Kinetics and Practical Significance of Biodegradation of Linear Alkylbenzene Sulfonate in the Environment

R.J. Larson[a,*], T.M. Rothgeb[a], R.J. Shimp[b], T.E. Ward[c] and R.M. Ventullo[d]

[a]Procter & Gamble Company, Ivorydale Technical Center, Cincinnati, Ohio 45217, [b]Procter & Gamble Company, Schwalbach Technical Center, Schwalbach, Germany, [c]Procter & Gamble Company, Sharon Woods Technical Center, Cincinnati, Ohio 45241 and [d]University of Dayton, Biology Department, Dayton, Ohio 45469

This paper reviews the kinetics of biodegradation of linear alkylbenzene sulfonate (LAS) in engineered (wastewater treatment) and natural environment systems, focusing on work conducted in our environmental laboratories over the past 10–15 yr. Biodegradation studies were conducted in laboratory microcosms in which pure-chainlength [^{14}C]-ring-labeled LAS homologs were used to allow complete mineralization to be assessed. In general, biodegradation rates for a series of LAS homologs (C10–C14) were comparable to each other and to values for naturally occurring materials such as sugars and fatty acids. Half-lives for LAS mineralization ranged from 1–2 d in aerobic and anaerobic sewage sludges, river water and sediments, to 1–3 wk in surface and subsurface soils and estuarine environments. The half-life for LAS degradation in different environmental compartments, relative to its residence time in these compartments, makes biodegradation a practically significant removal mechanism in a broad range of aquatic, benthic and terrestrial habitats.

KEY WORDS: Biodegradation, groundwater, kinetics, linear alkylbenzene sulfonate, marine, radiolabel, soil, subsurface soil, surface waters, wastewater treatment.

Due to their high-volume use in consumer products, detergent chemicals have the potential for broadscale release into aquatic and terrestrial environments. Linear alkylbenzene sulfonate (LAS), an anionic surfactant, is the major anionic surfactant used worldwide, accounting for an estimated 28% of all synthetic surfactants (1). Approximately 270,000 metric tons (600 million pounds) of LAS are used annually in the United States in laundry and cleaning products. Annual production volumes of LAS in the United States, Japan and Western Europe total about 1.3 million metric tons (2.8 billion pounds) (2). The large volumes of LAS used worldwide, coupled with its potential for broadscale distribution in the environment as a result of the use and disposal of consumer products, make biodegradation a key process for maintaining LAS exposure concentrations at acceptable levels.

Since its introduction over 25 years ago, an extensive database has been developed supporting the environmental safety and aerobic biodegradability of LAS. Much of the biodegradability database has been developed in standard laboratory screening tests, which are routinely used in North America and Europe to determine the biodegradation potential of organic substances (3). The potential for LAS degradation under anaerobic conditions is not as well studied, due primarily to a lack of validated standard test methods. The available data, however, suggest that LAS degradation is poor under anoxic conditions and affected by the same factors that govern the anaerobic degradation of other aliphatic hydrocarbons lacking oxidized substituents. In general, screening tests have led to the development of useful and cost-effective information on the aerobic biodegradability of LAS, and, more broadly, on the biodegradability of a variety of other organic chemicals under standard conditions. Two major factors, however, significantly impact the environmental relevance of screening test results and severely limit how accurately these results can be extrapolated to "real world" environmental systems: (i) Standard screening tests do not accurately simulate the physical, chemical and biological conditions found in actual environmental systems; and (ii) Standard screening tests typically ignore environmental compartments such as sediments, surface and subsurface soils and ground water, which may be an important contribution to the biodegradation of detergent chemicals in the environment.

Figure 1 presents a generalized flow diagram of the major routes of entry of LAS into the environment as a result of its use and disposal in consumer products. Clearly, LAS exposure can occur in a number of environmental compartments, many of which are not addressed by standard screening tests. To adequately assess the environmental safety of consumer product ingredients like LAS, accurate information on exposure levels in a variety of environmental compartments is needed. For LAS, and a number of other high-volume detergent chemicals, biodegradation plays a major role in controlling these environmental exposure levels.

This paper will review the results of some of the work conducted in our environmental laboratories over the past 10–15 yr to study the biodegradation of LAS in engineered (*i.e.*, wastewater treatment) and natural environmental systems. The work has utilized a variety of laboratory and field (*in situ*) microcosms to simulate natural environments exposed to LAS in wastewater effluents and sludges. These environments include river waters and sediments, sludge-amended surface soils, subsurface soils and groundwaters and estuarine/marine environments. This paper will focus primarily on the results of laboratory microcosm studies that characterize the kinetics of aerobic biodegradation of pure-chainlength LAS homologs in different environmental compartments. Additional information on the *in situ* and field work has recently been summarized and published elsewhere (4). In addition to reviewing aerobic biodegradation results for LAS, the biodegradation of LAS in anaerobic environments will also be addressed, with particular attention to the mechanism and environmental relevance of anaerobic LAS biodegradation. The paper concludes with a discussion of the practical significance of biodegradation as an environmental removal mechanism for LAS and the role of biodegradation in maintaining acceptable environmental safety margins.

BIODEGRADATION DEFINITIONS AND MEASUREMENTS

In broad terms, biodegradation can be defined as any process mediated by living organisms that results in the conversion of an organic chemical into organic and/or inorganic end-products that are chemically distinct from the parent material. A more precise technical definition of

*To whom correspondence should be addressed.

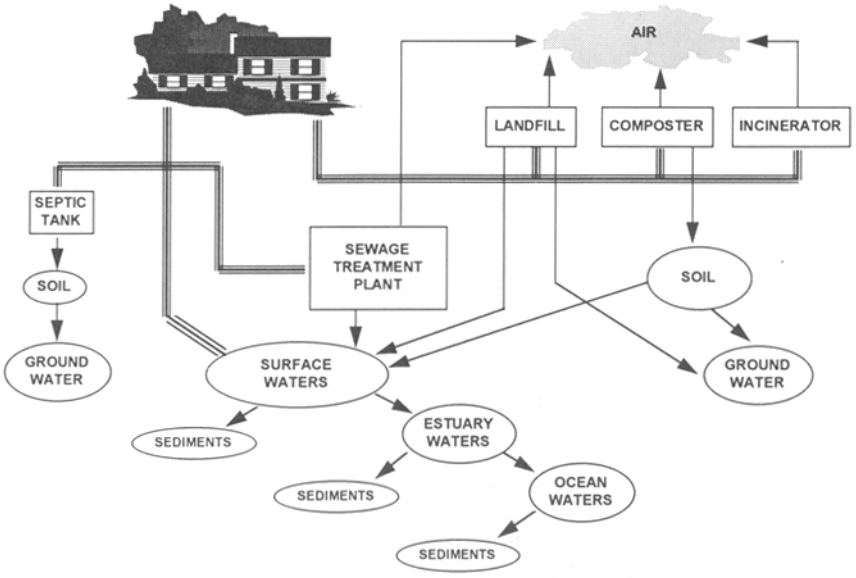

FIG. 1. Flow diagram showing the potential distribution of linear alkylbenzene sulfonate in various environmental compartments as a result of its use and disposal in consumer products.

biodegradation would be the metabolism of organic chemicals as sources of carbon and energy by heterotrophic microorganisms (primarily, bacteria and fungi) to form microbial biomass and inorganic and organic end-products, such as carbon dioxide or methane. Biodegradation processes can be grouped into two categories: primary biodegradation or biotransformation, and ultimate biodegradation or mineralization.

Primary biodegradation, which is generally assayed by a specific analytical technique, occurs when the structure of a chemical is altered such that basic physical and chemical properties are lost. This alteration results in a decreased sensitivity of the chemical to a specific method and a reduction in the analytical response relative to the parent chemical. Ultimate biodegradation or mineralization occurs when a chemical is completely broken down and metabolized to carbon dioxide (and methane under anaerobic conditions), water and other inorganic constituents. Complete mineralization is a highly significant endpoint from an environmental exposure standpoint. It results in a total loss of molecular identity and the conversion of a synthetic organic chemical into organic and inorganic end-products that are completely reassimilated into natural elemental cycles. For materials used in large volumes in particular, the most important criterion defining their true biodegradability and removal from the environment is their complete mineralization by microorganisms present in natural ecosystems. Biodegradation cannot be considered a true environmental removal mechanism for chemicals that undergo only primary biodegradation or that are partially degraded (biotransformed) to more persistent biodegradation intermediates.

The most accurate, direct and efficient methods to assess complete mineralization involve the use of specific radiolabeled (carbon-14, ^{14}C) materials. The use of ^{14}C-materials allows mineralization to be measured in complex environmental matrices at concentrations approximating realistic ($\mu g/L$) environmental levels. For most materials, it is not necessary to measure the metabolism of the entire carbon skeleton. Rather, only those structural moieties of the molecule that are known (or predicted) to be the most resistant to microbial attack need to be examined, because they represent the rate-limiting step for biodegradation. In the case of LAS, for example, the benzene ring is the last structural component to be metabolized. Biodegradation assays on this material, therefore, monitor the production of ^{14}C-gases from ^{14}C-ring-labeled LAS.

