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Dynamic response of naphthalene biodegradation in a continuous flow soil slurry reactor

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Abstract

Periodic perturbations were used to evaluate the system stability and robustness of naphthalene biodegradation in a continuous flow stirred tank reactor (CSTR) containing a soil slurry. The experimental design involved perturbing the test system using a sinusoidal input either of naphthalene or non-naphthalene organic carbon at different frequencies during steady state operation of the reactors. The response of the test system was determined by using time series off-gas analysis for naphthalene liquid phase concentration and degradation, total viable cell counts, and gene probe analysis of naphthalene degradative genotype, and by batch mineralization assays.

Naphthalene biodegradation rates were very high throughout the experimental run (95 to >99% removed) resulting in very low or undetectable levels of naphthalene in the off-gas and reactor effluent. Attempts to reduce the rate of naphthalene biotransformation by either reducing the reactor temperature from 20° C to 10° C or the dissolved oxygen level (>1 mg/L) were unsuccessful. Significant naphthalene biodegradation was observed at 4° C. While variable, the microbial community as measured by population densities was not significantly affected by temperature changes. In terms of naphthalene biotransformation, the system was able to adapt readily to all perturbations in the reactor.

Introduction

There is need to develop new analytical tools that improve understanding and prediction of the stability, robustness, and resilience of biological processes as well as the biotransformation rate activity (corrected for abiotic fates) in operating systems for hazardous waste remediation. While a wide variety of toxic organics can be biologically degraded (Gibson 1984; Leisinger et al. 1981; Rochkind et al. 1987), there are many factors which make it difficult to generalize on how a given biological population will perform in a given bioremediation scenario, thus making specific predictions on the rate and extent of biodegradation very difficult, if not impossible.

It is well known that specific populations in heterogeneous systems, often only a very small fraction of the total active population, are responsible for the biotransformation of specific organic compounds (Sayler et al. 1986). The ability to identify and quantify these specific populations and determine their activities as they relate to system processes is a requirement for reliable toxic removal

performance predictions. If the genes responsible for the activity of interest are known, gene probe techniques provide a valuable tool for discriminating and quantifying the degrading population (Jain et al. 1988). However, knowledge of the relative numbers of degraders to the total active population or genes is not sufficient information for catabolic biokinetic prediction of the behavior of such populations. Interactions with other populations, whether identified or not, may contribute to the overall behavior of the biodegrading population (Bender et al. 1984).

One approach to prediction of the population interaction effects might be to monitor all 'important' populations, and develop knowledge of how these groups interact. Structured, deterministic models for the prediction of the behavior of mixed microbial populations developed in this manner would provide invaluable information on general system behavior. However, the current inability to identify and measure all of the critical state population variables may make this type of constructionistic approach difficult.

Another approach which has been used to study complex communities is to consider the system holistically and use the response to imposed perturbations as an analysis tool. Experimentally this protocol involves the perturbation of the system by changing a critical system variable in a pre-determined manner, holding all other parameters constant, and monitoring an appropriate indicator variable to determine how this disturbance has affected system behavior.

Such analysis techniques have been applied to a number of complex systems in a variety of disciplines ranging from ecology to engineering (Beck & van Straten 1983; Granger & Newbold 1977, Malinvaud 1980; Markel & Gray 1976; Mendel 1983; Robinson & Trietel 1980). In particular, a number of investigators have used this approach to resolve the structures of complex communities in natural ecosystems, including mixed microbial communities (DiGrazia et al. 1990; Parkin & Speece 1983). Difficulties resolving the output signal of the system from the noisy response typical of biological systems has, however, been a problem (Bender et al. 1984). Periodic perturbation methods (Bode 1945) offer a convenient way to separate noise from the actual response to perturbations (Eisner 1971).

The objectives of this work were to 1) expand frequency response experimental methodology to organic biodegradation in soils, 2) investigate the stability and robustness of naphthalene biotransformation to perturbations in organic feed concentrations, and 3) use parameter estimation based on an unsteady-state naphthalene material balance (corrected for stripping and sorption losses) to estimate naphthalene biotransformation rates and rate constants. Naphthalene was selected for study because of successful prior attempts of naphthalene continuous reactor liquid analysis (Blackburn 1989) and to offer a basis for future comparisons in naphthalene-degrading systems.

Materials and methods

Experimental design

A continuous flow stirred tank reactor (CSTR) containing a soil slurry inoculated with a mixed microbial naphthalene degrading population was used as the test system. A frequency response protocol was used to establish the stability and robustness of naphthalene biotransformation in the soil system by sinusoidally varying the input naphthalene concentration to the reactor. The reactor naphthalene concentration was monitored to establish the effect of the perturbations on naphthalene biotransformation. In addition, gene probes were used to monitor the naphthalene-degrading population in the reactor to establish the effect of the perturbations on degrader numbers.

