PREFACE

Can nutrient-amended surfactant-modified zeolite (N-SMZ) be used as a microbial support system to enhance subsurface biodegradation of petroleum hydrocarbons? The manuscript, entitled "Biodegradation of Toluene Sorbed to Surfactant-Modified Zeolite," presents the results from aerobic solution-only and slurry-phase biodegradation experiments that address this question. Submitted to Environmental Science & Technology (ES&T), the article also discusses sorption and desorption properties of toluene on SMZ and N-SMZ and presents a biodegradation kinetic model used to simulate toluene transport through a N-SMZ permeable barrier. The manuscript was prepared in keeping with the editorial guidelines set by ES&T.

Following the ES&T manuscript, I briefly discuss preliminary studies, additional experiments, and important observations obtained in the course of this study that were not included in the manuscript. In particular, I discuss my initial aerobic experiments and an anaerobic biodegradation experiment that was unsuccessful and, therefore, not reported in the manuscript. Finally, I provide conclusions regarding my research and a brief discussion of future work that should be done.

BIODEGRADATION OF TOLUENE SORBED TO SURFACTANT-MODIFIED ZEOLITE

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ABSTRACT

We conducted laboratory studies to test if a reactive nutrient-amended subsurface microbial support system comprised of surfactant-modified zeolite (SMZ) could stimulate and support biodegradation of organic hydrocarbons by retaining the organic contaminant within a specific zone that provided essential nutrients for microbial growth. Toluene was used as a model hydrocarbon contaminant for all studies. Nutrient-amended SMZ (N-SMZ) was prepared by combining a manufactured zeolite product preloaded with N, P, and K with a cationic surfactant. Batch isotherm experiments showed that toluene sorption on SMZ and N-SMZ was similar, resulting in a K_d of 13.0 L kg^{-1}, and toluene sorption and desorption reached equilibrium within 2 h. A toluene-degrading culture was obtained by enrichment of an inoculum obtained from a wastewater treatment plant. The first-order toluene biodegradation rate in solution-only aerobic microcosms was 0.24 h^{-1}, while in both SMZ and N-SMZ slurry-phase cultures the mean biodegradation rate was 0.13 h^{-1}. Predictive models that combined the observed solution-phase biodegradation rate along with toluene desorption characteristics underestimated observed biodegradation in the presence of SMZ and N-SMZ. Transport simulations suggest that a 1-m wide N-SMZ permeable barrier could microbially degrade toluene to below the drinking water standard.

INTRODUCTION

The gasoline components benzene, toluene, ethyl benzene and xylene (BTEX) are major groundwater contaminants, primarily as a consequence of accidental hydrocarbon spills and leaking underground storage tanks (*1-2*). Once released into the subsurface, these contaminants are quite mobile relative to other gasoline components and are toxic even at low concentrations (*3*). Furthermore, natural processes only slowly degrade these hydrocarbons. Traditional *ex situ* remediation methods, such as groundwater pumping, are often slow, expensive, and ineffective (*4*).

New *in situ* technologies focus on the destruction of pollutants within the subsurface without the removal of aquifer material (*4*). Bioremediation, the use of microorganisms to degrade (transform or mineralize) environmental contaminants, is promising as a feasible, cost-effective technology for the remediation of subsurface contaminant plumes (*2, 4-6*). Enhanced *in situ* bioremediation attempts to optimize subsurface environmental conditions at contaminated sites to stimulate indigenous microbial growth and therefore accelerate biodegradation (*6*). However, enhanced *in situ* bioremediation is often hindered by the 1) difficulty and/or inability to supply nutrients and electron acceptors continuously to the microbial community, and 2) inhibition of microbial activities due to toxic concentrations of contaminants (*4-6*).

Several studies have focused on "*in situ* reaction systems" that combine a sorbent zone (permeable reactive barrier) in the subsurface with contaminant degradation mechanisms (biotic or abiotic) in order to enhance remediation (*7-8*). An *in situ* biological treatment system that allows establishment of an active microbial community, a continuous source of nutrients and electron acceptors, and protection against high

concentrations of toxic chemicals would eliminate many of the drawbacks of current bioremediation techniques.

This study investigates whether a nutrient-amended surfactant-modified zeolite (N-SMZ) can be used as a microbial support system to enhance subsurface biodegradation of petroleum hydrocarbons. Zeolites are naturally-occurring, hydrated aluminosilicate minerals characterized by cage-like molecular structures, high internal and external surface areas, and high cation exchange capacities (*9*). Bowman *et al.* (*10*) have shown that natural zeolites become excellent sorbents for inorganic anions and organic species when the negatively charged surface is modified with a cationic surfactant. The sorption capacity allows surfactant-modified zeolite (SMZ) to retain organic contaminants such as BTEX, thereby slowing contaminant migration in the subsurface and reducing contaminant concentrations in the flowing groundwater. SMZ also retains inorganic anions such as nitrate and sulfate that can serve as electron acceptors (*11*). In addition, because internal (zeolitic) cation exchange sites remain unaffected by surfactant-treatment, cationic nutrients such as ammonium and potassium can be preloaded onto SMZ. Li *et al.* (*12*) have shown that microorganisms can colonize SMZ but do not biodegrade the reactive surfactant coating.

Our research focused on a microbial support system made up of nutrient-amended SMZ (N-SMZ) that could be placed in the path of migrating subsurface contamination to slow contaminant transport and stimulate microbial degradation of dissolved petroleum products. We chose toluene as a model for a low molecular weight, aromatic petroleum component. We used laboratory experiments to investigate 1) toluene sorption on N-SMZ compared to SMZ; 2) toluene sorption/desorption kinetics; and 3) toluene

biodegradation in the presence of SMZ and N-SMZ compared to toluene biodegradation in solution-only microcosms.

MATERIALS AND METHODS

Zeolite

The starting material for SMZ experiments was a natural clinoptilolite-rich zeolitic tuff with a particle size of 8-14 mesh (2.4 to 1.4 mm) from St. Cloud Mining Co. (Winston, NM). The mineral content of the zeolite consists of 74% clinoptilolite, 5% smectite, 10% quartz plus cristobalite, 10% feldspar, and 1% illite. K^+ and Ca^{2+} are the major exchangeable cations (*13*).

The starting material for N-SMZ experiments was a nutrient-amended zeolite called ZeoPro™ from ZeoponiX, Inc. (Louisville, Co.) with a particle size of 8-14 mesh. Produced as slow-release fertilizer for horticultural applications, ZeoPro™ is manufactured using St. Cloud zeolite and contains by weight 0.1% N (as ammonium), 0.1% P (as hydroxyapatite), and 0.6% K.

SMZ and N-SMZ Preparation

The majority of SMZ was bulk-produced at the St. Cloud mine using hexadecyltrimethylammonium chloride (HDTMA-Cl). The details of the manufacturing process are described elsewhere (*14*). All N-SMZ was prepared in the laboratory using HDTMA-Br (>99% purity) from Aldrich (Milwaukee, WI). To achieve a target HDTMA loading of 130 mmol kg^{-1}, 100 g of ZeoPro™ and 250 mL of 52-mM aqueous HDTMA-

Br solution were put into 500-mL centrifuge bottles and placed into a New Brunswick rotary shaker at 80 rpm and 25 °C for 24 h, a period shown to be sufficient to attain HDTMA sorption equilibrium (*10*). The mixture was centrifuged and washed twice with purified water (reverse osmosis); then, the modified sample was air-dried. This procedure also was used to produce SMZ for the final biodegradation experiment.

For all biodegradation experiments, SMZ and N-SMZ were washed to remove any excess HDTMA (see Appendix F for more details). The SMZ or N-SMZ was packed in a column, flushed with approximately 50 pore volumes of purified water, removed from the column, and then oven-dried for 24 h at 120 °C. The SMZ or N-SMZ then was re-packed into the column, flushed with approximately 15 pore volumes of water, removed from the column, and allowed to dry at room temperature.

Toluene Sample Preparation

Unless otherwise noted, all toluene-containing samples were prepared in 27-mL glass headspace vials (crimp-cap). If samples were to contain SMZ or N-SMZ, the appropriate mass was placed into vials first. Toluene solutions then were dispensed into vials as prepared solutions from a collapsible Tedlar® bag (Alltech, Deerfield, Ill.) or prepared directly in vials by diluting a concentrated toluene solution (more detailed descriptions of the techniques used to prepare and transfer toluene solutions can be found in Appendices A and B). Following addition of the toluene solution, vials were sealed immediately with aluminum crimp caps fitted with Teflon-faced butyl rubber septa. All treatments were prepared in duplicate or triplicate. Using controls for each experiment tested mass conservation within the vials (no leakage).

Toluene Sorption/Desorption

Sorption Isotherms

We performed batch isotherm experiments to quantify sorption of toluene to SMZ and N-SMZ in comparison to untreated zeolite. To achieve a 1:4 soil/liquid ratio, each batch sample contained 4.0 g of SMZ, N-SMZ, or untreated zeolite and 16 mL toluene solution (aq) at concentrations ranging from 10 mg L^{-1} to 200 mg L^{-1}. In addition, a fourth treatment contained 4.0 g of SMZ and 16 mL toluene solution (10 mg L^{-1} to 200 mg L^{-1}) prepared with inorganic nutrient medium (Bushnell-Haas (B-H) broth (Difco, Detroit, MI)) to approximate the conditions of the biodegradation experiment more closely. Each liter of B-H broth contained 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g KNO_3, 0.05 g $FeCl_3$, 1.0 g $(NH_4)_2HPO_4$, and 1.0 g KH_2PO_4. All samples were placed into a rotary shaker at 80 rpm and 25 °C for 24 h, a period shown sufficient to attain sorption equilibrium (see Sorption and Desorption Kinetics section) and then were analyzed (see Gas Chromatographic Analysis of Toluene section).

Sorption and Desorption Kinetics

We performed sorption and desorption kinetic studies on SMZ to determine if and how the mass transfer rate would affect the biodegradation rate in our system. For both kinetic studies, the toluene solutions were prepared in B-H broth.

For sorption kinetics, each sample contained 4.0 g of SMZ and 16 mL toluene solution at an initial concentration of 120 mg L^{-1}. The samples were shaken at 80 rpm at 25 °C, and then sacrificed at various times over a 24-h period.

Two desorption kinetics experiments were conducted with initial toluene concentrations of 60 and 110 mg L^{-1}. Samples containing 4.0 g of SMZ and 16 mL

toluene solution were shaken at 80 rpm at 25 °C. After a 24-h equilibration period, 8 mL of equilibrium solution was removed with a sterile syringe and discarded. Then, 8 mL of toluene-free B-H broth was introduced into each vial with a syringe. The samples were shaken again, then sacrificed and analyzed over a 24-h period.

Culturing Toluene Degraders

We obtained a culture of aerobic, toluene-degrading microorganisms by a series of enrichment cultures initially inoculated with activated sludge from the wastewater treatment plant in Socorro, NM. The first enrichment culture was prepared in 70-mL glass serum vials using 50 mL B-H broth. Activated sludge (1 mL) was added to the vial and the culture was enriched for toluene degraders by adding 100 mg L^{-1} toluene as the sole carbon source. The culture was incubated in a shaker at 80 rpm and 25 °C for one week. A 1-mL aliquot of culture medium was removed, transferred to vials containing B-H broth and toluene (100 mg L^{-1}), and incubated for one week. The latter enrichment step was repeated three times and the final stock culture was stored at 4 °C.

Toluene Biodegradation

Unless noted otherwise, 24 h before each biodegradation experiment a 1-mL aliquot of stock culture was transferred to a 70-mL serum vial containing B-H broth and 100 mg L^{-1} toluene. This allowed all experimental treatments to be inoculated with exponential-growth-phase cells. Furthermore, throughout all of our biodegradation studies, we chose to consistently use an initial aqueous concentration of 100 mg L^{-1} toluene for comparison reasons.

Solution-Only Experiments

We quantified toluene depletion in aerobic solution-only batch cultures to determine the biodegradation rate in the absence of SMZ and N-SMZ. Each microcosm contained 16 mL B-H broth with a concentration of 100 mg L^{-1} toluene, and 0.2 mL inoculum. We also prepared uninoculated controls that contained 16 mL B-H broth with a concentration of 100 mg L^{-1} toluene. All samples were incubated in a shaker at 80 rpm and 25 °C and sacrificed at various times over a two-day period. A subsample of each was analyzed for toluene immediately and the remainder stored at 4 °C for subsequent 16s rDNA sequencing.

Effect of pH on Biodegradation

Preliminary monitoring showed that the pH in solution-only cultures remained between 6.9-7.1, while the pH in SMZ slurry-phase cultures dropped below 6.5 almost immediately. We concluded that the drop in pH was due to sorption of buffer components within the nutrient medium by the SMZ.

To better understand the effect of pH on microbial growth, several solution-only microcosms of varying pH were prepared in 40-mL glass vials. Each vial contained 30 mL B-H broth with a concentration of 100 mg L^{-1} toluene and 0.5 mL inoculum. The pH was regulated using dilute HCl solutions to achieve a pH range of 5.3-7.0. All samples were incubated in a shaker at 80 rpm and 25° for 2 days.

Varying the pH of solution-only cultures confirmed that pH affected the microorganisms' ability to grow and to degrade toluene. Batch cultures with a pH of 5.3-6.0 never exhibited any microbial growth, while cultures with a pH of 6.5-7.0 always

exhibited growth. The occurrence of growth varied in cultures with a pH range of 6.0-6.5 (see Appendix F for a more detailed description of the experiments and results).

Based on these observations, we concluded it was necessary to ensure that the initial pH within the slurry-phase cultures was above 7.0. Further studies indicated we needed to adjust the initial aqueous nutrient medium to a starting pH of 11.5 in order to maintain the requisite pH within the SMZ and N-SMZ slurry cultures (see Appendix F). This initial pH of 11.5 yielded a pH ≈ 8.0 after a 24-h equilibration period with the SMZ and/or N-SMZ. For SMZ slurry cultures, the pH-adjusted nutrient medium contained the same nutrients as the B-H broth, but replaced 1.0 g $(NH_4)_2HPO_4$ and 1.0 g KH_2PO_4 with 2.0 g K_3PO_4. For N-SMZ slurry cultures, we controlled the pH by adding a nutrient-free solution of 2.0 g K_3PO_4 L^{-1} H_20.

SMZ and N-SMZ Slurry-Phase Experiments

Each sample contained 4.0 g of SMZ pre-equilibrated with 16 mL of pH-controlled nutrient medium or 4.0 g of N-SMZ pre-equilibrated with 16 mL of nutrient-free solution. To each sample, neat toluene was added to achieve an aqueous concentration of 100 mg L^{-1} toluene and 0.2 mL inoculum were added. The microcosms were incubated in the shaker and sacrificed at various times over a three-day period. An aliquot of solution (removed with a sterile syringe) was analyzed for toluene immediately and the remainder stored at 4 °C following measurement of pH. We prepared and analyzed uninoculated controls in the same manner.

Gas Chromatographic Analysis of Toluene

For each experiment, an aliquot of equilibrium solution from sacrificed samples was removed with a sterile syringe, placed into 10 mL glass headspace vials, sealed, and then analyzed for toluene using a gas chromatograph (GC). The amount of toluene sorbed or degraded was calculated from the difference between initial and final solution concentrations.

Headspace toluene concentrations were measured using a Hewlett-Packard Model G1290A automated headspace analyzer connected to a Hewlett-Packard Model 5890A GC equipped with a 10 m x 0.53-mm I.D. HP-5 capillary column and flame ionization detector. Toluene was measured isothermally at 75 °C, with an injector port temperature of 120 °C and detector temperature of 250 °C. We used helium as the carrier gas and a split flow rate of 15 cm^3 min^{-1}.

Five milliliters of supernatant from each sample was transferred to 10-mL headspace vials for analysis. All samples were equilibrated in the automated headspace analyzer for 20 min at 60 °C (while shaken) prior to GC injection. This yielded a specific air-water partition coefficient (K_h) of 0.40 for toluene (see Appendix D for a more detailed description of the method used to determine (K_h). The injection volume was one milliliter. The retention time for toluene was approximately 1 min with a detection limit of 0.5 mg L^{-1} (aq) and a linear response range up to 250 mg L^{-1} (aq). See Appendices C and E for a more in depth discussion of static headspace chromatography principles and the gas chromatographic method used.

Identification of Toluene Degraders

DNA from the solution-only and SMZ slurry-phase cultures was extracted using the MO Bio Soil DNA Isolation kit (MO Bio Laboratories, Solana Beach, CA). 16S rDNA amplification and cloning were carried out using the methods of Chandler *et al.* (*15*). DNA sequencing was done at the University of Maine DNA Sequencing Facility (Orono, ME). DNA sequences were analyzed and edited using the methods described in Balkwill *et al.* (*16*). BLAST (*17*) analysis showed high similarity to *Pseudomonas veronii*. The GenBank/EMBL database accession number for the 16S rDNA sequence is AF313466.

RESULTS AND DISCUSSION

Toluene Sorption/Desorption

Sorption Isotherms

The toluene sorption isotherms for both SMZ and N-SMZ were linear, reflecting a partitioning type mechanism (Figure 1). Therefore, the equilibrium distribution of toluene between the solid and aqueous phases is described as

$$\frac{S_e}{C_e} = K_d \qquad [1]$$

where C_e (mg L^{-1}) and S_e (mg kg^{-1}) are the equilibrium toluene concentrations in the aqueous and sorbed phases, respectively, and K_d (L kg^{-1}) is the solid-water distribution coefficient. Linear sorption isotherms on SMZ have been observed for other nonpolar organics (*10*). A straight-line fit of the data resulted in the K_d values shown in Figure 1.

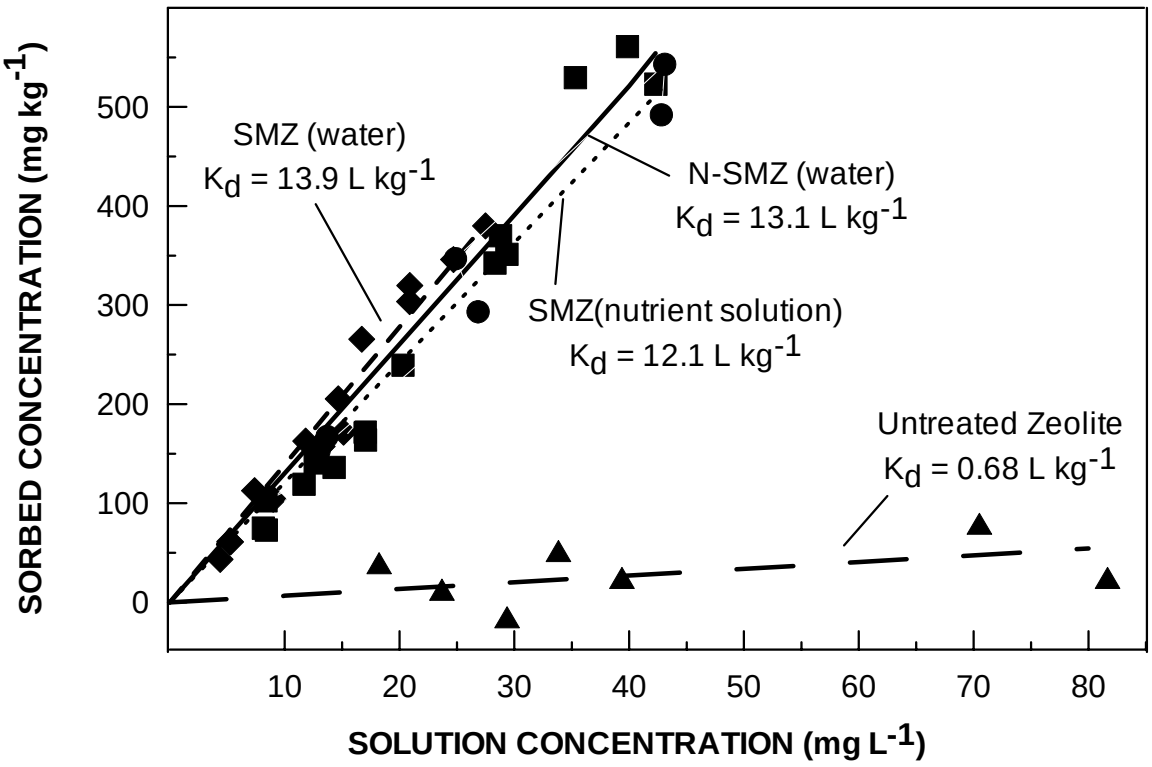

Figure 1. Sorption of toluene by SMZ, N-SMZ, and untreated zeolite. Fitted lines are based on linear regression through the origin. mg L^{-1} and 110 mg L^{-1} respectively (Figure 2b). The data used to create these figures are in Appendix J,

The three K_d's are not statistically different at the 95% confidence level. Therefore, a mean K_d of 13.0 L kg^{-1} was used to characterize toluene distribution between the solid and solution phases in the biodegradation studies. The data used to create this figure is in Appendix J, Table A-J1.

