Influence of Hydrophobe Type and Extent of Branching on Environmental Response Factors of Nonionic Surfactants[1]

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A number of ethoxylated nonionic surfactants differing in hydrophobe branching and chainlengths have been evaluated for environmental responses. Screening biodegradation tests show that those nonionics having more than one methyl group per hydrophobe degrade considerably slower than those having less extensive branching. Continuous flow-through activated sludge tests, simulating actual waste treatment, show that the more highly branched nonionics biodegrade more slowly and less extensively than those with less hydrophobe branching. In addition, treated effluents originating from influents containing the more highly branched nonionics tend to be more surface active and more toxic to aquatic species than those originating from influents containing surfactants with less hydrophobe branching. Under conditions simulating plant stress, such as high surfactant concentrations in the influent or low temperature, biodegradation of the highly branched nonionics was considerably less extensive, while biodegradation of the linear nonionics was not affected to any measurable degree compared to more normal operating conditions.

KEY WORDS: **Activated sludge, alcohol ethoxylates, aquatic toxicity, linear and branched hydrophobes, nonylphenol ethoxylates, primary and ultimate biodegradation.**

Surfactants are being used increasingly in household cleaning products, personal care and a variety of industrial and institutional applications (1). Owing to the tendency of these surfactants to foam and exhibit toxicity to aquatic organisms at relatively low concentrations, they must be removed prior to entering receiving waters in order to meet regulatory requirements. Secondary waste treatment, employing aerobic biodegradation, is the most widely used approach to reducing the undesirable properties of organic materials, such as surfactants, that enter domestic waste treatment plants. In this approach, bacteria provide enzymatic systems that utilize oxygen to convert organic materials to CO_2, H_2O and cellular mass. The process is complex, with biodegradation rates dependent on the structure of the organic material. Alcohol-based surfactants with essentially linear hydrophobes have been shown in extensive studies (2-9) to biodegrade rapidly to produce non-foaming, non-toxic products in treatment plant effluents. However, biodegradation of those alcohol-based surfactants with highly branched hydrophobes has been studied less extensively (10,11) than the linear surfactants, because demand for the highly branched surfactants has not been as great as for those based on linear hydrophobes. All surfactants must show acceptable biodegradability in order to meet effluent regulatory criteria.

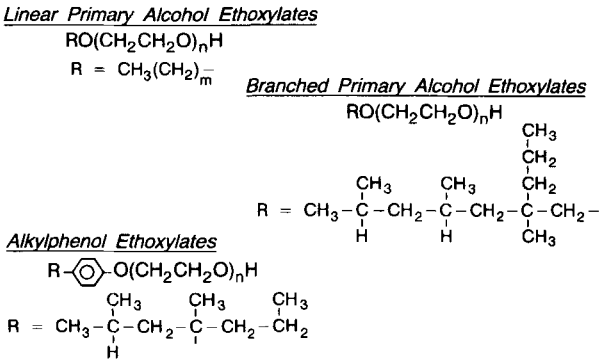

FIG. 1. Structural features of nonionic classes.

This paper discusses an environmental response study for three of the major types of surfactants derived from linear primary alcohols, highly branched primary alcohols and branched nonylphenols. Nonionics derived from the nonylphenols have also been studied extensively (3,12-19). Each of these nonionic classes is a complex mixture of homologs and isomers. Representative structural features are shown in Figure 1. The study encompasses ultimate biodegradability to CO_2 and H_2O, primary biodegradability in laboratory tests designed to simulate large-scale sewage treatment in summer and winter, aquatic toxicities of sewage effluents resulting from biotreatment of the three surfactant types, and identification of surfactant biodegradation intermediates.

In addition to ethoxylates of the linear and branched alcohols, the alcohol sulfates (AS) of these hydrophobes were also included in several of the biochemical oxygen demand (BOD) tests.

EXPERIMENTAL PROCEDURES

Synthesis of alcohol ethoxylates (AE) and alcohol sulfates (AS). AE synthesis was performed by ethylene oxide addition to the appropriate alcohol in an autoclave by means of conventional KOH catalysis. A modification of a chlorosulfonic acid procedure was used to prepare AS (20). In this modification, nitrogen gas was dried by passing through a 20 cm long × 3 cm wide glass column containing Ascarite. To ensure an essentially water-free alcohol reactant, the dried nitrogen was bubbled through 100 mmole of appropriate alcohol reactant dissolved in 50 mL of methylene chloride for 15 min prior to addition of chlorosulfonic acid. Chlorosulfonic acid at 1.05:1 molar ratio was then added dropwise with stirring with the

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nitrogen sparge tube placed above the surface of the liquid reaction medium. The addition of chlorosulfonic acid was controlled at a rate that maintained the exothermic reaction within a temperature range of 30–35°C. After addition of chlorosulfonic acid, the nitrogen sparge tube was placed below the surface of the reaction mixture with N_2 sparging continuing for 30 min under stirring. External heating was applied to maintain a 30–35°C temperature. This permitted removal of essentially all the HCl gas that had formed. The resulting AS in the acid form were neutralized with aqueous NaOH to produce 25% active AS at pH 8–9.

