Biodegradation of Nonionic Surfactants Containing Propylene Oxide[1]

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Insertion of oxypropylene groups into the polyoxyethylene chain of fatty and oxo alcohol ethoxylates decreased the rate and extent of biodegradation. The magnitude of the effects was proportional to the PO-block size and the extent of branching in the alcohol. Data are presented from a series of PO-containing nonionic surfactants in semi-continuous activated sludge reactors over test periods of up to 12 weeks. Bioassays performed on effluents from the SCAS units showed little or no toxicity due to residual degradation metabolities toward *Pimephales promelas* and *Daphnia pulex* and a stimulatory effect on *Selenastrum capricornutum* growth rate.

The ability of a surfactant to be biologically degraded is highly dependent on its structure. The more closely the surfactant hydrophobe resembles naturally occurring lipids, the more readily it is biodegraded. Hence, linear hydrocarbon-based surfactants degrade more rapidly and more completely with less acclimation time than those with branched or cyclic structures.

The polar portion of a surfactant also has an important influence on the course of biodegradation. The most commonly used nonionic surfactants have a polyoxyethylene (POE) chain as the hydrophile. POE chains have been shown to biodegrade, the rate and extent being inversely dependent on chain length (1). Two mechanisms of POE chain degradation have been proposed; cleavage at the α-carbon of the hydrophobe followed by breakdown of the polyethylene glycol (2), and step by step oxidation and hydrolysis of terminal glycol groups (3).

Oxypropylene groups, whether a part of the hydrophobe or hydrophile, introduce pendant methyl group branching when included in a surfactant. The poor or inconsistent biodegradability of propylene oxide-ethylene oxide (PO-EO) block copolymer surfactants in which the hydrophobe is a high molecular weight polypropylene glycol can be understood as a consequence of this high degree of branching (4).

Many specialty nonionic surfactants contain oxypropylene groups, either as a PO block or mixed with oxyethylene groups, within the hydrophile. The oxypropylene groups modify the physical and surface active properties of ethoxylates (5). For example, an oxypropylene block cap on an ethoxylate lowers its foaming power and cloud point. A PO block in the middle of the ethoxylate chain lowers its pour point.

PO-containing nonionics based on detergent alcohols are an important class of surfactant, with sales exceeding 20 million pounds/year in the U.S. (6). Because their usage is very small compared to alcohol ethoxylates, little attention has been focused on their environmental properties.

Quantitative information is sparse on the biodegradability of PO-containing nonionics. PO-EO block copolymers undergo only partial degradation (2). PO-capped ethoxylate low-foaming nonionics likewise fall short of the statutory requirement of the Organization for Economic Cooperation and Development of >80% primary degradation (7).

The most extensive data have been reported by Henkel (8). Measurement of ultimate degradation by biological oxygen demand (BOD) and dissolved organic carbon (DOC) assays showed that PO-capped ethoxylates having about 3.5 PO groups were about the upper limit in PO-block size for sufficient biodegradability. Other reports suggest similar limits, but provide less quantitative information (9–11).

The present study was undertaken to assess the biodegradability of alcohol ethoxylates containing oxypropylene blocks within the ethoxylate chain. The goal was to determine a quantitative correlation between PO block size and degradation.

EXPERIMENTAL

Primary and ultimate biodegradability tests were conducted in semi-continuous activated sludge (SCAS) bioreactors using the Soap and Detergent Association method (12-14). The SCAS method provides an inexact model of a sewage treatment plant. The small scale of operation and lack of infusion with fresh sewage culture during the test period are adequate to establish the inherent biodegradability of chemicals. But more resistant substances may have difficulty acclimating and achieving steady state degradation rates within the time limits of the test.

Assay of primary degradation was by foam height, while dissolved organic carbon (DOC) analysis was the method for determining ultimate degradation.

Toxicity testing of SCAS reactor effluents was performed on selected surfactant samples. Organisms used for the bioassays were *Pimephales promelas* (fathead minnow), *Daphnia pulex* (water flea), and *Selenastrum capricornutum* (a freshwater green alga).

