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Influence of Hydrophobe Structure on Biodegradation Pathways of Nonionic Ethoxylates

By

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Abstract
The structure of the hydrophobe in alcohol ethoxylates (AE) and alkylphenol ethoxylates (APE) has a significant influence on the biodegradation pathways and biodegradation rates of these nonionic surfactants in bacterial inocula present in waste treatment plants. APE undergo initial microbial attack at the terminal part of the polyoxyethylene (POE) chain followed by a shortening of the POE chain to leave intermediates which are slower to biodegrade than intact APE. Ethoxylates of alcohols derived from propylene or butene oligomerization appear to degrade by a similar mechanistic pathway. In contrast, ethoxylates of alcohols derived from linear olefins undergo initial attack which results in scission of the linear alkyl chain from the polyoxyethylene chain with rapid biodegradation of the hydrophobe to CO_2 and H_2O accompanied by slower degradation of the polyoxyethylene chain. The use of double radiolabeled nonionic surfactants has shed significant light on their biodegradation pathways by providing detailed information on degradation of the hydrophobic and hydrophilic moieties.

Introduction
Increasing quantities of surfactants are used in household products and such industrial and institutional applications as textile processing, pulp and paper deinking and deresination and hard surface cleaning.[1] Of the two largest classes of surfactants, anionics and nonionics, the latter have shown the fastest growth rate over the past twenty years. Such environmental issues as foaming and aquatic toxicity shown by all the major classes of surfactants may be faced by selecting those whose structural features permit rapid biodegradation to intermediates which have non-foaming and non-toxic properties under practical waste treatment conditions. In an era of increasing environmental awareness, a detailed understanding of biodegradation pathways is essential in developing an overview of the potential impact surfactants usage and treatment practices can have on aquatic life.

Secondary waste treatment, employing aerobic biodegradation, is the most widely used approach to reducing the undesired properties of organic materials, like surfactants, which enter domestic waste treatment plants. In this approach, bacteria provide enzymatic systems which utilize oxygen to convert organics to CO_2, H_2O and cellular mass. The process is complex with biodegradation proceeding through a series of intermediates produced by specific enzymes. Biodegradation rate is dependent upon the structure of the organic material assuming sufficient oxygen and viable bacteria are present. Those molecules having essentially linear structural features generally biodegrade in sewage treatment plants considerably faster than those having substantially branched carbon skeletons. A thorough review of the effect of structure on surfactant biodegradation has been written by Swisher.[2]

This paper will review the biodegradation of nonionic surfactants. The major focus will be on alcohol ethoxylates and alkylphenol ethoxylates — the two largest volume nonionics. In this paper the effect of hydrophobe structure will be discussed, since hydrophobe structure is considered more critical than that of the hydrophile in biodegradability of the largest volume nonionics. The influence of the hydrophobe on the biodegradation pathway will be examined with an emphasis on the use of radiolabeled nonionics.

Structural features of nonionic surfactants
Figure 1 summarizes the significant structural features of four classes of nonionic surfactants discussed in this paper. These are simplified representations of commercial surfactants which contain varying hydrophobe and hydrophile chain lengths. The hydrophiles are represented as polyoxyethylene chains, although such hydrophiles as polyglucosides and polyoxypropylene/polyoxyethylene chains are used to a lesser extent.

Linear Primary Alcohol Ethoxylates (LPAE)
The hydrophobes of LPAE are made in several ways:

1. Catalytic addition of CO and H_2 to linear detergent range olefins yields alcohols which generally contain 15-50% alkyl branches at the 2-carbon position.

2. Oligomerization of ethylene using aluminum alkyl technology followed by hydrolysis to alcohols which contain less than 5% branching.

3. Conversion of triglycerides, found in animal fats or vegetable oils, to corresponding alcohols which contain less than 1% branching.

Ethoxylates derived from essentially linear alcohols produced by the above processes have been shown in many extensive studies[3-10] to biodegrade rapidly to products which do not foam or show toxicity to aquatic life.

Branched Primary Alcohol Ethoxylates (BPAE)

The hydrophobes of BPAE are produced by oligomerization of propylene or butene followed by catalytic addition of CO and H_2 to yield highly branched alcohols. The ethoxylates of these alcohols biodegrade more slowly and less extensively than the linear alcohol ethoxylates.[11,12]

Linear Secondary Alcohol Ethoxylates (LSAE)

The hydrophobes of LSAE are made via borate-modified oxidation of n-paraffins to form inorganic esters followed by hydrolysis. The resulting alcohols contain secondary hydroxyl groups randomly located along the linear alkyl chain. LSAE biodegrade slightly slower than LPAE.[13,14]

Alkylphenol Ethoxylates (APE)

The hydrophobes of most commercial APE are made by reacting phenol with either propylene trimer or diisobutylene to form nonylphenol or octylphenol. These products contain an aromatic moiety and extensive branching in their alkyl chains. It has been shown that APE biodegrade more slowly and less extensively than LPAE.[3,15-20] The difference is more pronounced when the treatment system is operating under stress conditions such as low temperatures and high surfactant loadings.

Primary and ultimate biodegradation

As shown in Figure 2, biodegradation of nonionic surfactants to intermediates having different physical and chemical properties is termed primary biodegradation. In the example shown, a surface-active, aquatically toxic alcohol ethoxylate has degraded to less surface active, less toxic intermediates. Surfactant intermediates may be relatively resistant to further microbial attack when they arise from nonionics which contain significant branching and/or aromaticity. These intermediates, shown in Figure 3, are more toxic to aquatic life than their intact precursors.[21] The older methods used to measure primary biodegradation, such as foaming[3] and cobalt thiocyanate active substance (CTAS),[22] frequently do not determine the presence of these less biodegradable intermediates. Newer methods, such as high performance liquid chromatography (HPLC)[23-25] and gas chromatography (GC) coupled with mass spectroscopy (MS),[26,27] are useful for the determination of these intermediates.

