estimated that by 1990, the number of bills introduced in that biennium will have increased to 250,000, of which some 25,000 could affect the detergent industry; (d) state regulatory programs are growing rapidly, perhaps even faster than their federal counterparts, and they are taking on a more activist complexion.

The real concern for the detergent industry is not the narrow single-issue legislation, such as a proposed detergent phosphate ban, but rather the seemingly innocuous "good-guy" laws.

As an example, several Northeastern states recently have considered bills regulating the input of halogenated hydrocarbons into ground water. Specifically, the concern was related to certain septic tank cleaners and degreasers which contained potential carcinogens. Certainly, the intent of the legislation was sound, but the draftsmanship was so broad, that if enacted as written, it could have resulted in the banning of whole classes of household cleaning products.

Other legislation has been proposed requiring ingredient labeling and disclosure, packaging and a host of toxic chemical control bills.

For a national group such as the detergent industry, the danger of "Balkanization" should be readily apparent. As manufacturers of ubiquitous, relatively low-cost products, the imposition of regional, state or local laws could have a profound and costly effect with little benefit to the consuming public.

A discussion of state level activity would be incomplete without some mention of detergent phosphates. The situation has been essentially stable in recent years and only a few states have been seriously considering legislation. This is in contrast to conditions in the early 1970s when, in one year alone, 273 antiphosphate bills were introduced.

Pressure may continue for action in those areas around the Great Lakes that presently do not have bans and in other regions that have major fresh surface water resources. But, the trend is away from this kind of restriction, and there is even the possibility of repealing some bans as waste treatment facilities come on-stream.

The near future interaction between government at all levels and the soap and detergent industry is envisioned as follows: (a) with the exception of "superfund" or similar broad legislation aimed primarily at toxic chemicals, there is not likely to be new federal legislation which will directly impact the soap and detergent industry; (b) interaction with some federal regulatory agencies will increase significantly whereas involvement with others will decline. Regulations issued by the EPA under TSCA, RCRA, and Clean Water and Clean Air Acts will be of increasing importance to the industry. In the near term, TSCA regulations regarding the significant new use of existing chemicals will require careful analysis and comment; (c) while there will be a continuing dialog with the CPSC and FDA, no new activities generated by these agencies are predicted that will affect the soap and detergent industry; (d) it is improbable that the FTC will propose new trade regulation rules specifically for the soap and detergent industry; (e) state government—both legislatively and by the regulatory route—will take on added importance for the industry in the coming decade. States may become more active in the field of hazardous substance control, air and water quality standards, and packaging and labeling. It is unlikely that detergent phosphates will receive broad attention.

On balance, the soap and detergent industry appears well positioned to deal effectively with government in these areas. It is respected for technical competence, credibility and candor. The detergent industry has been forthright in addressing the issues which affect the industry and the American consumer, and this should lead to an improved climate of government and industry relations.

⁂Biodegradation of Nonionic Ethoxylates

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ABSTRACT

The biodegradation of alcohol ethoxylates (AE) and alkylphenol ethoxylates (APE) is reviewed. Biodegradation test methods, ranging from laboratory tests to full-scale waste treatment plant studies are described for these surfactants. A comparison is made between primary and ultimate biodegradability criteria and the limitations of the various analytical methods used in these determinations are discussed. The most recently published data suggest sewage bacteria degrade AE by a mechanism which is different from that by which APE degrades. The use of radiolabeled surfactants to elicit more detailed information about the biodegradation mechanisms of AE is described. The role of biodegradation on the impact of surfactants released to the environment is assessed, and future environmental concerns for nonionics are considered.

INTRODUCTION

Over the past 15 years, a number of factors caused significant changes in the detergent industry. The first of these took place in 1965, when the industry voluntarily switched its anionic workhorse surfactant, branched alkylbenzenesulfonate (ABS), to linear alkylbenzenesulfonate (LAS) upon the discovery that the less biodegradable ABS was largely responsible for excessive foaming in receiving waters. During the 1960s, phosphorus, present as phosphate builders in household laundry and some institutional detergents, was found to be a limiting nutrient in the eutrophication of lakes and streams. This finding has resulted in a number of states and municipalities enacting legislation limiting the use of phosphates in detergent products. Phosphates, in the form of sodium tripolyphosphate or potassium pyrophosphate, were the only low-cost builders capable of reducing water hardness concentrations to levels where hardness-sensitive LAS would perform a good cleaning job. An effective chelating builder, the sodium salt of nitrilotriacetic acid (NTA), was voluntarily abandoned in 1970 by U.S. detergent manufacturers and suppliers upon preliminary findings that NTA might act to increase the teratogenic activity of highly toxic heavy metals. The environmental Protection Agency (EPA) recently has reviewed all health data available on NTA and has decided that, pending any new data indicating adverse effects of NTA, they would not "take regulatory action against the resumed production and use of this substance for laundry detergents" (1). How soon production of NTA actually will resume for this end use is

somewhat in doubt.

Since an alternative builder that could perform well with LAS in laundry powders was unavailable, the detergent industry moved to unbuilt liquid and powder laundry products. The major thrust for this move occurred in those states having limited phosphate legislation. Nonionic ethoxylates, less hardness-sensitive than LAS, increased in use during the 1970s. These nonionics could be manufactured from readily available petrochemical feedstocks. In addition, the low hardness-sensitivity of nonionics permitted them to perform particularly well in removal of oily soils from synthetic fabric which has replaced cellulosics as the predominant apparel fabric.