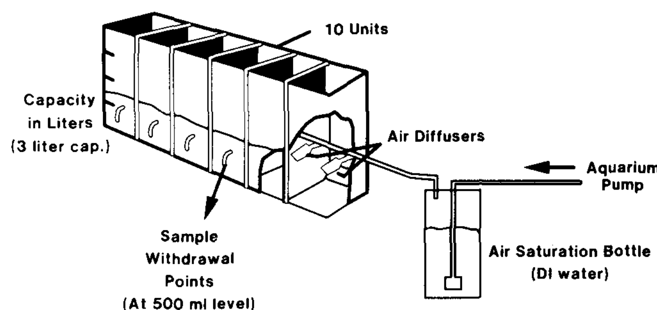

FIG. 1. Schematic of SCAS reactor test system.

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The experimental surfactants selected for these studies were linear and branched detergent alcohol-EO-PO adducts in which either the PO block was located between two EO blocks of equal length or PO was premixed with EO. They were prepared by base-catalyzed addition of ethylene oxide and propylene oxide (5). Cloud points were in the range 50° to 65°C. Primary degradation studies involved 10 alcohol ethoxylates and PO-containing ethoxylates; one was a commercial product (Surfonic® JL-80X, Texaco Chemical Co.). The study of ultimate biodegradability involved five surfactants, three of which are commercial products, including JL-80X.

Bioreactor test system. The experimental bioreactor test system used during the study of ultimate biodegradability is depicted in Figure 1. This apparatus was constructed from Plexiglas and consisted of individual test chambers of approximately three l capacity each. Each chamber was filled to contain a total of 1.5 l of combined sludge/test solution. Solution mixing and aeration were provided to each chamber by aquarium pumps connected to a manifold system that delivered air to a porous diffuser. Each chamber was fitted with a sampling port at the 500-ml volume level to allow daily withdrawal of one l of supernatant solution.

Ultimate biodegradation test program. The semicontinuous activated sludge (SCAS) procedure for assessing the biodegradation of surfactants is based on exposing the test compound in a synthetic sewage solution to a culture of activated sludge microorganisms over a period of several weeks or months duration and analyzing residual surfactant in the supernatant. Activated sludge from a sewage treatment plant is placed in a test chamber. The test compound and synthetic sewage are added, and the mixture is aerated for 23 hr. After the aeration period the sludge is allowed to settle and the supernatant is removed. The sludge remaining in the aeration chamber is then mixed with another aliquot of the test compound and synthetic sewage and the process is repeated. The organic carbon concentration in the supernatant is then determined. Test results are compared to organic carbon removal in a control reactor fed only the synthetic sewage solution. The formulation of the synthetic sewage is given in Table 1 (15).

The five test surfactants were handled as coded solutions and were treated as blind samples during the initial nine weeks of the test program (Table 2).

TABLE 1

Composition of Synthetic Sewage

Ingredient	Approximate concentration (mg/l)[a]
Bacto-peptone	160
Beef extract	110
Urea	30
NaCl	7
$CaCl_2 \bullet 2H_2O$	4
$MgSO_4 \bullet 7H_2O$	2
KH_2PO_4	2 as P
K_2HPO_4	2 as P

[a]Prepared in tap water.

During the start-up period of the procedure each of the reactor chambers was charged with 1.5 l of mixed liquor from a domestic waste-water treatment facility. These sludges were fed the synthetic sewage solution on a daily basis using the fill and draw technique previously described, until a clear supernatant was obtained. This required approximately two weeks. At the end of the two-week period, the settled sludges from all the chambers were mixed. A 500-ml aliquot of the resulting composite sludge was added back to each reactor chamber, and the surfactant testing was started.

During the surfactant testing period each reactor was filled on a daily basis with one l of surfactant/synthetic sewage solution. The initial concentration of the surfactant in each reactor was 32 mg/l of active ingredient, which was the concentration required to provide a 15-20 mg/l DOC contribution by the surfactant.