The aerobic biodegradation of a linear alcohol ethoxylate to its ultimate products CO_2 and H_2O is shown schematically in Figure 4. Also shown are methods for determination of ultimate biodegradability. Even the most rapidly biodegradable substrates such as glucose, are not completely mineralized to CO_2 and H_2O since some organic material is converted to cellular mass. Generally, an oxygen uptake of 50% or greater of the theoretical amount as measured by biochemical oxygen demand (BOD) in respirometry tests is considered sufficient to categorize the substrate as having "ready biodegradability".[28]

The ultimate biodegradability of a substrate, such as is depicted in Figure 4, may, in addition to oxygen uptake, be measured by disappearance of organic carbon, CO_2 evolution and the formation of water. A radiotracer approach provides a more accurate determination and is the only feasible way of measuring the formation of water in the aqueous medium required for all metabolic processes.

Potential points of initial attack for nonionic surfactants

Bacterial inocula have been shown to produce enzymes which can initiate degradation at any of three different locations[2] of the nonionic surfactant. As depicted in Figure 5, these are:

1. A central fission mechanism in which the hydrophobe is cleaved from the hydrophile. The well established β-oxidation mechanism then converts the linear chains to CO_2 and H_2O as indicated in Figure 6.
2. ω-hydrophobe attack in which the far end of the hydrophobe is first oxidized to a carboxylic acid. Biodegradation can then proceed inward, particularly in the case of linear alkyl chains, via β-oxidation.
3. ω-hydrophile attack in which the far end of the polyoxyethylene (POE) chain is oxidized initially to a carboxylic acid. Further conversion of the POE chain to CO_2 and H_2O then proceeds by a mechanism that has yet to be elucidated in detail.

Figure 6 depicts the β-oxidation process[2,29] which proceeds after initial oxidative attack by enzymes to split a linear alcohol ethoxylate into an alkyl carboxylic acid and polyethylene glycol (PEG). β-oxidation begins with esterification of the acid by a coenzyme called coenzyme A. The alkyl chain is then degraded to acetyl groups and another carboxylic acid with two less carbon atoms. This process continues two carbon atoms at a time to ultimately produce acetyl groups in the case of even carbon-numbered alcohols and propionyl groups in the case of odd carbon-numbered alcohols. The acetyl and propionyl groups can easily be incorporated into cellular mass or converted to CO_2. β-oxidation is an extremely fast process and does not leave any measurable quantities of biodegradation intermediates.

Ultimate biodegradation of the four types of nonionic ethoxylates

In a Gledhill-modified version[30] of the Sturm CO_2 evolution shake flask test,[31] the following nonionic ethoxylates were studied:

1. A C_{12-15} essentially linear primary alcohol ethoxylate having an average of 9 ethylene oxide (EO) units per mole of alcohol (C_{12-15}LPAE-9). The alcohol was prepared from C_{11-14} olefins using catalytic addition of CO and H_2. Approximately 80% of this alcohol contained linear alkyl chains. The 20% remaining contained 2-alkyl branches, mostly methyl.
2. A C_{11-16} linear secondary alcohol ethoxylate containing an average of 9 EO units per mole of alcohol (C_{11-16}LSAE-9).
3. A C_{13} branched alcohol ethoxylate containing an average of 7 EO units per mole of alcohol (C_{13}BAE-7). The alcohol precursor was made by catalytic addition of CO and H_2 to highly branched propylene tetramer.
4. A branched nonylphenol ethoxylate containing an average of 9 EO units per mole of nonylphenol (NPE-9).

Shake flasks were inoculated with mixed liquor suspended solids from activated sludge units in a Houston area domestic waste sewage treatment plant. Initial surfactant concentrations were 20 mg/l. CO_2 formed from biodegradation was trapped in aqueous $Ba(OH)_2$. The amount of CO_2 formed was determined by back-titrating residual $Ba(OH)_2$ with HCl at the end of each test period. Glucose was included as a positive biodegradation standard.

The results of this CO_2 evolution test are plotted in Figure 7. As shown, the two linear alcohol ethoxylates were converted to greater than 75% of their theoretical yields of CO_2 in 30 days with the C_{12-15}LPAE-9 biodegrading slightly faster then the C_{11-16}LSAE-9 and to a level of 88% — slightly less than glucose (92%). Conversely, the two highly branched nonionics, C_{13}BAE-7 and NPE-9 were converted to less than 50% of their theoretical yield of CO_2 in 30 days with the NPE-9 biodegrading somewhat slower than the C_{13}BAE-7. In these studies a slight induction period was observed for all the surfactants but not for the glucose. This induction period was significantly greater for the branched nonionics than for linear nonionics. It is likely that induction represents sorption of the hydrophobic portion of the surfactants onto sludge with later release as less surface active intermediates desorb from the sludge and are enzymatically attacked in solution.[13]

Another interesting feature of these CO_2 evolution tests is that CO_2 formation was still increasing for the linear alcohol ethoxylates but had reached a plateau for the branched nonionics. This suggests the formation of more bioresistant intermediates in the case of the branched surfactants.