The frequency response protocol involved a two week reactor acclimation period during which the system parameters were held constant, followed by a series of perturbation intervals at known cycle frequencies separated by relaxation intervals where no perturbations were induced. Two reactor systems were operated and maintained hydraulically at steady state during the perturbations. During each four-day perturbation phase, the naphthalene concentration in the feed to reactor #1 was

varied sinusoidally between 0 and 15 mg/L. In reactor # 2 the feed concentration of napthalene was held constant while the non-naphthalene organic carbon (NNOC) was varied sinusoidally between 0 and 50 mg/L. Each perturbation period was followed by a three day relaxation interval where the feed naphthalene concentration to reactor # 1 was held constant at 7.5 mg/L and the NNOC in the feed to reactor # 2 was held constant at 25 mg/L. This relaxation-perturbation sequence was repeated until all of the frequencies were tested. The sinusoidal frequencies administered were, in order, 0.0417, 0.0833, 0.125, 0.25, 0.5, and 1 cycles/hr, or in terms of cycle period, 24, 12, 8, 4, 2, and 1 hour, respectively.

The reactor liquid naphthalene concentration was monitored by sampling the reactor offgas no less than six times per cycle during the perturbation period, and once per hour during all relaxation periods. The liquid concentration of naphthalene was directly calculated from the measured offgas concentration using Henry's Law, Equation 3. Feed samples were taken daily for naphthalene analysis. Reactor samples were taken once per day and analyzed for total viable cells and probed using the colony hybridization technique to enumerate total degraders per reactor volume.

To determine the rate of biological degradation of the naphthalene, a mass balance for naphthalene in the reactor liquid was developed in terms of measured variables. The overall mass balance can be written as follows:

$$\frac{d(V_L C_A)}{dt} + \frac{V}{\varrho_s}\frac{d(WC_A^S)}{dt} + VX\frac{d(C_A^B)}{dt} =$$

$$FC_{in} - F\frac{V_L}{V}C_A - \frac{FWC_A^S}{\varrho_s} - FXC_A^B - QC_A^V - R \qquad (1)$$

Variable definitions and units are given in Table 1. The terms on the left side of Equation 1 represent accumulation of the naphthalene in liquid, soil, and biomass compartments, respectively. The first term on the right side of the equation represents

Table 1. Nomenclature.

Symbol	Definition	Units
C_A	Liquid phase naphthalene concentration	Milligrams per liter of liq.
C_A^B	Biomass naphthalene concentration	Milligrams per cell
C_A^S	Soil phase naphthalene concentration	Milligrams per liter of soil
C_A^V	Vapor phase naphthalene concentration	Milligrams per liter of air
C_{in}	Feed naphthalene concentration	Milligrams per liter of feed
D	Dilution rate, F/V	$Minute^{-1}$
f_L	Weight fraction of lipids in biomass	Unitless
f_{oc}	Weight fraction organic carbon in soil	Unitless
F	Feed flow rate	Liters of liquid per minute
H	Henry's Law Constant for naphthalene	Unitless
K_B	Partition coefficient, biomass to liquid	Liters per cell
K_{ow}	Octanol-water partition coefficient	Unitless
K_P	Partition coefficient, soil to liquid	Unitless
K_S	Soil sorption parameter	Liters per gram
K_G	Stripping constant, QH/F	Unitless
Q	Air flow rate	Liters per minute
R	Biological reaction rate	Milligrams per minute
t	Time	Minutes
V	Slurry volume in reactor	Liters of slurry
V_L	Liquid phase volume in reactor	Liters of liquid
W	Soil concentration in reactor	Grams per liter of slurry
X	Biomass concentration	Cells per liter of slurry
ϱ_B	Cell density	Grams per cell
ϱ_L	Lipid density	Grams per liter of cell
ϱ_S	Soil density	Grams per liter of soil

the input of naphthalene to the reactor and the following three terms represent the naphthalene output in the liquid, soil, and biomass compartments of the effluent respectively. The naphthalene removed in the offgas is accounted for in the fifth term on the right and the rate of biological removal of naphthalene is represented by the last term in Equation 1.

