure cumulative oxygen uptake in soil slurry reactors. The slurry reactor model is used to obtain the aqueous and soil phase Monod biokinetic parameters. (2) Measure cumulative oxygen uptake in soil wafer reactor. Use the biokinetic parameters determined from the slurry model and detailed wafer model to determine diffusivity of contaminant in soil matrix. (3) Measure cumulative oxygen uptake in porous tube reactor. Use the parameters determined from soil slurry and wafer models to calculate oxygen diffusivity in the slurry reactor which was also the highest when compared to the other reactors. The soil slurry reactor oxygen consumption attains a plateau value in about 1 day, while the wafer and tube reactor's cumulative oxygen uptake attains a plateau value in about 3.5 days.

The oxygen uptake data for the soil slurry, wafer and tube reactors were analyzed using a detailed mathematical model to determine the best-fit values for the model parameters. The slurry reactor was used to determine the biokinetic parameters for the suspended and soil immobilized microbiota in soil from porous tube reactor model. (4) Measure radiolabelled carbon dioxide evolution using uniformly labeled compound in soil slurry, wafer and porous tube reactors to verify that oxygen uptake was due to compound mineralization. The biokinetic parameters, compound and oxygen diffusivity in soil determined using the protocol can be used to model kinetics of *in-situ* bioremediation.

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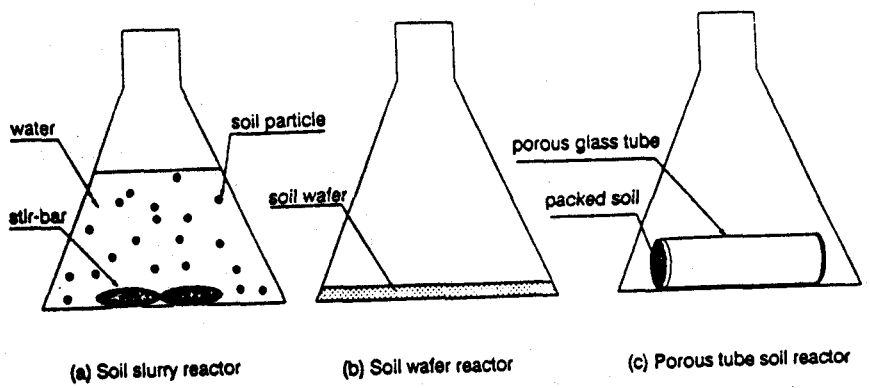

FIGURE 1 Schematic of Soil Slurry, Soil Wafer and Soil Porous Tube Respirometric Reactors

TABLE 1 Soil Characterization Data

Particle size		Soil pH	6.1
Clay (< 2 um)	24%		
Silt (2-44 um)	58%	Bulk density	1.06
Sand (> 44 um)	18%		
		1/3 bar moisture	30.7%
Classification	Silt Loam		
		15 bar moisture	14.8%
Organic Matter	2.9%		
		Nutrients in soil (ppm)	
Cation Exchange Capacity	6.5		
		Phosphorus	17 (h)
		Potassium	90 (m)
		Magnesium	80 (l)
		Calcium	1100 (m)
		Sodium	17 (v)

Note: h = high; m = medium; l = low; v = very low

TABLE 2 Adsorption/Desorption Soil Equilibrium and Soil Porosity and Density Parameters