Biodegradation of the linear products LPAE and LSAE have been shown to rapidly produce significant quantities of PEG[13,32] indicating the central fission pathway. However, biodegradation of the branched products NPE and BAE do not produce PEG, which suggests they biodegrade by the ω-hydrophile mechanism to give NPE's and BAE's with shorter POE chains.[11,33,34] These intermediates biodegrade more slowly by a mechanism which has yet to be elucidated.

Biodegradation pathways by radiotracer techniques

Somewhat more detailed mechanistic studies have been reported[13] using a double-labeled linear C_{14}AE-9, and a double-labeled NPE-9. As shown in Figure 8, these nonionics were labeled with tritium in selected positions of their hydrophobes and uniformly with carbon-14 in their POE chains. It should be noted that tritium labeling in the hydrophobe of the C_{14}AE-9 is concentrated in the alpha and gamma positions. Figure 9 shows the pattern of ultimate biodegradation for the hydrophobe of C_{14}AE-9 in a shake flask test similar to that used in the results discussed previously for the CO_2 evolution studies of the four types of nonionics. The alkyl CO_2 curve was obtained by subtracting $^{14}CO_2$ of the POE chain (determined by scintillation counting) from total CO_2 (determined by titration). The rapid appearance of ^3H_2O in solution was accompanied by very little CO_2 evolution. This indicates that the tritiated portion of the alkyl chain, located near the hydrophobe-hydrophile junction, was biodegrading at a much faster rate than the alkyl chain as a whole. Disappearance of the alkyl chain by HBr/GC is also shown and indicates that primary biodegradation proceeded at a rate which was parallel to that of ^3H_2O formation in the early stages of this study. 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 co-workers[32] 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 co-workers using ^{14}C-labeled AE at different locations in LPAE, indicate that the initial oxidation takes place at the terminal methyl group prior to hydrolytic cleavage of the alkyl group from the POE group.[35] It appears that different bacterial strains may exist with selective capabilities to initiate biodegradation of alcohol ethoxylates by more than one mechanism. However, the major pathway with non-selected bacterial strains such as are found in domestic waste treatment plants appears to be central fission.

In a separate study,[33] the double-labeled NPE-9, shown in Figure 8, was fed, along with a slipstream of a domestic waste treatment plant influent, to a bench-scale activated sludge unit. Table 1 lists data from this slipstream study showing the ratio of EO to hydrophobe in effluent samples rapidly went from 9 to approximately 2-3 within eight hours and remained at this ratio throughout the 28 day course of the study. These results are in line with APE biodegradation mechanisms proposed by other workers[11,34] who indicate that APE biodegradation is initiated by ω-attack at the far end of the POE chain with gradual shortening of the chain to a relatively bioresistant alkylphenoxy moeity.

Table 2 lists the radioactivity balance for the 28-day slipstream study[33] in which doublelabeled C_{12-15}LPAE-9 was also a substrate. The considerably greater amount of effluent ^3H_2O found at the completion of the study for C_{12-15}LPAE-9 (91.6%) compared to that found for NPE-9 (28.3%) indicates the ultimate biodegradation of the LPAE hydrophobe was more extensive than that of the NPE. Also, the much higher level of tritium found in the biomass (activated sludge) for NPE suggests that the biomass had sorbed significant quantities of the NPE-2 biodegradation intermediate. The sorption of an NPE with a short EO chain onto activated sludge has recently been observed in our laboratories using environmental samples from a waste treatment plant which was experiencing normal high loadings of NPE-9 from industrial point sources.[36] The results for influent, activated sludge and effluent samples from the plant are listed in Table 3. CTAS values, basis dewatered sludge, were 1300 mg/l. Since CTAS does not respond well to low EO-containing nonionics, the recently developed HPLC/MS procedure of Giger[23-25] was used and showed almost double the levels found by CTAS. In addition, the HPLC/MS procedure positively identified the sorbed material as an NPE mixture much more concentrated with NPE-2 than was present in the influent NPE-9. This sludge enrichment in shorter chain NPE's has also been observed by Giger and co-workers.[37]

The use of these radiolabeled C_{12-15}LPAE-9 and NPE-9 in continuous activated sludge benchscale units simulating winter conditions has been reported.[38] The results for biodegradation of the hydrophobes to ^3H_2O are shown in Figure 10 and suggest that under winter conditions biodegradation of the hydrophobe of linear alcohol ethoxylates is essentially unaffected while biodegradation of the hydrophobe of NPE is slowed significantly with decreasing temperature.

Aquatic toxicity of effluents

In a recent study,[39] bench-scale activated sludge units were fed up to 100 mg/l of LPAE and NPE to simulate influent concentrations from industrial sources. The effluents of these units were subjected to aquatic toxicity tests. The results, summarized in Figure 11, show the effluent from the NPE unit to be toxic at effluent dilutions as low as 7.3 percent. The LPAE effluent was rendered completely non-toxic under these conditions. The results of this study also showed that high loadings of surfactants which biodegrade slowly may have an adverse impact on the activated sludge process by causing poor biosolids growth and settling, impaired BOD removal and loss in nitrification capability. These intermediates sorb strongly to sludge and may interfere in the proper functioning of activated sludge treatment.

Summary

As discussed previously, the biodegradation pathways using non-selected bacterial inocula for nonionic ethoxylates may be divided into two distinct areas, each dependent on the structure of the surfactant.

1. **Linear Alcohol Ethoxylates**
   These biodegrade via central fission initiation in which the hydrophobe is cleaved from the hydrophile to produce non-surface active, non-toxic intermediates which readily biodegrade to CO_2 and H_2O.