The two principal classes of nonionics available for use are alcohol ethoxylates (AE), in which the alkyl group is predominantly linear, and alkylphenol ethoxylates (APE), in which the alkyl group is highly branched (Fig. 1). As a result of the branching and presence of a bioresistant aromatic group in APE, major detergent manufacturers selected the more rapidly biodegradable AE for use in household laundry products. Today, AE represents the fastest growing nonionic surfactant class. In the household detergent sector, it is the largest volume nonionic. In the industrial sector, APE currently is the major surfactant.

The passage of major federal legislation, such as the Toxic Substance Control Act (TSCA), the Water Pollution Control Act (WPCA) and the Resource Conservation and Recovery Act (RCRA), has made criteria for large volume surfactants like AE and APE more stringent than the foaming reduction required of the alkylbenzenesulfonates in the 1960s. Therefore, much work has been carried out both in industry and academia to assess the biodegradability and environmental safety of AE and APE. This paper reviews current knowledge, resulting from this work, of AE and APE biodegradation. The strengths and limitations of available biodegradation test methods and analytical procedures and the relationship of AE and APE biodegradability to an assessment of environmental acceptability are discussed. Finally, several environmental concerns are mentioned where future environmental regulatory activity might affect AE and APE surfactants. This review is not all-inclusive, but it is intended to show major significant areas of research in the biodegradation of two classes of nonionic ethoxylates.

Definition of General Terms

Biodegradation is the molecular breakdown of an organic substrate by the enzymatic action of living microorganisms which use the substrate for food. Conversion of substrate occurs stepwise with formation of metabolites and biodegradation intermediates which may degrade further at faster or slower rates compared to their precursors.

Primary biodegradation means biodegradation of a substrate to an extent sufficient to remove a characteristic property of the original intact molecule. For surfactants, this has been measured by loss of foaming capacity or ability to reduce surface tension. Primary biodegradation can leave high levels of organic residues altered in form from the original material.

Ultimate biodegradation is biodegradation which proceeds through a sequence of enzymatic attacks to ultimately produce the simplest structures possible in the biodegradation media. In aerobic biodegradation, such as that which consumes oxygen in the aeration sections of sewage treatment plants, CO_2, H_2O and mineral salts of other elements present are generated. In anaerobic septic tank systems in which microbial attack occurs with little oxygen present, methane is generated in addition to the products already mentioned. Today, ca. 75% of U.S. living units have their waste treated aerobically.

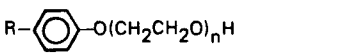
ALCOHOL ETHOXYLATES (AE)

$RO(CH_2CH_2O)_nH$

WHERE R = MIXED PREDOMINANTLY LINEAR ALKYL GROUPS IN C_8 TO C_{18} RANGE

n = AVERAGE ETHYLENE OXIDE UNITS PER MOLE

ALKYLPHENOL ETHOXYLATES (APE)



WHERE R = HIGHLY BRANCHED ALKYL GROUP

n = AVERAGE ETHYLENE OXIDE UNITS PER MOLE

FIG. 1. Principal classes of commercial nonionic surfactants.

PHYSICAL TESTS FOR BIODEGRADABILITY

Foaming

The capability of aqueous solutions of surfactants to foam is greatly reduced when they are subjected to microbial attack. A decrease of foam height is frequently used to measure the primary biodegradability of surfactants. However, foam measurements can be misleading criteria if surfactants biodegrade to chemical intermediates which are resistant to further biodegradation, yet foam readily. This has been reported for alkylphenol ethoxylates in which the polyoxyethylene (POE) chain was considerably shortened to produce products which still foamed (2). For surfactants like AE, where biodegradation is more rapid, foam data provide a better measurement of primary biodegradability than for APE. However, even rapidly biodegradable surfactants like AE do not provide very accurate primary biodegradability data when foaming is the criterion because of the interferences of low foaming biodegradation intermediates produced from organic materials present in sewage streams.

Surface Tension

Reduction of surface tension in aqueous media by surfactants can be used to follow their primary biodegradation. As the surfactants degrade, surface tension tends to rise. The limitations mentioned for foaming also apply to surface tension measurements.

ANALYTICAL TESTS

Cobalt Thiocyanate and Bismuth Iodide

These widely used primary biodegradability methods take advantage of the formation of metal complexes with the oxygen atoms in the POE structure of nonionic ethoxylates. Cobalt thiocyanate forms a blue-colored complex (CTAS) with nonionics which may be extracted with a chlorocarbon solvent and determined spectrophotometrically. Bismuth iodide forms a precipitate (BIAS) with nonionics which is dissolved and titrated for bismuth. Biodegradation reduces the complexing capabilities of the surfactants. The advantage of these techniques is that they can be used in laboratory-scale biodegradation tests as well as in sewage treatment plants and receiving waters where other organic materials are present. However, nonionic ethoxylates with very short (generally less than five) or very long (generally greater than 20) POE units do not complex with the cobalt and bismuth reagents. Hence, nonionics of this

type may go undetected by these analytical methods. Accuracy also is limited by interferences from other products found in environmental waters, particularly in sewage effluents and receiving waters where surfactant concentrations are low. The most serious limitation is the inability of the cobalt and bismuth methods to differentiate between different surfactants containing the POE chain.

INSTRUMENTAL AND CHROMATOGRAPHIC ANALYSES

Ultraviolet (UV) and infrared (IR) spectroscopy (3) have been used to determine nonionic ethoxylates and the rate at which they degrade. These primary biodegradability approaches are limited by interferences from other materials present in environmental samples and by their inability to identify the many varying chain length structures of AE and APE. Thin layer chromatography (TLC) has had considerable success in measuring the effect of nonionic structure on degradation rates (4). In recent years, a number of workers (5-7) have used a method in which nonionic ethoxylates and their reaction intermediates are extracted from environmental samples and treated with hydrobromic acid. The resulting chain scission products—ethylene bromide from the POE chain of AE or APE and alkylbromides from AE—are identified and quantified by gas chromatography. It is likely that such newer approaches as high performance liquid chromatography and gas chromatography, interfaced with mass spectrometry, will find increasing use in identifying nonionic ethoxylates and their biodegradation intermediates in environmental samples.

