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Biodegradation of the Surfactant Linear Alkylbenzenesulfonate in Sewage-Contaminated Groundwater: A Comparison of Column Experiments and Field Tracer Tests

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Transport and biodegradation of linear alkylbenzenesulfonate (LAS) in sewage-contaminated groundwater were investigated for a range of dissolved oxygen concentrations. Both laboratory column and an 80-day continuous injection tracer test field experiments were conducted. The rates of LAS biodegradation increased with increasing dissolved oxygen concentrations and indicated the preferential biodegradation of the longer alkyl chain LAS homologues (i.e., C_{12} and C_{13}) and external isomers (i.e., 2- and 3-phenyl). However, for similar dissolved oxygen concentrations, mass removal rates for LAS generally were 2−3 times greater in laboratory column experiments than in the field tracer test. Under low oxygen conditions (<1 mg/L) only a fraction of the LAS mixture biodegraded in both laboratory and field experiments. Biodegradation rate constants for the continuous injection field test (0.002−0.08 day^{-1}) were comparable to those estimated for a 3-h injection (pulsed) tracer test conducted under similar biogeochemical conditions, indicating that increasing the exposure time of aquifer sediments to LAS did not increase biodegradation rates.

Introduction

Linear alkylbenzenesulfonate (LAS) was introduced in 1965 as a biodegradable alternative to nonbiodegraded branch-chained alkylbenzenesulfonates (ABS) and since has become the most widely used anionic surfactant in commercial detergent formulations (*1*). Because of its widespread use and subsequent disposal in wastewater, LAS and its biodegradation products are present in domestic sewage influents and effluents discharged to surface waters or groundwater (*2*−*6*). As a result, the occurrence and fate of LAS in the environment have been the focus of numerous investigations (*3, 4, 7*−*16*). Several studies report that LAS is readily biodegraded under aerobic conditions such as those that exist during sewage treatment and in surface waters (*3, 9, 10, 12*−*14*) with half-lives in the range of 1−2 days for activated sludges, river waters, and sediments and 10−20 days for surface and subsurface soils (*10, 14*). In contrast, LAS persists in oxygen-depleted environments (<0.1 mg/L dissolved oxygen) which can occur in anaerobic sewage−sludge digestors (*17*) and sewage-contaminated groundwater (*4, 8*).

While LAS's fate in wastewater treatment systems and receiving surface waters is well-documented, relatively few studies describe LAS's fate in groundwater under in situ conditions. Many laboratory microcosm experiments have been conducted under aerobic conditions while many contaminated field sites are characterized by low oxygen conditions. In addition, microcosm experiments typically do not account for the effect of transport on biodegradation rates, which may be significant in groundwater systems. Field tracer tests offer the advantage of direct measurement of in situ processes and thus may provide more realistic estimates of LAS biodegradation rates in groundwater.

Earlier investigations of the fate and transport of LAS in sewage-contaminated groundwater conducted at the U.S. Geological Survey's Cape Cod Substances Hydrology Waste Research Site indicated that branched-chain alkylbenzene sulfonates (ABS) and LAS and LAS metabolites persisted in the groundwater (*4, 8*). Natural-gradient pulsed tracer tests were conducted by injecting LAS over a period of 3 h as a means for investigating LAS behavior in situ within distinct biogeochemical zones of the aquifer (*18, 19*). Single pulsed tracer tests were conducted in each of three zones of the aquifer. No biodegradation was observed in a sewage-contaminated suboxic zone (<0.1 mg/L dissolved oxygen) (*18*), whereas partial and selective biodegradation of the LAS mixture was observed in a zone with 1 mg/L dissolved oxygen (*19*). These single pulse experiments measured the short-term response of the subsurface microbial community. The contact time between the tracer cloud and the microbial community was minimal, so growth was not a major factor (*20*). Continuous injection experiments were needed to determine the potential of the microbial community to respond to an increase in exposure time to LAS or to increasing dissolved oxygen concentrations and the resulting affect on the rate or extent of LAS biodegradation. The field study described in this report was designed explicitly to investigate the effect of changing the injected dissolved oxygen concentration during an extended tracer test in which LAS was injected continuously over an 80-day period. Laboratory column experiments were conducted under conditions that simulated the field conditions to determine if similar rates of LAS biodegradation and changes in LAS mixture composition could be obtained for similar dissolved oxygen scenarios as those observed in the field.

Experimental Section

Site Description. This study was conducted in an unconfined sand and gravel glacial outwash aquifer at the U.S. Geological Survey's Cape Cod Toxic Substances Hydrology Research Site near Falmouth, MA. Disposal of secondary sewage effluent to infiltration beds at the site from 1936 to 1995 has resulted in a plume of sewage-contaminated groundwater that is approximately 6 km long, 1.5 km wide, and 30 m thick. The plume has been well-characterized (*21, 22*) and has been the site of numerous studies focused on the fate and transport of inorganic and organic constituents, surfactants, and microorganisms (*18, 19, 23*−*29*). The aquifer is characterized