2. **Highly Branched Alkylphenol Ethoxylates and Highly Branched Alcohol Ethoxylates**
   Biodegradation of these nonionics is initiated by ω-hydrophile attack in which the POE chain is shortened. The evidence for this pathway is extensive for APE. Although mechanistic pathways for branched AE need further study, it has been reported that their branched alkyl groups direct initial attack to occur by an ω-hydrophile mechanism similar to that for APE. This attack, followed by shortening of the POE chain to approximately 2-3 EO units per mole of hydrophobe, produces intermediates which de-

grade more slowly than their intact surfactant precursors, have higher aquatic toxicity, and sorb strongly onto activated sludge thereby having the capability of upsetting the activated sludge process.

Currently, those surfactants with highly branched alkyl chains and those with aromaticity do not have an appreciable impact on domestic waste treatment from household sources since they are not used extensively in household laundry and dishwashing which are the largest volume end-uses for surfactants. However, these highly branched surfactants are used in many industrial applications such as textiles and pulp and paper. When used in the industrial sector, they generally enter domestic waste treatment plants at much higher loadings than are found for surfactants from household sources. The fate of these surfactants in effluents, sludges, receiving waters and sediments will require further studies to ascertain their environmental impact.

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Figure 1/**Structural features of nonionic classes**

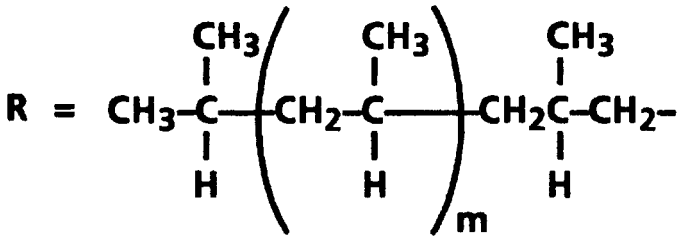
Linear Primary Alcohol Ethoxylates

$$RO(CH_2CH_2O)_nH$$

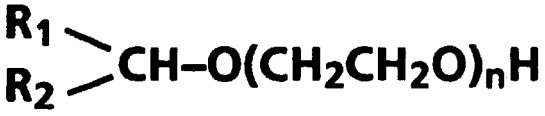
$$R = CH_3(CH_2)_m-$$

Branched Primary Alcohol Ethoxylates

$$RO(CH_2CH_2O)_nH$$



Linear Secondary Alcohol Ethoxylates



$$R_1 = CH_3(CH_2)_m- \; ; \quad R_2 = CH_3(CH_2)_p-$$

Alkylphenol Ethoxylates

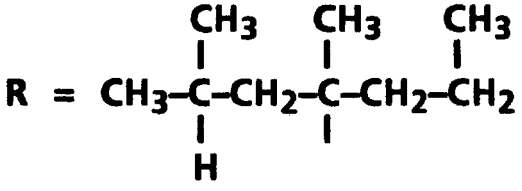


Figure 2/**Primary biodegradation**

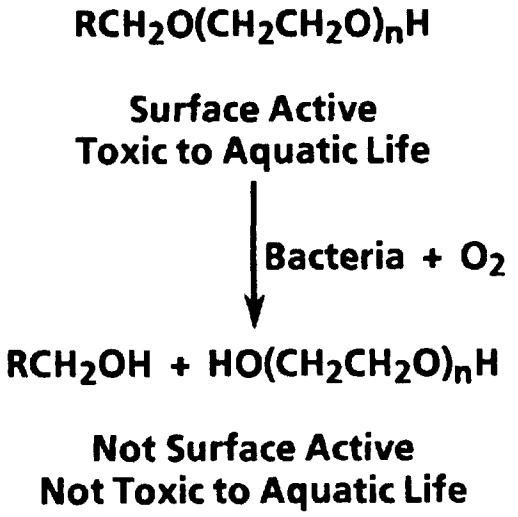


Figure 3/**Biodegradation intermediates**

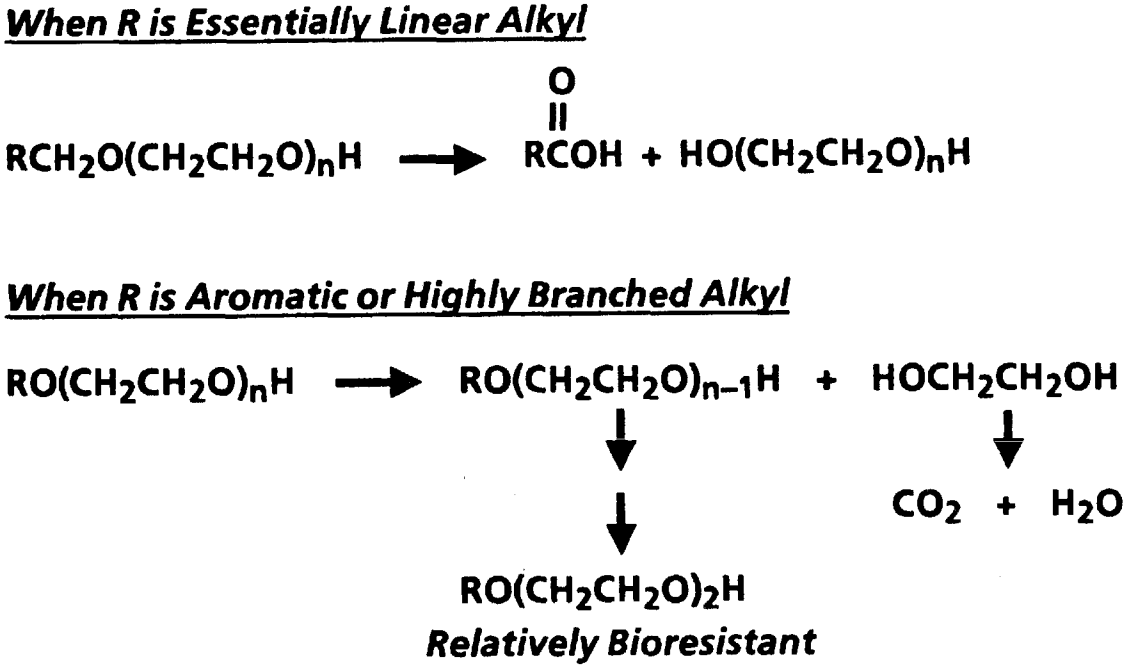
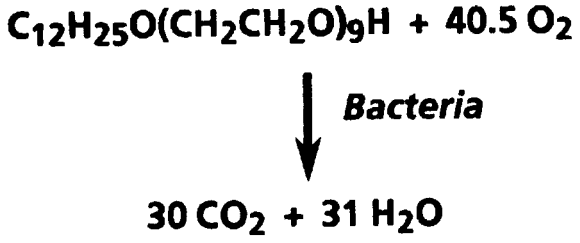