Because SMZ and N-SMZ exhibited such a high sorption capacity for toluene, a microbial support system comprised of SMZ or N-SMZ would provide protection from high toluene concentrations in the groundwater by significantly reducing the mass of toluene in the aqueous phase. We did not test at what concentration toluene becomes toxic to microbial populations, and 100 mg L^{-1} toluene is a fairly high concentration that is unlikely to occur within contaminated groundwater plumes. The high sorption capacity for organics, however, could be beneficial with respect to other organic contaminants that are toxic to microorganisms at lower concentrations.

Sorption and Desorption Kinetics

Figure 2 shows rates of toluene sorption and desorption on SMZ. Figure 2a indicates that sorption was rapid, with complete sorption occurring in approximately 1 h. Figure 2b shows that desorption was slightly slower, with complete desorption occurring in less than 2 h. An expression for a first-order desorption rate is

$$\frac{dS}{dt} = -k_r S \qquad [2]$$

where k_r (h^{-1}) is the desorption (or reverse sorption) rate coefficient and S (mg kg^{-1}) is the amount of toluene sorbed at time t. Linear regression of $ln\ (S/S_o) = -\ k_r\ t$ resulted in a k_r of 0.07 (± 0.01) h^{-1} and 0.06 (± 0.01) h^{-1} (numbers in parentheses represent standard error

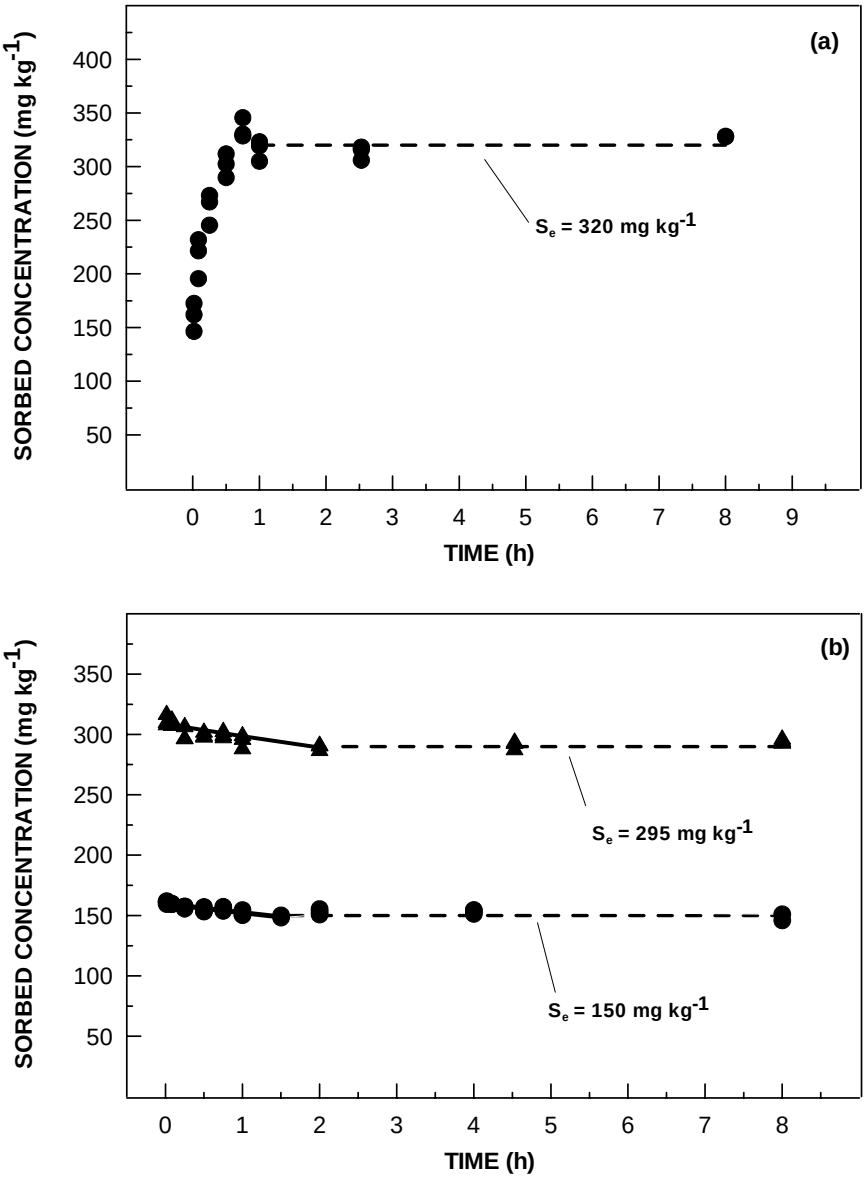

Figure 2. Toluene sorption/desorption kinetics on SMZ. **a)** Sorption kinetics. Initial toluene concentration in solution was 120 mg L^{-1}; **b)** Desorption kinetics. Initial toluene concentrations for the sorption step were 60 mg L^{-1} (circles) and 110 mg L^{-1} (triangles). Solid lines are based on linear regression of data by plotting $ln\ (S/S_o)$ *vs. t*.

at the 95% confidence level) for the initial solution concentrations of 60 mg L^{-1} and 100 mg L^{-1}, respectively. The data used to create these figures is in Appendix J, Tables A-J2 (a-c). The two k_r's are not statistically different at the 95% confidence level. Therefore, a mean k_r of 0.06 h^{-1} was used to characterize toluene desorption in the biodegradation studies.

The final sorption equilibrium concentrations, S_e (shown in Figure 5), for both desorption experiments were plotted versus their respective equilibrium solution concentrations (calculated from mass balance equations). Both of these desorption equilibrium points coincide with the sorption isotherms shown in Figure 1 (data not shown here, but can be found in Appendix I). This indicates that sorption of toluene by SMZ is a fully reversible process and suggests that a microbial support system comprised of SMZ could protect against high contaminant concentrations in the groundwater without limiting access to the toluene for biodegradation. Although it was not tested, since the sorption isotherms for SMZ and N-SMZ were very similar (Figure1), sorption/desorption kinetics for SMZ and N-SMZ are also likely similar and the above observations should hold true for N-SMZ.

Toluene Biodegradation

Solution-Only Experiments

Toluene was rapidly depleted in aerobic solution-only batch cultures (Figure 3). The data used to create this figure is in Appendix J, Table A-J3. The added aqueous concentration of 100 mg L^{-1} dropped in concentration to 86 mg L^{-1} at t = 0 due to partitioning between the solution and gas phases, as expected from the K_h of 0.25-0.26 at 25 °C (*21-22*). Figure 3 clearly indicates a lag phase of about 8 h followed by a period of biodegradation

until t = 21 h. Uninoculated controls showed no decrease in toluene concentration over 45 h (data not shown). Microbial biomass (indicated by total protein concentrations) increased concomitant with toluene disappearance (data not shown), demonstrating that toluene biodegradation was coupled to microbial growth.

We observed less than 100% biodegradation of toluene within the solution-only microcosms (Figure 3). The samples incubated for more than 21 h maintained a mean toluene concentration in solution of 6 (± 2) mg L^{-1}. Even up to 45 h, no downward trend in concentration was observed. Therefore, the microorganisms were able to degrade 92 (±2) % of the toluene. This incomplete degradation, which appeared throughout our experiments, is discussed in more detail later.

Biodegradation of organic contaminants is often described by first-order kinetics. The following equation was used to calculate the first-order biodegradation rate constant in solution, $k_{w,}$ over the period t = 8 to 21 h:

$$C_t = C_0 \exp^{\left[-k_w\left(t-t_{lag}\right)\right]} \qquad [3]$$

where C_0 = initial concentration in solution (mg L^{-1}), C_t = concentration in solution at time t (mg L^{-1}), and t_{lag} = lag time (h) (*23*). A plot of $ln\ (C_t / C_0)$ vs. $(t - t_{lag})$ resulted in a k_w of 0.24 (± 0.05) h^{-1} (numbers in parentheses represent standard error at the 90% confidence level).

SMZ and N-SMZ Slurry-Phase Experiments

Figure 4 shows the depletion of toluene in slurry-phase batch cultures. The data used to create this figure is in Appendix J, Tables A-J4. The added concentration of 100 mg L^{-1} dropped immediately due to partitioning between the solution, sorbed, and gas phases.

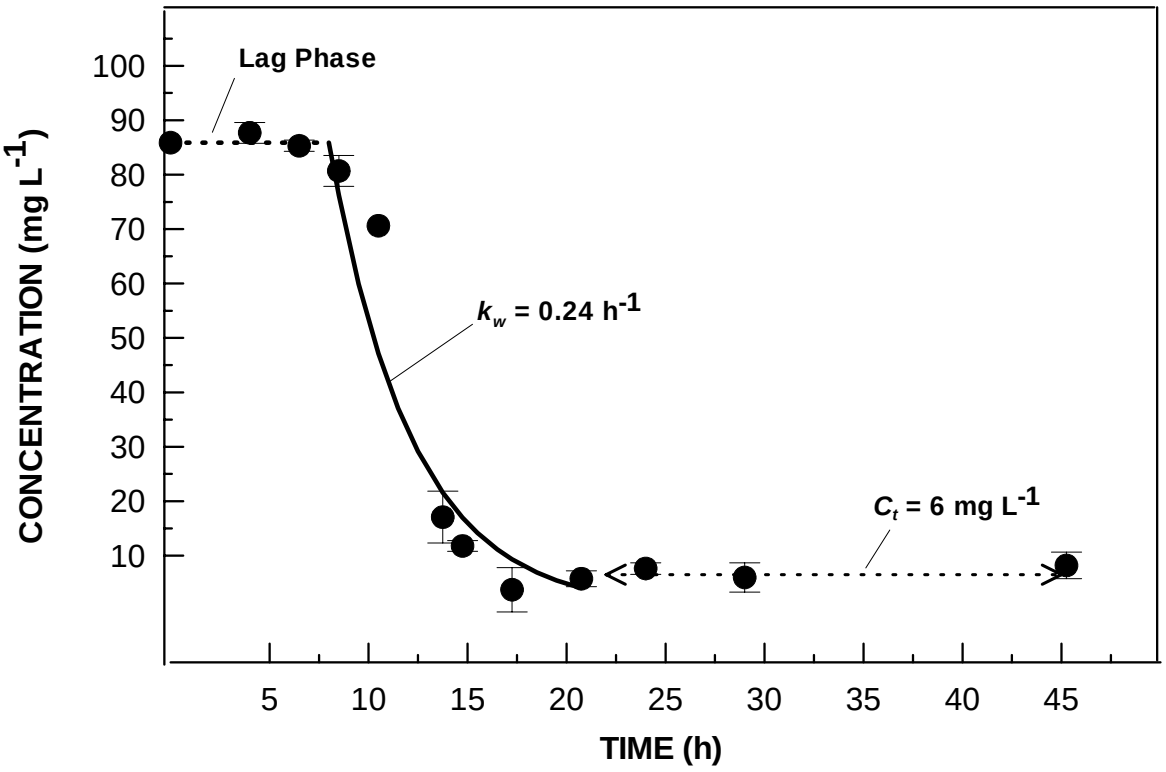

Figure 3. First-order biodegradation kinetics of toluene in solution-only batch cultures. Points are means of triplicates; error bars represent standard deviation. Solid line based on linear regression of data according to $ln\ (C_t / C_o) = -\ k_w\ (t\text{-}t_{lag})$ where t_{lag} = 8 h.

The uninoculated controls maintained a mean concentration of 22 (± 1) mg L^{-1} throughout the experiment. For SMZ-inoculated samples, we observed a lag phase of approximately 8 h followed by a biodegradation period. The lag phase for N-SMZ samples was longer, about 22 h (Figure 4). Consistent with the solution-only cultures, we did not observe 100% biodegradation of toluene within either of the slurry-phase microcosms (Figure 4). After a 25-h incubation period, toluene degradation ceased within the SMZ samples and the toluene concentration in solution increased, reaching a plateau of 4 (± 1) mg L^{-1}. The final concentration was equivalent to an 80 (± 7) % degradation of toluene originally present. The toluene concentration in the N-SMZ samples incubated for more than 32 h also increased and then maintained a mean concentration of 10 (± 1) mg L^{-1}, resulting in a 54 (± 5) % degradation of toluene. No downward trend in concentration was observed for either treatment after the initial biodegradation period. The pH within the microcosms did not reach the critical value of 6.5 that was previously found to inhibit microbial growth. Therefore, we concluded that pH was not inhibiting the biodegradation of toluene within the microcosms.

Previous research showed similar biodegradation results to those we found here. Robinson *et al*. (1990) studied the availability of sorbed toluene for biodegradation. They observed a similar elevated plateau in the aqueous concentration of toluene due to slowly desorbing toluene once microbial activity had ceased within the microcosms. In their case, however, they forced microbial degradation to cease by adding a lethal dose of hydrogen peroxide, while in our case, it is not clear whether microbial activity ceased within our microcosms due to limitations in nutrients, oxygen, or substrate levels.

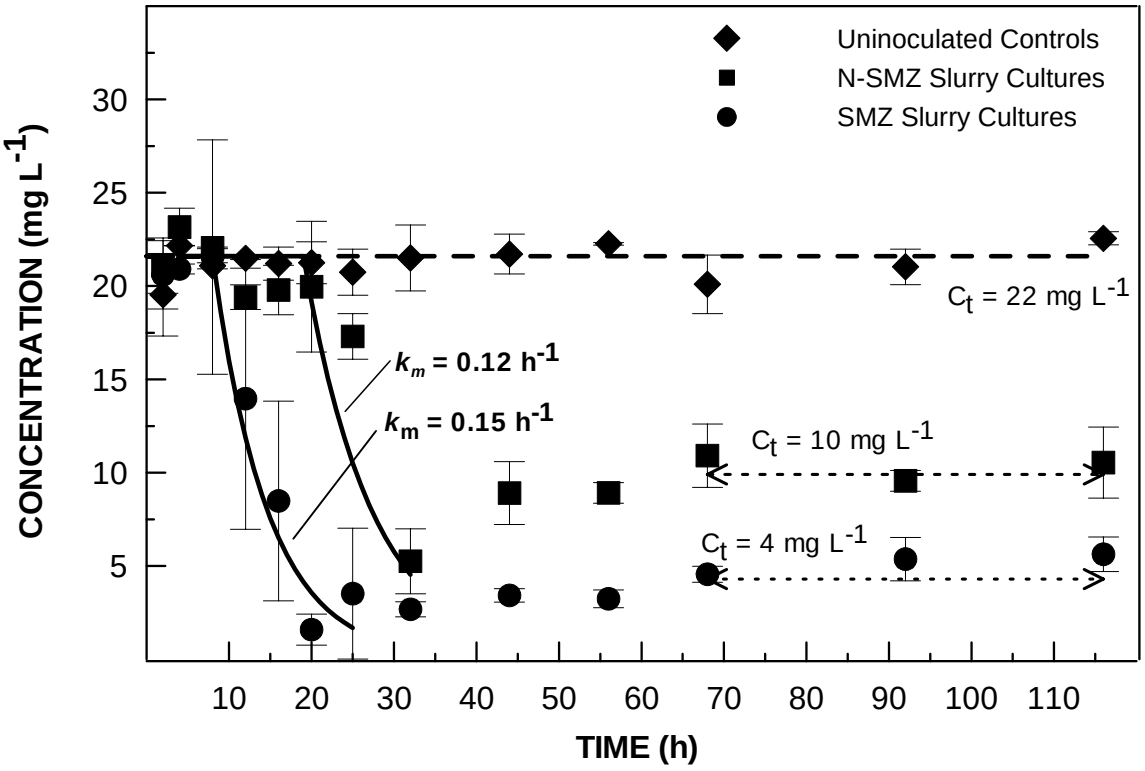

Figure 4. First-order biodegradation kinetics of toluene in slurry-phase batch cultures. Points are means of triplicates; error bars represent standard deviation. Solid lines are based on linear regression of data according to $ln(C_t / C_o) = - k_m(t-t_{lag})$ where t_{lag} = 8 h for SMZ cultures and 22 h for N-SMZ cultures.

Figure 4 shows that, although the N-SMZ samples had a longer lag time, biodegradation occurred at a similar rate in the SMZ and N-SMZ samples. Applying a first-order biodegradation model to the data, the mixed biodegradation-desorption rate constant (k_m) for the SMZ cultures was determined to be 0.15 (± 0.07) h^{-1} for t = 8 to 25 h. For the N-SMZ cultures, k_m was determined to be 0.12 (± 0.05) h^{-1} for t = 20 to 32 h. Statistical analysis confirmed that the k_m values for the SMZ and N-SMZ samples are not statistically different from each other, but are lower than the rate constant for solution-only cultures (k_w= 0.24 h^{-1}) at the 90% confidence level. The slower biodegradation rates in the presence of a sorbing solid phase are consistent with studies that have found biodegradation rates of organic contaminants to be slower in the presence of soil (*2,20,24*).

These experiments show that organic hydrocarbons such as toluene can be degraded in the presence of SMZ and N-SMZ when the pH is properly controlled. In addition, the use of N-SMZ eliminated the need to add nutrients to the system. Although we do not know what caused the incomplete degradation within the microcosms, one possibility is the lack of an unidentified nutrient or slow diffusion of a nutrient. This is the most likely explanation for the incomplete degradation of toluene in the N-SMZ cultures, and possibly the other cultures. Depletion of a required nutrient would cause biodegradation to stop at a certain point, followed by an increase in the toluene concentration in solution due to desorption. Another possible explanation is that once the concentration of toluene in solution becomes sufficiently low, the microorganisms may initiate or increase secretion of surfactants thereby increasing the toluene solubility. Because this would alter the distribution coefficient for toluene on SMZ or N-SMZ, as

well as the air-water partition coefficient for toluene, it could explain the apparent slight increase in the toluene concentration once biodegradation ceases. These are, however, only possible explanations and further experiments are required in order to explain the incomplete degradation within the microcosms.

Biodegradation Rate Model

To determine if the observed biodegradation rate in the slurry-phase system could be predicted from solution-only biodegradation kinetics and abiotic sorption/desorption characteristics, we combined the aqueous first-order biodegradation rate constant with desorption-related mass transfer parameters. Assuming that biodegradation only occurred in the solution phase and that nutrient, electron acceptor, and temperature limitations did not exist, we should be able to characterize biodegradation in the slurry-phase cultures with knowledge of the solution concentration and solution-only biodegradation rate constant (*24*).

First-order kinetics in the slurry-phase system can be expressed as

$$\frac{dC_T}{dt} = -k_m C_T \qquad [4]$$

where k_m is the "mixed" biodegradation-desorption rate constant and C_T (mg L^{-1}) is the total concentration of toluene in the system (*6, 24*). If we assume that only aqueous toluene is degraded, the appropriate rate expression is

$$\frac{dC_w}{dt} = -k_w C_w \qquad [5]$$

where k_w is the biodegradation rate constant in solution and C_w (mg L^{-1}) is the toluene concentration in solution (*24).* If B_f is defined as the mass fraction of toluene present in the aqueous solution, $C_w = B_f C_T$ and Equation 5 becomes

$$\frac{dC_w}{dt} = -B_f k_w C_T \qquad [6]$$

We then can define the mixed biodegradation-desorption rate constant, k_m, as $B_f k_w$.

Two potential models can be used to characterize the effect of desorption. Applying a desorption-equilibrium model, we assume that sorption is linear and desorption is instantaneous. Therefore, sorbed phase concentrations are assumed to be at equilibrium with the aqueous phase at all times ($S = K_d C_w$) and

$$B_f = \frac{1}{1 + K_d R_{s/w}} \qquad [7]$$

where $R_{s/w}$ is the solid-water ratio (mass: volume) (*6*, *24*). The biodegradation-desorption rate constant for the slurry-phase system is thus:

$$k_m = \frac{k_w}{1 + K_d R_{s/w}} \qquad [8]$$

Equation [8] is similar to the rate constant defined in Zhang *et al* (*6*) and Gamerdinger *et al* (*24*).

A desorption-limited model assumes that desorption is rate-limited and, therefore, takes into account the desorption rate coefficient, k_r. According to this model (*6*),

$$B_f = \frac{1}{1 + K_d R_{s/w}\left(1 + \frac{k_w}{k_r K_d R_{s/w}}\right)} \qquad [9]$$

and the mixed biodegradation-desorption rate constant becomes:

$$k_m = \frac{k_w}{1 + K_d R_{s/w}\left(1 + \frac{k_w}{k_r K_d R_{s/w}}\right)} \qquad [10]$$

Both approaches were used to estimate k_m for our slurry-phase system, using the measured parameters K_d = 13.0 L kg^{-1}, k_w = 0.24 h^{-1}, k_r = 0.06 h^{-1}, and $R_{s/w}$ = 0.25. Applying Eqs. [8] and [10] resulted in a k_m of 0.06 h^{-1} for the desorption-equilibrium model and 0.03 h^{-1} for the desorption-limited model. Application of the desorption-equilibrium model (Eq. 8) slightly underestimated the observed biodegradation-desorption rate constant for both the SMZ and N-SMZ slurry-phase systems where the measured k_m = 0.13 h^{-1} (Figure 5). The data used to create this figure is in Appendix J, Table A-J5. The desorption-limited model, however, greatly underestimated the measured toluene biodegradation in the slurry-phase microcosms. This suggests that, in our particular system, it is not necessary to account for rate-limited desorption in order to predict the biodegradation of toluene in SMZ or N-SMZ slurry systems. In addition, researchers recently have concluded that observed biodegradation rates higher than those predicted by coupled desorption/biodegradation models is evidence that sorbed substrates are available to the microorganisms for degradation (*26*).