The liquid volume can be expressed in terms of soil concentration in the reactor by

$$V_L = V\left(1 - \frac{W}{\varrho_s}\right) \tag{2}$$

where the slurry volume is constant and the soil concentration in the reactor is measured. The concentrations of naphthalene in the liquid, soil, and biomass compartments can be expressed in terms of the reactor liquid concentration by using equilibrium partition coefficients as follows:

$$C_A^V = HC_A \tag{3}$$

$$C_A^S = 0.62 f_{oc} K_{ow} C_A = K_P C_A \tag{4}$$

$$C_A^B = \frac{f_L \varrho_B K_{ow}}{\varrho_L} C_A = K_B C_A \tag{5}$$

The experimentally determined values of H and K_P are 0.0079 and 12.1, respectively. Substitution of Equations 2, 3, 4, and 5 into Equation 1 yields:

$$(1 + K_S W + K_B X)\frac{dC_A}{dt} + (K_S C_A)\frac{dW}{dt} =$$
$$DC_{in} - DC_A(1 + K_S W + K_B X + K_G) - \frac{R}{V} \tag{6}$$

where

$$K_S = \frac{(K_P - 1)}{\varrho_S} \tag{7}$$

The differential terms of Equation 6 can be expressed as difference equations:

$$\frac{d(C_A)}{dt} \approx \frac{C_A|_{t+1} - C_A|_{t-1}}{2\Delta t} \tag{8a}$$

$$\frac{d(W)}{dt} \approx \frac{W|_{t+1} - W|_{tm1}}{2\Delta t} \tag{8b}$$

Rearrangement of Equation 6 to express the rate of biological degradation in terms of measured variables and physical constants yields:

$$R = FC_{in} - FC_A(1 + K_S W + K_B X + K_G) +$$
$$\frac{V(1 + K_S W + K_B X)}{2\Delta}(C_A|_{t-1} - C_A|_{t+1}) +$$
$$+ \left(\frac{VK_S C_A}{2\Delta}\right)(W|_{t-1} - W|_{t+1}) \tag{9}$$

Simply stated, this is a representation of Equation 1 in the form

$$R = f(F, C_{in}, C_A, W, X, \Delta t) \tag{10}$$

which relates the rate of biological degradation to experimentally measured variables. Equation 9 was used to calculate the naphthalene degradation rates for all of the experimental results presented. At this point no kinetic rate equation for naphthalene biodegradation has been assumed and the results are, subject to model assumptions, general.

Reactor system

The experimental apparatus used in this experiment is shown schematically in Fig. 1. The reactor system consisted of an L.H. Fermentation Series 500 continuous flow bioreactor with 1 L glass reaction vessel, direct drive agitator, dissolved oxygen measurement, temperature control and air flow control modules. The reactor slurry effluent was withdrawn through the top of the reactor with a timed Masterflex peristaltic pump and received in a 1 L glass vessel. The reactor liquid slurry volume was maintained at 0.75 L. Once per day, the reactor slurry effluent was centrifuged and the solids were returned to the reactor. Typical reactor operating conditions for this experiment are shown in Table 2.

The feed solutions were contained in two 6 L stainless steel containers and refrigerated at 4° C. The first feed solution was saturated with naphthalene and the second solution had no naphthalene. The feed was continuously delivered to the reactor with two Gilson 302 stainless steel positive dis-

placement pumps. All surfaces in contact with the feed solutions were either stainless steel or teflon. Buffer solution composed of (g/L): $NaNO_3$, 4; KH_2PO_4, 1.5; Na_2HPO_4, 0.5; $FeCl_3 \cdot 6H_2O$), 0.0005; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.01; was periodically fed to the reactor with a timed peristaltic pump. The pH of the reactor liquid was measured daily and adjusted to 7.0 if necessary with 1 M K_2HPO_4.

Reactor offgas was routed to a computer-controlled Valco sampling valve with a 7.7 mL sample loop. The lines, valve, and sample loop were maintained at 140° C by means of heating tape and a solid state temperature controller (Omega). Offgas samples were automatically sent to a dedicated, on-line gas chromatograph. The offgas sampling frequency, feed flow rate, and feed concentration were all controlled by an I.B.M. PC personal computer. The feed concentration of naphthalene to the reactor was controlled by varying the relative flow rates of the two feed pumps while keeping the total flow rate constant. The reactor liquid naphthalene concentration was periodically determined by automatically sampling the reactor offgas and relating the measured gas concentration of naphthalene to the liquid concentration using Equation 3.

The reactors were started by adding 300 g of soil to approximately 600 mL of water. The inoculum was centrifuged to give a concentrated cell solution with a total cell count of 10^{12} cells. The cells were added and the reactors run for approximately two weeks with steady naphthalene feed before perturbation experiments began.

Table 2. Reactor operating conditions.

