Determination of Nonionic Surfactant Biodegradability: Physical Properties vs. Colorimetry[1]

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Abstract

Biodegradation studies with three alkyl phenol nonionic products have revealed that loss of cobaltothiocyanate colorimetric sensitivity may not necessarily correlate with loss of surface tension lowering and foaming properties when these type structures are exposed to microbial attack. Where this type of disagreement between analytical methods is present, only the surface tension and foam data are valid measurements of biodegradation.

Introduction

THE PROBLEMS ATTRIBUTED to the presence of detergents in water distribution systems have, for the most part, resulted from the inability of the microbial flora associated with these systems to rapidly destroy the foam and surface tension lowering properties of the detergents. Thus, for a detergent to be defined as biodegradable and effective in eliminating these problems, it must be rapidly decomposable by microorganisms to the extent that it is no longer capable of lowering surface tension or producing foam. Analytical methods used to evaluate detergent biodegradability must provide data commensurate with this definition.

Until about one year ago the only analytical tools generally used to ascertain the biodegradability of nonionic detergents were surface tension, foam, and oxygen uptake measurements. Although surface tension and foam characteristics provided valid biodegradation data, a more specific analytical method was sought. Almost simultaneously, at least two laboratories developed colorimetric tests based upon a complexing reaction between ethylene oxide adducts and ammonium cobaltothiocyanate (1,2). IR and UV spectroscopy have also been used by some in biodegradation mechanism studies of certain nonionic structures (3).

Since the advent of these colorimetric tests, they have been used almost exclusively to characterize the biodegradation properties of nonionic detergents. There has been general agreement that primary and secondary straight chain alcohol adducts are readily biodegradable as demonstrated by all applicable analytical tests. However, whether or not the alkyl phenol ethoxy adducts qualify as biodegradable detergents has become a controversial point (4–6). Recent findings suggests that this controversy has resulted from biodegradation conclusions based on tests, that when applied to certain alkyl phenol nonionic products, do not provide data equivalent to loss of surface tension and foam properties.

Experimental Methods

Three alkyl phenol nonionic products were selected for detailed biodegradability studies; an isoctyl phenol polyethoxy adduct containing 9 to 10 moles of EO, a straight chain nonyl phenol polyethoxy adduct containing 9 moles of EO, and a straight chain dodecyl phenol polyethoxy adduct containing 12 moles of EO. For comparative purposes, a straight chain alcohol (C_{10-12}) adduct containing 6 moles of EO was included in each test system. All four of these nonionics are commercial products and the descriptions given represent the average compositions.

[1] Presented at the AOCS meeting, Houston, Texas, April 1965.

TABLE I

Shake Culture Alkyl Phenol Nonionic Biodegradation Summary

Product	Method of Analysis		
	Colorimetry, ppm	Foam, ml	Surface tension dynes/cm
St. ch. C_{10-12} alkyl NI	30→0	17→0	39→72
Br. ch. C_8 alkyl Φ NI	30→27.5	18→16	42→44
St. ch. C_9 alkyl Φ NI	30→17.4	19→18	35→44
St. ch. C_{12} alkyl Φ NI	30→12.6	17→13	38→46
Medium blank		0→0	59→72

Three well-known and widely used biological test systems for evaluating detergent biodegradability were employed. They were the shake culture test (7), the semicontinuous activated sludge test (8), and the river die-away test (9). These tests have all been described in detail as referenced and it should suffice here to say that they are laboratory scale systems representing biological environments likely to be encountered following household or industrial use of detergents. Each surfactant was run in triplicate in each test system, and entire studies in each test system were performed at least two times. In addition to the ordinary one week of culture adaptation for the shake flask method, extensive adaptation studies were made with the shake flask culture and fresh activated sludge to the isooctyl phenol nonionic product. Weekly transfers for a total of three months were made in flasks containing this particular nonionic.

During biodegradation studies in each test system, surface tension measurements were taken with a Du-Noüy tensiometer. Foamability of the nonionics while subjected to degradation was determined by pouring 50 ml samples of the test medium into a 100 ml glass-stoppered graduated cylinder, shaking for 15 seconds, and after 15 additional seconds reading the amount of foam present.