This study indicated that toluene biodegradation occurs in the presence of SMZ and/or N-SMZ, but at a slower rate than in aqueous systems. The observed biodegradation in the slurry-phase systems is, however, faster than predicted by accounting for equilibrium toluene desorption. Thus, a minimum slurry-phase degradation rate can be estimated using the solution-only degradation rate and Eq. [8]. Desorption kinetics need not be considered.

One potential application of N-SMZ would be as a reactive permeable barrier that could be placed in the path of a groundwater contaminant plume. The expectation is that the

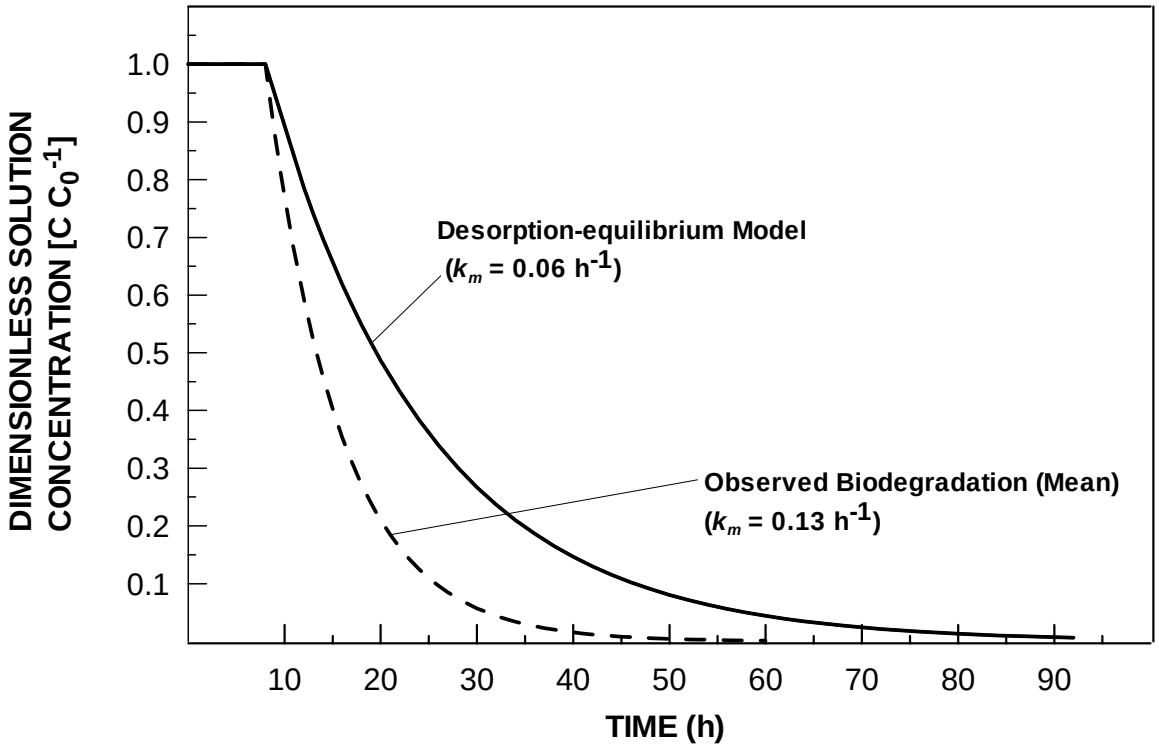

Figure 5. Comparison of predicted biodegradation-desorption rate (desorption-equilibrium model only) and observed biodegradation-desorption rate. Solid line based on k_m calculated using Eq. 8; broken line based on the observed average k_m values for SMZ and N-SMZ slurry cultures.

contaminated groundwater would emerge from the barrier with acceptable contaminant levels. To test the implementation of an *in situ* permeable reactive barrier, the 1-D modeling code CXTFIT2 (*27*) was used to predict the biodegradation of a groundwater toluene plume within a 1-m thick N-SMZ barrier. The simulation assumed the biodegradation rate constants were equal to the biodegradation rate constant we observed in the slurry-phase biodegradation experiments (k_m = 0.13 h^{-1} for SMZ and N-SMZ). We used additional input data consistent with a permeable barrier pilot test by Bowman *et al* (*14*) (a dispersion coefficient (D) of 0.23 m^2 d^{-1}; a pore-water velocity (v) of 0.23 m d^{-1}; a volumetric water content (θ) of 0.6; and a bulk density (ρ_b) of 1.0 kg L^{-1}).

The CXTFIT2 simulations are shown in Figure 6. The data used to create this figure is in Appendix J, Table A-J6. The code predicted that a groundwater contaminant plume with an input concentration of 100 mg L^{-1} toluene would emerge from a 1-m N-SMZ barrier with a concentration < 1 mg L^{-1} once steady-state was attained (Figure 6). Federal drinking water standards allow a maximum concentration of1 mg L^{-1} for toluene (*28*). Therefore, a N-SMZ barrier would remediate such a toluene plume below the federal requirement.

N-SMZ has the potential to be used as the material for a permeable reactive barrier placed in the path of a contaminant plume. Because N-SMZ has a strong affinity for toluene, the microbial community will be protected from high contaminant concentrations. In addition, the reversible nature of toluene sorption should allow sustained degradation of toluene as it desorbs from N-SMZ. The nutrient portion of N-SMZ can also be customized to optimize biodegradation of various contaminants for site-specific conditions.

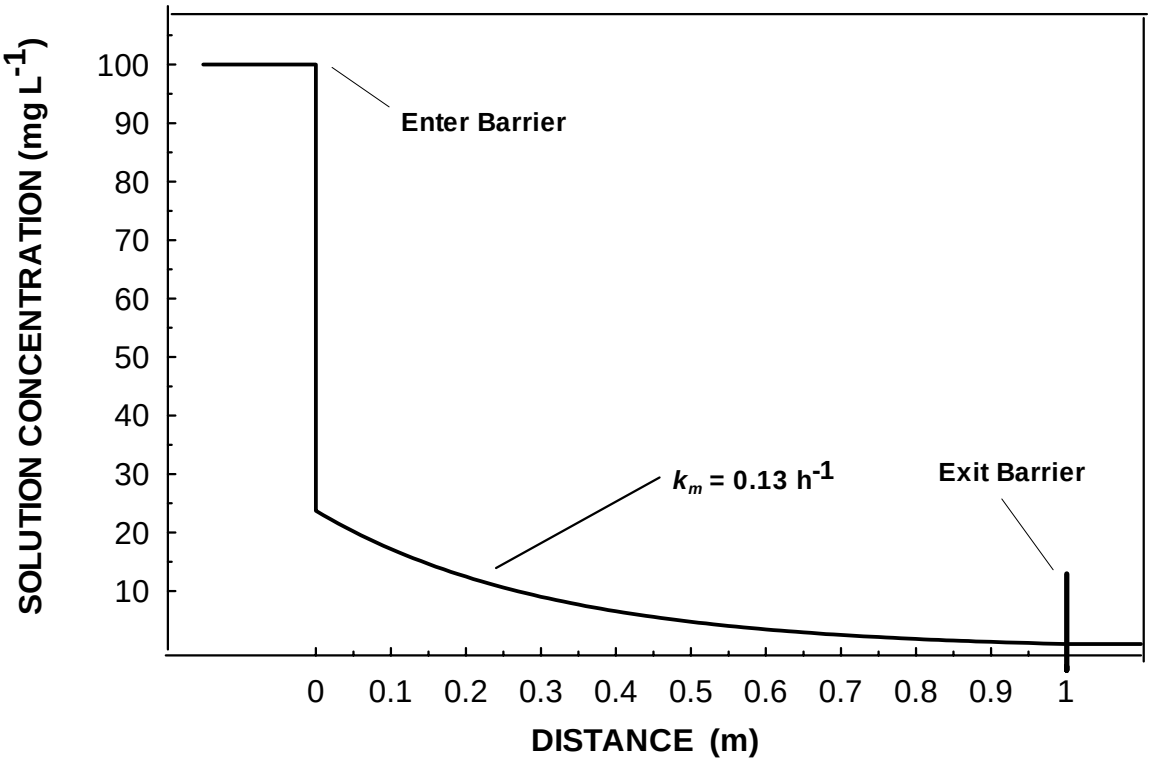

Figure 6. Predicted steady-state concentration profile of a 100 mg L^{-1} toluene plume as it passes through a 1-m N-SMZ reactive barrier. Simulation assumes the following: a dispersion coefficient (D) of 0.23 m^2 d^{-1}; a pore-water velocity (v) of 0.23 m d^{-1}; a volumetric water content (θ) of 0.6; and a bulk density (ρ_b) of 1.0 kg L^{-1} (*14*).

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Mary McHale and Bridget Rutz performed DNA and protein analysis, respectively; Doug McGhee assisted with biodegradation and sorption-desorption kinetic experiments. This research was supported by a grant from the New Mexico Waste-management Education and Research Consortium.

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ADDITIONAL EXPERIMENTS AND CONCLUSIONS

AEROBIC SLURRY-PHASE DEGRADATION EXPERIMENTS

Prior to the biodegradation experiments reported in the manuscript, aerobic and anaerobic continuous cultures of toluene-degrading microorganisms were isolated from three different petroleum-hydrocarbon contaminated soils (See Appendix F). Using the aerobic continuous culture and SMZ (unwashed), I conducted a concurrent solution-only and SMZ slurry-phase biodegradation experiment under aerobic conditions. This initial experiment resulted in no apparent toluene degradation or microbial growth within the SMZ microcosms. The details of this experiment can be found in Appendix F.

Due to the unsuccessful results, I performed several experiments to determine if excess HDTMA in solution or sorption of nutrients by SMZ was limiting the microorganisms' growth. The studies indicated that if the SMZ was not washed prior to inoculation, excess surfactant would be released into solution and would indeed prohibit microbial growth within the microcosms. The experiments were inconclusive with respect to whether or not microbial growth was inhibited by sorption of the nutrients (See Appendix F). Therefore, for the aerobic slurry-phase experiment reported in the manuscript, I obtained an activated sludge sample from the WWTP and I column-washed the SMZ to eliminate excess HDTMA in solution.

Despite these changes, I still did not observe consistent microbial growth within SMZ slurry-phase microcosms. I conducted pH experiments to determine the effect a decreasing pH would have on microbial growth. The results indicated it was necessary to control pH during slurry-phase biodegradation experiments because inconsistent microbial growth was observed when the pH was less than 6.5. The specifics and results of the pH experiments are found in Appendix F.

I then performed a successful aerobic biodegradation experiment, the results of which are found in the previous manuscript. However, due to the incomplete degradation of toluene within the microcosms, I decided to repeat the aerobic SMZ slurry-phase experiment with closer pH monitoring. The details and results of this experiment, found in Appendix G, indicate that the pH within the pH-controlled microcosms never reached a pH low enough to cause incomplete biodegradation of toluene.

Therefore, it remains unclear why the biodegradation of toluene appeared to cease within the slurry-phase microcosms. Further investigation into this area is needed.

ANAEROBIC SLURRY-PHASE DEGRADATION EXPERIMENTS

I also performed an anoxic SMZ slurry-phase biodegradation experiment that used nitrate as the electron acceptor instead of O_2. Because the experiment was inconclusive, however, the results were not included in the manuscript. The experiment was set up in a similar manner as the aerobic experiments except that 1) all solutions and SMZ were purged with N_2 prior to the experiment to provide an anoxic environment; and 2) no headspace was allowed to remain within the microcosms. Although I observed what appeared to be complete degradation of toluene within the solution-only treatments,

I did not have sufficient time samples to determine if there was any degradation of toluene within the SMZ slurry-phase samples. Appendix H contains a very detailed description of the procedures used and the results obtained from this sole anaerobic experiment. Due to insufficient time, the anaerobic experiment was never repeated or investigated further.

SUMMARY

Results from batch isotherm experiments confirmed that HDTMA-modified St. Cloud zeolite and ZeoPro™ have a similar sorption capacity for toluene, while raw (natural) zeolite has almost no ability to sorb toluene from solution. The sorption isotherms for SMZ and N-SMZ were linear with a mean K_d of 13.0 L kg^{-1}. Subsequent sorption and desorption kinetic experiments with SMZ provided evidence that toluene sorption and desorption on SMZ reached equilibrium within 1h and 2 h, respectively. Linear regression of the desorption data collected from two separate experiments with distinct initial toluene concentrations resulted in a K_r of 0.04 h^{-1}.

Aerobic and anaerobic continuous cultures of toluene-degrading microorganisms were isolated from petroleum-hydrocarbon contaminated soils. These cultures, however, seemed to have trouble growing in the presence of SMZ. Therefore, an aerobic toluene-degrading culture was obtained by enrichment of an inoculum obtained from a wastewater treatment plant. This culture was used to inoculate the samples for the reported aerobic biodegradation experiments. Subsequent 16S rDNA analysis showed that the toluene-degrading culture was dominated by *Pseudomonas veronii*.

An aerobic solution-only biodegradation experiment provided evidence that the first-order toluene biodegradation rate was 0.24 h^{-1} in solution-only microcosms. Preliminary studies necessitated that I wash the SMZ/N-SMZ to remove excess HDTMA and control the pH of the slurry-phase microcosms in order to achieve microbial growth in the presence of SMZ and/or N-SMZ. Subsequent SMZ and N-SMZ slurry-phase biodegradation experiments resulted in a reduced mean biodegradation rate of 0.13 h^{-1}. Although similar biodegradation rates were observed within the SMZ and N-SMZ microcosms, the N-SMZ slurry cultures had a longer lag time.

I attempted to establish a model that could predict the toluene biodegradation rate in the presence of SMZ or N-SMZ based on the observed solution-phase biodegradation rate and toluene desorption rate. However, the predictive model underestimated observed biodegradation rates in both the SMZ and N-SMZ slurry-phase cultures. Finally, CXTFIT2 direct simulations suggest that if a 1-m wide N-SMZ subsurface permeable barrier is placed in the path of a 100-ppm toluene contaminated plume, toluene will be degraded to below the Federal Drinking Water Standard.

CONCLUSIONS

From the evidence presented herein, it is possible to derive the following conclusions:

- Surfactant-modified zeolite and ZeoPro™ show enhanced sorption of toluene from solution and, therefore, have the ability to retain toluene in the subsurface and protect microbial communities from high contaminant concentrations.

- The linearity of the sorption isotherms suggests that partitioning is the likely mechanism for the sorption of toluene on both SMZ and N-SMZ.
- Sorption and desorption of toluene on SMZ is rapid and reversible. The reversible nature suggests that sorption by N-SMZ will not limit microbial access to carbon sources such as toluene and the rapid desorption rate suggests that sorption by N-SMZ will not limit the rate of microbial degradation.
- Biodegradation of toluene will occur in the presence of SMZ if the pH within the system is properly monitored and controlled (when necessary).
- SMZ preloaded with nutrients (N-SMZ) can support substantial biodegradation of toluene without adding an aqueous nutrient medium.

SUGGESTIONS FOR FUTURE WORK

Based on the findings of this research, the following are recommended as future studies:

- Biodegradation experiments with SMZ and N-SMZ should be repeated with the goal of determining why the toluene concentration within the microcosms reached a plateau. Instead of analyzing the concentration of toluene in solution only, the total mass of toluene within the microcosms (sorbed and in solution) should be analyzed with a total extraction technique.
- A study should be conducted that focuses on the isolation of anaerobic toluene-degraders and the feasibility of using a microbial support system in an anoxic environment. This is significant because microorganisms rapidly deplete oxygen within the subsurface, causing the bioremediation of contaminant plumes to be limited by the

absence of an electron acceptor(s). I am currently working on a continuation of this project that is moving forward with this work.

- In light of the original goals of this project, the production and use of nutrient and nitrate-amended SMZ (NN-SMZ) should be investigated. Further biodegradation batch studies, similar to the experiments reported here, should be conducted with the NN-SMZ.
- In addition to the batch studies reported herein, column studies should be conducted to investigate the biodegradation of hydrocarbon contaminants flowing through N-SMZ/NN-SMZ. This should also provide information on whether pH will ultimately inhibit microbial growth within a permeable reactive barrier.
- Based on the results of these experiments, a pilot study should be conducted at a field site contaminated with gasoline in order to examine the feasibility of *in situ* bioremediation of contaminated groundwater with amended SMZ.

INTRODUCTION TO APPENDICES

The following appendices provide further information on preliminary and unreported studies, more detailed descriptions of experimental conditions, and significant observations.

Appendix A contains a description of the two methods I used to prepare toluene solutions for each of my experiments.

Appendix B contains a description of the technique used to transfer toluene (neat or in solution) into individual sample vials. The technique was used throughout my research and is referred to as the "rapid open-vial transfer technique."

Appendix C presents a detailed discussion of the headspace autosampler used throughout this research, including how I determined the proper equilibration time for the samples to be thermostatted in the oven.

Appendix D contains the method used to determine the specific air-water partition coefficient (K_h) for toluene in the relevant solvents.

Appendix E discusses the gas chromatographic method used to quantify toluene in the aqueous samples including calibration of the gas chromatograph.

Appendix F discusses the first aerobic slurry-phase SMZ biodegradation experiment I attempted and the problems that arose. It also contains the troubleshooting

experiments that were conducted to isolate what was inhibiting microbial growth within the SMZ slurry-phase microcosms.

Appendix G discusses the final aerobic slurry-phase SMZ biodegradation experiment with pH monitoring.

Appendix H contains a discussion of the anaerobic SMZ slurry-phase biodegradation experiment I attempted.

Appendix I provides more details regarding the desorption kinetic experiments.

Appendix J contains the experimental data that was used to create Figures 1-5 in Chapter 2 (manuscript submitted to ES&T).

Appendix K contains a list of literature citations used in the Appendix section.

APPENDIX A. PREPARATION OF TOLUENE SOLUTIONS

In the preliminary stages of this project, the volatilization of toluene needed to be addressed. Because toluene has a high Henry's Constant at room temperature (K_h of 0.25-0.26 at 25 °C), any prepared toluene solutions would be subject to significant mass loss due to partitioning between the solution and gas phases (Mackay, 1979; National Academy of Sciences, 1980). Therefore, it was necessary to develop methods for dispensing toluene solutions with constant concentrations into multiple sample vials (by minimizing toluene loss due to volatilization). Depending on the circumstances of the experiment, two distinct methods were developed to prepare samples with a uniform initial toluene concentration. For either method, I used a "rapid open-vial transfer technique" to dispense the toluene solution or neat toluene into individual sample vials (See Appendix B).

One method used was to prepare and contain a toluene solution in a 10-L (12" x 19") Tedlar® Gas Sampling Bag (Alltech, Deerfield, Ill.) with a barbed on/off valve. First, I siphoned approximately 5 L of reverse osmosis (RO) water into the bag through the on/off valve. If the experiment involved the use of nutrient medium (i.e., the biodegradation or kinetic experiments), I first prepared 5 L of B-H broth in volumetric flasks and then siphoned it into the bag. I extracted any large air bubbles in the bag with a glass 10-mL syringe through the on/off valve. I then added the appropriate volume of neat toluene with a glass 2.5-mL Hamilton gas-tight syringe through the on/off valve and distributed the toluene throughout the bag using manual agitation. Because 20-30 % of the toluene sorbed to the inside of the gas-sampling bag, the solution was allowed to equilibrate in the bag for 24 hours with frequent agitation. Although the initial toluene

concentration was lowered by sorption to the bag, the main concern was that the samples all received a uniform toluene concentration.

After the equilibration period, a glass 10-mL Hamilton gas-tight syringe was used to withdraw 4 or 5 mL of solution from the on/off valve. The bag contracted as the toluene solution was extracted with the syringe, thereby minimizing toluene loss due to volatilization. The solution was transferred from the syringe (see Appendix B) to a 10-mL headspace vial and immediately sealed with an aluminum crimp-cap fitted with a Teflon-faced butyl rubber septum (Supelco, Inc., Bellefonte, PA). Several samples of equal volume were collected in this way over a 2 to 4-hour period and analyzed with the GC immediately to ensure that the toluene concentration had stabilized.

Once it was confirmed that equilibrium had been reached within the gas-sampling bag, the appropriate volume of toluene solution was transferred (in the same manner) into the requisite number of sample vials for a particular experiment. If necessary, a larger Hamilton glass gas-tight syringe was used to extract and transfer the toluene solution into the sample vials. Throughout the sample preparation, I would periodically prepare blanks by transferring 4 or 5 mL of solution to 10-mL headspace vials and immediately analyze the blanks with the GC to ensure that the toluene concentration in the gas-sampling bag was not changing.