Parameter	Set point
Agitation	750 r.p.m.
Air flow	75 mL/min
Feed flow	1 L/day
Buffer/nutrient flow	50 mL/day
Soil concentration	400 g/L
Feed naphthalene concentration	15 mg/L
Temperature	4° C, 10° C, or 20° C
Dissolved oxygen	20 mg/L
pH	7.0

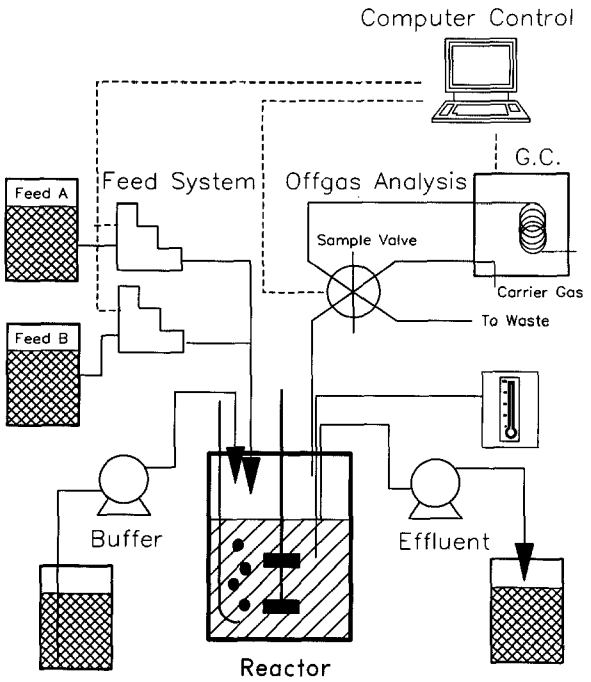


Fig. 1. Schematic of CSTR naphthalene bioreactor system used in periodic perturbation response analysis.

Analytical methods

Feed samples for naphthalene determination were taken daily by withdrawing 1.5 mL sample from each feed line with a glass syringe. Samples were refrigerated in 2 mL glass vials with teflon lined caps prior to analysis.

Feed and offgas samples were analyzed using a Shimadzu GC-9AM gas chromatograph equipped with flame ionization detector, Supelco 10% SP-2100 on 100/120 mesh Supelcoport 1/8" × 6 ft packed column, and Shimadzu CR501 integrator. Gas chromatograph operating conditions are as follows: carrier gas flow, 40 mL/min; air flow, 500 mL/min; hydrogen flow, 40 mL/min; column temperature, 140° C; detector temperature, 225° C; injector temperature, 225° C.

Total suspended solids in the reactor was determined by withdrawing 2 mL of reactor slurry and drying for two hours in an oven at 80° C. Preliminary experiments showed that longer drying times and/or higher temperatures did not affect results.

Dry weight of the solids was then used to calculate the total solids in the reactor.

Bacterial cultures and strains

E. coli BHB2600 carrying the plasmid pDTG113 was used for source plasmid DNA in generating [^{32}P]-DNA probes. The strain was obtained from D.T. Gibson and contains the upper pathway genes coding for initial naphthalene biodegradation (~ 15 kB EcoR1 fragment of a NAH7 homologous plasmid) cloned into the vector pKT230 to give plasmid pDTG113. An *E. coli* strain carrying the vector pKT230 minus the NAH upper pathway genes was used for obtaining purified control vector DNA.

The inoculum used in the reactors was prepared from a series of batch enrichments in one-tenth strength yeast extract peptone glucose (YEPG) medium (Sayler et al. 1979) supplemented with 100 mg/L each of naphthalene, anthracene, and phenanthrene. Soil from a manufactured gas plant site was added to the primary enrichment together with slurries from a set of freshwater microcosms (Sayler & Sherrill 1983) exposed to polycyclic aromatic hydrocarbons (PAHs). To provide inocula for future studies, YEPG broth cultures were inoculated from the final enrichment culture and were spiked with additional PAH-degrading strains obtained from a variety of environmental sources. Frozen stocks of the final inoculum culture were prepared by mixing equal volumes of glycerol and the fresh culture. The stocks were maintained at − 90° C. Prior to addition to the reactors, the inoculum was pre-grown in batch from the frozen stock in YEPG medium.

Media

The feed solution to the reactors was prepared by hot water extraction from uncontaminated soil (Table 3). Approximately 10 kg in 20 L of water was autoclaved for at least 2 hours, shaken, and allowed to cool to room temperature. The solids were then removed by continuous centrifugation followed by pressure filtration through a 0.45 μm membrane filter. Two 55 gallon drums of feed material were prepared and stored at 4° C to eliminate batch-wise variation over the course of the experiment. The total organic carbon in the feed averaged 50 mg/L. Naphthalene was added to the feed by saturating the solution at 50° C and allowing naphthalene to crystallize out of solution while cooling to 4° C. Naphthalene saturates water at 15 mg/L at 4° C.

E. coli strains were grown in Luria-Bertani (LB) medium (Maniatis et al. 1982) supplemented with 50 μg/mL kanamycin (Sigma Chemical Co., St. Louis, MO) at 37° C. Environmental isolates were grown on YEPG medium at 20° C.