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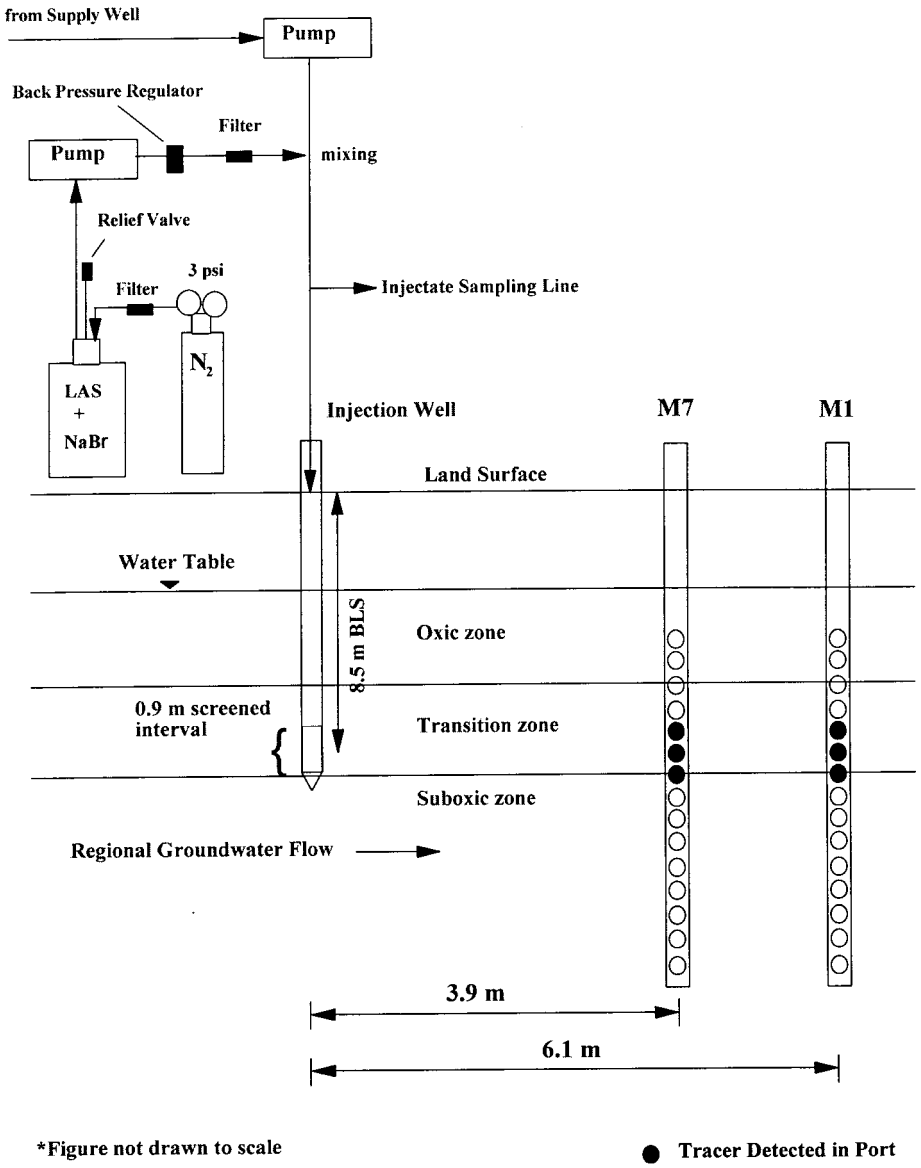

FIGURE 1. Schematic of the field tracer experiment including the location of the injection well and downgradient multilevel monitoring wells (M7 and M1) and sampling ports.

by an average hydraulic conductivity of 1.3×10^{-3} m/s and a porosity of 0.38 with average porewater velocities ranging from 0.3 to 0.5 m/day (*21, 22*).

The continuous injection tracer test was conducted at a multilevel sampling well array used for the previous pulsed tracer tests (*18, 19*) located approximately 300 m downgradient from the infiltration beds. The multilevel monitoring wells each have 15 discrete sampling ports vertically spaced at 0.6 m intervals that span a vertical distance of approximately 8 m, from 5.6 to 14.4 m below land surface (BLS) (Figure 1). At this location the aquifer has a vertical geochemical gradieint due to uncontaminated groundwater from recharge overlying the contaminant plume (*22, 24*). There is an ~2-m-thick transition zone between the uncontaminated and contaminated groundwater characterized by a sharp increase in specific conductivity and a decrease in dissolved oxygen concentration. The tracer test was conducted in the transition zone at approximately 8.5 m BLS with background dissolved oxygen concentration of 1 mg/L and specific conductivity of 180 μS/cm. The background LAS concentration was at the limit of detection (0.01 mg/L).

Column Experiments. The relationship between dissolved oxygen concentration and LAS biodegradation was investigated in column experiments conducted under variable oxygen concentrations. Aquifer sediments were collected from ~8.5 m BLS at the tracer test site in 1.5-m-long × 5 cm-diameter aluminum liners by hollow-stem auger drilling and a wireline−piston core barrel. Groundwater was pumped from a sampling port also located 8.5 m BLS into 1-L glass bottles. Sediments and groundwater were stored at 4 °C.

