Biodegradation of Sodium Alkyl Poly(oxyalkylene)sulfates

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ABSTRACT

The biodegradability of sodium alkylpoly(oxyalkylene)sulfates was studied under aerobic conditions by oxygen consumption, total organic carbon (TOC) and methylene blue active substance (MBAS) measurements. MBAS of linear alkylpoly(oxyalkylene)sulfates with propylene oxide (APS) or ethylene oxide (AES) disappeared within 5 days, whereas AES with branched alkyl chains were degraded less than linear AES. APS with propylene oxide from 1 to 3 mol showed BOD/ThOD values of more than 40% after 6 days. Therefore, these surfactants are considered to be readily biodegradable. In comparison to the biodegradability of APS and AES, the existence of propylene oxide groups resulted in a slight decreasing in oxygen consumption and TOC removal. Linear APS with PO of 1-3 mol were degraded according to Swisher's distance principle up to a C_{16} alkyl chain length. That is, increasing distance between sulfate and chain end increased the rate of biodegradation of these surfactants. Furthermore, from the biodegradation test of ^{35}S-$C_{12}E_3S$ and the fish toxicity test in the course of biodegradation of $C_{12}E_3S$, it is suggested that the initial step of biodegradation is attack on the terminal methyl group.

INTRODUCTION

Alkyl poly(oxyethylene)sulfates (AES) are widely used as components of liquid dishwashing products, shampoo and other household speciality products. Alkyl poly(oxypropylene)sulfates (APS) with propylene oxide instead of ethylene oxide groups are new anionic surfactants and their potential applications are under investigation. Murata et al. (1) reported that the physicochemical and colloid chemical properties of these surfactants are applicable to low-phosphate and low-temperature detergents. Linear AES has been established as readily biodegradable (2), but the biodegradability of APS has rarely been reported, except for nonylphenoltrioxypropylene sulfate by Asahara et al. (3).

This paper describes the biodegradability of sodium poly(oxyalkylene)sulfates containing propylene oxide (PO) or ethylene oxide (EO) from 1 to 4 mol. Furthermore, the biodegradation mechanism of AES is discussed in this paper.

EXPERIMENTAL PROCEDURES

Materials

Surfactants. Samples of surfactants used are shown in Table I. These surfactants were prepared by the oxyalkylation of alcohols with ethylene oxide or propylene oxide with subsequent sulfation with a 5% molar excess of $ClSO_3H$. $SnCl_4$ was used as the catalyst for ethoxylation and KOH was used for oxypropylation. The distribution of poly-oxyethylene or propylene adducts was analyzed by gas liquid chromatography (see Table II). The ^{35}S-labeled sodium dodecyltrioxyethylene sulfate (^{35}S-$C_{12}E_3S$) was prepared by sulfation of dodecyltrioxyethylene ether with ^{35}S-labeled sulfuric acid and purification with preparative thin layer chromatography (TLC). The specific radioactivity of the ^{35}S-$C_{12}E_3S$ was 10.12 mCi/mmol (radiopurity, 95.5%). In this paper, the structure of surfactants is abbreviated as: $C_lP_mE_nS$, where l is the averaged carbon number, m is the averaged mol of PO, n is the averaged mol of EO, and S is a sulfate group.

Fish. Adult Japanese killifish (*Oryzias latipes*), 3.0-3.5 cm body length, were obtained from a fish farm in Tokyo. The fish were acclimated to dechlorinated tap water and fed daily with commercial dry fish food until 2 days before the beginning of the experiment, but were not fed during bioassay.