For quality control purposes one surfactant (Sample A) was tested in triplicate.

The SCAS biodegration tests were performed over a 12-week period. The daily supernatant catch from four of the reactors was retained and composited during the final four weeks of testing to provide the four test effluents for the aquatic toxicity tests.

Primary biodegradability studies. A similar system was used for the primary biodegradation studies previous to the ultimte degradation studies described above. Activated sludge was obtained from a domestic sewage treatment plant. Test period was 32 days using a surfactant dosage of 20 ppm. Assay was by foam height.

TABLE 2

Surfactants Used for Study of Ultimate Biodegradability

Sample	Chemical type	Equivalent EO	/ PO	/ EO	Cloud point	Primary hydroxyl	Product name
A	Fatty alcohol-EO/PO/EO	3.6	1.4	3.6	58°C	75%	Surfonic® JL-80X
B	Fatty alcohol-EO/PO/EO	3.7	2.8	3.7	56°C	73%	
C	Fatty alcohol-EO/PO/EO	3.8	4.2	5.6	54°C		
AE-6.5	Fatty alcohol ethoxylate	6.5	0	0	50°C	100%	Neodol® 23-6.5
LF	PO-capped fatty alcohol ethoxylate	9	8	0	32°C	0%	Surfonic LF-17

Analytical procedures. Each test reactor supernatant was monitored three times per week for dissolved organic carbon (DOC) and foaming properties. Samples were filtered through 0.45-μm filters to remove any residual microbial cells. The techniques for these tests are described briefly in the following paragraphs.

Dissolved organic carbon (DOC). DOC was determined as the difference in the concentration of total carbon and inorganic carbon in the filtered solution samples as measured with a Beckman Model 915B carbon analyzer.

Foaming properties. The foaming properties of the supernatant solutions were also determined. In this procedure a 25-ml solution was shaken vigorously for 10 seconds in a 100-ml glass stoppered, graduated cylinder. The solution was allowed to stand for 30 seconds, and the foam height was measured.

Plotting of curves. Curves are drawn through the data points from DOC and foam die-away assays. The shape of the curves was selected from the choices in the plotting software (fitted to first order to sixth order regression polynomials) by judging the best match to the data points, or were drawn by connecting the data points using a spline fit. Data points were omitted from the plots.

The plotting system used was a Tektronix Model 4113 plotting terminal with a Model 4663 S interactive digital plotter interfaced with a Data General MV/10000 mainframe computer.

Toxicity testing of biologically treated surfactants. Four composite effluent samples were selected for toxicity testing based on the apparent biodegradability of four of the five test compounds during the first eight weeks of the SCAS procedure. The four test samples selected were the effluents from Samples A, B and C and AE-6.5 reactors. Effluents from these reactors were collected daily and composited for four to six weeks. Each daily effluent catch was filtered through a 0.45-μm filter to remove residual microbial cells prior to addition to the composite holding containers. All composites were maintained at 4°C during the six week collection period. Immediately prior to initiation of a bioassay, a sufficent quantity of effluent for the procedure was brought up to ambient temperature. Each solution was also pH adjusted to 7.0-7.5 prior to use.

Fathead minnow bioassays. Bioassays of the freshwater fish *Pimephales promelas* (fathead minnow) were performed using EPA methods (16). The fish were held

TABLE 3

Composition of Algal Nutrient Broth

Compound	Concentration (mg/l)
$NaNO_3$	25.5
$MgCl_2 \cdot 6H_2O$	12.2
$CaCl_2 \cdot 2H_2O$	4.2
H_3BO_3	0.19
$MnCl_2 \cdot 4H_2O$	0.42
$ZnCl_2$	0.003
$FeCl_2 \cdot 6H_2O$	0.16
$CoCl_2 \cdot 6H_2O$	0.001
$Na_2MnO_4 \cdot 2H_2O$	0.007
$CuCl_2 \cdot 2H_2O$	trace
$Na_2EDTA \cdot 2H_2O$	0.3
$MgSO_4 \cdot 7H_2O$	14.7
K_2HPO_4	1.04
$NaHCO_3$	15

