Biodegradation of Sodium Linear Alkylbenzenesulfonates Evaluated with a Soil Perfusion Method

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In order to explain biodegradation behavior of sodium alkylbenzenesulfonate (LAS) in the soil environment, a soil perfusion method has been applied. This method is one of continuous enrichment cultures with soil. The degree of degradation of LAS was evaluated by measuring the amount of ferroin reagent active substances (FRAS) instead of methylene blue active substances (MBAS) and/or of total organic carbon (TOC) in the perfusion fluid. The biodegradation of LAS started after adsorption onto soils. At high LAS concentrations, the biodegradation occurred after some lag phase, but further added LAS was degraded readily without a lag phase. These results of biodegradation are in good agreement with those obtained from the biodegradation tests on river water and activated sludge, and reproducible results were obtained for the soil perfusion with the same soil. This proves the soil perfusion method to be a useful method for evaluation of biodegradation in the soil environment.

Many studies have been reported on biodegradation of alkylbenzenesulfonate (ABS) in river water, activated sludge and others (1-5), but only a few have been presented on its biodegradation in the soil environment (6-9). The disappearance of surfactants in soils could be attributed to adsorption onto soil particles and decomposition by soil microorganisms.

Robeck et al. (6) suggested from experimental studies on the fate of ABS by the use of a soil lysimeter that adsorption of ABS is followed by biodegradation. Klein et al. (7) also studied the disappearance behavior of ABS in the soil by using soil columns, and observed that degradation of ABS occurs only in biologically active soils under water-unsaturated conditions. Their method might be considered one of the continuous enrichment cultures with soil. Another method of the enrichment cultures with soil has been established by Quaster and Lees (10) as a soil perfusion technique. This method allows us to examine biological actions of soil microorganisms under in situ conditions; it has been used for biodegradation tests of agricultural chemicals and other organics.

In a previous paper (11) the author and her collaborators applied this method to explain the disappearance behavior of some anionic surfactants in the soil under water-unsaturated conditions, and proved that this method is useful for estimation of the biodegradability of surfactants. In this paper, we examine further details of the degradation behavior of sodium linear alkylbenzenesulfonate (LAS) in the soil columns to make sure this method is one of the most suitable for biodegradation tests in the soil.

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EXPERIMENTAL

Surfactants. The surfactants used were mostly linear alkylbenzenesulfonate (LAS) and linear dodecylbenzenesulfonate (C_{12}LAS). The compositions of LAS and C_{12}LAS, determined by high performance liquid chromatography, are given in Table 1. Branched alkylbenzenesulfonate (ABS) and sodium dodecyl sulfonate (SDS) were also used for references.

Soils. Soils were collected from cultivated fields (surface soil) at Koganei, Tokyo, and at Saku, Nagano. The collected samples were immediately dried in air, sieved (between 1 and 2 mm) and stored at 5 C. The physical properties of these soils are shown in Table 2.

Biodegradation test by soil perfusion. A soil column was prepared by filling a glass chromatographic tube (30 mm i.d. and 300 mm long) with 50 g of the air dried soil. Then, the perfusion was conducted by circulating 1,000 ml of a perfusion fluid drop by drop through the column at the rate of 80 ml/hr. The perfusion fluid was prepared as follows: sterilized water was perfused for 24 hr at the above mentioned rate and then a surfactant was added to have a given concentration before perfusion started. At definite intervals, a given amount of the perfusion fluid was sampled to determine the surfactant concentration as the ferroin reagent active substances (FRAS) or the total dissolved organic carbon (TOC).

Detection of LAS in soil. Five g perfused soil was placed in a centrifugal tube, and 40 ml of methanol

TABLE 1

Compositions of LAS and C_{12}LAS Used by HPLC

Surfactant	Homologue or isomer	mol %
C_{12}LAS	2-phenyl C_{12}	30.0
	3-phenyl C_{12}	19.3
	4-phenyl C_{12}	16.7
	5+6-phenyl C_{12}	30.0
LAS	2-phenyl C_{10}	4.8
	3-phenyl C_{10}	1.9
	4+5-phenyl C_{10}	3.3
	2-phenyl C_{11}	12.0
	3-phenyl C_{11}	7.4
	4+5-phenyl C_{11}	19.8
	2-phenyl C_{12}	8.5
	3-phenyl C_{12}	5.5
	4-phenyl C_{12}	4.9
	5+6-phenyl C_{12}	12.6
	2-phenyl C_{13}	4.4
	3-phenyl C_{13}	3.2
	4-phenyl C_{13}	2.9
	5+6-phenyl C_{13}	8.9