The sediments were packed as a groundwater slurry into sterilized glass columns 25 cm long with internal diameters of 2.5 cm. The columns were designed so that the sediments and column effluent were not in contact with the ambient atmosphere and were exposed only to oxygen supplied in the influent solution (Figure 2). The packed columns had a porosity of ~0.40 based on the measured sediment bulk density (1.64 g/cm^3), sediment mass, and column volume. Influent solutions of 13 mg/L LAS (Condea Vista, Austin, TX) and 35 mg/L Br^- (Aldrich Chemical Co., Milwaukee, WI) in groundwater were continuously delivered to the columns with a Beckman model 110A HPLC pump (Beckman Instruments, Inc., Fullerton, CA). The homologue distribution of the LAS mixture was 22% C_{10}, 39% C_{11}, 29% C_{12}, and 9% C_{13}. New influent solutions were prepared every 1−2 days to avoid

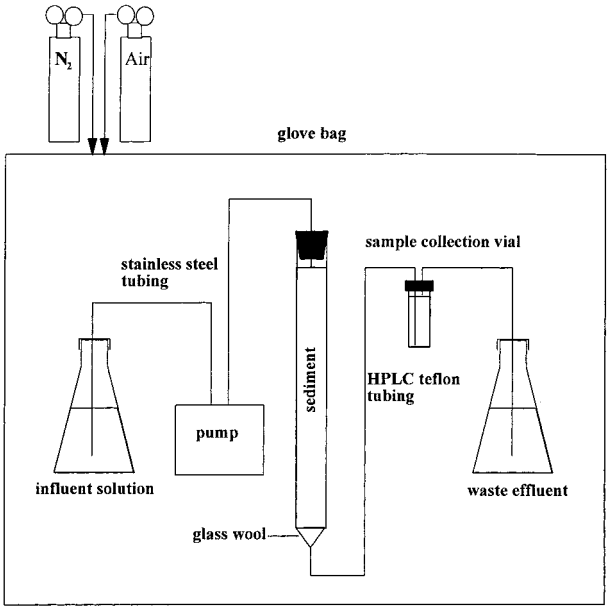

FIGURE 2. Schematic of the laboratory column experiments.

contamination and biodegradation of LAS. The flow rate of 3.3 mL/h corresponded to a porewater velocity of 0.44 m/d calculated from the column porosity and cross-sectional area and was comparable to values calculated for the aquifer in the vicinity of the tracer test. The column experiments were performed at 25 °C.

Influent dissolved oxygen concentrations were controlled by varying the air−nitrogen ratio in the glovebag atmosphere and by allowing the influent solution to equilibrate with the atmosphere in the glovebag. The aerobic column was operated for 10 days at 8 mg/L dissolved oxygen. The variable-oxygen experiment was conducted over a 83-day period with influent dissolved oxygen concentrations ranging from 0.5 to 8.0 mg/L. Dissolved oxygen concentrations in the column influent and effluent were measured colorimetrically (CHEMetrics, Calverton, VA). Influent and effluent samples were collected in 15-mL glass vials, preserved with 1% v/v formalin, and stored at 4 °C. Bromide concentrations were determined with an ion-selective electrode and meter (Orion model 250A) after the samples had equilibrated to 25 °C. Quantitative LAS concentrations were determined by the method of Krueger and Field (*6*). Enumerations of total free-living bacteria were determined by the method of Harvey et al. (*30*). Determinations of active cell concentrations were made on unpreserved samples using fluorescein diacetate (FDA) coupled with epifluorescent microscopy (*31*). Sediment-bound biomass was determined before and after the experiments by means of phospholipid analysis (*32, 33*).

Continuous Injection Tracer Test. A continuous injection tracer test was conducted in a sewage-contaminated aquifer with 1 mg/L dissolved oxygen where partial biodegradation of the LAS mixture had been observed previously during a pulsed tracer test (*19*). The continuous injection tracer test was conducted between June 18 and October 26, 1995. A stock solution of NaBr (16 g/L) and a commercial mixture of LAS (4 g/L) was prepared in degassed distilled water. The homologue distribution of the LAS mixture was the same as for the column experiments. The solution was filtered through a 0.2-μm polycap filter (Whatman International Ltd., Maidstone, England) and pumped into an autoclaved, nitrogen-purged 5-gallon glass carboy fitted with a rubber stopper.

During the injection, the stock solution was stirred constantly and maintained under N_2 (2−3 psi) that had passed through a 0.2-μm bacterial air filter (Gelman Sciences, Ann Arbor, MI). The stock solution was pumped with a peristaltic pump (Manostat Corporation, New York, NY) at a flow rate of 0.5 mL/min and mixed in-line with groundwater from the transition zone that was pumped from a nearby supply well with a GeoTech peristaltic pump (Environmental Equipment, Inc., Denver, CO) (Figure 1). In-line 0.2-μm filters (Gelman Sciences, Ann Arbor, MI) were also connected between the mixing junction and the stock solution, and an HPLC back-pressure regulator (Scientific Systems, Inc., State College, PA) was installed to prevent groundwater from entering the carboy. All tubing used in the injection system was either copper or stainless steel except the peristaltic pumps, which were fitted with low gas-permeability Norprene tubing (Cole-Parmer Instrument Company, Chicago, IL).

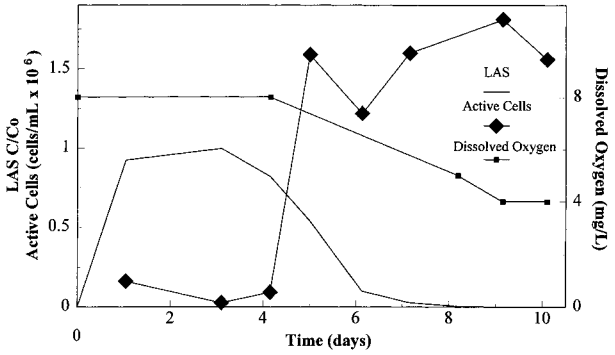

FIGURE 3. Breakthrough curves for total LAS, dissolved oxygen, and active cells in the aerobic column.

