Biodegradation **12:** 31–37, 2001.

How adaptation and mass transfer control the biodegradation of linear alkylbenzene sulfonate by activated sludge

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Accepted 8 November 2000

Key words: Activated sludge, adaptation, linear alkylbenzene sulfonate (LAS), mass transport, sorption.

Abstract

We use a nonsteady-state model to evaluate the effects of community adaptation and sorption kinetics on the fate of linear alkylbenzene sulfonate (LAS) in batch experiments conducted with activated sludge that was continuously fed different concentrations of LAS. We observed a sharp decrease in the biodegradation rate between 30 and 60 minutes and the presence of an LAS residual at the end of the batch experiments. The modeling analysis indicates that these phenomena were caused by relatively slow inter-phase mass transport of LAS. The modeling analyses also showed that the amount of LAS-degrading biomass increased when the continuous activated sludge was fed a higher LAS concentration. Although community adaptation to LAS involved accumulation of more LAS degraders, the increase was not proportional to the feed concentration of LAS, which supports the concept that LAS degraders also utilized portions of the general biochemical oxygen demand (BOD) fed to the continuous activated sludge systems.

Introduction

The biodegradation of hydrophobic molecules in biological-treatment processes such as activated sludge is controlled by the competition between biodegradation and sorption. Biodegradation is favored when the hydrophobic compound is rapidly transformed by a significant fraction of the biomass. Sorption is favored when the compound has a large partition coefficient, the rate of mass transfer from the liquid phase to the sorbed phase is rapid, and the rate of sludge wasting is high, which correlates with a short solids retention time (Lee et al. 1998; Namkung & Rittmann 1987; Rittmann et al. 1988; Enviromega 1994).

This work addresses two factors affecting the balance between biodegradation and sorption when linear alkylbenzene sulfonate (LAS) is the target hydrophobic material. The first is the fraction of the biomass able to rapidly transform LAS. In particular, we evaluate whether or not the long-term feeding of LAS to an activated sludge process enriches the community in LAS degraders. The second factor is the kinetics of mass transfer of LAS between the bulk liquid and the biomass-solid phase. We investigate whether or not mass transfer is a significant limitation for biodegradation of LAS by activated sludge.

Herein, we report new experimental results that accentuate our ability to define mass-transfer kinetics between the two phases and that offer an opportunity for different levels of community adaptation to LAS. We then apply a non-steady-state mathematical model that includes the biodegradation of dissolved-phase LAS by LAS-degrading bacteria, hydrophobic sorption of LAS to all the volatile suspended solids (VSS), and mass-transport resistance for the transfer of LAS between the biomass and dissolved phases.

We use the model to separate the effects of sorption from biodegradation in the experimental results. Specifically, we determine whether or not long-term feeding of LAS caused community adaptation to enhance LAS biodegradation and the approximate rate of LAS mass-transport between the two phases.

Experimental methods

We obtained ^{14}C-labelled LAS from New England Nuclear (Boston, MA). The ring was uniformly ^{14}C labeled, and the specific activity was 68 mCi/mg. Radiochemical purity exceeded 98% based on thin layer chromatography (TLC). Working solutions of the radiolabeled LAS were mixed with commercial (unlabeled) LAS in water.

We collected activated sludge from the Polk Run Municipal Sewage Treatment Plant in Loveland, Ohio, USA. This plant receives primarily domestic sewage. We placed the sludge inocula in 3-liter continuous activated sludge (CAS) test systems (OECD, 1993) and fed domestic wastewater amended with 1, 3, or 10 mg/l of commercial LAS. We operated the CAS units with a hydraulic residence time of 6 h, a solids retention time of 10 d, and a temperature of 20–24 °C. The systems came to steady state before we removed sludge for batch biodegradation tests.

We conducted batch biodegradation experiments using a modification of the procedures described by Federle & Itrich (1997). We placed activated sludge mixed liquor obtained from the CAS units into Erlenmeyer flasks. We slowly added the LAS working solutions drop-wise as the contents were mixed with a magnetic stirrer. Following addition of all materials, we stirred the sludge vigorously for 1 min prior to taking the first sample. Subsequently, we incubated the flasks on a shaker table (150-rpm) in a controlled-temperature room at 20 ± 2 °C. During sampling, the flasks were mixed vigorously with a magnetic stirrer.