Biodegradation Tests

Biodegradation tests were done by the oxygen consumption method or by the die-away method. The basal medium of both methods was BOD diluent water prepared by adding 3 mL each of solutions A, B, C and D to 988 mL of deionized water (Table III). The oxygen consumption method was based on the MITI's (The Ministry of International & Industry in Japan) biodegradation test method. In this report, the concentration of test substances was changed to 30 mg/L from 100 mg/L. Activated sludge was obtained from the Chemical Biotesting Center in Japan. It had been incubated with synthetic sewage (1 g of peptones, 1 g of glucose and 1 g of KH_2PO_4 per L, pH 7.0) according to MITI's test method. The die-away test was done at static conditions (25 C). Initial surfactant concentration was 30 mg/L and, as an inoculum, 0.5 mL of the supernatant of activated sludge was added to 100 mL of the basal medium. The activated sluge used in the die-away test was obtained from a municipal sewage treatment plant in Tokyo. The water used in river die-away test was obtained from the Tama River (Maruko-bashi) in Tokyo. The biodegradation of ^{35}S-$C_{12}E_3S$ was added to the basal medium containing 0.5% supernatant of activated sludge used in the die-away test already mentioned. The surfactant concentration, after dilution of ^{35}S-$C_{12}E_3S$ with unlabeled $C_{12}E_3S$, was 100 mg/L. The test medium was incubated at 37 C.

Fish Toxicity Test

Biodegradation samples were prepared by the same method as the die-away test just described. The initial concentration of $C_{12}E_3S$ was changed from 30 mg/L to 100 mg/L. The fish bioassay of $C_{12}E_3S$ during the course of biodegradation was done in 1.0 L of the daily biodegraded solution using 4 fish/sample. The 24-hr survival and TLm values of adult Japanese killifish were evaluated according to a standard Japanese testing procedure described in JIS K 0102.

Analytical Methods

Oxygen consumption was measured with an Ohkura Coulometer as described in a previous paper (4). Surfactant concentration was measured using Abbott's methylene blue method (MBAS). TOC measurement was with a Sumitomo TC-TN analyzer, after removing microorganisms by centrifugation at 3,000 rpm for 15 min. Qualitative analysis of ^{35}S-$C_{12}E_3S$ intermediates was done using radio-TLC. Initial ^{35}S-$C_{12}E_3S$ and its intermediates were developed on Merck's silica gel plate (Kiesel Gel 60) with chloroform/methanol (3:1) containing 5% of 1 N H_2SO_4. This was followed by exposure to a Sakura X-ray film for 30 hr.

RESULTS AND DISCUSSION

Effect of Hydrophobe Structure on Biodegradation

In order to investigate the effect of hydrophobe structure on biodegradation, 6 kinds of AES with different hydro-

TABLE I

Structures of Sodium Alkyl Poly(oxyalkylene)sulfates

$R[OCH_2CH(CH_3)]_m[OCH_2CH_2]_nSO_4Na$				Abbr.
R		m[a]	n[a]	
n-$CH_{12}H_{25}$		0	0	$C_{12}AS$
		0	1	$C_{12}E_1S$
C_{12-14} (Coconut alcohol)		0	3	$C_{12-14}E_3S$
n-$C_{12}H_{25}$		0	3	$C_{12}E_3S$
$C_{11-15}H_{23-31}$ (Oxo alcohol)		0	3	Oxo-$C_{11-15}E_3S$
sec-$C_{12}H_{25}$		0	3	sec-$C_{12}E_3S$
$C_6H_{13}CHC_4H_9$ CH_2		0	3	br-$C_{12}E_3S$
$C_7H_{15}CHC_9H_{19}$ CH_2	(A)	0	0	br-$C_{18}AS$
		0	1	br-$C_{18}E_1S$
		0	3	br-$C_{18}E_3S$
		0	5	br-$C_{18}E_5S$
$C_7H_{15}CHCH(CH_3)C_7H_{15}$ CH_2 (A:B=5:1)	(B)			
n-C_8H_{17}		1	0	C_8P_1S
n-$C_{10}H_{21}$		1	0	$C_{10}P_1S$
n-$C_{12}H_{25}$		1	0	$C_{12}P_1S$
n-$C_{12}H_{25}$		1	1	$C_{12}P_1E_1S$
n-$C_{12}H_{25}$		3	0	$C_{12}P_3S$
n-$C_{12}H_{25}$		3	1	$C_{12}P_3E_1S$
n-$C_{12}H_{25}$		3	5	$C_{12}P_3E_5S$
n-$C_{12}H_{25}$		5	3	$C_{12}P_5E_3S$
n-$C_{16}H_{33}$		3	0	$C_{16}P_3S$
n-$C_{18}H_{37}$		3	0	$C_{18}P_3S$
n-$C_{18}H_{37}$		5	3	$C_{18}P_5E_3S$

[a]Average number.