Emphasis on mineralization of the most resistant carbon positions in a particular molecule (*e.g.*, the benzene ring in LAS) is a fairly conservative approach for two reasons: (i) It focuses on the key structural component that is least susceptible to microbial attack and therefore degraded at the slowest rate. (ii) It measures only the production of end-products like carbon dioxide or methane, which are a function of the slowest, rate-limiting step in the biodegradation pathway. The major advantage of this approach, however, is that it prevents the over-extrapolation of laboratory data to actual environmental systems, and it guards against the generation of false-positive (as opposed to false-negative) results. Ideally, biodegradation results measured in the laboratory should provide a good simulation of what actually occurs in a specific environmental compartment. They should not, however, overestimate the rate and extent of degradation possible under realistic exposure conditions.

EXPERIMENTAL PROCEDURES

LAS samples. Figure 2 shows the structure of radiolabeled LAS homologs used in biodegradation studies. All homologs were pure-chainlength materials with alkyl chains of C10, C11, C12, C13 or C14, and a mixed phenyl composition typical of commercial LAS. The homologs were uniformly labeled with carbon-14 in the benzene ring, with

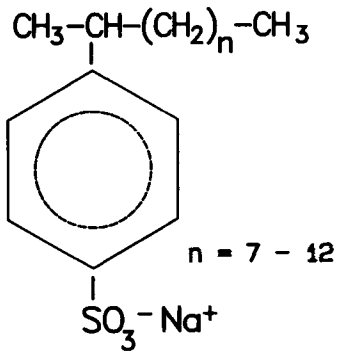

FIG. 2. Structure of linear alkylbenzene sulfonate. The average chainlength used in commercial detergent formulations is approximately C12.

radiochemical purities >97% and specific activities of 8.5 to 12.9 mCi/mmol (5).

Biodegradation test systems. Removal and biodegradation of LAS in engineered systems was determined in laboratory-scale continuous activated sludge (CAS) treatment systems by using modifications of the standard Organization for Economic Cooperation and Development (OECD) protocol (6). The CAS units (Fig. 3) were largely operated as described in the OECD protocol with a hydraulic retention time (HRT) of about 6 h and a solids retention time (SRT) of about 7 d. These values approximate operating conditions found in U.S. treatment plants. However, they are significantly less than the HRT and SRT values in European treatment plants, which can range to as much as threefold higher. Trace levels (20 μg/L) of a radiolabeled LAS (C13 homolog) were continuously dosed to the CAS units in influent sewage containing a background of about 5 mg/L mixed-chain commercial LAS. The influent sewage was obtained from a municipal treatment plant (Avondale, PA) treating predominately domestic wastewater. Removal and mineralization of LAS in the CAS system were measured daily after a three-week stabilization or acclimation period to achieve steady-state operating conditions. A control unit receiving wastewater only (no radiolabeled LAS) was included to monitor CAS operating parameters and to ensure that the units were operating normally. Radiolabel in the LAS test units was quantitated as a function of time in influent, effluent and mixed-liquor suspended solids to determine LAS removal during treatment and incorporation into sludge solids. The amount of removal due to biodegradation (mineralization) was determined by the difference between the total amount of ^{14}C dosed and that recovered in influent, effluent and sludge fractions after a total mass inventory of radioactivity.

Biodegradation assays in laboratory microcosms were conducted as previously described (7,8) by using environmental samples collected from the compartment of interest. In all studies, the kinetics of mineralization of various ring-labeled LAS homologs were determined in time-course studies at LAS concentrations approximating realistic environmental levels (μg/L in aqueous samples, μg/g in particulate samples). Briefly, ^{14}C-LAS was added to microcosms containing water, sludge, soil or sediment samples and incubated at ambient or *in situ* temperatures

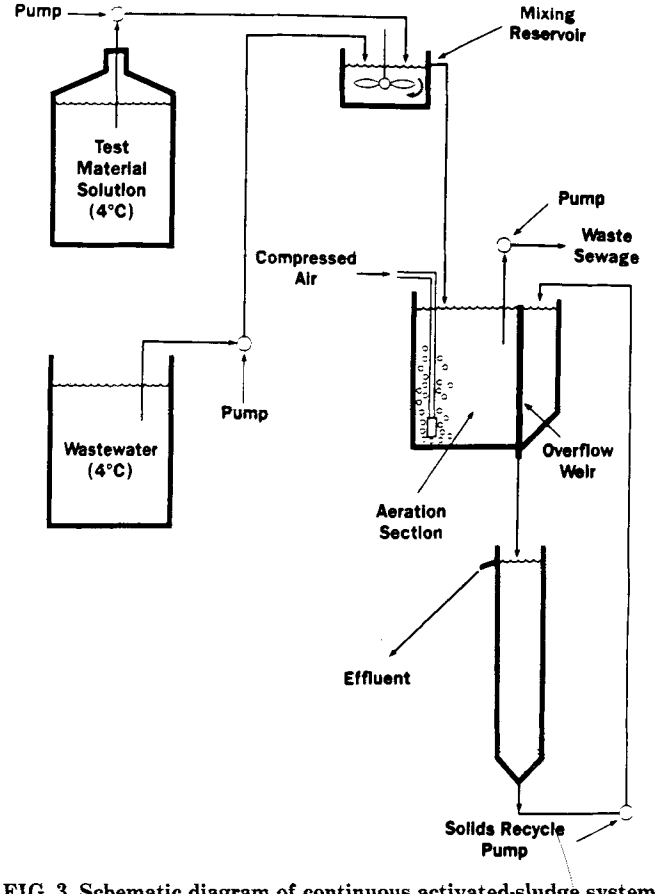

FIG. 3. Schematic diagram of continuous activated-sludge system used to characterize the removal and biodegradation of linear alkylbenzene sulfonate during wastewater treatment.

(Fig. 4). For water and sludge studies, samples were removed at various intervals, and radioactivity was determined in three fractions [$^{14}CO_2$ produced, ^{14}C associated with particulates (biomass) and ^{14}C in solution] by liquid scintillation spectrometry. Mass balances of radioactivity were obtained at each sampling point and routinely exceeded 90%. In high particulate soil and sediment studies, biodegradation determinations were based on $^{14}CO_2$ evolution measurements only, and mass balances (generally >90%) were obtained by combustion techniques (9). Data for cumulative $^{14}CO_2$ production or ^{14}C removal from solution were analyzed by nonlinear regression techniques to estimate first-order rate constants for degradation (k_1), normalized for the extent of degradation observed. Half-lives ($t_{1/2}$) were determined from the relationship $t_{1/2} = \ln 2/k_1 = 0.693/k_1$. All LAS homologs were tested over a wide concentration range (5–5000 μg/L in water and sludge samples, 0.01–250 μg/g in sediment and soil samples) to verify that degradation was first-order with respect to LAS concentration.

RESULTS AND DISCUSSION

Biodegradation in activated sludge. Activated sludge is the major form of secondary wastewater treatment in the United States, accounting for more than 90% of the total municipal wastewater flow treated in this country (10). We

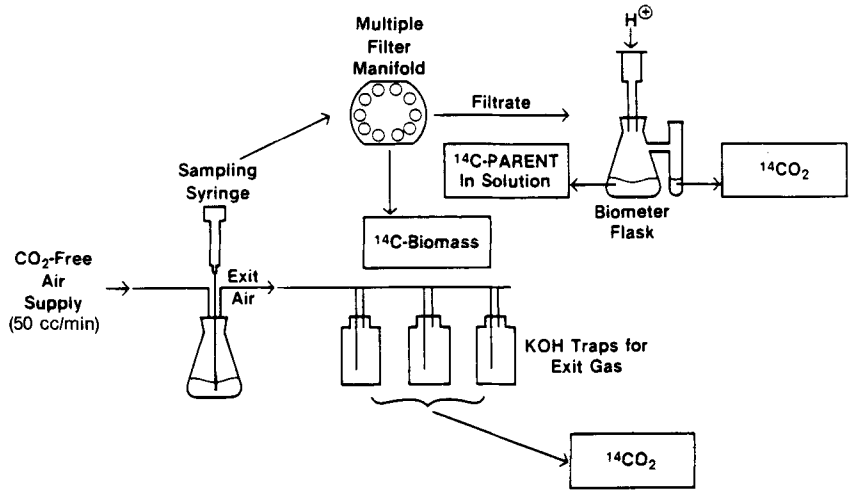

FIG. 4. Schematic diagram of typical assay system used to measure the biodegradability of linear alkylbenzene sulfonate homologs in laboratory microcosms.

have investigated the aerobic biodegradation and removal of LAS homologs in activated sludge under both batch and continuous-flow conditions. To dimension the potential for LAS degradation under anaerobic conditions, we have also measured the kinetics of LAS mineralization at low redox potentials in methanogenic digester sludge. Aerobic studies were typically conducted with sludges collected from the secondary aeration chamber of activated sludge systems treating predominantly domestic wastewater. Anaerobic studies were conducted with sludge collected from the secondary digester of municipal treatment plants having a combination of conventional activated sludge and anaerobic digester treatment. Digester sludge was collected and transported to minimize exposure to oxygen and to maintain microbial viability. All anaerobic studies were conducted in a Forma Anaerobic Incubation System (Model 1024) under a reducing atmosphere (nitrogen/hydrogen/carbon dioxide, 85:10:5) with both chemical and electronic redox indicators to ensure the maintenance of strict anaerobic conditions (≤ -200 mV).