On the basis of the current estimated hydraulic gradient and the predicted tracer plume path, the injection well was installed just prior to the test in a location such that the tracer plume would intersect wells that had not been used in previous LAS tracer experiments. The supply and injection wells consisted of 0.9-m screened stainless steel drive points with a PTFE mesh liner (Solinst Canada Ltd., Georgetown, Ontario) connected to copper tubing. The screened intervals of the supply and injection wells were centered at 8.5 m BLS in the transition zone and had comparable groundwater chemistry (~1 mg/L dissolved oxygen and 180 μS/cm specific conductivity). The bromide, LAS, and groundwater injectate solution was injected continuously for 61 days from June 18 to August 18, 1995 at an average flowrate of 100 ± 5 mL/min.

Daily analysis of injectate samples verified constant bromide (93 mg/L) and LAS (23 mg/L) concentrations. Dissolved oxygen concentration in the injectate was increased from 1 to 2.5 mg/L on day 30 by splicing a 15-m-long section of silicone tubing, which has a greater gas permeability than the Norprene tubing, into the injection system, allowing oxygen to diffuse into the injectate. Approximately 8000 L of injectate containing 740 g bromide and 191 g LAS were injected during the experiment.

Wells located downgradient from the injection well (Figure 1) were monitored for 130 days from the beginning of the tracer test until October 26, 1995. Groundwater samples for bromide, LAS, and bacteria analysis were collected in polyethylene bottles daily from sampling ports with a peristaltic pump fitted with Norprene tubing, and preserved and analyzed according to methods previously described for the column experiments. Dissolved oxygen concentrations were determined colorimetrically (CHEMetrics, Calverton, VA) in the discharge line of the peristaltic pump during sample collection.

Results and Discussion

Column Experiments. Nearly complete breakthrough of the LAS mixture occurred within 1 day in the aerobic column experiment indicated by a relative concentration (C/C_0) of

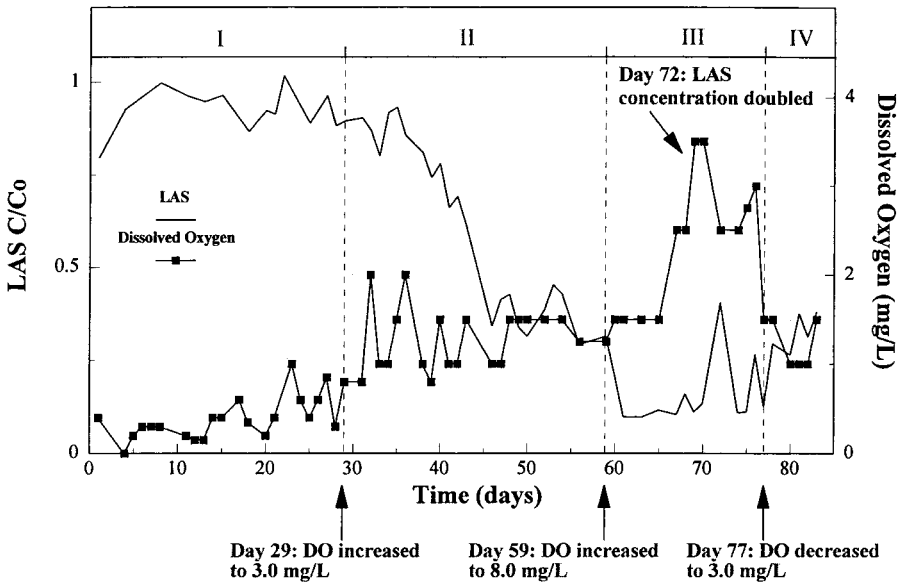

FIGURE 4. Breakthrough curve for total LAS and dissolved oxygen in the variable-oxygen column experiment. Regions correspond to the following dissolved oxygen concentrations: I, −0.5 mg/L; II, −3 mg/L; III, −8 mg/L; IV, −3 mg/L.

unity where C is the observed concentration and C_0 is the injectate solute concentration (Figure 3). After a 3-day lag, the LAS concentration steadily decreased to below detection (0.01 mg/L) by day 8. Note that the decrease in LAS concentration occurred while LAS was continuously being applied to the column. The increasing rates of LAS removal during the 10-day experiment suggest acclimation or perhaps an increase in microbial biomass. Although no increase in the total number of free-living bacteria was observed in the column effluent during the test, the concentration of active cells increased 10-fold as the biodegradation rate increased. After completion of the experiment, phospholipid analysis of the column sediments determined that sediment-bound biomass also increased 2.4 times over the background concentration of 1×10^7 cell/g sediment but only in the top 3 cm of the column. The longer alkyl chain LAS homologues and external isomers initially were preferentially biodegraded and had higher removal rates (data not shown), which is consistent with previous laboratory studies (*9*, *12*, *34*). Dissolved oxygen concentration in the column effluent decreased from 8 to 4 mg/L.

In contrast to the aerobic column, little biodegradation occurred during the first 29 days (region I) of the variable-oxygen column experiment when the influent dissolved oxygen concentration was 0.5 mg/L (Figure 4). The LAS relative concentration averaged 0.93 ± 0.06, and a small but measurable quantity of the 2-phenyl C_{12} and C_{13} isomers was degraded (Figure 5a). The relative mass, M_{rel}, for region I was estimated as the amount of LAS observed in the effluent normalized to the amount injected (Table 1). LAS mass removal rates, k_{mass}, (mg/(L day)) for the C_{10}−C_{13} 2-phenyl isomers were calculated from M_{rel} and a column residence time of 0.7 days (Table 1). The mass removal rate for total LAS was 1 mg/(L day) in region I.