TABLE 2

Properties of Soils Used

Symbol	Source	Soil texture	Clay mineral	% Clay content	Total-c (%)	Humus (%)	CEC[a] (m eq/100 g)	pH (H_2O)
A	Koganei, Tokyo	Clay loam	Allophane	22.5	6.42	11.0	55.4	6.7
B	Saku, Nagano	Sandy loam	Allophane	14.4	3.00	5.2	30.3	5.1

[a]Cation-exchange capacity (CEC) was measured by Schollenberger's method (17).

containing 1% formaldehyde was added. After LAS was eluted by shaking the tube for 20 min at 50 C, the eluate was separated by centrifugation. Another 35 ml methanol was added to repeat elution in the same manner, and the eluate was mixed with the first one. Then, methanol was distilled off at reduced pressure, and the residue was heated with hot water for dissolution. The solution was diluted to 25 ml and filtered through a 0.45 μm membrane filter to analyze LAS with HPLC.

Determination of surfactants. Following the biodegradation of anionic sufactants, the methylene blue method is most widely used. But, in this study, the ferroin reagent method developed by Taylor and Fryer (12) was applied because it is used with a more rapid and simpler extraction technique than the methylene blue method. According to this method, the complex of the ferroin reagent with anionic surfactant was extracted with chloroform to measure its absorbance at 512 nm. For the quantitative analyses of LAS and ABS, C_{12}LAS was used as a standard.

Determination of TOC. The total organic carbon was determined by the use of a Total Organic Carbon Analyzer (Shimazu TOC-10B). Since organic carbon was detected in the soil perfusion only with sterilized water, the TOC value was corrected by subtracting a value of TOC, which was determined from the perfusion with water only during 24 hr, from the measured value.

Analysis by HPLC. A Shimazu LC3A Liquid Chromatograph with a UV detector (Shimazu SPD-2A) was used. The determination was carried out under the following conditions: Column, Hitachi Gel #3056, 4.0 × 100 mm; flow rate, 1.0 ml/min; pressure, 70 kg/cm^2; eluent, 0.1M sodium perchlorate in acetonitrile/water (45/55); column temperature, 40 C; detector, UV 225 nm.

RESULTS AND DISCUSSION

Biodegradation of LAS by the soil perfusion method. Figures 1-3 summarize the results of biodegradation tests of LAS, C_{12}LAS, ABS and SDS, respectively, on soil A (Koganei soil) through the detection of FRAS. The initial concentrations of the perfusion fluids were about 50 and 100 mg/l for LAS and C_{12}LAS, and 50 mg/l for ABS and SDS. After FRAS was no longer detected, a surfactant was added to make nearly the same concentration as the first for the second perfusion.

In all cases, the ferroin reagent active substances (FRAS) decreased sharply immediately after the

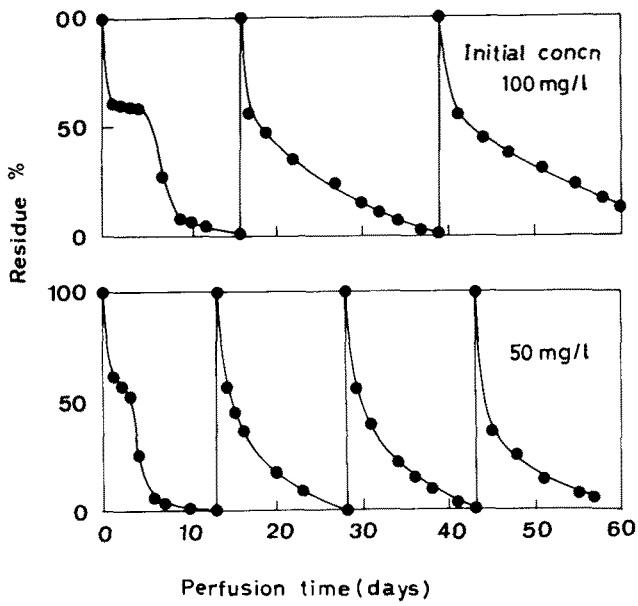

FIG. 1. Disappearance of LAS measured by FRAS, at 25 C.

