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Development of a Differential Volume Reactor System for Soil Biodegradation Studies

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ABSTRACT

A bench scale experimental system was developed for the analysis of polycyclic aromatic hydrocarbon (PAH) degradation by mixed microbial cultures in PAH contaminated Manufactured Gas Plant (MGP) soils and on sand. The reactor system was chosen in order to provide a fundamental protocol capable for evaluating the performance of specific mixed microbial cultures on specific soil systems by elucidating the important system variables and their interactions. The reactor design and peripherals are described. A plug flow differential volume reactor (DVR) was used in order to remove performance effects related to reactor type, as opposed to system structure. This reactor system could be well represented mathematically. Methods were developed for on-line quantitative determination of PAH liquid phase concentrations. The mathematical models and experimental data are presented for the biodegradation of naphthalene on artificial and MGP soils.

Index Entries: Biodegradation kinetics; soil systems; reactor design; polycyclic aromatic hydrocarbons; gene probes; on-line detection.

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NOMENCLATURE

A_c	Cross sectional area of reactor m^2
$C_{naphthalene_c}$	Feed concentration of naphthalene moles/m^3
De	Dispersion Coefficient cm^2/s
$F_{naphthalene}$	Molar flow rate of naphthalene moles/h
k	reaction rate constant h^{-1}
U	Velocity meters/h
V	Volume of reactor m^3
X	Conversion dimensionless
z	Reactor length m
$E(\theta)$	Dimensionless Residence Time Distribution
θ	Dimensionless Time

INTRODUCTION

Protocols are needed for evaluating bioremediation technologies in soil systems contaminated with hazardous waste. Present remediation methods are expensive as a result of high energy costs. Treatment costs could be substantially reduced if reliable means were available for bioremediation. The complexities of soil systems with respect to ecological, physical, and microbial factors are substantial. The development of tools to evaluate the effects and applicability of bioremediation in soil is desirable.

Past production of a synthetic natural gas (town gas) from the pyrolysis of coal has produced several thousand sites nationwide that are contaminated with polycyclic aromatic hydrocarbons (PAH). These sites range in size from less than one acre to over 100 acres, with contamination at depths exceeding 15 ft. Much concern exists over these sites since many PAH compounds, such as benzo(a)pyrene, benzo(j)fluoranthene, benzo(b)fluoranthene, benzo(e)pyrene, and others, are believed to be carcinogenic (*1*). PAH compounds are relatively insoluble in water (*2*), and are quite stable in the environment (*3*). PAH compounds readily sorb to sediments, and therefore, pose a threat to groundwater (*4*). These xenobiotic compounds are difficult to degrade biologically, however, PAH degrading organisms have been isolated (*5*). A fundamental understanding of interactions occurring in a soil matrix is required when using microbial systems for remediation of contaminated sites.

Heterogeneous catalytic reactions take place in two or more phases. These reactions can be slowed by fluid diffusional mass transfer, adsorption onto the catalyst surface, or solid phase diffusional processes. In contaminated soil, the biodegradation of xenobiotic chemicals by microorganisms may be influenced by one or more of these processes. A hydrophobic compound, such as naphthalene, predominantly adsorbs onto the organic components of the soil (*6*). At equilibrium, the naphthalene will be preferentially partitioned throughout the solid matrix of the organic

matter, on the surface of the organic matter, and in the liquid and gas phases. The bioavailability of naphthalene and, hence, the biodegradation of naphthalene may be influenced by one or all of these processes. A plug flow reactor (PFR) is useful for providing fluid diffusional and sorption mass transfer information on heterogeneous reactions (*7*), such as microbial degradation, in soil systems. A differential element of a PFR will facilitate the evaluation of kinetics and sorption properties.

The objective of this work was the development and analysis of a differential volume reactor (DVR) system suitable for abiotic and microbial degradation studies. The reactor was characterized with respect to fluid dynamic characteristics and tested, using different soil types with naphthalene as the model compound for abiotic and biological studies. On-line analysis capabilities were developed to monitor feed and effluent PAH concentrations. Experiments with naphthalene degrading communities were performed to demonstrate the utility of the reactor design. This paper concerns the development and analysis of a DVR suitable for studying contaminated soil systems.

MATERIALS AND METHODS

A feed rate of 0.3 cc/min was used, since this approached fluid transport rates through sandy soils (*8*). A soil wafer thickness of 0.8 cm was used, since this allowed spatial analysis of bacterial populations and was the minimum thickness available. The DVR used in this work was not designed to represent a true differential element of a soil column, and actually operated as an integral volume reactor during some experiments. Two reactor variables were available for controlling the PAH conversion to the desired range, soil thickness, and flow rate. The soil wafer thickness was limited to 0.8–5.0 cm, and flow rate from 0.1 to 2.0 cc/min by pressure drop considerations.