TABLE II

Distribution of Products in the Oxyalkylation of Alcohols

	$R(OCH_2CH_2)_nSO_4Na$			$R'[OCH_2CH(CH_3)]_nSO_4Na$		
	$C_{12}E_1S$	$C_{12}E_3S$	$C_{12}P_1S$	$C_{12}P_3S$	$C_{16}P_3S$	$C_{18}P_3S$
n= 0	31.52[a]	2.79	21.96	0.44	0.53	0.51
1	43.22	16.38	58.10	18.70	19.58	17.56
2	18.35	22.69	16.77	29.62	30.84	28.58
3	5.45	21.60	3.17	23.88	25.06	25.57
4	1.25	16.36	–	13.62	13.86	15.09
5	0.21	10.36	–	7.69	6.53	7.57
6	–	5.64	–	3.42	2.71	3.39
7	–	2.67	–	1.64	0.89	1.30
8	–	1.09	–	0.76	–	0.43
9	–	0.36	–	0.23	–	–
10	–	0.06	–	–	–	–
$\tilde{P}$[b]	1.02	3.09	1.01	2.83	2.67	2.83

[a]After the hydrolysis of sulfates, the resulting alcohols were acetylated by acetic anhydride/pyridine. The mole ratio (%) was determined by GLC analysis of the acetate derivatives.
[b]Average number of propylene or ethylene oxide groups.

TABLE III

Reagents for the Standard Japan BOD Diluent

Reagent	Substrate	Water (g/L)
A	K_2HPO_4	21.75
	KH_2PO_4	8.5
	$Na_2HPO_4 \cdot 10H_2O$	44.6
	NH_4Cl	1.7
B	$MgSO_4 \cdot 7H_2O$	22.5
C	$CaCl_2$ (anhydride)	27.5
D	$FeCl_3$	0.25

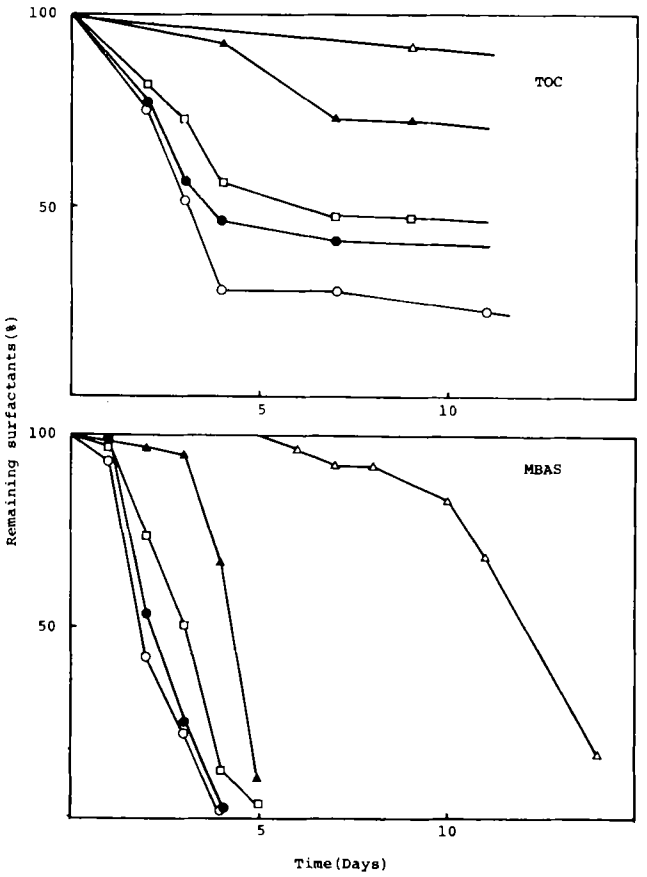

FIG. 1. Effect of hydrophobe structure on the biodegradation of AES. Conditions: inoculum, 0.5 mL of activated sludge supernatant/100 mL BOD diluent; surfactants, 30 mg/L; temp., 25 C. Symbols: (○), $C_{12\text{-}14}E_3S$; (●), n-$C_{12}E_3S$; (□), oxo-$C_{11\text{-}15}E_3S$; (▲), sec-$C_{12}E_3S$; (△), br-$C_{12}E_3S$.

