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Ultimate biodegradability of detergent range alcohol ethoxylates

by

L. Kravetz, H. Chung, J.C. Rapean, K.F. Guin, W.T. Shebs
Shell Development Company

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Introduction

Alcohol ethoxylates, the fastest growing major surfactant today[1], have been reported to be readily biodegradable. However, the biodegradability criteria used in many of the earlier studies generally were limited to surface tension, foam height measurements or loss of capability to react with a chemical reagent specific for undergraded surfactant.[2,3,4] While these primary biodegradability studies were adequate during the surface water foaming controversies of the 1960's, today more stringent criteria are expected by regulatory agencies who are questioning the possible formation of persistent, environmentally harmful, biodegradation intermediates of chemicals to which the general population may be exposed. Consequently, recent studies have focused on ultimate biodegradability[5,6,7] and have shown at least 60 percent biodegradation of alcohol ethoxylates to CO_2 and water in shake flask, activated sludge or river die-away conditions.

More recently, Tobin and co-workers[8,9,10] have reported results which suggest that the polyoxy ethylene (POE) chain of primary alcohol ethoxylates (AE) may be more resistant to microbial degradation than had been indicated in previous studies. These workers used a technique in which ether linkages in residual (POE) were cleaved with hydrobromic acid followed by gas chromatographic analysis of the resulting dibromethane and alkyl bromides. The levels of residual POE and alkyl chain from intact and partially degraded alcohol ethoxylate were then calculated. Using the HBr-GC approach these workers showed that only 20-25 percent degradation of POE and almost 100 percent degradation of the alkyl chain had occurred. In contrast, Nooi and co-workers[11] have reported 49 percent biodegradation of the POE chain to CO_2 and H_2O using radiolabeled linear primary alcohol ethoxylates. Wickbold[7] has reported that a C_{12} AE with an average of 13 oxyethylene units per mole of alcohol was 63 percent biodegraded to CO_2 and water. Analysis of the biodegradation products showed 13 percent POE as determined by hydriodic acid cleavage on aqueous solutions which had previously been foam-fractionated to separate intact surfactant.

In order to study the effect of nonionic surfactant structure on ultimate biodegradability, a shake flask study has been carried out using commercial nonionic ethoxylates which varied in hydrophobe type, degree of hydrophobe branching, and oxyethylene content. A major focus of this study was to determine the extent of POE chain degradation using the HBr-GC procedure. As an alternative approach the use of an alcohol ethoxylate labeled with tritium in the hydrophobe and ^{14}C in the hydrophile permitted a comparison of the relative biodegradabilities of the alkyl and POE chains. Carbon dioxide evolution and dissolved organic carbon measurements were used to determine ultimate biodegradability for all substrates whide cobaltothiocyanate complexing was used to determine primary biodegradability for the nonionic substrates.

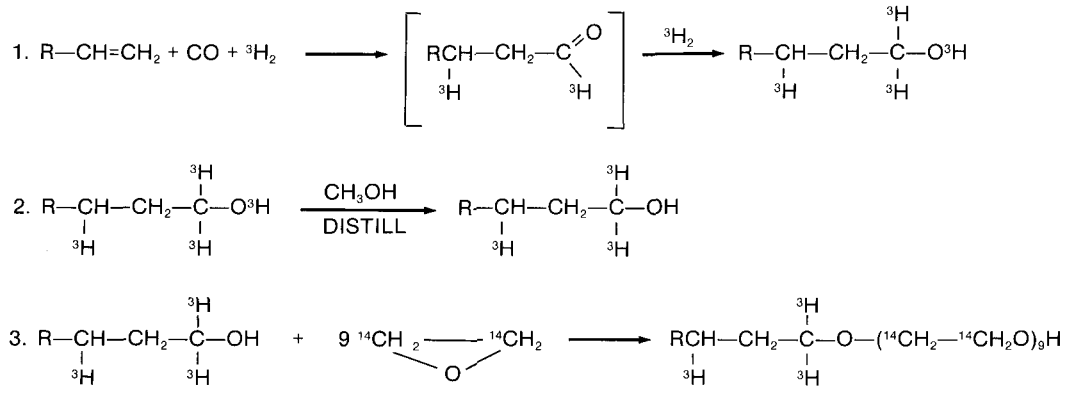
Experimental

Substrates

Normal alcohol ethoxylates having 75-80 percent linearity were manufactured from mixtures of synthetic primary alcohols consisting of 75-80 percent normal alcohols and 20-25 percent isomeric 2-alkyl (predominantly 2-methyl) primary alcohols. These alcohols were manufactured by hydroformylation of olefins derived from linear paraffins using proprietary technology. NAE 91, NAE 25, and NAE 4 were C_{9-11}, C_{12-15} and C_{14} alcohols having average carbon numbers of 10.1, 13.5, and 14, respectively. They were ethoxylated to average oxyethylene chain lengths ranging from 6-12 to produce NAE 91-6, NAE 25-7, NAE 25-9, NAE 25-12, and NAE 4-9 alcohol ethoxylates.

The 45 percent normal alcohol ethoxylate (45% NAE 25-9), 100 percent linear secondary alcohol ethoxylate (LSAE 15-9), branched octylphenol ethoxylate (OPE 10) and C_{13} linear alkylbenzene sulfonate (C_{13} LAS) are described in Table 1 and were obtained from the sources indicated.

Figure 1/**Preparation of labeled alcohol ethoxylate**



Preparation of labeled surfactant NAE 4-9

The labeled surfactant was made by condensing 1,2-^{14}C-ethylene oxide with ^{3}H-tetradecyl alcohol. The overall reaction scheme is shown in Figure 1.

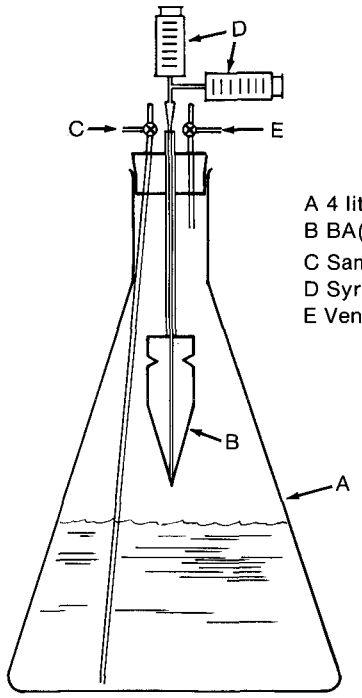
The alcohol was prepared from normal 1-tridecene by hydroformylation using commercial proprietary technology in a miniature pilot plant. Tritium gas in the CO/H_2 synthesis gas provided the label. From the hydroformylation reactions the expected positions of the label (after removal of the labile hydroxyl tritium by exchange) are the alpha and gamma carbons in the ratio of 2:1. However, substantial amounts of additional tritium are incorporated into the molecule by exchange, and the ratios are approximately 2:1:1, alpha: gamma: general. The alcohol was purified by vacuum distillation followed by preparative scale GLC. Analysis by gas-liquid radiochromatography (GLRC) showed no radioactive impurities present. The alcohol contained 65% normal alcohols and 35% 2-alkyl isomers.