Figure 4/**Ultimate biodegradation**



Determination	Method
• Oxygen Uptake	Respirometry
• Loss of Organic Carbon	DOC/^{14}C
• CO_2 Evolution	Titration/^{14}C
• Formation of H_2O	^{3}H Only

Figure 5/**Points of initial attack**

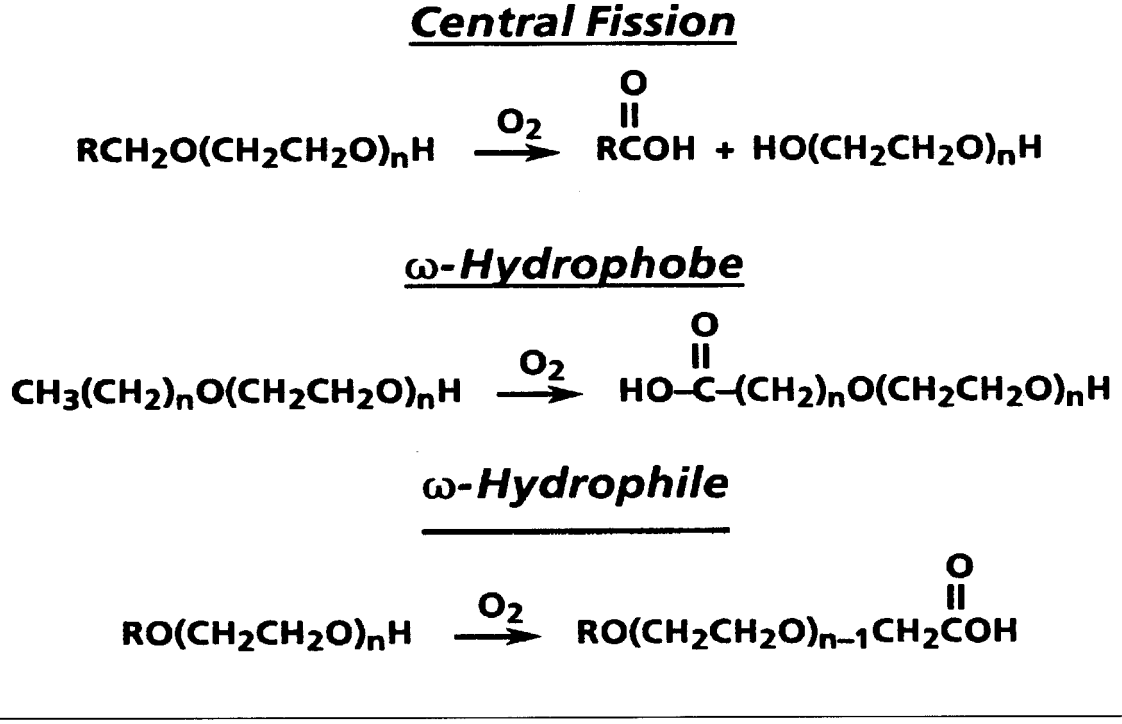


Figure 6/**Suggested biodegradation pathway for hydrophobe of linear primary alcohol ethoxylates**