phobe structures were prepared. As shown in Figure 1, their biodegradation behavior varied widely, depending on the structure of hydrophobe. MBAS of $C_{12\text{-}14}E_3S$, n-$C_{12}E_3S$ and oxo-$C_{11\text{-}15}E_3S$ derived from linear primary alcohol disappeared within 5 days, whereas about 75% of branched AES(br-$C_{12}E_3S$) remained after 10 days. Based on MBAS disappearance, AES(sec-$C_{12}E_3S$) derived from linear secondary alcohol degraded a little slower than linear primary AES. The biodegradability of oxo-$C_{11\text{-}15}E_3S$ was between that of linear primary AES and linear secondary AES. This result was also observed based on TOC removal. Furthermore, similar results are also obtained by the river die-away test (Fig. 2). From these results, it appears that AES with a linear hydrophobe is readily biodegradable, whereas branched AES is much more resistant. The effect of branching is considered to be caused by hindrance to enzymic attack. This result has been found for the alkyl sulfates and the alkylbenzene sulfonates (2).

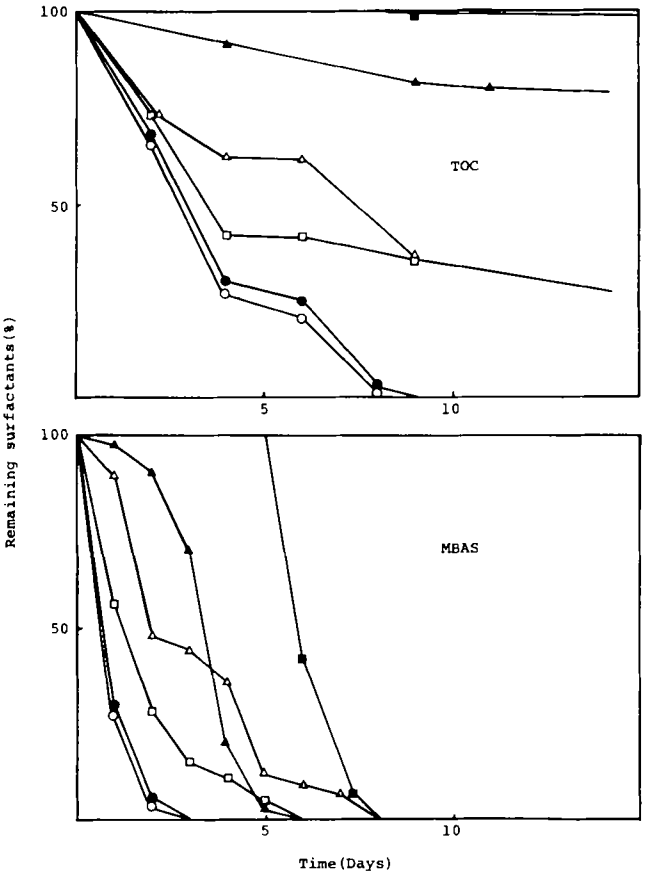

FIG. 2. Effect of hydrophobe structure on the biodegradation of AES in river water. Conditions: river water, Tama River (Marukobashi) in Tokyo; surfactants, 30 mg/L; temp., 25 C. Symbols: (○), $C_{12\text{-}14}E_3S$; (●), n-$C_{12}E_3S$; (□), oxo-$C_{11\text{-}15}E_3S$; (▲), sec-$C_{12}E_3S$; (△), br-$C_{12}E_3S$; (■), br-$C_{18}E_3S$.

