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Influence of substrate exposure history on biodegradation in a porous medium

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Abstract

This study investigates the influence of fluctuating toluene concentrations on aerobic toluene degradation in a sandy porous medium colonized with *Ralstonia pickettii* PKO1. Column effluent toluene concentrations were found to increase after a temporary decrease in influent toluene concentration. Subsequent examination of the spatial gradient of toluene degradative activity in the column suggested that the observed increase in effluent toluene concentrations was attributable to an adverse effect of toluene limitation on the biodegradative activity of attached cells. The traditional Michaelis–Menten-type biodegradation equation associated with batch-measured V_{max} (2.26 $mg_{toluene}/mg_{living\ cell}/day$) and K_S (1.20 $mg_{toluene}/l$) of nonstarved cells was unable to predict the observed toluene breakthrough behavior when the column had been previously exposed to no-toluene conditions. An alternative modeling approach was developed based upon the assumptions that (i) degradative activity was completely deactivated within the no-toluene exposure period (53.5 h) and (ii) a lag-phase was present prior to the subsequent reactivation of degradative activity in previously toluene-starved cells. These assumptions were independently verified by batch microbial investigations, and the modified model provided a good fit to the same observed toluene breakthrough curve. Application of single lag-time and threshold concentration values, however, failed to predict observed toluene breakthrough under different toluene exposure conditions. Results of this experimental and modeling investigation suggested that substrate exposure history, including the length of the starvation period and the level of substrate concentration, affected the induction of biodegradation in the porous medium.

Keywords: Toluene; Biodegradation kinetics; Porous media; Contaminant transport modeling

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1. Introduction

Potential biodegradative capacity, as assessed by treatability studies performed in laboratory batch experimental systems, is often not fully realized in contaminated subsurface environments (e.g., Alexander, 1985). The reduced degradation rates observed in field studies may be attributed, in part, to the fact that laboratory batch experimental systems are not fully representative of the field conditions to which their extrapolated parameters are applied. For example, field conditions typically exhibit physical and chemical heterogeneity (e.g., Ronen et al., 1986; Frind et al., 1990; Murphy et al., 1997), complexity in microbial community structure and population dynamics (e.g., Fries et al., 1997), mass transfer and diffusion limitations (e.g., Bosma et al., 1997), and reduced availability of mineral nutrients and electron acceptors (e.g., Cobb and Bouwer, 1991).

In particular, microbes in the field may have experienced a different substrate exposure history than those used in batch experiments. Field conditions are often characterized by widely fluctuating substrate concentrations resulting from the presence of groundwater flow and heterogeneous source distributions (e.g., National Research Council, 1993; Vroblesky and Chapelle, 1994). In addition, subsurface microbes, believed to exist primarily in an attached state (e.g., Aakra et al., 2000), are generally in dormant or starvation conditions, due to a lack of carbon and energy source (e.g., Morita, 1993). In contrast, batch studies generally employ suspended cells, grown at high substrate concentration levels and harvested during exponential growth. Furthermore, results of some laboratory batch studies that have used suspended-growth microbes suggest that variations in preculture conditions may cause variations in batch-measured biodegradation kinetic parameters (Grady et al., 1996; Sommer et al., 1998). If the biodegradative activity of attached-growth subsurface microbes is affected by prior substrate exposure, one would expect that Michaelis–Menten kinetic parameters derived under typical batch conditions may be inappropriate for the prediction of field scale bioremediation.

For most subsurface microorganisms, biodegradative activity is inducible, i.e., the presence of a particular substrate results in an induction of synthesis of enzymes needed to degrade the substrate via specific gene expression. Important examples of such inducible enzyme systems are bacterial toluene oxygenase pathways that have the ability to degrade a variety of organic contaminants, including aromatic hydrocarbons and trichloroethylene (TCE) (Wackett and Gibson, 1988; Shields et al., 1991; Mikesell et al., 1993; Leahy et al., 1996; Fries et al., 1997; McCarty et al., 1998). When toluene acts as a growth substrate, as well as an exogenous molecular effector, i.e., an inducing agent that enhances specific gene expression for synthesis of enzymes required for the toluene oxygenase pathway (Byrne and Olsen, 1996; Shingleton et al., 1998), it is reasonable to expect toluene limitation to have a significant effect on biodegradation. However, the effect of toluene limitation on biodegradative activity of attached bacterial cells has not yet been examined for any inducible toluene oxygenase system.

In view of the considerations set forth above, a laboratory column study was conducted using the well-characterized toluene-3-monooxygenase (T3MO) expressing bacterium, *Ralstonia pickettii* PKO1 (Olsen et al., 1994; Byrne and Olsen, 1996) in a

defined system (i.e., in a homogenous saturated sandy porous medium, and in the presence of sufficient oxygen and mineral nutrients) in order to explore the influence of toluene exposure history on toluene degradation and transport. For a mechanistic understanding of the effects of physiological state on biodegradation and transport, the use of an inert homogenous porous medium that is colonized by a specific bacterium or bacterial consortium exhibiting the phenomena of interest is an effective experimental approach (Bouwer and McCarty, 1983; Truex et al., 1992). Although such an approach is less representative of field conditions than the use of a soil column packed with indigenous aquifer material (Kuhn et al., 1985; van der Meer et al., 1987; Allen-King et al., 1996), use of indigenous aquifer material often precludes mechanistic analysis due to the inherent physical and microbial complexity of such systems (Truex et al., 1992). Furthermore, use of a well-defined system permitted data interpretation through the application of mathematical modeling associated with experimentally determined parameters. In addition to the column studies, microbial investigations were also conducted with PKO1 suspended-growth cells in order to examine whether toluene exposure history of the magnitude observed in the column system had an effect on toluene degradation behavior in strain PKO1.

The specific objectives of this study were to (i) examine whether the response of attached cell biodegradative activity to fluctuating toluene concentrations followed a Michaelis–Menten or Monod-type behavior, (ii) examine the effect of toluene limitation on attached cell biodegradative activity, and (iii) examine the applicability of batch-measured biodegradation kinetic parameters for the prediction of column biodegradation under fluctuating toluene concentrations.

2. Materials and methods

2.1. Materials

For all batch and column investigations, a basal salts medium (BM; Leahy et al., 1996) was employed as the mineral nutrient medium. Toluene (HPLC-grade, Aldrich Chemical) was used as the sole carbon and energy source. Ottawa Foundry Sand F95 (U.S. Silica), a uniform quartz sand, was selected as the porous medium, based upon its low sorption capacity. The mean particle diameter (d_{50}) and the uniformity coefficient (or Hazen's coefficient, $C_u = d_{60}/d_{10}$) of this sand are 0.13 ± 0.02 mm and 1.36 ± 0.06, respectively.

2.2. Culture conditions

Prior to batch and column experiments, cells were grown in 4-l culture flasks containing 500 ml BM, in the presence of toluene vapors. To ensure adequate enzymatic induction, toluene vapor was continuously provided to each culture from 0.5 ml of liquid toluene placed in a 1.8-ml GC vial attached to the inside of the stopper in each 4-l culture flask. In a similar setup, Leahy and Olsen (1997) have reported that the toluene concentrations in the culture flasks were about 230 mg/l. The prepared 4-l culture flasks

were placed on a platform shaker (270 rpm) in a controlled-temperature room (30°C). After 72 h of incubation, cells were harvested from the spent medium by centrifugation (8000 rpm at 4°C for 10 min). The cell pellet was washed once with fresh BM and then resuspended in 200 ml of fresh BM prior to use. In addition, whenever toluene-starved cells (suspended growth) were needed, the prepared 200-ml cell suspensions in 1-l culture flasks were stirred using magnetic stirrers. The cells were then incubated for the desired period of toluene starvation under a laminar flow chamber.

2.3. Analytical techniques

A viable plate count method was used to quantify the biomass of living (viable and culturable) cells. Tryptone nutrient agar (TNA; Olsen and Hansen, 1976) medium was used for the plate counts. In this study, specific toluene degradation rates for batch and column experiments are reported on a wet weight basis. To convert colony forming units (cfu) to milligrams of wet weight of living cells, a conversion factor of 5.8×10^{-10} mg_{cell} per living cell was used. This conversion factor was estimated using the microscopically measured size of toluene-grown PKO1 cells (length $= 2.05 \pm 0.15$ μm, width $= 0.6 \pm 0.1$ μm) and by assuming that one living cell forms a colony on a TNA plate and that the density of a living cell is approximately 1 g/ml at 25°C. Our previous microscopic examination has supported the validity of the first assumption (i.e., a single cell forms one colony); most of suspended PKO1 cells do not coalesce into groups but rather remain separate from one another.