An ideal PFR has radial mixing with the reactant stream flowing in a flat velocity profile through the reactor. Axial mixing caused by dispersion and diffusion in the experimental DVR caused departure from the ideal case. A residence time distribution (RTD) (*9*) was used to ascertain the departure from ideal behavior. The RTD was used to produce an effective dispersion coefficient that accounted not only for axial dispersion but also for diffusion effects (*10*). Performing a mass balance around a section of the soil bed in the DVR with appropriate mathematical treatment, produced a second order differential equation that could be solved for reactant concentration as a function of time (*11*).

Stripping Column

An on-line method was developed for monitoring PAH concentrations in the DVR effluent stream. A stripping column was designed and

optimized to remove the volatiles for gas chromatography analysis. A 0.635 cm outside diameter and 0.533 cm inside diameter tube packed with 3 mm glass beads was used for the analytical stripping column. A constant molar flow rate was assumed, since the naphthalene solution was dilute and the column operated isothermally. A Henry's Law Constant of 327.8 torr-L/gmole (*12*) was used to model naphthalene equilibrium partitioning between the liquid and vapor phases. Coupled with an estimate of the overall mass transfer coefficient from the Yoshida correlation (*13*), an approximate column height was determined. After inclusion of a safety factor, a 20 cm column was built. Countercurrent flow was not used, since free two-phase flow does not occur (*14*) under required conditions. Gas and liquid streams entered through the bottom of the column and passed upward through the packing. The two streams separated in a 1.90 cm tee where the volatiles passed through a heated gas line into a gas chromatograph (GC). The GC used an automatically controlled gas sampling valve. The temperature of the gas sampling valve was controlled by the GC, ensuring a constant amount of sample for injection. Hardware for automated analysis of volatile compounds was also developed.

Experimental System

Figure 1 is a diagram of the DVR which simulates a section of an ideal PFR. The reactor consisted of a top "plunger like" section, and a lower annulus sealed at the bottom. An *n*-butyl rubber o-ring was used to produce a gas tight seal between the two sections. The inner diameter was 5.08 cm, the height could be varied from 0.8–5.08 cm. Fluid entered at the base and proceeded upwards through a set of channels (*see* Fig. 1), etched in the base of the DVR. The channels distributed the fluid to a porous metal frit (Pall Metal Company), 0.32 cm thickness, 316 stainless steel with 0.4-4 μ pores. The metal frit supported a soil wafer with attached bacteria, and distributed the flow evenly over the soil wafer. The fluid exited the wafer passing through two 0.2 μ Teflon filters (Gelman TF-200) that were constructed with a nylon support mesh. The Teflon filter protected the top metal frit from plugging caused by small particulates from the soil. The fluid passed through a second metal frit (0.32 cm thickness, 316 stainless steel, 0.4-4 μm pores) and into a second set of channels, etched in the top portion of the reactor, which collected the fluid. The frits were welded to the reactor over the channels to assist in developing a plug flow type profile. All reactor parts were made of 316 stainless steel and manufactured in the Department of Chemical Engineering machine shop at the University of Tennessee.

All DVR system equipment was rated at or above 8,500 kPa. A pressure relief valve was installed to assure maintenance of safe operating pressures. Gilson 301 HPLC pumps, controlled by serial port communications to a personal computer, were used to control the feed of substrate to the reactor system. The personal computer was programmed so that substrate

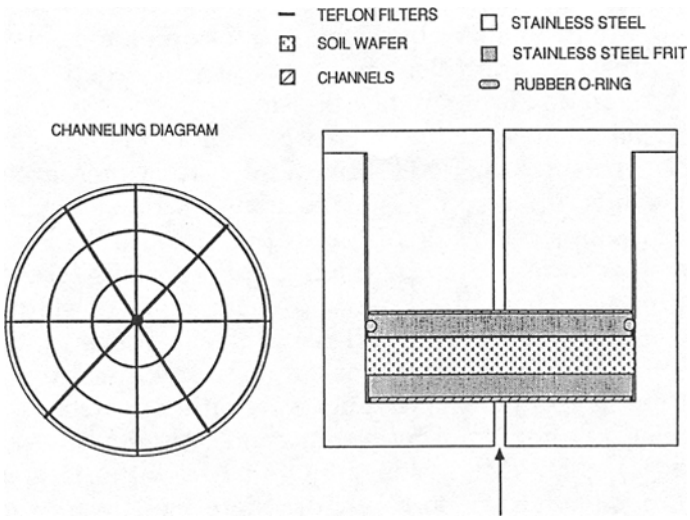

Fig. 1. Diagram of the DVR. The reactor consisted of a top "plunger like" section and a lower annulus sealed at the bottom. Medium entered at the base and was distributed through a set of channels etched in the base of the DVR to a porous metal frit. The metal frit supported a soil wafer with attached bacteria. The fluid exited the wafer passing through two 0.2 μ Teflon™ filters, a second metal frit, and into a second set of channels.