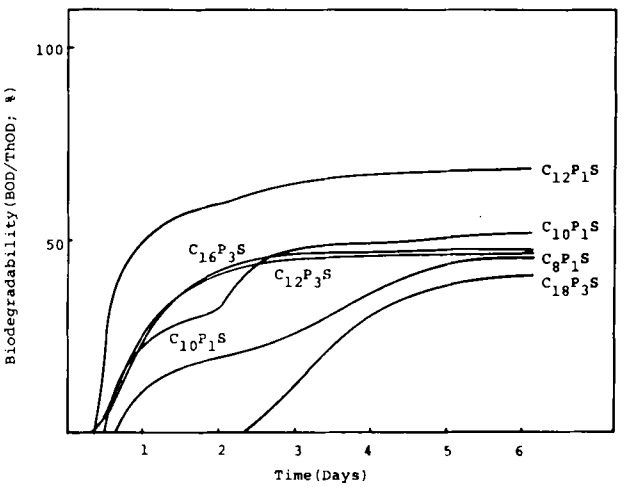

FIG. 3. Effect of alkyl chain length on the biodegradation of APS, detected by oxygen consumption. Conditions: surfactants, 30 mg/L; activated sludge, 30 mg/L; temp., 25 C.

Figure 3 shows the effect of alkyl chain length on the biodegradation of APS. No difference was observed in the MBAS biodegradability of AP_1S and AP_3S. Both were more

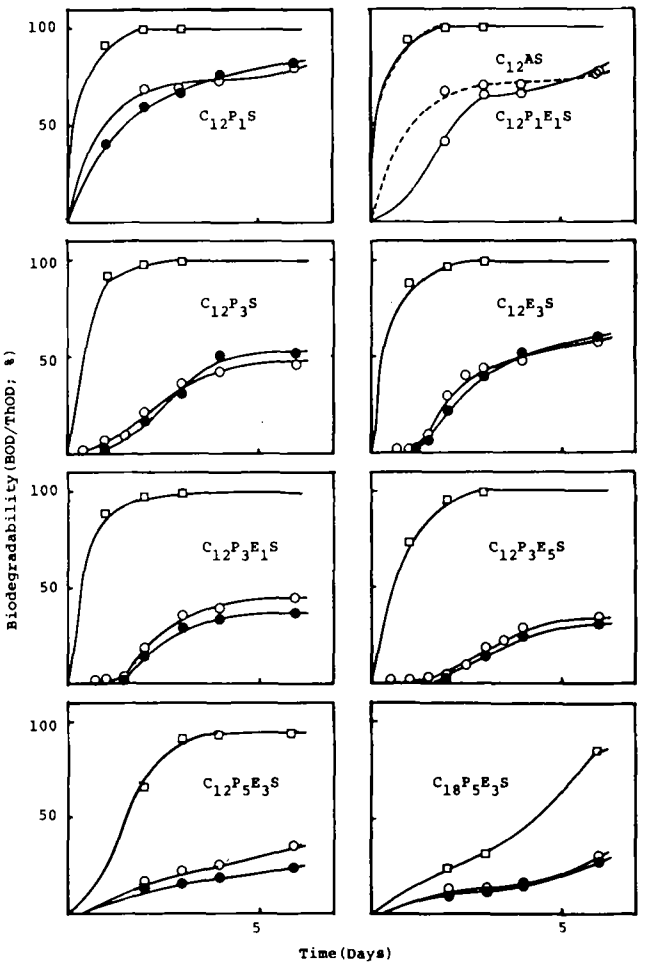

FIG. 4. Biodegradation of APS, APES and AES. Conditions: activated sludge, 30 mg/L; surfactants, 30 mg/L; temp., 25 C. Symbols: (○), BOD/ThOD (%); (□), MBAS; (●), TOC.