Nuclear magnetic resonance (NMR) analysis for branched hydrophobes. Carbon-13 NMR analysis for branching in alcohols and AEs was performed on a Bruker AM-500 spectrometer (Bruker Analytische, Karlsruhe, Germany). A 50% vol solution was made from each sample and deuterochloroform solvent, which contained 0.2 molar TEMPO relaxation reagent. Two milliliters were placed into a 10-mm NMR tube. The NMR acquisition was done by using a 45° pulse with a 10-second relaxation delay, and 2000 scans were acquired for each experiment. Two experiments were required for each analysis. A normal NMR acquisition with NOE (Nuclear Overhauser Enhancement) suppression and a j-modulated spin echo experiment (JMSE) were used to identify the carbons according to the number of protons attached. Spectral editing was then used to identify and quantify the methyl groups.

Gas chromatography/mass spectroscopy (GC/MS) for hydrophobe carbon number distribution (CND). GC/MS was used to determine CNDs for highly branched alcohols, because GC and liquid chromatography were unsuccessful due to extensive branching that led to significant carbon number overlap. GC/MS data were obtained in the VG-Tritech TS-250 GC mass spectrometer (VG Analytical, Manchester, U.K.). Trimethylsilyl (TMS) ether derivatives of the branched alcohols were prepared with n,o-*bis* (trimethylsilyl) trifluoroacetamide (BSTFA) and 1% trimethylchlorosilane (TMCS). This catalyzed formulation was used to silylate any slightly hindered hydroxyls that might be present. This formulation is stronger than BSTFA alone. BSTFA reacts quantitatively with free OH-groups to form stable TMS derivatives for GC analysis. Replacement of the acidic hydrogen with $Si(CH_3)_3$ increases the molecular weight of the free alcohol by 72 daltons. For example, the molecular weight of a C_{12} alcohol (M.W.186) is shifted to 258. The molecular ion is frequently weak or absent in the electron impact mass spectrum of aliphatic trimethylsilyl ethers. However, the intense $(M - CH_3)^+$ peak can be used for purposes of molecular weight determination and quantification. The spectrum of the trimethylsilyl ether derived from a C_{12} alcohol ($M^+ = 258$) would therefore exhibit an intense ion occurring at *m/z* 243. A normalized percent carbon number distribution was obtained by summing the characteristic $(M - CH_3)^+$ fragment ions, in the appropriate GC retention time window, for each carbon chainlength.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) for polyoxyethylene (POE) distributions. POE chainlengths from 1–6 were determined using a Varian 3500 capillary GC (Varian Associates, Palo Alto, CA) fitted with a 15-meter narrow bore DB-5 column. AEs were injected as their trimethylsilyl ether derivatives. Pure dodecyl AEs of specific POE chainlengths ranging from 0–8 were used as calibration standards. Pure decyl alcohol was used as internal standard. Detection was by flame ionization. A Varian 5500 HPLC was used to determine POE chainlengths greater than five. AEs were injected as their phenylurethane derivatives into a silicon column with a ternary gradient system composed of methanol, isopropanol and acetonitrile. Detection was by UV at 254 nm. Data from the GC and HPLC were combined, and POE distributions were calculated *via* a spreadsheet program in a PET 2001 computer.

Surfactant substrates. Nonionic and anionic surfactant substrates used in the biodegradation and/or aquatic toxicity tests discussed below are listed with their major structural features in Table 1. They will be referred to by the acronyms listed in column 2. Parenthetical suffix letters L and B refer to essentially linear and highly branched, respectively. Methyl group branching is defined

TABLE 1

Surfactants Tested

Surfactant	Acronym	Alkyl carbon no.		Internal methyl groups/hydrophobe
		Distribution range	Average	
Linear C_{12-15}AE-7 EO[a]	C_{12-15}AE-7(L)	12–15	13.5	0.1
Linear C_{12-15}AE-9 EO[a]	C_{12-15}AE-9(L)	12–15	13.5	0.1
Linear $C_{14,15}$AE-7 EO[a]	$C_{14,15}$AE-7(L)	14 15	14.5	0.1
Branched C_{12}AE-7 EO[b]	C_{12}AE-7(B)	10–14	11.9	2.9
Branched C_{13}AE-7 EO[c]	C_{13}AE-7(B)	11–15	13.4	4.0
Branched NPE-9 EO[d]	NPE-9(B)	9	9	1.7
Linear $C_{14,15}$ alcohol sulfate[e]	$C_{14,15}$AS(L)	14, 15	14.5	0.1
Branched C_{13} alcohol sulfate[f]	C_{13}AS(B)	11–15	13.4	4.0

[a]NEODOL® 25 and NEODOL 45 ethoxylates (Shell Chemical Co.).

[b]Made by lab-scale ethoxylation of Exxal 12 alcohol (Exxon Chemical Co.).