input concentration could be controlled in a sinusoidal manner, using two HPLC pumps for each reactor. A constant hydraulic flow to the reactor was maintained by operating the pumps 180° out of phase. Substrate was stored in stainless steel bombs (Millipore) rated to 690 kPa. After flowing through the reactor, the effluent then entered the stripping column, mixed with helium, and passed out of the column. The volatiles in the helium stream entered the GC (Hewlett Packard 5890 Series II) through a heated sample line. Injection and GC operation was controlled by a second computer and support software (Hewlett Packard computer, HP Chem Station software). The system was equipped with a reactor bypass valve for calibration of the stripping column. Sample valves upstream and downstream of the reactor were also used for liquid analysis and calibration. Manual measurement of oxygen concentration was made by attachment of a four-way valve and sample cell to the effluent sample port by diverting flow through the sample port. Tracers were introduced upstream of the DVR via a six-port valve with sample loop. The reactor temperature was controlled by a Lauda RMT-6 recirculating bath and heat transfer coil.

Reactor Methods

The DVR was an enclosed system, with all nutrients and oxygen entering in the liquid stream. The soil extract contained an organic carbon content of 10 mg/L. The total oxygen demand was calculated such that enough

oxygen was supplied to insure aerobic conditions (DO > 1.0 mg/L in effluent). By filling the head space of the substrate containers with oxygen at atmospheric pressure, the calculated amount of oxygen would enable organisms to degrade all organic carbon in the feed stream to CO_2 and H_2O. Oxygen partial pressure was increased to 230 kPa for the biodegradation experiments using MGP soil. Minimum system pressure was maintained by installing a Nupro pressure regulator with a cracking pressure of 170 kPa on the upstream side of the stripper for the MGP experiment. Perturbation runs were started by operating the reactor for 2–5 d with a constant naphthalene feed of 7.5 mg/L to acclimate the system. Perturbations were then induced in the feed naphthalene concentration cycling from 0 to 15 mg/L for seven cycles, or a minimum time of two days.

Support media for the experiments were either characterized uncontaminated soils, chromatography grade sand (Aldrich 50–70 mesh), or manufactured gas plant (MGP) soil, contaminated with a large number of PAH compounds from a site in New York state. Soils were prepared for use by wet sieving. Soil particle sizes used were between 0.18 and 1.0 mm. Chromatography grade sand was used without treatment.

Media consisted of either basal salts/soil extract having a total organic carbon content of 10 mg/L or basal salts/soil extract plus naphthalene, having a total organic carbon content of 25 mg/L. Soil extract was prepared by autoclaving 20 L distilled water containing 4 L soil. After cooling, the liquid was separated by continuous centrifugation. The supernatant soil extract was then passed through a 0.2 micron filter. The medium was stored at 4°C, with the naphthalene concentration at 15 mg/L.

Cultures were attached to soil. Sand was sterilized by autoclaving. Medium was then inoculated with culture isolated from MGP soil and incubated for 48 h. Sand was added to the medium and incubated for an additional 48 h. The sand was removed from the broth, rinsed with 50 mL phosphate buffered salts, and placed in the reactor. MGP soil was prepared as above, except the soil was not autoclaved. Uncontaminated soil was used for hydrodynamic characterization of the DVR.

Flow distributions through reactors were characterized, using a Br^- or Cl^- tracer. 100 μL of one molar potassium salt tracer was introduced to the reactor through a six-port valve directly upstream of the reactor. Effluent samples were analyzed for tracer, using a Waters Ion chromatography system with a series 510 HPLC pump, IC-PAK anion exchange column, and 431 conductance detector (Waters, Cambridge, MA).

Biological

Biological analyses were prepared from DVR samples by adding phosphate buffered saline (pH 7.0) and sodium pyrophosphate (0.1% w/v), and votexing for 2 min. Total viable cells were enumerated by the serial dilution technique and spread plate inoculation onto nonselective YEPG agar. All plates were incubated for at least 5 d at 20°C.