ULTIMATE BIODEGRADABILITY

The ultimate biodegradation of AE and APE under aerobic conditions may be represented by the following stoichiometry:

(a) $C_{13}H_{27}O(CH_2CH_2O)_9H + 42O_2 \rightarrow 31\ CO_2 + 32\ H_2O$, for AE having 13 carbon atoms in the alkyl chain and an average of 9 EO units/mol;

(b) $C_{15}H_{23}O(CH_2CH_2O)_9H + 43\ O_2 \rightarrow 33\ CO_2 + 30\ H_2O$, for APE having 9 carbon atoms in the alkyl chain and an average of 9 EO units/mol.

The AE and APE just listed are highly simplistic average structures. Commercial samples of these surfactants are actually complex mixtures of single chemical entities.

It also should be noted that the ultimate biodegradation sequences a and b are theoretical. Even the most biodegradable compounds, like glucose, do not oxidize completely to CO_2 and water since a portion of the organic matter is used by biodegrading bacteria to form new bacterial cells.

Theoretical equations a and b, however, are useful in determining the extent of ultimate biodegradation of the respective substrates. If analytical methods are available, determination of oxygen uptake, disappearance of organic carbon, CO_2 evolution and the formation of water from a known substrate will measure the extent to which that substrate biodegrades to CO_2 and water. The following analytical methods are used to determine ultimate biodegradability.

Biochemical Oxygen Demand (BOD)

This is one of the oldest methods used to measure oxygen uptake. Substrate, bacterial inoculum and oxygen are generally placed in a glass vessel and O_2 uptake determined by chemical analysis, manometrically or by an oxygen electrode. However, BOD tests to determine the ultimate biodegradability of a single substrate do not simulate realistic sewage plant conditions where organic materials other than the substrate to be tested are present. Other limitations include use of unacclimated inocula, possible interference from inorganics like sulfur compounds which also can consume oxygen, and from materials which can destroy bacteria and give false negative values on the biodegradability of a substrate.

Total Organic Carbon (TOC) and Chemical Oxygen Demand (COD) Analyses

These methods determine residual organic carbon in biodegradation media. In TOC, the organics in an aqueous sample are pyrolyzed to CO_2 in the presence of a catalyst. CO_2 levels are then determined in an IR spectrophotometer interfaced with the combustion unit. In the COD method, the sample is oxidized by a mixture of potassium dichromate and sulfuric acid. The quantity of dichromate used is calculated as oxygen equivalents.

TOC and COD are useful in dilute bacterial laboratory media where a test substrate is the major organic present, and in environmenal samples where the cumulative TOC contribution from all organics present might be desired. A major limitation is the inability of these methods to determine organic carbon from a specific substrate in environmental samples.

CO_2 Evolution

In this method, CO_2 evolving from a closed biodegradation system is trapped in a basic medium. The carbonates produced are titrated with acid to determine CO_2 evolved. This method is finding increasing use in laboratory experiments having a single surfactant present as the major substrate (7,8). It suffers from the same limitations as the TOC and COD methods since it cannot be used to differentiate between specific substrates simultaneously present in environmental samples.

Radiotracers

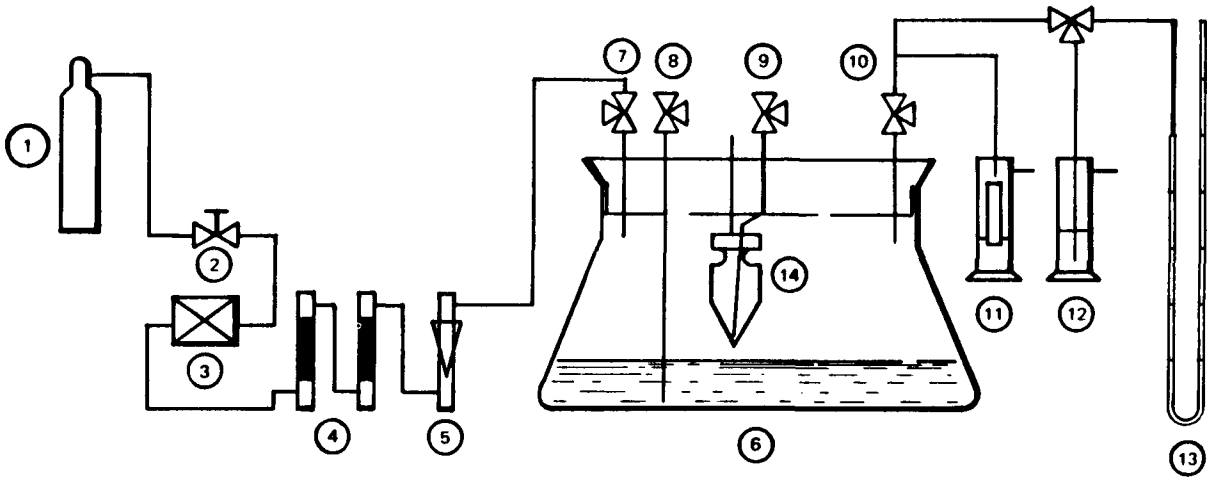
Use of radiolabeled compounds (7) provides an extremely sensitive method for determining the presence of low levels of substrate. When coupled with organic carbon and/or CO_2 evolution tests, it becomes a most powerful technique to determine ultimate biodegradability and the presence of intermediate biodegradation products. The use of radiolabeled compounds eliminates the major limitations of the organic carbon and CO_2 evolution tests already discussed, since the radiolabeled substrate and its biodegradation products can be followed accurately in the presence of much higher levels of other organics. Limitations to the use of radiolabeled substrates include the high cost and complexity of synthesis, the difficulty of interpreting results without undertaking a detailed study requiring additional sophisticated analytical techniques and the virtual impossibility of using radiolabeled substrates in large-scale plant studies because of the large quantities required. For these reasons, radiotracers cannot be considered for use in routine biodegradability testing.