The specific activity of the alcohol was 3.14 μCi/g. The alcohol was diluted with carrier tetradecyl alcohol made by the hydroformylation process and having an overall composition similar to the labeled alcohol.

To obtain a labeled ethoxylate with a composition similar to the commercial product, the ethoxylation was done on a relatively large scale (80 grams) in an apparatus with known operation characteristics. This also allowed the use of low specific activity ethylene oxide, so dilution of the final product with inactive carrier was not necessary.

The labeled ethylene oxide was purified by preparative scale GLC and its radiochemical purity verified by GLRC - before dilution with inactive ethylene oxide. Ethoxylation was carried out in a stainless steel autoclave using the commercial conditions. The ethoxylate product was shown to contain 9.1 and 8.8 EO groups/mole of alcohol by radioactivity and NMR measurements, respectively. Analysis also showed the final product contained 3.5% free alcohol and 2.8% polyethylene glycol (PEG). The specific activity was 0.0297 μ Ci ^{3}H per mg and 0.0070 μ Ci ^{14}C per mg surfactant.

Figure 2/**Shake—flask CO_2 evolution reactor**



A 4 liter Erlenmeyer flask
B $BA(OH)_2$ reservoir
C Sampling tube
D Syringe
E Vent tube

Test method

Surfactant biodegradation was studied using a modification of Gledhill's shake-flask CO_2-evolution test method.[12] Larger reactor test units (4-liter Erlenmeyer flasks) shown in Figure 2 were used due to the increased volume of reaction medium required to monitor the degradation progress. All of the substrates were tested in duplicate using a common acclimated innoculum (24 test units), which required the use of two identical gyrotory shakers (New Brunswick Scientific, Model G10-21). The influence of agitation variability of the two shakers was minimized by deploying the test units on the shaker platforms at random.

Fresh raw sewage and unchlorinated secondary wastewater effluent were obtained from the Houston Sims Bayou Sewage Treatment plant and used as the inoculum source. To insure that the bacteria were viable, we determined, qualitatively, their capability to metabolize dextrose using the dissolved oxygen uptake bottle test.

The microorganisms were acclimated to each test compound in a minimal salts-vitamin (MSV) solution supplemented with Difco vitamin-free casamino acids and yeast extract.[12] This medium was inoculated with 5%w of fresh raw sewage which had been clarified by gravity-settling prior to use. Each test compound was added in increments (1.5 mg/1 on day 0, 4.5 mg/1 on day 3, 9.0 mg/1 on day 7 and 15.0mg/1 on day 11) during a 14 day incubation period at room temperature in the dark and under quiescent conditions. The acclimated inocula were pooled on the 14th day, and five volumes of the pooled inocula were mixed with one volume of fresh unchlorinated secondary wastewater effluent to form the inoculm used for the subsequent biodegradation experiments. Unlabeled NAE 4-9 was used during the acclimation phase to avoid the presence of radioactivity in the pooled inocula.

The reactor test unit was charged with 240 ml of freshly prepared acclimated inoculum and 1760 ml of MSV solution. The test compound (20 ml, 1.5 g/1 surfactant) was added to obtain a surfactant concentration of 15 mg/l. A repipet dispenser (Lab Industries) was used to charge a constant 40 ml volume of 0.2N $Ba(OH)_2$ solution into the central reservoir of the test unit to absorb the evolved CO_2. In the case of the radiolabeled surfactant 0.2N LiOH was used as the CO_2 absorber to facilitate radiotracer analysis. The test unit was purged with 70% O_2/N_2 gas to provide sufficient oxygen for complete surfactant oxidation to CO_2 and then sealed. Biodegradation was carried out at 24 ± 1° C in the dark to minimize algal growth. The test units were agitated on a gyrotory shaker at 120 rpm for 29 days or longer where required.

Biodegradation progress was monitored by sampling the reaction media and removing the entire CO_2 absorber solution for analysis on run days 1, 3, 7, 14, 21, 28, and 29. Fresh CO_2 absorber solution was recharged after every sampling period. On the day prior to the end of the test, 20 ml of 20% H_2SO_4 was added to each medium to convert carbonates to CO_2.

All sampling operations were carried out with the test unit under 70% O_2/N_2 atmosphere. Nitrogen purged bottled distilled water was used for reducing the hold-up of the CO_2 absorber solution in the central reservoirs of the test units. Sample containers for TOC and DOC analysis were cleaned by thermal treatment at 550° C for one hour. Other glassware was cleaned by rinsing in dionized water, then in methanol, and finally in deionized water. Drying was at 105° C.

After sample removal, the CO_2 absorber reservoir was rinsed twice with a constant volume of 50 ml distilled water. The rinse water was recovered and combined with the reacted absorber solution sample for analysis. Fresh 0.2N $Ba(OH)_2$ or 0.2N LiOH was recharged into the central reservoir. After purging with 70% O_2/N_2 gas, the test unit was sealed and the test was resumed.

Along with the test compounds, duplicate control blank runs were included which contained only acclimated innoculum and MSV solution. The biodegradation results from the analysis of these controls were substracted from the corresponding values of the test compound. The net result is the measure of surfactant biodegradation.

The biodegradation solution samples were preserved with $HgCl_2$ at a concentration of 50 mg/l immediately after sampling to avoid further sample degradation before analysis. However, for MBAS and CTAS analysis, one percent by volume of 40% aqueous formaldehyde solution was added as the preservative to avoid interference from $HgCl_2$.

Biochemical oxygen demand (BOD) tests were run separately on the substrates listed in Table 1 using an established test procedure.[13] For BOD studies, bacterial inocula had been obtained from a seed which was unacclimated and different from that used for the CO_2 evolution and DOC tests. BOD measurements were made by titrating for dissolved oxygen using the azide modification of the Winkler method.[14] Chemical oxygen demand (COD) measurements were made using the silver sulfate-mercuric sulfate modification of the dichromate method.[15]

Table 1/**Surfactant substrates tested**

Product	Molecular weight	
80% NAE 91-6[1]	425	C_{9-11} $(EO)_6$ 80% Normal alcohol ethoxylate
75% NAE 25 7[1]	515	C_{12-15} $(EO)_7$ 75% Normal alcohol ethoxylate
75% NAE 25-9[1]	603	C_{12-15} $(EO)_9$ 75% Normal alcohol ethoxylate
75% NAE 25-12[1]	748	C_{12-15} $(EO)_{12}$ 75% Normal alcohol ethoxylate
75% NAE 4-9[1]		
(^{3}H- & ^{14}C-Labeled)	601	C_{14} $(EO)_9$ 75% Normal alcohol ethoxylate
45% NAE 25-9[2]	591	C_{12-15} $(EO)_9$ 45% Normal alcohol ethoxylate
100% LSAE 15-9[2]	609	C_{11-15} $(EO)_9$ 100% Linear secondary alcohol ethoxylate
OPE 10[3]	628	Branched C_8 octyl phenol $(EO)_{10}$
C_{13} LAS[4]	362	Average C_{13} linear alkyl benzene sulfonate