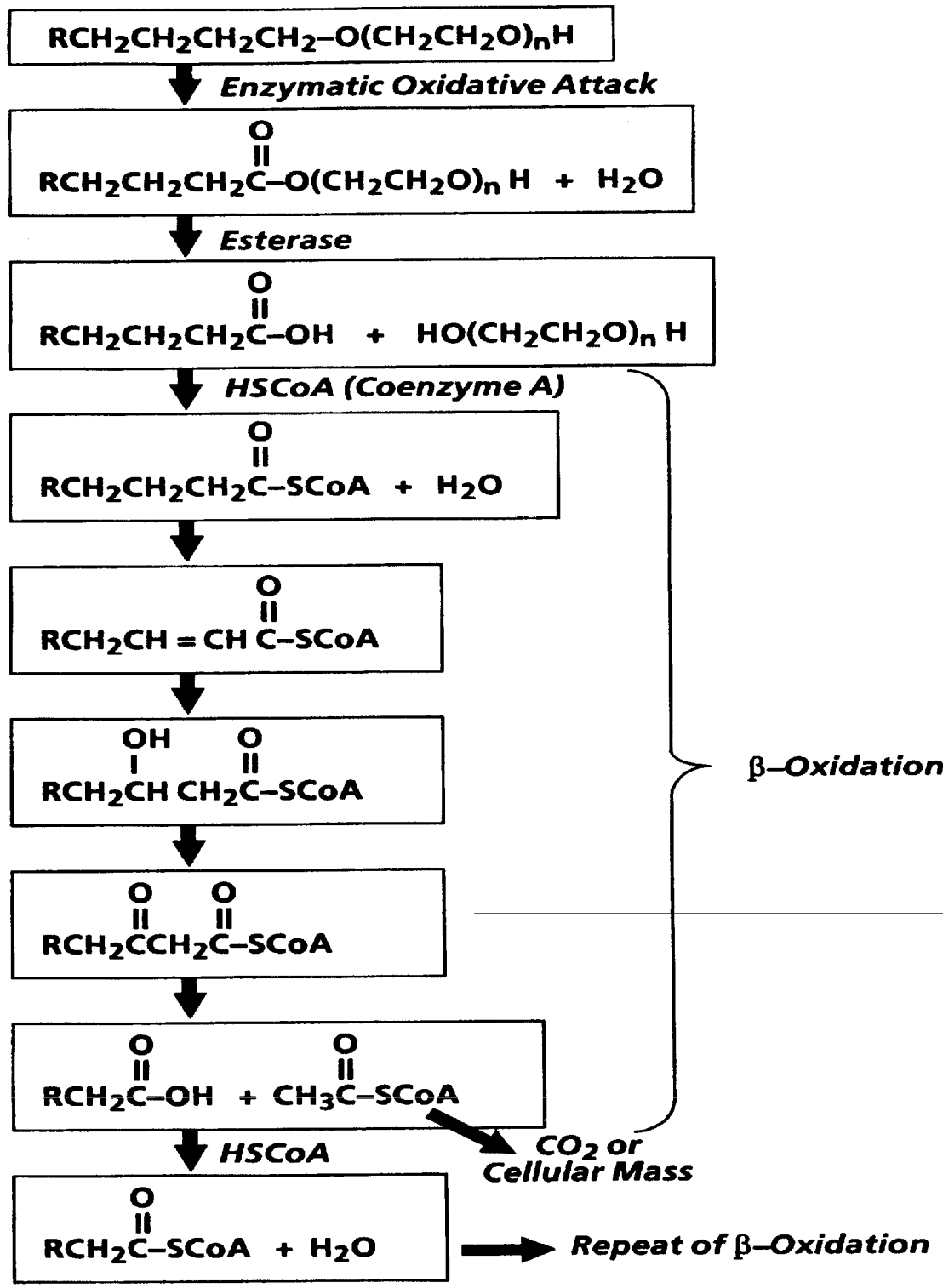


Figure 7/**Ultimate biodegradation of nonionic surfactants by CO_2 evolution**

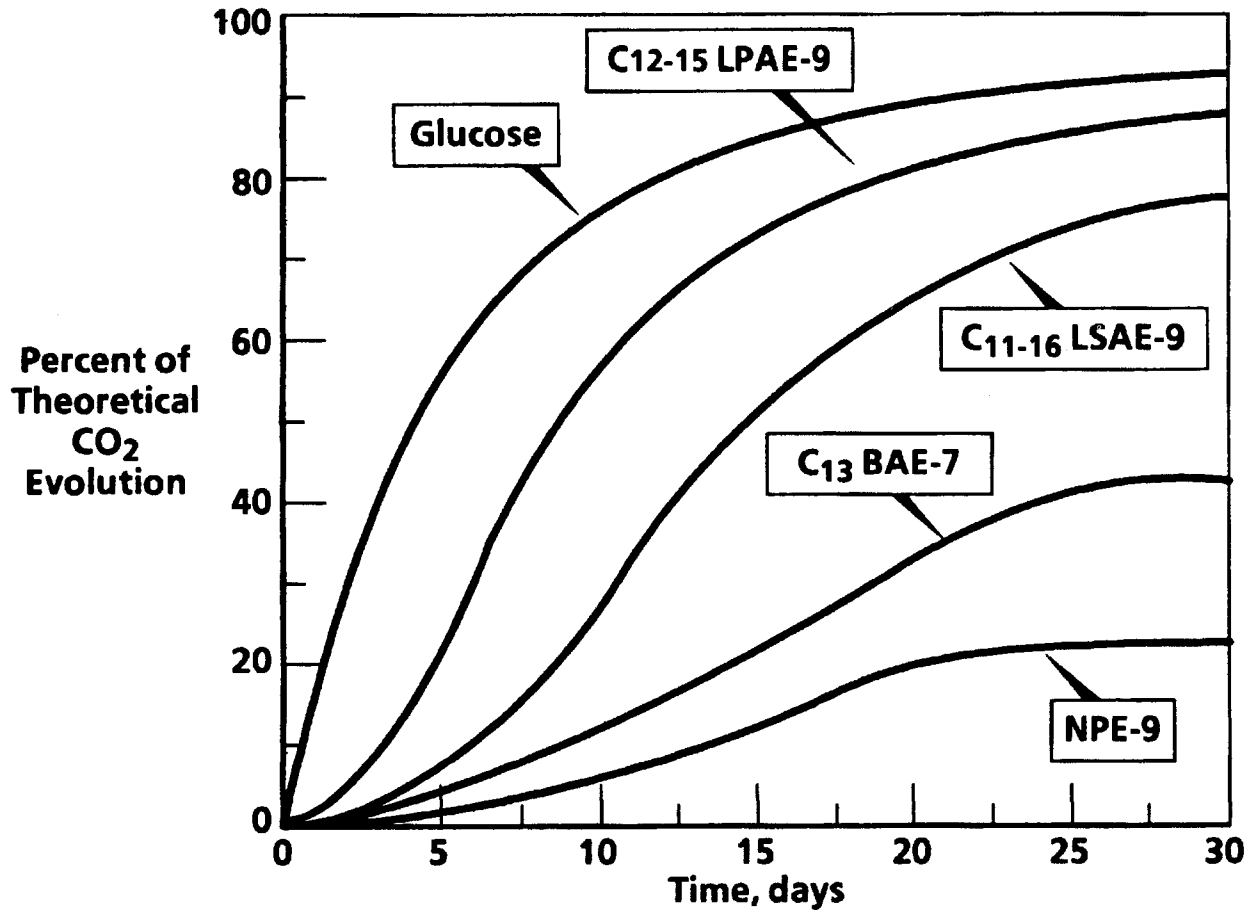


Figure 8/**Double radiolabeled surfactants**

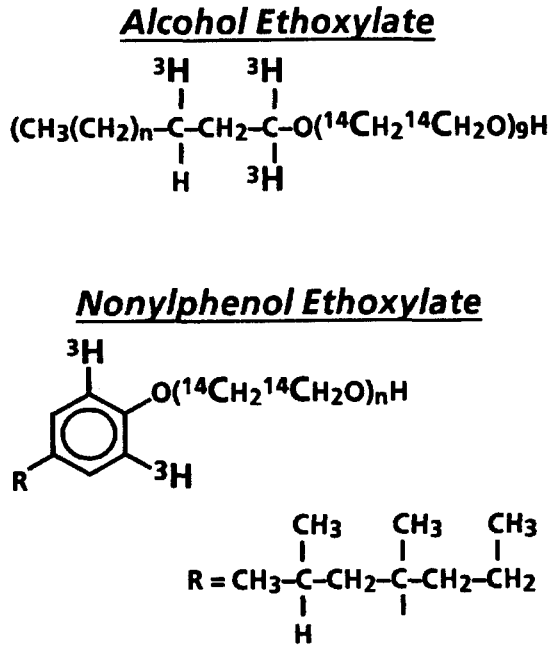