On day 29 of the experiment, the influent dissolved oxygen concentration was increased from 0.5 to 3.0 mg/L. After a 10-day lag, which was determined as no significant ($P = 0.01$) change in the LAS concentrations between days 29 and 39, biodegradation rates increased until day 46 when the rate became constant (LAS $C/C_0 = 0.37 \pm 0.06$) (region II, Figure 4). In this region, the effluent dissolved oxygen concentration was 1 mg/L, indicating that 1.5−2.0 mg/L dissolved oxygen was consumed. Mass removal rates for the individual LAS homologues (Table 1) and total LAS (9.3 mg/(L day) increased in region II and resulted in increased depletion of the 2-phenyl isomers of all homologues and enrichment of the short alkyl chain internal isomers (Figure 5b).

The influent dissolved oxygen concentration was increased from 3.0 to 8.0 mg/L on day 59 and LAS concentrations declined rapidly with no observable lag to an average C/C_0 of 0.12 ± 0.02 over the period from 61 to 70 days (region III, Figure 4). The removal rate for total LAS mass was 18 mg/(L day). The only LAS components present in the effluent were the short alkyl chain internal isomers (e.g., 5- and 4-phenyl C_{10} and 5-phenyl C_{11}) (Figure 5c). Interestingly, these are the same LAS components that persist in the sewage-contaminated groundwater at the field site that is characterized by an average residence time of >3 years (*4*). Within region III, the effluent dissolved oxygen concentration increased to only 3 mg/L, indicating that oxygen consumption and biodegradation increased concomitantly with an increase in influent dissolved oxygen concentration. The temporary increase in LAS concentration on day 72 was a result of a 2-fold increase in influent LAS concentration during days 71−76 (Figure 4). The system responded quickly to reduce LAS concentration back to the levels observed prior to the increase in influent LAS concentration, which is a typical response of biologically-active systems (*9*).

On day 77, the influent dissolved oxygen concentration was decreased to 3.0 mg/L. Subsequently, the LAS C/C_0 increased to 0.33 ± 0.05 with a total LAS mass removal rate of 12 mg/(L day) during days 78−83 (region IV, Figure 4), which is comparable to rates computed for region II that had similar dissolved oxygen concentrations. In addition, similar LAS mixture compositions were observed in regions II and IV (Figure 5b,d). Rates of oxygen consumption (1.5−2.0 mg/L) also were similar in regions II and IV. An increase in biodegradation rates and a change in LAS mixture composition with increasing dissolved oxygen suggest a relation exists between dissolved oxygen concentration and LAS homologue biodegradation rates. Furthermore, these results suggest that the selectivity for the longer alkyl chain external LAS isomers in the mixture is retained under all oxygen conditions, which is in contrast to reports of laboratory studies operated under fully aerobic conditions in which biodegradation rates did not vary significantly for the different LAS homologues and isomers (*13*, *14*).

The longer lag in the variable-oxygen column (10 days) relative to the aerobic column (3 days) suggests that the low

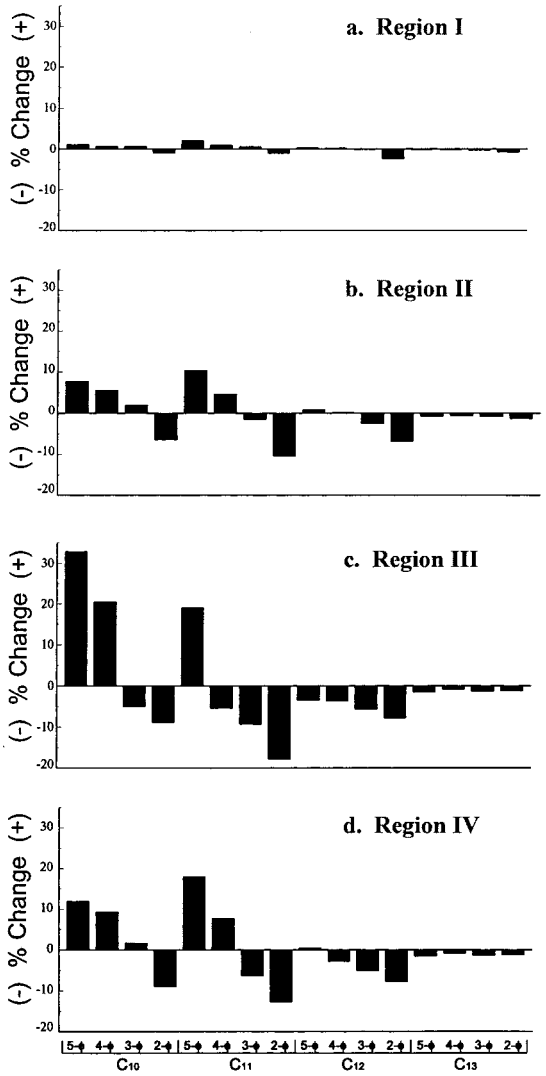

FIGURE 5. Change in LAS mixture composition represented as the difference between the % of total mass of each LAS component in the column influent and effluent in the four regions (averaged over each region) of the variable-oxygen column experiment. The symbol ϕ denotes the sulfophenyl group.