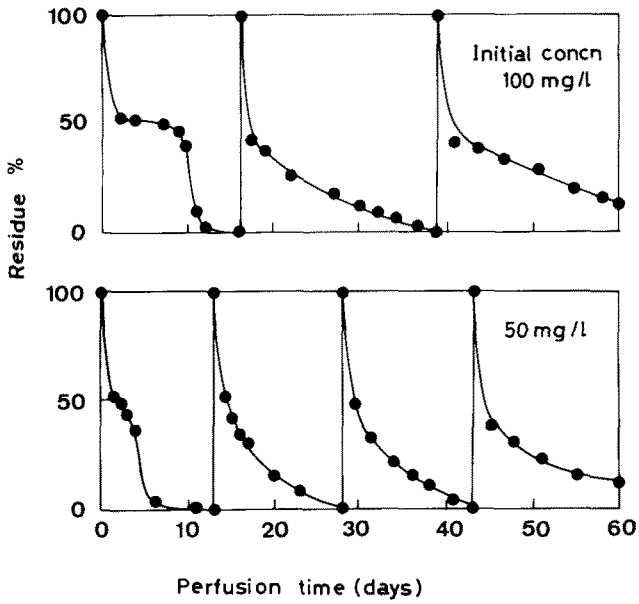

FIG. 2. Disappearance of C_{12}LAS measured by FRAS, at 25 C.

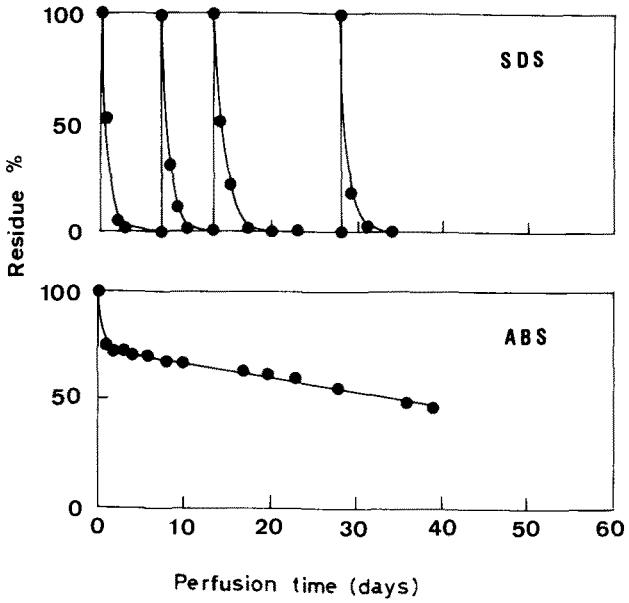

FIG. 3. Disappearance of SDS and ABS measured by FRAS, at 25 C.

initiation of perfusion. As the initial decrease was observed for the perfusion of soil sterilized with an autoclave treatment (13), it is considered to be due to adsorption of surfactant onto soil. For SDS the adsorption onto soil was followed immediately by biodegradation, resulting in rapid disappearance of FRAS. LAS and C_{12}LAS decomposed biologically showing a relatively short lag phase after adsorption, and the lag phase became more distinct and longer with higher concentrations. Swisher (1) suggested that the inhibition of activities of microorganism enzymes by surfactants results in a longer lag time and a lower rate of disappearance of surfactants with higher concentrations. Sekiguchi et al. (3) reported a similar result in a river die-away test. LAS and C_{12}LAS were added again for the perfusion after the disappearance of FRAS; an immediate decrease in FRAS was observed by the decomposition following adsorption, as in the case of SDS. This behavior is considered to be due to the acclimation of soil microorganisms by the surfactant. The rate of disappearance of LAS was slow compared to that of SDS, and the continuing perfusion with added LAS resulted in gradual retention of FRAS, about 7% retention of FRAS for the third addition, while in the case of SDS, the repeated perfusions did not give any sign of FRAS retention. This shows that the decomposition of LAS takes a longer time, and that the partially decomposed products can be detected as FRAS.

Because of its lower biodegradability, unlike LAS, ABS showed only a gradual decrease in FRAS after adsorption onto the soil, and the acclimation of the perfused soil was not detected with ABS.

These results coincide essentially with the data of biodegradation tests obtained for river water and activated sludge (3,4,13) and indicate that soil perfusion could provide an effective method to evaluate biodegradabilities of surfactants.