The naphthalene genotype population was enumerated by using DNA:DNA colony hybridization (*15*), using a gene probe for iron-sulfur protein of the naphthalene dioxygenase. Primers suitable for use in the polymerase chain reaction were determined from the DNA sequence of the iron-sulfur protein subunit provided by Bert Ensley (personal communication). The oligonucleotides for the amplification were obtained commercially from Genetic Designs, Inc. (Woodlands, TX). The amplified fragment was subsequently subjected to a one-sided polymerase reaction, using only one primer and Taq polymerase (*16*). The nucleotide mix for this reaction contained P^{32} dCTP. The resulting single stranded probe had a specific activity of around 108 dpm/μg of DNA.

$HgCl_2$ (0.05%) was used as an inhibitor for abiotic experiments performed, using MGP soil. Carbon monoxide was used as an inhibitor for aerobic respiration. The usual pathway for biological degradation of PAH compounds is an aerobic pathway (*17*) involving mono- or dioxygenases. A third inhibition route was to reduce the feed to anaerobic conditions blocking aerobic degradation pathways.

Analytic Methods

High purity grade helium gas and the reactor effluent entered the base of a stripping column. The column consisted of a 0.635 cm outside diameter, 0.533 cm inside diameter and 20 cm long stainless steel tube packed with 3 mm glass beads. Reactor effluent liquid entered the stripping column at a rate of 0.30 mL/min. Helium gas flow at a rate of 2 mL/min was maintained by flow restriction, using a needle valve and regulating the upstream pressure at 14 kPa. The gas and liquid streams separated in a 5.08 cm diameter tee at the top of the column. The liquid stream entered a 0.32 cm outside diameter and 0.22 cm inside diameter stainless steel tube maintained at temperatures above 100°C. The gas stream then passed through a 7.0 micron frit and entered a heated zone of the GC containing the six-port gas sampling valve. The heated zone was kept at a constant temperature of 142°C, volatilizing organics and maintaining a constant sample size for injection.

High performance liquid chromatography (HPLC) with fluorescent detection was employed as an additional method for analysis of PAH compounds. The HPLC system consisted of a beckman 110A HPLC pump, a Phenomenex Spherex 5, C-18 column, and a Perkin Elmer programmable FL-4 fluorometer equipped with a 3 μL flow sample cell. A mobile phase of 90% acetonitrile and 10% water was used.

Capillary Gas Chromatography (HP 5890, series II) was used as the separation method for the compounds of interest. A 5% phenylmethylsilicon crosslinked 25 m, 0.33 mm inside diameter, 0.5 micron film thickness column (*18*) was used for separation of the volatiles from the reactor effluent. Helium was used as the carrier gas at 4 mL/min. The GC was programmed with an initial temperature of 35°C, an initial time of 2 min,

a temperature program rate of 40°C/min to a temperature of 115°C, 1 minute at 115°C, a second ramp rate of 5°C/min to 155°C, then a rate of 40°C/min to 250°C, with a final 2 min at 250°C. The GC was equipped with a flame ionization detector (FID).

Gas Chromatography—Mass Spectroscopy (GC-MS) was used to identify compounds desorping from the soil. The effluent passed through a column containing 0.1 g Tenax-GC (*19*). The Tenax-GC was prepared for use by heating in a muffle furnace for 2 h at 375°C under a stream of Nitrogen. The Tenax-GC was then extracted with GC-MS grade isooctane (Baxter). One microliter of the isooctane solution was injected onto a GC-MS (Hewlett Packard 5996), using a HP1 methylsilicon crosslinked, 50 m, 0.2 mm, 20 micron film thickness column. Tenax-GC was generally used as an adsorbent for aromatic and PAH compounds in the atmosphere, and in ground and drinking water. Retention times and mass spectra were identified, using Standard Reference Material 1491 obtained from the National Institute of Standards and Technology.

RESULTS AND DISCUSSION

Analytic

Several methods were used for on-line and off-line detection, identification, and quantification of PAH compounds. The detection limit for the Tenex-GC with GC-MS method was not determined, since detection was a function of the amount of gas or liquid sample passed through the Tenax-GC column. The Tenax-GC method was quite sensitive for PAH compounds and was routinely used as a method for isolation and identification of PAH compounds occurring in gases or in the aqueous phase. The detection limit for a compound using the GC stripping method was dependent on the solubility of the compound of interest and the Henry's Law constant of the compound in water. Solubility of most PAH compounds ranged from several mg/L to low μg/L. For a compound to be detectable by the stripping technique, the Henry's Law constant of the compound must compensate for the low solubility of most PAH compounds. One-, two-, and some three-ring aromatic compounds were detectable by the stripping technique developed in this work. The stripping technique was used to selectively analyze lower mol wt PAH compounds. For naphthalene, the limit of detection, using the stripping method, was approx several μg/L. Combining the selectivity of the stripping technique with the high resolution available from a capillary column provided a functional method for monitoring low mol wt PAH compounds. HPLC with fluorescence was used as an off-line method for verifying effluent concentration and feed concentration GC stripping measurements. The HPLC with fluorescent detection method was quite sensitive for naphthalene, and had a detection limit of approx 20 ηg/L, using the maximum