[1]NEODOL® Ethoxylates marketed by Shell Chemical Company
[2]TERGITOL® Ethoxylates marketed by Union Carbide Corporation
[3]TRITON® Ethoxylates marketed by Rohm and Haas Company
[4]A blend of two commercial C_{13} LAS products obtained from the Soap and Detergent Association; active matter, 45%; 2-phenyl isomer, 22%

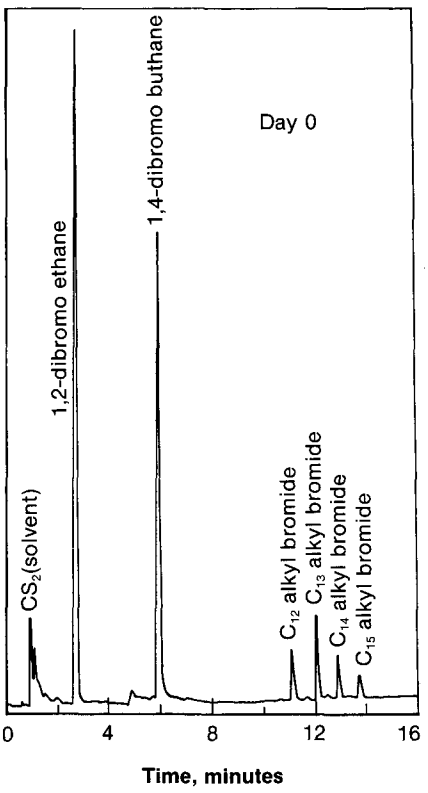
Analytical methods

Hydrogen bromide ether cleavage - GLC determination

Biodegradation of the POE hydrophilic and alkyl hydrophobic portions of the nonionic surfactants was studied by gas chromatography of the alkyl bromides and dibromides formed from the ether cleavage of the surfactant ether linkages with hydrogen bromide. The procedure[16] used for these analyses consisted of three steps. 1) chloroform extraction of the undegraded material from the aqueous biodegradation media, 2) cleavage of the ether linkages with hydrogen bromide, and 3) gas chromatographic determination of the alkyl bromides and dibromides in the fission product. Slight modifications of the literature procedure were used in which chloroform extractions were made from 70 ml of aqueous sample in which 21 gm of $MgSO_4.7H_20$ had been dissolved. A 30 ml chloroform extraction was followed by four 15 ml extractions. Prior to HBr cleavage, 0.5 ml of internal standard solution (1.0 mg POLYMEG 1000 per ml of dichloromethane) was added to the reaction vessel. Reaction of POLYMEG 1000 with HBr produced 1, 4-dibromobutane.

The concentration of the undegraded polyoxyethylene and alkyl portions of each biodegradability sample was calculated from the chromatogram of the alkyl bromides and dibromides. The method was calibrated with pure dodecyl ether of pentaethylene glycol ($C_{12}EO_5$) and with each of the nonionic surfactants used in the biodegradability study. Aqueous standards containing 1000, 500, and 200 micrograms of the nonionic surfactants were extracted and treated in the same manner as the test samples. These standards were run along with each set of nonionic biodegradability samples. A chromatogram of the HBr cleavage products of 75% NAE 25-9 is shown in Figure 3.

Figure 3/**Chromatogram of the hydrogen bromide cleavage products of 75% NAE 25-9**



The limit of detection for the polyoxyethylene materials in the biodegradability samples is about 0.2 ppm and for the alkyl compounds is about 0.5 ppm. The repeatability of the method was equal to the detectable limit below the 1 ppm level and about 10 percent at higher levels.

Since the polyoxyethylene content is determined from the hydrogen bromide fission products, the nature of the undegraded materials, i.e., intact surfactant, partially degraded surfactant, polyethylene glycols, etc., is not established. Also, only those materials which are extractable from an aqueous 1.2M $MgSO_4$ solution with chloroform are determined. Anthony and Tobin[10] report 90-100 percent recoveries from aqueous solutions of linear alcohol ethoxylates, alkylphenol ethoxylates, PEG copolymers and long chain (> 7 EO units) polyglycols. Lower molecular weight polyglycols give lower recoveries. Compounds such as ethylene glycol, diethylene glycol and triethylene glycol are not recovered in the chloroform extract. Recoveries of alkyl and POE chains from standard solutions of nonionic ethoxylates were found to be greater than 80 percent as shown in Table 2. It should be noted that the alkyl chain of secondary alcohol ethoxylates and the alkylaryl chain of alkylphenolethoxylates were not recoverable by the above procedure.

Table 2/**Recovery of nonionic surfactants from standard solutions chloroform extraction — HBr/gc***

Substrate**	Recovery, % Polyoxyethylene	Alkyl
80% NAE 91-6	94	86
75% NAE 25-7	93,86	85,81
75% NAE 25-9	95,81	85
75% NAE 25-12	103,113	85,90
75% NAE 4-9	103	80
45% NAE 25-9	78,88	82
100% LSAE 15-9	101,97	—
OPE 10	80	—
PEG 400	70,71	—

*150° C, 3 Hr
**15 PPM in aqueous solution

Organic carbon

Prior to running organic carbon measurements, samples were centrifuged at 45,000 xg for 20 minutes at 10° C using an IEC B-20A centrifuge. Total organic carbon (TOC) and dissolved organic carbon (DOC) measurements were made using an Oceanography TOC Analyzer, Model 0524B which could detect organic carbon to the 0.1 ppm level. The following procedure was used with particular emphasis on using scrupulously clean sampling and work-up procedures in order to minimize carbon contamination.

1) One ml of sample is charged into a precombusted sterile ampoule and diluted with 4.0 ml purified distilled H_20 (Super Q Ultrapure water millipore system).
2) Inorganic carbon is removed by acidification with persulfuric acid and oxygen sparging.
3) After sealing, the ampoules are placed in an autoclave at 120° C for four hours to effect the oxidation.
4) After cooling to room temperature, the ampoules are opened in a closed chamber and nitrogen is used to sparge out the CO_2 for measurement using a nondispersive infrared analyzer.

Determination of radioactivity in biodegradation samples

All radioactivity measurements were made by liquid scintillation counting in a Packard Tricarb Model 3314 or 3375 liquid scintillation counter, Packard Instrument Company, Downers Grove, Illinois. The operating characteristics for these instruments are similar and when net count rates are converted to disintegration rates, no instrumental bias is seen.