Effect of soils on disappearance behavior of LAS. In order to investigate whether biodegradation and adsorption behaviors are affected by kinds of soil or not, the soil perfusion tests were done with two kinds of soils, A soil (clay loam) and B soil (sandy loam). As discussed in the previous section, an increasing tendency in concentration of partial decomposition products was observed, when the perfusion is continued with repeated additions of LAS. This suggests it takes a long time for the ultimate biodegradation. Then, the disappearance behavior of TOC also was examined to determine the extent of degradation during the perfusion.

Figure 4 depicts the disappearance processes of FRAS and TOC, when A and B soil were perfused with 50 mg/l LAS solutions, respectively. Some differences in adsorption are recognized between these two soils. Adsorption of LAS onto soil greatly depends on the physical and chemical properties of the soil. Klein et al. (7) and Kawashima et al. (14) reported higher adsorption onto soils with higher contents of humus and clay minerals, while Fink et al. (15) showed a lower extent of LAS adsorption onto sandy soil. Because of the lower humus and clay contents of B soil, it shows a lower degree of adsorption of LAS. The lag phases for both TOC and FRAS of B soil were slightly longer than those of A soil, and this difference is also assumed to be attributable to the difference in adsorption. After 14 days of perfusion for A soil and 20 days for B soil, at which time LAS remaining in the fluid after initial decrease was about 50% degraded, TOC decrease became rather moderate. Such a behavior also was observed in the biodegradation tests for river water and activated sludge. In this regard, Sekiguchi et al. (3) assumed that a certain period of acclimation is necessary for the steady decrease in TOC value. For A soil 100% disappearance of TOC is observed by 50 days' perfusion, while for B soil about 10% of TOC remained after 60 days.

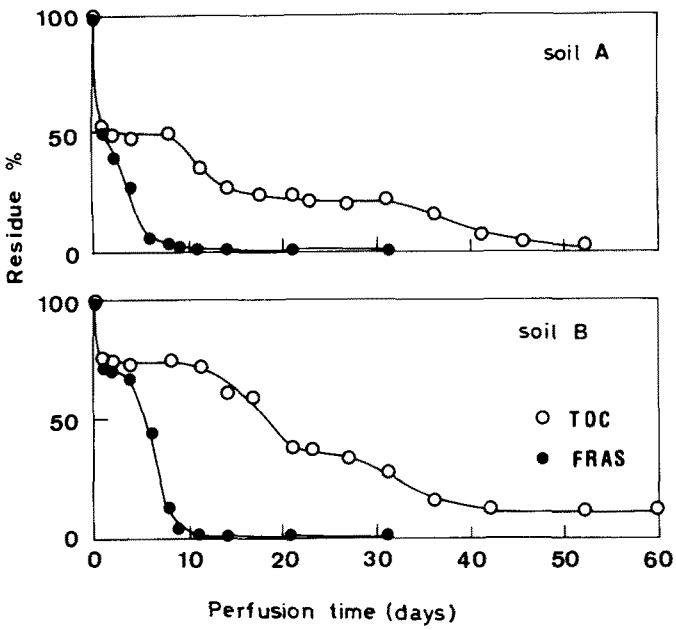

FIG. 4. Effect of soils on biodegradation of LAS.

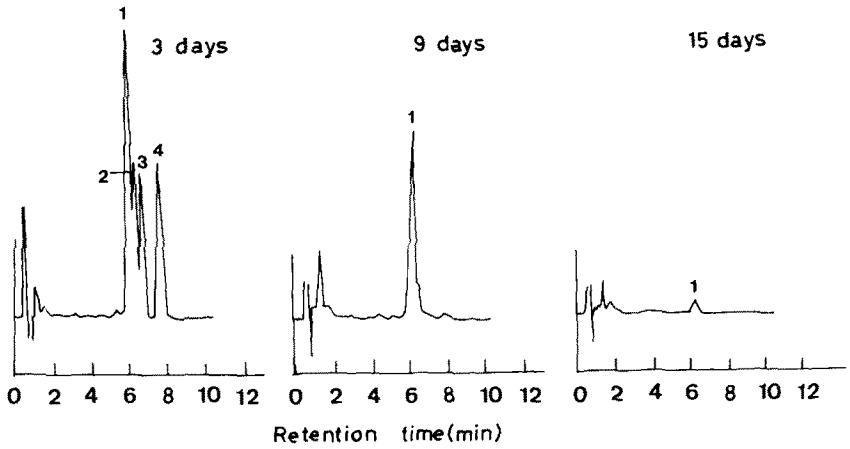

FIG. 5. HPLC chromatograms of C_{12}LAS eluted from the perfusion soil, (1) 5+6-phenyl; (2) 4-phenyl; (3) 3-phenyl; (4) 2-phenyl isomer. Perfusion experiment was done at 20 C.