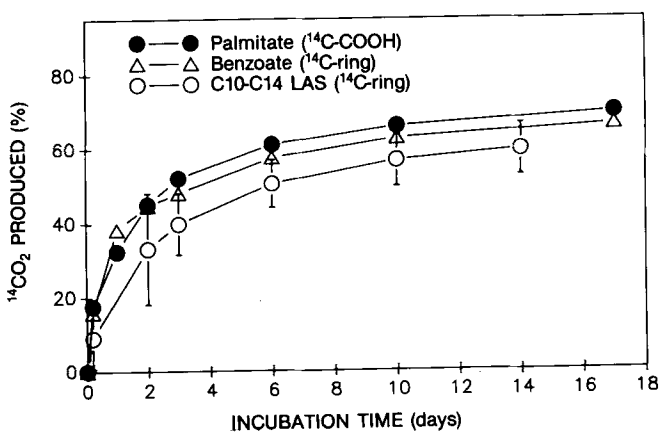

FIG. 5. Kinetics of mineralization of linear alkylbenzene sulfonate (LAS) homologs (C10–C14) in batch activated-sludge systems incubated under aerobic conditions. Palmitate and benzoate were tested as positive controls in the same study to characterize the kinetics and mechanism of LAS mineralization. Figure illustrates aerobic biodegradation of LAS homologs.

Figure 5 shows cumulative mineralization curves during the biodegradation of LAS homologs in batch activated-sludge (BAS) systems under aerobic conditions. Plotted are mean values for $^{14}CO_2$ production at a specific time point, averaged across several replicate experiments for all five homologs. The error bars represent two standard deviations. Also plotted are $^{14}CO_2$ evolution curves for the degradation of two naturally derived organic compounds having structural similarities to the alkyl and benzene sulfonic acid groups of LAS. The two natural compounds, palmitic acid (a sixteen-carbon fatty acid) and benzoic acid (a short-chain aromatic acid), were tested in the same series of experiments as the LAS homologs and served as positive controls.

In general, the kinetics of biodegradation for all five LAS homologs were quite comparable and reproducible across different sludge samples and experiments. The rate and extent of mineralization were not significantly different (95% confidence level) for any LAS homolog over the range of alkyl chainlengths tested (C10–C14). Degradation results for individual LAS homologs in BAS systems were also quite comparable to the results obtained for the two positive controls, palmitate and benzoate. Half-lives for LAS mineralization, based on k_1 values determined by nonlinear regression analysis, ranged from 1.5 to 2.2 d and showed no consistent trend as a function of alkyl chainlength (Fig. 6). The mean half-life for degradation of all five LAS homologs (1.8 d) was also comparable to the mean half-life value for palmitate and benzoate (1.2 d). A key conclusion from biodegradation studies in BAS systems is that LAS mineralization is independent of alkyl chainlength and equivalent to that observed for naturally occurring structural analogs. The comparable half-lives obtained for degradation of individual LAS homologs, benzoate and palmitate also confirm that alkyl chain degradation occurs rapidly in sludge and that mineralization of the benzene ring is the rate-limiting step for LAS degradation.

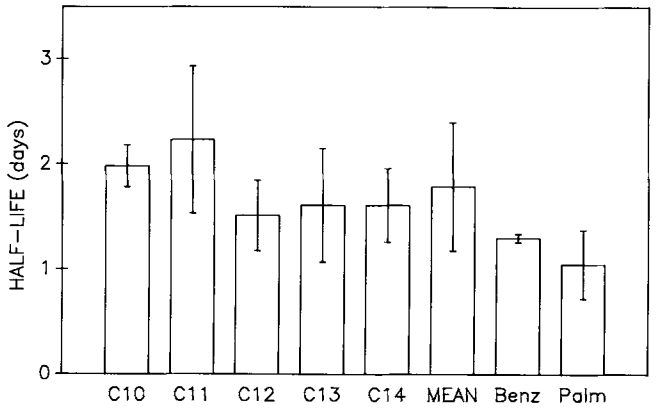

FIG. 6. Half-lives for aerobic biodegradation of linear alkylbenzene sulfonate homologs in batch activated-sludge systems. The error bars represent two standard deviations. Benz, benzoate; Palm, palmitate.

To validate the comparative biodegradation results obtained for LAS in BAS systems, the amount of LAS removal and biodegradation has also been quantified under more realistic continuous-flow treatment conditions in laboratory-scale CAS systems. These studies, which are summarized in Figure 7, confirm that the removal of LAS is high and stable in CAS systems during steady-state operation. Over a 5-d test period, for example, ^{14}C removal averaged about 90% with a relative standard deviation (error bars in Fig. 7) of less than 5%. Over 90% of this ^{14}C removal was due to mineralization of the LAS benzene ring to $^{14}CO_2$, and biodegradation accounted for more than 80% of the total LAS removal observed throughout the CAS system. The levels of radioactivity in sludge solids were low (<10%) and primarily reflected incorporation of LAS carbon into biomass components. The levels of radioactivity remaining in CAS effluents were also low (<10%) and not associated with parent material. Given the radiochemical detection limits of the specific CAS study shown, residual effluent levels were insufficient to allow further fractionation or characterization by radioanalytical techniques. However, based on subsequent work in our laboratories and information reported in the literature, this residual effluent ^{14}C activity represents either partially oxidized biodegradation intermediates (sulfophenylcarboxylates) and/or soluble microbial products (11).

In general, the CAS work indicates that mineralization plays an important role in the removal of LAS during wastewater treatment. Mineralization is typically considered a conservative estimate of the total biodegradation occurring in activated sludge because it does not account for material incorporated into sludge biomass or released as soluble microbial products. Extensive monitoring studies indicate that removal of parent LAS in activated sludge exceeds 98%, with the majority of this removal (77–99%) due to biodegradation (2). Biodegradation, therefore, clearly represents a major removal mechanism for LAS in sludge and plays a significant role in reducing the amount of LAS released to aquatic and terrestrial environments.

Anaerobic biodegradation. Few studies have directly examined the biodegradability of LAS in anaerobic environments. Indirect support for the lack of anaerobic LAS degradation comes mainly from monitoring studies that show high concentrations of LAS on anaerobic digester sludges (12,13). More definitive work by Federle and Schwab (9) has recently shown that LAS is not mineralized in anaerobic lake sediments that have active methanogenic and sulfate-reducing microbial communities and a long history of exposure to LAS and other detergent chemicals. Subsequent work in our laboratory has also confirmed a low potential for degradation of parent LAS in anaerobic digester sludge. This work, however, also indicates that LAS degradation can proceed under anaerobic conditions if preceded by a period of aerobic exposure.

Figures 8 and 9 show the results of studies in which a series of ^{14}C-LAS homologs (C10–C14) were incubated aerobically for 5–6 h in activated sludge and then transferred to digester sludge and incubated under strictly anaerobic (methanogenic) conditions. The 6-h aerobic preincubation period approximates one HRT in a typical wastewater treatment plant and reflects the minimum amount of time secondary sludge is exposed to aerobic

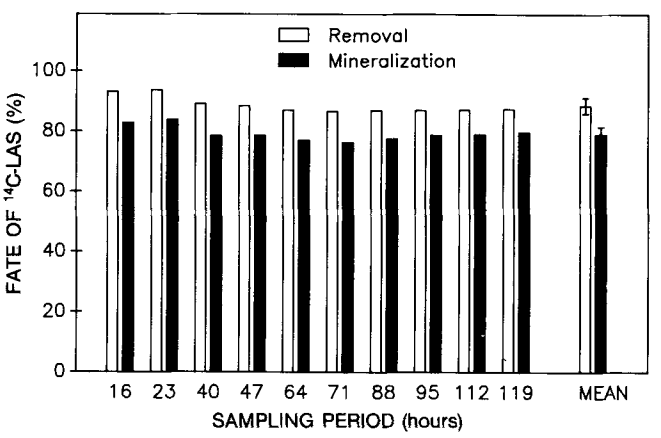

FIG. 7. Removal and mineralization of linear alkylbenzene sulfonate (LAS) in continuous activated-sludge systems. The error bars represent two standard deviations.

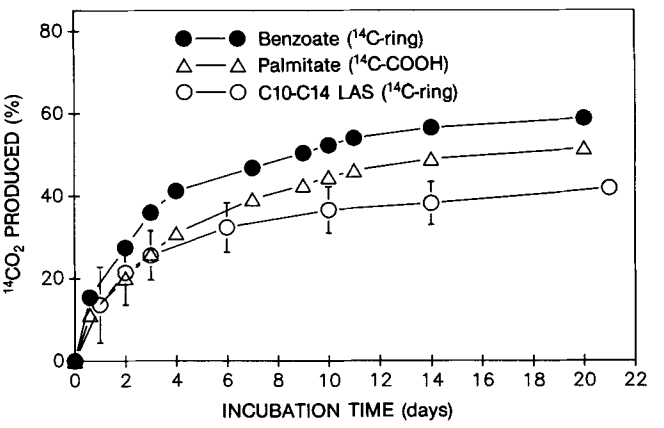

FIG. 8. Kinetics of mineralization of linear alkylbenzene sulfonate (LAS) homologs (C10–C14) in batch activated-sludge systems incubated under anaerobic conditions after a period of aerobic exposure simulating one hydraulic residence time (6 h). Palmitate and benzoate were tested as positive controls in the same study to characterize the kinetics and mechanism of LAS mineralization.

conditions prior to wasting to an anaerobic digester. Figure 8 plots mean values for cumulative mineralization at a specific time point averaged across all five homologs. The error bars represent two standard deviations. Also plotted are $^{14}CO_2$ evolution curves for two structurally related materials, benzoate and palmitate, which were tested concurrently with the LAS samples and exposed to the same conditions. Production of ^{14}C-methane was not specifically quantitated in this study for each of the five homologs tested. However, based on the results of other studies with individual homologs where radiolabeled methane was measured, ^{14}C-methane production would increase total ^{14}C-gas yields at least 50%.