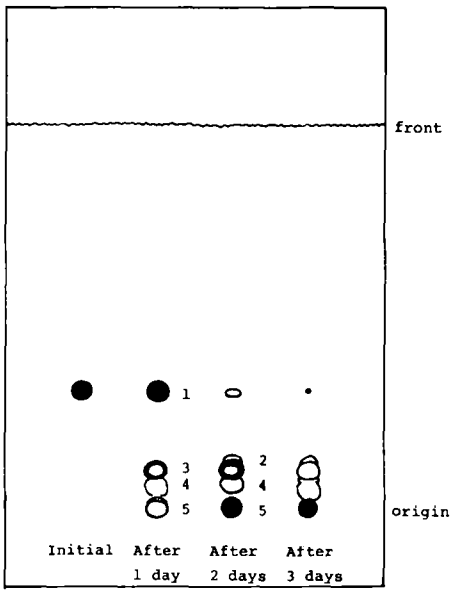

FIG. 5. Radio-thin-layer chromatogram of ^{35}S-$C_{12}E_3S$ and its intermediates on silica gel plate developed with $CHCl_3$/MeOH (3:1) containing 5% of 1 N H_2SO_4. Surfactant: 100 mg/L. R_f values: spot 1, 0.31; spot 2, 0.14; spot 3, 0.11; spot 4, 0.05; spot 5, 0.

than 95% degraded after 5 days. Oxygen consumption (BOD/ThOD) of the AP_1S increased when the alkyl chain length increased from C_8 to C_{12}. In the case of AP_3S with alkyl chain length from C_{12} to C_{18}, the BOD/ThOD values for $C_{18}P_3S$ were lower than for $C_{12}P_3S$ and $C_{16}P_3S$. However, $C_{12}P_3S$ showed almost the same oxygen consumption as $C_{16}P_3S$. There has been little study of the effect of alkyl chain length of AES or APS on biodegradation. Judging from these results, it seems that APS with PO 1-3 mol is degraded according to Swisher's distance to principle, i.e., "Increased distance between the sulfonate group and the far end of the hydrophobe group increases the speed of primary biodegradation of ABS and possibly of other surfactants" up to about C_{16} alkyl chain length.

Effect of Hydrophilic Structure on Biodegradation

The biodegradability curves of APS, APES, AES and AS are shown in Figure 4. MBAS of these surfactants, with the exception of $C_{12}P_5E_3S$ and $C_{18}P_5E_3S$, disappeared within 3 days, whereas MBAS removals were 92 and 31%, respectively, for this time period. As shown in Figure 4, the rate of oxygen consumption and TOC removal varied appreciably and differed from MBAS removal. Therefore, the effect of the hydrophilic group must be evaluated by MBAS removal and by oxygen consumption (BOD/ThOD) and TOC removal. The BOD/ThOD values of $C_{12}P_1S$ and $C_{12}P_1E_1S$ were almost the same as that for $C_{12}AS$, but $C_{12}P_1E_1S$ showed a little retardation at the initial stage of incubation. The BOD/ThOD values of $C_{12}P_3S$ were lower than for $C_{12}P_1S$ and $C_{12}P_1E_1S$. In comparing $C_{12}P_3S$ with the corresponding AES ($C_{12}E_3S$), the BOD/ThOD values and TOC removal of $C_{12}P_3S$ was slightly lower than for $C_{12}E_3S$. This result suggests that APS is a little more resistant to biodegradation than AES. This is likely due to the inclusion of a secondary carbon atom in the structure of the propylene oxide group. From the BOD/ThOD values and TOC removals in Figure 4, the biodegradability of APS tended to deteriorate with increasing PO units. The biodegradability of surfactants containing both PO and EO was lower than for the corresponding AES or APS. In the range of PO 1-3 mol, however, the effect of PO attachment was not greater than might be anticipated (Figs. 3 and 4). Furthermore, because APS with PO 1-3 mol and $C_{12}E_3S$ showed BOD/ThOD values of more than 40 or 50% after 6 days, these surfactants are considered to be readily biodegradable. Miura et al. (5) obtained similar results with $C_{12}AES$. That is, when the biodegradation tests of various surfactants were done according to MITI's method, MBAS of 100 mg/L $C_{12}AES$ disappeared within 5 days; the BOD/ThOD values and TOC removals were between 50-70% in 5-10 days.

Biodegradation Mechanism of AES

In order to investigate the biodegradation mechanism of $C_{12}E_3S$, ^{35}S-labeled $C_{12}E_3S$ was used in the biodegradation test. Figure 5 shows the radio-thin layer chromatogram of ^{35}S-$C_{12}E_3S$ and its intermediates. Initial ^{35}S-$C_{12}E_3S$ almost disappeared after 3 days; then, several spots appeared below the spot of ^{35}S-$C_{12}E_3S$. The spots at the origin are considered to be inorganic sulfuric salts or intermediates with high polarity. Therefore, the 3 or 4 spots with R_f values of 0.05-0.14 are considered intermediates having ^{35}S-sulfate groups. In general, it can be assumed that the AES is degraded through either of the following 2 pathways.