We transferred 10-ml samples for analysis of parent LAS to screw-top test tubes and immediately flash froze the samples by submersion in a dry ice/acetone bath. These samples were then stored in a freezer (−80 °C) until being lyophilised using a Virtis Benchtop Freeze Dryer. For analysis, we added 5 ml of methanol to the lyophilized solids, vortexed the mixture for 5 min, centrifuged the mixture, and reserved the solvent supernatant for subsequent analysis. We repeated the entire extraction process three times. We then combined the extracts, transferred sub-samples to scintillation vials, added Ultima Gold XR cocktail, and analyzed them by liquid scintillation counting (LSC) to obtain the total counts.

Methanol extracts also were dried under nitrogen and reconstituted in a minimal amount of methanol. We spotted sub-samples onto Silica Gel 60 TLC plates (Merck) with 20 pre-channeled lanes and diatomaceous-earth concentrating zones. We developed the plates in chloroform:methanol:water:formic acid (80:25:3:1) and allowed them to dry. We scanned each lane of the plates using a Bioscan Imaging 200 System (Bioscan, Washington, DC) to determine the fraction that was parent LAS. Finally, we corrected the recovery of parent LAS based on recovery from abiotic controls, which averaged 95.3%.

The batch experiments examined the effect of varying influent LAS concentration to the CAS systems and the effect of LAS concentration during the batch tests. Sludge was removed from each CAS unit and dosed with ^{14}C-labeled LAS. The initial concentration of total-LAS was varied between 0.1 to 20 mg/L. The disappearance of parent LAS was measured over time, as described in the preceding paragraphs.

Nonsteady-state model

The nonsteady-state model used to analyze the results of the batch experiments was derived from the activated sludge model of Lee et al. (1998), but four changes were required to make the model conform to the batch experiments. First, since the batch tests had no hydraulic flows, all flow rates (Q values) in the model were set to zero. Second, since the only substrate added for the batch tests was LAS, the utilization of non-specific biochemical oxygen demand (BOD) and growth of bacteria utilizing BOD (but not LAS) was neglected. Third, sorption of LAS, which was present initially in the liquid phase, was described with mass- transport kinetics instead of being at equilibrium between the two phases. Finally, the biodegradation of LAS was limited to only LAS in the dissolved phase.

The nonsteady-state model has five mass-balance differential equations: non- specific active biomass (X_a); active biomass able to utilize LAS ($X_{a,j}$); total volatile suspended solids (X_v), dissolved-phase LAS (C_j); and sorbed LAS ($q_j X_v$, where q_j is the sorption density of LAS on the volatile suspended solids). The five equations are presented below, and new parameters are defined as they appear. The units are mgVSS

for all types of biomass, mgLAS for LAS, liters (L) for volume, and days (d) for time.

Nonspecific Active Biomass (X_a)

$$V\frac{\mathrm{d}X_a}{\mathrm{d}t} = -bX_aV, \tag{1}$$

in which V = reactor volume (L), b = first-order decay coefficient (d^{-1}), and t = time (d).

LAS-Utilizing Biomass ($X_{a,j}$)

$$V\frac{\mathrm{d}X_{a,j}}{\mathrm{d}t} = Y_j q_{\max 1}\frac{C_j}{K_{s1}+C_j}X_{a,j}V - bX_{a,j},V, \tag{2}$$

in which Y_i = biomass true yield for LAS utilization (mgVSS/mgLAS), $q_{\max i}$ = maximum specific rate of LAS utilization from the dissolved phase (mgVSS/mgLAS-d), and K_{SI} = half-maximum-rate concentration for dissolved-phase LAS (mgLAS/L).