Enumeration of bacteria

Samples of slurry and effluent from the reactors were added to phosphate buffered saline (pH 7.0) and sodium pyrophosphate (0.1% w/v) and vortexed for two minutes at high speed. Total cells were enumerated by the serial dilution technique and spread plate inoculation of YEPG agar plates which were incubated at 20° C for five days. The NAH genotype population was enumerated by DNA:DNA colony hybridization using the plasmid probe pDTG113.

Table 3. Physical and chemical properties of soil.

pH (1:1 soil:water)	5.9
Total carbon	0.8%
CEC, (pH 7)	6.5 meq/100 g
Mechanical analysis	
Sand	24%
Coarse silt	18%
Silt	33%
Clay	25%
Fe, as free Fe oxide	1.9%
Clay sized minerals[a]	HKMQG

[a] K = Kaolinite, H = Hydroxyinterlayer vermiculite, M = Mica, Q = Quartz, G = Gibbsite

Isolation of DNA and probe preparation

Plasmid DNA preparations were performed by alkaline lysis method (Maniatis et al. 1982). Purified plasmid DNA for probe preparation was obtained by dye-buoyant density ultracentrifugation in cesium chloride and ethidium bromide. [^{32}P]DNA probe was prepared with a nick-translation kit (Bethesda Research Lab., Gaithersburg, MA) by following the manufacturers protocol. [^{32}P]dCTP (New England Nuclear Corp., Boston, MA) was used as the sole labeling nucleotide. The labeled probe was purified by using a spin-column procedure (Maniatis et al. 1982) and the activity was quantified by liquid scintillation counting. Specific activity of the labeled DNA probe was approximately 10^8 dpm/μg of DNA.

Colony hybridization

Bacterial colonies on plates were directly transferred to Biotrans nylon membranes by overlaying the membranes on the agar surface and lifting off the colonies. Conditions for colony lysis, pre-hybridization, hybridization washing, and autoradiography have been previously described (Sayler et al. 1985). An additional hybridization step was included in which unlabeled pKT230 vector DNA (single stranded) was added prior to the labeled probe DNA. This was done to prevent false positive responses due to the binding of the labeled vector DNA of the probe to homologous bacterial DNA on the membranes. Hybridization washings were conducted at low-salt conditions (10 mM NaCl) to give a stringency of approximately 95%.

Results

A frequency response experiment with a 24 h cycle period was initiated after the acclimation period. The response of reactor # 1 to this disturbance is shown in Fig. 2. A similar response was seen in reactor # 2 (not shown). The reactor liquid naphthalene concentrations varied sinusoidally, in phase with the feed cycle, just above the limit of

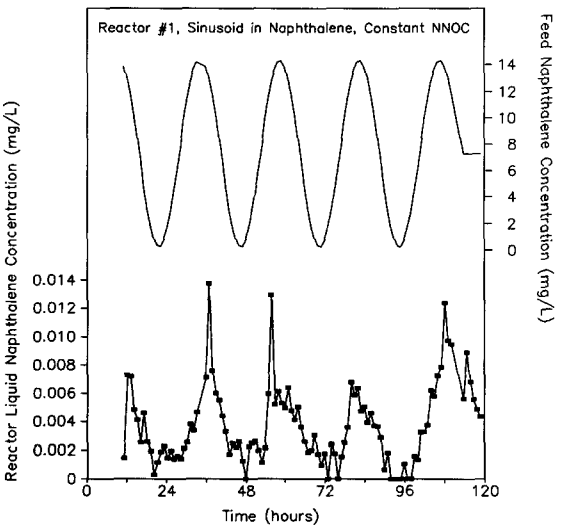
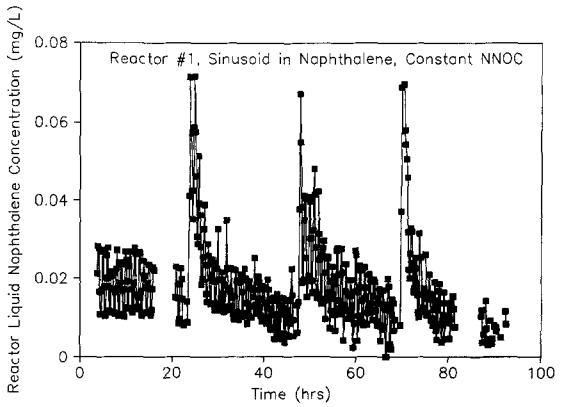


Fig. 2. Reactor liquid phase naphthalene frequency response to 24 h periodic disturbance in feed naphthalene concentration with constant NNOC at 20° C. (—) Feed naphthalene concentration; (■) Reactor liquid naphthalene concentration.

detection of 1 μg/L naphthalene, representing greater than 99% naphthalene removal. During the relaxation interval following the 24 h period perturbation interval (18 days after inoculation) the liquid naphthalene concentration in both reactors fell below the limit of detection. All subsequent attempts to upset the system at 20° C had no detectable effect on reactor performance indicating that the system was very stable with respect to naphthalene and non-naphthalene organic carbon (NNOC) feed perturbations.