The ability of viable cells to grow on toluene was examined using a modified toluene-plate method (Mars et al., 1996). Colonies previously grown on TNA plates were resuspended in 1 ml of fresh BM, and then serial dilutions were carried out. The cells from these serial dilutions were spread onto solid BM medium to which vapors of toluene were continuously supplied by adding one drop of pure toluene on a filter paper disk in the cover of the petri dish (toluene plates). The toluene plates were stored in air-tight glass containers for 5 days and the number of colonies was then determined.

Toluene and its transformation products, *m*-cresol and 4-methyl catechol, were analyzed by reverse phase high performance liquid chromatography (HPLC) using a Hewlett-Packard 1090 series II system with a hypersil 5C18 column and ultraviolet (210 nm) and fluorescence detectors. Under these conditions, the detection limits were 10 ppb for toluene, 1 ppb for *m*-cresol and 100 ppb for 4-methyl catechol. Aqueous phase samples (0.5 ml) for HPLC analysis were taken with a gas-tight 1-ml syringe, mixed with an equal volume of methanol in 1.8-ml GC vials and stored at 4°C until analyzed.

Dissolved oxygen (DO) was analyzed in batch experiments using a needle electrode (Diamond General Development) coupled to a Diamond General Chemical Microsensor. For the column experiment, DO was measured in-line with a 1-ml chamber equipped with a removable cap with Clark style polarographic electrode (model 125/05, Instech Laboratory). To minimize mass-transfer limitations through the membrane of the electrode, samples in the 1-ml chamber were vigorously mixed using a mini-magnetic stirrer (Fisher Scientific). Calibration curves for DO measurements were constructed using BM saturated with 15%, 21%, and 30% oxygen. Bromide concentration (detection

limit 0.1 μM) was measured by ion chromatography using a Dionex series 4500 I system equipped with a Dionex Ionpac column.

2.4. Batch experimental procedures

In order to monitor toluene degradation in cell suspensions, 15-ml CMBRs (completely mixed batch reactors) equipped with sampling ports containing GC septa (Alltech Associates) and 160-ml serum bottles with teflon-lined septa were used. Prior to measuring toluene degradation, cell suspensions in BM were preoxygenated for 30 min. When 15-ml CMBRs were used, the reactors were first filled with cell suspensions without head-space, then crimp-sealed and toluene was added from a stock solution prepared in $N'N'$-dimethylformamide through the GC septa into the reactors. When 160-ml serum bottles were used, 15 ml of each cell suspension was first transferred into each bottle. Toluene was then added from the same stock into the bottle, and the bottle was immediately crimp-sealed. Aqueous phase samples for toluene HPLC analysis were taken using a microsyringe through either the GC septa of 15-ml CMBRs or the teflon-lined seals of 160-ml serum bottles, and then stored in 50% (v/v) methanol prior to HPLC analysis as described above. To minimize volatilization losses in the 160-ml serum bottles, the maximum number of samples taken from one bottle was limited to five. Abiotic disappearance in the reactors was investigated through control experiments conducted in the same manner as described above. Controls consisted of cells that had been previously killed by exposure to 0.2 N NaOH or of bottles containing media and toluene but no cells. Duplicate plate counts were conducted before and after each experiment to quantify the biomass. The final DO concentrations were measured to ensure that aerobic conditions (i.e., above 2.5 mg/l; Leahy and Olsen, 1997) had been maintained through the experiments.

To measure initial toluene degradation rates, toluene degradation was monitored every 30 min for 2 h using the 15-ml CMBRs as described above. The initial rates were calculated using the linear portion of the toluene disappearance curve (R^2 was larger than 0.9). Since viable biomass during the initial rate measurements did not change significantly, the measured initial viable biomass was used to report specific degradation rate.

Determination of microbial parameters was undertaken with nonstarved cells, i.e., the suspended cells that had been precultured in BM in the presence of toluene vapors as described above. After the cell suspension in BM containing the nonstarved cells was first exposed to no-toluene conditions, the viable biomass in the cell suspension was determined daily by duplicate plate counts and these viable biomass data were used to estimate a first-order cell decay coefficient. To determine Michaelis–Menten kinetic parameters (V_{max} and K_S) for aerobic toluene degradation, both a single-batch method and an initial rate method were used with 15-ml CMBRs. For the single-batch method, toluene degradation was monitored in one reactor every 15 min for a period shorter than the doubling time of PKO1 on toluene (approximately 6 h). For the initial rate method, the initial rates were measured as described above for 15-ml CMBRs with varying initial toluene concentrations. All batch experiments were performed at 24.0 ± 2.0°C.

2.5. Column experimental procedures

The column experimental setup is shown in Fig. 1. A cylindrical borosilicate glass column (inner diameter 4.8 cm, effective bed length 14.1 cm, Chromaflex Organic Column, Konetes) with a water jacket was used as the porous medium container. To minimize the sorptive losses of toluene, Kalrez *O*-rings and inert teflon end-fittings with a woven mesh bed support (10 μm opening size, Konetes) were used. Stainless steel tubes installed in the HPLC pump (Rainin) were flushed with methanol and then rinsed with sterile 40 mM phosphate buffer to minimize bacterial contamination. Other parts of the experimental system that would be exposed to cells were autoclaved prior to use. Sand was prepared by washing several times with Milli-Q water, and oven-drying at 110°C overnight. The prepared sand was autoclaved before the column was packed.

Packing and inoculation were conducted simultaneously in a laminar flow chamber using a protocol developed for this study. The procedure was initiated by adding 5 ml of the prepared cell suspension to the empty porous medium container. The autoclaved sand was then gradually added to the cell suspension so that the sand became saturated. The column was vibrated with the aid of a touch mixer (model 134, Fisher Scientific) to uniformly distribute the sand and cell suspension. This procedure was repeated until the

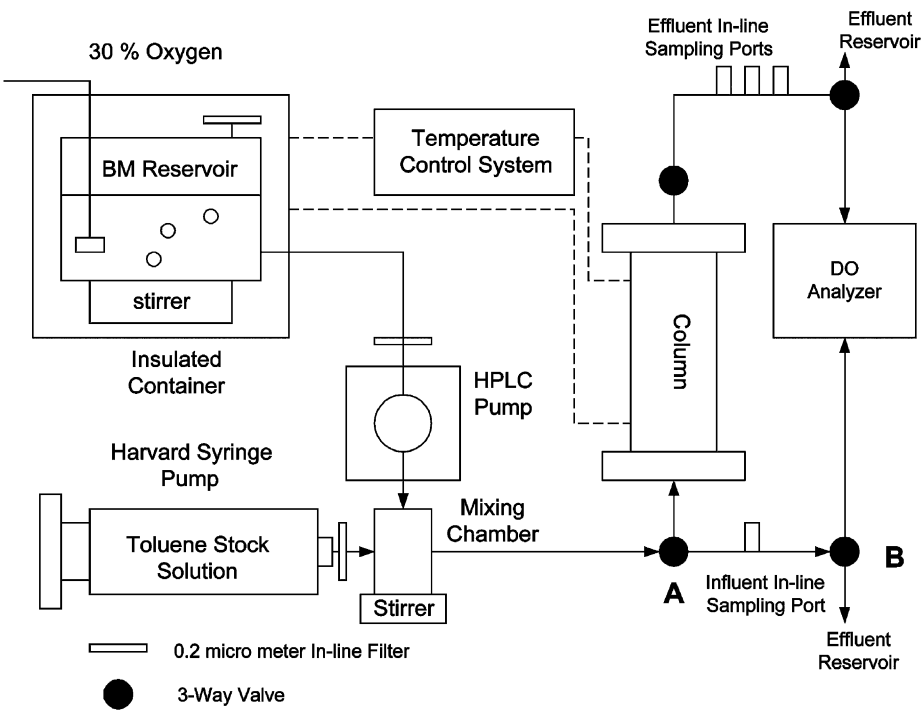

Fig. 1. Column apparatus schematic. The temperature of the basal salts medium (BM) reservoir and column was controlled at 25 ± 0.5°C.