The samples were counted in "instagel" scintillator solution, a Packard proprietary mixture of aromatic solvent, nonionic surfactants and fluors capable of keeping water in suspension. In all cases, 6 ml of sample were added to 14 ml of Instagel. At this composition the samples formed a translucent gel which was stable for more than 72 hours. After 2 hours equilibration at the counting temperature of 12° C no shifts in count rate were seen for at least 72 hours. Calibration standards of the same composition were run with the samples to determine sample counting efficiencies.

Counter settings were: RED channel, 70% gain, 35-500 window; GREEN channel, 7% gain, 100-1000 window. Under these conditions the counting efficiencies for tritium were approximately 20.9% and 0.1% in the RED and GREEN channels, respectively, and for carbon-14, 7.9% and 58.8%. Background count rates were about 15 cpm in the RED and 24 cpm in the GREEN channel.

Samples were counted sequentially for 20 minute periods and then the group was recycled, counted, etc., for at least 3 cycles. No count rate changes due to decay of chemiluminescence were observed in any of the water samples or the LiOH absorber solution samples.

The sludge remaining at the termination of the experiment was collected by centrifugation and burned in a packed-tube combustion unit. The combustion products were trapped in 10 ml of 0.2N of LiOH, which was diluted to 0.08N before

Table 3/**Ultimate biodegradation of nonionic and anionic surfactants**

CO_2 evolution method

Conditions: Acclimated inoculum
surfactant concentration, 15mg/l
test medium volume = 2l

Surfactant	Test No.	CO_2 evolution[1], accumulative, mg day 1	3	7	14	21	28	29	35	42	50
80% NAE 91-6	1	0.36	12.96	18.02	33.31	42.71	45.15	45.14			
	2	1.37	16.17	30.91	47.66	57.41	62.76	62.76			
75% NAE 25-7	1	1.87	12.28	27.02	49.23	54.48	56.39	62.82			
	2	2.62	10.84	21.36	43.21	47.21	48.64	48.64			
75% NAE 25-9	1	1.37	8.21	22.30	48.52	54.60	55.55	55.55			
	2	0.62	6.92	19.71	46.29	54.03	56.91	56.91			
75% NAE 25-12	1	3.38	9.68	27.66	49.87	57.61	60.49	62.78			
	2	0.87	6.07	26.00	46.39	52.47	54.87	54.87			
75% NAE 4-9	1	2.04	10.07	28.67	54.97	57.89	58.47	58.47			
	2	0.87	8.21	28.13	54.03	55.82	55.82	55.82			
45% NAE 25-9	1	2.37	8.94	19.14	38.07	43.32	45.72	54.22			
	2	2.37	10.04	27.69	49.90	56.31	57.86	63.08			
100% LSAE 15-9	1	0	4.10	15.92	35.10	48.90	55.38	55.64			
	2	0	3.55	15.04	33.03	44.78	49.02	49.66			
OPE 10	1	0	0	0	0.14	1.92	8.60		28.67	42.56	62.14
	2	0	0	0	0.14	2.27	6.56		8.49	26.95	49.79
C_{13} LAS	1	0	0	0	0	25.81	32.00		31.68	40.70	45.46
	2	0	0	0	9.29	25.98	33.04		37.79	43.60	50.13
Dextrose	1	8.90	21.21	30.41	35.21	37.24	36.76	38.35			
	2	8.90	21.73	31.46	36.53	36.02	36.42	40.98			

[1]Corrected for blank control

counting. Substantial chemiluminescence interferred with the tritium analysis on the combustion samples but the carbon-14 determination was unaffected.

To determine tritiated water, the solution samples from the NAE 4-9 test unit were passed through a column of Witco 718 activated carbon to remove dissolved organic materials. The column consisted of a funnel attached to a U-tube which was fitted to the bulb end of a 150 mm drying tube. The column was filled with approximately 14 gms of activated carbon. The water sample was added to the funnel and allowed to flow slowly upward through the carbon bed. A fresh, dry column was used for each sample. An elution rate of 2 ml/min was employed, and the first 10 ml of eluted solution contained carbon fines which was discarded. The collected sample was analyzed for tritium activity and DOC. The absence of the soluble organic products and the presence of radioactivity indicated the amount of $^3H_20$ present.

Primary biodegradability and aromaticity

Primary biodegradability of the nonionic and anionic substrates was monitored by cobaltothiocyanato[17] (CTAS) and methylene blue active substance[18] (MBAS) analysis, respectively.

Benzene ring degradation of the reference substrates was determined by monitoring the intense ring absorption band in the UV at 223 nm following the procedure of Swisher.[19] A Cary Model 15 spectrophotometer was used.

Results and discussion

CO_2 evolution, DOC, and BOD results

Data on CO_2 evolution, DOC, BOD, and CTAS measurements are listed in Tables 3, 4, 5, and 6, respectively. These data were then calculated as percent biodegradability using the following stoichiometry for ultimate biodegradation:

1) **For alcohol ethoxylates**

$$C_nH_{2n+1}O(CH_2CH_2O)_mH + \frac{5m+3n}{2} O_2 \rightarrow (2m+n)CO_2 + (2m+n+1)H_2O$$

Table 4/**Ultimate biodegradation of nonionic and anionic surfactants**

DOC method

Conditions: Acclimated inoculum
surfactant concentration. 15 mg/l
test medium volume = 2l

Surfactant	Test no.	DOC[1], mg/l Day 0	1	3	7	14	21	28
80% NAE 91-6	1	7.5	5.2	3.7	4.3	2.0	1.2	1.3
	2	7.4	4.7	4.2	3.6	2.3	1.1	0.6
75% NAE 25-7	1	7.7	7.3,6.3	4.9	6.2	5.2	3.5	3.5
	2	7.6	5.9	5.9	6.2	4.4	3.3	3.2
75% NAE 25-9	1	6.9	4.8	3.9	3.8	1.4	0.8	0.8
	2	6.9	4.8	4.2	3.8	0.7	1.1	0.6
75% NAE 25-12	1	6.5	4.8	4.0	3.5	1.2	0.9	0.1
	2	7.0	4.8	4.6	5.0	1.3	0.5	0.8
75% NAE 4-9	1	6.8	5.3	4.4	3.7	1.5	2.2	0.5
	2	6.0	5.0	4.2	3.6	1.1	0.5	0.6
45% NAE 25-9	1	6.7	5.3	4.1	2.5	1.3	1.3	0.8
	2	6.8	5.6	4.1	2.8	1.2	2.4	0.7
100% LSAE 15-9	1	6.8	6.4	5.4	5.6	3.3	2.3	1.3
	2	6.9	6.4	5.6	5.8	3.7	2.3	1.8
OPE 10	1	7.8	6.7	5.6	7.2	7.0	6.0	6.0
	2	7.1	6.7	5.8	7.4	6.5	6.3	6.3
C_{13} LAS	1	6.0	6.3	5.5	5.3	5.6	4.1	3.3
	2	6.5	6.6	5.0	7.0	5.8	4.3	3.0
Dextrose	1	5.9	0.3	0.8	0.8	0.8	0.2	0.4
	2	5.6	0.5	0	0.6	0.6	0.6	0.5