Figure 9/**Ultimate biodegradation of alkyl portion of radiolabeled C_{14}LPAE-9**

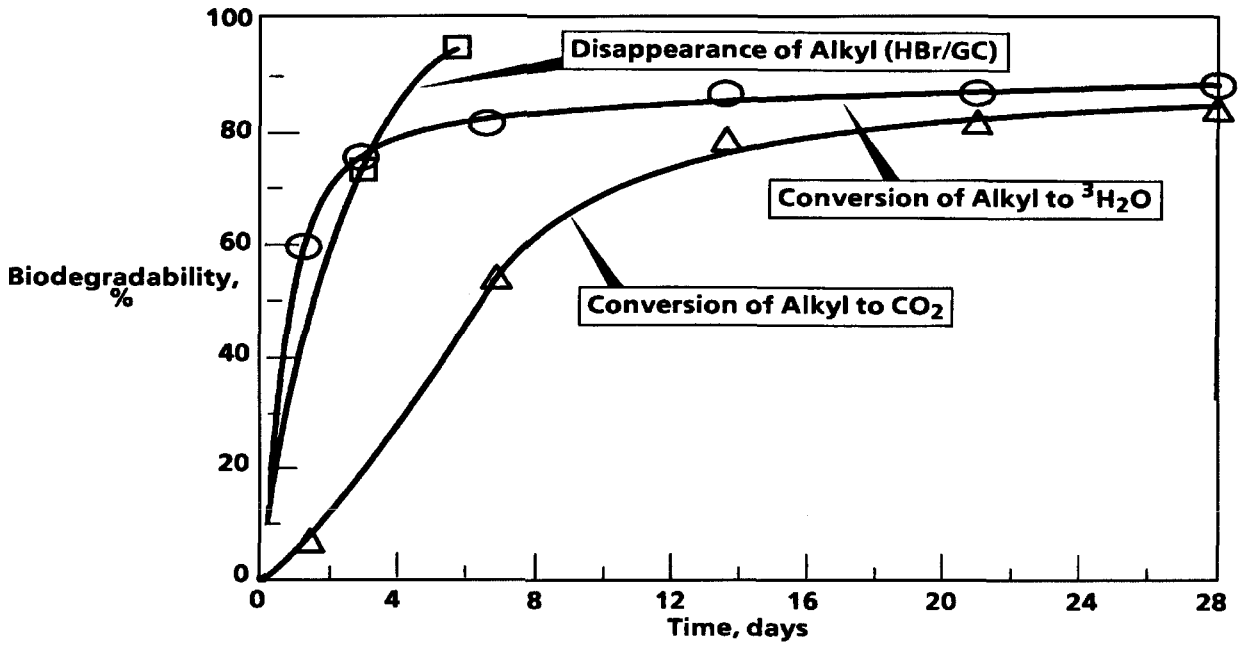


Figure 10/**Biodegradation of ^{3}H-hydrophobe groups of C_{12-15} LPAE-9 and NPE-9 to ^3H_2O**