effective bed was filled with saturated sand. The total weight of the added sand and the total volume of the added cell suspension were measured to calculate average bulk density (ρ_b) and porosity (n). After a 4-h acclimation period, to facilitate adhesion of cells onto the sand, the column was flushed with fresh BM, containing 3 mM bromide for approximately 24 h (retention time was 11.8 h) at a constant flow rate of 0.123 ± 0.02 ml/min. During flushing, the fresh BM was aerated aseptically to provide aerobic conditions for the cells and effluent collected. The concentration of viable cells in the collected effluent was determined using replicate plate counts. The total number of viable cells remaining in the column was estimated by subtracting the number of cumulative viable cells in the collected effluent from the total number of added viable cells. The initial column cell concentration was then reported as colony forming units per bulk column volume ($\text{cfu/ml}_{\text{column}}$). To ascertain the accuracy of biomass estimates and the uniformity of inoculation, preliminary tests were conducted by sacrificing columns that had been prepared using the same packing and inoculation procedure. The columns were opened and the spatial distribution of viable biomass was quantified using replicate plate counts. Cells were removed from the sand by repeated extraction with 40 mM phosphate buffer, as described below. Estimated biomass concentrations in the columns were within the error bars of measured values, and the measured biomass was spatially uniform.

Toluene was supplied to the column from a stock solution prepared in a 50-ml stainless steel syringe, which was pumped by a Harvard syringe pump (model 55-5722) at a constant flow rate (0.004 ml/min). Mineral nutrients (BM) and oxygen were supplied by a HPLC pump (Rainin) from a sterile 4-l reservoir saturated with an aerobic gas mixture (30% oxygen, 5% carbon dioxide and 65% nitrogen) at a constant flow rate of approximately 0.127 ml/min. The temperature in the reservoir bottle and the column was controlled at 25 ± 0.5°C by circulating water from a water-bath (NESLAB).

To monitor toluene disappearance, both the influent and effluent streams were sampled in-line. Influent samples were analyzed daily for toluene, and effluent samples were analyzed for toluene, *m*-cresol and 4-methyl catechol using the HPLC analytical technique described above. In order to ensure that aerobic conditions had been maintained, DO was measured periodically in both the influent and effluent streams. Viable cell concentration in the effluent was quantified daily from duplicate plate count analyses. Flow rates were verified by periodic effluent volume (mass) measurement.

The spatial gradients of viable cell density and biodegradative activity in the column were examined after flushing. To determine viable biomass concentration gradients, bulk samples of approximately 3 ml were taken at different column locations and transferred into 35-ml glass vials. Cells were removed from the sand by shaking with 10 ml of sterile 40 mM phosphate buffer at 300 rpm for 20 min. This extraction was repeated five times with separate 10 ml aliquots of the same buffer and the combined extracts were pooled in a tared 100-ml glass bottle. The remaining bulk samples in the 35-ml glass vials were oven-dried at 110°C for 24 h, and the weight of the dried sand measured. The total number of viable cells in the combined extracts was calculated based upon the viable cell concentration and the volume of the combined extracts. The measured column cell concentration is reported in units of $\text{cfu/ml}_{\text{column}}$ based upon the number of total cells, the weight of dried sand and the column bulk density (ρ_b). To examine the

spatial gradient of biodegradative activity, cells were repeatedly extracted with 40 mM phosphate buffer from different zones in the column, and the levels of biodegradative activity for the extracted cells were quantitatively compared using the modified initial rate method as described above. In order to detect toluene degradation by the extracted cells, the cells in the extracts were concentrated by centrifugation (8000 rpm for 10 min at 4°C) and cell pellets were resuspended in 50 ml of preoxygenated fresh BM.

To estimate dispersivity, a tracer test was conducted simultaneously with the first injection of the BM/toluene solution. As previously described, the column had been saturated with 3 mM bromide solution during the packing and inoculation procedure. Subsequent to the initiation of the BM/toluene injection, effluent bromide concentrations were measured every hour for the first 27 h. A separate control column experiment was also conducted with a sterilized column prepared by the same procedure. In this control experiment, about 2.75 pore volumes of influent containing both 0.9 ppm toluene and 3 mM bromide were pulsed, and effluent toluene and bromide concentrations were monitored together.

3. Results

3.1. Batch-measured kinetic parameters of toluene degradation by nonstarved cells

The Michaelis–Menten kinetic parameters (V_{max} and K_S) for aerobic toluene degradation by suspended cells of strain PKO1 determined in this study are presented in Table 1, along with parameters determined by other investigators who have worked with this strain. To estimate these parameters, a nonlinear regression was performed on the data obtained from the single-batch kinetic measurements using an integrated form of the Michaelis–Menten model (Robinson and Tiedje, 1983). A differentiated form of the model was fit to data from the initial rate measurements. In both cases, only data obtained under conditions of excess oxygen were used for parameter estimation, based upon a previous study that showed that toluene degradation kinetics for PKO1 are not affected by oxygen levels when the dissolved oxygen concentration was above 2.5 ppm (Leahy and Olsen, 1997).

Table 1 reveals considerable variation among available estimates of aerobic toluene degradation kinetic parameters for PKO1 (one and two orders of magnitude for K_S and V_{max}, respectively). Parameters derived using two different estimation approaches in this study, however, varied by no more than 40%. Moreover, the estimates obtained in this study are of the same magnitude as those obtained previously by Leahy et al. In contrast, the estimates of Massol-Deyá et al. (1997) differ significantly from those obtained by this study and by Leahy et al. It is difficult to conclusively explain the reasons for this discrepancy because the studies were based on different experimental conditions. Nevertheless, it is possible to postulate that previous toluene exposure history may have influenced toluene degradation kinetic behavior. Massol-Deyá et al. used relatively low initial toluene concentrations (50 mg/l) for T3MO induction conditions prior to their batch kinetic measurements, while other laboratories used higher toluene concentrations (i.e., 230 mg/l).

Table 1
Batch-measured kinetic parameters of aerobic toluene degradation of *R. pickettii* PKO1

Measurement method	Toluene concentration for induction ($mg_{toluene}$ /l)	V_{max} ($mg_{toluene}$ / mg_{cell} /day)[a]	K_S ($mg_{toluene}$ /l)	R^2 (observed versus predicted)	Reference
Single-batch[b]	In the presence of toluene vapor	2.26 (1.82–2.96)[c]	1.20[d]	$R^2 = 0.980$	This study
Single-batch	50	0.041	0.20	NR[e]	Massol-Deyá et al. (1997)
Initial rates	In the presence of toluene vapor	3.14 (1.14–5.15)[f]	1.41 (0.34–2.48)[f]	$R^2 = 0.850$	This study
Initial rates	230	1.06 (0.97–1.15)[g]	1.20 (1.17–1.23)[g]	NR	Leahy (personal communication)

[a]The unit is milligram of toluene per milligram of wet weight of living cell per day. To compare the reported values from different sources, the units for the documented V_{max} values were converted from reported units of nanomoles per minute per optical density at 425 nm (Leahy, personal communication) or milligram of toluene per 10^{10} colony forming units per hour (Massol-Deyá et al., 1997, and this study), assuming that one living cell forms one colony and a living toluene-grown cells is 5.8×10^{-10} mg.

[b]The initial toluene concentrations were 2.32 and 2.61 mg/l in the duplicated single-batch measurements.

[c]Numbers in parentheses indicate a range of the estimated parameter values when uncertainty of biomass measurements is incorporated in the parameter estimation. The range is reported because the variation due to uncertainty in biomass measurements is wider than the 95% confidence interval of the reported parameter value (2.02–2.49).

[d]The parameter, K_S, was fixed at 1.20 when nonlinear regression analysis was performed with single-batch kinetic data attained from this study.

[e]NR, not reported.

[f]The numbers in the parentheses indicate 95% confidence intervals of the reported parameter values.

[g]The numbers in the parentheses represent one standard deviation of the reported parameter values.