[c]Made by lab-scale ethoxylation of Exxal 13 alcohol (Exxon Chemical Co.).

[d]Igepal CO-630 (GAF Corp.).

[e]Made by lab-scale sulfation of NEODOL 45 alcohol (Shell Chemical Co.).

[f]Made by lab-scale sulfation of Exxal 13 alcohol (Exxon Chemical Co.).

by the number of methyl groups pendant to the alkyl chain, and they are listed as internal methyl groups/hydrophobe.

Biodegradation via *biochemical oxygen demand (BOD) tests.* BOD tests were run at Edna Wood Laboratories, Inc. (Houston, TX) by means of an established test procedure (21). For BOD studies, bacterial inocula had been obtained from a seed that was unacclimated and different from that used for the CO_2 evolution tests. BOD measurements were made by titrating for dissolved oxygen following the azide modification of the Winkler method. Chemical oxygen demand (COD) measurements were made by the silver sulfate-mercuric sulfate modification of the dichromate method.

Ultimate biodegradation via *CO_2 evolution batch tests.* CO_2 evolution resulting from aerobic microbial attack on surfactants was performed by means of an OECD modification (22) of the Sturm Test (23). Closed one-liter biotreatment units with 500 mL liquid volume contained Sturm mineral salts solution, 10, 20 or 50 mg/L surfactant or sodium benzoate (positive control), antifoam agent and composited mixed liquor suspended solids (MLSS) from acclimated activated sludge cultures. Activated sludge was obtained from a Houston domestic waste treatment plant. Viable bacterial counts were 10^7–10^8/mL initially, and 10^6–10^7/mL after the 28-day test.

Continuous activated sludge biotreatment tests. These laboratory tests, designed to simulate the secondary treatment section of a full-scale sewage treatment plant, have been described previously (24). Low temperatures, simulating winter conditions, were generated in an environmental chamber equipped with a refrigeration unit. A schematic of the bench-scale units is shown in Figure 2.

Aquatic toxicity tests. Intact surfactants and effluents from biotreaters degrading 50 mg/L surfactants at 25°C were subjected to acute and chronic aquatic toxicity testing using waterfleas (*Daphnia magna*) and fathead minnows (*Pimephales promelas*) according to established EPA procedures (21,25,26). Testing was done by TRAC Laboratories, Inc. (Denton, TX).

Primary biodegradation. The extent of removal of intact surfactant (primary biodegradation) in continuous activated sludge biotreaters was followed by the cobalt thiocyanate active substance (CTAS) procedure (27). An approximation of the extent of surfactant primary biodegradation was also made by measuring foam heights generated from 50 mL of effluent manually shaken in a stoppered 100-mL graduate cylinder.

RESULTS AND DISCUSSION

Chemical structure of surfactants. Each nonionic ethoxylate shown in Table 1 was found to contain an average of 7 or 9 (±0.2) EO units/mole of alcohol or nonylphenol. Alcohol sulfates contained 1–2% unreacted organic matter based on active matter present. Alcohol ethoxylates (AE), and alcohol sulfates (AS) and each contained hydrophobes of varying chainlengths as indicated. Approximately 20% of the essentially linear AEs derived from the C_{12-15} and $C_{14,15}$ alcohols contained 2-alkyl monobranching—mostly methyl. The highly branched C_{12} and C_{13} alcohols appear to have been derived from propylene oligomers. The structural characteristics of the highly branched surfactants are quite complex. However, it is instructive to characterized branching by the number of internal methyl groups per hydrophobe as determined by NMR. In a comparison of the biodegradation of linear with branched alcohols, Swisher (28) indicated that a single internal methyl group has no appreciable effect on the biodegradation rate compared to a 100% linear alcohol. However, incorporation of a second methyl group decreases the biodegradation rate significantly, particularly if the second methyl group is located on the same carbon atom to produce a quaternary carbon structure.

Biodegradation via *BOD tests.* The results of a 30-day BOD test on the C_{12-15}AE-9(L), C_{13}AE-7(B) and NPE-9(B) are shown in Figure 3. The linear AE biodegraded considerably faster and more extensively (88%) as compared to the branched AE (44%) and the branched NPE (31%). In this test, at least 50% biodegradation is required in order to categorize the test substrate as having "ready biodegradability" according to standards set by the Organization for Economic Cooperation and Development (OECD) (29).

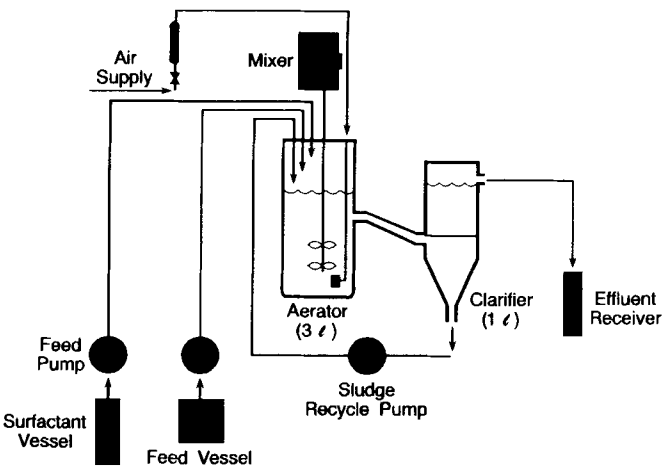

FIG. 2. Bench-scale continuous activated sludge biotreater.