TABLE 4

Primary Biodegradation of Oxo Alcohol-Based EO-PO Adducts

	Structure	Biodegradation after 32 days %[d]	Branching of alcohol	Typical isomer
C_{13} Oxo alcohol[a]	+ 9 EO	>99	Linear + 2-methyl branched	$CH_3(CH_2)_{11}CH_2OH$
	+ 4.5 EO + 2.0 PO + 4.5 EO	>90		
	+ 5.2 EO + 3.5 PO + 5.4 EO	>90		
	+ 4.9 EO + 5.0 PO + 6.9 EO	<80		
C_{13} Oxo alcohol[b]	+ 11 EO	99	3-Alkyl branched, both	CH_3 $(CH_2)_4$
	+ 5.0 EO + 2.0 PO + 5.8 EO	80–90	tails linear	$CH_3(CH_2)_5CH\text{-}CH_2CH_2OH$
C_{14} Aldol alcohol[c]	+ 9 EO	>90	2-Alkyl branched, tails	CH_3
	+ 3 EO + (9.3 EO, 0.2 PO mixed)	>90	have some methyl	CH_3 $(CH_2)_4$
	+ 3 EO + (4.3 EO, 0.4 PO mixed)	80–90	branching	$CH_3(CH_2)_3\text{-}CH\text{-}CH_2\text{-}CHCH_2OH$
	+ (10.6 EO, 0.7 PO mixed)	<80		

[a]Prepared from 1-dodecene. Estimated 80% linear.
[b]Prepared from 2-butyl-1-octene.
[c]Prepared from 1-hexene via the oxo reaction, aldol condensation and hydrogenation.
[d]Foam height method, SCAS system, surfactant dosage 20 ppm.

for 14 days prior to beginning the tests and were maintained under a photo period of 16 hr light and eight hr darkenss. During the holding period the fish were fed daily a dry, pelleted food, *ad libitum*, except during the 48 hr prior to testing. There was no mortality in the test fish population during this 48-hr pretest period.

Ten fish were randomly distributed to each of the test jars within 30 min after the test solutions had been prepared. Loading of fish in each test jar equalled 0.55 g/l. Each test was conducted in duplicate. The pH, dissolved oxygen, conductivity and temperature of each test solution were measured at 0, 24, 48, 72 and 96 hr of exposure period. Mortalities were recorded and removed from each jar at these time periods.

Water-flea bioassays. The water fleas *(Daphnia pulex)* used in these tests were obtained from laboratory stocks from the University of Texas, Austin, Texas. The *D. pulex* were cultured in hard water reconstituted from deionized water (16).

Ten water fleas were distributed to each test container within 30 min of preparation of the test solutions. Mortalities in the test solutions were recorded at 24 and 48 hr of exposure. The pH, dissolved oxygen, temperature and conductivity of the test solutions were measured at zero hour on a separate portion of the test solutions.

Algal bioassays. The toxicity tests with the freshwater alga *Selenastrum capricornutum* were performed using EPA guidelines (17). Stock cultures for bioassay tests were grown in a synthetic algal nutrient medium. The formulation of this medium is given in Table 3.

Test flasks were incubated at 24°C under ca. 4000 lux illumination. Flasks were shaken at about 100 rpm on an orbital shaker. Cell counts were made on the stock culture solution used for inoculating the test solutions and on the test flasks after seven days of exposure. Cell counts were performed using a hemacytometer and a compound microscope at 430X magnification. For each cell count a minimum of 13 fields were counted and the average of the cell counts reported.

Acute toxicity testing of undegraded surfactant. Acute toxicity of undegraded Surfonic® JL-80X toward aquatic organisms was determined by Espey, Houston and Associates, Inc., Galveston, Texas, using standard protocols (12-14, 18). Test organisms were the water flea (*Daphnia magna*) and bluegill fish (*Lepomis macrochirus*).