After a period of aerobic exposure simulating one HRT, mineralization of individual LAS homologs in anaerobic sludge was comparable to that observed in aerobic sludge. The kinetics of anaerobic degradation of individual homologs was quite comparable over the C10–C14 range and also similar to the degradation kinetics observed for palmitate and benzoate incubated under the same conditions. Half-life values for anaerobic degradation of the various homologs ranged from 2.1 to 2.6 d and showed no significant difference (95% confidence level) as a function of alkyl chainlength (Fig. 9). The mean half-life value for LAS anaerobic degradation (2.5 d) was identical to the mean value obtained for anaerobic degradation of palmitate and benzoate tested in the same study and was also similar to the mean half-life obtained for LAS degradation under aerobic conditions (1.8 d). Mineralization efficiencies in anaerobic sludge (measured as $^{14}CO_2$) were less than values in aerobic sludge. This reduced efficiency is due to the production of ^{14}C-methane, which represents a significant fraction (50–75%) of the total gas produced in digester sludge. After normalizing for this reduction in total gas yields, however, the kinetics of aerobic degradation and anaerobic degradation with aerobic preexposure were quite similar.

In general, the results of the LAS anaerobic studies indicate that LAS biodegradation can occur under strictly anaerobic (methanogenic) conditions after an initial period of aerobic exposure. This aerobic exposure allows ω-oxidation of the terminal carbon of the alkyl sidechain, resulting in a carboxylated intermediate (sulfophenylcarboxylate), which is subject to further degradation by

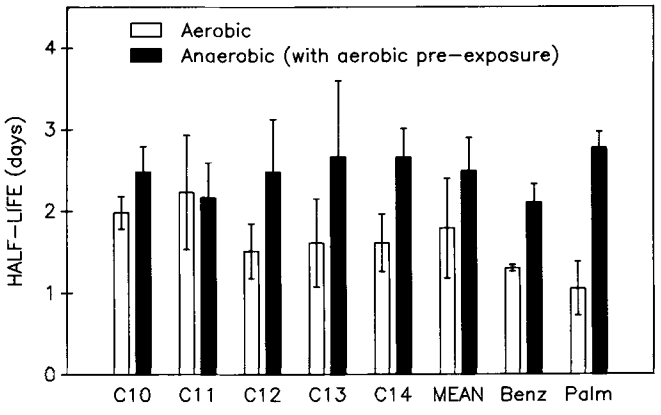

FIG. 9. Half-lives for aerobic and anaerobic biodegradation of linear alkylbenzene sulfonate homologs in batch activated-sludge systems. The error bars represent two standard deviations. Abbreviations as in Figure 6.

β-oxidation. The initial oxidative attack on the LAS alkyl sidechain is the only step that requires molecular oxygen. Once formed, the sulfophenylcarboxylates can be biodegraded *via* β-oxidation and ring hydroxylation/cleavage under strictly anaerobic conditions. The rates of mineralization of individual LAS homologs are comparable to those obtained for benzoate and palmitate, which again indicates that alkyl chain degradation is rapid after ω-oxidation has occurred. As in aerobic systems, therefore, mineralization of the benzene ring is the rate-limiting step for LAS degradation under anaerobic conditions. Parent LAS that has not undergone ω-oxidation of the alkyl chain terminus cannot undergo direct oxidation of the benzene ring and, therefore, is not subject to complete mineralization under anaerobic conditions.

The requirement for molecular oxygen in the initial oxidation of the alkyl chain is the key factor limiting the degradation of LAS under anaerobic conditions. This limitation is not unique to LAS but applies to all saturated hydrocarbons and unsubstituted aromatic compounds lacking oxidized substituents. The practical significance of this restriction in anaerobic degradation has not been well established for LAS or other detergent chemicals, but it is probably limited to anoxic sediments or anaerobic treatment systems highly impacted by other organic materials. Degradation of LAS in anaerobic digesters is poor due the limited potential for ω-oxidation. However, degradation of LAS in sediments and subsurface soil environments is more favorable than in digesters because variable exposure to oxygen and ω-oxidation can occur. In general, the environmental compartments exposed to LAS and other consumer product chemicals are usually aerobic. Although intermittent exposure to anaerobic conditions in wastewater treatment systems and selected receiving environments does occur, chronic exposure to anaerobic conditions is limited and not likely to significantly impact LAS environmental concentrations. This is clearly supported by monitoring studies, which indicate that environmental concentrations of LAS are comparable to those of other detergent chemicals, even those known to undergo rapid anaerobic degradation (14).

Biodegradation in freshwater aquatic environments. After secondary treatment, the majority of wastewater effluents in the United States are discharged into freshwater receiving environments, *i.e.*, rivers and streams. In-stream biodegradation can be a significant removal mechanism for rapidly degraded detergent chemicals, as illustrated for LAS in Figures 10–12. Figure 10 is a typical plot of LAS biodegradation in river water, showing the distribution of radiolabel during first-order degradation of LAS over a tenfold concentration range. Figure 11 depicts the kinetics of biodegradation of a range of LAS homologs in river water. Plotted are the mean values for cumulative mineralization of five different LAS homologs (C10–C14) at a specific time point, averaged across all five homologs tested concurrently in the same experiment. The error bars represent two standard deviations. Biodegradation data in both of these experiments were obtained in river water samples collected from Rapid Creek, SD, at a site approximately 7 km downstream from the discharge of the Rapid City Municipal plant. Rapid Creek has been the subject of several comprehensive modeling and monitoring studies to characterize the fate of LAS in aquatic environments (15).

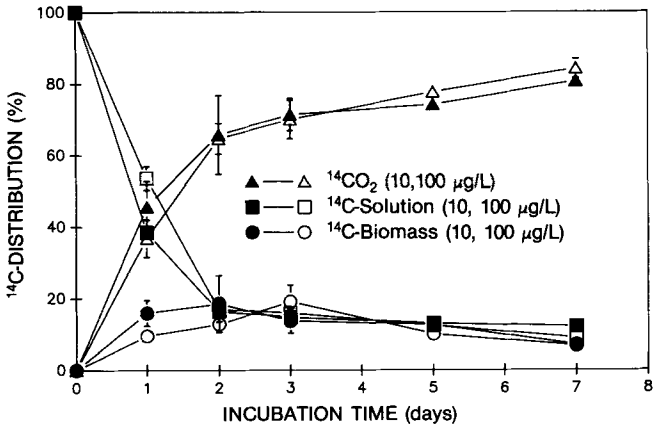

FIG. 10. Kinetics of mineralization and removal for 2-phenyl C12 linear alkylbenzene sulfonate in Rapid Creek river water at initial concentrations of 10 and 100 μg/L. The error bars represent two standard deviations.

Based on the $^{14}CO_2$ evolution curves produced during ring mineralization of individual LAS homologs at two initial concentrations, 10 and 100 μg/L, the biodegradation of LAS in river water followed a typical first-order pattern (Fig. 10). The rate and extent of cumulative $^{14}CO_2$ production were similar for all of the homologs tested and showed little variation as a function of alkyl chainlength over the range tested (Fig. 11). Radiolabel not converted to $^{14}CO_2$ was incorporated primarily into microbial biomass, with low residual levels remaining in solution at the end of testing. These low residual levels were not parent material, did not vary with the initial LAS starting concentrations and were radiochemically indistinguishable from the level of radiochemical impurities present in the original starting material. This indicates that degradation of individual LAS homologs was radiochemically complete in river water at the detection limits of the radioassay system used (about 10 ng/L).

Half-lives for mineralization of the C10–C14 homologs in river water ranged from 21 to 31 h and were not significantly different (95% confidence level) for the five homologs tested (Fig. 12). The mean half-life for degradation averaged about 24 h, which is comparable to values obtained in BAS systems and is consistent with a first-order relationship for LAS degradation. The low half-life values in river water indicate that microbial communities in Rapid Creek are well acclimated to LAS and that in-stream biodegradation can play a major role in controlling environmental exposure levels. In-stream biodegradation is particularly important in situations where secondary wastewater treatment is minimal and direct exposure of wastewater to receiving waters occurs.

The biodegradation results obtained for LAS in Rapid Creek have been confirmed in different river waters. Figure 13 shows the distribution of ^{14}C activity into various fractions during biodegradation of a mixture of radiolabeled ^{14}C-LAS homologs (average C12) in river water collected from the Miami River near Dayton, OH. The mass balance data shown are mean values, ±1 standard deviation, for triplicate units. As in the Rapid Creek studies, LAS degradation was rapid and complete in Miami river water. The majority of radiolabel was converted to $^{14}CO_2$ and biomass, and only low levels of radioactivity remained free in solution as transient biodegradation intermediates or parent material. At day 7, for example, only about 6% of the initial radioactivity remained as parent LAS. This level is consistent with a half-life of 1 to 2 d and predictable based on first-order degradation kinetics. The remaining ^{14}C activity was primarily associated with cell biomass, with a small fraction (2%) as water-soluble components. These water-soluble components represent transient biodegradation intermediates (sulfophenylcarboxylates) or non-LAS radiochemical impurities (11).