Volatile Suspended Solids (X_v)

$$\begin{aligned} V\frac{\mathrm{d}X_v}{\mathrm{d}t} &= Y_j q_{\max 1}\frac{C_j}{K_{s1}+C_j}X_{a,j}V \\ &\quad -(1-f_d)b(X_a+X_{a,j})V, \end{aligned} \tag{3}$$

in which $X_v = X_a + X_{a,j} + X_i$, X_i = inert volatile suspended solids (mgVSS/L) and f_d = the biodegradable fraction of the active biomass.

Dissolved-Phase LAS (C_j)

$$\begin{aligned} V\frac{\mathrm{d}C_j}{\mathrm{d}t} &= -q_{\max 1}\frac{C_j}{K_{s1}+C_j}X_{a,j}V \\ &\quad -k_{\mathrm{MT}}(K_d-q_j)X_vV, \end{aligned} \tag{4}$$

in which k_{MT} = the interphase mass-transfer rate coefficient (d^{-1}), K_d = linear partitioning coefficient for LAS between the dissolved phase and sorbed to the VSS (L/mgVSS), and q_j = the sorbed-phase density of LAS (mgLAS/mgVSS-d). Equation (4) underscores that biodegradation (first term on right side) and sorption (second term) are competing sinks for dissolved-phase LAS.

Sorbed-Phase LAS (q_jX_v)

$$V\frac{\mathrm{d}q_jX_v}{\mathrm{d}t} = k_{\mathrm{MT}}(K_d-q_j)X_vV. \tag{5}$$

Equation (5) indicates that sorbed-phase LAS can exchange with the dissolved phase, but no other sinks for sorbed-phase LAS are present in the batch die-away experiments.

The five mass-balance equations, which are ordinary differential equations, are solved simultaneously from user-provided initial conditions for C_j, q_j, X_a, X_v, and $X_{a,j}$. We solved them using MATLAB (The Mathworks, Inc.), which employs a Runge–Kutta–Feldberg method with an order of accuracy of (4, 5) (Pärt-Erender et al. 1996; Ascher et al. 1988).

Figure 1 illustrates the kind of response the model gives for the general conditions of the batch experiments. Figure 1 presents the fraction of the initial hydrophobic compound (identified as LAS, although the example is generic) that is present in the dissolved phase (C_j), the sorbed phase (q_jX_v), and in total ($C_j + q_jX_v$). The concentrations of the three forms of biomass changed negligibly during the 7 h and are not shown. The top panel is for a low initial LAS concentration (0.1 mg/L), while the bottom panel is for a higher initial concentration (20 mg/L).

The top panel in Figure 1 shows that loss of total mass is rapid in the first one-half hour. Biodegradation decreases the total concentration to about 25% in that time. By about one hour, however, the loss of total mass slows because the C_j is very small, and almost all of the mass is present in the sorbed phase, which is not available for biodegradation. Initially rapid biodegradation followed by nearly complete partitioning to the unavailable sorbed phase gives the characteristic "elbow" shape to the total-mass curve. The elbow and residual total mass present almost totally in the sorbed phase are key signs that the sorption kinetics are slow enough to affect the fate of the hydrophobic compound. Both can be clearly observed when the initial concentration is small, the situation shown in the top panel.

The bottom panel in Figure 1 shows that the *relative* removal rate of total mass is slowed when C_j is large compared to K_{S1}. Biodegradation during the first hour removes only about 20% of the total mass, since the biodegradation kinetics have a reaction order less than one in C_j. Sorption is less dominant than in the top panel, although q_jX_v is much greater than C_j after about one hour. Although the rate of removal of total mass continually slows, the sharp elbow disappears when the initial C_j is large compared to K_{s1}.