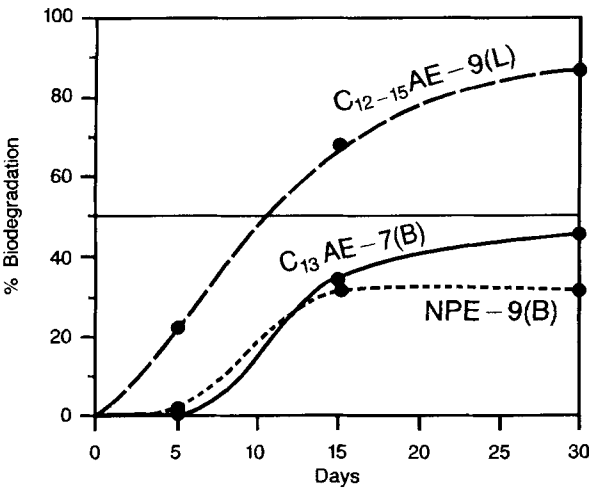

FIG. 3. Effect of hydrophobe on biodegradation of nonionic surfactants *via* BOD test.

A comparison of the effect of hydrophobe chainlength on the ultimate biodegradability of linear and branched AEs as determined by BOD tests is shown in Figure 4. The linear AEs again showed much faster and more extensive biodegradation over 30 days than the highly branched AEs. In addition, hydrophobe chainlength appeared to have a slight but perceptible effect on the linear AEs, with the $C_{14,15}$AE-7(L) biodegrading to 83% while the lower chainlength C_{12-15}AE-7(L) biodegraded at a slightly faster rate to 92%. In contrast, the C_{12}AE-7(B) and C_{13}AE-7(B) showed no appreciable differences in rate or extent of biodegradation.

Figure 5 shows 30-day BOD results for $C_{14,15}$AS(L) as compared to branched C_{13}AS(B). The $C_{14,15}$AS(L) biodegraded to near the theoretical amount (98%), while the C_{13}AS(B) biodegraded considerably less extensively (41%). These results are qualitatively in line with literature values (30), which show 30% biodegradation for a C_{13}AS(B) and 86% biodegradation for a C_{11-15}AS(L) in a 20-day BOD test.

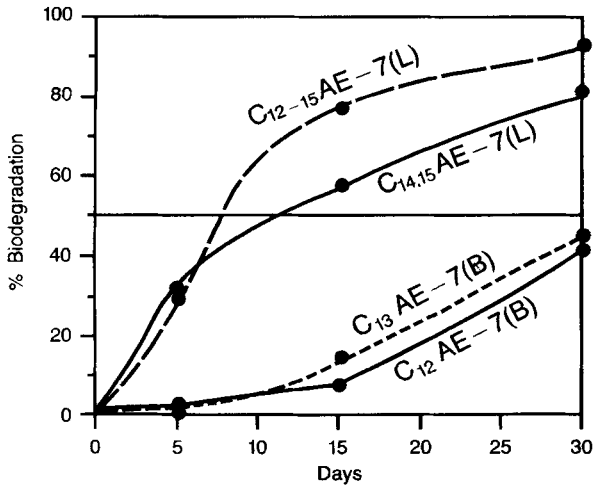

FIG. 4. Effect of hydrophobe chainlength on biodegradation of branched nonionic surfactants *via* BOD test.

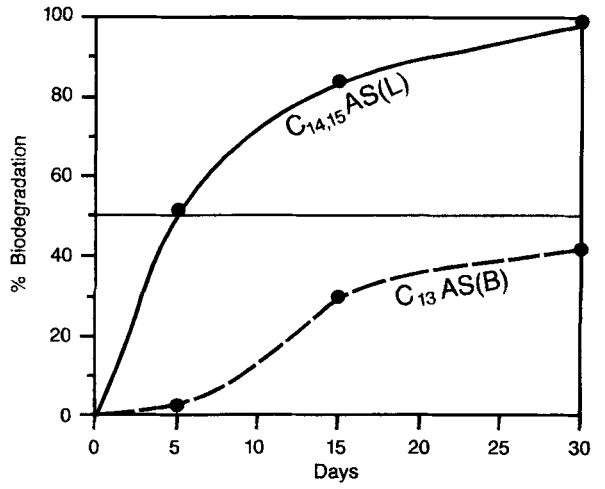

FIG. 5. Effect of hydrophobe on biodegradation of alcohol sulfates *via* BOD test.