Biodegradation in benthic environments. Several monitoring studies have reported the presence of LAS in freshwater sediments (16,17). The average LAS concentrations are quite variable, ranging from less than 1 to more than 100 μg/g. These measured levels could reflect either accumulation of LAS in sediments due to reduced biodegradation rates or steady-state LAS concentrations dictated by sorption, usage and environmental removal

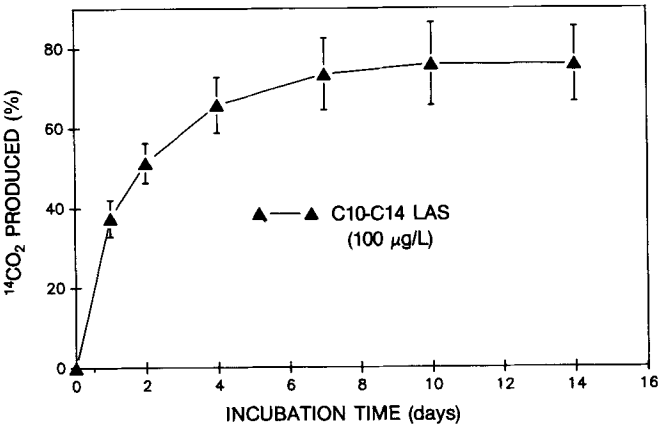

FIG. 11. Kinetics of mineralization of C10–C14 linear alkylbenzene sulfonate (LAS) homologs in Rapid Creek river water. Mineralization data for all five homologs are averaged at a single time point and the error bars represent two standard deviations of the mean values.

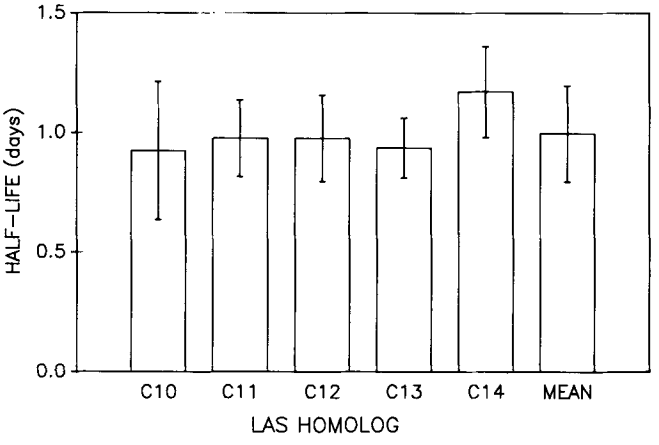

FIG. 12. Half-lives for mineralization of linear alkylbenzene sulfonate (LAS) homologs in Rapid Creek river water. The error bars represent two standard deviations.

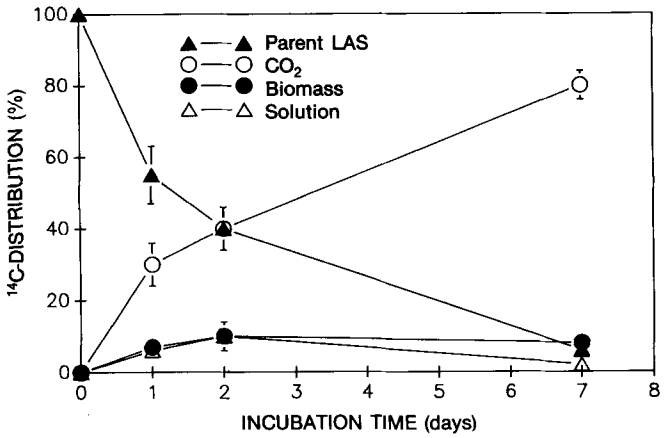

FIG. 13. Distribution of radiolabel during biodegradation of a mixture of ^{14}C-linear alkylbenzene sulfonate (LAS) homologs (average chainlength C12) in the Miami River water. The error bars represent one standard deviation from triplicate vessels.

rates. Biodegradation studies for a range of LAS homologs with widely different sorption properties are most consistent with the steady-state explanation, as described below.

Figure 14 indicates the results of a typical study to characterize the rate and extent of LAS degradation in aerobic freshwater sediments (Rapid Creek) at initial concentrations covering the high end of values measured in monitoring studies (100 μg/g). The sediments were exposed to gentle agitation and incubated with a series of LAS homologs (C10–C14) whose sorption coefficients varied almost two orders of magnitude over the four-carbon chainlength (5). Plotted are mean values for $^{14}CO_2$ production at a specific time point, averaged across all five homologs tested in a single experiment. Similar $^{14}CO_2$ evolution data for river water without sediments is also plotted for comparison. The error bars represent two standard deviations.

In general, degradation of LAS was comparable in river water and sediments and proceeded at similar rates for all of the homologs tested. Cumulative $^{14}C_2$ production in sediments exceeded 80% of the total ^{14}C added and was consistent across a range of homologs with significant differences (log-fold) in sorption properties. Half-life values for mineralization in sediments ranged from 19 to 23 h and showed no consistent trend as a function of alkyl chainlength (Fig. 15). The mean half-life for mineralization averaged about 20 h in sediment systems, which is quite comparable to the value obtained in river water alone (24 h). At the ratios of LAS to sediment tested, the amount of LAS sorbed to sediment varied from about 5% for the C10 homolog to >75% for the C14 homolog, with no apparent effect on the kinetics of degradation. Sorption to sediment, therefore, did not significantly affect the rate or extent of LAS mineralization, even at LAS and sediment concentrations where a substantial fraction of the LAS was bound. These results are consistent with previous work, which showed that LAS sorption is rapid and reversible (18). The reversible nature of LAS sorption, coupled with rapid sorption/desorption kinetics, allows degradation of either the bound or unbound form to occur. As long as the kinetics of sorption and desorption are more rapid than the kinetics of biodegradation, degradation of sorbed LAS and free LAS (*i.e.*, LAS in solution) can occur equally well.

Monitoring studies provide additional support for the importance of sediment biodegradation in reducing LAS exposure concentrations in benthic environments. DeHenau *et al.* (17) have shown that sediment LAS concentrations decrease significantly as the distance between the sampling location and sewage treatment plant outfall increases, ranging from a maximum of 275 μg/kg approximately 1 km below the outfall to less than 2 μg/g at a distance of 48 km downstream. As shown in Figure 16, this decrease in LAS sediment concentration is almost two orders of magnitude greater than the corresponding decrease in overlying water concentrations. Measured concentrations of LAS in sediment are also significantly less than LAS sediment concentrations calculated from equilibrium sorption coefficients by using measured water concentrations and suspended solids levels. The significant difference in measured *vs.* calculated sediment levels clearly indicates that LAS is readily degraded in sediment systems. Sediment biodegradation, therefore, is an

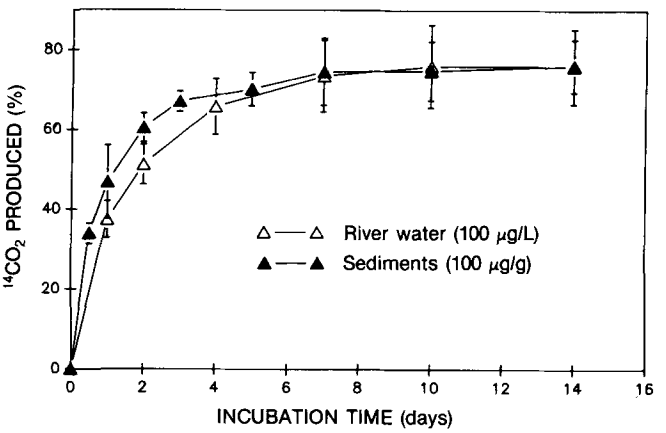

FIG. 14. Kinetics of mineralization of C10–C14 linear alkylbenzene sulfonate homologs in Rapid Creek river water and sediments. Mineralization data for all five homologs are averaged at a single time point, and the error bars represent two standard deviations of the mean values.

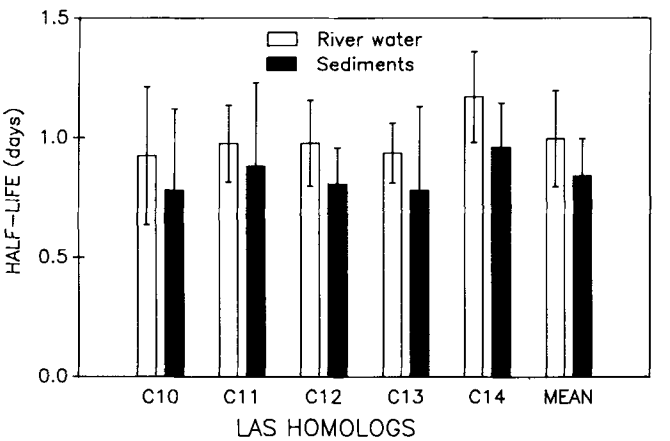

FIG. 15. Half-lives for mineralization of linear alkylbenzene sulfonate (LAS) homologs in Rapid Creek river water and sediments. The error bars represent two standard deviations.