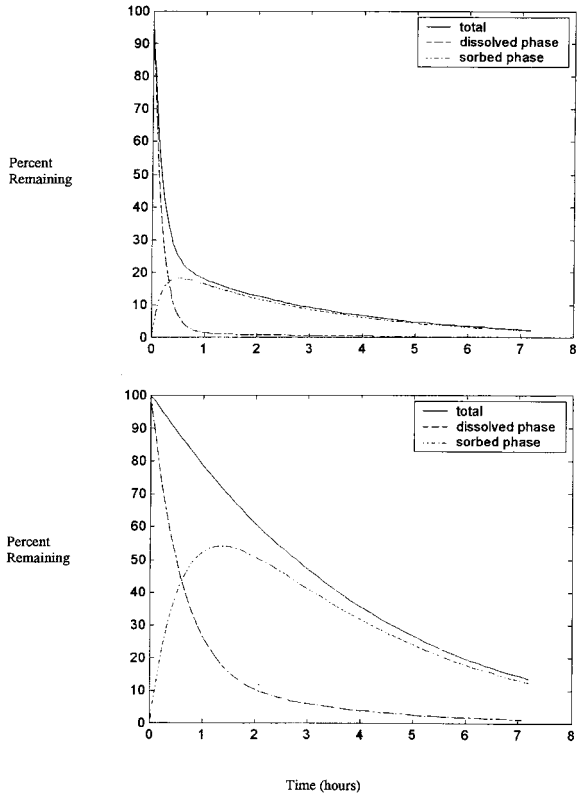

Figure 1. Characteristic model-generated distributions of dissolved (C_j), sorbed ($q_j X_v$), and total ($C_j + q_j X_v$) LAS during the course of a batch tests. The top panel is for a low initial concentration (0.1 mg/L), while the bottom panel is for a high initial concentration (20 mg/L). Common parameters are $q_{\max j}$ = 0.1 mgLAS/mgVSS-d, K_{S1} = 1 mg LAS/L, k_{MT} = 10 d^{-1}, K_d = 0.002 L/mgVSS, Y_j = 0.35 mgVSS/mgLAS, b = 0.15/d^{-1}, f_d = 0.8, and V = 6 L. Common initial conditions are: X_v = 2000 mgVSS/L, X_a = 788 mgVSS/L, X_i = 375 mgVSS/L, and $X_{a,j}$ = 337 mgVSS/L.

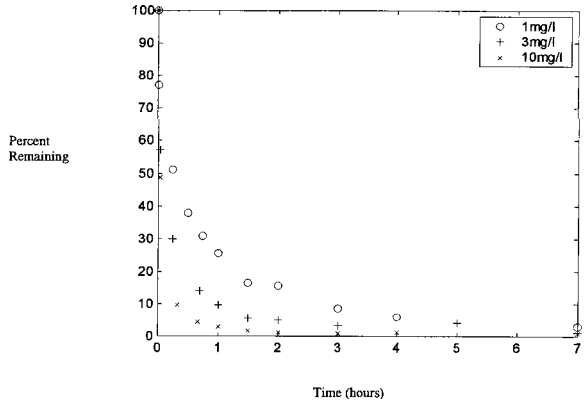

Figure 2. Experimental results for the loss of LAS initially present at 0.1 mg/L in batch systems containing activated sludge acclimated to wastewater with 1, 3, or 10 mg/L of LAS amended to the influent. All data sets have a 100% value at time = 0 h.

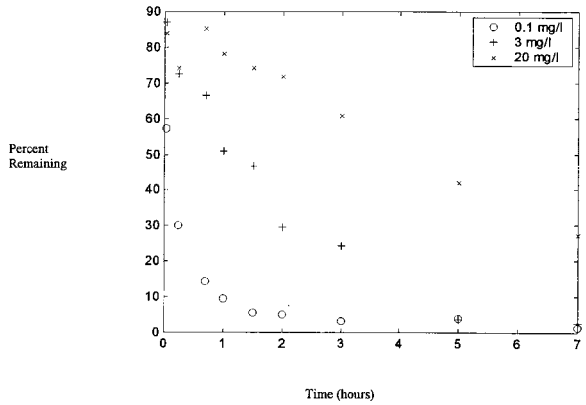

Figure 3. Experimental results for the loss of LAS for the activated sludge continuously fed 3 mg/L LAS. The batch experiments were conducted with initial LAS concentrations of 0.1, 3, or 20 mg/L. All data sets have a 100% value at time = 0 h.