Ultimate biodegradation via *CO_2 evolution.* The results of a 28-day CO_2 evolution test at initial surfactant concentrations of 20 mg/L are shown in Figure 6 for C_{12-15}AE-7(L), C_{13}AE-7(B) and NPE-9(B). The results are qualitatively similar to those found in the BOD test (Fig. 3), with the linear AE biodegrading faster and more extensively than the branched AE and NPE. Twenty-eight-day CO_2 evolution test results for these surfactants at varying initial concentrations (10, 20 and 50 mg/L) are shown in Table 2, along with sodium benzoate added as a positive biodegradation standard. Similar biodegradation patterns were observed for all concentrations, with the linear AE showing considerably greater biodegradation than the branched nonionics.

Continuous activated sludge bench-scale biotreater test. Bench-scale biotreater tests run at 25°C simulating summer conditions were run in separate units with C_{12-15}AE-9(L), C_{13}AE-7(B), NPE-9(B) and a sodium benzoate control. Bacterial inoculum in each unit was adapted to the appropriate surfactant or sodium benzoate at increasing concentrations (0–50 mg/L) over a period of 5–6 months. The minimum adaptation time for any given substrate concentration was 40 days. The lower concentrations are similar to influent loadings from domestic waste, while the higher concentrations are typical of influent loading from industrial waste. Operating parameters are summarized in Table 3.

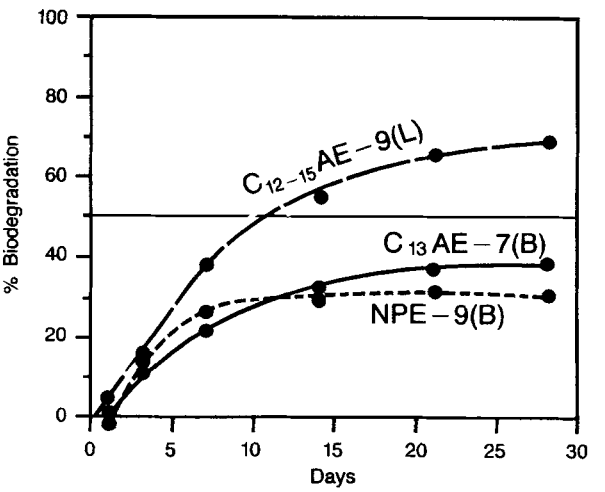

FIG. 6. Effect of hydrophobe on ultimate biodegradation of nonionic surfactants *via* CO_2 evolution test.

TABLE 2

Effect of Hydrophobe on Ultimate Biodegradation of Nonionic Surfactants *via* CO_2 Evolution Test

Surfactant	% Biodegradation at initial concentration		
	10 mg/L	20 mg/L	50 mg/L
C_{13} AE-7(B)	48	37	50
NPE-9(B)	14	28	34
C_{12-15} AE-9(L)	64	67	79
Na Benzoate	88	85	94

TABLE 3

Biotreater Operating Conditions

Parameter	Condition
Synthetic waste	Modified Hussmann (OECD/EEC) sewage
Activated sludge	Houston domestic waste treatment plant
Sludge residence time	45-60 days
Aerator MLSS	3500-4500 mg/L
pH	7-7.5
Dissolved oxygen	2-5 mg/L

Effluent CTAS values for each unit are shown in Figures 7-9 for C_{12-15}AE-9(L), C_{13}AE-7(B) and NPE-9(B), respectively. Effluent foam heights for these surfactants are shown in Figures 10-12. These CTAS and foam height primary biodegradation criteria show that the C_{12-15} AE-9(L) unit yielded less intact surfactant in the effluent than the C_{13}AE-7(B) and the NPE-9(B). The many CTAS and foam height peaks and valleys, particularly for the branched surfactants, are probably due to sludge desorption and sorption of intact surfactants and/or their partially degraded metabolites as fresh surfactant is continuously fed to the biotreaters. What is clear from these studies is that the highly branched nonionics do not biodegrade as readily as the linear nonionics, particularly at the higher influent concentrations. The continuous activated sludge biotreater results discussed above are in line with the few continuous or semicontinuous activated sludge tests reported for highly branched AEs (31,32) under normal operating conditions.

The results of operating under simulated winter conditions at 10 mg/L influent concentrations are shown in Figures 13-15, for effluent CTAS response of the C_{12-15} AE-7(L), C_{13}AE-7(B) and NPE-9 units, respectively. CTAS response for all three surfactant units were similar at 25°C. As the operating temperature was lowered sequentially from 25°C to 8°C, significant CTAS breakthrough for the C_{13}AE-7(B) and NPE-9(B) units occurred near 8°C (Figs. 14 and 15), indicating that biodegradation had slowed considerably for these branched surfactants. In contrast, C_{12-15}AE-9(L) showed no significant CTAS breakthrough even at temperatures as low as 8°C (Fig. 13).