Quantitative analysis of the blanks substantiated that the gas-sampling bag transfer method resulted in uniform initial toluene concentrations among replicate samples. The blanks from an aerobic and anaerobic biodegradation experiment, as well as a desorption kinetics experiment, can be seen in Figures A.A1-A.A3. The data used to create these figures is found in Tables A.A1-A.A3.

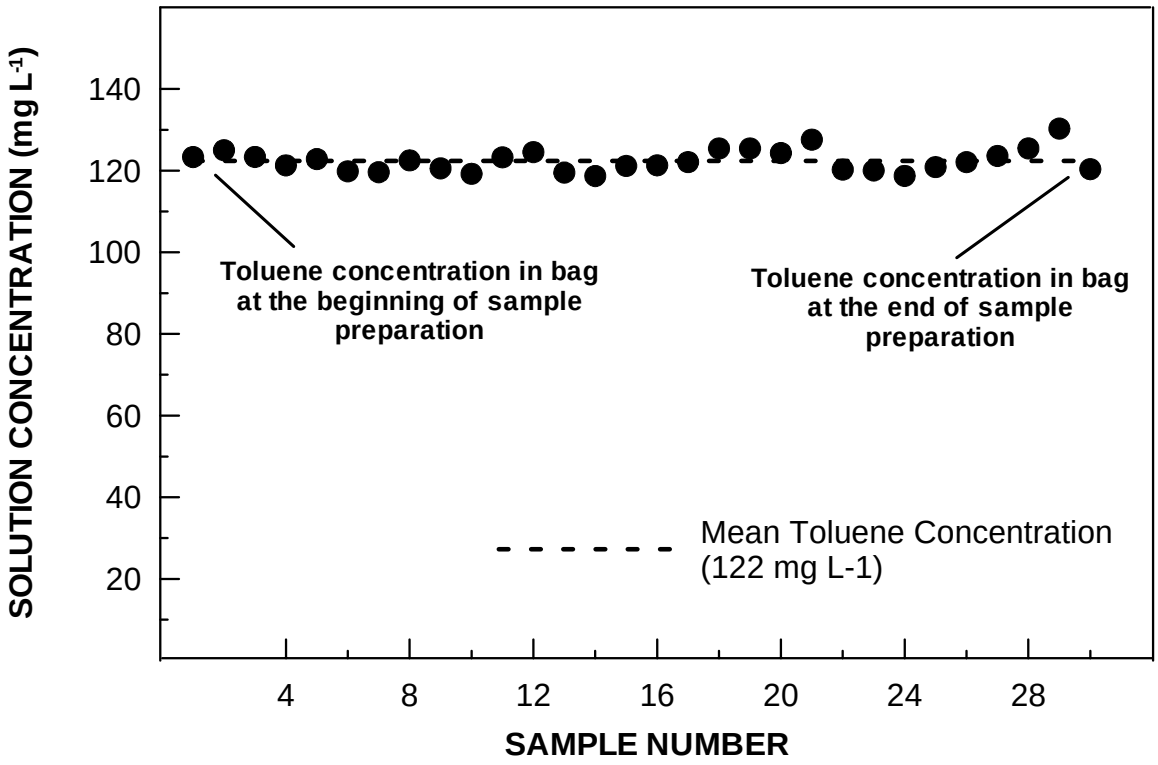


Figure A-A1. Toluene concentration in 5-mL blanks prepared over a time period of approximately 3 h while dispensing solution from Tedlar® gas-sampling bag into sample vials for aerobic biodegradation experiment.

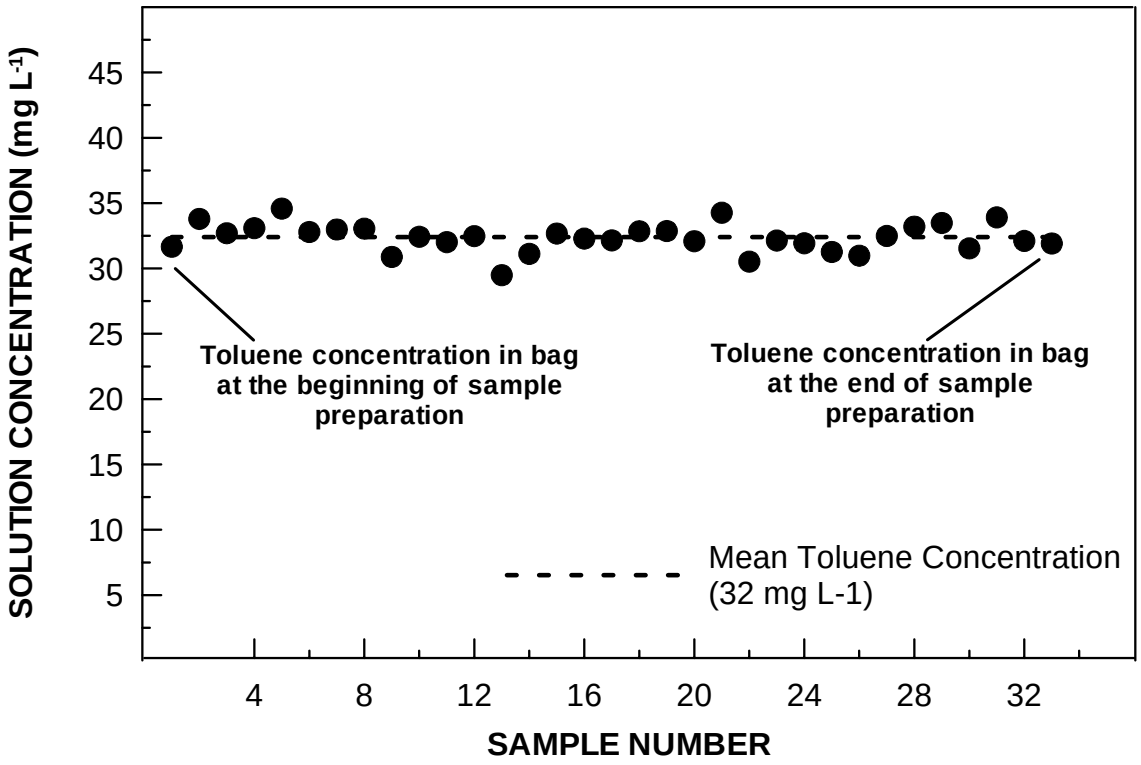


Figure A-A2. Toluene concentration in 4-mL blanks prepared over a time period of approximately 3 h while dispensing solution from Tedlar® gas-sampling bag into sample vials for anaerobic biodegradation experiment.

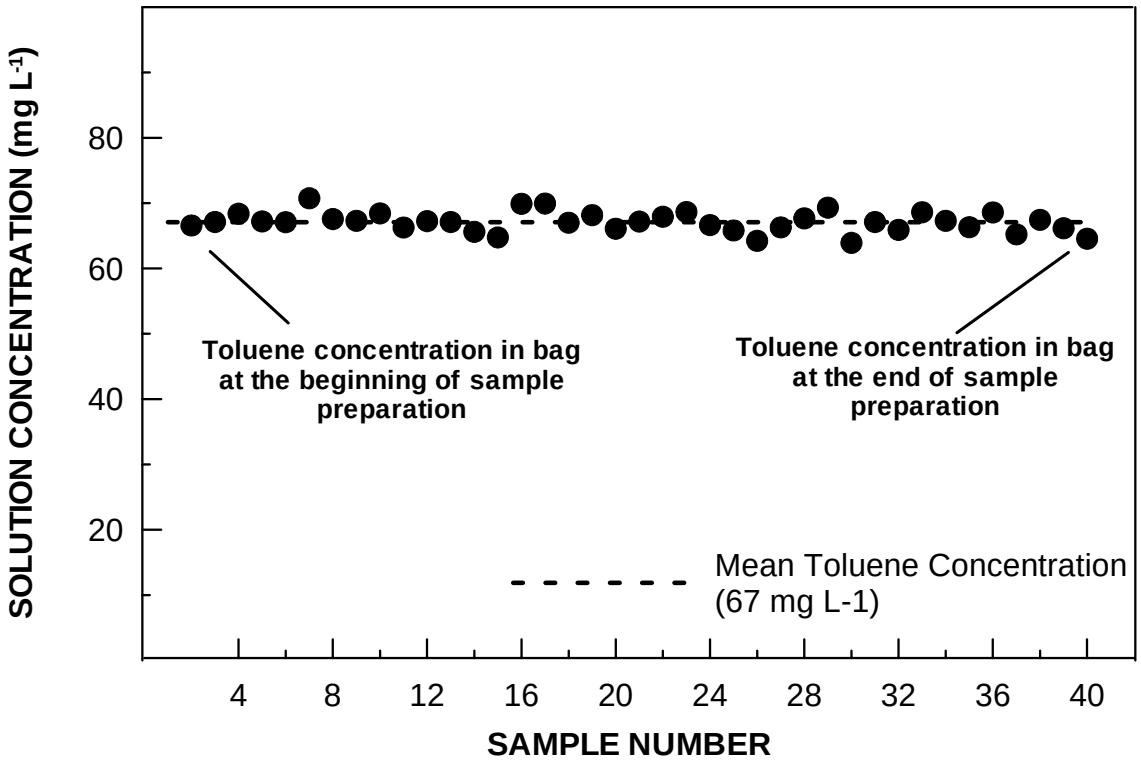


Figure A-A3. Toluene concentration in 5-mL blanks prepared over a time period of approximately 2 h while dispensing solution from Tedlar® gas-sampling bag into sample vials for desorption kinetics experiment.

For the aerobic biodegradation experiment, the mean toluene concentration was 122.4 (±1.0) mg L^{-1} with a standard deviation of 2.7 mg L^{-1}; for the anaerobic biodegradation experiment, the mean toluene concentration was 32.4 (±0.4) mg L^{-1} with a standard deviation of 1.1 mg L^{-1}; and for the shown desorption kinetics experiment, the mean toluene concentration was 67.3 (± 0.6) mg L^{-1} with a standard deviation of 1.8 mg L^{-1} (numbers in parentheses indicate 95% confidence intervals for the means).

If this method was not suitable for a particular experiment, I prepared the toluene solutions individually in each sample vial by first adding the appropriate volume of water or nutrient medium to the vial and then adding toluene (neat) to each vial with a Hamilton glass microliter syringe. For example, the gas-sampling bag transfer method could not be used for the final aerobic biodegradation experiments because it was necessary to equilibrate the SMZ with the pH-controlled nutrient medium 24 hours prior to adding the toluene or inoculum. The consistent toluene concentration of the uninoculated controls in Figure 4 (Chapter 2) and Figure A-G1 (Appendix G) provide evidence that this technique resulted in uniform initial toluene concentrations among replicate samples.

APPENDIX B. TOLUENE TRANSFER TECHNIQUE

I used an open-vial transfer technique to dispense any toluene or toluene-containing solution into sample vials, instead of piercing the Teflon-faced butyl rubber septa of already closed vials (Kolb, 1997). I used this technique when preparing experimental samples, as well as when I needed to transfer supernatant to headspace vials for GC analysis (See Appendix C). This was done because piercing the septum could introduce errors when a volatile sample remained in a vial for any length of time. In particular, once the inert Teflon protective layer had been pierced, the butyl rubber material would be accessible to sorb toluene from the vial.

To minimize toluene loss, the transfer needed to be done as quickly as possible. Therefore, I will refer to it as the "rapid open-vial transfer technique." First, the aluminum crimp-cap fitted with a Teflon-faced butyl rubber septum was loosely placed on top of the headspace vial, but was not crimped. A small gap remained open, through which the syringe needle was inserted towards the bottom of the vial. If injecting neat toluene, I would place the needle's tip below the surface of the solution. As quickly as possible, the sample was injected, the needle was withdrawn, and the crimp-cap was pressed onto the vial and crimped (See Figure A-B1).

Figure A-B1. Sketch of “rapid open-vial transfer technique” immediately after injecting neat toluene sample, prior to rapidly withdrawing syringe needle (adapted from Kolb, 1997).

APPENDIX C. STATIC HEADSPACE CHROMATOGRAPHY METHOD

Because toluene, a highly volatile compound, was our compound of interest, I used static headspace-gas chromatography (HS-GC) for all analyses. During the initial stages of this project, we procured a stand-alone headspace autosampler (Hewlett-Packard Model G1290A) to aid in the static headspace chromatographic analysis of toluene. Therefore, it was necessary to determine the proper operating conditions for the headspace autosampler and develop a static headspace chromatographic method.

Unlike purge-and-trap (dynamic headspace) analyses, where the total mass of a volatile compound is purged from a liquid/solid sample by a continuous flow of an inert gas, static or equilibrium headspace analysis is conducted by placing the liquid/solid sample in a headspace vial and sealing it, heating the vial at a constant temperature until the liquid and gas phases have reached equilibrium, and then introducing an aliquot of the gas phase into the GC. (Kolb, 1997). In our case, the gas sample transfer was carried out automatically by means of a headspace autosampler pressure/loop system.

There are four steps to the pressure/loop system for headspace introduction into the GC. First, the headspace vial is placed in the temperature-controlled oven for the required equilibration time. While in the oven, the vial can be agitated. Second, when the equilibration time is over, a needle or probe is pierced through the vial's septum and the sample vial is pressurized by the carrier gas. Third, the pressurized headspace sample is vented to a sample loop. Fourth, once the sample loop is filled and equilibrated, the contents of the loop is carried to the GC injector through a heated inert transfer line. (Kolb, 1997).

Equilibration Time Determination

The required equilibration time in the oven depends on the sample (both the sample matrix and the analyte), the thermostatting temperature, the sample volume (i.e., the phase ratio), and whether or not the sample is shaken while it is heated. (Kolb, 1997). For this project, the time required for each sample to equilibrate within the oven was established by preparing several identical 5-mL aqueous toluene samples in 10-mL glass headspace vials. The samples were placed in the autosampler, under identical conditions, and were thermostatted at 65 °C for increasing times. I chose 65 °C because I wanted a temperature high enough to increase my headspace sensitivity, but low enough to ensure a short equilibration time.

The resulting GC peak areas were plotted against the thermostatting time. The equilibration time was the shortest time the sample had to be heated for a constant peak area to be obtained. In other words, even if the sample were thermostatted longer than the determined equilibration time, the peak area generated by the chromatogram would not change.

Results and data from this experiment can be seen in Figure A-C1 and Table A-C1. At first, I did not shake my samples while they were being heated in the oven. Although there was no apparent disadvantage to shaking the vials, I was not sure that it would be necessary. I found, however, that my equilibration time was inconveniently long without shaking. I ran the equilibration experiment again, but this time I agitated the sample vials during the thermostatting process. This equilibration experiment indicated that my samples should be equilibrated for 20 minutes at 65 °C (while shaken) prior to GC injection.

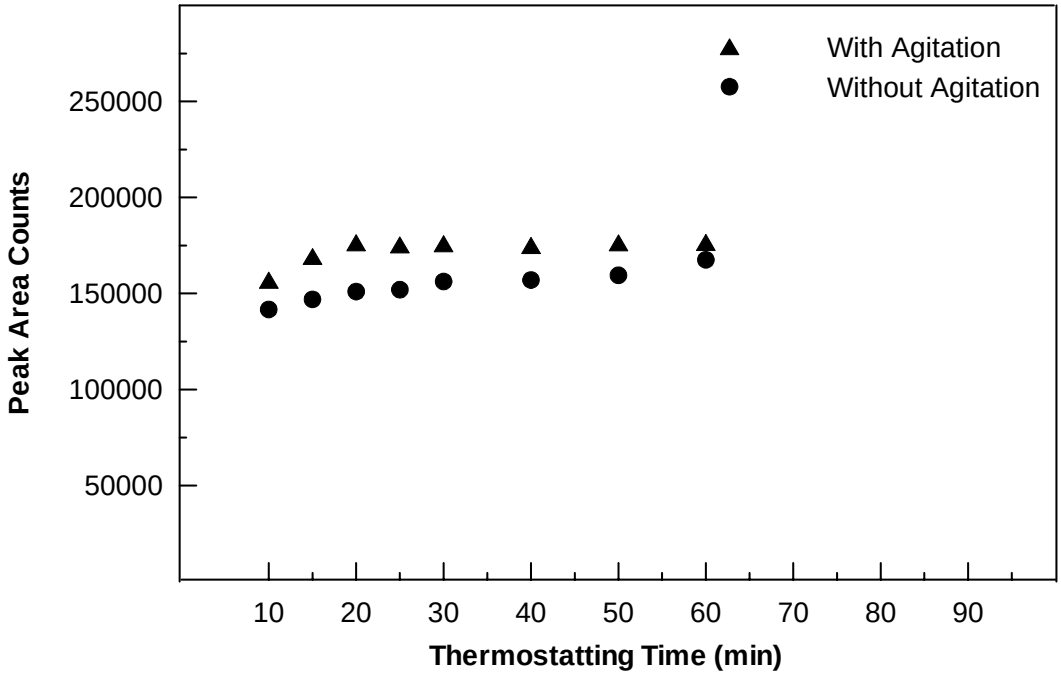


Figure A-C1. Equilibration time experiment. Each sample was 5 mL aqueous toluene solution (5 mg L^{-1}) in a 10 mL headspace vial that was thermostatted at 65° C.

Table A-C1. Data used for headspace autosampler equilibration experiment (used to create Figure A-C1).

Time (min) (no agitation)	Area	Time (min) (agitated)	Area
10	141701.6	10	156956.0
15	146956.0	15	169252.3
20	151012.3	20	176437.8
25	151998.7	25	175278.9
30	156252.3	30	175937.6
40	157039.8	40	175028.9
50	159489.3	50	176393.8
60	167553.2	60	176637.0

Static Headspace Chromatographic Method

The headspace autosampler operating conditions and parameters used for each experiment can be seen in Table A-C2.

Table A-C2. HP Model G1290A headspace autosampler operating conditions and parameters for toluene analysis.

Zone Temperatures		**°C**
	Oven	65
	Loop	70
	Transfer Line	70
Event Times		**Minutes**
	Vial Equilibration	20
	GC Cycle	2.5
	Pressurization	0.2
	Loop Fill	0.2
	Loop Equilibration	0.05
	Injection	0.5
Vial Parameters		
	Shake	High
Autosampler Parameters		
	Sample Loop Size	1
	Transfer Line Length	

For all experiments, each treatment sample was sacrificed and analyzed for toluene in the same manner. To achieve a sufficiently high analyte concentration in the headspace and, thereby, increase headspace sensitivity, I needed to transfer an adequate volume of the treatment sample for analysis. I transferred a 5-mL aliquot of each treatment sample into a 10-mL headspace vial. This resulted in a sample/vial phase ratio of approximately 0.4 because the average volume of the 10-mL vials is actually 12.5 mL.

With this phase ratio and equilibration temperature, I was able to detect toluene concentrations down to 0.5 mg L^{-1}. Because my biodegradation experiments started at 100 mg L^{-1} toluene and the drinking water standard for toluene is 1 mg L^{-1}, this phase ratio was appropriate.

The 5 mL of supernatant was withdrawn from each sacrificed treatment vial using a disposable 10-mL sterile syringe and 26s gauge needle. An additional 26s gauge needle was used as a vent. The solution quickly was transferred, using the "rapid open-vial transfer technique," into a 10-mL glass crimp-top headspace vial. I did not evaluate the potential sorption of toluene by the plastic syringe. The withdrawal and transfer of the supernatant, however, was completed within a few seconds. Therefore, although this was a potential source of error, I do not feel that it was significant.

The transferred subsamples were immediately placed in the headspace autosampler for analysis, resulting in a very short residence time for the toluene solutions in the headspace vials. However, I used the open-vial technique to transfer the solutions (instead of piercing the septum of an already closed vial) because if the septum was punctured prior to the thermostatting and pressurization steps of the headspace autosampler system, toluene volatilization or sorption to the septum could occur. In addition, a second piercing by the probe could deform the septum and result in leakage during the pressurization step.

APPENDIX D. K_h DETERMINATION METHOD

Based on the above headspace autosampler operating conditions, I experimentally determined a specific air-water partition coefficient, K_h, for toluene in water, nutrient medium (B-H broth), and water with HDTMA in solution. To do this, I used the Vapor Phase Calibration (VPC) method outlined by Kolb (1997). This method is based on the total vaporization of a small amount of the pure analyte in a headspace vial Because there is only one phase (vapor) within the headspace vial, the sample can then used as a calibration standard.

First, neat toluene (10 μl) was transferred into a 10-mL headspace vial. The sample was thermostatted so that the entire amount of the toluene evaporated and was analyzed with the GC. Then, a second sample was prepared in a 10-mL headspace vial with 5 mL of the relevant solvent and the same amount of toluene (10 μl). This sample was thermostatted for the proper equilibration time and was analyzed with the GC.

The partition coefficient, K_h, was calculated according to the following equation:

$$K_h = \frac{A_C \; x \; V_V \quad - \quad A_G \; x \; V_G}{A_G \; x \; V_S} \qquad [D1]$$

where V_V was the total volume of the headspace vial; V_S was the volume of the solvent added to the vial; V_G was the volume of the headspace in the solvent-containing vial; A_G was the peak area obtained for the solvent-containing vial; and A_C was the peak area obtained for the "calibration vial" (vial with no solvent) (Kolb, 1997).