[1]Corrected for blank control

Table 5/**BOD results on surfactant substrates**

Substrate	Micrograms O_2/gram substrate x 1000 5-day BOD	17-day BOD	29-day BOD	COD Found	COD Theoretical
80% NAE 91-6	772	1590	1900	2160	2424
75% NAE 25-7	765	1610	1960	2220	2346
75% NAE 25-9	644	1490	1620	2040	2269
75% NAE 4-9	706	1460	1620	2150	2316
75% NAE 25-12	430	1380	1740	2070	2150
45% NAE 25-9	601	1490	1560	2100	2312
100% LSAE 15-9	445	1130	1440	2090	2207
OPE 10	573	1010	1060	2030	2242
C_{13} LAS	345	392	606	2326	2387
Dextrose	694	704	794	1140	1067

2) **For OPE 10**

$C_{14}H_{21}O(CH_2CH_2O)_{10}H + 44\ O_2 \quad 34\ CO_2 + 31\ H_2O$

3) **For C_{13} LAS**

$C_{19}H_{31}SO_3Na + 27\ O_2 \quad 19\ CO_2 + 15\ H_2O + NaHSO_4$

4) **For dextrose**

$C_6H_{12}O_6 + 6\ O_2 \quad 6\ CO_2 + 6\ H_2O$

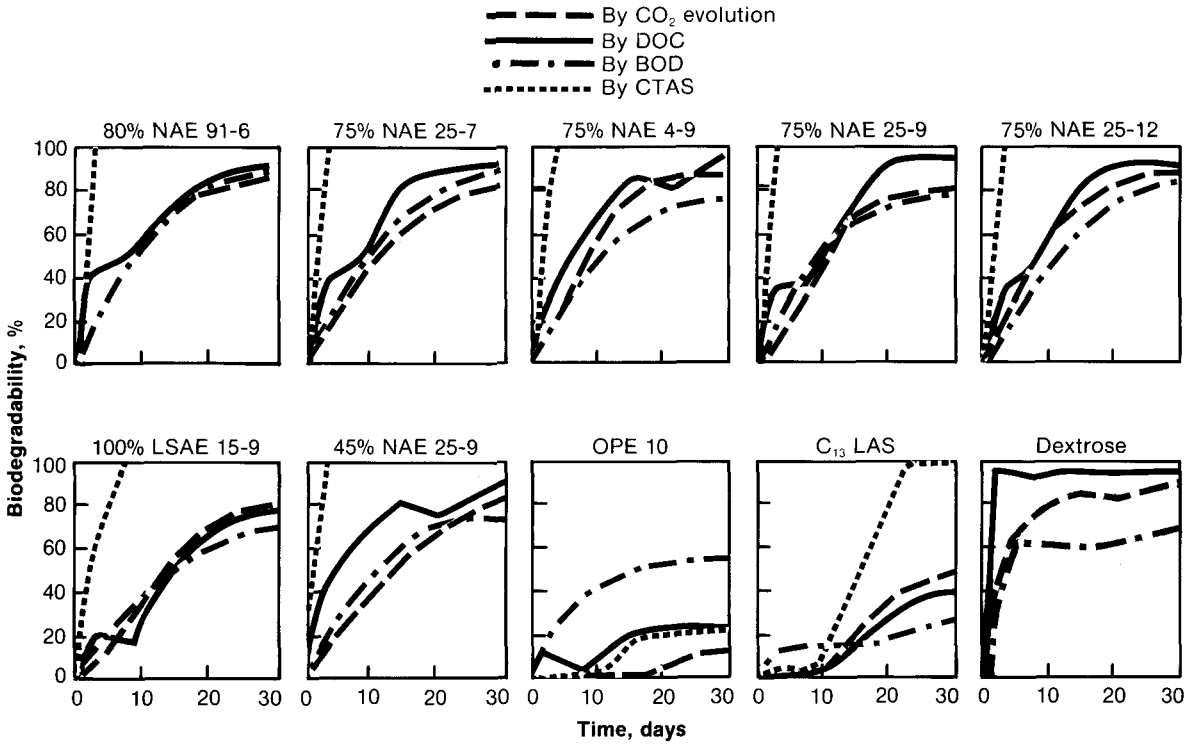
Table 6/**Primary biodegradation of nonionic and anionic surfactants**

Conditions: Acclimated inoculum
surfactant concentration, 15 mg/l
Test medium volume = 2l

Surfactant	Test no.	CTAS, mg/l Day 0[2]	1	3	7	14	21	28	50
80% NAE 91-6	1	14.0	6.7	0.5					
	2	14.8	5.6	0.5					
75% NAE 25-7	1	12.7	0.8	0.9					
	2	13.2	0.9	0.5					
75% NAE 25-9	1	12.8	1.7	0.6					
	2	11.7	1.8	0.5					
75% NAE 25-12	1	13.8	0.5	0.8					
	2	13.2	1.7	0.6					
75% NAE 4-9	1	13.6	0.9	0.5					
	2	13.8	0.8	0.5					
45% NAE 25-9	1	13.0	1.9	0.5					
	2	12.5	0.5	0.5					
100% LSAE 15-9	1	15.2	11.0	1.0	0.5				
	2	14.3	14.2	1.1	0.5				
OPE 10	1	15.2	13.9	15.2,16.0	17.0	12.9		10.7	0.5
	2	16.0	14.9	15.5	17.0	12.3		13.9	0.5
C_{13} LAS[1]	1	13.8	11.3[3]	14.3	12.5	13.2	1.3	0.1	
	2	14.3	11.5[3]	14.2	13.2	3.9	1.1	0.1	
Blank	1	0.5							

[1]Basis MBAS analysis.
[2]A Lag time of 0.5 to 4 hr was required for sampling all of the test units.
[3]Inoculum removed from sample by centrifugation.

Figure 4/**Biodegradability of surfactant substrates**



The results are plotted in Figure 4. As shown, all of the alcohol ethoxylates biodegraded to CO_2 to the extent of 80-88 percent over 28 days. As expected, dextrose biodegraded faster than the surfactants. The 45% NAE 25-9 biodegraded at about the same rate as the 75% linear NAE 25-9 indicating that this range of branching in these oxo-derived alcohol ethoxylates has no appreciable effect on biodegradation rate. These results are in line with data reported by Borsari *et al*[20] who used primary biodegradability tests to show that oxo-derived primary alcohol ethoxylates with 58 percent branching biodegrade at rates which are not appreciably different from primary alcohol ethoxylates with virtually zero branching. The 100% linear secondary ethoxylate, listed as 100% LSAE 15-9 in Figure 4, biodegraded slightly slower than the primary alcohol ethoxylates in line with CO_2 evolution results reported by Sturm.[5]

OPE 10, a branched alkylphenol ethoxylate, biodegraded considerably slower and to a lesser extent over the 28-day duration of the test than did the alcohol ethoxylates. Laboratory and field trial studies by Mann and Reid[21] have shown that a branched OPE 9 biodegraded considerably slower than the NAE 25-9 used in this work with biodegradability differences considerably greater during winter than during summer. These results are in agreement with those reported by Borstlap and Kortland[22] and Sturm[5] who have shown that multibranching, aromaticity, and relatively long ethylene oxide chain length tend to decrease biodegradability. More recently, aquatic toxicity studies by Reiff[23] have shown that branched alkyl phenol ethoxylates lose their toxicity to rainbow trout in a river die-away test much more slowly than 75 percent linear primary alcohol ethoxylates.