Two additional observations on the physical conditions of the biotreater units deserve mentioning: i) Those units treating the highly branched surfactants tended to foam and required periodic addition of antifoaming agents, while little foam was observed in the unit treating the linear surfactant. The foaming was worse when the branched surfactants were treated under stress conditions, *i.e.*, at higher influent concentrations or at lower temperatures. ii) Increased solids carryover was periodically observed in the clarifier overflow for the branched surfactant units but not for the linear surfactant unit. Solids carryover was worse when the branched surfactants were treated at the higher influent loadings or at lower temperatures. The solids carryover is probably due to the

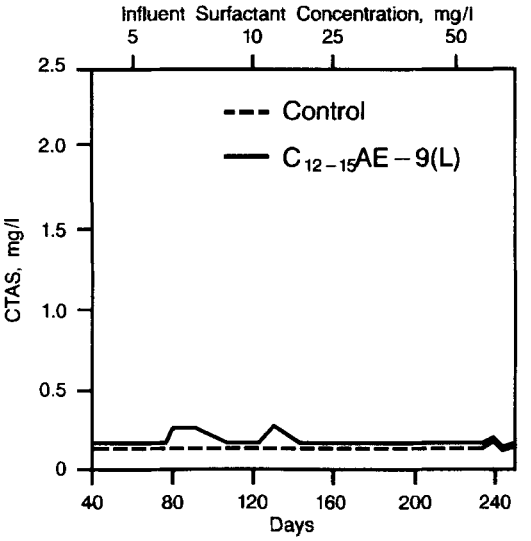

FIG. 7. Effluent CTAS at 25°C biotreatment.

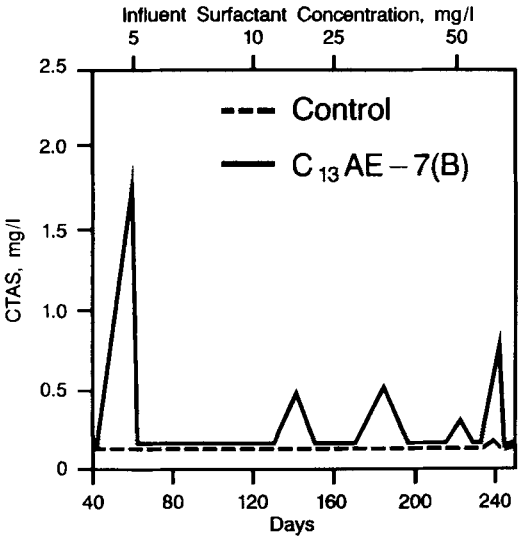

FIG. 8. Effluent CTAS at 25°C biotreatment.

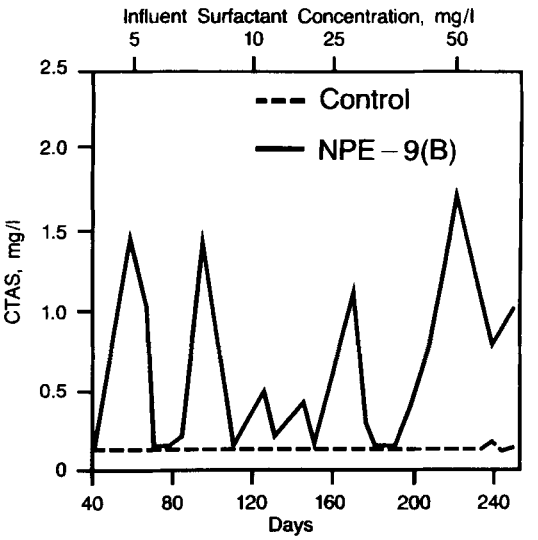

FIG. 9. Effluent CTAS at 25°C biotreatment.

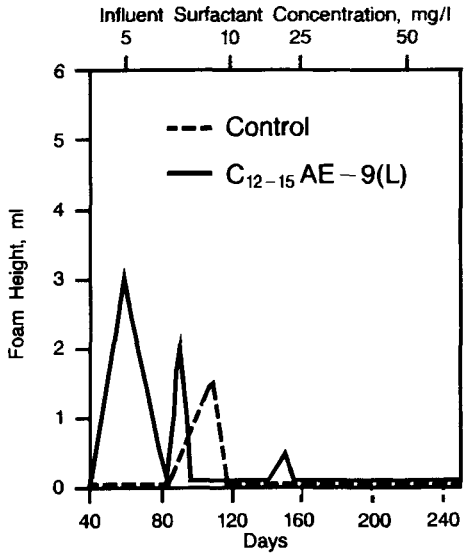

FIG. 10. Effluent foaming at 25°C biotreatment.

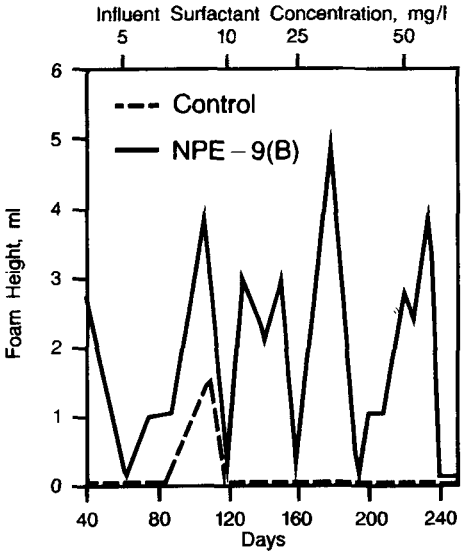

FIG. 12. Effluent foaming at 25°C biotreatment.