I completed triplicate measurements for three different solvents (water, B-H broth, and dissolved HDTMA). The HDTMA-solution sample was prepared by

extracting the aqueous supernatant from a 1:4 slurry of SMZ (target loading of 130 mmol kg^{-1} HDTMA) and water following a 24-hour equilibration period. The exact concentration of HDTMA in solution was not measured. However, at the time, I believed that it would represent the HDTMA concentration we would observe within our SMZ slurry-phase samples. Based on these measurements, the mean K_h values for toluene were 0.42 (± 0.04) in water, 0.43 (± 0.04) in B-H broth, and 0.40 (± 0.02) in the HDTMA-solution sample. Analysis of variance confirmed that the three K_h values were not statistically different, resulting in a mean K_h value of 0.42.

APPENDIX E. CHROMATOGRAPHIC METHOD

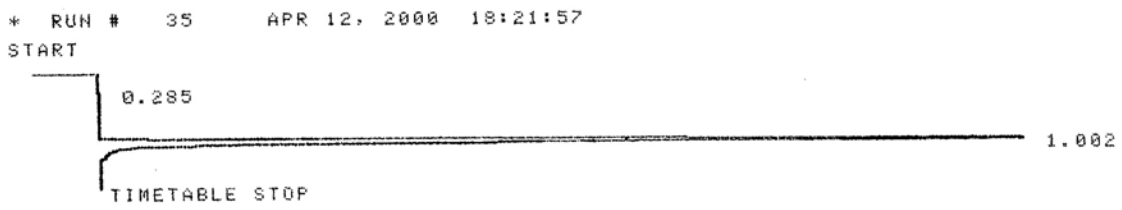
All chromatographic analyses performed during this project were carried out using the method outlined in the Materials and Methods section of the ES&T manuscript (Chapter 2).

The GC settings can be seen in more detail in Table A-E1. All chromatographic data was recorded using an integrator (HP Model 3396A). Attenuation was set at 5 and the chart speed was set at 2. The integrator was programmed to stop recording the GC run at 1.7 minutes. An example of a typical chromatogram can be seen in Figure A-E1.

I calibrated the flame ionization detector (FID) response by running samples with increasing toluene concentrations from 5 to 250 mg L^{-1} (aq). An example of one of my calibration curves can be seen in Figure A-E2. The data used to create this figure is found in Table A-E2. I consistently observed a linear response up to 250 mg L^{-1} toluene. Because I did not analyze samples with a concentration greater than 250 mg L^{-1} toluene for any of my experiments, I did not test the linearity of the detector response above this concentration (see Table A-E2).

Temperatures		**°C**
	Oven (Isothermal)	75
	Detector	250
	Injector	120
Flow		**mL/min**
	Column (He)	3-4
	Split Flow (He)	15-16
	He + Auxiliary (He)	~ 30
	He + Air	~350
	He + H_2	~ 40
	Split Ratio	1:5
	Retention Time (min)	0.9-1.0
	Column Head Press (psi)	2.2-2.3
	Total Run Time (min)	2

Table A-E1. Temperature and gas flow settings for FID gas chromatograph.



RUN# 35 APR 12, 2000 18:21:57

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.285	1125	VV	.044	.13772
1.002	815761	PB	.059	99.86227

TOTAL AREA= 816886
MUL FACTOR=1.0000E+00

Figure A-E1. Example of a typical chromatogram for toluene analysis.

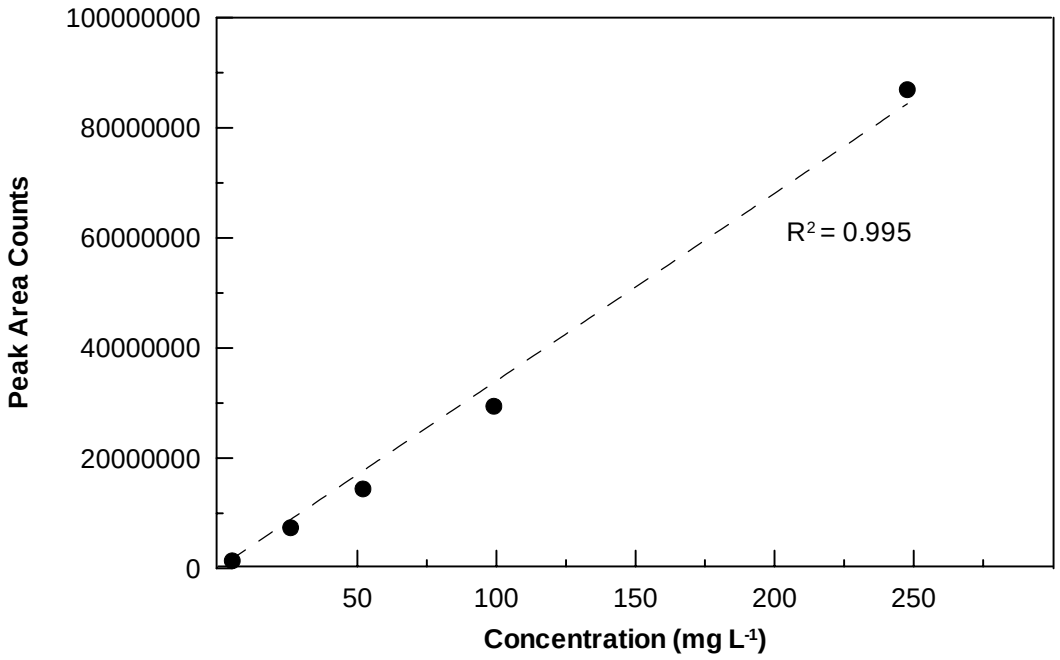


Figure A-E2. Example of a typical calibration curve for toluene.

Table A-E2. Calibration data for FID gas chromatograph (used to create Figure A-E2).

Sample	Intended concen (mg/L)	Volume Toluene Added (μl)	Concentration Solution (mg/L)	Concentration Headspace (mg/L)	Peak Area
1	5	5.8	5.029	2.413728	1371010
2	25	30	26.010	12.4848	7380227
3	50	60	52.020	24.9696	14422464
4	100	80	99.086	47.56114286	29430320
5	250	200	247.714	118.9028571	86928576

APPENDIX F. INITIAL AEROBIC SMZ SLURRY-PHASE DEGRADATION EXPERIMENTS

I originally isolated a continuous culture of aerobic toluene-degraders by obtaining three different hydrocarbon-contaminated soil samples: one from the Conoco Station in Socorro, NM; one from a gasoline-contaminated site in Los Cruces, NM; and one from a contaminated site in New Jersey. The procedure used to obtain the toluene-degrading culture was identical to that described in the Materials and Methods section of the ES&T manuscript (Chapter 2).

SMZ Slurry-phase Biodegradation Experiment

For my first aerobic SMZ slurry-phase biodegradation experiment, each microcosm was prepared in a 27-mL glass headspace vial containing 4.0 g SMZ. Inoculum from the exponential growth phase (0.2 mL) was distributed to the samples, followed by 16 mL of nutrient medium with a concentration of 122 mg/L via the gas-sampling bag method described above. The samples immediately were sealed with aluminum crimp caps fitted with Teflon-faced butyl rubber septa. All samples were incubated in a shaker at 80 rpm and 25 °C and sacrificed at various times over a 7-day period. At the specified time, the samples were analyzed for toluene using the GC method described above. I also prepared 1) solution-only microcosms containing 16 mL toluene/nutrient solution and 0.2 mL inoculum; 2) SMZ uninoculated controls containing 4.0 g SMZ and 16 mL toluene/nutrient solution; and 3) solution-only uninoculated blanks containing 16 mL toluene/nutrient solution. Within each treatment, duplicate samples were prepared for each time period.

This biodegradation experiment resulted in no significant depletion of toluene within the SMZ slurry-phase microcosms (data not shown).

I ran a series of "troubleshooting" experiments to determine why the microorganisms were unable to grow (degrade the toluene) within the SMZ slurry-phase microcosms. The first tests I ran were to determine the effect of the surfactant HDTMA on the viability of the microorganisms and to determine if sorption of the nutrients was limiting the microorganisms' growth. Finally, I monitored the pH of the microcosms and evaluated the effect of pH on the system.

HDTMA and Nutrient Sorption Effect on Microbial Growth

I conducted a series of experiments to test the microorganisms' ability to exhibit growth in the presence of HDTMA in solution. Although previous work showed that HDTMA sorbed to zeolite was relatively biostable over a 3-month period (Li, 1998), it was not certain whether HDTMA released into solution would have a toxic affect on the microorganisms. The SMZ used in this initial biodegradation experiment was not pre-washed and, therefore, excess HDTMA could have been released into solution once the SMZ and nutrient solution were combined. An HDTMA increase in solution could have been acutely toxic to the toluene-degraders. Observed "foaming" within the microcosms appeared to support this hypothesis.

In addition, I tested whether the SMZ was adsorbing the B-H broth to such an extent that the microorganisms did not have access to nutrients for growth.

First, I placed 50 g of SMZ and 50 g of raw zeolite in 500 mL centrifuge bottles. I added 200 mL of nutrient medium to each centrifuge bottle to achieve a 1:4 ratio (same as the slurry-phase microcosms). The centrifuge bottles were placed in the shaker

overnight at 100 rpm at 25 °C. I then centrifuged the mixtures at 3000 rpm for 15 minutes. I decanted the supernatant from each into several 50 mL centrifuge bottles. I centrifuged the smaller bottles once more at 8500 rpm for 15 minutes. I used a syringe to extract 9 mL of clear supernatant. The supernatant was transferred to 20-mL glass headspace vials. I prepared eight samples with the SMZ extract and eight samples with the raw zeolite extract. I also prepared eight samples with 9 mL of fresh nutrient medium.

I transferred 0.5 mL of an aerobic toluene-degrading culture from the exponential-growth phase into each of the vials using a 1-mL sterile disposable syringe. Finally, I transferred 1 μl of toluene using a glass microliter syringe into each sample and immediately sealed the vials. All of the samples were incubated in a shaker at 100 rpm at 25 °C.

Within 24 hours, all of the samples had turned turbid (were exhibiting growth) except for the samples prepared with the SMZ extract. Subsequent GC analysis confirmed that 1) toluene had been completely depleted within the samples prepared with raw zeolite extract and fresh nutrient medium, and 2) there was no depletion of toluene within the samples prepared with SMZ extract.

Based on this experiment, I determined that it was either the presence of HDTMA in solution or the sorption of the nutrient medium that was inhibiting or killing the toluene-degrading microorganisms. To determine if it was definitely the presence of the surfactant, I repeated the above experiment with the SMZ extract. This time, however, I added more nutrients to the supernatant after it had been extracted from the SMZ. Again,

no turbidity was observed within the samples and subsequent GC analysis confirmed that toluene had not been depleted.

Therefore, I concluded that it was necessary to wash the SMZ prior to setting up further biodegradation experiments in order to reduce the concentration of HDTMA in solution. In addition, I decided to isolate a toluene-degrading culture from the wastewater treatment plant in Socorro, NM. It was believed that microorganisms from the activated sludge stage of the treatment process would be acclimated to surfactants and therefore the HDTMA would be less likely to have a toxic effect.

pH Effect on Microbial Growth

Although I began to wash the SMZ prior to preparing slurry-phase cultures, I still was not observing significant toluene depletion within the microcosms. Therefore, I ran several tests to determine the effect a decreasing pH within the microcosms would have on microbial growth. This experiment was discussed in the ES&T manuscript (Chapter 2), but I have included a chart here to summarize my findings (Table A-F1).

pH	**Growth**
7.05	Yes
6.8	Yes
6.56	?
6.0	No
5.52	No
5.37	No

Table A-F1. pH effect on microbial growth**.**

Once I had confirmed that a decreasing pH was in fact inhibiting the microorganisms' growth, it was necessary to determine an appropriate buffer system for future biodegradation experiments. I prepared several different samples of my own nutrient medium with the same ingredients as the B-H broth, except I replaced the 1.0 g $(NH_4)_2HPO_4$ and 1.0 g KH_2PO_4 found in the B-H broth with 2.0 g of KH_2PO_4, K_2HPO_4, or K_3PO_4 as a buffer. I recorded the starting pH of each nutrient solution (pH before contact with SMZ).

I added 20 mL of the nutrient solutions to 40-mL vials containing 5 g of SMZ. I placed the samples in the shaker and allowed them to equilibrate over night. I then removed the samples from the shaker and recorded the pH of the slurry-mixtures (Figure A-F2). Finally, I inoculated the samples with 1 mL of a stock toluene-degrading culture and added 100-mg/L toluene as a carbon source. I incubated the samples in a shaker at 100 rpm and 25 °C for three days. The results are shown in Figure A-F2.

As a result of this experiment, I concluded that it was best to replace the 1.0 g $(NH_4)_2HPO_4$ and 1.0 g KH_2PO_4 found in the B-H broth with 2.0 g K_3PO_4.

Buffer System	pH Before Contact With SMZ	pH After Contact With SMZ	Growth
BH Medium	7.3	5.2-5.8	No
Monobasic Potassium Phosphate	7.2	5.0-5.4	No
Dibasic Potassium Phosphate	7.7	6.1-6.5	Yes
Tribasic Potassium Phosphate	11.5	7.2	Yes

Table A-F2. Results from pH experiment showing 1) how the pH of "buffered" systems is affected by SMZ cationic sorption; and 2) which buffer system supported microbial growth.

APPENDIX G. FINAL AEROBIC SMZ SLURRY-PHASE DEGRADATION EXPERIMENT

To eliminate the possibility that a decreasing pH or lack of a nutrient was causing the incomplete biodegradation of toluene within the microcosms, I repeated the aerobic SMZ slurry-phase biodegradation experiment presented in the ES&T manuscript (Chapter 2). The experiment was set up in the exact same manner as the previous slurry-phase experiment that was described in the Materials and Methods section of the manuscript.

For this experiment, however, I closely monitored the pH of the microcosms throughout the course of the experiment by recording the pH of each microcosm using a Beckman Φ™ 45 pH meter immediately following toluene analysis. Once biodegradation had ceased within the microcosms and the toluene concentration had reached a plateau, I intended to add more nutrients to the microcosms to see if the addition would generate further biodegradation.

Figure A-G1a depicts the depletion of toluene within the SMZ slurry-phase batch cultures. The data used to create this figure can be found in Tables A-G1a. Although the initial solution concentration was 100 mg L^{-1}, partitioning resulted in an actual initial solution concentration of 25 mg L^{-1}. The controls maintained a mean concentration of 25 (± 1) mg L^{-1} (numbers in parentheses represent standard deviation). Similar to the previous aerobic experiment, I observed a lag phase of about 8 hours followed by a fairly rapid period of biodegradation. Applying the first-order biodegradation model, k_m was determined to be -0.13 (± 0.01) h^{-1} for t = 8 to 18.5 hours. If the data through t = 25 hours was included, a reasonable fit to the data could not be achieved using the first-order rate model. Statistical analysis showed that the k_m value for these SMZ cultures were

statistically the same as the k_m values for the previous SMZ and N-SMZ cultures but statistically different than the k_m values for the solution-only samples.

Here, the toluene concentration reached 0 mg L^{-1} and stayed there for several hours. Therefore, I did not add nutrients to see if biodegradation would continue. However, two days later, the toluene concentration rose slightly to an equilibrium concentration of 2 (± 1) mg L^{-1}. The final concentration was equivalent to a 91 (±2) % degradation of the toluene originally present. No further decrease in concentration was observed throughout the 85-hour incubation period.

Figure A-G1b plots the pH in the SMZ cultures and controls along with the toluene concentration. The data used to create this figure can be found in Table A-G1b. The pH of the cultures decreased with time along with the toluene concentration, while the pH of the controls remained at 8.0 (± 0.1). Although the pH of the cultures decreased, it never reached the critical pH value (< 6.5) that our previous pH experiments showed to be inhibitory. This indicated that the pH in the microcosms was not limiting the microorganisms' ability to degrade the toluene as long as I adjusted the nutrient medium to a starting pH of 11.5.

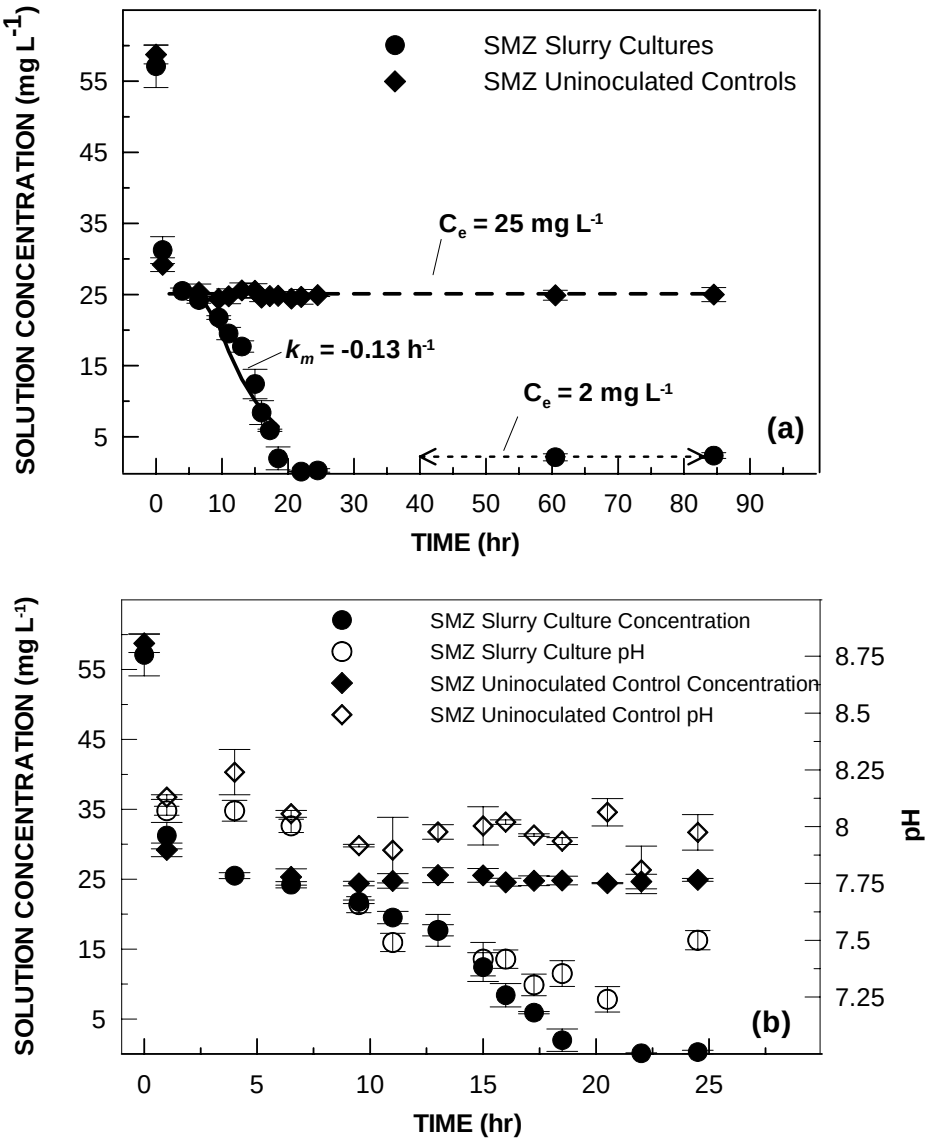


Figure A-G1. First-order biodegradation kinetics of toluene in slurry-phase batch cultures. Points are means of triplicates; error bars represent standard deviation. **a)** Solid line is based on linear regression of data according to $ln(C_t/C_o) = - k_m(t-t_{lag})$ where t_{lag} = 8 h; **b)** pH and toluene solution concentration of the microcosms plotted together.

Table A-G1a. SMZ slurry-culture data from second aerobic biodegradation experiment (used to create Figure A-G1).

Sample	Time (h)	pH	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration of Solution in HS Vial (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Solution Concentration in Slurry Cultures (mg/L)
X1	0.00	8.38	22905968	15.32	108.77	38.69	193.43	302.21	60.44
X2	0.00	8.08	21328784	14.27	101.28	36.02	180.11	281.40	56.28
X3	0.00	8.14	20688864	13.84	98.24	34.94	174.71	272.95	54.59
X4	1.00	8.06	12220104	8.17	58.03	20.64	103.19	161.22	32.24
X5	1.00	8.07	12286664	8.22	58.34	20.75	103.76	162.10	32.42
X6	1.00	8.09	11018064	7.37	52.32	18.61	93.04	145.36	29.07
X7	4.00	8.12	9481280	6.34	45.02	16.01	80.07	125.09	25.02
X8	4.00	8.06	9783270	6.54	46.46	16.52	82.62	129.07	25.81
X9	4.00	8.03	9733658	6.51	46.22	16.44	82.20	128.42	25.68
X10	6.50	8.02	9147098	6.12	43.44	15.45	77.24	120.68	24.14
X11	6.50	8.02	9364435	6.26	44.47	15.82	79.08	123.55	24.71
X12	6.50	7.97	9029318	6.04	42.88	15.25	76.25	119.13	23.83
X13	9.50	7.66	8168800	5.46	38.79	13.80	68.98	107.77	21.55
X14	9.50	7.62	8219904	5.50	39.03	13.88	69.41	108.45	21.69
X15	9.50	7.69	8354496	5.59	39.67	14.11	70.55	110.22	22.04
X16	11.00	7.49	7654483	5.12	36.35	12.93	64.64	100.99	20.20
X17	11.00	7.45	7024576	4.70	33.36	11.86	59.32	92.68	18.54
X18	11.00	7.53	7511546	5.02	35.67	12.69	63.43	99.10	19.82
X19	13.00	7.61	7025987	4.70	33.36	11.87	59.33	92.70	18.54
X20	13.00	7.47	6420029	4.29	30.49	10.84	54.22	84.70	16.94
X21	13.00	7.55	6671837	4.46	31.68	11.27	56.34	88.02	17.60
X22	15.00	7.39	5025405	3.36	23.86	8.49	42.44	66.30	13.26
X23	15.00	7.36	3818194	2.55	18.13	6.45	32.24	50.37	10.07
X24	15.00	7.50	5288019	3.54	25.11	8.93	44.66	69.77	13.95
X25	16.00	7.41	3808872	2.55	18.09	6.43	32.16	50.25	10.05

Table A-G1a (cont.).