The cobaltothiocyanate colorimetric method was essentially that reported by Greff, Setzkorn and Leslie (1) except that an initial ether extraction step was incorporated. The extraction step was found not to change the overall degradation picture but significantly improved the reproducibility of the technique.

Chloroform extracts of acidified river die-away media and semicontinuous activated sludge effluent media were used to obtain IR and NMR spectroscopic evaluations of the four nonionic products exposed to

TABLE II

Mississippi River Water Alkyl Phenol Biodegradation Summary

Product	Method of analysis		
	Colorimetry ppm	Foam ml	Surface tension dynes/cm
St. ch. C_{10-12} alkyl NI	10→0	16→0	58→72
Br. ch. C_8 alkyl Φ NI	10→0.5	14→2	51→52
St. ch. C_9 alkyl Φ NI	10→0.6	15→2	44→62
St. ch. C_{12} alkyl Φ NI	10→0.7	6→4	49→65
Medium blank		0→0	72→72

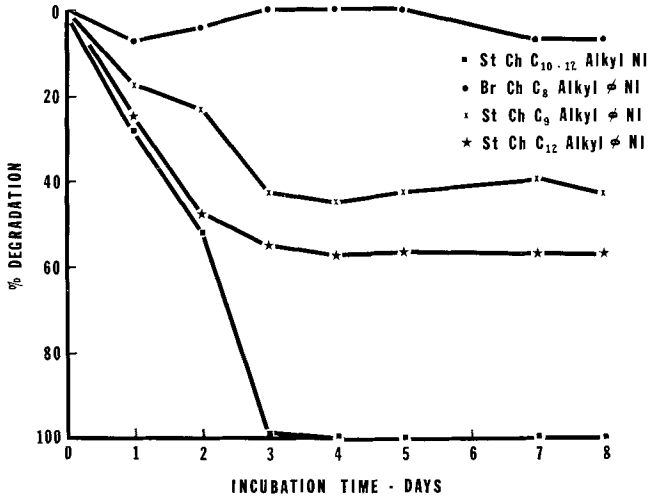

FIG. 1. Biodegradation of alkyl phenol nonionics in the shake culture system, colorimetric measurements. Initial NI levels, 30 ppm.

microbial attack. The basic IR methods employed were those reported by Frazee, Osburn and Crisler (3).

Experimental Results

Biodegradation of the four nonionic products in the shake culture system is shown in Figures 1, 2, and 3 and summarized in Table I. Only the straight chain alcohol adduct was found biodegradable. The straight chain C_9 and C_{12} alkyl phenol products were 41% and 58% decomposed as measured by colorimetry. Surface tension and foam values were not in disagreement with these levels of biodegradation. The isooctyl phenol ethoxy product was only 8% degraded by colorimetry and practically no changes in surface tension and foam levels were observed. Adaptation of the shake flask culture and fresh activated sludge for up to 3 months did not produce more than 10% degradation of this product.

Colorimetric and foam measurements in the river die-away system (Fig. 4 and 5) demonstrated rapid and complete biodegradation of the straight chain alcohol derived nonionic. After 15 days of incubation these methods of analyses also showed major degradation of the three aromatic nonionic products. Surface tension levels in the die-away studies are given in Figure 6 and a summary of die-away degradation findings is presented in Table II. The surface tension changes observed in jars containing the alkyl phenol

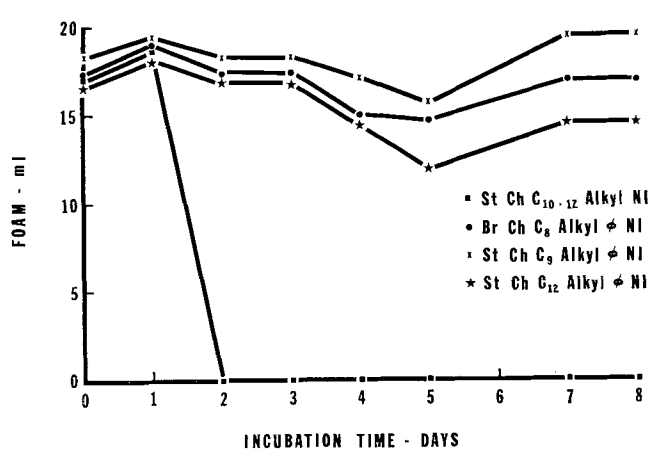

FIG. 2. Biodegradation of alkyl phenol nonionics in the shake culture system, foam measurements. Initial NI levels, 30 ppm. Medium blank, no foam.