dissolved oxygen concentration in the variable-oxygen column initially may have slowed rates of microbial growth because oxygen concentration was the only difference between the two column experiments. It is not known to what extent oxygen limitations affected microbial growth and metabolism in the presence of high (>10 mg/L) concentrations of LAS. Phospholipid analysis of the variable-oxygen column sediments after day 83 indicated that the sediment-bound biomass increased 3.5 times over the background value (1×10^7 cells/g sediment) during the 83-day experiment but only in the first 3 cm of the column, as was observed in the aerobic column. Other laboratory studies reported that bacterial acclimation to LAS is necessary before biodegradation begins; the induction of enzymes necessary for ω-oxidation is presumed to occur during the acclimation period (*9, 12*). However, the sewage-contaminated sediments and groundwater used in these experiments had prior exposure to low LAS concentrations (10 μg/L) at 1 mg/L dissolved oxygen concentration, which suggests that the observed lag was more likely due to an initially low number of LAS-degrading bacteria rather than to enzyme induction. Therefore, the increasing LAS removal rates in regions II and III, which led to a decline in LAS concentration, may have resulted from increased biomass near the column inlet once sufficient oxygen was supplied. The observation that growth occurred only in the top portion of the column and that dissolved oxygen (regions I, II, and IV) and LAS (region III) potentially were depleted before the column outlet suggests that biodegradation did not occur throughout the entire length of the column. For this reason the calculated rates may underestimate the actual rates and therefore should be considered to be minimum estimates.

Continuous Injection Tracer Test. The tracer plume intersected downgradient monitoring wells M7 and M1 in sampling ports located between 7.9 and 9.1 m BLS (Figure 1). The largest concentrations were observed in wells M7 and M1 at 7.9 m BLS, indicating that the plume passed most directly through these sampling ports. Maximum values of C/C_0 approached 1.0 indicating that little dilution occurred in the plume's interior.

The following discussion will focus on results from well M7 at a depth of 7.9 m BLS, although similar results were observed at the other wells and depths. Breakthrough curves for LAS components were constructed by normalizing the concentrations of each component to its concentration in the injectate (Figure 6). Separation of the surfactant mixture components was observed during transport. The leading edges of the breakthrough curves for bromide and the more hydrophilic shorter alkyl chain LAS components (i.e., C_{10} and C_{11}) were nearly coincident, whereas the more hydrophobic LAS components (i.e., C_{12} and C_{13}) were noticeably retarded relative to bromide (region I, Figure 6). Retardation factors, R, and sediment partition coefficients, K_d, for each LAS component were estimated from the breakthrough curves using the first temporal moment, M_1 (*19, 35, 36*). Values of R (1.0−1.4) and K_d (0.00−0.14 L/kg) were comparable to values calculated in pulsed tracer tests conducted at this site (*18, 19*) and varied as a function of alkyl chain length and phenyl position such that sorption increased with increasing hydrophobicity (*18, 19, 37*).

Unlike the shorter alkyl chain homologues and bromide, the longer alkyl chain homologues were attenuated and never attained C/C_0 values near 1.0. In addition, the tail edges of the breakthrough curves of the more hydrophobic, longer alkyl chain homologues were not retarded relative to bromide nor the shorter alkyl chain homologues (region II, Figure 6). If partitioning to sediment were the only process affecting LAS, the tailing edges of the more hydrophobic LAS homologues should be retarded relative to bromide, as has been previously observed during pulsed tracer tests conducted at this site (*18, 19*). Since sorption is an equilibrium process (i.e., reversible), the shape of the breakthrough curves suggest that additional processes (e.g., biodegradation) are occurring that result in the removal of the long alkyl chain homologues.

The total mass of bromide and LAS passing the monitoring well were estimated from the zeroeth temporal moments, M_0, of the breakthrough curves (*19, 35, 36*). Relative mass, M_{rel}, which is the value of M_0 for a given solute normalized to that of the conservative bromide tracer, was used to determine whether the mass of a given LAS component was conserved ($M_{rel} \cong 1$) or removed ($M_{rel} < 1$) during the test (*19, 26, 35*). Relative mass estimates for total LAS over the entire test were less than unity at wells M7 (0.85) and M1 (0.86), indicating significant mass removal within the first 3.9 m of transport with no significant additional removal between 3.9 and 6.1 m downgradient. This is in contrast to the pulsed test previously conducted in the transition zone in which relative mass progressively decreased over the entire 9.4-m transport interval (*19*).

Region I for the field experiment is defined as the first 41 days of the injection conducted with an injectate dissolved oxygen concentration of 1.0 mg/L (Figure 6). The dissolved oxygen concentration increased from a background concentration of 0.5 mg/L to 1.0 mg/L as the tracer plume arrived

TABLE 1. LAS Homologue (2-phenyl isomers) Relative Masses, M_{rel}, and Mass Removal Rates, k_{mass}, (mg/(L day) from the Continuous Tracer Test and Variable-Oxygen Column

	region I				region II			
	field tracer test[a]		column		field tracer test		column	
homologue	M_{rel}	k_{mass}	M_{rel}	k_{mass}	M_{rel}	k_{mass}	M_{rel}	k_{mass}
C_{10} 2-ϕ	0.97	0.005	0.98	0.038	0.76	0.039	0.30	1.22
C_{11} 2-ϕ	0.83	0.061	0.96	0.15	0.65	0.125	0.34	2.39
C_{12} 2-ϕ	0.61	0.056	0.85	0.19	0.38	0.089	0.15	1.06
C_{13} 2-ϕ	0.22	0.019	0.61	0.069	0.26	0.018	0.07	0.16

[a] Results from well M7.