3.2. Continuous-flow column experiments

Significant degradation of toluene was observed during the column experiment. As shown in Fig. 2, toluene was not detected in the effluent except during the three breakthrough periods that occurred between days 0–1, days 6–8, and on day 13. The first and second degradation intermediates in the toluene-3-monooxygenase pathway of strain PKO1, *m*-cresol and 4-methylcatechol (Olsen et al., 1994), were not detected in effluent samples. In the control column experiment, insignificant abiotic losses were observed, i.e., approximately 99% of the applied influent toluene concentrations were recovered in the effluent. These results support the conclusion that toluene was completely mineralized in the column experiment.

An examination of Fig. 2 reveals that the three bell-shaped toluene breakthroughs detected in the effluent were linked to temporary decreases in the influent toluene concentrations. Taking into consideration the hydraulic retention time of 11.6 ± 0.1 h, the first detected breakthrough (peak A) corresponds to the initial toluene influent concentration after a 53.5-h (2.2-day) period of no-toluene exposure, and peaks B and C correspond to the two perturbations in the level of influent toluene concentration. This

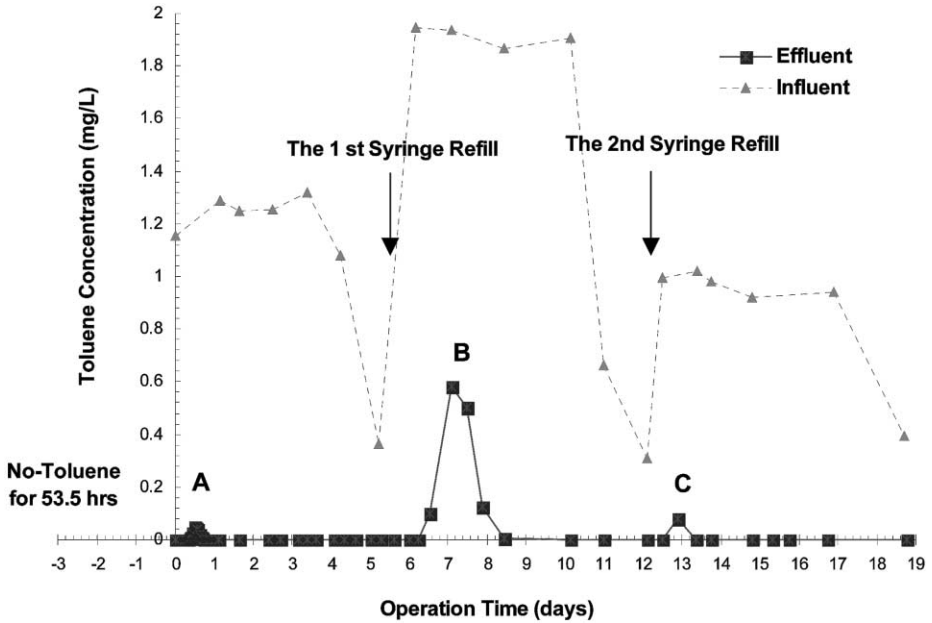

Fig. 2. Influent and effluent toluene concentrations in the column experiment. The measured hydraulic retention time was 11.6 ± 0.1 h.

behavior could not result from factors other than the fluctuating toluene concentrations. No significant increase in detachment of cells was observed prior to each peak and all the levels of influent toluene concentration were lower than the observed inhibitory concentration level for strain PKO1 (about 18 mg/l, Leahy, personal communication). An insignificant change in flow rates (less than 3%) was observed during the experiment and all measured dissolved oxygen concentrations in the column were above 2.5 mg/l, ensuring that there was excess oxygen for toluene degradation by strain PKO1 (Leahy and Olsen, 1997).

The appearance of peak C in Fig. 2 indicates that the toluene degradative activity of PKO1 cells in the column depended upon the level of toluene influent concentration. If substrate concentration dependence did not exist, the lower toluene influent concentration (about 0.9 mg/l after the second syringe refill) would have been completely degraded within the column on the 13th day, since a higher toluene influent concentration (about 1.85 mg/l) had been completely degraded prior to the 10th day in the experiment. It is possible to explain the reduced degradation capacity on the 13th day of the experiment by a Michaelis–Menten-type substrate concentration dependence since the influent toluene concentration also decreased prior to the 13th day. However, the Michaelis–Menten-type substrate concentration dependence cannot explain the complete degradation that occurred subsequent to the 13th day of the experiment. Thus, the concentration dependence manifested in the appearance of peak C did not follow a Michaelis–Menten-type equation.

An examination of the spatial gradient of toluene degradative activity in the column provides evidence that toluene limitation had a negative effect on the biodegradative activity of the attached cells. Fig. 3 shows the batch experimental results for toluene degradation measurements on core samples extracted at the end of the column experiment. The slopes of these curves represent specific (viable biomass normalized) toluene degradation rates of the cells extracted from the column core samples. Only the upstream sample (0–3-cm core) exhibited statistically significant toluene degradation for the 60-min measurement made after a lag-time of approximately 30 min. Since a nearly identical concentration of toluene (0.3 mg/l) was applied initially in the toluene degradation measurements, differences in the intrinsic biodegradative activity for attached cells along the length of the column can be compared directly from the slopes in Fig. 3. For attached cells in the downstream core, i.e., 9–12-cm core in Fig. 3, the absence of biodegradative activity was associated with toluene limitation, since toluene was not detected at the effluent during the last 5 days in the column experiment. Further evidence for this toluene limitation in the 9–12-cm downstream core is provided by the fact that no growth was observed at the corresponding column locations as described below (Fig. 4). The observed spatial gradient of biodegradative activity in the column suggests that biodegradative activity depends upon prior exposure history, and reveals that history-independent Michaelis–Menten parameters cannot predict observed degradation rates.

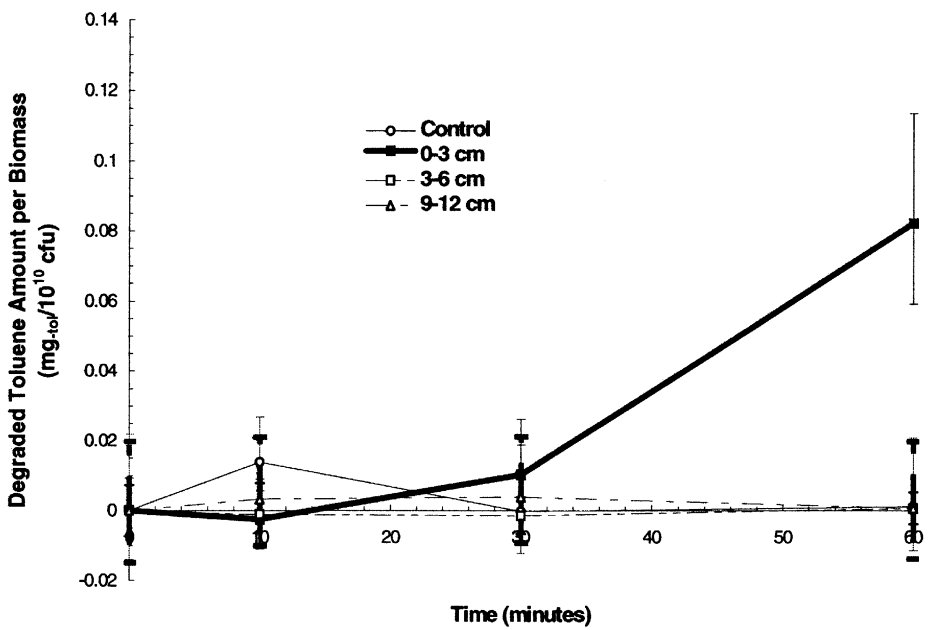

Fig. 3. Biomass normalized toluene degradation (initial toluene concentration = 0.3 mg/l) in cells extracted from column core samples at the end of column experiment. The numbers in the annotation indicate distance from the column inlet. The uncertainty in biomass quantification and the differences between duplicate degradation measurements were incorporated into the calculation of the *Y*-error bars.