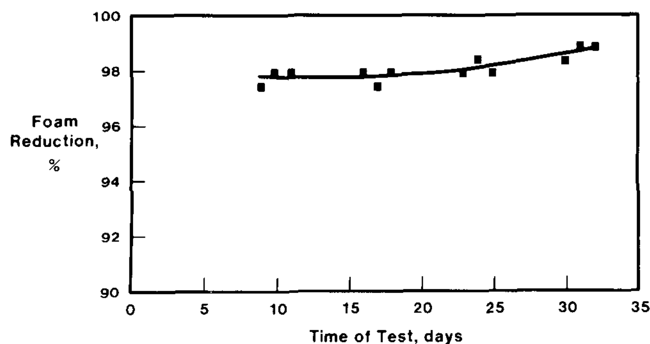

FIG. 2. Primary biodegradation of Surfonic® JL-80X. SCAS system, 20 ppm initial surfactant concentration.

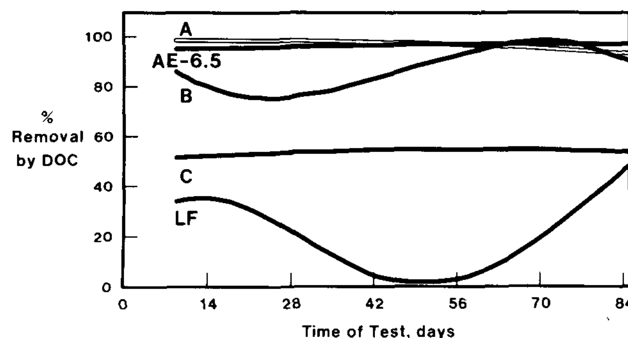

FIG. 3. Ultimate biodegradation of PO-containing surfactants. SCAS system, 32 ppm initial surfactant concentration; surfactants of Table 2.

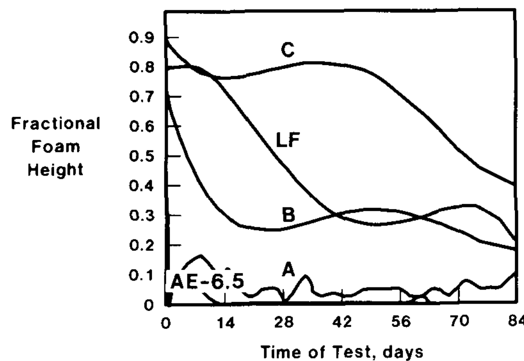

FIG. 4. Primary biodegradation of PO-containing surfactants. SCAS system, 32 ppm initial surfactant concentration; surfactants of Table 2.

RESULTS AND DISCUSSION

Oxo alcohol-PO/EO adducts primary biodegradability. The first phase of this work was a screening of oxo alcohol-EO/PO adducts. The alcohols were of three types (Table 4):

(i) C_{13} alcohol from C_{12} α-olefin (1-dodecene) via the Oxo process; linearity was about 80%.

(ii) C_{13} alcohol from C_{12} vinylidene olefin (2-butyl-1-octene) via the Oxo process.

(iii) C_{14} alcohol from 1-hexene via a three-step process: oxo, aldol condensation and hydrogenation.

The extent of branching increases in the order (i)<(ii)<(iii).

The structures were selected to show the impact of minor amounts of branching in the hydrophobe on the biodegradability of PO-containing nonionics. Assay for primary biodegradation was by foam height compared to standard solutions of undegraded surfactant. Data in Table 4 are semi-quantitative because of the nonlinear response of foam height to concentration and scatter of the measurements.