Reproducible results were obtained for the soil perfusion test with the same soil, and these results confirmed that the disappearance behavior was affected by the kind of soil.

Adsorption onto soil and disappearance. It was shown in the previous sections that the disappearance of LAS observed in the early stage of perfusion is ascribed to the adsorption onto soil, but the fate of the adsorbed LAS is not clear. Thus, the disappearance process of LAS on soil was traced by the elution technique followed by HPLC test. Several methods have been reported on LAS elution from soil (7,14,16), and in the present study an elution method with methanol was adopted due to its shortest time for elution. The usefulness of this method was checked in an adsorption experiment with a batch technique; the adsorption data obtained by the methanol elution were compared with those estimated from the analysis on the residual solution. The result of the preliminary test showed there is good agreement between both the results on distribution patterns of isomers in the adsorbed LAS and that the reproducibilities of the results were

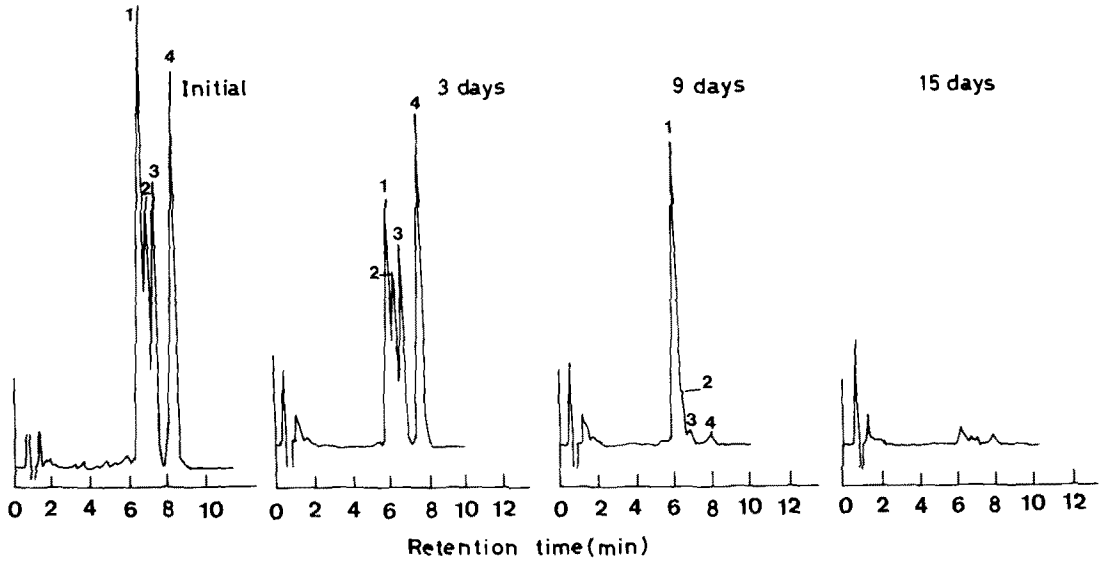

FIG. 6. HPLC chromatograms of C_{12}LAS remaining in the perfusion fluid, (1) 5+6-phenyl; (2) 4-phenyl; (3) 3-phenyl; (4) 2-phenyl isomer.

adequately high. In the case of the perfusion experiment conducted with a C_{12}LAS solution of 100 mg/l initial concentration on A soil, the elution was made for upper, middle and bottom layers of a soil column. Since all of these three layers showed nearly the same result, only the data on the middle layer are shown in Figure 5. Figure 6 also shows chromatograms of LAS in the perfusion fluid.

During three days of perfusion, only adsorption takes place and the adsorption ratio is nearly 50%. Each isomer of LAS indicates different characteristics of adsorption. In particular, the 2-phenyl isomer shows a very high adsorptive property. After nine days, a decrease in LAS concentration due to decomposition was observed; the survival ratio at the ninth day was 16.6%. The chromatogram for LAS eluted from the soil (Fig. 5) is similar to that remaining in the fluid (Fig. 6), and this result suggests biodegradation of LAS in the adsorbed state. After 15 days, both the chromatograms of LAS indicate almost complete disappearance of LAS with no detection of any isomers.

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