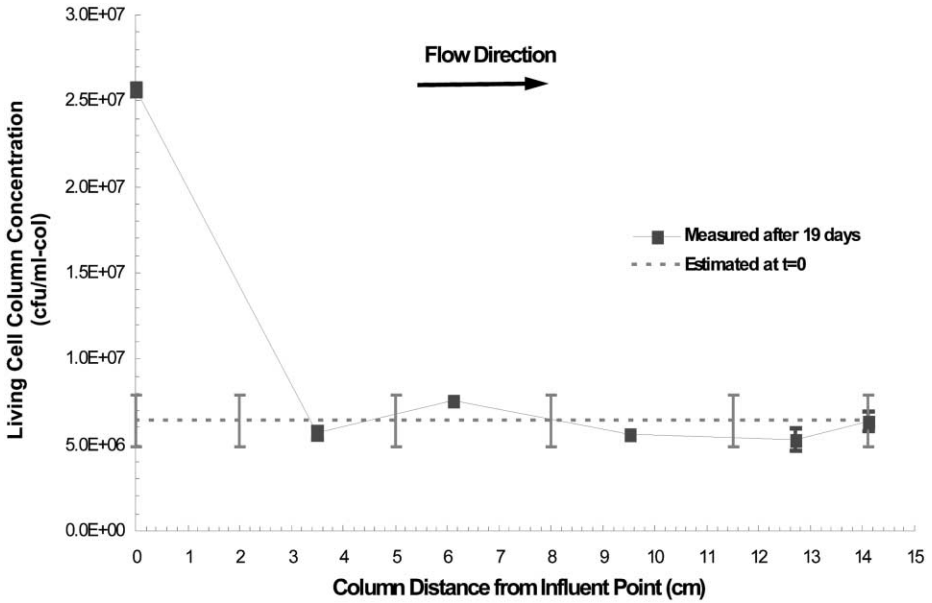

Fig. 4. Spatial distributions of living cell concentrations before and after the column experiment. The filled squares and the dashed line represent the average values. The *Y*-error bar represents one standard error of the measured average value.

It could be speculated that the observed lag-time of about 30 min in the upstream sample (0–3-cm core, Fig. 3.) may be due to changes in microbial physiology caused by destructive sampling and repeated extraction. However, an examination of culturability on TNA and toluene plates suggests otherwise, since the culturability (i.e., the numbers of colonies on the plates after 3 days [for TNA plates] and 5 days [for toluene plates]) was not significantly different for these cells than for nonstarved cells that had been shaken with sand and centrifuged in a manner identical to that used for the sampling and extraction. Instead, a plausible explanation for the observed lag-time of about 30 min in the upstream sample is the existence of a microbial lag-phase associated with the production or activity of the toluene metabolic system. Since the cells extracted from this part of the column had not been exposed to toluene for approximately 10 h during sampling and preparation for toluene degradation measurements, it is reasonable to postulate that the toluene degradative activity declined for the extracted cells during the 10-h no-toluene exposure period, and that cell activity was reactivated after a lag-time of approximately 30 min, during which the cells were continuously exposed to toluene applied in the batch experiments for degradation measurements.

The spatial distribution of viable cells quantified at the end of the column experiment is presented in Fig. 4. The highest viable cell concentration of $2.57 \pm 0.04 \times 10^7$ $\mathrm{cfu/ml_{column}}$ was observed at the influent end, while the measured viable cell concentrations at other column distances were close to the initial cell concentration of $6.42 \pm 1.51 \times 10^6$ $\mathrm{cfu/ml_{column}}$. Because extraction efficiency could not be determined in this experimental system, the actual viable cell concentrations may deviate from the reported

values. However, extraction efficiency per se should not affect the observed trend seen in Fig. 4, since the same extraction procedure was consistently used for all the samples. The observed trend in Fig. 4 indicates a spatial gradient of viable cell density in the column, and this trend is consistent with observations from previous column studies (e.g., van der Meer et al., 1987; Allen-King et al., 1996; Kelsey and Alexander, 1995). Together with the data plotted in Fig. 3, the results from Fig. 4 indicate that the biologically active zone that had a relatively large viable biomass and biodegradative activity was confined to the first 3 cm of the column, near the influent.

3.3. Model simulations

A one-dimensional finite element simulator (Chen et al., 1992) was used to examine the applicability of batch-measured Michealis–Menten parameters of nonstarved and suspended cells to the prediction of the observed column behavior under fluctuating toluene concentrations. Major processes incorporated in the simulator include advective and dispersive solute transport, equilibrium linear sorption, substrate utilization, microbial growth and cell decay. For the column experiments considered here, model equations were simplified to a coupled system of two differential equations, a partial differential equation governing transport and transformation of toluene:

$$\left(1+\frac{\rho_b K_D}{n}\right)\frac{\partial C}{\partial t}=-\frac{\partial}{\partial x}(vC)+\frac{\partial}{\partial x}\left(\alpha_L|v|\frac{\partial C}{\partial x}\right)+\left(\frac{V_{max}C}{K_S+C}\right)\left(\frac{X_m}{n}\right) \quad (1)$$

and an ordinary differential equation governing microbial mass balance:

$$\frac{dX_m}{dt}=Y\left(\frac{V_{max}C}{K_S+C}\right)X_m-bX_m \quad (2)$$

Here, C represents the aqueous phase toluene concentration; t, time; x, distance along the column; and X_m, the bulk phase biomass (viable cell) concentration. The parameters used in Eqs. (1) and (2) are defined in Table 2. Consistent with the observed aerobic conditions in the column experiment (the DO never dropped below 2.5 mg/l), an excess oxygen condition was assumed. Thus, no equation was solved for oxygen transport and transformation.

In addition, potential substrate and oxygen limitations to the cells in the column were not considered in the model because the estimated rates of diffusion to cells were greater than or close to the predicted utilization rates in the cells for the entire range of the experimental toluene and oxygen concentrations (i.e., from the lowest effluent concentration to the highest influent concentration), as estimated by a Michaelis–Menten equation and batch-measured V_{max} and K_S values (see the second column in Table 2). In this calculation, the rate of diffusion to a cell was estimated using the following flux (N) equation (Eq. (3)), based upon a packed bed mass transfer correlation (Eq. (4); Gupta and Thodos, 1996).

$$N=k_c(C_b-C_{a.s.})A_{surface} \quad (3)$$

where

$$k_c=1.17\left(d_{50}v/\nu_{H_2O}\right)^{-0.42}\left(\nu_{H_2O}/D\right)^{-0.67}v \quad (4)$$

Table 2
Parameters used in the model simulations

Parameter	Independent estimation		Modified model fit	Unit
	Average	Range		
Maximum specific toluene degradation rate (V_{max})	2.26[a]	1.82–2.96[a]	2.8	$mg_{toluene}/mg_{cell}/day$
Half-saturation toluene concentration (K_S)	1.20[b]	1.17–1.23[b]	1.2	$mg_{toluene}/l$
Cell yield coefficient (Y)	0.5[c]	0.4–0.6[c]	0.5	$mg_{cell}/mg_{toluene}$
Cell decay coefficient (b)	0.06	0.03–0.09[d]	0.06	1/day
Initial bulk phase biomass concentration (X_m at $t=0$)	3.72[e]	2.85–4.60[e]	4.2	mg_{cell}/l
Interstitial velocity (v)	1.21	1.20–1.22[f]		cm/h
Dispersivity (α_L)	0.18	0.14–0.27[d]		cm
Linear sorption coefficient (K_D)	0.020	0.003–0.039[d]		ml/g
Porosity (n)	0.35			
Bulk phase column density (ρ_b)	2.03			g/ml
Lag-time (T_{lag})			1.68	h
Threshold toluene concentration for activation (C_{th})			0.06	$mg_{toluene}/l$

[a] The values were obtained from single-batch kinetic measurement result (Table 1).
[b] Leahy, personal communication.
[c] The reported values are assumed based upon literature (Chen et al., 1992).
[d] The range represents the 95% confidence interval of the reported average value.
[e] The unit of the reported value was converted from colony per milliliter based upon cell size measurement (conversion factor $= 5.8\times10^{-10}$ mg/cell, assuming cell density is 1 g/ml). The uncertainty in biomass measurements was incorporated in the range of the reported value.
[f] The range represents one standard deviation of the reported average value.