The trends strongly support some general conclusions:

- Branching in the alcohol hydrophobes does not affect the complete loss of surfactant behavior of their all-EO alkoxylates.
- Branching does diminish the level of PO in the alkoxylate which can be tolerated before incomplete degradation is noted.
- The primary biodegradation of the most linear alco-

TABLE 5

Average Surfactant Removal Over 85-Day Test Period

Sample	DOC Removal ± 95% confidence limits (days 8-85, except as noted)	Foam reduction (days 20-85, except as noted)
A (1)[a]	96 ± 6%	90 ± 2%
A (2)[a]	95 ± 7	99 ± 1
A (3)[a]	97 ± 5	99 ± 1
B	95 ± 4 (days 43-85)	77 ± 4
C	52 ± 11	53 ± 10 (days 57-85)
AE	97 ± 5	99.8 ± 0.4
LF	40 ± 5 (days 71-85)	71 ± 6 (days 41-85)

[a]Sample A tested in triplicate.

hol (from α-olefin) can accommodate 3.5 PO, followed by up to 2 PO for vinylidene olefin-derived alcohol and no more than 0.4 PO for oxo-aldol process alcohol.

Fatty alcohol-EO-PO-EO adduct. Studied under the same system was a commercial product, Surfonic® JL-80X (Texaco Chemical Co.). It is described (19) as an alcohol alkoxylate with a small block of PO (about 1.5 equivalents) in the middle of the EO chain. Figure 2 demonstrates that primary degradation of JL-80X reached ca. 97% within nine days and 99% after 31 days in the SCAS bioreactor. This result of essentially complete loss of surface activity is consistent with the earlier findings (Table 4). The small PO-block size of this product is well within the limit which can be degraded.

Primary and ultimate biodegradability of fatty alcohol-EO/PO/EO adducts. The five surfactants (Table 2) subjected to a 12-week SCAS study displayed a wide range of biodegration rates as monitored by dissolved-organic-carbon (Fig. 3) and foam height (Fig. 4) assays. Statistical averages are summarized in Table 5.

Samples A and AE-6.5 both gave essentially complete DOC removal. However, Sample A, in one of the three bioreactors, continued to have some foaming (about 10% of initial foam height) throughout the test period, while the other A reactors and AE-6.5 had virtually none after the first two weeks. The curve for Sample A in Figure 4 connects the average foam height data points from all three reactors.

Samples B and C, containing twice and three times the amount of PO as Sample A, respectively, showed less DOC removal and more foaming. Sample B attained the same high DOC removal rate as Samples A and AE during the second half of the test period. Sample C gave an average 52% DOC removal over the entire test period. Both retained considerable foaming ability, especially Sample C. Foam heights thus followed only a rough correlation with DOC removal, indicating that foaming did not decrease linearly with decreasing surfactant concentration.

The surfactant LF did not maintain stable DOC removal, declining to very low values during the middle weeks of the test before recovering to earlier levels (about 40%). Foaming power of LF remained about 30% of its initial level during the latter half of the test.

During the period of upset in the LF reactor pH measurements were made to investigate whether the observed upset could be related to pH drop. Similar studies using the SCAS method reported operational difficulties due to significant declines in pH (20). Initially, pH measurements were made only on the control and LF effluents; beginning with the tenth week pH measurements were performed on all effluents. The

TABLE 6

Surface Tension of Degraded Surfactants

Sample[a]	Initial PO, Equivalents	Surface tension, 25°C (dynes/cm)
A, Degraded effluent	1.4	63.6
B, Degraded effluent	2.8	60.7
C, Degraded effluent	4.2	62.0
AE-6.5, Degraded effluent	0	70.3
Tap water		72.0
A, 1 ppm reference		57.1
A, 0.1 ppm reference		62.6

[a]Surfactant concentrations initially 32 ppm.