BIODEGRADATION TEST METHODS

Swisher (9) and Gilbert and Watson (10) have reviewed many test methods which have been used for determining the biodegradability of surfactants in aqueous media. Those used most frequently are summarized next.

Shake Flask

Substrate, dilute bacterial inoculum, usually obtained from a sewage treatment plant, and inorganic supplements are added to Erlenmeyer-type flasks. The flask is mounted,



① G as Cylinder, 70% O_2/30% N_2, CO_2 Free Biodegradation Atmosphere
② Shut Off Valve
③ Pressure Relief Valve
④ Ascarite Scrubbers to Remove CO_2
⑤ Purge Rotameter - 1 for Each Unit
⑥ 4 Liter Erlenmeyer Flask with No. 12 Rubber Stopper, Covered with Black Polyethylene Tape and Aluminum Foil
⑦ O_2/N_2 Purge Gas Inlet Valve
⑧ Test Media Sample Valve and Line
⑨ Base Sample/Charge Line
⑩ O_2/N_2 Purge Gas Outlet Valve
⑪ Bubbler to Monitor Purge Gas Flow During Sampling
⑫ LiOH Scrubber Used While Sampling ^{14}C Containing Units
⑬ Manometer to Monitor Internal Pressure on 1000 mg Units
⑭ Base (LiOH) Reservoir

FIG. 2. Schematic diagram of a shake flask test system designed to determine ultimate biodegradability of radiolabeled surfactants.

open-mouthed, on a reciprocating or oscillating shaker to permit air to enter the medium. Samples are withdrawn at intervals and analyzed for the presence of surfactant by the techniques already discussed. Shake flask systems vary from simple Erlenmeyer flasks, when only primary biodegradability is to be measured, to very complicated equipment (Fig. 2), when ultimate biodegradation data are desired and radiolabeled surfactants are used. The dilute bacterial media present in shake flask systems do not simulate sewage treatment plant conditions. However, the system is excellent for screening a variety of substrates.

Activated Sludge

These systems use concentrated biological solids obtained in aeration units of sewage treatment plants. Laboratory-scale activated sludge reactor systems with forced air introduction simulate sewage treatment plant conditions more closely than shake flask systems. Generally, they are used to obtain primary biodegradability data but may be modified to obtain CO_2 evolution data. Activated sludge tests for surfactants are practiced in semicontinuous units mostly in the U.S., and in continuous units mostly in Europe. Limitations of laboratory activated sludge systems include the frequent absence of several realistic sewage conditions such as influent parameters and sludge wasting.

River Dieaway

This is an attempt to simulate the action of a receiving water on a substrate. It is similar to a shake flask system except that it is under static conditions. Its limitations are the same as those of the shake flask method—somewhat unrealistic in accurately simulating a river dieaway situation. In addition, the use of a single substrate in static river dieaway tests does not simulate the dynamic flow-through system of an environmental stream where bacteria feed on many substrates which change in type and concentration with time.

Sewage Treatment Plant Study

This is a highly realistic test in which the influent of a sewage treatment plant is dosed with surfactant at a specific feed rate at levels considerably above background. Various sections of the plant are then analyzed for the presence of surfactant. The method is limited by the fact that it can be used to obtain primary biodegradability data only, it requires a plant to operate without upsets during the course of the study and depends upon cooperative attitudes of plant personnel.

Monitoring Study

This is the most realistic study possible since data are obtained under normal operating conditions. The substrate to be studied must be present in the influent through normal conditions of home use. A monitoring study is limited by the availability of specific and sensitive analytical methods for determining the substrate in its course through the plant and in the receiving waters. No extensive monitoring of sewage plants and receiving waters has been reported for nonionic surfactants primarily because specific analytical methods for differentiating between AE and APE have been unavailable.

CURRENT STATUS OF AE AND APE BIODEGRADABILITY

Primary Biodegradability

The primary biodegradability of AE and APE has been investigated extensively, generally in separate studies, for these two nonionic classes. In those studies where both AE and APE have been compared directly, AE with predomi-

nantly linear alkyl chains biodegraded considerably faster than APE (4,11-13). An example (Fig. 3) from recent studies (7) shows an essentially linear AE undergoing complete primary biodegradation within 3 days, as measured by CTAS and HBr-GC whereas a branched chain APE having EO content essentially equivalent to the AE showed only 20% primary biodegradation in 30 days. Bacterial inocula previously had been acclimated to both of these substrates for 14 days.

The importance of adequate acclimation of bacterial inocula in studying the biodegradation of surfactants has been mentioned in the literature (9,14,15). River dieaway studies by Reiff (16) have shown slower bacterial acclimation times as well as slower primary biodegradation rates for a commercial APE compared to two commercial AE. Slower biodegrading surfactants may require considerably longer acclimation times. In a sewage treatment plant which has experienced a period of upset, such as a bacterial kill or excess storm runoff, reacclimation and recovery of the plant to normal operating conditions would be expected to depend on acclimation time.