Experimental results

Figure 2 shows disappearance of a low level (0.1 mg/L) of radiolabeled LAS in batch systems containing activated sludge that was acclimated with three different concentration of LAS fed to the CAS systems. In all three cases, disappearance of LAS exhibited the characteristic elbow pattern consisting of an initial rapid loss followed by a very slow rate. LAS was detected after 24 h of incubation (not shown in the figure), but values were less than 5% of what was originally added to the flasks. These patterns are similar to the top panel of Figure 1 and suggest that two pools of LAS were degraded at vastly different rates: the rapidly degraded dissolved pool and the slowly degraded sorbed pool. The patterns support the concept that slow desorption kinetics were keeping the sorbed LAS in an unavailable state. Increasing LAS concentration to the CAS moved the elbow to a lower concentration and an earlier time, which indicate an increasing ability to biodegrade the dissolved LAS before it is sorbed by the biomass solids.

Figure 3 shows disappearance of different concentrations of radiolabeled LAS in batch experiments containing activated sludge that was acclimated to an influent containing 3 mg/L of LAS. These results show two key trends:

1. The slope, which represents the relative biodegradation rate, is smaller for larger initial LAS concentrations. This is the trend illustrated in Figure 1 and suggests that K_{S1} is small compared to 20 mg/L.
2. The 0.1-mg/L initial concentration shows a sharp elbow at around 1 h, followed by a slower rate and

continued presence of LAS at 7 h. These suggest that sorbed LAS is unavailable and that mass transport of sorbed LAS to the liquid phase is limiting biodegradation after about one hour.

Evaluation of experimental results

We used the nonsteady-state model to analyze in detail the experimental results. Our evaluation centered on estimating values of K_{SI}, $q_{\max j} X_{a,j}$, k_{MT}, and K_d. The goal was to achieve reasonable and representative values of each parameter so that we could characterize the effect of adaptation to continuous feeding of LAS and assess the importance of desorption kinetics. Because the experiments involved the same mixing conditions and activated sludge grown mainly through the oxidation of sewage BOD, we determined common values of k_{MT}, K_d, and K_{SI}. On the other hand, unique values of $q_{\max j} X_{a,j}$ were determined for each set of experiments using activated sludge continuously fed 1, 3, or 10 mg/L LAS.

The selected values of k_{MT} and K_d were 10 d^{-1} and 6×10^{-4} L/mgVSS, respectively. Literature-reported values of K_d for LAS range from 3×10^{-6} L/mgVSS to 2.6×10^{-2} L/mgVSS (DiToro et al. 1990; Cowan et al. 1993; Hand & Williams 1987), and we evaluated K_d values in the range 1×10^{-4} to 2×10^{-3} L/mgVSS. No previous work provides guidance on k_{MT} values. When combined, $K_d = 6 \times 10^{-4}$ L/mgVSS and $k_{MT} = 10$ d^{-1} properly captured the elbow effect for the batch experiments with 0.1 mg/L LAS (Figures 2 and 3). Larger k_{MT} or smaller K_d values precluded having the slowed loss rate after about $\frac{1}{2}$ h and residual LAS at 7 h when the initial concentration of LAS was 0.1 mg/L. Smaller k_{MT} or larger K_d made the elbow too sharp, indicating that the loss rate of LAS was too slow.

To estimate the K_{SI} and $q_{\max j} X_{a,j}$ values, we first fit the results for the 0.1-mg/L batch experiments (Figure 2). We first fixed $K_{SI} = 2$ mg/L and found the best-fit $q_{\max j} X_{a,j}$. This gave a best-fit ratio $q_{\max j} X_{a,j} / K_{SI}$ for each series. We then held that ratio constant for each series as we varied $q_{\max j} X_{a,j}$ and K_{SI} to match the data as well as possible for the 3-mg/L experiments (Figure 3). Some compromises had to be made, and we used our best judgment to get a common K_{SI} value for all series and unique $q_{\max i} X_{a,j}$ values for the three series.