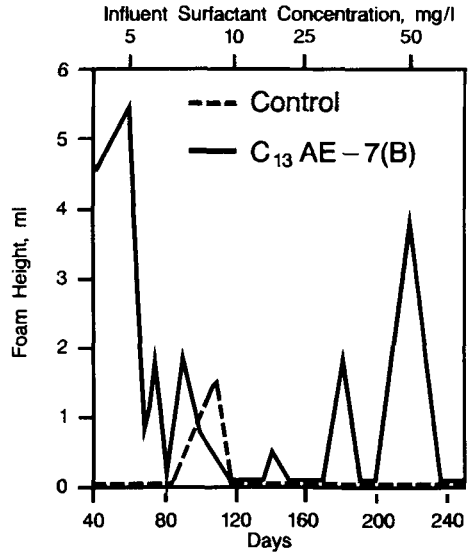

FIG. 11. Effluent foaming at 25°C biotreatment.

presence of significant levels of surface-active material in the branched surfactant units. This surface-active material is likely due to blends of intact surfactant and their partially degraded metabolites. Movement of these surface-active components to the sludge/liquor interfaces likely prevents good sludge flocs from forming and results in solids carryover into the clarifier. The linear nonionic undergoes sufficiently rapid biodegradation and does not permit accumulation of surface-active material that can sorb onto sludge and interfere with solids separation (18,24).

Aquatic toxicity tests on biotreated effluents. Acute and chronic aquatic toxicity test results for *Pimephales promelas* (fathead minnow) and *Daphnia magna* (waterflea) exposed to intact surfactants used in the continuous biotreater studies (50 ppm at 25°C) are listed in Table 4. It is apparent that the intact linear AE was somewhat more acutely and chronically toxic than the intact branched AE and branched NPE. However, Figure 16 shows that acute toxicity was present only in the effluent from the NPE-9(B) unit (20–40% LC-50s), indicating that the C_{12-15}AE-9(L) and C_{13}AE-7(B) surfactants had

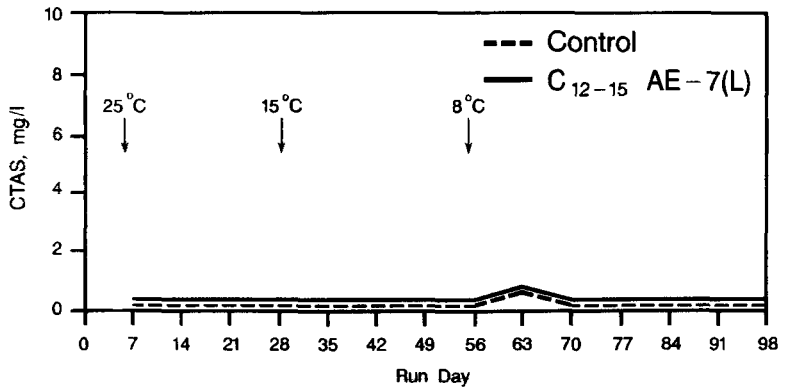

FIG. 13. Effluent CTAS at low temperature biotreatment.

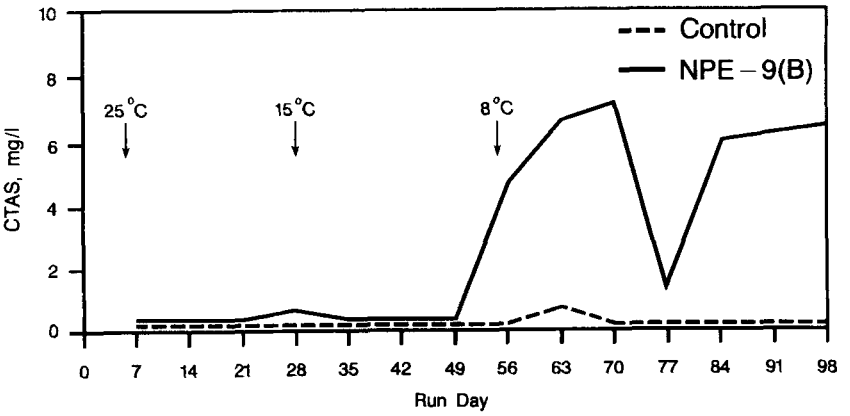

FIG. 14. Effluent CTAS at low temperature biotreatment.