Ultimate Biodegradability

In recent studies, the rate at which surfactants are converted to their ultimate biodegradation products, CO_2 and water, has been examined. A pioneering study of the biodegradation of nonionic surfactants by CO_2 evolution has been published by Sturm (17). This work showed AE with predominantly linear alkyl chains were converted to CO_2 more rapidly than either branched or linear APE in aerated BOD water-containing sewage inocula. Shake flask results from more recent studies (7) showed the much slower rate at which a branched octylphenol ethoxylate evolves CO_2 compared to an essentially linear AE containing an alkyl chain in the C_{12-15} range and approximately the same average ethoxylate chain length as the octylphenol ethoxy-

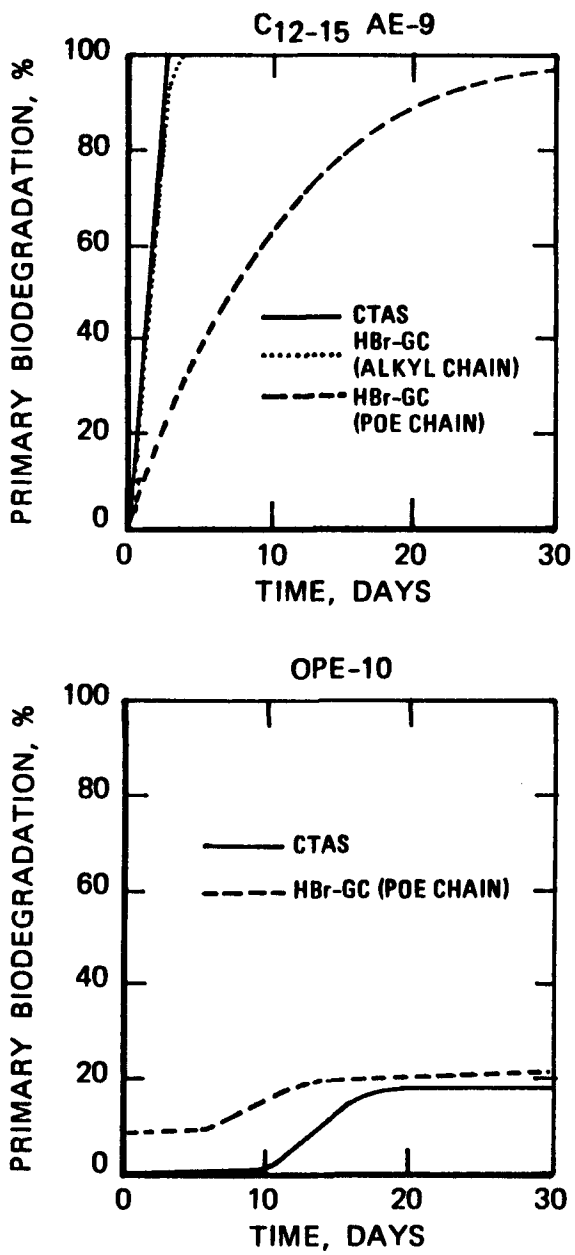


FIG. 3. Primary biodegradation determined by CTAS and by HBr-GC method.

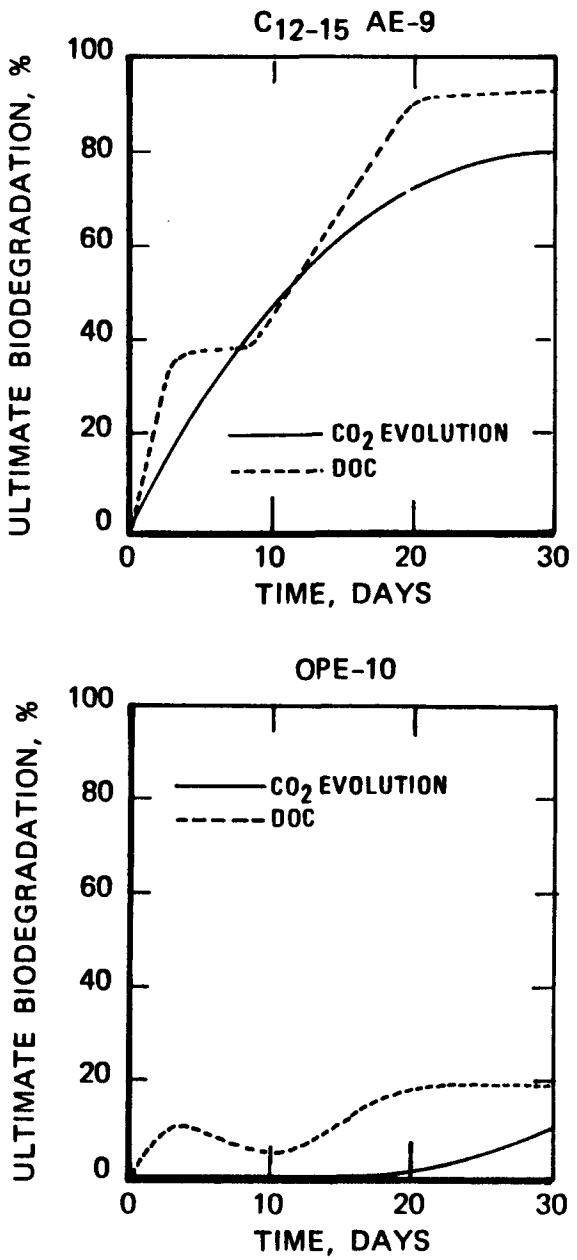


FIG. 4. Ultimate biodegradation determined by CO_2 evolution and by dissolved organic carbon (DOC).

late (Fig. 4). Disappearance of organic carbon from the aqueous medium also showed the much slower rate at which the APE biodegraded. Gledhill (8) has found less than 20% biodegradability of a branched octylphenol ethoxylate as measured by CO_2 evolution in a modified shake flask test.