TABLE 7

Acute Toxicity of Undegraded Surfonic® JL-80X[a]

Aquatic fauna	Concentration, ppm	Percent survival	LC_{50}, ppm (14)
Daphnia magna, 48 hr	1.0	98	
(water flea, 24 hr old)	1.8	92	
	3.2	22	2.6 ± 0.3
	5.6	4	
	10.0	0	
Lepomis macrochirus, 96 hr	1.0	93	
(bluegill sunfish, 1-2 g)	1.8	90	2.3 ± 0.3
	3.2	13	
	5.6	0	
	10.0	0	

[a]Conditions, 22 ± 2°C; pH 7.3-8.0 for *D. magna*, 7.9 to 8.4 for *L. macrochirus*; dissolved oxygen 7-8 ppm, aeration as needed; 50 specimens for *D. magna*, 30 for *L. macrochirus*.

majority of the reactor effluents showed decreases in pH to a level between 3.5 and 5.0 after 23 hr of aeration (the initial pH of the surfactant/synthetic sewage solutions were 7.5 to 7.8). No apparent relationship was found between pH drop and dissolved organic carbon (DOC) removal efficiency. Test solutions showing greater than 70% DOC removal showed pH decreases similar to those observed for test solutions showing less than 70% DOC removal.

SCAS reactor effluents of four surfactants were measured for surface tension at the end of the test period (Table 6). Based on the reference sample analyses, the undegraded surfactant concentrations of Samples A and AE-6.5 were less than 0.1 ppm. Primary degradation was estimated to be at least 99% for all four surfactants.

The response of the various surfactant solutions to the SCAS biodegradation test suggests three groupings for the experimental data:

- Compounds that are completely biodegradable under the test conditions (i.e., Samples A, B and AE-6.5).
- Compounds that are partially biodegradable and exhibit consistent removal characteristics for the microbially active fraction (i.e., Sample C).
- Compounds that are partially biodegradable but exhibit inconsistent removal characteristics for the microbially active fraction (i.e., LF).

The biodegradation properties of the first two groups are characteristic of most microbially active substances that are either completely or partially degraded by organic assimilating bacteria. However, Sample LF seems to exhibit the combined properties of biodegradation and bioaccumulation where the surfactant and/or its metabolites may adsorb onto the biofloc before being actively biodegraded. If the surfactant and/or metabolites accumulate in the biofloc to a concentration that is inhibitory to the organic assimilating bacteria, biodegradation could cease. This may, in part, describe the observed effect of reduced biological activity with the surfactant LF. It is possible that the physical conditions that control the removal of this surfactant are that of adsorption/desorption which ultimately effects the rate of biodegradation. The question is whether the data follow a cyclic pattern of surfactant removal or whether the bacteria will eventually acclimate to the wastewater and exhibit a consistent treatment performance. Too few test data are available to assess the actual trend.

Biodegradation of nonionic surfactants containing oxypropylene groups is clearly inversely proportional to the amount of propylene oxide incorporated into the surfactant. For linear alcohol (fatty or oxo) EO-PO adducts the maximum PO block size which allows complete biodegradation is about four equivalents if located in the middle of the ethoxylate chain. The 2.8 PO adduct (Sample B) gave >90% DOC removal after sufficient acclimation time, while the 4.2 PO adduct (Sample C) gave 50 to 55% DOC removal (Fig. 3). The 3.5 PO adduct (Table 4) had >90% primary degradation. Less PO can be accommodated if the hydrophobe is branched (Table 4).

Acute toxicity of undegraded surfactant. Acute aquatic toxicity of undegraded Surfonic® JL-80X toward *L. macrochirus* and the *D. magna* were performed using conventional methods. Results are summarized in Table 7. LC_{50} values, the concentrations of surfactant lethal to half the test organisms, were about 2.5 ppm at 22°C for both animals (18). They are very similar to LC_{50}'s of alcohol ethoxylates of similar cloud points (21).

TABLE 8

Percent Survival of *Pimephales promelas* (fathead minnow) in Surfactant SCAS Effluents

Compound	Percent survival[b]			
	24 hr	48 hr	72 hr	96 hr
A	100	80	70	70
A, repeat[a]	100	100	100	100
B	100	100	90	90
C	100	80	75	75
AE-6.5	100	100	100	100
Control	100	100	100	100

[a]Reduced fish population.