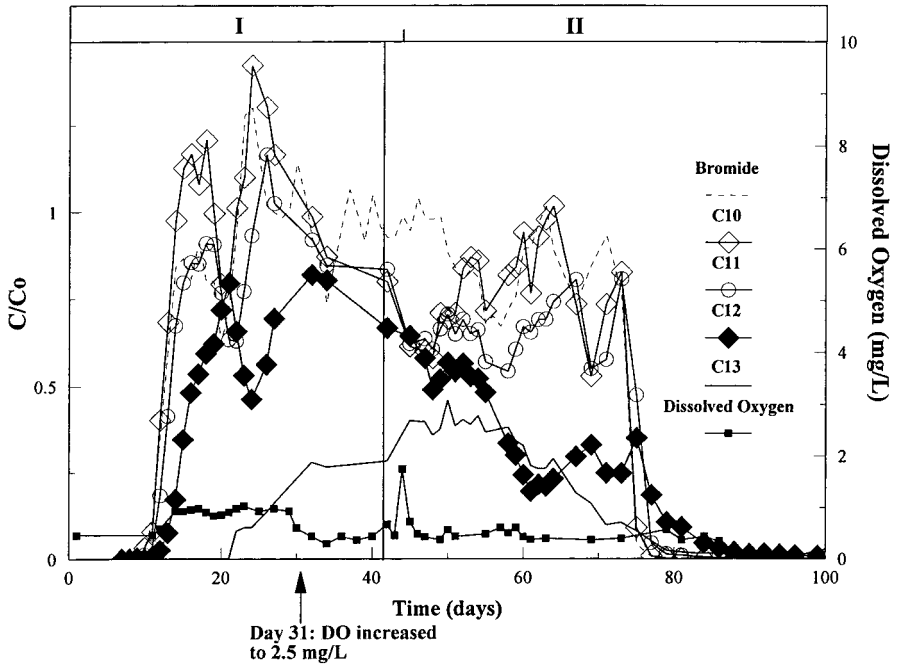

FIGURE 6. Breakthrough curves for bromide, the individual LAS homologues (2-phenyl isomers), and dissolved oxygen at well M7 during the continuous injection tracer test.

at well M7 on day 12 and then declined rapidly to 0.4 mg/L on day 30. The decline in dissolved oxygen concentration on day 30, which is indicative of biodegradation, coincided with the breakthrough of the C_{13} 2-phenyl isomer, suggesting that both sorption and biodegradation processes were responsible for the apparent retardation and attenuation relative to the more hydrophilic LAS components. Biodegradation in region I, indicated by decreasing M_{rel} values (Table 1), resulted in a mixture depleted in the longer alkyl chain homologues and external isomers (Figure 7a).

Region II is defined as days 42−74 with an injectate dissolved oxygen concentration of 2.5 mg/L. Dissolved oxygen concentrations increased to 1.8 mg/L on day 43 and rapidly decreased to 0.5 mg/L for the remainder of the test (Figure 6). In addition, a 10-fold increase in the number of free-living bacteria was observed in region II. Mass removal rates were calculated from M_{rel} values and an estimated residence time of 12.5 days. Greater removal of the LAS homologues was observed in the higher-oxygen region II than in region I, demonstrated by lower M_{rel} values and higher mass removal rates in region II (Table 1). In addition, the LAS mixture in region II was enriched in internal isomers (i.e., 4- and 5-phenyl) and short alkyl chain homologues (Figure 7b). Again, it should be emphasized that these rates most likely are minimum estimates due to the possibility of a nonhomogeneously distributed increase in biomass over the transport interval and that dissolved oxygen was consumed before reaching the first downgradient sampling well. Thus, as in the case of the column experiments, LAS biodegradation may have been limited to the intitial portion only of the transport interval, which corresponds to a shorter residence time, such that the calculated rates should be considered to be minimum estimates. However, this does not undermine the utility of the estimated rates because they allow for direct comparison of the different regions in both the laboratory and field studies.

Comparison of Column and Field Experiments. The experimental conditions in regions I and II in the field test were comparable both in duration and dissolved oxygen concentration to those in the variable-oxygen column experiment. Lower M_{rel} values and greater mass removal rates of the LAS components were observed in region II compared to region I in both the field and laboratory (Table 1). In addition, similar LAS mixture compositions were observed for region I (Figures 5a and 7a) and region II (Figures 5b and 7b) of the variable-oxygen column experiment and the field tracer test. Note that greater depletion of the longer alkyl chain homologues and external isomers is exhibited in region II which had greater oxygen concentrations. In contrast to LAS biodegradation under aerobic conditions, only a fraction of the LAS mixture biodegraded under the low oxygen conditions in both column and field experiments.

According to the established biodegradation pathway for LAS, molecular oxygen only is required for primary degradation of LAS by ω-oxidation while subsequent chain-shortening steps by β-oxidation do not require molecular oxygen

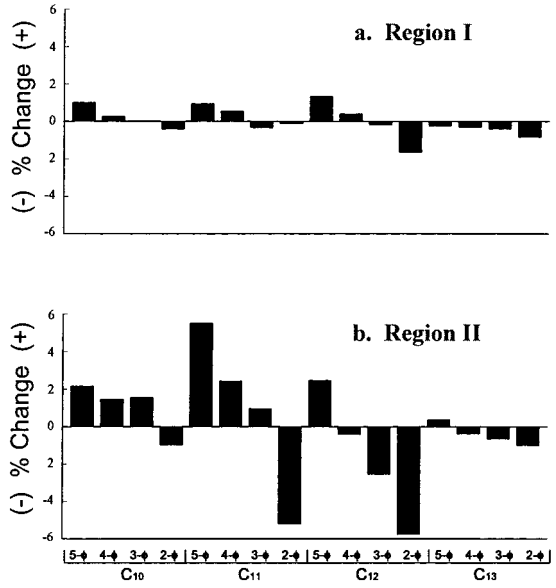

FIGURE 7. Change in LAS mixture composition represented as the difference between the % of total mass of each LAS component in the influent and effluent in the two regions of the continuous tracer test. The symbol ϕ denotes the sulfophenyl group.