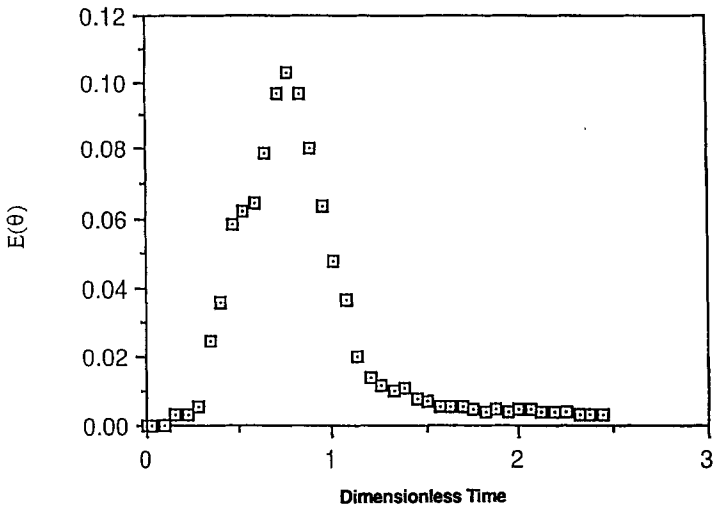

Fig. 2. RTD for the DVR containing sand. The distribution approached a normal shape, indicating a good flow pattern within the DVR, with minimal dispersion effects.

excitation wavelength of 272 nm and the maximum emission wavelength of 330 nm.

Sand

Abiotic

Figure 2 presents the dimensionless RTD for the DVR containing sand. The distribution approached a Gaussian shape, indicating a good flow pattern within the DVR with minimal dispersion effects. The calculated dispersion coefficient was 4.4×10^{-4} cm^2/s. The pressure drop across the reactor for the experiment shown in Fig. 2 was 50 kPa. The reactor pressure drop was an important operating variable for developing a good flow pattern. Initial experiments conducted before installation of the two Gelman TF-2000 filters produced a pressure drop of less than 7 kPa, and a RTD distribution that indicated substantial deviation from ideal plug flow.

A naphthalene mass balance indicated sorption effects from the metal reactor parts were negligible. The reactor effluent concentration tracked the inlet when the feed was diverted through the reactor bypass valve while varying the naphthalene concentration in a sinusoidal manner, indicating once again that sorption effects were negligible with the metal parts of the reactor.

Biological

The oxygen concentration in the reactor effluent was greater than 2.5 mg/L, indicating aerobic conditions. The reactor effluent naphthalene concentration went below the detection limit of 1 μg/L in this experiment. If a

first order rate equation for the disappearance of naphthalene in the DVR was assumed, and a differential mole balance was written over the reactor length, appropriate mathematical manipulation would yield the following equation for conversion in the reactor as a function of volume:

$$V = F_{naphthalene_0} \int_0^x dx \,/\, -r_{naphthalene} \quad (1)$$

Assuming the rate of disappearance of naphthalene is first order in naphthalene concentration, the rate equation for the disappearance of naphthalene would be:

$$-r_{naphthalene} = k\, C_{naphthalene_0} (1 - X) \quad (2)$$

These equations could be combined, integrated, and solved for the rate constant. The resulting rate constant was $>20\ h^{-1}$. The soil depth required to achieve this result was less than 0.8 cm. Populations of cells in the bottom part of the reactor were 3.0×10^5 cells/mL, with 7.3% probing positive for the NAH genotype. Populations of cells in the top part of the reactor were 1.0×10^7 cells/mL, with 4.0% NAH genotype. Total populations of cells on the filters of the reactor were 3.7×10^5 cells, with 5.4% NAH genotype.

To prove that the disappearance of naphthalene was the result of biotransformation, two inhibition experiments were conducted. Both inhibition experiments used the same actively degrading microbial communities described above. Prior to the beginning of the experiment, conversion was greater than 0.999, using a naphthalene feed concentration of >5 mg/L and assuming disappearance was caused by biotransformation. Carbon monoxide, a competitive inhibitor of aerobic respiration, was added to the 5 mg/L naphthalene feed. Figure 3 presents results with time zero, indicating the start of the CO saturated feed. The outlet concentration became equal to the inlet concentration 9 h after the start of the experiment, indicating that aerobic biotransformation was inhibited. An anaerobic feed mix was prepared by purging with nitrogen. Figure 4 depicts the results from the anaerobic experiment, indicating that the behavior of the system was similar to the behavior observed in the CO experiment. The inlet and outlet naphthalene concentrations became nearly equal after 10 h. On addition of oxygen to the feed stream, the naphthalene effluent concentration dropped below detection levels. These two experiments verified that the observed naphthalene disappearance was caused by aerobic biotransformation.