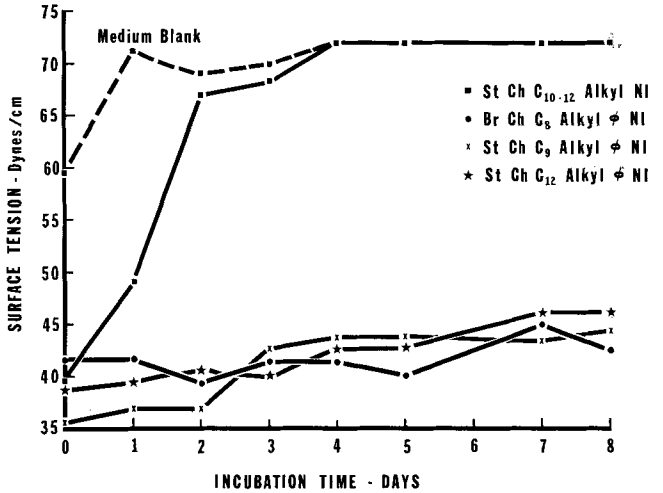

FIG. 3. Biodegradation of alkyl phenol nonionics in the shake flask system, surface tension measurements. Initial NI levels, 30 ppm.

products were not commensurate with the high degradation levels shown by colorimetry.

Degradation in the semicontinuous activated sludge test system is shown in Table III. As in the other two test systems only the straight chain alcohol ethoxylate was completely biodegradable by all three methods of analyses. Again the surface tension and foam changes exerted by the alkyl phenol nonionics did not support the high level degradations indicated by the colorimetric data.

Chloroform extracts of river die-away and semicontinuous samples were weighed and the resultant findings are shown in Table IV. As predictable from the surface tension data, biodegradation of the alkyl phenol nonionics was not as extensive as the colorimetric determinations indicated. These extracts were then examined by IR and NMR spectroscopy and the materials present characterized. Alterations found in the polyethoxy chains are shown in Table V.

Discussion

Since the definition of a biodegradable detergent implies loss of foam and surface tension properties, analytical methods such as the cobaltothiocyanate test and oxygen uptake measurements, are indirect measures of detergent biodegradation. As long as degradation conclusions, derived with indirect analytical techniques, are also reflected in loss of surface tension and foam properties of the detergent in question, the conclusions are valid. Unless this correlation is shown

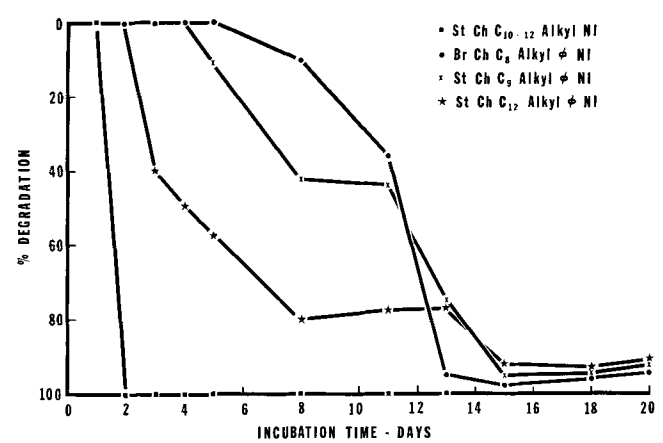

FIG. 4. Biodegradation of alkyl phenol nonionics in Mississippi River water, colorimetry. Initial NI levels, 10 ppm.