Field Tests

Studies in a trickling filter plant have indicated only 20% removal of APE compared to greater-than-90% removal for AE under cool water conditions as measured by primary biodegradation criteria (15). Although the removal of APE increased after the onset of warmer summer months to ca. 80%, it never consistently reached the greater-than-90% levels of the AE. Abram et al. (18) have reported greater-than-95% primary biodegradation of AE which had been fed to a trickling filter plant at 5-10 C at concentration levels of 10 and 25 mg/ℓ.

The results of a field test on the effect of an AE on the operation of an activated sludge treatment plant in Ohio recently have been reported (19,20). In this test, the plant influent was dosed with 10 mg/ℓ of the AE under both summer and winter conditions. Plant performance was followed by sampling specific locations throughout the treatment facility before, during and after dosing. Results of analyses for such parameters as surfactant concentration, 5-day BOD, COD, sludge volume index and sludge retention time indicated that the AE was 90% removed and its presence had no adverse effects on plant performance or on aquatic life in a receiving stream.

Effect of Structure on Biodegradability

The effect of up to 55% branching in the alkyl chain apparently has a marginal negative effect on the biodegradability rates of primary alcohol ethoxylates (7) at 25 C. A slight decrease has been observed in the initial rate of CO_2 evolution for a 55% 2-alkyl branched primary AE-9 compared to a 25% 2-alkyl branched AE-9 (Fig. 5). A 100% linear secondary AE having approximately the same alkyl and polyoxyethylene chain lengths degraded at a slightly slower rate than the branched primary AE products already discussed (Fig. 6).

The effect of POE chain length on the biodegradabilities of linear primary alcohol ethoxylates containing an average of 7, 18, 30 and 100 oxyethylene units/mol has been evaluated (21) using CO_2 evolution, DOC, CTAS and HBr-GC criteria (Fig. 6). The results of this study indicate a significantly lower level of biodegradation only for the AE containing 100 EO units/mol.

Replacing the branched nonyl chain found in most commercial APE with a linear nonyl chain has been reported to increase the biodegradation rate to some extent (17). The branched APE had reached a 5% theoretical yield of CO_2 evolution during 26 days in a laboratory activated sludge system whereas the linear APE had attained a 40% yield. AE having essentially equivalent oxyethylene content produced greater than 65% CO_2 yields in equivalent tests. These results indicate that both the branching and the aromatic structure of APE decrease its biodegradation rates.

Effect of Temperature

Mann and Reid (15) have reported that low winter temperatures have a significant negative effect on the biodegradation of branched APE but little effect on a 25% branched primary AE. A primary biodegradation study (22) using commercial samples of 55% branched AE and a branched APE showed both of these products biodegraded slower at 3-4 C than at 20-23 C. However, the negative effect of

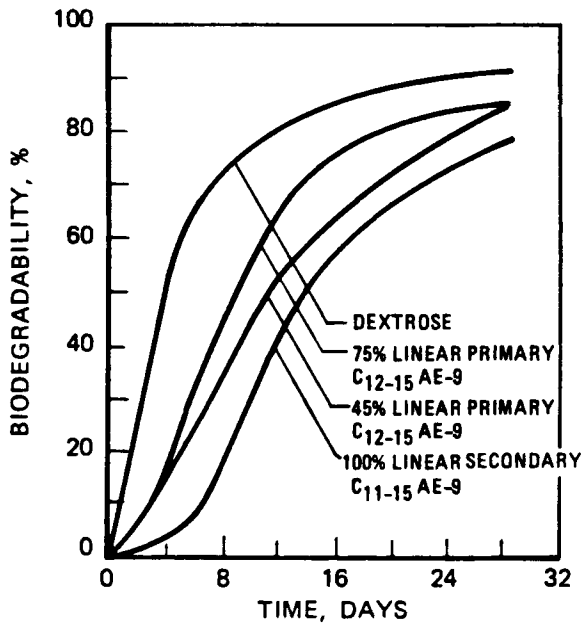


FIG. 5. Effect of alkyl structure on AE ultimate biodegradation by CO_2 evolution.

temperature was much more pronounced for the APE. It would appear from these results that low temperatures begin to exert a pronounced negative biodegradation effect for AE which have branching in excess of 25%.

Aquatic Toxicity

Commercial surfactants used commonly in the home and in industrial applications exhibit little toxicity to mammals but are relatively toxic to fish (23). Since these surfactants generally go through sewage treatment plants before entering receiving waters containing aquatic life, the ability of the surfactants to be rendered harmless as a result of treatment is of major concern.

The effect of AE on a sewage treatment plant and the

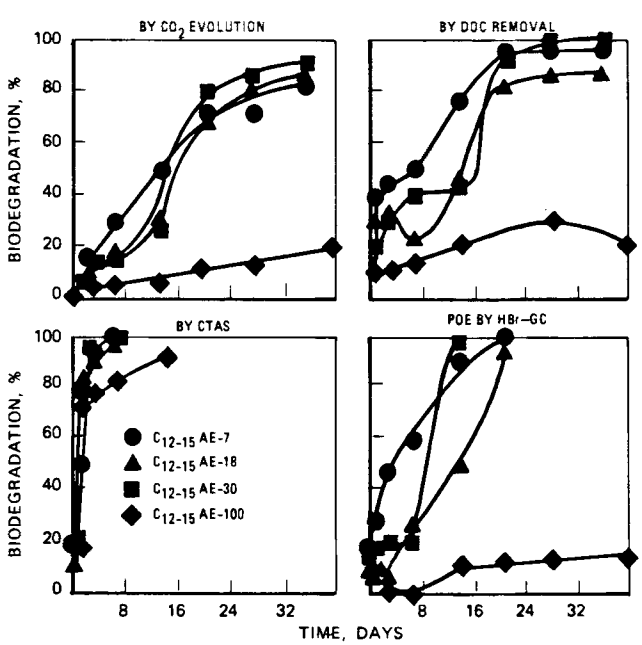


FIG. 6. Effect of polyoxyethylene chain length on biodegradation of 75% linear primary C_{12-15} AE.