Sample	Time (h)	pH	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration of Solution in HS Vial (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Solution Concentration in Slurry Cultures (mg/L)
X26	16.00	7.46	3212966	2.15	15.26	5.43	27.13	42.39	8.48
X27	16.00	7.38	2538216	1.70	12.05	4.29	21.43	33.49	6.70
X28	17.25	7.34	2297360	1.54	10.91	3.88	19.40	30.31	6.06
X29	17.25	7.25	2179205	1.46	10.35	3.68	18.40	28.75	5.75
X30	17.25	7.32	2242144	1.50	10.65	3.79	18.93	29.58	5.92
X31	18.50	7.37	1213272	0.81	5.76	2.05	10.25	16.01	3.20
X32	18.50	7.29	959036	0.64	4.55	1.62	8.10	12.65	2.53
X33	18.50	7.40	62127	0.04	0.30	0.10	0.52	0.82	0.16
X34	20.50	7.29	44782	0.03	0.21	0.08	0.38	0.59	0.12
X35	20.50	7.18	31394	0.02	0.15	0.05	0.27	0.41	0.08
X36	20.50	7.25	23867	0.02	0.11	0.04	0.20	0.31	0.06
X37	22.00	7.24	12224	0.01	0.06	0.02	0.10	0.16	0.03
X38	22.00	7.18	58774	0.04	0.28	0.10	0.50	0.78	0.16
X39	22.00	7.17	42723	0.03	0.20	0.07	0.36	0.56	0.11
X40	24.50	7.53	34447	0.02	0.16	0.06	0.29	0.45	0.09
X41	24.50	7.47	169256	0.11	0.80	0.29	1.43	2.23	0.45
X42	24.50	7.60	N/A	N/A	N/A	N/A	N/A	N/A	N/A
X46	60.50	7.21	855376	0.57	4.06	1.44	7.22	11.29	2.26
X47	60.50	7.31	965259	0.65	4.58	1.63	8.15	12.73	2.55
X48	60.50	7.36	597891	0.40	2.84	1.01	5.05	7.89	1.58
X70	84.50	7.33	981709	0.66	4.66	1.66	8.29	12.95	2.59
X71	84.50	7.45	710459	0.48	3.37	1.20	6.00	9.37	1.87
X72	84.50	7.19	981014	0.66	4.66	1.66	8.28	12.94	2.59

Table A-G1b. SMZ uninoculated control data from second aerobic biodegradation experiment (used to create Figure A-G1).

Sample	Time (h)	pH	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration of Solution in HS Vial (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Solution Concentration in Slurry Cultures (mg/L)
W1	0.00	8.13	21972224	14.70	104.34	37.11	185.55	289.89	57.98
W2	0.00	7.97	22830544	15.27	108.41	38.56	192.80	301.21	60.24
W3	0.00	7.98	21981120	14.70	104.38	37.12	185.62	290.00	58.00
W4	1.00	8.13	11332600	7.58	53.81	19.14	95.70	149.51	29.90
W5	1.00	8.14	10814712	7.23	51.35	18.27	91.33	142.68	28.54
W6	1.00	8.12	NA	NA	NA	NA	NA	NA	NA
W7	4.00	8.53	9350650	6.25	44.40	15.79	78.96	123.37	24.67
W8	4.00	8.09	9690611	6.48	46.02	16.37	81.83	127.85	25.57
W9	4.00	8.10	8677856	5.80	41.21	14.66	73.28	114.49	22.90
W10	6.50	8.06	9764294	6.53	46.37	16.49	82.46	128.82	25.76
W11	6.50	8.07	9928883	6.64	47.15	16.77	83.85	130.99	26.20
W12	6.50	8.04	9095322	6.08	43.19	15.36	76.81	120.00	24.00
W13	9.50	7.91	9318202	6.23	44.25	15.74	78.69	122.94	24.59
W14	9.50	7.92	9104954	6.09	43.24	15.38	76.89	120.12	24.02
W15	9.50	7.92	9313568	6.23	44.23	15.73	78.65	122.88	24.58
W16	11.00	7.73	9818144	6.57	46.62	16.58	82.91	129.53	25.91
W17	11.00	7.97	9050650	6.05	42.98	15.29	76.43	119.41	23.88
W18	11.00	7.99	9284352	6.21	44.09	15.68	78.40	122.49	24.50
W19	13.00	7.94	9243034	6.18	43.89	15.61	78.05	121.95	24.39
W20	13.00	8.00	10016936	6.70	47.57	16.92	84.59	132.16	26.43
W21	13.00	7.99	9827398	6.57	46.67	16.60	82.99	129.66	25.93
W22	15.00	7.95	9264448	6.20	43.99	15.65	78.24	122.23	24.45
W23	15.00	7.96	9998534	6.69	47.48	16.89	84.43	131.91	26.38
W24	15.00	8.10	9779520	6.54	46.44	16.52	82.58	129.02	25.80
W25	16.00	8.02	9405120	6.29	44.66	15.88	79.42	124.08	24.82

Table A-G1b (cont.).

Sample	Time (h)	pH	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration of Solution in HS Vial (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Solution Concentration in Slurry Cultures (mg/L)
W26	16.00	8.03	9069504	6.07	43.07	15.32	76.59	119.66	23.93
W27	16.00	8.01	9454899	6.32	44.90	15.97	79.84	124.74	24.95
W28	17.25	7.97	9133818	6.11	43.37	15.43	77.13	120.51	24.10
W29	17.25	7.96	9659782	6.46	45.87	16.31	81.57	127.44	25.49
W30	17.25	7.96	9388224	6.28	44.58	15.86	79.28	123.86	24.77
W31	18.50	7.92	9641491	6.45	45.78	16.28	81.42	127.20	25.44
W32	18.50	7.94	9395520	6.28	44.62	15.87	79.34	123.96	24.79
W33	18.50	7.95	9182714	6.14	43.61	15.51	77.55	121.15	24.23
W34	20.50	8.57	9231283	6.17	43.84	15.59	77.96	121.79	24.36
W35	20.50	7.77	9281747	6.21	44.08	15.68	78.38	122.46	24.49
W36	20.50	7.85	9267424	6.20	44.01	15.65	78.26	122.27	24.45
W37	22.00	7.93	9565082	6.40	45.42	16.15	80.77	126.19	25.24
W38	22.00	7.76	8892390	5.95	42.23	15.02	75.09	117.32	23.46
W39	22.00	7.74	9585011	6.41	45.52	16.19	80.94	126.46	25.29
W40	24.50	8.03	9490470	6.35	45.07	16.03	80.14	125.21	25.04
W41	24.50	7.92	9388826	6.28	44.58	15.86	79.29	123.87	24.77
W42	24.50	7.96	9782451	6.54	46.45	16.52	82.61	129.06	25.81
W46	60.50	7.75	9310618	6.23	44.21	15.73	78.63	122.84	24.57
W47	60.50	7.73	9250822	6.19	43.93	15.62	78.12	122.05	24.41
W48	60.50	7.68	9744058	6.52	46.27	16.46	82.29	128.56	25.71
W46	84.50	7.90	9612611	6.43	45.65	16.24	81.18	126.82	25.36
W47	84.50	7.77	9110442	6.09	43.26	15.39	76.93	120.20	24.04
W48	84.50	7.63	9789015	6.55	46.48	16.53	82.67	129.15	25.83

APPENDIX H. ANAEROBIC SMZ SLURRY-PHASE DEGRADATION EXPERIMENT

Concomitant with obtaining the cultures of aerobic, toluene-degrading microorganisms, I also obtained cultures of anaerobic, toluene-degrading microorganisms by a series of enrichment cultures. As with the aerobic cultures, I initially inoculated the anaerobic cultures with three different hydrocarbon-contaminated soil samples. I then switched to inoculating the cultures with activated sludge from the wastewater treatment plant in Socorro, NM for the same reasons discussed above.

To provide an anoxic environment in which to prepare the cultures and samples, I purchased an anaerobic glove bag (Instruments for Research & Industry I^2R Inc., Cheltenham, PA). The glove bag had two integral gloves and an equipment sleeve in between, through which I could introduce any glassware or equipment I was using. Across from the equipment sleeve was a small opening to connect the glove bag to a N_2 source and a length of tubing. The inflatable workspace measured 17" x 17" x 11". Figure A-H1 is a diagram of the anaerobic glove bag setup.

First, I purged with N_2 all equipment and/or solutions I intended to use within the bag and inserted them through the sleeve. I sealed the equipment sleeve using a clamp provided with the bag and then inflated the bag. Although, in theory, the use of the anaerobic glove bag was a good idea, it was very clumsy to work with and often times sample vials or volumetric flasks would spill over inside the bag due to its instability. It was also difficult to work quickly while transferring toluene or toluene-containing solutions.

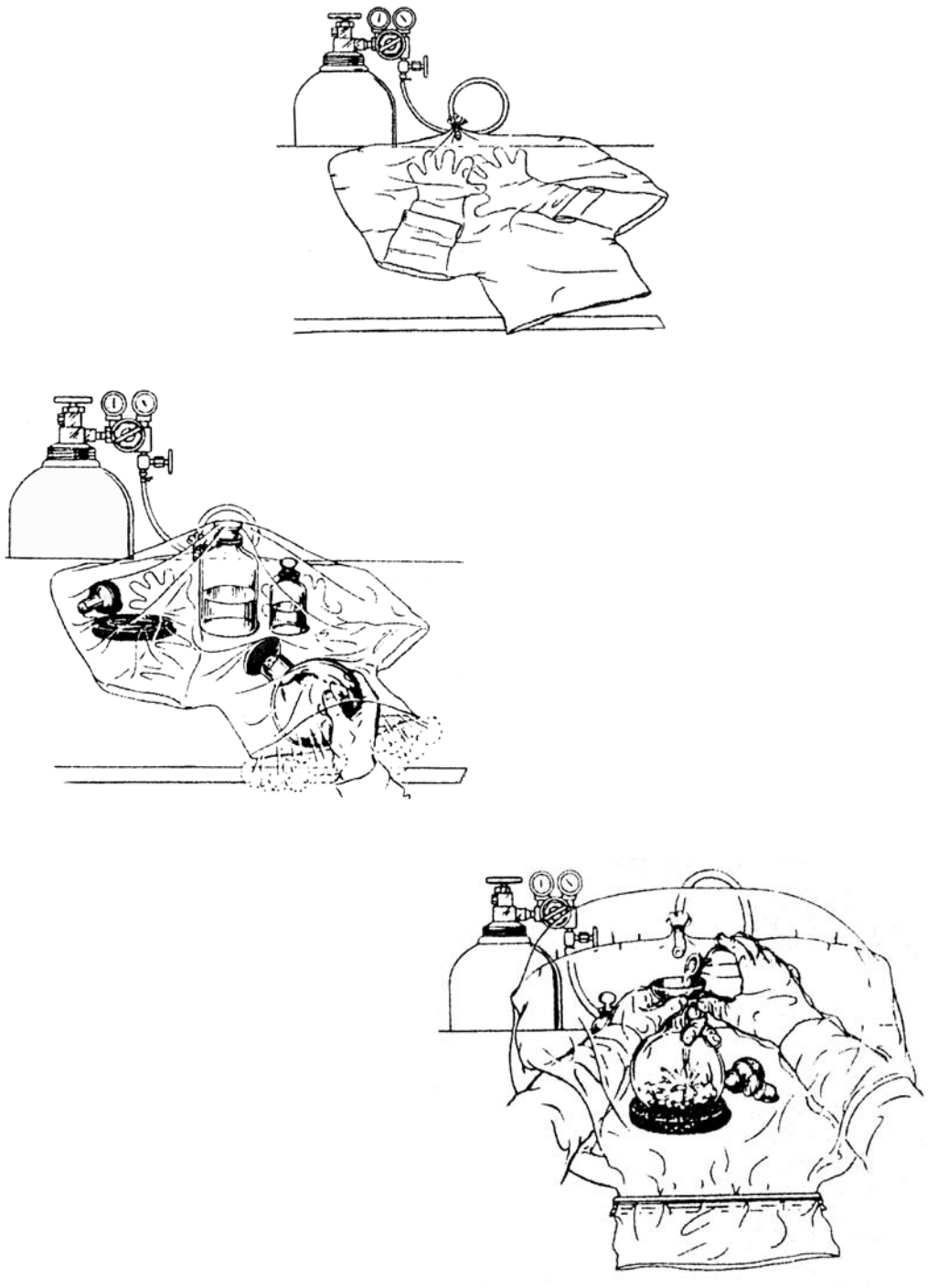


Figure A-H1. Anaerobic glove bag illustration (adapted from catalog provided by Instruments for Research & Industry I^2R Inc.).

Isolating a Culture of Anaerobic Toluene Degraders

The first enrichment culture was prepared in 70-mL glass serum vial filled with approximately 70 mL B-H broth. To increase the likelihood of an anaerobic environment, as little headspace as possible was allowed to remain in the vial. N_2 gas was bubbled into the vial through a long needle/probe for a few minutes to remove any O_2 dissolved in the solution. The serum vial was then placed inside the anaerobic glove bag that was filled with N_2 and the bag was sealed. While inside the glove bag, 1 mL of inoculum from the activated sludge was added to the bottom of the vial with a 1-mL sterile disposable syringe. Then, using a technique similar to the "rapid open-vial transfer technique," the appropriate volume of toluene was added to the vial using a glass microliter syringe and the vial was immediately sealed. As with the aerobic cultures, the goal was to provide 100 mg L^{-1} toluene as the sole carbon source.

The initial culture was incubated in a shaker at 80 rpm and 25 °C for one week. Subcultures were then prepared in the same way: a serum vial was filled with B-H broth, purged with N_2, and a 1-mL aliquot of the preceding culture medium and 100 mg L^{-1} toluene were transferred to the vial as described previously. The anaerobic subcultures were incubated for seven to ten days. I repeated this subculturing step two more times, each time allowing the culture to incubate for seven to ten days. I stored the anaerobic stock culture at 4 °C.

After this initial isolation step, I maintained a continuous culture by repeating the subculturing step every four to seven days. Throughout my experiments, a 4-day incubation period consistently was sufficient time for turbidity (evidence of microbial

growth) to be observed in solution-only anaerobic cultures. Each of these cultures was stored at 4 °C after growth was observed and they had been subcultured.

Toluene Anaerobic Biodegradation Experiments

Four days before the anaerobic biodegradation experiment, I prepared my inoculum by transferring a 1-mL aliquot of the continuous culture to a 70-mL serum vial filled with B-H broth (purged with N_2) and 100 mg L^{-1} toluene. In this way, I hoped to inoculate all treatments with exponential-growth-phase cells.

Three days before the anaerobic biodegradation experiment, I filled 27-mL glass headspace vials with 6.0 g of SMZ. The vials were then placed inside the anaerobic glove bag filled with N_2. The vials were left open inside the sealed N_2-filled bag to allow any O_2 to diffuse out of the zeolite. After 24 hours, the samples were shaken to redistribute the SMZ within the vials, the bag was resealed, and the anaerobic glove bag was purged with fresh N_2 gas. This step was repeated one more time before the biodegradation experiment.

Aluminum crimp-caps fitted with septa were placed on top of the vials, but not crimped and the vials were removed from the glove bag because it was necessary to complete the next step outside of the bag (due to problems with the toluene-solution transfer). Inoculum (0.2 mL) from the exponential-growth phase was distributed to the vials, immediately followed by 25.5 mL of nutrient medium (that had been purged with N_2 gas) with an initial concentration of 32-mg/L toluene.* The toluene/nutrient solution

* The intended initial concentration was 100-mg/L toluene. However, perhaps due to sorption by the gas-sampling bag and/or volatilization while introducing the neat toluene into the bag, the concentration within the gas-sampling bag was severely lowered. Although I was aware of the lower concentration, I proceeded with the experiment due to time constraints and because I did not think the lower concentration would cause any problems. What was more important was that the samples all received a uniform toluene concentration. This was achieved, as discussed in Appendix A and shown in Figure A-A2.

was introduced via the gas-sampling bag and rapid open-vial transfer methods described above.

I prepared SMZ uninoculated controls in the same manner, except that no inoculum was added prior to addition of the toluene/nutrient solution. I also prepared 1) solution-only microcosms containing 28 mL toluene/nutrient solution and 0.2 mL inoculum and 2) solution-only uninoculated blanks containing 28 mL toluene/nutrient solution.

All samples were incubated in a shaker at 80 rpm and 25°C and sacrificed in pairs at various times over a 12-day period. This provided me with a total of ten sampling times. A subsample of each was analyzed for toluene at the appropriate time using the GC method described above.

The results from this experiment can be seen in Figure A-H2. The data used to create this figure can be found in Table A-H1. The difference in initial concentration between the solution-only and SMZ cultures was due to partitioning between the solution and sorbed phases. No significant depletion in toluene concentration was observed in either the solution-only or SMZ cultures over the first seven days. However, the pure aqueous phase microcosms became turbid approximately eight days after inoculation. At this time, I only had one time-sample remaining. Therefore, I allowed the solution-only and SMZ cultures to incubate for four more days before I sacrificed them for analysis.

In the solution-only microcosms, the toluene was completely depleted on the 12^{th} day. Because no samples were analyzed between the 7^{th} and 12^{th} day, it is not clear what occurred within these microcosms during this time period.

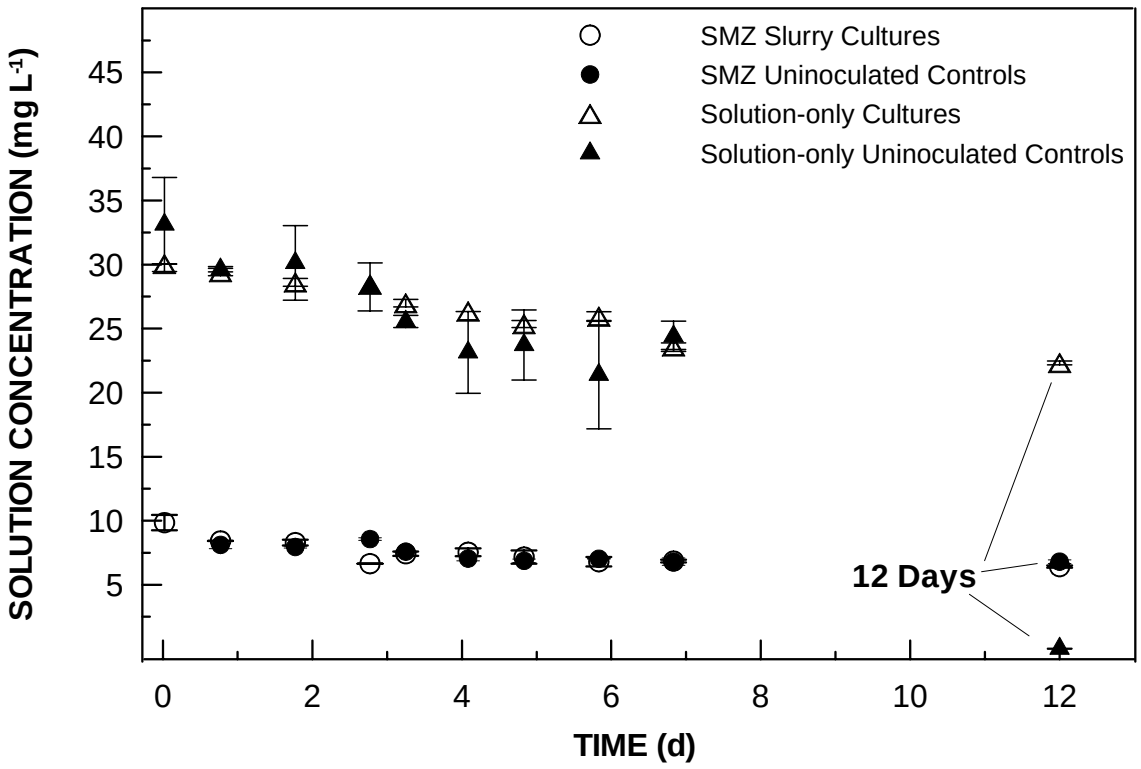


Figure A-H2. Anaerobic biodegradation of toluene in solution-only and slurry-phase batch cultures. Points are means of duplicates; error bars represent standard deviation.