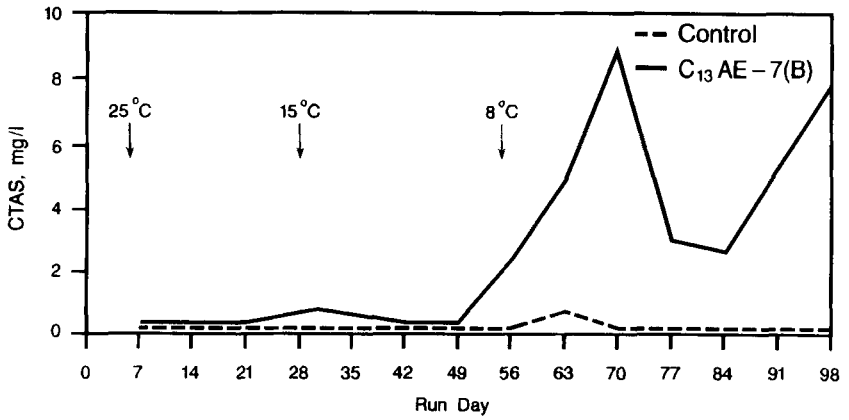

FIG. 15. Effluent CTAS at low temperature biotreatment.

TABLE 4

Acute and Chronic Aquatic Toxicity of Neat Surfactants

	Acute (mg/L)		Chronic (mg/L) growth			
			Fathead minnow		*Daphnia magna*	
Surfactant	96-hr LC50 Fathead minnow	48-hr LC50 *Daphnia magna*	NOEC	LOEC	NOEC	LOEC
C_{12-15} AE-9(L)	1.6	1.3	0.4	1.0	1.0	1.0
C_{13} AE-7(B)	6.1	11.6	1.0	2.0	4.0	4.0
NPE-9(B)	4.6	14.0	1.0	2.0	10.0	10.0

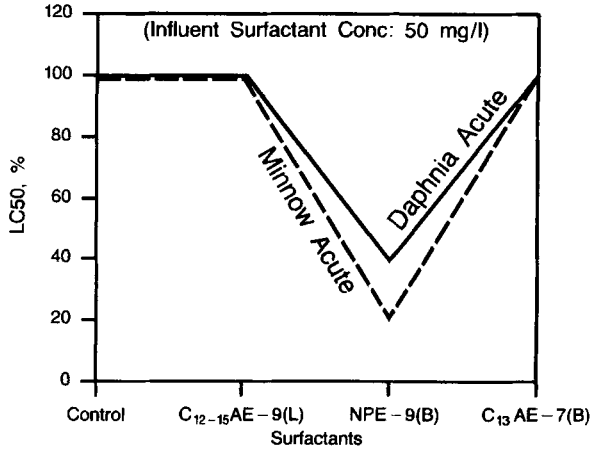

FIG. 16. Acute toxicity of biotreated surfactant effluents. Biotreated at 25°C.

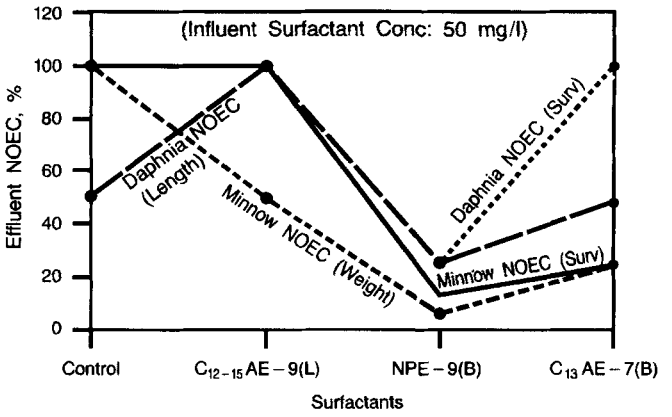

FIG. 17. Chronic aquatic toxicity of biotreated surfactant effluents. Biotreated at 25°C.

biodegraded to products that were not acutely toxic. Effluent chronic toxicity data, plotted in Figure 17, show least toxicity for the effluents from the C_{12-15}AE-9(L) unit and significant levels of toxicity for effluents from the two branched surfactant units.

Correlation of structure with environmental response. Those surfactants with more than one internal methyl branch per hydrophobe tend to biodegrade slower than surfactants based on linear hydrophobes. The lower aquatic toxicities shown by intact surfactants with more than one methyl branch is offset by their slower biodegradability, which tends to leave treatments effluents more toxic than those derived from the more linear hydrophobes.

The mechanism for biodegradation of linear AEs is known to involve rapid cleavage of hydrophobe from hydrophile (6,33) to produce non-toxic products. For biodegradation of branched NPEs, the mechanism reportedly involves a slower stepwise shortening of the hydrophilic portion to yield NPEs with shorter POE chains (18,19,33). For highly branched AEs, the few reported mechanistic studies point to a POE chain-shortening pathway similar to that for NPE (33,34). The importance here is that these NPEs and AEs with short POE chains may biodegrade more slowly and show greater aquatic toxicity (35,36) than their longer POE chain precursors. Also, due to their hydrophobic character they may tend to accumulate in effluents and on sludges and sediments (18,24,37). Analytical methodology to identify and determine AE and NPE metabolites on preserved sludges and effluents from the continuous biotreater study is underway and will be reported at a later date.

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