resulting loss of its aquatic toxicity (20) have been mentioned. In an aquatic toxicity study comparing AE with APE under unacclimated river dieaway conditions, Reiff (16) reported the AE, at a 20 mg/ℓ initial concentration, became nontoxic to rainbow trout *(Salmo gairdneri)* in 10-14 days. Under these conditions the APE required ca. 70 days to be rendered nontoxic to trout. Loss of toxicity of both these surfactants was accompanied by decreasing response to analysis for primary biodegradation (BIAS). The intermediate products of AE and APE biodegradation were less toxic than their respective intact precursors.

Mechanisms of AE and APE Biodegradation

Efforts to elucidate detailed mechanistic pathways for AE and APE biodegradation have been limited by the complexity of these commercial products. Based on TLC results, Patterson (24) has suggested that APE biodegradation proceeds through simultaneous slow oxidation of the alkyl chain, the aromatic moeity and the POE chain, whereas AE biodegradation proceeds by initial fission into alkyl and POE chains followed by rapid oxidation of the alkyl chain and a somewhat slower oxidation of the POE chain. Rudling and Solyom (25) have found that APE biodegrades to moderately stable APE intermediates containing two EO units. Using AE radiolabeled with carbon-14 at selected sites in the molecule, Nooi et al. (26) reported results which indicate the terminal methyl group of the alkyl chain is the point of initial attack for AE. A somewhat more detailed mechanistic study for AE recently has been reported (7). For this study, double-labeled C_{14} AE-9 containing tritium in the alkyl chain and carbon-14 uniformly in the POE chain was synthesized (Fig. 7). Tritium in the alkyl chain was concentrated mostly in the alpha and gamma positions. CO_2 evolution studies in a modified shake flask showed the rapid appearance of ^3H_2O in the early stages of biodegradation accompanied by very little CO_2 evolution (Fig. 8). This implies that the tritiated portion of the alkyl chain biodegraded faster than the alkyl chain as a whole. The CO_2 evolution data show the alkyl chain degrades at a faster rate than the POE chain.

The rapid formation of ^3H_2O with little CO_2 evolution suggests a mechanism in which the α- carbon of the alkyl chain is oxidized initially to form an ester (Fig. 9). Enzymatic hydrolytic cleavage would then give a fatty acyl group and a POE moeity. Next, the fatty acyl group would biodegrade rapidly by a well-established biological process called β-oxidation (27). The POE degrades more slowly by a mechanism that apparently involves POE carboxylates (28).

The mechanism in which the α- carbon atom is the initial point of attack is consistent with the molecular fission approach suggested by Patterson et al. (24), but not with studies of Nooi et al. (26) in which the terminal methyl carbon atom appears to be attacked initially. These apparently conflicting results indicate that AE may be degraded by different bacterial systems, each having varying capabilities to initiate the biodegradation.

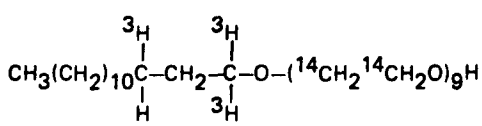


FIG. 7. Radiolabeled C_{14} AE-9.

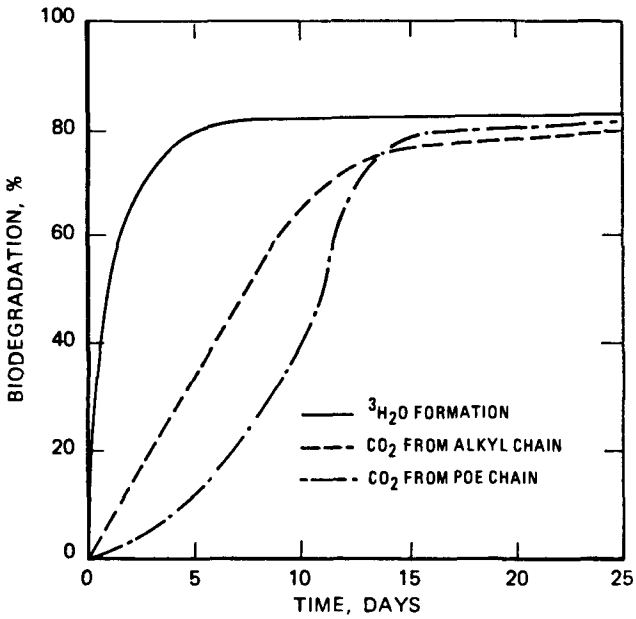


FIG. 8. Biodegradation of double-labeled C_{14} AE-9.