There was no significant depletion of toluene within the SMZ slurry-phase microcosms on the 12th day and no more time-samples remained for further evaluation. Therefore it is not clear if the SMZ cultures simply were experiencing a longer lag time than expected or if there was no growth at all in the microcosms. Turbidity observations in the slurry-cultures proved to be futile as the presence of the SMZ created a cloudy solution.

Previous inoculation of anaerobic cultures had indicated that the anaerobes reached full culture populations within four to five days. Based on this lag time, I believed I had prepared sufficient samples for measuring toluene biodegradation over time. In hindsight, however, I had not prepared sufficient samples and, as a result, this experiment proved inconclusive with respect to anaerobic toluene biodegradation. Unfortunately, I never had a chance to repeat the anaerobic experiments with more time samples.

Table A-H1. Toluene data from anaerobic biodegradation experiment (used to create Figure A-H2).

Solution-Only Uninoculated Controls

Time (h)	Sample	Time (d)	Final Concentration in Solution (mg/L)
0.5	E1	0.02	30.21
0.5	E2	0.02	31.31
18.5	E3	0.77	29.82
18.5	E4	0.77	30.25
42.5	E5	1.77	30.66
42.5	E6	1.77	30.32
66.5	E7	2.77	27.57
66.5	E8	2.77	27.87
78	E9	3.25	27.14
78	E10	3.25	27.71
90	E11	3.75	27.25
98	E13	4.08	26.60
98	E14	4.08	27.84
116	E15	4.83	27.10
116	E16	4.83	26.50
140	E12	5.83	26.07
164	E17	6.83	25.98
164	E18	6.83	26.23
288	E19	12.00	25.58
288	E20	12.00	26.38

Solution-Only Cultures

Time (h)	Sample	Time (d)	Final Concentration in Solution (mg/L)
0.5	D1	0.02	30.53
0.5	D2	0.02	35.72
18.5	D3	0.77	29.78
18.5	D4	0.77	29.49
42.5	D5	1.77	28.08
42.5	D6	1.77	32.18
66.5	D7	2.77	26.93
66.5	D8	2.77	29.58
78	D9	3.25	25.22
78	D10	3.25	25.90
90	D11	3.75	25.40
98	D13	4.08	20.89
98	D14	4.08	21.79
116	D15	4.83	25.66
116	D16	4.83	18.42
140	D12	5.83	24.39
164	D17	6.83	23.57
164	D18	6.83	25.24
288	D19	12.00	0.03
288	D20	12.00	0.00

Table A-H1 (cont.).

SMZ Slurry Cultures

Time (h)	Sample	Time (d)	Final Concentration in Solution (mg/L)
0.5	A1	0.02	9.42
0.5	A2	0.02	10.27
18.5	A3	0.77	8.41
18.5	A4	0.77	8.42
42.5	A5	1.77	8.45
42.5	A6	1.77	8.13
66.5	A7	2.77	6.65
66.5	A8	2.77	n/a
78	A9	3.25	7.54
78	A10	3.25	7.30
98	A13	4.08	7.76
98	A14	4.08	7.32
116	A15	4.83	6.79
116	A16	4.83	7.53
140	A11	5.83	6.54
140	A12	5.83	7.06
164	A17	6.83	6.78
164	A18	6.83	6.92
288	A19	12.00	6.35
288	A20	12.00	6.48

SMZ Uninoculated Controls

Time (h)	Sample	Time (d)	Final Concentration in Solution (mg/L)
0.5	B1	0.02	10.01
0.5	B2	0.02	10.58
18.5	B3	0.77	7.90
18.5	B4	0.77	8.32
42.5	B5	1.77	7.90
42.5	B6	1.77	8.00
66.5	B7	2.77	8.50
66.5	B8	2.77	8.63
78	B9	3.25	7.60
78	B10	3.25	7.56
98	B13	4.08	7.17
98	B14	4.08	6.91
116	B15	4.83	6.78
116	B16	4.83	6.96
140	B11	5.83	7.04
140	B12	5.83	7.01
164	B17	6.83	7.00
164	B18	6.83	6.61
288	B19	12.00	6.70
288	B20	12.00	6.90

APPENDIX I. TOLUENE DESORPTION FROM SMZ

Availability of sorbed organic substrates, such as petroleum hydrocarbons, for microbial degradation can greatly influence the potential for bioremediation. (Feng, 2000; Robinson, 1990). Research has shown both that sorption of a contaminant to soil surfaces greatly reduces its availability and thus reduces its biodegradation rate, as well as that sorbed compounds can be readily available for microbial degradation (Alexander, 1994; Guerin, 1992; Robinson, 1990). For this research, it could not be determined if the microbes utilized the toluene while it was sorbed to the SMZ/N-SMZ or if it was necessary for the toluene to desorb before it could be degraded. Generally, it is considered that sorbed organic substrates are unavailable for biodegradation unless desorption to the aqueous phase occurs (Smith, 1992). Therefore, I was concerned that a slow rate of toluene desorption from SMZ, or incomplete desorption from SMZ, would severely limit the microorganisms' ability to degrade toluene.

As discussed in the ES&T manuscript (Chapter 2), the mass transfer kinetic experiments indicated that both sorption and desorption of toluene by SMZ occur very rapidly. In fact, in both cases the system reached equilibrium within the first 1-2 hours. The manuscript also mentions that the equilibrium points from the desorption experiments coincided with the previously determined sorption isotherm for SMZ, indicating that the sorption of toluene by SMZ is a fully reversible process. The data for this observation was not included in the manuscript; therefore, I have included it here in Figure A-I1 and Table A-I1.

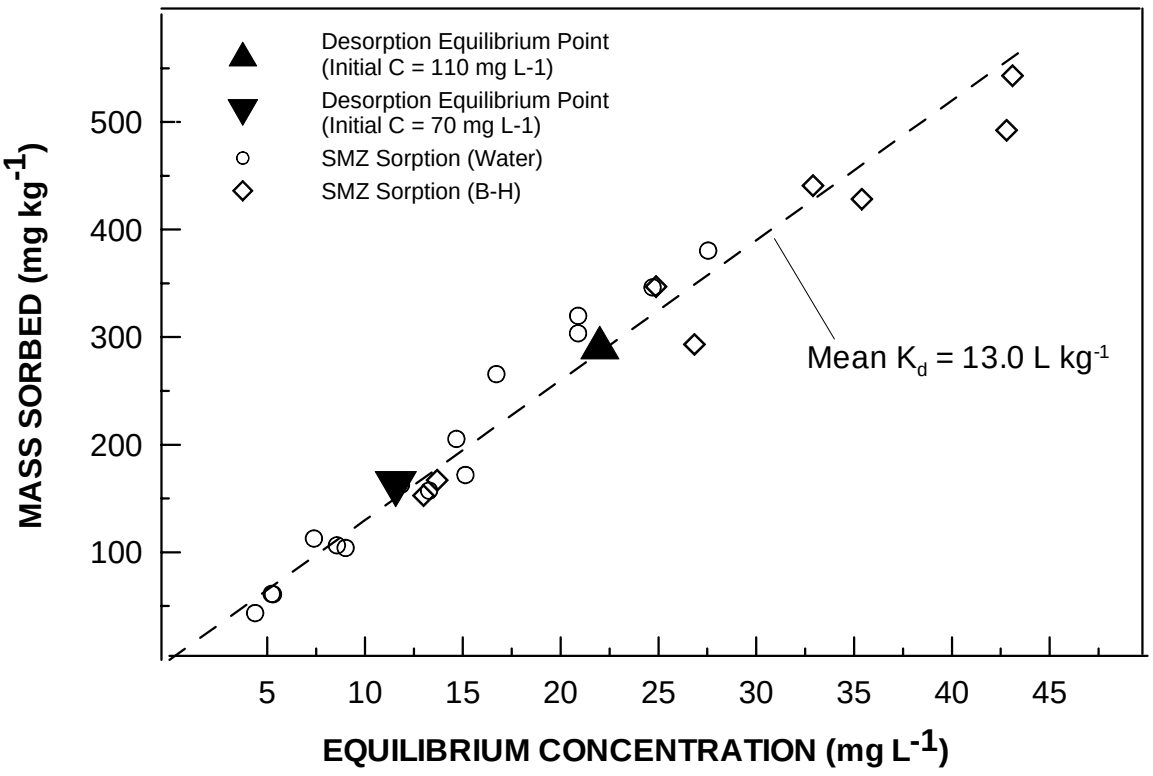


Figure A-I1. Desorption equilibrium points plotted with sorption isotherm for SMZ. Dashed line represents linear sorption isotherm with a mean K_d of 13.0 L kg^{-1}.

Table A-I1. Isotherm Data from sorption experiment and equilibrium data from desorption kinetics experiment (used to create Figure A-I1).

Sorption Isotherm (SMZ and water):

Equilibrium Solution Concentration (mg/L)	Mass Toluene Sorbed (mg/kg)
13.274	157.311
11.839	162.787
20.895	319.788
20.890	303.551
4.383	43.461
5.297	60.930
5.231	61.287
9.007	104.060
7.389	112.793
8.565	106.448
14.671	205.362
16.715	265.605
15.132	171.823
27.540	380.405
24.700	346.248

Sorption Isotherm (SMZ and BH medium):

Equilibrium Solution Concentration (mg/L)	Mass Toluene Sorbed (mg/kg)
13.69	167.00
12.99	152.70
26.84	293.30
24.88	346.90
43.10	543.10
42.80	492.20
35.40	428.34
32.90	440.86

Table A-I1 (cont.).

Equilibrium data from desorption experiment:

Final Equilibrium Solution Concentration for Desorption Exp. (mg/L)	Final Mass Sorbed to SMZ for Desorption Exp. (mg/L)
11.57	158.70
22.00	349.50

APPENDIX J. RAW DATA USED TO CREATE FIGURES IN CHAPTER 2

The tables found on the following pages contain the experimental data used to create all figures found in the ES&T manuscript.

Table A-J1. Isotherm data for SMZ and N-SMZ (used to create Figure 1 in Chapter 2).

SMZ (water)		SMZ (nutrient solution)		N-SMZ (water)	
Equil. Solution Concentration (mg/L)	Sorbed Concentration (mg/kg)	Equil. Solution Concentration (mg/L)	Sorbed Concentration (mg/kg)	Equil. Solution Concentration (mg/L)	Sorbed Concentration (mg/kg)
4.38	43.46	13.69	167.37	8.41	72.92
5.30	60.93	12.99	152.66	8.15	74.96
5.23	61.29	26.84	293.30	5.46	53.38
9.01	104.06	24.88	346.89	12.66	140.81
7.39	112.79	43.10	543.00	14.31	136.17
8.57	106.45	42.80	492.00	8.37	102.37
13.27	157.31			17.04	164.00
11.84	162.79			17.01	171.72
14.67	205.36			11.69	119.15
16.72	265.60			28.31	342.54
15.13	171.82			29.35	351.38
20.90	319.79			20.31	239.34
20.89	303.55			42.33	523.09
27.50	380.00			39.85	560.89
24.70	346.00			28.79	370.10
				35.32	529.49

Table A-J2a. Sorption kinetic data for initial solution concentration of 120 mg L^{-1} toluene (used to create Figure 2a in Chapter 2).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Slurry Solution (mg/L)
Z108	0.017	23831264	15.939	113.165	40.250	201.248	314.413	62.883
Z109	0.017	22569136	15.095	107.172	38.118	190.589	297.761	59.552
Z110	0.017	21711200	14.521	103.098	36.669	183.344	286.442	57.288
Z90	0.083	19839120	13.269	94.208	33.507	167.535	261.743	52.349
Z91	0.083	16891136	11.297	80.210	28.528	142.640	222.850	44.570
Z92	0.083	17736352	11.862	84.223	29.956	149.778	234.001	46.800
Z105	0.250	15794928	10.564	75.004	26.677	133.383	208.387	41.677
Z106	0.250	13545288	9.059	64.321	22.877	114.386	178.707	35.741
Z107	0.250	14039400	9.390	66.668	23.712	118.558	185.226	37.045
Z96	0.500	11166176	7.468	53.024	18.859	94.295	147.319	29.464
Z97	0.500	12190688	8.153	57.889	20.589	102.947	160.835	32.167
Z98	0.500	10404592	6.959	49.407	17.573	87.863	137.271	27.454
Z99	0.750	7663952	5.126	36.393	12.944	64.720	101.113	20.223
Z100	0.750	9039507	6.046	42.925	15.267	76.336	119.261	23.852
Z101	0.750	8906502	5.957	42.294	15.043	75.213	117.506	23.501
Z93	1.000	9480922	6.341	45.021	16.013	80.063	125.085	25.017
Z94	1.000	9793555	6.550	46.506	16.541	82.703	129.209	25.842
Z95	1.000	10957432	7.329	52.033	18.506	92.532	144.565	28.913
Z102	2.530	10878184	7.276	51.656	18.373	91.863	143.519	28.704
Z103	2.530	9890234	6.615	46.965	16.704	83.520	130.485	26.097
Z104	2.530	10076400	6.739	47.849	17.018	85.092	132.941	26.588
Z87	8.000	9103686	6.089	43.230	15.376	76.878	120.108	24.022
Z88	8.000	9069683	6.066	43.068	15.318	76.591	119.659	23.932

Table A-J2a(cont.).

Time (hours)	Mass in Slurry Solution (μg)	Mass in Slurry HS (μg)	Mass Sorbed in Slurry (μg)	Mass Sorbed (mg/kg)
0.017	1.006	0.168	0.586	146.417
0.017	0.953	0.159	0.648	161.965
0.017	0.917	0.153	0.690	172.534
0.083	0.838	0.140	0.782	195.597
0.083	0.713	0.119	0.928	231.914
0.083	0.749	0.125	0.886	221.502
0.250	0.667	0.111	0.982	245.418
0.250	0.572	0.096	1.093	273.132
0.250	0.593	0.099	1.068	267.045
0.500	0.471	0.079	1.210	302.441
0.500	0.515	0.086	1.159	289.820
0.500	0.439	0.073	1.247	311.823
0.750	0.324	0.054	1.382	345.586
0.750	0.382	0.064	1.315	328.640
0.750	0.376	0.063	1.321	330.279
1.000	0.400	0.067	1.293	323.202
1.000	0.413	0.069	1.277	319.351
1.000	0.463	0.077	1.220	305.013
2.530	0.459	0.077	1.224	305.989
2.530	0.418	0.070	1.273	318.160
2.530	0.425	0.071	1.263	315.866
8.000	0.384	0.064	1.311	327.849
8.000	0.383	0.064	1.313	328.268

Table A-J2b. Desorption kinetic data for initial solution concentration of 60 mg L^{-1} toluene (used to create Figure 2b in Chapter 2).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Slurry Solution (mg/L)
Z40	0.0167	3534824	2.364	16.786	5.970	29.850	46.636	9.327
Z41	0.0167	3694208	2.471	17.542	6.239	31.196	48.739	9.748
Z42	0.0167	3498781	2.340	16.614	5.909	29.546	46.160	9.232
Z37	0.083	3698128	2.473	17.561	6.246	31.230	48.791	9.758
Z38	0.083	3692333	2.470	17.533	6.236	31.181	48.714	9.743
Z39	0.083	3665669	2.452	17.407	6.191	30.955	48.362	9.672
Z4	0.25	4005861	2.679	19.022	6.766	33.828	52.851	10.570
Z5	0.25	3851880	2.576	18.291	6.506	32.528	50.819	10.164
Z6	0.25	3861402	2.583	18.336	6.522	32.608	50.945	10.189
Z7	0.5	4175056	2.792	19.826	7.051	35.257	55.083	11.017
Z8	0.5	4196522	2.807	19.928	7.088	35.438	55.366	11.073
Z9	0.5	3890640	2.602	18.475	6.571	32.855	51.330	10.266
Z10	0.75	4026573	2.693	19.121	6.801	34.003	53.124	10.625
Z11	0.75	3876952	2.593	18.410	6.548	32.740	51.150	10.230
Z12	0.75	4154934	2.779	19.730	7.017	35.087	54.817	10.963
Z13	1	4403482	2.945	20.910	7.437	37.186	58.096	11.619
Z14	1	4097240	2.740	19.456	6.920	34.600	54.056	10.811
Z15	1	4442970	2.972	21.098	7.504	37.519	58.617	11.723
Z1	1.5	4456406	2.981	21.162	7.527	37.633	58.795	11.759
Z2	1.5	4605325	3.080	21.869	7.778	38.891	60.759	12.152
Z16	2	4400803	2.943	20.898	7.433	37.163	58.061	11.612
Z17	2	4031976	2.697	19.146	6.810	34.049	53.195	10.639
Z18	2	4093502	2.738	19.438	6.914	34.568	54.007	10.801
Z31	4	4356986	2.914	20.690	7.359	36.793	57.483	11.497
Z32	4	4199987	2.809	19.944	7.094	35.468	55.412	11.082
Z33	4	4382013	2.931	20.808	7.401	37.005	57.813	11.563
Z34	8	4796010	3.208	22.774	8.100	40.501	63.275	12.655
Z35	8	4729555	3.163	22.459	7.988	39.940	62.398	12.480

Table A-J2b(cont.).

Time (hours)	Mass in Slurry Solution (μg)	Mass in Slurry HS (μg)	Mass Sorbed in Slurry (μg)	Mass Sorbed (mg/kg)
0.0167	0.149	0.025	0.646	161.454
0.0167	0.156	0.026	0.638	159.490
0.0167	0.148	0.025	0.648	161.898
0.083	0.156	0.026	0.638	159.442
0.083	0.156	0.026	0.638	159.513
0.083	0.155	0.026	0.639	159.842
0.25	0.169	0.028	0.623	155.651
0.25	0.163	0.027	0.630	157.548
0.25	0.163	0.027	0.630	157.430
0.5	0.176	0.029	0.614	153.566
0.5	0.177	0.030	0.613	153.302
0.5	0.164	0.027	0.628	157.070
0.75	0.170	0.028	0.622	155.396
0.75	0.164	0.027	0.629	157.239
0.75	0.175	0.029	0.615	153.814
1	0.186	0.031	0.603	150.752
1	0.173	0.029	0.618	154.525
1	0.188	0.031	0.601	150.266
1.5	0.188	0.031	0.600	150.100
1.5	0.194	0.033	0.593	148.266
2	0.186	0.031	0.603	150.785
2	0.170	0.028	0.621	155.329
2	0.173	0.029	0.618	154.571
4	0.184	0.031	0.605	151.325
4	0.177	0.030	0.613	153.259
4	0.185	0.031	0.604	151.017
8	0.202	0.034	0.584	145.917
8	0.200	0.033	0.587	146.735

Table A-J2c. Desorption kinetic data for initial solution concentration of 110 mg L^{-1} toluene (used to create Figure 2b in Chapter 2).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Slurry Solution (mg/L)
Z64	0.017	6725856	4.498	31.939	11.360	56.798	88.736	17.747
Z65	0.017	7266250	4.860	34.505	12.272	61.361	95.866	19.173
Z66	0.017	7418765	4.962	35.229	12.530	62.649	97.878	19.576
Z67	0.083	6797680	4.546	32.280	11.481	57.404	89.684	17.937
Z68	0.083	7464512	4.992	35.446	12.607	63.035	98.482	19.696
Z69	0.083	7108147	4.754	33.754	12.005	60.026	93.780	18.756
Z52	0.250	8356547	5.589	39.682	14.114	70.568	110.250	22.050
Z53	0.250	7548698	5.049	35.846	12.749	63.746	99.592	19.918
Z54	0.250	8348384	5.584	39.643	14.100	70.499	110.143	22.029
Z55	0.500	7921901	5.298	37.618	13.380	66.898	104.516	20.903
Z56	0.500	8176579	5.469	38.827	13.810	69.049	107.876	21.575
Z57	0.500	8255133	5.521	39.200	13.942	69.712	108.912	21.782
Z58	0.750	7876877	5.268	37.404	13.304	66.518	103.922	20.784
Z59	0.750	8291613	5.546	39.374	14.004	70.020	109.394	21.879
Z60	0.750	8131536	5.439	38.614	13.734	68.668	107.282	21.456
Z61	1.000	9035386	6.043	42.906	15.260	76.301	119.207	23.841
Z62	1.000	8159216	5.457	38.745	13.780	68.902	107.647	21.529
Z63	1.000	8394509	5.614	39.862	14.178	70.889	110.751	22.150
Z73	2.000	9163136	6.128	43.512	15.476	77.380	120.892	24.178
Z74	2.000	8848787	5.918	42.019	14.945	74.725	116.745	23.349
Z75	2.000	8806490	5.890	41.819	14.874	74.368	116.187	23.237
Z70	4.530	8597254	5.750	40.825	14.520	72.601	113.426	22.685
Z71	4.530	8681638	5.806	41.226	14.663	73.314	114.539	22.908
Z72	4.530	8719622	5.832	41.406	14.727	73.634	115.041	23.008
Z76	8.000	8661626	5.793	41.131	14.629	73.145	114.275	22.855
Z77	8.000	8392390	5.613	39.852	14.174	70.871	110.723	22.145
Z78	8.000	8539142	5.711	40.549	14.422	72.110	112.659	22.532

Table A-J2c(cont.).