Table 1 summarizes the best-fit parameters we obtained. Figures 4 and 5 compare the experimental

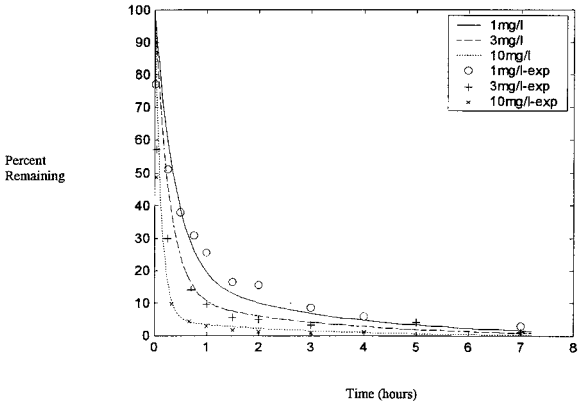

Figure 4. Comparison of the experimental measurements for total LAS with the model simulations for the activated sludge continuously fed 1, 3, or 10 mg/L LAS and with an initial batch concentration of 0.1 mg/L LAS. All data sets have a 100% value for time = 0 h.

results to the simulations using the best-fit parameters. The fitting of the 0.1-mg/L results is excellent using the common values of $k_{MT} = 10$ d^{-1}, $K_d = 6 \times 10^{-4}$ L/mgVSS, and $K_{SI} = 0.64$ mgLAS/L, along with the series-specific values of $q_{\max j} X_{a,j}$ shown in Table 1. In particular, the model simulations reproduce the rapid decrease in total LAS for the first 30 to 60 minutes, the elbow, and the residual of 1–2% at the end of the 7 h (Figure 4). The same parameters capture all the major trends for the batch experiments with higher initial concentrations (Figure 5). Most important is the systematically decreasing slope, which is determined mainly by the K_{SI} value, which is substantially smaller than the initial concentrations of dissolved-phase LAS for the higher concentration experiments (recall Figure 1).

Table 1 shows that $q_{\max j} X_{a,j}$ increased as the LAS concentration fed continuously to the activated-sludge unit increased. This indicates that the long-term availability of LAS as a primary substrate is an important factor in controlling the number of bacteria capable of degrading LAS. The table also computes a concentration of LAS degraders by assuming that $q_{\max i} = 6$ mgLAS/mgVSS-d, which converts to 16 mgCOD/mgVSS-d, a normal maximum rate for simple organic molecules whose utilization kinetics are controlled by electron transport to the terminal electron acceptor (Rittmann & McCarty 2001). This estimate procedure gives minimum concentrations of LAS degraders, which range from 6 to 21 mgVSS/L, or 0.5 to 1.9% of the total active biomass ($X_a + X_{a,j}$). If the actual maximum specific rate of LAS utilization

Table 1. Best-fit parameters describing the fate of LAS in the batch experiments

Parameters and units	LAS concentration fed continuously to the activated sludge (C_{j0}) 1 mgLAS/L	3 mgLAS/L	10 mgLAS/L
Common to all experiments			
Y_j, mgVSS/mgLAS	0.35	0.35	0.35
b, d^{-1}	0.15	0.15	0.15
f_d	0.8	0.8	0.8
X_v, mgVSS/L	1500*	1500*	1500*
K_d, L/mgVSS	6×10^{-4}	6×10^{-4}	6×10^{-4}
k_{MT}, d^{-1}	10	10	10
K_{S1}, mgLAS/L	0.64	0.64	0.64
Specific to each steady-state LAS feed concentration (C_{j0})			
$q_{\max j} X_{a,j}$, mgLAS/L-d	36	54	126
$X_{a,j}$ when $q_{\max j}$ = 6 mgLAS/mgVSS-d, mgVSS/L	6	9	21
% of $X_a + X_{a,j}$	0.5	0.8	1.9
$q_{max\,j} X_{a,j}/C_{j0}$, d^{-1}	36	18	12.6

* VSS = 1500 mg/l was measured in the CAS reactors.