(a) Enzymic hydrolysis of the sulfate group takes place to give sulfate and alcohol ethoxylate, and then the latter is

TABLE IV

Fish Toxicity of $C_{12}E_3S$, $C_{12}H_{25}O(EO)_3H$, $C_{12}H_{25}OH$ and Biodegraded $C_{12}E_3S$[a]

Chemicals	TLm (24 hr)
$C_{12}E_3S$	30-35 mg/L
$C_{12}H_{25}O(EO)_3H$	2-3
$C_{12}H_{25}OH$	20
Biodegraded $C_{12}E_3S$[a]	43

[a]After 6 days.

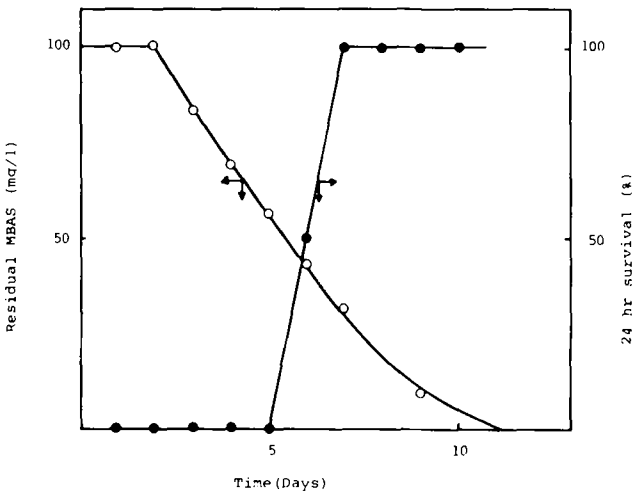

FIG. 6. Acute toxicity to Japanese killifish of $C_{12}E_3S$ in the course of biodegradation.

degraded through the biodegradation pathway of a primary alcohol ethoxylate.

(b) The initial step is attack of the terminal methyl group, followed by β-oxidation.

These results suggest the possibility that ^{35}S-$C_{12}E_3S$ is degraded by pathway b. If $C_{12}E_3S$ were degraded through pathway a, the corresponding alcohols ($C_{12}H_{25}O(EO)_3$-H or $C_{12}H_{25}OH$) are produced as intermediates. As shown in Table IV, the fish toxicity of these intermediates is stronger than that of the initial surfactant. However, Figure 6 shows no tendency toward increased fish toxicity in the course of the biodegradation of $C_{12}E_3S$. This result further supports the possibility of pathway b.

The biodegradability of singly or doubly branched AES increased with increasing ethylene oxide content (Table V). This result is different from Berger's observation (6) that tetrapropylene oxo AES was somewhat more resistant than the corresponding unethoxylated sulfates (alkyl sulfates). This deviation from Berger's result is not understood. However, as similar results were reported for branched alkylphenol ethoxylates (2), the above result is considered to be one of a few exceptions. As shown in Table V, this branched hydrophobe is very resistant to biodegradation, so introduction of an EO group seems to be favorable for attack by sulfatase enzyme on the hydrophilic chain. Therefore, these surfactants are considered to be degraded through pathway a.

TABLE V

Comparative Biodegradability of Branched AS and AES

	MBAS decrease (%)	
R[a] $(OCH_2CH_2)_nSO_4Na$	b	c
n=0	0	5
1	15	5
3	72	48
5	100	100

[a]The structure of R is as same as that of br-$C_{18}E_3S$ (Table I).

[b]River die-away test; river water: Jikken River in Tokyo; surfactant: 30 mg/L; temp.: 25 C; after 15 days.

[c]Die-away test; inoculum: supernatant of laboratory activated sludge (0.5 mL/100 mL BOD diluent water); surfactant: 30 mg/L; temp.: 25 C; after 14 days.

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