In order to perform frequency response analysis it was necessary to make changes in reactor parameters to reduce naphthalene biotransformation rates and bring the liquid concentration in the reactors above the limit of detection. First, the reactor temperature was lowered to 10° C and, as expected, the rate of degradation was correspondingly reduced bringing the liquid naphthalene concentration in both reactors above the limit of detection. A one hour cycle period was then started and the response of the two systems was measured (Fig. 3). Again, the reactor liquid naphthalene concentration followed the sinusoidal pattern of the feed disturbance with reactor # 1 having a larger amplitude than that of reactor # 2. It is important to



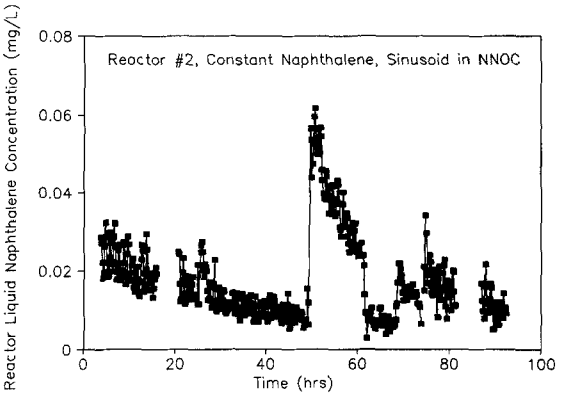
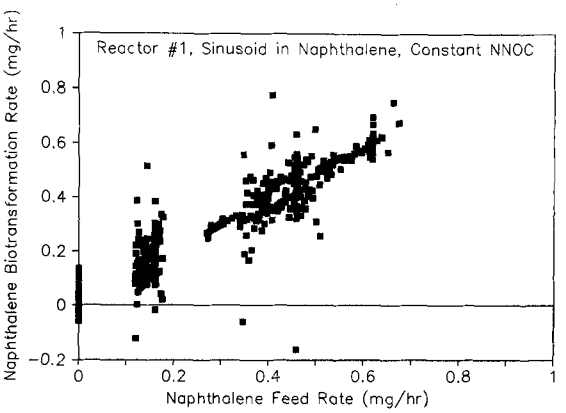


Fig. 3. Response of reactor liquid phase naphthalene concentration to sinusoidal feed perturbations at 10° C. Reactor # 1 (Panel A): sinusoid in naphthalene feed concentration, feed NNOC constant. Reactor # 2 (Panel B): sinusoid in feed NNOC, constant naphthalene feed concentration. (■) Reactor liquid naphthalene concentration.



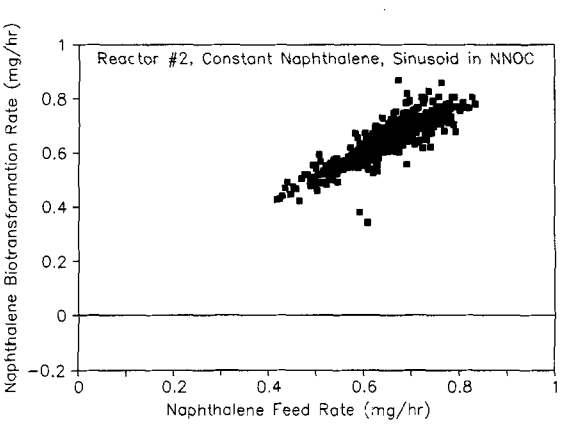


Fig. 4. Relationship between naphthalene feed rate to the reactors and naphthalene biotransformation at 10° C. Reactor # 1 (Panel A): sinusoid in naphthalene feed concentration, feed NNOC constant. Reactor # 2 (Panel B): sinusoid in feed NNOC, constant naphthalene feed concentration.

note that there was an increase in reactor liquid concentration around 25, 50, and 72 h after the initiation of the perturbations. These times correspond to the daily maintenance periods. After the start of the relaxation period, the reactor liquid concentration fell below detection and further attempts at 10° C to upset the system and bring the concentration into the detection range were unsuccessful.