Time (hours)	Mass in Slurry Solution (μg)	Mass in Slurry HS (μg)	Mass Sorbed in Slurry (μg)	Mass Sorbed (mg/kg)
0.017	0.284	0.047	1.274	318.394
0.017	0.307	0.051	1.247	311.736
0.017	0.313	0.052	1.239	309.855
0.083	0.315	0.053	1.237	309.294
0.083	0.300	0.050	1.255	313.683
0.250	0.353	0.059	1.193	298.304
0.250	0.319	0.053	1.233	308.258
0.250	0.352	0.059	1.194	298.402
0.500	0.334	0.056	1.215	303.659
0.500	0.345	0.058	1.202	300.522
0.500	0.349	0.058	1.198	299.555
0.750	0.333	0.056	1.217	304.215
0.750	0.350	0.059	1.196	299.102
0.750	0.343	0.057	1.204	301.077
1.000	0.381	0.064	1.160	289.942
1.000	0.344	0.058	1.203	300.736
1.000	0.354	0.059	1.191	297.837
2.000	0.387	0.065	1.153	288.369
2.000	0.374	0.062	1.169	292.239
2.000	0.372	0.062	1.171	292.762
4.530	0.363	0.061	1.181	295.363
4.530	0.367	0.061	1.177	294.289
4.530	0.384	0.064	1.157	289.153
8.000	0.366	0.061	1.178	294.569
8.000	0.354	0.059	1.192	297.884
8.000	0.360	0.060	1.184	296.063

Table A-J3. Solution-only biodegradation data (used to create Figure 3 in Chapter 2).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
3	0	32540096	21.763	154.520	54.958	274.791	429.311	85.862
4	4	33935136	22.696	161.145	57.314	286.572	447.716	89.543
5	4	32491872	21.731	154.291	54.877	274.384	428.675	85.735
6	4	33275376	22.255	158.012	56.200	281.000	439.012	87.802
7	6.5	32555760	21.774	154.595	54.985	274.923	429.518	85.904
8	6.5	32546944	21.768	154.553	54.970	274.849	429.402	85.880
9	6.5	31883472	21.324	151.402	53.849	269.246	420.648	84.130
10	8.5	31372224	20.982	148.975	52.986	264.929	413.903	82.781
11	8.5	31004816	20.737	147.230	52.365	261.826	409.056	81.811
12	8.5	29349392	19.629	139.369	49.569	247.846	387.215	77.443
13	10.5	26762512	17.899	127.085	45.200	226.001	353.086	70.617
14	10.5	na	na	na	na	na	na	na
15	10.5	na	na	na	na	na	na	na
16	13.75	5194922	3.474	24.669	8.774	43.869	68.538	13.708
17	13.75	8544243	5.715	40.573	14.431	72.153	112.727	22.545
18	13.75	5682035	3.800	26.982	9.597	47.983	74.965	14.993
19	14.75	4695450	3.140	22.297	7.930	39.652	61.948	12.390
20	14.75	4676154	3.128	22.205	7.898	39.489	61.694	12.339
21	14.75	4031366	2.696	19.143	6.809	34.044	53.187	10.637
22	17.25	na	na	na	na	na	na	na
23	17.25	3067749	2.052	14.568	5.181	25.906	40.474	8.095
24	17.25	1177110	0.787	5.590	1.988	9.940	15.530	3.106
25	20.75	1790135	1.197	8.501	3.023	15.117	23.618	4.724
26	20.75	2810470	1.880	13.346	4.747	23.734	37.079	7.416
27	20.75	1955519	1.308	9.286	3.303	16.514	25.800	5.160
28	24	2543445	1.701	12.078	4.296	21.479	33.556	6.711
29	24	2815754	1.883	13.371	4.756	23.778	37.149	7.430

Table A-J3(cont.).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
30	24	3331824	2.228	15.822	5.627	28.136	43.958	8.792
31	29	2718682	1.818	12.910	4.592	22.958	35.868	7.174
32	29	1102895	0.738	5.237	1.863	9.314	14.551	2.910
33	29	2987848	1.998	14.188	5.046	25.231	39.420	7.884
34	45.25	2367613	1.584	11.243	3.999	19.994	31.237	6.247
35	45.25	2806357	1.877	13.326	4.740	23.699	37.025	7.405
36	45.25	na	na	na	na	na	na	na

Table A-J4a. SMZ slurry-culture biodegradation data (used to create Figure 4 in Chapter 2).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
D1	0	10071568	6.736	47.826	17.010	85.051	132.877	26.575
D2	0	10342792	6.917	49.114	17.468	87.342	136.456	27.291
D3	2	7316707	4.894	34.744	12.357	61.787	96.532	19.306
D4	2	8296653	5.549	39.398	14.013	70.063	109.460	21.892
D5	4	7995920	5.348	37.970	13.505	67.523	105.493	21.099
D6	4	7855939	5.254	37.305	13.268	66.341	103.646	20.729
D7	8	9848698	6.587	46.768	16.634	83.169	129.937	25.987
D8	8	6483773	4.336	30.789	10.951	54.753	85.542	17.108
D9	12	7167427	4.794	34.035	12.105	60.527	94.562	18.912
D10	12	3417240	2.286	16.227	5.772	28.858	45.085	9.017
D11	16	1781740	1.192	8.461	3.009	15.046	23.507	4.701
D12	16	4649834	3.110	22.080	7.853	39.266	61.347	12.269
D13	20	823872	0.551	3.912	1.391	6.957	10.870	2.174
D14	20	379267	0.254	1.801	0.641	3.203	5.004	1.001
D15	25	393179	0.263	1.867	0.664	3.320	5.187	1.037
D16	25	2272856	1.520	10.793	3.839	19.194	29.986	5.997
D17	32	906562	0.606	4.305	1.531	7.656	11.961	2.392
D18	32	1123320	0.751	5.334	1.897	9.486	14.820	2.964
D19	44	1200398	0.803	5.700	2.027	10.137	15.837	3.167
D20	44	1395320	0.933	6.626	2.357	11.783	18.409	3.682
D21	56	1355658	0.907	6.437	2.290	11.448	17.886	3.577
D22	56	1100592	0.736	5.226	1.859	9.294	14.520	2.904
D23	68	1611596	1.078	7.653	2.722	13.609	21.262	4.252
D24	68	1842447	1.232	8.749	3.112	15.559	24.308	4.862
D25	92	1722528	1.152	8.180	2.909	14.546	22.726	4.545

Table A-J4a(cont.)

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
D26	92	2347094	1.570	11.145	3.964	19.820	30.966	6.193
D27	116	1884718	1.261	8.950	3.183	15.916	24.866	4.973
D28	116	2380890	1.592	11.306	4.021	20.106	31.412	6.282
D29	140	1627979	1.089	7.731	2.750	13.748	21.478	4.296
D30	140	2165616	1.448	10.284	3.658	18.288	28.572	5.714
D31	164	1259273	0.842	5.980	2.127	10.634	16.614	3.323
D32	164	2405002	1.609	11.420	4.062	20.309	31.730	6.346
D33	212	2285624	1.529	10.854	3.860	19.301	30.155	6.031
D34	212	1662600	1.112	7.895	2.808	14.040	21.935	4.387
D35	260	1309982	0.876	6.221	2.212	11.062	17.283	3.457
D36	260	na	na	na	na	na	na	na
D37	284	2144838	1.435	10.185	3.622	18.112	28.297	5.659
D38	284	1104642	0.739	5.246	1.866	9.328	14.574	2.915
D39	na	1908201	1.276	9.061	3.223	16.114	25.175	5.035
D40	na	1338067	0.895	6.354	2.260	11.300	17.654	3.531

Table A-J4b. N-SMZ slurry culture biodegradation data (used to create Figure 4 in Chapter 2).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
F1	0	8446554	5.649	40.109	14.266	71.328	111.438	22.288
F2	0	8231011	5.505	39.086	13.902	69.508	108.594	21.719
F3	2	8390112	5.611	39.841	14.170	70.852	110.693	22.139
F4	2	7598816	5.082	36.084	12.834	64.170	100.253	20.051
F5	4	8511296	5.693	40.417	14.375	71.875	112.292	22.458
F6	4	9047456	6.051	42.963	15.281	76.403	119.366	23.873
F7	8	8343917	5.581	39.622	14.092	70.462	110.084	22.017
F8	8	8369117	5.597	39.742	14.135	70.675	110.416	22.083
F9	12	7179179	4.802	34.091	12.125	60.626	94.717	18.943
F10	12	7532426	5.038	35.769	12.722	63.609	99.378	19.876
F11	16	7142771	4.777	33.918	12.064	60.318	94.237	18.847
F12	16	7854157	5.253	37.296	13.265	66.326	103.622	20.724
F13	20	8505043	5.688	40.387	14.364	71.822	112.210	22.442
F14	20	6628605	4.433	31.477	11.195	55.976	87.453	17.491
F15	25	6885424	4.605	32.696	11.629	58.145	90.841	18.168
F16	25	6229562	4.166	29.582	10.521	52.607	82.188	16.438
F17	32	1523552	1.019	7.235	2.573	12.866	20.101	4.020
F18	32	2456174	1.643	11.663	4.148	20.742	32.405	6.481
F19	44	2922558	1.955	13.878	4.936	24.680	38.558	7.712
F20	44	3827438	2.560	18.175	6.464	32.322	50.497	10.099
F21	56	3231878	2.162	15.347	5.458	27.292	42.639	8.528
F22	56	3528186	2.360	16.754	5.959	29.794	46.548	9.310
F23	68	3680160	2.461	17.476	6.216	31.078	48.553	9.711
F24	68	4589392	3.069	21.793	7.751	38.756	60.549	12.110
F25	92	3474582	2.324	16.499	5.868	29.342	45.841	9.168

Table A-J4b (cont).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
F26	92	3773013	2.523	17.917	6.372	31.862	49.778	9.956
F27	116	3485394	2.331	16.551	5.887	29.433	45.984	9.197
F28	116	4504029	3.012	21.388	7.607	38.035	59.423	11.885
F29	140	4143325	2.771	19.675	6.998	34.989	54.664	10.933
F30	140	4245046	2.839	20.158	7.170	35.848	56.006	11.201
F31	164	3123346	2.089	14.832	5.275	26.376	41.207	8.241
F32	164	4063686	2.718	19.297	6.863	34.317	53.613	10.723
F33	212	4235597	2.833	20.113	7.154	35.768	55.881	11.176
F34	212	3825112	2.558	18.164	6.460	32.302	50.466	10.093
F35	260	3897461	2.607	18.508	6.583	32.913	51.420	10.284
F36	260	3838418	2.567	18.227	6.483	32.414	50.641	10.128
F37	284	3556112	2.378	16.887	6.006	30.030	46.917	9.383
F38	284	3305637	2.211	15.697	5.583	27.915	43.612	8.722
F39	548	3837274	2.566	18.222	6.481	32.405	50.626	10.125
F40	548	3575776	2.392	16.980	6.039	30.196	47.176	9.435

Table A-J4c. Uninoculated control biodegradation data (used to create Figure 4 in the ES&T manuscript).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
C1	0	9235123	6.177	43.854	15.598	77.988	121.842	24.368
C2	0	7734704	5.173	36.729	13.063	65.317	102.046	20.409
C3	2	7993043	5.346	37.956	13.500	67.499	105.455	21.091
C4	2	6808525	4.554	32.331	11.499	57.496	89.827	17.965
C5	4	8402016	5.619	39.898	14.190	70.952	110.850	22.170
C6	4	8395405	5.615	39.867	14.179	70.897	110.763	22.153
C7	8	7945744	5.314	37.731	13.420	67.099	104.831	20.966
C8	8	8034064	5.373	38.151	13.569	67.845	105.996	21.199
C9	12	8162432	5.459	38.760	13.786	68.929	107.689	21.538
C10	12	8125747	5.435	38.586	13.724	68.619	107.205	21.441
C11	16	7798531	5.216	37.032	13.171	65.856	102.888	20.578
C12	16	8274269	5.534	39.291	13.975	69.874	109.165	21.833
C13	20	8354163	5.587	39.671	14.110	70.548	110.219	22.044
C14	20	7752960	5.185	36.816	13.094	65.471	102.287	20.457
C15	25	7531213	5.037	35.763	12.720	63.599	99.362	19.872
C16	25	8193619	5.480	38.908	13.839	69.193	108.101	21.620
C17	32	8625958	5.769	40.961	14.569	72.843	113.805	22.761
C18	32	7675840	5.134	36.450	12.964	64.820	101.270	20.254
C19	44	7944707	5.314	37.726	13.418	67.091	104.817	20.963
C20	44	8515290	5.695	40.436	14.382	71.909	112.345	22.469
C21	56	8419187	5.631	39.979	14.219	71.097	111.077	22.215
C22	56	8455738	5.655	40.153	14.281	71.406	111.559	22.312
C23	68	8035498	5.374	38.157	13.571	67.857	106.015	21.203
C24	68	7194768	4.812	34.165	12.152	60.758	94.923	18.985
C25	92	7714189	5.159	36.632	13.029	65.144	101.776	20.355

Table A-J4c(cont.).

Sample	Time (hours)	Peak Area	Concentration in HS (mg/L)	Mass in HS (μg)	Concentration in Solution (mg/L)	Mass in Solution (μg)	Total Mass (μg)	Concentration of Initial Solution (mg/L)
C26	92	8225395	5.501	39.059	13.892	69.461	108.520	21.704
C27	116	8454214	5.654	40.146	14.279	71.393	111.539	22.308
C28	116	8643578	5.781	41.045	14.598	72.992	114.037	22.807
C29	140	8285376	5.541	39.344	13.993	69.967	109.311	21.862
C30	140	8148202	5.450	38.693	13.762	68.809	107.502	21.500
C31	164	8166090	5.462	38.778	13.792	68.960	107.738	21.548
C32	164	8544250	5.715	40.573	14.431	72.153	112.727	22.545
C33	212	8735866	5.843	41.483	14.754	73.772	115.255	23.051
C34	212	8511814	5.693	40.419	14.376	71.880	112.299	22.460
C35	260	8918918	5.965	42.353	15.063	75.317	117.670	23.534
C36	260	8221770	5.499	39.042	13.886	69.430	108.472	21.694
C37	284	8545446	5.715	40.579	14.433	72.164	112.743	22.549
C38	284	8263418	5.527	39.240	13.956	69.782	109.022	21.804
C39	na	na	na	na	na	na	na	na
C40	na	na	na	na	na	na	na	na

Table A-J5. Data used to create Figure 5 in Chapter 2.

Observed Biodegradation Model (Km = -0.13 1/h)				Desorption-Equilibrium Model (Km = -0.06 1/h)			
Time (h)	**Time-T(lag) (h)**	**[T*Km]**	**C/Co [EXP(T*Km)]**	**Time (h)**	**Time-T(lag) (h)**	**[T*Km]**	**C/Co [EXP(T*Km)]**
0	0	0.00	1.000				
1	0	0.00	1.000	0	0	0.00	1.000
4	0	0.00	1.000	8	0	0.00	1.000
8	0	0.00	1.000	12	4	-0.24	0.787
8.1	0.1	-0.01	0.987	13	5	-0.30	0.741
9	1	-0.13	0.878	14	6	-0.36	0.698
10	2	-0.26	0.771	15	7	-0.42	0.657
11	3	-0.39	0.677	16	8	-0.48	0.619
12	4	-0.52	0.595	17	9	-0.54	0.583
13	5	-0.65	0.522	18	10	-0.60	0.549
14	6	-0.78	0.458	19	11	-0.66	0.517
15	7	-0.91	0.403	20	12	-0.72	0.487
16	8	-1.04	0.353	22	14	-0.84	0.432
17	9	-1.17	0.310	24	16	-0.96	0.383
18	10	-1.30	0.273	26	18	-1.08	0.340
19	11	-1.43	0.239	28	20	-1.20	0.301
20	12	-1.56	0.210	30	22	-1.32	0.267
21	13	-1.69	0.185	32	24	-1.44	0.237
22	14	-1.82	0.162	34	26	-1.56	0.210
23	15	-1.95	0.142	36	28	-1.68	0.186
24	16	-2.08	0.125	38	30	-1.80	0.165
25	17	-2.21	0.110	40	32	-1.92	0.147
26	18	-2.34	0.096	42	34	-2.04	0.130
27	19	-2.47	0.085	44	36	-2.16	0.115
28	20	-2.60	0.074	46	38	-2.28	0.102
29	21	-2.73	0.065	48	40	-2.40	0.091
30	22	-2.86	0.057	50	42	-2.52	0.080

Table A-J5 (cont.).

Observed Biodegradation Model (Km = -0.13 1/h)				Desorption-Equilibrium Model (Km = -0.06 1/h)			
Time (h)	Time-T(lag) (h)	[T*Km]	C/Co [EXP(T*Km)]	Time (h)	Time-T(lag) (h)	[T*Km]	C/Co [EXP(T*Km)]
31	23	-2.99	0.050	52	44	-2.64	0.071
32	24	-3.12	0.044	54	46	-2.76	0.063
33	25	-3.25	0.039	56	48	-2.88	0.056
34	26	-3.38	0.034	58	50	-3.00	0.050
35	27	-3.51	0.030	60	52	-3.12	0.044
36	28	-3.64	0.026	62	54	-3.24	0.039
37	29	-3.77	0.023	64	56	-3.36	0.035
38	30	-3.90	0.020	68	60	-3.60	0.027
39	31	-4.03	0.018	72	64	-3.84	0.021
40	32	-4.16	0.016	76	68	-4.08	0.017
41	33	-4.29	0.014	80	72	-4.32	0.013
42	34	-4.42	0.012	84	76	-4.56	0.010
43	35	-4.55	0.011	92	84	-5.04	0.006
44	36	-4.68	0.009				
45	37	-4.81	0.008				
46	38	-4.94	0.007				
47	39	-5.07	0.006				
48	40	-5.20	0.006				
49	41	-5.33	0.005				
50	42	-5.46	0.004				
51	43	-5.59	0.004				
52	44	-5.72	0.003				
53	45	-5.85	0.003				
54	46	-5.98	0.003				
55	47	-6.11	0.002				
56	48	-6.24	0.002				
57	49	-6.37	0.002				
58	50	-6.50	0.002				
59	51	-6.63	0.001				
60	52	-6.76	0.001				

Table A-J6. CXTFIT2 data (used to create Figure 6 in Chapter 2).

Distance through Barrier (m)	Concentration (mg/L)	Distance through Barrier (m)	Concentration (mg/L)	Distance through Barrier (m)	Concentration (mg/L)
-0.15	100.00	0.37	7.21	0.78	1.93
-0.05	100.00	0.38	6.98	0.79	1.87
-0.01	100.00	0.39	6.76	0.8	1.81
-9E-13	100.00	0.4	6.55	0.81	1.75
0	23.71	0.41	6.34	0.82	1.70
0.01	22.96	0.42	6.14	0.83	1.64
0.02	22.24	0.43	5.95	0.84	1.59
0.03	21.53	0.44	5.76	0.85	1.54
0.04	20.85	0.45	5.58	0.86	1.49
0.05	20.19	0.46	5.40	0.87	1.44
0.06	19.55	0.47	5.23	0.88	1.40
0.07	18.93	0.48	5.06	0.89	1.35
0.08	18.33	0.49	4.90	0.9	1.31
0.09	17.75	0.5	4.75	0.91	1.27
0.1	17.19	0.51	4.60	0.92	1.23
0.11	16.65	0.52	4.45	0.93	1.19
0.12	16.12	0.53	4.31	0.94	1.15
0.13	15.61	0.54	4.17	0.95	1.12
0.14	15.12	0.55	4.04	0.96	1.08
0.15	14.64	0.56	3.91	0.97	1.05
0.16	14.17	0.57	3.79	0.98	1.01
0.17	13.73	0.58	3.67	0.99	0.98
0.18	13.29	0.59	3.55	1	0.95
0.19	12.87	0.6	3.44	1.01	0.95
0.2	12.46	0.61	3.33	1.05	0.95
0.21	12.07	0.62	3.23	1.1	0.95
0.22	11.69	0.63	3.13		
0.23	11.32	0.64	3.03		
0.24	10.96	0.65	2.93		
0.25	10.61	0.66	2.84		
0.26	10.28	0.67	2.75		
0.27	9.95	0.68	2.66		
0.28	9.63	0.69	2.58		
0.29	9.33	0.7	2.49		
0.3	9.03	0.71	2.42		
0.31	8.75	0.72	2.34		
0.32	8.47	0.73	2.27		
0.33	8.20	0.74	2.19		
0.34	7.94	0.75	2.12		
0.35	7.69	0.76	2.06		
0.36	7.45	0.77	1.99		

APPENDIX K. REFERENCES

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