K_s	1/n	K_d	1/n'	ϵ	ρ(g/L)	R(mm)
0.0105	0.84	0.01259	0.77*	0.06525	2250	0.334

* Only for the slurry model

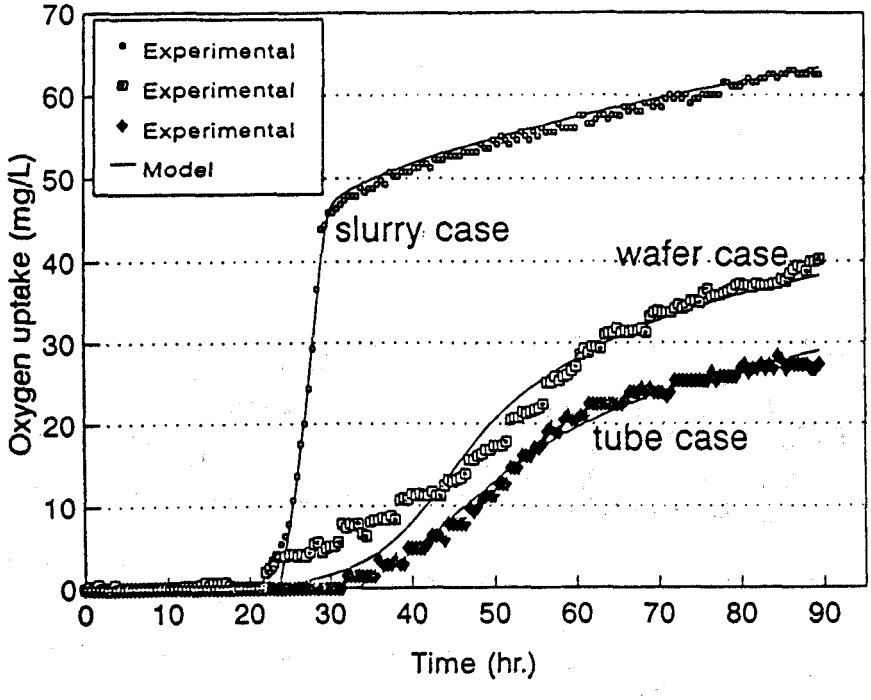

FIGURE 2 Oxygen Uptake Curves for Soil Slurry, Wafer and Porous Tube Reactors for Phenol. The line indicates the Fit by the Mathematical Model Developed for each Reactor Type

DESIGN AND TESTING OF AN EXPERIMENTAL IN-VESSEL COMPOSTING SYSTEM

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INTRODUCTION

Composting has received much attention as a potential technology for treating solid waste. Most of that attention has been focused on treatment of municipal solid waste, sewage sludge, yard trimmings, and agricultural wastes. More recently, composting has been investigated as a remediation technology for hazardous wastes. Laboratory and field-scale work has been conducted to determine the fate of pesticides, hydrocarbons, and explosives in the composting environment (Ziegenfuss *et al.*, 1991).

In the composting of non-hazardous materials, the objectives are to stabilize and oxidize organic materials, reduce the volume of waste, reduce the moisture content of waste, and destroy pathogens. Composting of hazardous waste includes the same objectives plus detoxification of hazardous substances into innocuous end-products.

Optimal application of biotechnology to large-scale compost systems is based on a working understanding of processes and mechanisms involved in composting of organic material. Currently, commercial compost operations are operated as black-box systems where optimization is largely achieved through trial and error. Large-scale treatment of hazardous waste will require optimal controls to meet the specified end points.

Some proponents of compost treatment have claimed significant success in destruction of hazardous wastes without strong data to support their claims. Disappearance of parent compounds has been used to claim that microorganisms successfully degraded waste chemicals. However, some toxic chemicals could potentially adsorb to, or react with, humic substances in the compost and become undetectable by chemical analysis. Such toxicants might later desorb from humus and migrate to the biosphere. This emphasizes the need for well controlled studies to rigorously document degradation rates and identify metabolic products of hazardous chemicals, metabolically active microbial species, and mechanisms of hazardous chemical transformation in compost systems.

We have designed and tested closed bench-scale compost reactors to evaluate composting processes using contaminated soils. Identification of suitable co-compost and bulking agents, appropriate ratios of soil to organic components and effective aeration strategies and rates have been selected as major factors requiring investigation.

This research program is designed to develop a thorough engineering analysis and optimization of composting as a process to treat soil contaminated with hazardous waste. Bench-scale composters serve as diagnostic tools to predict treatment effectiveness of larger systems. Fully enclosed, insulated reactors permit reliable data collection on microbial population dynamics and fate of toxic chemicals during soil composting.