MGP Soil

Abiotic

Initial RTD experiments using MGP soil indicated a substantial amount of distortion in the reactor flow pattern, as compared to RTD of sand system. The RTD was quite broad and exhibited substantial tailing, indicating channeling and/or axial dispersion. The initial pressure drop across

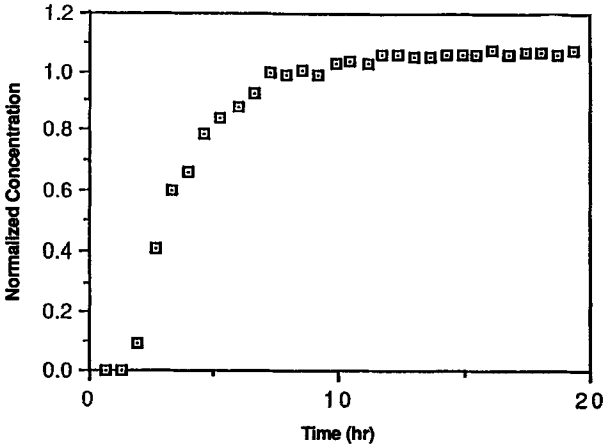

Fig. 3. Inhibition of an actively degrading biofilm by the addition of dissolved carbon monoxide in the feed. The outlet concentration became equal to the inlet concentration 9 h after the start of the experiment, indicating that aerobic biotransformation was inhibited. For a period of a week prior to the start of the experiment, disappearance of naphthalene was greater than 99.9%, as measured by the GC-stripping method and by the fluorescence method. The feed concentration is normalized to 5 mg/L of naphthalene.

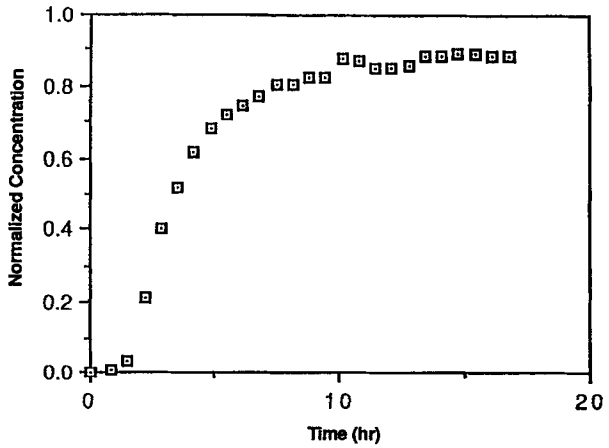

Fig. 4. Inhibition of an actively degrading biofilm by the addition of anaerobic feed. The outlet concentration became equal to the inlet concentration 10 h after the start of the experiment, indicating that aerobic biotransformation was inhibited. For a period of several weeks prior to the start of the experiment, disappearance of naphthalene was greater than 99.9%, as measured by the GC-stripping method and by the fluorescence method. On addition of oxygen to the feed stream, the naphthalene effluent concentration dropped back below detection levels of less than 1 μg/L.

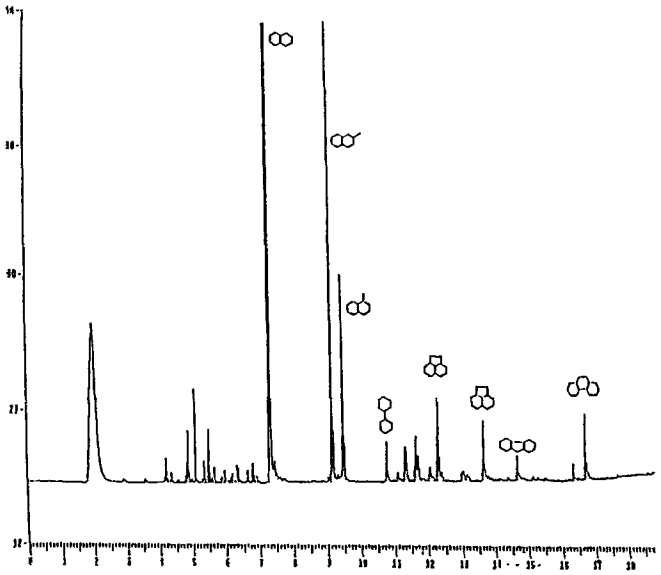

Fig. 5. Chromatogram from $HgCl_2$ inhibition experiment, using contaminated MGP soil. The only PAH added in the feed was naphthalene.

the reactor was only 7–14 kPa, typically. It was postulated that the hydrophobic nature of the Teflon filters was somehow altered by the MGP soil. Typically, the pressure drop across the reactor increased to 40–70 kPa after several days of operation, with some improvement in the reactor's dispersion coefficient.