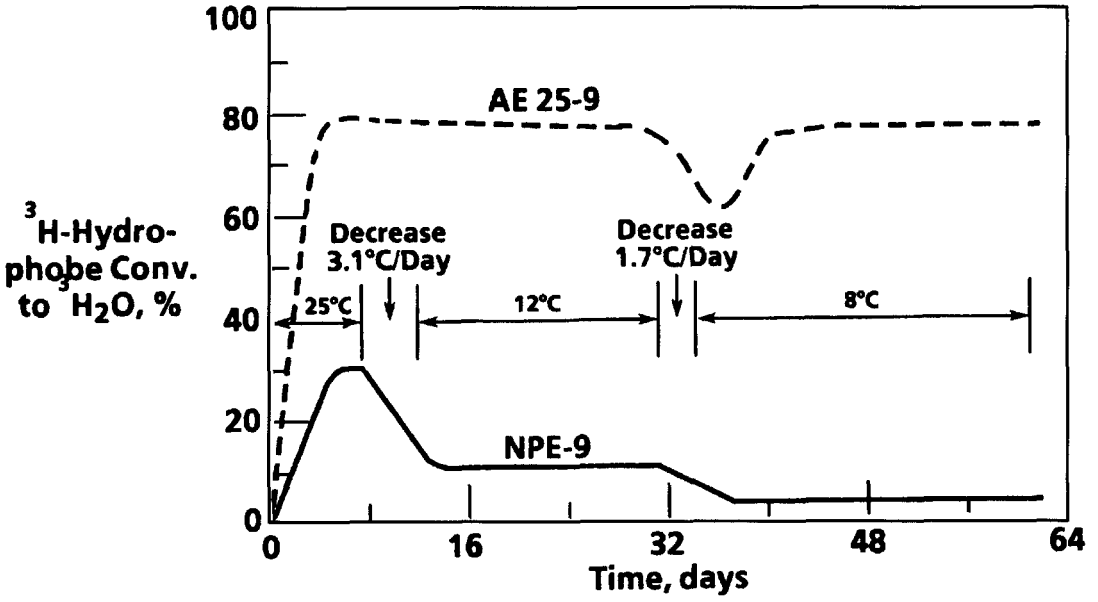
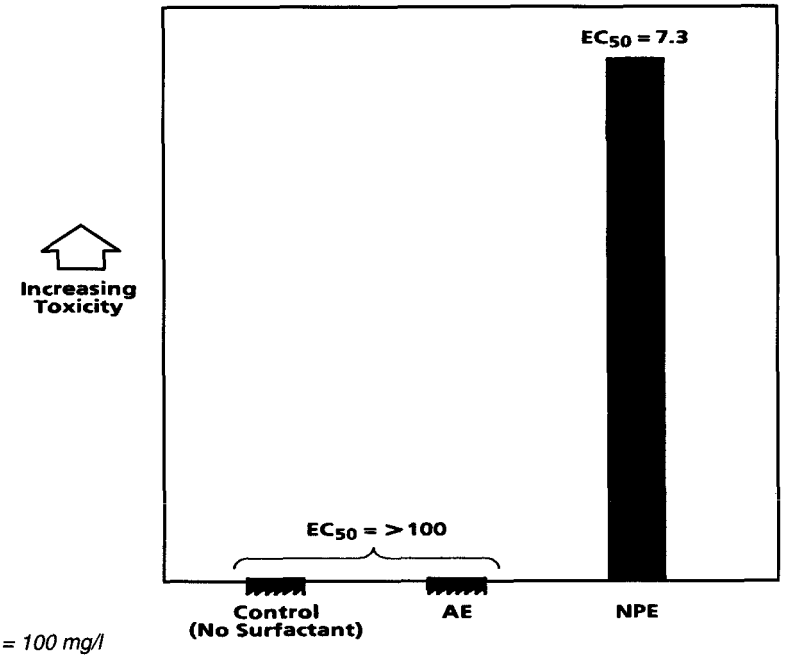


Figure 11/**Acute aquatic toxicities (% effluent) of biotreated effluents* to fathead minnow**



**Influent Surfactant Conc. = 100 mg/l*

Table 1/**Ratio EO/Hydrophobe during biodegradation of NPE-9**

Time, days	Ratio, EO/Hydrophobe*
0.08 (2 hr)	5.4
0.33 (8 hr)	2.4
1	2.2
18	2.4
24	2.7
28	2.2

*Basis, $^{14}C/^{3}H$ Activity in Effluent

Table 2/**Radiochemical product distribution**

Product	Distribution, %		
	C_{12-15} LPAE-9	NPE-9	Glucose
$^{3}H_2O$	91.6	28.3	--
Soluble ^{3}H-Metabolites	1.5	36.7	--
^{3}H in Biomass	4.1	31.5	--
Total	97.2	96.5	
$^{14}CO_2$	65.4	54.0	42.8
Soluble ^{14}C-Metabolites	1.3	8.5	1.4
^{14}C in Biomass	24.7	27.6	52.0
Total	91.4	90.1	96.2

Table 3/**NPE fate in biotreatment**

Influent, mg/ℓ		Activated Sludge, mg/ℓ[2]		Effluent, mg/ℓ	
CTAS	HPLC	CTAS	HPLC	CTAS	HPLC
63	55[1]	1300	2340[3]	5.4	4.6[1]

[1]Identified as NPE-9
[2]Basis, dewatered Sludge
[3]Identified as NPE heavily enriched with NPE-2

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