The rate of biological degradation of naphthalene was calculated at each sample point using Equation 9. Reactor naphthalene concentrations in the low ppb range indicate that essentially all of the naphthalene fed to the reactor was degraded. The relationship between the mass flow rate of naphthalene into the reactor and the rate of degradation is shown in Fig. 4. The data clearly form a cluster around a straight line with a slope of one, indicating that the rate of naphthalene biotransformation is, in fact, nearly equal to the rate of naphthalene influx to the reactor. When the rate of naphthalene biotransformation is plotted versus reactor naphthalene concentration (Fig. 5) it appears that there is no simple functional relationship between the two variables. This makes interpretation of the data difficult based upon elementary rate equations (zero, first and second order, Monod, etc).

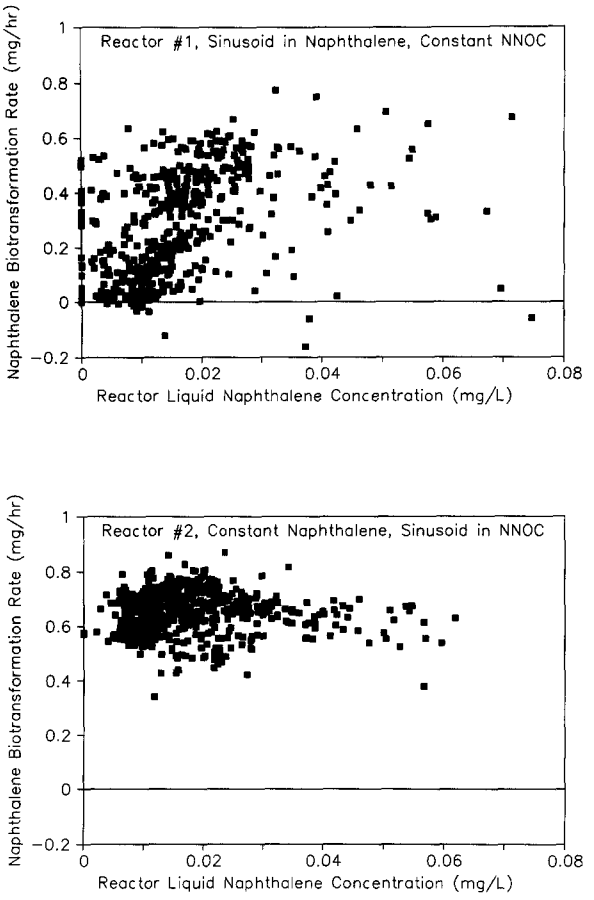


Fig. 5. Relationship between reactor liquid naphthalene concentration and naphthalene biotransformation at 10° C. Reactor # 1 (Panel A): sinusoid in naphthalene feed concentration, feed NNOC constant. Reactor # 2 (Panel B): sinusoid in feed NNOC, constant naphthalene feed concentration.

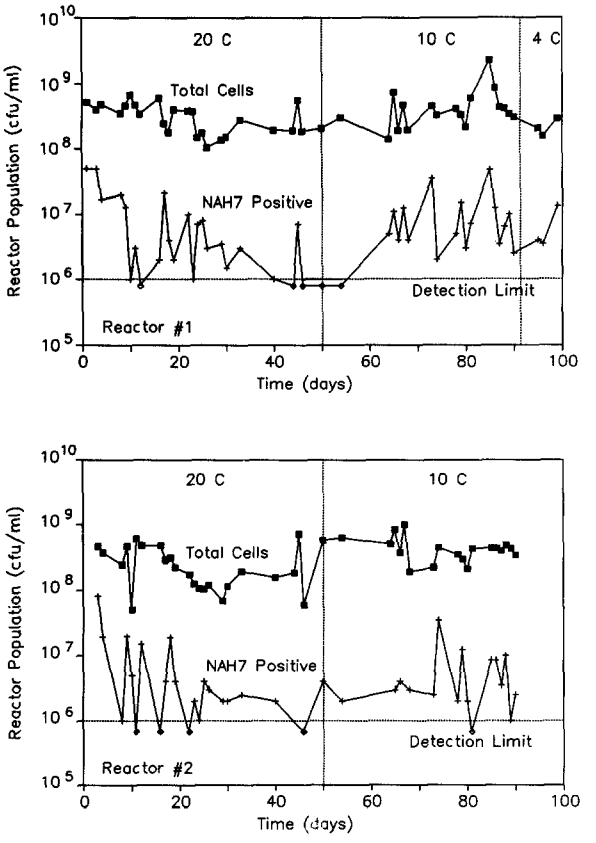


Fig. 6. Dynamics of total enumerable cells and naphthalene-degrading genotype during periodic perturbation response at 20° C, 10° C, and 4° C. Reactor # 1 (Panel A): sinusoid in naphthalene, constant NNOC; Reactor # 2 (Panel B) constant naphthalene, sinusoid in NNOC. (■) Total viable cell count, YEPG; (+) NAH7 genotype; (◇) samples taken with no detectable NAH7 genotype.