Several PAH and hydrocarbon compounds were identified as leaching from the MGP soil into the feed medium. Not all of these compounds were volatile enough to be detected with the stripping column-GC system. Biphenyl, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, acenaphthalene, acenaphthene, 9-h-flourene, phenanthrene, and benzo(e)pyrene were identified with GC-MS and compared with compound retention times, using Standard Reference Material 1647 from the National Institute of Standards and Technology. 1,3,5-trimethylbenzene, ethenylmethylbenzene. 1,6-dimethylnaphthalene, and dibenzofuran were identified, using GC-MS only. Two ring compounds and benzene derivatives were generally volatile enough to be detected by the stripping-GC method. Figure 5 shows a typical chromatogram of the compounds stripped from the effluent using MGP soils. After several days of the desorption from the soil, concentrations of the leachates decreased, as indicated by decreasing peak areas. Decreases in concentrations were the result of depletion in the soil, since microbial activity was inhibited by continuous addition of 0.05% $HgCl_2$.

Naphthalene was pulsed to the reactor in a sinusoidal manner, using $HgCl_2$ as an inhibitor. Figure 6 illustrates the differences between input and output naphthalene concentration waves from the MGP soil. Initial naphthalene effluent concentrations were above feed levels, indicating

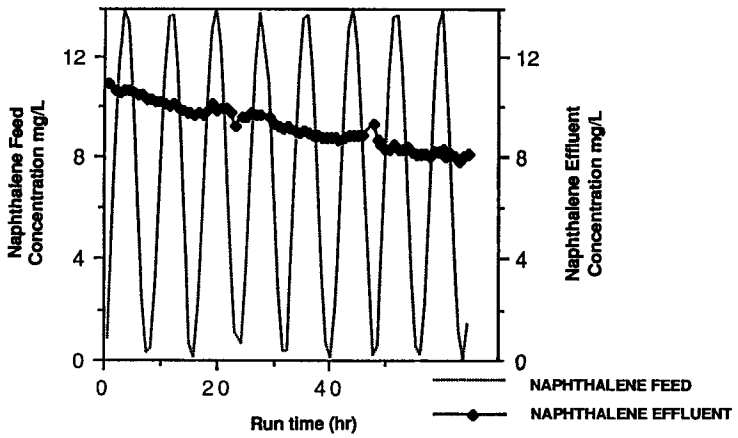

Fig. 6. Sorption of naphthalene in the MGP soil system. The effluent naphthalene wave from the MGP soil was severely dampened. The frequency of the input sine wave was 8 h with a maximum naphthalene concentration of 14 μg/L, which was about 70% of saturation at the temperature of this experiment. The output sine wave was severely damped by sorption processes, and by a large amount of preexisting naphthalene leaching from the soil. $HgCl_2$ inhibited.

desorption of preexisting naphthalene from the soil matrix. The frequency of the input sine wave was 8 h, with a maximum naphthalene concentration of 14 mg/L. The output sine wave was severely damped by sorption processes, and by a large amount of preexisting naphthalene leaching from the soil.

Biological

Initially, the oxygen feed concentration was maintained at 24 mg/L; however, the effluent oxygen concentration was <1 mg/L. Under these oxygen limited conditions, disappearance of the feed naphthalene and leachates was not complete. The feed oxygen concentration was increased to 140 mg/L, remedying aerobic biotransformation inhibition. Total disappearance of feed naphthalene and all leachates occurred, as monitored by the GC-stripping column analysis and HPLC fluorescence methods. An excitation wavelength of 272 nm and an emission wavelength of 330 nm was used in the fluorescent technique. The effluent oxygen concentration was in excess of 24.0 mg/L. The total cell count at the conclusion of the experiment was 3.3×10^8 cells/mL in the soil, with a total of 1.23×10^9 cells attached to the filters with the NAH genotype population at 10% of total population in the soil and on the filters. It is important to realize that if channeling occurs, the effluent oxygen concentration may not be a good measure of aerobic conditions throughout the reactor. The inhibition experiments using sand indicate that aerobic conditions are an important factor in degradation of these compounds. The calculated first order rate constant for naphthalene degradation was $>20\ h^{-1}$.