(*9*). The theoretical oxygen demand required to achieve the primary oxidation of the LAS mixture that was removed during region I of the field experiment was calculated as 3.2 mg/L. In comparison, the dissolved oxygen concentration supplied during the injection was only 1 mg/L, which suggests that the observed biodegradation rates may have been limited by oxygen availability. The rapid and complete biodegradation observed in the aerobic column and region III of the variable-oxygen column indicates that increased dissolved oxygen concentrations could potentially stimulate further LAS biodegradation. These laboratory results suggest that an additional increase in the injected dissolved oxygen concentration during the continuous field test would have resulted in increased biodegradation rates.

The differences in the biodegradation rates and mixture composition observed between regions I and II in the field and laboratory indicate that increased oxygen availability resulted in increased rate and extent of LAS biodegradation. However, the mass removal rates in regions I and II of the variable-oxygen experiment were generally at least 2−3 times greater than those observed in the field (Table 1). For example, in regions I and II combined, 26% of the injected LAS was biodegraded in the column, whereas 15% of the injected LAS was degraded in the field.

Differences between the LAS biodegradation in laboratory and field experiments may be attributed to the potential inhibition of microbial activity at LAS concentrations > 10 mg/L (*38*). Because the column and field studies were conducted with 13 and 20 mg/L LAS, respectively, select populations of LAS degraders and not necessarily all potential degraders may have been enriched in these experiments. Furthermore, the LAS concentrations used in the column study might have enriched a population with biodegradation kinetics and capabilities that differed with respect to oxygen concentrations than those enriched during the field test conducted with higher LAS concentrations. The lower temperature in the field (12 °C) compared to the laboratory experiments (25 °C) also may have led to lower rates of biodegradation in the field experiment. For example, previous laboratory studies conducted with river waters reported inhibition of LAS biodegradation at 10 °C with maximum rates observed at 25 °C (*39*). However, both types of experiments described in this study exhibited preferential biodegradation of the LAS mixture with the removal of select LAS mixture components under low oxygen conditions and increased biodegradation rates with increased dissolved oxygen concentrations.

The parallel between region I of the continuous test and the pulsed tracer test previously reported (*19*), both of which were conducted in the transition zone under similar biogeochemical conditions, allows for comparison of the two types of field tests. The field conditions, including injected LAS and dissolved oxygen concentrations, residence time (12 days), and duration (30 days), were comparable in both the pulsed test and region I of the continuous test (*19*). However, LAS was continuously injected for 30 days in the continuous test whereas in the pulsed tracer test LAS was injected as a 3-h pulse resulting in a pulse width of 8 days (based on width at half-maximum peak height). It should be noted that the pulse width will change with time due to dispersion. In addition, there exists a concentration gradient within the pulse such that the aquifer exposure to LAS over the pulse width varies. Thus, while the residence times between the injection well and the first downgradient monitoring well were similar in the two tracer tests, the overall exposure time of the aquifer sediment to LAS concentrations greater than 1 mg/L was greater during the continuous test. In both the continuous and pulsed tracer tests, dissolved oxygen concentrations declined to <0.5 mg/L as a result of LAS biodegradation. Estimated values of M_{rel} for the C_{10}−C_{13} 2-phenyl isomers from the pulsed test and corresponding mass removal rates were comparable to those in region I of the continuous tracer test (Table 1). Similar results from the two tests indicate that increased exposure time did not increase biodegradation rates and that LAS biodegradation proceeds only slowly under oxygen-limited groundwater conditions, which is supported further by the results of the low oxygen region I in the variable-oxygen column experiment.

Previous studies have reported LAS biodegradation in subsurface sediments to follow first-order kinetics (*10, 14*), which assumes that no microbial growth occurred. Reported first-order rate constants for LAS mixture mineralization range from 0.03 to 0.06 day-1 (*10, 14*). Assuming first-order kinetics in the low oxygen region I of the continuous tracer test, first-order rate constants ranging from 0.002 to 0.08 day^{-1} for the C_{10}−C_{13} 2-phenyl isomers were estimated from M_{rel} and the residence time (12.5 days) as previously described (*19*) and were comparable to those estimated for the pulsed test (0.002−0.09 day^{-1}) (*19*). It should be noted that only the rates for the more biodegradable LAS components (e.g., the C_{12} and C_{13} 2-phenyl isomers) were comparable to reported literature values. First-order rate constants estimated from the low oxygen region I of the variable-oxygen column experiment (0.03−0.7 day^{-1}) were greater by an order of magnitude than those from the continuous or pulsed tests, further demonstrating the tendency for laboratory experiments to predict greater rates than are observed under in situ conditions. Increasing rates of biodegradation and measurements of increased biomass for column experiments indicate that bacterial growth occurred; thus, it may not be appropriate to determine first-order rate constants for these conditions. For this reason, a model that incorporates bacterial growth will be considered in the future to describe these regions.

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