Selected Bacterial Strains

Recent work (28,29) has permitted isolation of several selective bacteria that are specific in their ability to degrade AE and polyoxyethylene glycols having varying POE chain lengths. Selective bacterial strains seem promising in attempts to elucidate the biodegradation mechanisms and

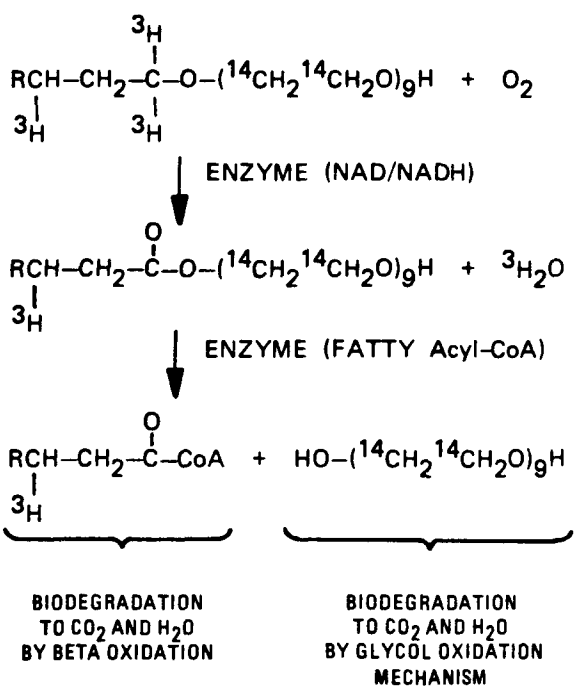


FIG. 9. Proposed biodegradation mechanism for AE.

identify nonionic biodegradation intermediates. It is doubtful whether selected strains will find extensive use in sewage treatment plants in the foreseeable future because of the difficulty of preparing and maintaining them in the large quantities which would be required.

ENVIRONMENTAL CONCERNS FOR NONIONIC SURFACTANTS

With increasing regulatory implementation of environmental legislation by the EPA, there is active interest in the environmental fate of nonionics. The Soap and Detergent Association (SDA), through its technical committee structure, is expending considerable effort to ascertain the effect of surfactants on the environment. Areas being considered for further study include the following.

Monitoring of Nonionics in Sewage Plant Outfalls and Receiving Waters by Primary Biodegradation Criteria Such As CTAS

A project to accomplish this study is currently underway by the SDA. Methodology is being developed for monitoring LAS and AE under Federal Good Laboratory Practices guidelines. A selective analytical method for AE is required in order to properly understand the primary biodegradation monitoring results.

Standardization of Biodegradation Test Methodology

The EPA has already suggested test protocol in a guidance document issued in 1979. The European Economic Community recently has issued a draft directive in which test methodology and a minimal standard of at least 80% primary biodegradability for nonionic surfactants has been stated.

Coupling of Biodegradation Tests to Aquatic Toxicity Tests

Few results based on testing nonionic surfactants by biodegradability/toxicity coupled tests have been reported. The effects of intact surfactants and their biodegradation intermediates on aquatic organisms is an area of continuing study.

Effect of Surfactants on Amended Soils Derived from Waste Sludge

Increasing amounts of waste sludge are being used for agricultural purposes. Data may be required on the effects of nonionics and their biodegradation products which are present in these sludges.

REFERENCES

1. *The Wall Street Journal*, May 29, 1980, p. 10.
2. Huddleston, R.L., and R.C. Allred, JAOCS 42:983 (1965).
3. Osburn, Q.W., and J.H. Benedict, Ibid. 43:141 (1966).
4. Patterson, S.J., C.C. Scott and K.B.E. Tucker, Ibid. 44:407 (1967).
5. Anthony, D.H.J., and R.S. Tobin, Anal. Chem., 49:398 (1977).
6. Cook, K.A., Water Res. 13:259 (1979).
7. Kravetz, L., H. Chung, J.C. Rapean, K.F. Guin and W.T. Shebs, Proccedings, Am. Oil Chem. Soc. 69th Annual Meeting, St. Louis, MO, May 1978, in press.
8. Gledhill, W.E., Appl. Microbiol, 30:922 (1975).
9. Swisher, R.D., Surfactant Biodegradation, Marcel Dekker, New York, 1970.
10. Gilbert, P.A., and G.K. Watson, Tenside Deterg. 14:171 (1977).
11. Scharer, D.H., L. Kravetz and J.B. Carr, Tappi 62:75 (1979).
12. Huddleston, R.L., and R.C. Allred, JAOCS 41:723 (1964).
13. Patterson, S.J., C.C. Scott and K.B.E. Tucker, Ibid. 45:528 (1968).
14. Lashen, E.S., F.A. Blakenship, K.A.. Booman and J.J. Dupree, Ibid. 43:371 (1966).
15. Mann, A.H., and V.W. Reid, Ibid. 48:794 (1971).
16. Reiff, B., Seventh International Congress on Surface Active Substances, Moscow, September 1976.
17. Sturm, R.N., JAOCS. 50:159 (1973).
18. Abram, F.S.H., V.M. Brown, H.A. Painter and A.H. Turner, Fourth Symposium on Surface Active Substances, Yugoslavia, October 1977.
19. Sykes, R.M., A.J. Rubin, S.A. Rath and M.C. Chang, J. Water Poll. Control Fed., 51:71 (1979).
20. Maki, A.W., A.J. Rubin, R.M. Sykes and R.L. Shank, Ibid. 51:2301 (1979).
21. Kravetz, L., H. Chung, K.F. Guin and W.T. Shebs, Am. Oil Chem. Soc. 70th Annual Meeting, San Francisco, CA, May 1979, in press.
22. Schoeberl, P., and H. Mann, Arch, Fisch. Wiss, 27:149 (1976).
23. Macek, K.J., and S.F. Krzeminski, Bull. Env. Contam. and Toxicol., 13:377 (1975).
24. Patterson, S.C., C.C. Scott and K.B.E. Tucker, JAOCS 47:37 (1970).
25. Rudling, L., and P. Solyom, Water Res., 8:115 (1974).
26. Nooi, J.R., M.C. Testa and S. Willemse, Tenside Detergents, 7:61 (1970).
27. Gottschalk, G., Bacterial Metabolism, Springer-Verlag, N.Y. (1979).
28. Watson, G.K., and N. Jones, Water Research, 11:95 (1977).
29. Cook, K.A., J. of Appl. Microbiol., 44:297 (1978).