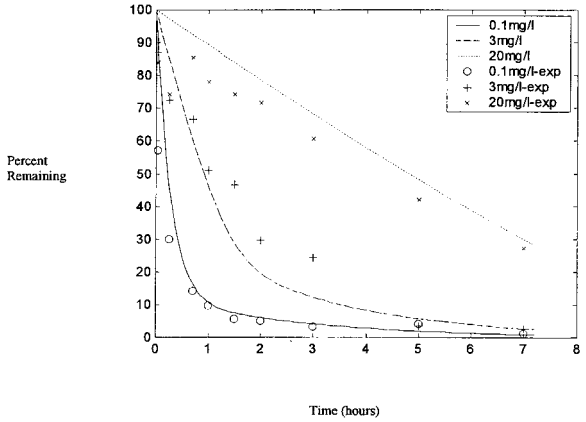

Figure 5. Comparison of the experimental measurements for total LAS with the model simulations for the activated sludge continuously fed 3 mg/L LAS. Initial concentrations in the batch experiments are 0.1, 3, or 20 mg/l. All data sets have a 100% value for time = 0 h.

were smaller than 6 mgLAS/mgVSS-d, the percentages would be larger; however, the relative values would remain the same as shown in Table 1.

Table 1 also provides the ratio of $q_{\max j} X_{a,j}$ to the continuous-feed concentration of LAS, C_j. If the growth of LAS degraders in the continuous activated sludge system were based only on LAS degradation, this ratio would be a constant. However, the ratio is largest for smallest feed concentration of LAS. This trend suggests that LAS degraders gain energy and support synthesis through the utilization of some fraction of the non-LAS BOD. Lee et al. (1998) modeled the synthesis rate of specific-compound degraders as being the sum of growth through utilization of the specific compound and of a fraction f_j of the general BOD. The ratio trend in Table 1 supports that f_j was significantly greater than zero.

Conclusions

We developed and applied a dynamic model to analyze the effects of sorption and biodegradation kinetics on the fate of LAS in batch experiments with activated sludge adapted to different feeding levels of LAS. The model assumes that only the dissolved- phase LAS is available for biodegradation and mass-transport resistance makes sorption kinetically controlled.

The sharp elbow and the slowed loss rate of total LAS in the batch experiments, particularly for lower initial LAS concentrations, support the model assumptions concerning the availability of dissolved-phase LAS and the importance of mass-transport resistance. For the activated sludge and the batch reaction conditions studied, we found that k_{MT} = 10 d^{-1} and K_d = 6×10^{-4} L/mgVSS represented the sorption phenomena well. In the batch experiments, mass-transport kinetics were slow enough that equilibrium was not reached, and the long-term biodegradation of LAS was limited by the desorption rate from the unavailable pool of sorbed LAS.

We estimated that K_{SI} was about 0.64 mgLAS/L for the sludge studied, while the product $q_{\max j} X_{a,j}$ increased from 36 to 126 mgLAS/L-d as the feed concentration of LAS to the continuous activated sludge unit increased from 1 to 10 mg/L. The rising values of $q_{\max j} X_{a,j}$ indicate that community adaptation involved added growth of LAS degraders in response to increasing LAS inputs. However, the increase in $q_{\max j} X_{a,j}$ was not proportional to the increase in input LAS, which supports the concept of Lee et al. (1998) that the synthesis of specific-compound degraders also is supported by their utilization of part of the general BOD.

Our results demonstrate that the kinetics of sorption and desorption can play an important role in controlling the biodegradation rate of hydrophobic compounds. Although this is a very important trend, care should be taken when applying them to activated sludge treatment processes, which normally are continuously fed. The batch experiments we performed accentuated the effects of mass-transport kinetics, and mass-transport rates (e.g., k_{MT}) may vary significantly from batch experiments to full-scale treatment systems. Our results also document that the long-term feeding of LAS affects the number of LAS-degrading bacteria in the system, although it is not the sole control. This finding underscores the importance of knowing the history of the biomass being used in a batch die-away test so that the experiment can be experiment is design properly and the results interpreted appropriately. Finally, our results indicate that biodegradation kinetics should be evaluated in such a way that the effects of sorption kinetics can be separated.

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