Comparison of Systems

Use of sand facilitated the characterization of a well-behaved system that was isotropic with a good RTD. In contrast, the MGP soil system was anisotropic with strong interactions present between PAH's in the aqueous phase and the soil matrix. Both systems gave similar degradation kinetics if aerobic conditions were maintained. The chromatograms from MGP soil were far more complex, and considerably more difficult to interpret, than those from the sand system.

CONCLUSIONS

The experimental system developed in this work was suited for examining biological activity and mass transfer in soil systems. The flow properties of the DVR were measured, and can easily be modeled, mathematically. The small residence time could allow physical perturbation analysis and rapid evaluation of kinetics. It is postulated that mathematical separation of mass transfer processes from biological processes is possible. Since there was no gas phase present in the reactor, the concentration of dissolved gases fed to the reactor could be easily manipulated by controlling gas partial pressures in the feed vessel. The system had a wide range of on- and off-line analytic tools for monitoring purposes.

The DVR was also suited for studying sorption effects in soil systems. A distinct advantage was gained for the study of soil systems under unsteady state conditions. The effects of changing concentrations of materials leaching from contaminated soil could be measured in real time.

A stripping column was developed for on-line analysis of the reactor effluent. The system provided an easily maintained tool for analyzing reactor effluent concentrations of volatile organics.

The DVR was used to characterize PAH and PAH-related compounds leaching from contaminated MGP soil, indirectly addressing the question of the bioavailability of compounds in soil systems, since the system is easily modified for various types of perturbation analysis. Whereas many techniques were available for determining the total amount of PAHs in the soil, the total concentration of any PAH in the soil sample may not correlate well with the bioavailability of that compound.

Disappearance of naphthalene below detection limits, as measured by fluorescence and the GC stripping method, occurred under aerobic conditions for both the sand and MGP soil systems. Inhibition experiments confirmed this disappearance was caused by aerobic biological activity. The first order rate constant for biotransformation was $>20\ h^{-1}$. The DVR system is now being used for evaluating the biotransformation kinetics of the higher PAHs that degrade at much slower rates, allowing the DVR to represent a differential element of a soil column.

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REFERENCES

1. Harrison, R. M., Perry, R., and Wellings, R. A. (1974), *Water Res.* **9,** 331–346.
2. Mackay, D. and Shiu, W. Y. (1977), *J. Chem. Eng. Data* **22,** 399–402.
3. Lee, M. L., Novotny, M. V., and Bartle, K. D. (1981), *Analytic Chemistry of Polycyclic Aromatic Hydrocarbons,* Academic, New York, pp. 11–14.
4. Karickhoff, Sl. W., Brown, D. S., and Scott, T. A. (1979), *Water Res.* **13,** 241–248.
5. Blackburn, J. W. (1987), *Environ. Sci. Technol.* **21,** 884–890.
6. Karickhoff, S. W. (1984), *J. Hydraulic Eng.* **110,** 707–733.
7. Levenspiel, O. (1972), *Chemical Reaction Engineering,* Wiley, New York, pp. 483–491.
8. Freeze, R. A. and Cherry, J. A. (1979), *Ground Water.* Prentice-Hall, Englewood Cliffs, NJ, p. 29.
9. Danckwerts, P. V. (1953), *Chem. Eng. Sci.* **2,** 1–13.
10. Aris, R. (1956), *Proc. R. Soc.* (London), **A235,** 67–77.
11. Fogler, H. A. (1986), *Elements of Chemical Engineering,* Prentice-Hall, Englewood Cliffs, NJ, p. 701.
12. Dunbar, P. D., MS Thesis, University of Tennessee, p. 43.
13. Yoshida, F., Ramaswami, D., and Hougen, O. A. (1962), *A. I. Ch. E. Journal* **8,** 5–11.
14. Cliff, R., Grace, J. R., and Weber, M. E. (1978), *Bubbles Drops and Particles,* Academic, New York, pp. 11–14.
15. Applegate, B. M. and Uphoff, R. A. (1990), *Modified Protocol for Colony Hybridization,* submitted to *Biotechniques.*
16. Werner, C., Applegate, B. A., and Corcoran, C., Modification of DNA Extraction Method and Screening of the DNA Polymerase Chain Reaction-Generated Single-Strand Probe (1990), Presented at the National Meeting of the American Society for Microbiology, Anaheim CA, May 17, 1990.
17. Cerniglia, C. E. (1984), *Adv. Appl. Microbiol.* **30,** 31–71.
18. Lee, M. L., Vassllaros, D. L., White, C. M., and Novotny, Milos (1979), *Anal. Chem.* **51,** 768–773.
19. Lee, M. L., Novotny, M. V., and Bartle, K. D., *Analytic Chemistry of Polycyclic Aromatic Hydrocarbons* Academic Press, New York, 79–91.