Here C_b represents the bulk concentration and $C_{a.s}$ the concentration at the cell surface ($C_{a.s}=0$ for a fast reaction at the cell surface). k_c is a mass transfer coefficient. The mean particle size (d_{50}) and interstitial velocity (v) were based upon column conditions. The cell surface area ($A_{surface}$) was calculated using the measured size of a toluene-grown cell and assuming the shape of a cell is cylindrical. The values of toluene diffusivity ($D_{toluene}$), oxygen diffusivity (D_{O_2}), and kinematic viscosity (ν_{H_2O}) were 0.95×10^{-5} (Hayduk, 1986), 2.29×10^{-5} (Hayduk, 1986), and 9.38×10^{-4} cm^2/s (Reid et al., 1977), respectively. The oxygen utilization rate was estimated based upon the estimated toluene utilization rate and a theoretical metabolic oxygen requirement of 2.33 $mg_{O_2}/mg_{toluene}$. This theoretical metabolic oxygen requirement was calculated based on cellular toluene oxidation stoichiometry utilizing a cell yield of 0.5 $mg_{living\ cell}/mg_{toluene}$ and assuming complete oxidation, as described by Sawyer and McCarty (1978).

Numerical simulations were conducted only with respect to the first toluene breakthrough curve (peak A in Fig. 2) since (i) the initial X_m was quantified, (ii) toluene exposure history was well defined (no-toluene exposure for 53.3 h and then continuous injection to the column of toluene with concentration at approximately 1.25 mg/l), (iii) more frequent effluent concentration measurements were available, and (iv) a bromide tracer test was conducted simultaneously. The values of the parameters used in the

model simulations and their ranges of uncertainty are presented in the second and third columns of Table 2. Except for the Y value, all parameters were measured. For the model simulation in this study, the V_{max} and K_S values obtained by the single-batch kinetic measurement method (Table 1) were selected based upon the relatively high R^2 value. The Y value was assumed based upon the literature (Chen et al., 1992). The cell decay coefficient (b) was estimated by fitting a first-order decay equation to the cell decay data quantified by TNA plate counts. Effluent bromide concentration data from the column experiment were fit to an analytical solution of the advection–dispersion equation using CXTFIT 2.0 (Toride et al., 1995) to estimate α_L ($R^2 = 0.995$) and K_D ($R^2 = 0.984$). Bulk density and porosity were estimated by weight and volume determinations. For the simulations, a third-type flux condition was imposed at the column inlet, and a second-type (no dispersive flux) condition was imposed at the outlet.

Fig. 5 compares numerical model predictions (indicated by the solid line) with the experimental observations (indicated by the filled squares) for the first toluene breakthrough curve (peak A in Fig. 2). The simulation substantially under-predicts the magnitude of the peak of the breakthrough curve. Variation of all parameters within their ranges of uncertainty failed to capture the observed behavior. These results indicate that the large observed effluent concentrations are inconsistent with a model prediction based upon the batch-measured Michaelis–Menten parameters.

An alternative modeling approach was developed to simulate the appearance of the first toluene breakthrough curve (peak A) in Fig. 2. In this approach, all cells were

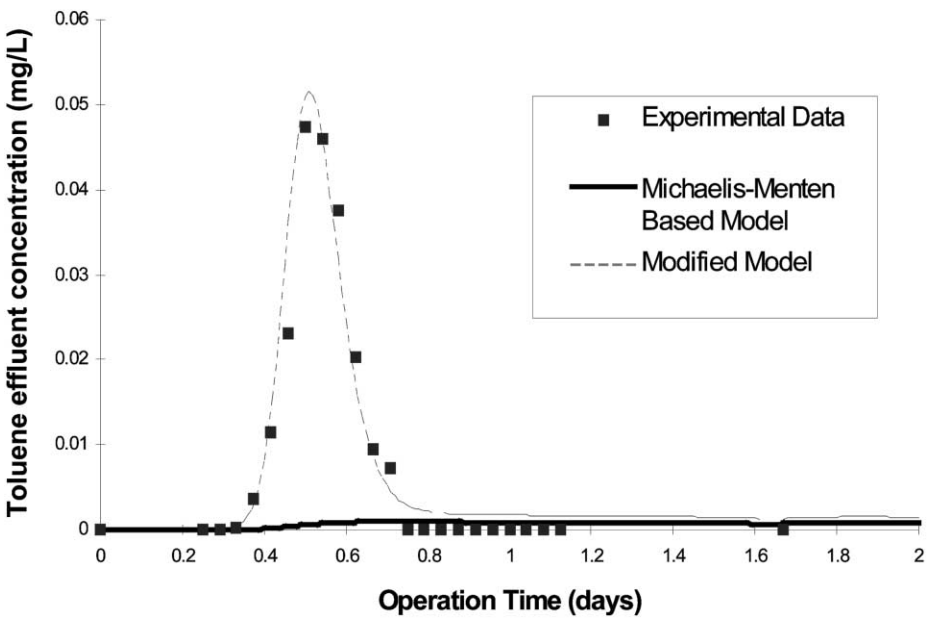

Fig. 5. Comparison of different model simulations with the first toluene breakthrough curve (peak A in Fig. 2) observed in the column experiment.

assumed to be completely deactivated within the 53.5-h period of no-toluene exposure prior to the initialization of toluene injection. The subsequent reactivation was assumed to occur following a finite lag-time (T_{lag}), after continuous exposure to toluene above a threshold concentration (C_{th}). These assumptions were made based upon the independent observations presented in Fig. 3. Deactivation was assumed based upon the observed lack of toluene degradative activity in attached cells in the downstream core sample (9–12 cm) where toluene concentrations were below the HPLC detection limit (0.01 mg/l). Reactivation after a lag-phase was assumed based upon the observed biodegradative activity after a lag-time of 30 min in the upstream core sample (0–3 cm).

The new model parameters, T_{lag} and C_{th}, were treated as fitting parameters. The T_{lag} parameter value was constrained within the range of 0.5 h to the column hydraulic retention time (11.6 h). The lower limit of 0.5 h was based upon the previous batch experimental results from the upstream column sample (Fig. 3), since the cells in the column were exposed to no-toluene conditions for a longer period (53.5 h) than those in the batch experiment (10 h). The upper limit reflects the fact that significant toluene degradation was observed subsequent to toluene injection (peak A in Fig. 2). The C_{th} parameter value was constrained between 0.02 and 0.18 mg/l. To obtain this constraint, the minimum and maximum threshold concentrations for growth (S_{min}) were calculated using an equation derived from the Monod model: $S_{min} = bK_S/[YV_{max} - b]$ (Rittmann and McCarty, 1980; Button, 1985; Tros et al., 1996) and the minimum and maximum parameter values within the ranges presented in Table 2 (see the second and third columns).

Fig. 5 illustrates that the modified model, reflecting a deactivation and a reactivation after a certain lag-time, was able to capture the observed characteristics of peak A. The microbial parameter values that were employed in the modified model simulation are presented in Table 2 (see the fourth column). Here, fitted T_{lag} (1.68 h) and C_{th} (0.06 mg/l) values are within the defined constraints. As seen in Table 2, the biodegradation kinetic parameters (V_{max} and K_S) used in the modified model simulation were consistent with the batch-measured values from nonstarved cells. The threshold concentration for activation, C_{th}, estimated from the column observations, was close to the calculated minimum substrate concentration for growth, S_{min} (0.067 mg/l), as extrapolated from the independently determined batch-measured microbial parameters from nonstarved cells.

A threshold concentration for deactivation can be determined by measuring the residual concentration under steady-state conditions (Rittmann and McCarty, 1980; Tros et al., 1996). Since the residual concentration observed in the effluent was below the detection limit for HPLC analysis, it is reasonable to conclude that the threshold concentration for deactivation in the attached cells in the column was below 0.01 mg/l. The measured threshold concentration for deactivation is lower than the estimated threshold for activation, C_{th} (0.06 mg/l). This difference between the threshold concentrations suggests that deactivation and its subsequent reactivation are not exactly reversible.

Although the modified model provided a good fit with peak A, predictions of subsequent toluene breakthrough curves (peak B in Fig. 2) with the same lag-time and threshold values failed to match experimental observations, in that predicted concentra-

tions were lower than those observed. This under-prediction indicates that the reactivation behavior of the attached cells in the column may have changed during the period prior to the continuous application of the higher influent toluene concentrations (i.e., approximately 5.5 days prior to the first syringe refill).