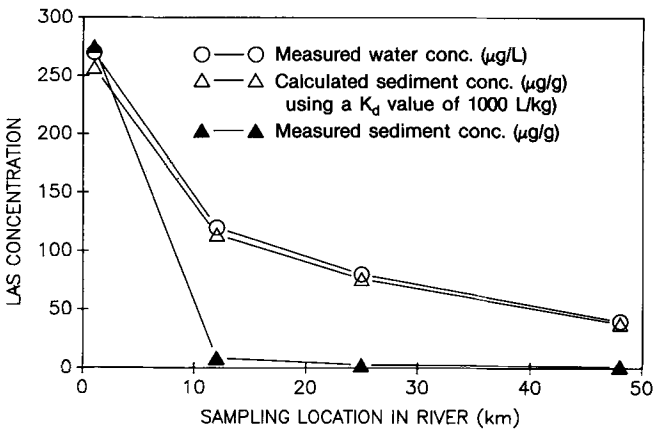

FIG. 16. Calculated and measured disappearance of linear alkylbenzene sulfonate (LAS) in river water and sediments. Calculated sediment concentrations use the measured LAS water column concentrations indicated and assume an adsorption coefficient of 1000 L/kg with a river water suspended solids concentration of 30 mg/L.

effective removal mechanism that limits the accumulation of LAS in benthic environments.

Biodegradation in estuarine environments. Approximately 50% of the total U.S. population lives within 50 miles of a coastline. Of this number, another 50% live in metropolitan areas directly located on and discharging treated wastewater effluents to coastal environments (19). Coastal estuarine environments receive approximately 780 million cubic meters (3 trillion gallons) of domestic wastewater a year, representing 20 to 30% of the total domestic wastewater flow of this country. Exposure of estuarine environments to wastewater discharges has significant environmental implications because these environments are among the most productive ecosystems on earth.

Although geographic factors have complicated the study of marine biodegradation processes in our laboratories, we have conducted several studies to characterize the biodegradation of LAS in estuarine environments. Figure 17 illustrates the results of one study to characterize the kinetics of LAS degradation in estuarine water and sediments from the Newport River Estuary near Morehead City, NC. Water and sediment samples were collected from two sites, a pristine (unacclimated) site with no known exposure to LAS-containing wastewater (Crab Point) and an acclimated site located below the discharge of the Morehead City Wastewater Treatment Plant (Calico Creek). A single homolog (C13) was tested in this particular study at concentrations of 20 μg/L and 20 μg/g in water and sediments, respectively. [U-^{14}C]-Glucose was the positive control.

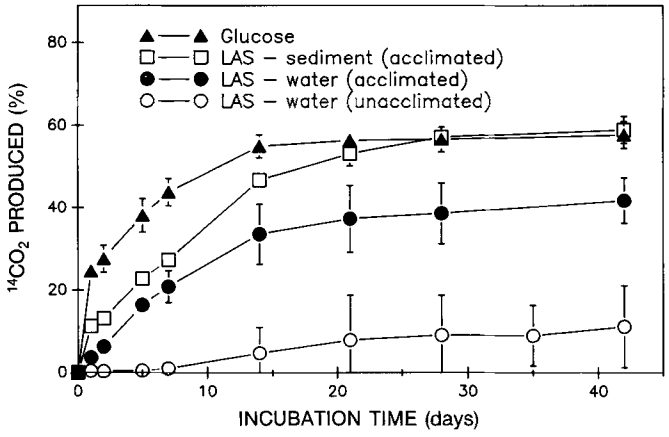

FIG. 17. Effect of acclimation on the kinetics of biodegradation of linear alkylbenzene sulfonate (LAS) in estuarine water and sediments. The error bars indicate one standard deviation from triplicate vessels.

In general, the kinetic patterns observed for LAS degradation in estuarine water and sediments were comparable to those observed in freshwater systems. The rate and extent of degradation were most extensive in sediment samples collected from the acclimated site, closely approximating that of the glucose control. Degradation was least extensive in water samples collected from the pristine site, indicating the importance of prior exposure and acclimation on the kinetics of LAS degradation in estuarine environments. Acclimation has also been shown to increase LAS degradation rates in freshwater environments previously unexposed to LAS (7). The presence of sediments had a positive effect on the extent of LAS mineralization, and the high levels of $^{14}CO_2$ produced clearly indicate that sorbed material was available for degradation. The half-life for degradation in both water and sediment was about 7 d, which is somewhat longer than the value measured in freshwater systems. Lower biodegradation rates were also observed for the glucose control, which had a half-life of approximately 2 d. Reduced degradation activity has been noted for a number of natural and synthetic chemicals in estuarine environments and is indicative of general reductions in the overall metabolic activity of estuarine microbial communities (20). This reduced activity, however, does not limit the effectiveness of biodegradation in removing chemicals from estuarine systems (19).

Biodegradation in terrestrial environments. The large-scale application of wastewater effluents and sludges to terrestrial environments has lead to increased concerns about the potential accumulation of ecotoxic organic chemicals in soil environments. Given the high volumes of LAS used in detergent products and its extensive removal during wastewater treatment, the steady-state concentrations of LAS in sewage sludges can reach significant levels, ranging from 0.3 to 1.2% on a dry-weight basis (12). Because a large percentage (>50%) of sewage sludge is applied to land in the United States and Europe, soil biodegradation is an important process for minimizing the exposure concentrations of LAS and other sludge-associated chemicals in terrestrial environments.

The results of two studies to characterize the kinetics of biodegradation of LAS homologs in sludge-amended soil are shown in Figure 18. Soil samples were obtained from agricultural fields in Rapid City, SD, and Harleysville, PA, which differed primarily in the interval and rate of application of sewage sludges. The Rapid City soil had much higher concentrations of nutrients and heavy metals than the Harleysville soil and had received sludge approximately twice as long (~10 yr). Plotted are mean values for cumulative mineralization for all five homologs

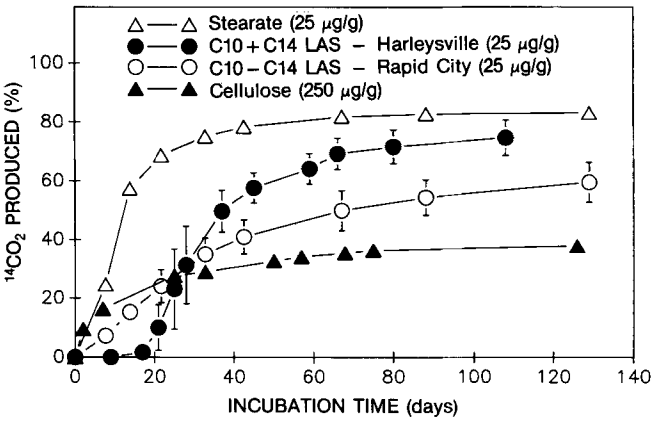

FIG. 18. Kinetics of mineralization of C10–C14 linear alkylbenzene sulfonate (LAS) homologs in sludge-amended surface soils. Mineralization data for all five homologs are averaged at a single time point, and the error bars indicate one standard deviation.

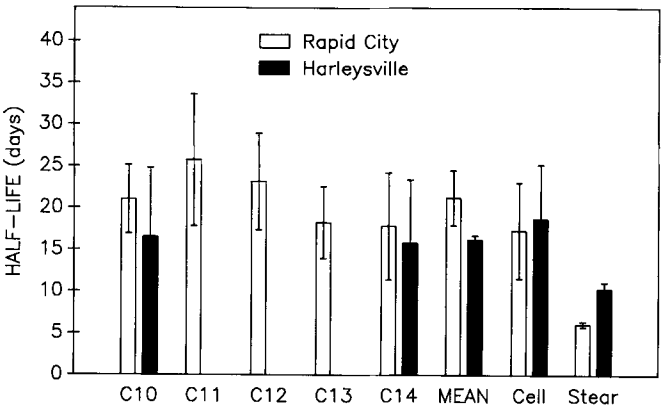

FIG. 19. Half-lives for mineralization of linear alkylbenzene sulfonate homologs in sludge-amended surface soils. The error bars represent one standard deviation. Cell, cellulose; Stear, stearate.

(C10–C14) in the Rapid City soil and the C10 and C14 LAS homologs in Harleysville soil. The error bars represent one standard deviation. Uniformly labeled ^{14}C-cellulose and ^{14}C-stearate were tested as positive controls to gauge the efficiency of $^{14}CO_2$ production yields on naturally occurring soil constituents.

In general, the rate and extent of degradation of LAS homologs were comparable in the two soils. A short lag phase preceded degradation in the Harleysville soil, which was not noticeable in Rapid City soil. This lag is consistent with the reduced frequency of sludge loading at Harleysville and may indicate a less stable LAS-degrading microbial community. Mineralization efficiencies for the various LAS homologs ranged from 60–70%, compared to about 40% for cellulose and about 80% for stearate. This wide variation in CO_2 yields is typical of soil systems in general and reflects the utilization of substrate carbon in different metabolic pathways. The lower yields reflect incorporation of substrate carbon into both cell biomass (assimilation) and soil humic materials (humification). The higher yields indicate utilization of substrate carbon primarily as an energy source in dissimilatory (energy-yielding) metabolic pathways. The $^{14}CO_2$ yields observed for LAS were relatively high and comparable to those for stearate. The LAS ring carbon, therefore, is primarily being utilized as an energy source by soil microorganisms and is being metabolized by dissimilatory pathways.