[b]Temperature range, 21-23°C; conductivity range, 510-610 μmhos/cm (control = 120-130 μmhos/cm); pH 6.5-7.1 (control = 6.9 -7.4).

TABLE 9

Percent Survival of *Daphnia pulex* (water flea) in SCAS Effluents[a]

Compound	24-hr survival	48-hr survival
A	100%	93%
B	100%	100%
C	100%	100%
AE-6.5	100%	98%
Control	100%	100%

[a]Initial water quality characteristics: pH, 7.1-7.2 (control 8.0); dissolved oxygen, 5.8-6.2 mg/l; conductivity, 450-520 μmhos/cm; temperature, 22°C.

TABLE 10

Results of 7-Day Exposure of the *Selenestrum capricornutum* (freshwater alga) to SCAS Effluents

Compound	% Change[a]			
	100%[b]	75%[b]	50%[b]	25%[b]
A	390	400	410	630
B	360	330	350	580
C	400	450	560	670
AE-6.5	140	230	340	230
Control	100	100	100	100

[a]Percent change is ratio of cell number in exposed cultures to control at day 7.

[b]Percent effluent volume in test sample.

Acute toxicity of degraded surfactants. Bioassay testing for ecological effects was performed to determine aquatic toxicity of the biodegradation products of surfactants A, B and C. Testing also was performed using AE-6.5 as a reference material. Screening tests were conducted using *P. promelas* (fathead minnow), *D. pulex* (water flea) and *S. capricornutum* (freshwater alga).

Bioassays with Pimephales promelas. The results of the toxicity test performed with *P. promelas* are presented in Table 8. Each effluent was tested without dilution under static conditions. The observed mortalities in the effluents of PO-containing surfactants show only a slight toxic effect. These initial tests did not produce sufficient mortality to require tests with diluted effluents or to allow the calculation of an LC_{50} for any of the samples.

The mortalities that did occur may have resulted from oxygen deficiency. The solutions were not aerated during the tests, and by the end of the 96-hr test period the dissolved oxygen content had fallen to extremely low concentrations (<1.0 mg/l). The acute toxicity test procedure recommends a minimum concentration of dissolved oxygen of 40% of saturation to maintain healthy fish populations during bioassay. In most test chambers, the dissolved oxygen concentration was below this level after 48 hr of bioassay testing. The observed drop in dissolved oxygen may have been due to the residual chemical oxygen demand of the degradation products or to a slight overloading of fish in the assays.

Generally the assays are conducted with 15 l of solution and 10 test fish. For this program, there was only sufficient effluent available to use nine l of solution for the tests for the majority of the compounds. Sufficient effluent of Sample A was available to repeat the bioassay for this compound with 20 l of solution, thereby reducing the fish loading and giving a residual dissolved oxygen concentration after 96 hr of ca. 4.0 mg/l, which is above 40% of saturation. The results of the retest with Sample A (0% mortality) indicate that low oxygen concentration was the likely cause of the observed mortalities during the first test.

Bioassays with Daphnia pulex. The results of the toxicity tests performed with the aquatic invertebrate, *Daphnia pulex*, are presented in Table 9. The results of these test show that the biologically treated surfactants did not exhibit toxicity.

Algal bioassays. Table 10 presents the results of the toxicity tests with *S. capricornutum.* For each concentration tested the resulting increase in cell numbers during the seven-day test period was compared to the resulting increase for the control samples during the same period.

No toxic effects were observed during the seven-day period for any of the effluents tested. In the growth tests performed with *S. capricornutum* all effluents assayed showed an apparent enhancement in the growth of this freshwater algae. The observed increase in cell growth with the percent decrease in effluent concentration is most likely due to an optimization of the utilization of carbon, nitrogen and phosphorus by the microalgae. The growth stimulation is attributed to the metabolites being readily available as nutrients. Sample C had the largest effect, AE-6.5 the smallest.

ACKNOWLEDGMENT

The primary biodegradation studies were conducted by R.D. Matson of Texaco Chemical Co.

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