DOC data generally was in good agreement with CO_2 evolution data. The slightly greater levels of biodegradability indicated by DOC results at various stages in the biodegradation probably results from sorption-desorption of surfactants and their biodegradation intermediates. It is of interest to note that the rate of DOC disappearance generally was suppressed between days 1-7 for most of the nonionic ethoxylates studied. This may be caused by diauxic growth[24] due to the buildup of hydrolytic products to which bacteria must acclimate before cell growth resumes.

BOD tests were performed using an unacclimated industrial seed that was different from that used in the CO_2 evolution and DOC studies. Despite differences in bacterial types and population, BOD data generally paralleled most of the CO_2 and DOC results. A notable exception was OPE 10 for which BOD values were considerably greater than CO_2 or DOC values. Apparently the industrial inoculum used in the BOD test was somewhat more acclimated to alkyphenol ethoxylates than was the inoculum used in the DOC-CO_2 evolution studies.

Primary biodegradability, as measured by response of intact surfactant to cobaltothiocyanate reagent in the case of nonionics and to methylene blue in the case of C_{13} LAS, showed all surfactant substrates except OPE 10 to biodegrade faster than was indicated by the ultimate biodegradability criteria. In the case of OPE 10, CTAS and CO_2 data are similar probably because the level of biodegradation reached with this substrate over 28 days was insufficient to prevent reaction with CTAS.

During the early stages, the large differences generally observed between primary biodegradability criteria like CTAS and ultimate criteria like CO_2 evolution is an indication of the presence of biodegradation intermediates which in the case of the alcohol ethoxylates used in this study were essentially biodegradable to CO_2 and H_2O.

The biodegradability of C_{13} LAS, added as a "soft" surfactant standard was found to be considerably poorer than anticipated over the 28 day test period. On the assumption that C_{13} LAS and OPE 10 had not been afforded sufficient acclimation time, the test was extended to 50 days for these substrates. As measured by CO_2 evolution and loss of aromaticity, the biodegradation rates of C_{13} LAS and OPE 10 increased significantly over this extended time period.

The biodegradability of nonionic ethoxylates containing 9-10 oxyethylene units per mole of hydrophobe, as determined by CO_2 evolution, is shown in Figure 5. Dextrose and C_{13} LAS are shown for comparison. The rate of substrate biodegradation was found to proceed in the following descending order.

Dextrose > 75% NAE 25-9 45% NAE 25-9
100% LSAE 15-9 C_{13} LAS >> OPE 10.

Figure 5/**Ultimate biodegradation of commercial surfactants by CO_2 evolution**

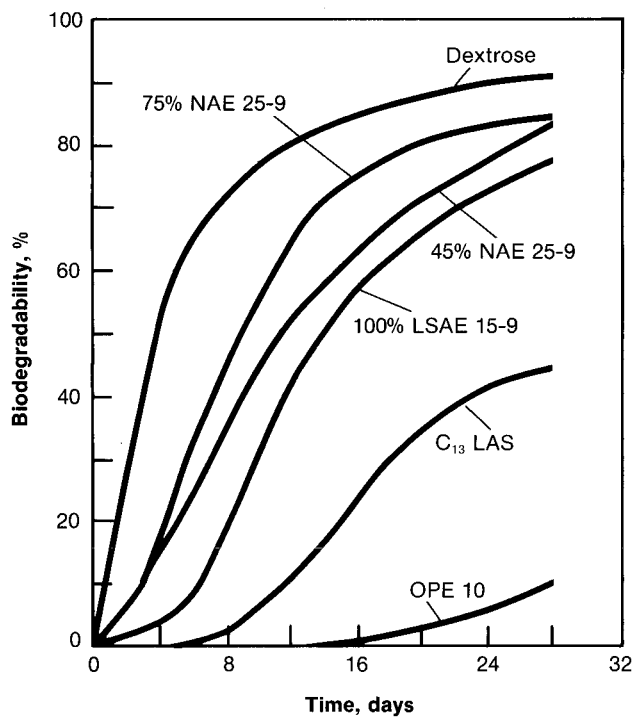


Table 7/**Ultimate biodegradation of nonionic surfactants**

HBr ether cleavage and gas chromatographic analysis

Conditions: Acclimated inoculum
surfactant concentration, 15 mg/l
Test medium volume = 2l

	Content in test media, mg/l Day											
	0		1		3		7		21		28	
Surfactant	**POE**	**Alkyl**	**POE**	**Alkyl**	**POE**	**Alkyl**	**POE**	**Alkyl**	**POE**	**Alkyl**	**POE**	**Alkyl**
80% NAE 91-6	10.1	5.5	7.2	2.9	5.3		4.3		0.6		0.3	
75% NAE 25-7	8.9	4.9	5.8	0.4	4.5		2.7		0.2		0.1	
75% NAE 25-9	10.3	4.8	9.9	0.6	6.6		4.9		0.4		0.2	
75% NAE 25-12	11.1	4.3	10.5	0.8	8.6	0.3	3.2		0.3		0.2	
75% NAE 4-9	9.8	5.3	—	—	7.2	1.5	1.5		0.5		0.5	
45% NAE 25-9	8.1	4.0	7.1	0.7	6.3		0.6		1.3		0.3	
100% LSAE 15-9	10.0		9.2		6.3		4.2		0.5		0.3	
OPE 10	9.6		9.0		9.1		8.7		7.7		7.6	

HBr-GC studies

HBr ether cleavage and gas chromatographic analysis data of the nonionics studied during biodegradation are listed in Table 7. POE and alkyl degredation by HBr-GC are shown in Figure 6 for several of the nonionics containing nearly equivalent POE chains but different hydrophobes. All nonionics except OPE 10 indicated POE had biodegraded to levels which exceeded 90 percent by the 28th day. For OPE 10, POE biodegradation had proceeded to just 20 percent. These results are slightly higher but parallel the CO_2 evolution data for total surfactant. This is expected since low molecular weight POE is poorly extracted into chloroform[10], causing results which are misleadingly high.