3.4. Microbial investigations with suspended cells

Additional batch experiments were conducted with suspended-growth PKO1 cells to examine the critical assumptions made in the modified model that simulated peak A (see Fig. 5). It was necessary to examine these assumptions since the effect of no-toluene exposure on biodegradative activity and the induction of toluene degradation in strain PKO1 had not been experimentally determined. In particular, the critical assumptions examined here were (i) the complete deactivation of biodegradative activity at some point during 53.5 h (2.2 days) of no-toluene exposure and (ii) the existence of a lag-phase required for the subsequent reactivation of biodegradation in previously toluene-starved cells.

To examine whether the biodegradative activity of PKO1 cells previously induced in the presence of toluene remains constant during toluene starvation, cells were precultured in the presence of toluene using the same method utilized for the column experiment and then the response of the specific biodegradative activity to varying lengths of no-toluene exposure was observed. Specific biodegradative activity was quantified by measuring initial toluene degradation rates and viable cell concentrations. The observed degradative activity (the *Y*-axis value in Fig. 6) exponentially declined with time during the period of no-toluene exposure. The loss of toluene degradative activity resulting from toluene limitation was consistent with the column observations and model simulations (i.e., the observation of no activity in the downstream core samples as seen in Fig. 3 and the under-prediction based upon the Michaelis–Menten model associated with the batch-measured kinetic parameters for nonstarved cells as seen in Fig. 5). Since no biodegradative activity was observed after approximately 30 h of no-toluene exposure, the deactivation observed in strain PKO1 was consistent with the first assumption made in the modified model.

It is possible that the observed loss of toluene degradative activity was attributable to changes in population structure. The examination of this issue is important in identifying the factors that might cause the observed deactivation during toluene starvation. A genetic event (i.e., a mutation) would be expected to have consequences that affect population structure. However, even after 102 h of toluene starvation, all of the colonies grown on TNA plates were able to grow on toluene when subsequently transferred to toluene plates. Similarly, a previous study reported that toluene starvation did not result in the mutational loss of toluene degradative ability for *Burkholderia cepacia* G4 (Mars et al., 1996). Taken together, these findings suggest that the loss of toluene degradative activity was not the result of a mutation but rather that the observed deactivation in strain PKO1 was a consequence of intracellular physiological changes during toluene starvation. A first-order decay expression was able to describe the observed intracellular deactivation phenomenon (Fig. 6). The first-order rate for the observed deactivation in suspended-growth PKO1 cells was 4.68 ± 0.91 day^{-1} ($R^2 = 0.992$).

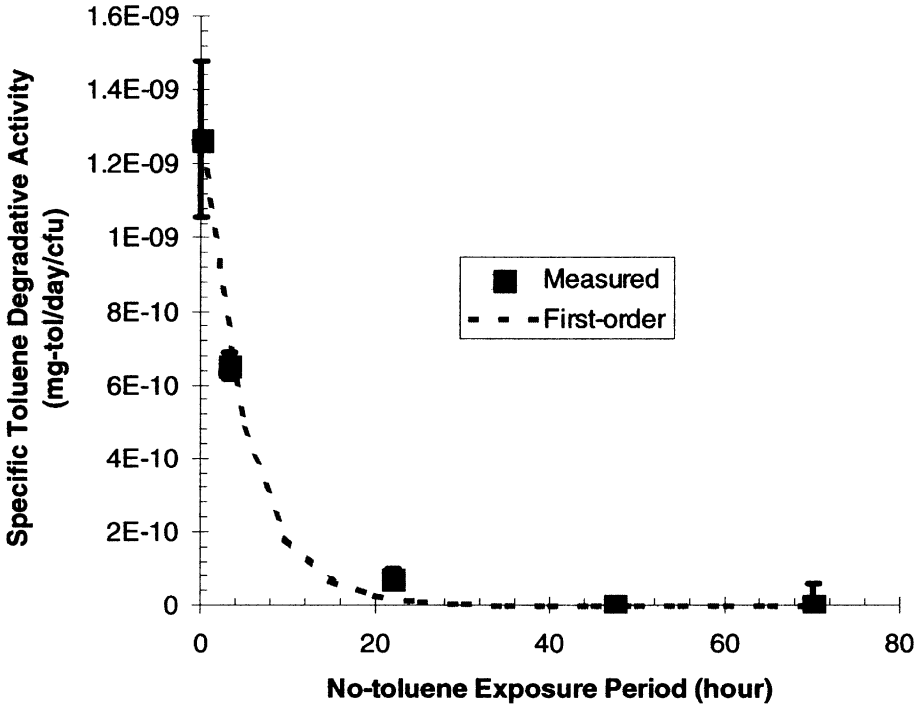

Fig. 6. Specific toluene degradative activity (initial toluene concentration = 1.4 mg/l) versus no-toluene exposure period. Each filled square represents the average value, and the *Y*-error bar represents one standard error of the average value.

To examine the second assumption made in the modified model, the toluene degradation behavior of previously toluene-starved cells of strain PKO1 was monitored using the batch experimental procedure with 160-ml serum bottles, as described above. To ensure that the toluene exposure history was similar to that corresponding to the appearance of peak A, the cells were precultured in the same manner as used in the column experiment and were exposed for approximately 2.5 days to no-toluene conditions and then exposed to an initial toluene concentration of 1.4 mg/l. The toluene (aqueous phase) concentrations in 160-ml serum bottles containing previously toluene-starved cells were measured with time. In Fig. 7, the toluene concentration data for toluene-starved cells are presented together with those for nonstarved cells. A significant lag was observed in the previously toluene-starved cells compared to nonstarved cells. Because relatively high viable cell concentrations ($1.0–1.3 \times 10^8$ cfu/ml) were used in the batch experiments, it is likely that the observed lag represents a "true" microbial lag rather than an "apparent" lag due to the detection limit of the applied batch method (Linkfield et al., 1989). These observations of the microbial lag required for subsequent reactivation are consistent with the second assumption made in the modified model.

To examine whether the reactivation in suspended-growth PKO1 cells is quantitatively consistent with the findings from the modified model simulation for peak A, the

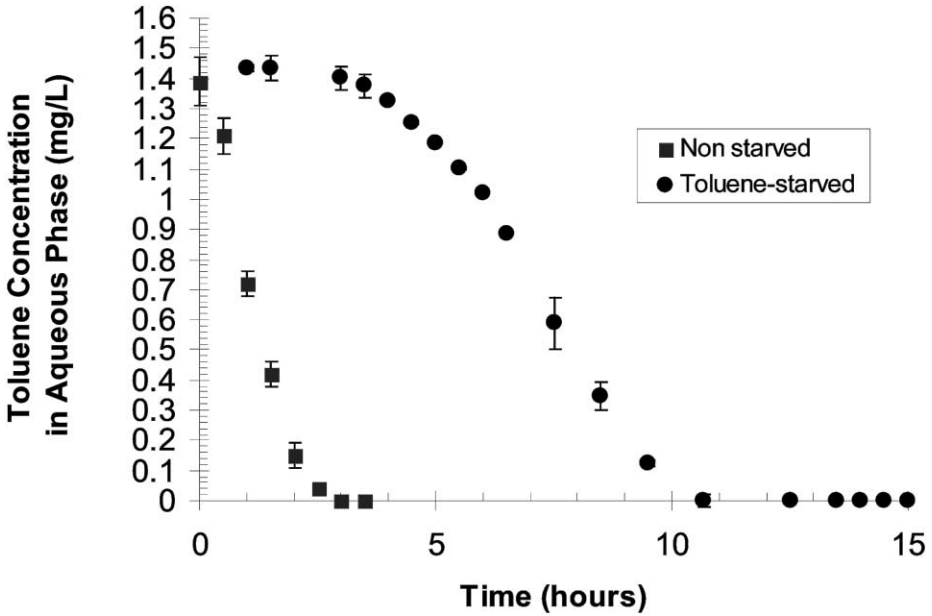

Fig. 7. Toluene (aqueous phase) concentration versus time in the toluene degradation batch experiments with nonstarved and toluene-starved PKO1 cells (suspended growth). The filled squares and the filled circles represent the average values, and the *Y*-error bar represents one standard error of each average value.

lag-time and the kinetic parameters for toluene degradation following the lag-time were quantified based upon the data presented in Fig. 7, and then compared with those employed in the best model fit to peak A (see the fourth column in Table 2). The lag-time for suspended cells was experimentally determined at 3.5 ± 0.5 h in the batch experiments since, at a time between 3 and 4 h, the observed toluene concentrations decreased significantly relative to the detection limit of toluene degradation in the batch experimental technique with 160-ml serum bottles (i.e., 0.1 mg/l of toluene in the aqueous phase). This lag-time for suspended cells was longer than the model-fitted lag-time value from the column experiment. Based upon the toluene degradation data following the lag-time (i.e., the filled circles after 5.5 h in Fig. 7), V_{max} and K_S values were estimated at 0.68 ± 0.16 $mg_{toluene}/mg_{cell}/day$ and 0.50 ± 0.24 $mg_{toluene}/l$, respectively ($R^2 = 0.963$). These kinetic parameter values are lower than the model-fitted values from the column experiment.