Half-lives for mineralization of the C10–C14 homologs ranged from about 16 d in the Harleysville soil to 21 d in Rapid City soil. These half-lives were not significantly different (95% confidence level) over the range of homologs tested (Fig. 19). The mean half-life across both soils averaged about 20 d, which is in the range of values (1 to 27 d) reported in the literature (5,21,22). Interestingly, the 20-d half-life is much longer than values measured in pristine soil systems with no previous exposure to surfactants (1–4 d) (23). These results indicate that prior exposure to LAS in sewage sludge is not required for rapid LAS degradation to occur. They are also consistent with reports that LAS degradation capability is present in both sludge-amended and unamended surface soils and is mediated by native soil microbial communities (21–23). In general, the results of our soil studies indicate that LAS biodegradation capability is ubiquitous in soil environments and represents an important mechanism for preventing the accumulation of LAS in terrestrial environments.

Biodegradation in subsurface environments. As indicated earlier (Fig. 1), approximately 25% of the LAS used in laundry and cleaning products in the United States is disposed of to home or on-site disposal systems (OSDS). These systems discharge their effluents to drainage fields and, therefore, have the potential to directly impact groundwater and subsurface soils. Since the early 1980s, we have had an active program to characterize the biodegradation and fate of LAS and other detergent chemicals in groundwater/subsurface soil systems. Figures 20 and 21 show the results of a typical study to measure the acclimation and biodegradation response of subsurface microbial communities to individual LAS homologs.

Subsurface soil samples with no prior history of exposure to detergent chemicals were collected aseptically from the saturated zone (17 m) of an unconfined aquifer

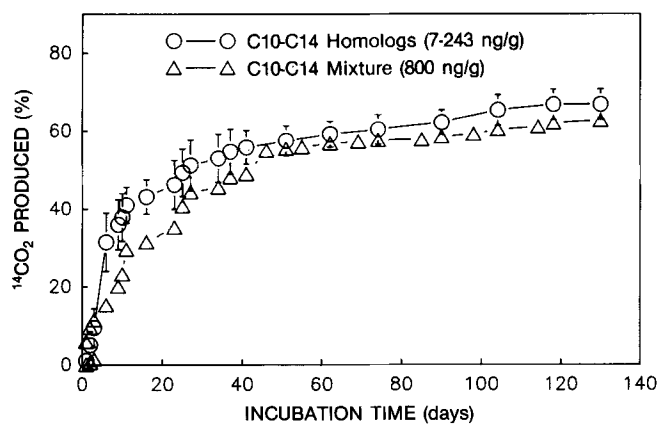

FIG. 20. Kinetics of mineralization of individual C10–C14 linear alkylbenzene sulfonate homologs and a mixture of homologs (average chainlength C12) in subsurface soil collected near Summit Lake. Mineralization data for all five individual homologs are averaged at a single time point, and the error bars indicate two standard deviations.

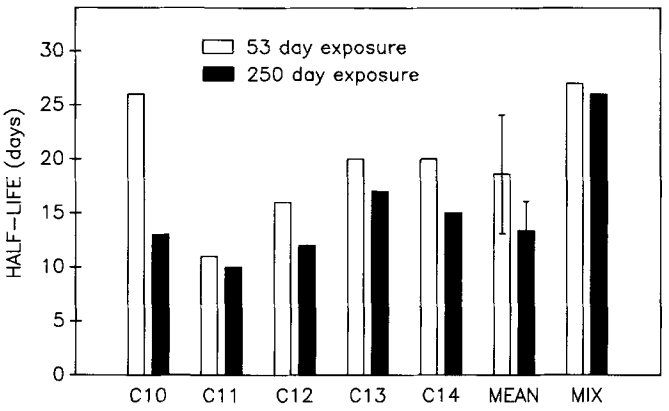

FIG. 21. Half-lives for mineralization of linear alkylbenzene sulfonate (LAS) homologs in Summit Lake subsurface soil samples exposed to LAS for 53 to 250 d. The error bars represent one standard deviation.

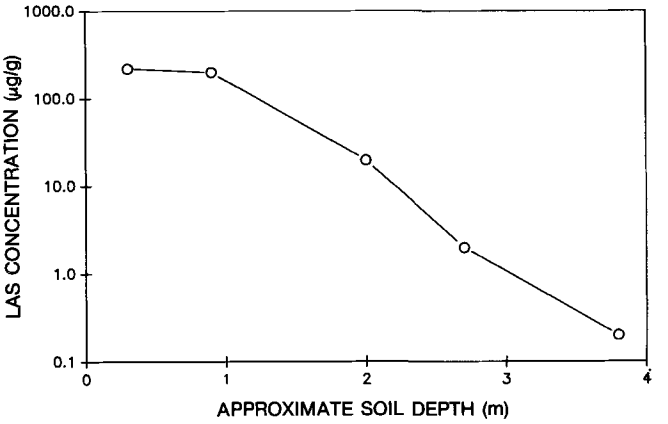

FIG. 22. Decrease in linear alkylbenzene sulfonate (LAS) concentrations as a function of depth in subsurface soil samples collected near Summit Lake.

near Summit Lake, WI. The Summit Lake site has been the subject of intensive study by Federle and co-workers (24–26) to characterize the fate of LAS and other detergent chemicals in subsurface environments. The samples were exposed to a trace quantity (200 ng/g) of a commercial LAS mixture (average chainlength C12) and were incubated for up to 250 d with no additional amendments. At selected intervals, subsamples were collected and spiked with various concentrations (7 to 243 ng/g) of pure-chainlength homologs (C10–C14), and the rate and extent of mineralization of individual homologs were measured. In addition to the pure-chainlength materials, a mixture of individual homologs (800 ng/g) was prepared to represent a commercial formulation (average chainlength C12) and tested concurrently with the pure homologs.

Figure 20 shows cumulative mineralization curves for the biodegradation of pure-chainlength LAS homologs and a C12 LAS mixture in subsurface soil exposed to LAS for 53 d. Mineralization data for the LAS homologs are plotted as mean values for $^{14}CO_2$ production at a specific time point, averaged across all five homologs. The error bars represent one standard deviation. Figure 21 shows half-life values for individual LAS homologs at different exposure periods (53 and 250 d), with the error bars for the mean homolog values representing two standard deviations.

In general, the kinetics of biodegradation of individual LAS homologs were comparable to each other and to the LAS mixture. No significant differences (95% confidence level) in the rate and extent of mineralization were apparent for the pure homologs over the range of alkyl chainlengths tested (Fig. 20). The kinetics of degradation of individual LAS homologs in subsurface soil was also quite comparable to the kinetics obtained in surface soil systems. Half-lives for mineralization ranged from 10 to 27 d and showed no consistent trend as a function of alkyl chainlength. The mean half-life for degradation of LAS homologs in subsurface soils (~16 d) was similar to the mean value in surface soils (~20 d) and remained constant for up to 250 d after the initial exposure (Fig. 21). In general, the results of studies in subsurface microcosms indicate that prior exposure of subsurface microbial communities to trace quantities of LAS leads to development of a significant biodegradation response. This response is maintained for extended periods of time without subsequent re-exposure and is comparable to the response observed in surface soil environments.

Monitoring studies at the Summit Lake site have confirmed the importance of biodegradation processes in reducing LAS exposure concentration in subsurface environments. These studies indicate that LAS concentrations decrease significantly with depth, from more than 200 µg/g to less than 2 µg/g, over a distance of less than 3 m (Fig. 22). This significant decrease is not unique to the Summit Lake site, where high LAS concentrations are present as a result of exposure to wastewater effluent from a commercial laundromat operation. Similar decreases are also observed in soil drainage fields exposed to high concentrations of LAS in wastewater effluents from domestic on-site disposal system (27). Levels of LAS decrease several orders of magnitude (from ~14 mg/L to <10 µg/L) within a few meters of the title field. In general, the results of subsurface monitoring studies clearly indicate that biodegradation is an effective removal mechanism which limits the accumulation of LAS in groundwater and subsurface soil environments.

LAS biodegradation—a practical environmental removal mechanism. The preceding sections of this paper have reviewed the kinetics of biodegradation of LAS during wastewater treatment and in aquatic, benthic, subsurface and terrestrial environments. Biodegradation measurements were based on mineralization of the benzene ring, which is the slowest (rate-limiting) step for LAS mineralization. From these studies, it is apparent that mineralization of LAS is rapid in a variety of environmental compartments and relatively unaffected by structural (alkyl chainlength) or environmental factors. However, the most important factor determining whether biodegradation will be a practical environmental removal mechanism is not the rate or extent of biodegradation over an arbitrary time period in a laboratory test. Rather, it is the rate and extent of mineralization occurring in a particular environmental compartment relative to the time the compounds are present in these environments (residence time). Reduction in total mass is the most important factor controlling environmental exposure concentrations. This

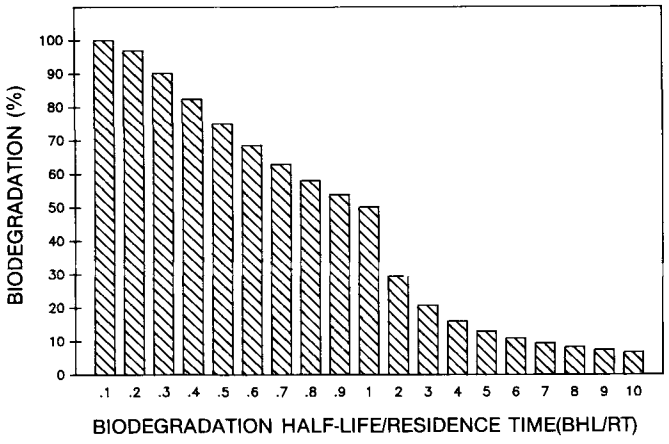

FIG. 23. Generic diagram of the amount of biodegradation occurring in a given environmental compartment as a function of the biodegradation half-life to residence time (BHL/RT) ratio.