The data plotted in Figure 6 also show that alkyl degradation is extremely rapid as determined by the HBr-GC method. These results may be misleadingly high since all alkyl biodegradation intermediates may not be extracted into chloroform under the conditions used. Also, GC conditions may be insufficient to determine those intermediates which are extracted. Efforts are underway to determine the presence of alkyl chain biodegradation intermediates which may be unresponsive to the HBr-GC method.

Figure 6/**Biodegradability measured by HBr/GC method**

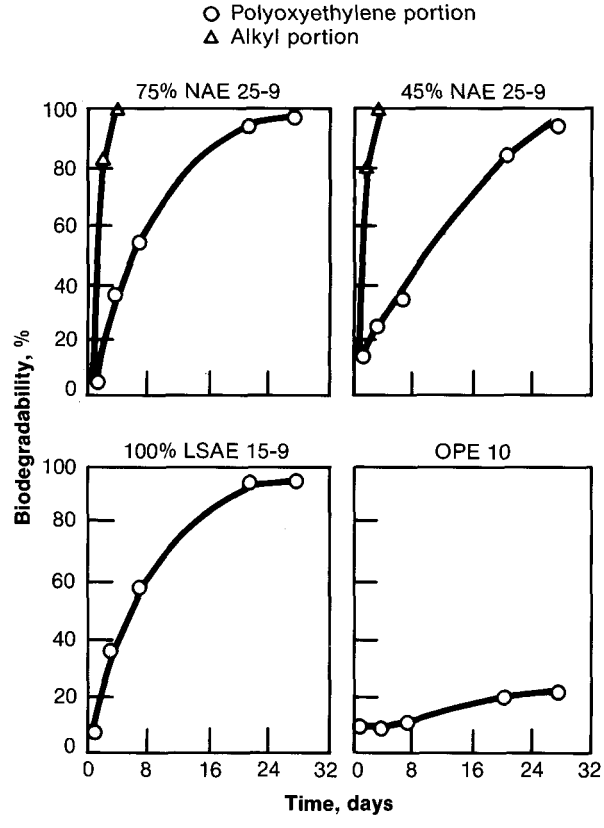


Table 8/**Ultimate biodegradation of NAE 4-9 (^3H & ^{14}C labeled) radiotracer data**

Condition: Acclimated inoculum
Neodol 4-9, 15mg/l
Test medium volume = 2l

Analysis	Day 0	1	3	7	14	21	28	29
				Test no. 1				
^{14}C activity[1] in solution, %	86.3	94.9	85.4	43.3	13.9	7.6	7.6	5.7
^{14}C activity[1] in solution, centrifuged[5]	85.0	94.9	84.5	34.3	7.4	5.4	4.5	
^3H activity[2] in solution, %	76.7	81.5	85.2	87.1	97.4	93.6	91.4	83.5
^3H activity[2] in solution, centrifuged[5]	73.2	75.5	83.1	86.8	97.6	90.5	91.0	
^3H_2O in solution, %		61.4	76.3	80.1	88.1	87.7	89.9	
$^{14}CO_2$ evolved, accumulative[4], mg		0.23	1.89	11.35	30.15	31.32	31.77	31.82
$^{14}CO_2$ from pyrolysis of sludge[3], mg								1.50
				Test no. 2				
^{14}C activity[1] in solution, %	88.4	94.4	87.1	38.2	13.2	7.2	6.6	6.4
^{14}C activity[1] in solution, centrifuged[5]	88.0	93.6	86.3	27.5	7.5	5.4	4.5	
^3H activity[2] in solution, %	77.0	80.2	84.1	86.2	98.7	92.7	91.8	87.3
^3H activity[2] in solution, centrifuged[5]	76.5	78.4	82.0	84.7	97.5	91.5	90.6	
^3H_2O in solution, %		59.4	73.8	80.7	89.1	85.4	85.9	
DOC of ^3H_2O solution, mg/l		0.3	1.2	1.0	0.6			
$^{14}CO_2$ evolved, accumulative[4], mg		0.17	1.55	12.11	30.71	31.83	32.21	32.25
$^{14}CO_2$ from pyrolysis of sludge[3], mg								1.37

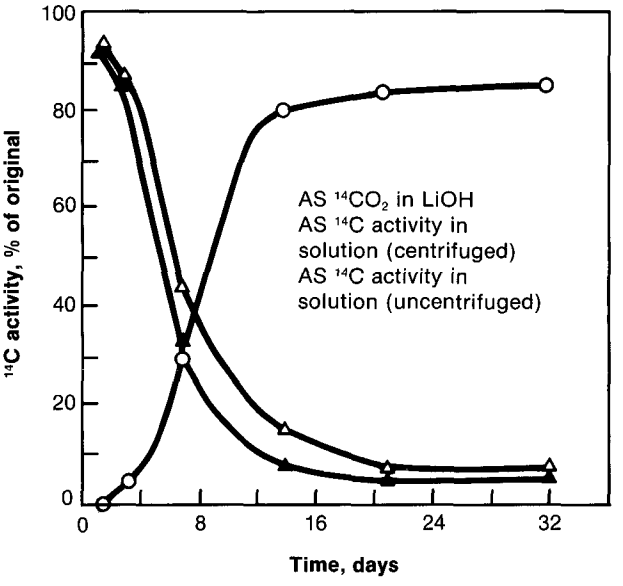
[1]Basis total initial ^{14}C activity at 15 mg NAE 4-9 (labeled)/l
[2]Basis total initial ^3H activity at 15 mg NAE 4-9 (labeled)/l
[3]Basis sludge after 29 days reaction time
[4]Corrected for blank control
[5]Inoculum removed by centrifugation

Radiotracer studies

Radiotracer data are tabulated in Table 8. As shown in Figure 7, $^{14}CO_2$ evolution from the POE chain of NAE 4-9 was slightly inhibited during the first three days but increased rapidly to a maximum of about 80 percent of the original activity by the 14th day. Also shown is the disappearance of ^{14}C activity from solution. Differences between centrifuged and uncentrifuged results in the intermediate stages of the experiement are likely due to differences in the sorption of biodegradation intermediates on bacterial media. The sludge remaining at the end of the experiment was collected, pyrolyzed, and ^{14}C content of the pyrolysis products determined. A total of 92.8% of the original activity was accounted for as follows:

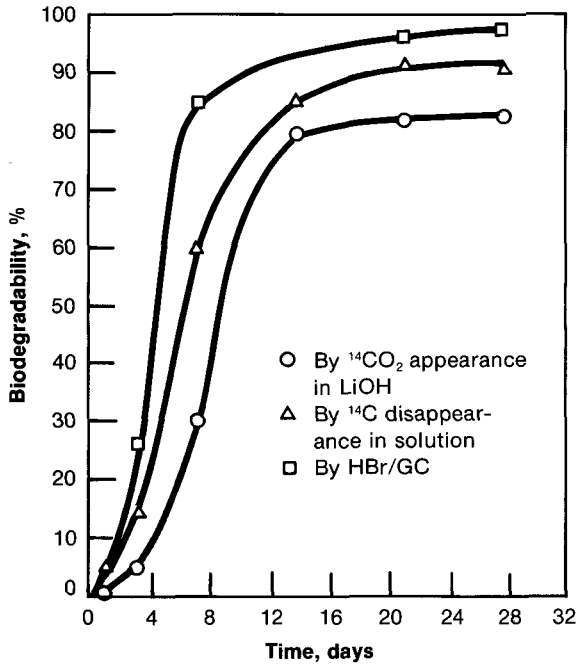
Distribution	^{14}C Activity (% of original activity)
In solution	6.1
Evolved $^{14}CO_2$	83.0
In $^{14}CO_2$ produced by pyrolysis of sludge	3.7
Recovery	92.8

Figure 7/**Biodegradation of polyoxyethylene portion of radiolabeled NAE 4-9**



The biodegradability of the POE chain of NAE 4-9 as determined by $^{14}CO_2$ evolution, ^{14}C disappearance in solution and by HBr-GC is compared in Figure 8. Each of these methods indicates greater than 80 percent biodegradability of the POE chain. Higher POE values as determined by ^{14}C in solution are probably due to uptake by the bio-mass. HBr-GC results are highest, undoubtedly because of the insensitivity of this method to lower molecular weight POE.

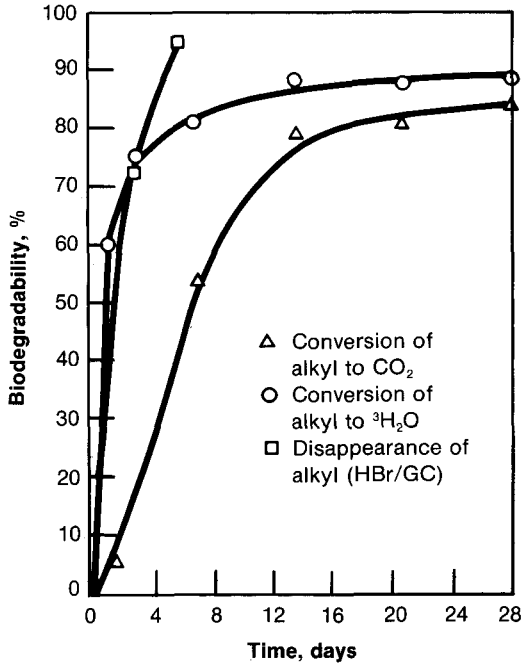
Figure 8/**Biodegradation of polyoxyethylene of radiolabeled NAE 4-9**



The 80 percent plus levels of POE biodegradability found by radiotracer and HBr-GC studies are in marked contrast to the much lower levels recently reported[10] using only the HBr-GC method. Differences in the types and populations of bacteria and acclimation time might be responsible for these disparate results.

Figure 9 compares biodegradability of the alkyl chain of NAE 4-9 by CO_2 evolution, by appearance of ^3H_2O and by HBr-GC. The alkyl CO_2 curve was obtained by substracting $^{14}CO_2$ of the POE chain from the total CO_2 determined by titration. Interestingly, in the early stages of the experiment considerably greater levels of alkyl biodegradability resulted from ^3H_2O appearance than from CO_2 evolution. These results are consistent with a biodegradation mechanism in which the initial step is cleavage of the molecule to form hydrophobic and hydrophilic products followed by alkyl degradation to CO_2 beginning at the functional group rather than at the terminal methyl group. The above mechanism is in line with studies by Patterson and coworkers[25] which indicate an initial cleavage of the alkyl material from the POE material followed by alkyl degradation. However, Patterson's studies did not indicate whether biodegradation proceeded from the functional portion of the alkyl chain or from the terminal methyl group. In contrast, Nooi and coworkers[11] indicate that the initial biooxidation takes place at the terminal methyl group prior to hydrolytic cleavage of the alkyl group from the POE group. It appears that different bacterial strains may exist with selective capability to initiate biodegradation of alcohol ethoxylates by more than one mechanism.

Figure 9/**Ultimate biodegradation of alkyl portion of radiolabeled NAE 4-9**



References

[1]Von Hennig, D. H., Household & Personal Products Industry 14, 48(1977).
[2]Frazee, C. D., Q. E. Osburn, and R. O. Crisler, JAOCS 41, 808 (1964).
[3]Huddleston, R. L. and R. C. Allred, Ibid. 42, 983 (1965).
[4]Wickbold, R., Vom Wasser 33, 229 (1966).
[5]Sturm, R. N., J. Am. Oil Chem. Soc. 50, 159 (1973).
[6]Kurata, N. and K. Koshida, Yukagaku, 24 (12) 879 (1975).
[7]Wickhold, R., Tenside, 11 (3) 137 (1974).
[8]Tobin, R. S., F. I. Onuska, D. H. J. Anthony, and M. E. Comba, Ambio 5, 30 (1976).
[9]Tobin, R. S., F. I. Onuska, B. G. Brownlee, D. H. J. Anthony, and M. E. Comba, Water Research 10, 529 (1976).
[10]Anthony, D. H. J. and R. S. Tobin, Analyt. Chem 49, 398 (1977).
[11]Nooi, J. R., M. C. Testa, and S. Willemse, Tenside 7, 61 (1970).
[12]Gledhill, W. E., Appl. Microbiol., 30, 922 (1975).
[13]Standard Methods for the Examination of Water and Wastewater, 13th Edition, p. 489 (1971).
[14]Ibid, p. 477.
[15]Ibid, p. 495.
[16]Kaduji, I. I. and J. B. Stead, Analyst 101, 728 (1976).
[17]Boyer, S. L., K. F. Guin, R. M. Kelley, M. L. Mausner, H. F. Robinson, T. R. Schmitt, C. R. Stahl, and E. A. Setzkorn, Env. Science and Technology 11, 1167 (1977).
[18]Standard Methods for the Examination of Water and Wastewater, 13th Edition, p. 339 (1971).
[19]Swisher, R. D., Yukagaku 21, 130 (1972).
[20]Borsari, G. B., F. Buosi, and E. P. Fuochi, LaRivista Italiana delle Sostanze Grasse 52, 1 (1975).
[21]Mann, A. H. and V. W. Reid, J. Am. Oil Chem. Soc. 48, 794 (1971).
[22]Borstlap, C. and C. Kortland, FSA 69, 736 (1967).
[23]Reiff, B., "The Effect of Biodegradation of Three Nonionic Surfactants on Their Toxicity to Rainbow Trout," VIIth International Congress on Surface Active Substances, Moscow, USSR, September 1976.
[24]Brock, T. D., Biology of Microorganisms, 2d Edition, p. 260 (1974).
[25]Patterson, S., C. C. Scott, and K. B. E. Tucker, J. Am. Oil Chem. Soc. 47, 37 (1970).

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