To completely stop biological degradation, the temperature of reactor # 1 was reduced to 4° C. Biodegradation decreased significantly (reactor naphthalene concentration $\simeq 100$ BDg/L) but greater than 98% of the naphthalene fed to the reactor was still being degraded and not sorbed, stripped, or flushed from the reactor. After the reactor had run for nearly a week at 4° C without any significant change in degradation rate, the air flow system was modified to allow the manipulation of the O_2 to N_2 ratio. The results of experimenting with the air-to-nitrogen feed ratio to the reactor indicated that there was no reduction in biodegradation rate unless the dissolved oxygen level in the reactor liquid feel below (1 mg/L). Degradation in the reactor ceased when pure nitrogen was fed to the reactor in place of air, demonstrating that at least during this experiment, naphthalene biotransformation was an enzymatically aerobic as anticipated.

Over the entire experimental time course there were no significant population changes between temperature ranges as indicated by analysis of variance (95% confidence interval). Fig. 6 shows the population data for reactors # 1 and # 2.

Discussion

Rates of biological degradation of naphthalene in the soil slurry reactors were very high during the entire experimental run (resulting in 95 to >99% removed), indicating that the population involved in the degradation process is very efficient and competes well with sorption and stripping in removing naphthalene from the liquid feed stream. Results from the plate counts and gene probe analysis indicate that the microbial population is unaffected by perturbations in substrate concentration, temperature, and dissolved oxygen concentration (95% confidence intervals). There was, however, significant fluctuation in population data, especially during the first and final thirds of the experimental run. In general, when there was a large increase in total viable cells, the fraction of naphthalene degrading genotype remained relatively constant.

Figure 5 demonstrates the calculated rates of naphthalene biotransformation in the CSTR reactors. Equation 9 was used to calculate the rate of degradation at each point that offgas sample was calculated. Several terms in Equation 9 could have been neglected due to the low naphthalene concentration in the reactor, however, a rigorous computer calculation was used thus eliminating the need to neglect any terms.

It is curious that there are several negative rate values calculated using Equation 9. This behavior has been reported earlier (Blackburn 1989). There may be several contributing sources of this behavior. First, error is introduced through the use of the discrete time approximation of the differential terms of the mass balance. Another possible source of error would be if the naphthalene on the soil and in the reactor liquid are not in equilibrium at times on the order of the sampling intervals (10 min). If there is an abiotic or biotic rate process involved in the sorption/desorption of naphthalene from the soil, the naphthalene in the liquid and on the soil will not be in equilibrium. This, however, is not likely since the equilibrium relations have been proven experimentally valid for this system (DiGrazia et al. 1990).

First-order rate constants can be used for comparison of the different reactor systems (# 1 and # 2). These constants are indicators of the efficiency of naphthalene removal rather than actual rate 'constants' for modeling system performance. Comparing the results from the two reactors, one perturbed in naphthalene and the other perturbed with NNOC, it is observed that the qualitative response of the two systems is similar. Both reactors show changing naphthalene concentrations with changes in feed, bacterial populations in the two systems react similarly, and naphthalene degradation rates are similar throughout the experimental run. The variation seen in reactor # 2 could be attributed to changes in feed NNOC since the naphthalene level in the feed to this reactor was relatively constant. However, there were slight differences in the naphthalene concentrations in the two feed reservoirs for reactor # 2 which resulted in a slight perturbation in the feed concentration in addition to NNOC perturbations. So, although the experiment was designed to have fixed naphthalene feed, there was slight variation which complicates interpretation of the data.

Added information on system structure and properties can be gained from the input/output data from a series of frequency response experiments. This perturbation protocol proved to be useful in testing the stability and robustness of the system. The results clearly indicate that perturbations in feed naphthalene concentration did not cause naphthalene biotransformation upsets.

Several conclusions may be drawn from this investigation. This new application of periodic perturbation response measurement proved to be useful in assessing system robustness and adaptability to changes in system parameters. The naphthalene biodegrading soil system exhibited very stable, adaptive response to gross pertubations in feed concentration of naphthalene, temperature, and dissolved oxygen as indicated by the continued ability of the system to biotransform naphthalene at high rates and efficiencies. The high rates of degradation and the stability of the system establish bioremediation as a reasonable approach for the remediation of PAH-contaminated soils.

Frequency response analysis of biodegradation systems may offer an approach for improved rate

estimates if analytical detection does not limit the investigation. This experimental system did not produce such data due to the very stable nature of the system. Further studies must probe biodegrading soil systems by forcing the system to respond to gross perturbations in some critical variable which would allow one to resolve the structure of these systems.

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