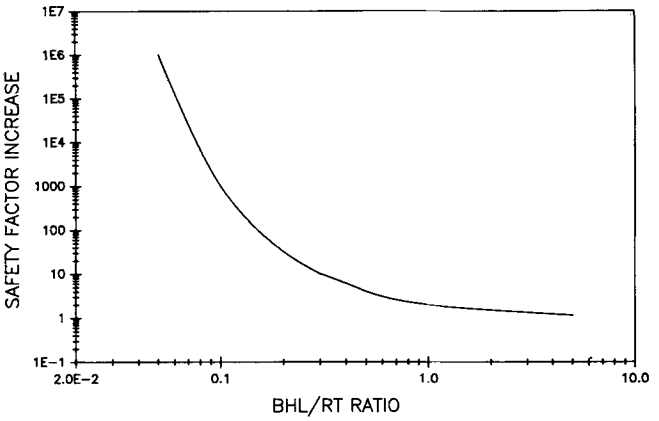

FIG. 24. Generic diagram showing the rate of increase in linear alkylbenzene sulfonate safety factors as a function of the biodegradation half-life to residence time (BHL/RT) ratio.

reduction in total mass must occur over a time frame that is relevant for a particular environmental compartment for biodegradation to represent a meaningful removal mechanism.

Figure 23 shows the amount of biodegradation that can occur in a given environmental compartment as a function of the biodegradation half-life to residence time (BHL/RT) ratio. RT is defined quite simply as the time available for biodegradation to occur relative to chemical usage, application or loading rates and transport velocities within an environmental compartment. Clearly, significant biodegradation (50%) occurs when the BHL for a chemical equals its RT in a particular environment (BHL/RT = 1). The amount of biodegradation increases still further as the BHL becomes a smaller fraction of the chemical RT (BHL/RT < 1). At BHL/RT ratios of 0.1 or less (ten BHLs within a given RT), biodegradation is essentially complete, *i.e.,* >99.9%. By contrast, biodegradation is much less effective as a removal mechanism when the BHL exceeds the RT of a chemical in a given environmental compartment, *i.e.,* BHL/RT > 1. At BHL/RT ratios of about five or greater, less than 15% removal occurs due to biodegradation. Materials that exhibit this range of BHL/RT ratios are nondegradable in a practical sense and will accumulate and persist in environmental compartments in direct proportion to their usage rates.

Based on the half-life values determined for LAS in engineered systems and natural environmental compartments, biodegradation is a significant removal mechanism for LAS in all environmental compartments tested. Half-lives in activated sludge treatment systems, where the sludge RT is one to two weeks, are one to two days. Half-lives in aquatic and benthic compartments, where the RT can vary from days to weeks, are one day or less. Half-lives in terrestrial and subsurface compartments, where RTs can vary from months to years, range from less than one day to a few weeks. Conservative estimates of the BHL/RT ratio for LAS range from 0.5 to less than 0.1. These ratios are sufficient to reduce LAS exposure concentrations by several orders of magnitude, *i.e.,* levels that are well below those having adverse environmental impacts.

Figure 24 illustrates the above points more directly and shows the importance of biodegradation in reducing LAS exposure concentrations and in increasing environmental safety factors. Environmental safety factors are dimensionless numbers that relate the ecotoxicity of a chemical to its known ore predicted exposure level. For consumer product chemicals such as LAS, these safety factors are typically calculated from annual usage volumes, removal during wastewater treatment and dilution/distribution into receiving environments. Figure 24 is a generic plot, which relates the relative increase in environmental safety factor for LAS to its biodegradation half-life to RT ratio. The plot is dimensionless and shows how biodegradation can increase preexisting safety factors in any environmental compartment by significantly decreasing exposure concentrations. Clearly, relative safety factors for LAS multiply sharply as the BHL/RT ratio becomes less than 1. At a BHL/RT ratio of 0.3, for example, safety factors are multiplied by a factor of ten. At a ratio of 0.1, they are multiplied a thousandfold, and at 0.05, they are more than six orders of magnitude higher than at BHL/RT ratios where biodegradation is not a practical removal mechanism (*i.e.,* BHL/RT $\geq$ 5). These increased safety factors clearly indicate the importance of biodegradation as a removal mechanism for LAS and underscore its role in maintaining LAS exposure concentrations well below those predicted to have adverse environmental impacts.

This paper has reviewed a variety of studies to characterize the kinetics of LAS mineralization in aquatic, benthic and terrestrial environments. Degradation measurements were based on mineralization of the benzene ring, the slowest step in the LAS biodegradation pathway. Based on the results of these studies, several key points emerge: (i) Biodegradation rates for a range of LAS homologs (C10–C14) are comparable to each other and to values observed for naturally occurring materials such as sugars and fatty acids. (ii) Half-lives for LAS mineralization range from a day or less in sewage sludges, river water and sediments, to 1–3 wks in surface and subsurface soils and estuarine environments. (iii) The half-life for LAS degradation in different environmental compartments, relative to its RT in these compartments, resulted in BHL/RT ratios

of 0.5 to less than 0.1. (iv) Biodegradation represents a significant removal mechanism for LAS in a variety of environmental compartments, decreasing exposure levels and increasing environmental safety factors by several orders of magnitude.

REFERENCES

1. Chemical Information Services, in *Chemical Economics Handbook,* Menlo Park, Report No. 583.8001H, 1988.
2. Rapaport, R.A., and W.S. Eckhoff, *Environ. Toxicol. Chem. 9:*1245 (1990).
3. Organization for Economic Cooperation and Development, *Guidelines for Testing of Chemicals: Section 3—Degradation and Accumulation,* OECD, Paris, 1981.
4. *Tenside Deterg. 26:*63 (1989).
5. Hand, V.C., and G.K. Williams, *Environ. Sci. Technol. 21:*370 (1987).
6. Organization for Economic Cooperation and Development, *Guidelines for Testing of Chemicals: Test Guideline 303A,* OECD, Paris, 1981.
7. Larson, R.J., and A.G. Payne, *Appl. Environ. Microbiol. 41:*621 (1981).
8. Ward, T.E., and R.J. Larson, *Ecotoxicol. Environ. Saf. 17:*119 (1989).
9. Federle, T.W., and B.S. Schwab, *Water Res. 26:*123 (1992).
10. *EPA,* U.S. Environmental Protection Agency, 430/9-84-011, Washington, D.C., 1985.
11. Steber, J., *Tenside Deterg. 16:*140 (1979).
12. McEvoy, J., and W. Giger, *Environ. Sci. Technol. 20:*376 (1986).
13. Giger, W., P.H. Brunner, M. Ahel, J. McEvoy, A. Marcomini and C. Schaffner, *Gas-Wasser-Abwasser 67:*111 (1987).
14. Rapaport, R.A., and W.S. Eckhoff, *Environ. Toxicol. Chem. 9:*1245 (1990).
15. Games, L.M., in *Modeling the Fate of Chemicals in the Aquatic Environment,* Ann Arbor Science, Ann Arbor, 1982.
16. Takada, H., and R. Ishiwatari, *Environ. Sci. Tech. 21:*875 (1987).
17. DeHenau, H., E. Matthijs and W.D. Hopping, *Int. J. Environ. Anal. Chem. 26:*279 (1986).
18. Larson, R.J., *Environ. Toxicol. Chem. 24:*1241 (1990).
19. Lung, W.-S., R.A. Rapaport and A.C. Franco, *Ibid. 9:*1127 (1990).
20. Pfaender, F.K., R.J. Shimp and R.J. Larson, *Ibid. 4:*587 (1985).
21. Holt, M.S., E. Matthijis and J. Waters, *Water Res. 23:*749 (1989).
22. Litz, N., H.W. Doering, M. Thiele and H.-P. Blume, *Ecotoxicol. Environ. Saf. 14:*103 (1987).
23. Knaebel, D.B., T.W. Federle and J. Robie Vestal, *Environ. Tox. Chem. 9:*981 (1990).
24. Federle, T.W., and G.M. Pastwa, *Ground Water 26:*761 (1988).
25. Federle, T.W., and B.S. Schwab, *Appl. Environ. Microbiol. 55:*2092 (1989).
26. Federle, T.W., and R.M. Ventullo, *Ibid. 56:*333 (1990).
27. Larson, R.J., T.W. Federle, R.J. Shimp and R.M. Ventullo, *Tenside Deterg. 26:*116 (1989).

[Received March 26, 1992; accepted March 26, 1993]