The potential influence of abiotic factors (e.g., mass transfer limitation in the 160-ml serum bottles) on the estimated kinetic parameters was also examined. For this examination, toluene degradation kinetic parameters were determined based upon the toluene degradation data for nonstarved cells in Fig. 7 (see the filled squares), and then compared with those determined using the non-head spaced and completely mixed 15-ml CMBR method. Since the kinetic parameter values determined using the 160-ml serum bottle method ($V_{max} = 2.42 \pm 0.62$ $mg_{toluene}/mg_{cell}/day$, $K_S = 1.27 \pm 0.54$ $mg_{toluene}/l$, $R^2 = 0.981$) were consistent with those determined using 15-ml CMBR

method (see the values on the second and third columns in Table 2), it seems reasonable to conclude that the quantification of kinetic parameters using the 160-ml serum bottle method was not affected by any abiotic factors.

4. Discussion and conclusions

Column observations and model simulations revealed that batch-measured biodegradation kinetic parameters and their associated Michaelis–Menten-type biodegradation kinetic model could not accurately predict the rate of toluene biodegradation when toluene concentrations fluctuated. The toluene concentration dependence observed in the column (peak C in Fig. 2) did not follow a Michaelis–Menten-type substrate concentration dependence. Examination of the spatial gradient of biodegradative activity for attached cells in the column (Fig. 3) further showed that degradative activity for these cells depends upon prior toluene exposure (i.e., history), as well as current exposure. Because the Michaelis–Menten model does not incorporate such history dependence, toluene transport resulting from fluctuating toluene concentrations could not be predicted by the traditional biodegradation model and its associated batch-measured kinetic parameters. Moreover, subsequent microbial investigations with suspended cells revealed that the observed history dependence in the column experiment was attributed to delayed responses in microbial behavior towards substrate concentration, i.e., deactivation during toluene starvation and a lag-phase required for subsequent reinduction of biodegradation in previously toluene-starved cells.

By quantifying the specific (i.e., viable biomass normalized) toluene degradative activity of the cells extracted from different column distances, this study provided evidence that toluene limitation had an adverse effect on biodegradation by attached PKO1 cells. Based upon subsequent microbial investigations, this observed negative effect on biodegradative activity would appear to be a consequence of physiological changes during toluene starvation rather than a change of population structure. Because toluene was the sole exogenous molecular effector (i.e., inducer) provided to induce toluene degradative activity in the column experimental system, the lack of toluene (i.e., below a threshold concentration) should have resulted in a reduced level of the induction of toluene degradation by strain PKO1 (Byrne and Olsen, 1996). Such a reduction, in turn, would be expected to cause the observed loss of toluene degradative activity in the strain when toluene concentrations were below a certain threshold (i.e., below 0.01 mg/l). However, it is important to note that the observed deactivation is attributable not only to the lack of an exogenous molecular effector but also to the reduction in the intracellular pool of essential building blocks needed for anabolic and catabolic processes, inasmuch as toluene was the sole carbon and energy source in this experimental system, as well as the exogenous molecular effector. Furthermore, consistent observations of exponential deactivation, even for some noninducible catabolic enzyme systems, such as the methane monooxygenase system of methanotrophs (i.e., a consistent exponential decline of specific methane utilizing activity with time during methane starvation, Roslev and King, 1994), suggest that growth state may be a factor controlling the observed deactivation in strain PKO1.

Despite the deactivation driven by toluene starvation, toluene degradative activity in strain PKO1 was subsequently reactivated when the cells were reexposed to toluene concentrations above a certain threshold. In a bacterial population carrying an inducible catabolic enzyme system, possible explanations for an increase in apparent substrate utilization are induction, cellular growth, and genetic (i.e., mutational) change (Linkfield et al., 1989). In strain PKO1, a genetic change is an unlikely explanation for the observed reactivation since a mutational loss of toluene degradative ability was not observed in toluene-starved cells. In addition, the subsequent batch experiments with previously deactivated PKO1 cells showed that the observed reactivation was not attributable to cellular growth because no significant increase in viable biomass was observed in the batch experiments in which relatively high viable cell concentrations were applied initially. Thus, the induction of toluene metabolism in strain PKO1 becomes the most probable explanation for the observed reactivation.

In their modeling of aerobic benzene degradation in a soil column, Wood and Ginn (1995) incorporated a microbial lag to capture the characteristics of the observed effluent behavior. Their use of a microbial lag, however, was not experimentally verified, since their study did not identify the microbial phenomena that caused the observed history dependence in the porous medium. In contrast, the present study identified the microbial phenomena causing the observed history dependence in the column experiment, viz., deactivation during prior toluene starvation and a lag-phase required for subsequent reinduction in previously toluene-starved cells.

The appearance of peak B in Fig. 2 illustrates that substrate exposure history had a significant impact on the biodegradation and transport of toluene in the column. At the time that peak B appeared, the microbial population along the column had experienced a varied toluene exposure history, in that (i) the column downstream of a biologically active zone was likely to have been exposed to toluene-limited conditions for a longer period of time (approximately 5.5 days), and (ii) the influent toluene concentration was approximately 1.4 mg/l, which is much lower than the toluene concentration in preculture prior to column packing (i.e., 230 mg/l). The inability of the modified model to capture peak B when parameterized using the same lag-time and threshold values used for the simulation for peak A suggests that toluene exposure history affected cellular reactivation behavior in the column. The observed increase in effluent toluene concentration in peak B may be interpreted as a consequence of the increase in lag-time required for subsequent reinduction for cells that had been starved for a longer period of time (Truex et al., 1992) and/or a consequence of a lower induction level (quantified by specific degradation rate) at lower toluene concentrations (Robertson and Button, 1987; Shingleton et al., 1998).

The influence of toluene exposure history on reactivation could explain why the lag-time and toluene degradation kinetic parameter values derived from the best model fit to the observed toluene breakthrough curve (Fig. 5) were inconsistent with those experimentally determined in the microbial investigations. The model assumed a single lag-time along the column, which may not be representative of the actual lag and degradation kinetics in the column because it is likely that toluene exposure history (e.g., toluene starvation period in the case of peak A in Fig. 2) varied along the column. Unfortunately, there is very little quantitative information about the effects of starvation

and substrate concentration level on the induction of biodegradative activity in attached-growth cells, and further research is needed to better understand the effect of substrate exposure history on induction of biodegradation.

The results of this study illustrate that substrate exposure history dependent microbial behavior has a significant impact on the transport of contaminants and that disregarding a threshold and a microbial lag, when they exist, can lead to an overestimation of contaminant biodegradation in situ. Since a first-order decay expression and a modified Michaelis–Menten-type expression that incorporates a lag-time and a threshold concentration were able to describe the toluene exposure history dependent microbial behavior in strain PKO1 (i.e., the deactivation phenomenon and the subsequent reinduction of biodegradation), these results provide a basis for the construction of a predictive model for inducible biodegradation. In addition, the results from model simulations and microbial investigations suggest that quantifying the influence of substrate exposure history on induction of biodegradation is of paramount importance to the proper application of batch-measured biodegradation kinetic parameters for the prediction of field biodegradation rates. These findings may have significant implications for assessing microbial decontamination capacity in natural attenuation or in situ bioremediation applications, in particular, when substrate concentrations fluctuate.

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