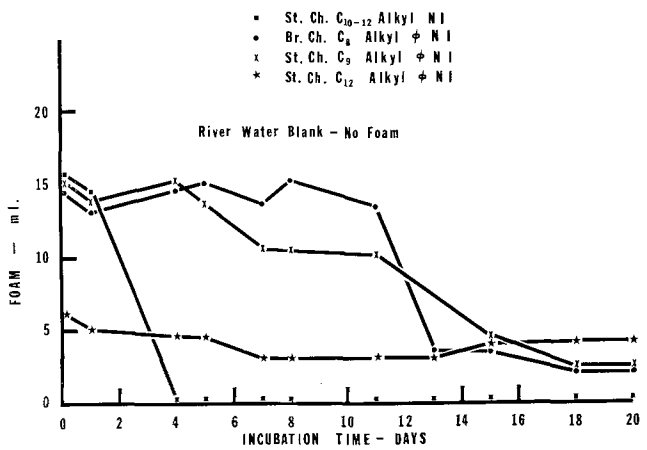

FIG. 5. Biodegradation of alkyl phenol nonionics in Mississippi River water, foam measurement. Initial NI levels, 10 ppm.

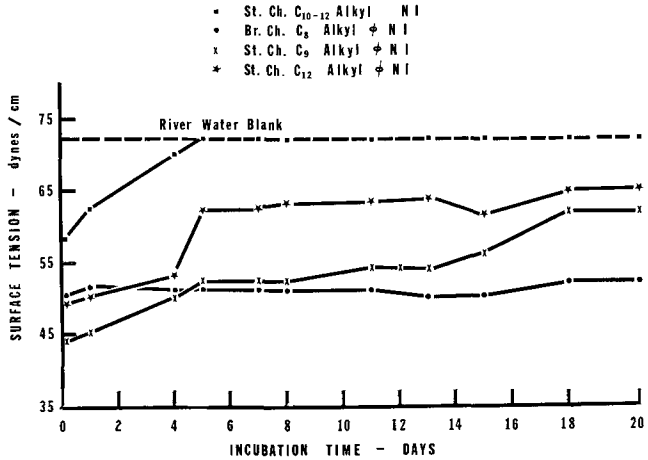

FIG. 6. Biodegradation of alkyl phenol nonionics in Mississippi River water, surface tension. Initial NI levels, 10 ppm.

to exist, the use of indirect analytical methods may create a false impression of biodegradability.

From Table VI it is apparent that the cobaltothiocyanate test may be validly employed to ascertain biodegradability properties of straight chain alcohol nonionics. It is equally apparent that the colorimetric test should not be applied alone to evaluate biodegradabilities of at least some alkyl phenol products. It appears that during the degradation of these products intermediate structures are produced that continue to exert foam and surface tension lowering effects. Degradation of the alkyl phenol nonionics in the shake culture test was so poor that biodegradation to these intermediate forms did not occur.

The loss of colorimetric sensitivity, particularly in the case of the isooctyl phenol nonionic product, may be explained by the partial removal of ethylene oxide by the microorganisms. Adducts containing 3–4 moles of EO or less are insensitive to the test. Loss of these properties by microbial attack on the hydrophobic end of the molecule is theoretically possible, but appears to have been hindered by the highly branched alkyl chain. The bioresistance of a branched alkyl chain and partial removal of ethylene oxide from this type of structure was also reported by Frazee et al.

The spectroscopic evidence indicates that the straight chain alkyl phenols possess enough ethylene oxide units, even after prolonged biodegradation, that the intermediate structures should remain sensitive to colorimetry. One might conclude then, that the alkyl chain of these structures has been sufficiently modified to render them less sensitive to the colorimetric test.

Biodegradation of the straight chain alcohol adducts appears to result in essentially no residue. Degradation was so rapid that no identifiable intermediates could be found. Probably these types of nonionics are easily biodegraded from both ends of the molecules. Essentially these same conclusions were reported by Frazee et al. These workers did find evidence of some polyethylene glycol present during degradation of a C_{14} straight chain alcohol adduct after 5 days of river die-away incubation. Further incubation probably would have resulted in loss of the glycol.

While it is important to have a reliable and rapid analytical tool to follow biodegradation of the alkyl phenol type of nonionic detergents, it is imperative that the method provide results commensurate with

TABLE III

Biodegradation of Alkyl Phenol Nonionics in the Semicontinuous Activated Sludge System

NI feed level—20 ppm

Values based on uniform operation between 5 and 24 days of system operation

	Color (% Biodeg.)	Foam (ml) Influent	Foam (ml) Effluent	Sur. Ten. (dynes/cm) Influent	Sur. Ten. (dynes/cm) Effluent
St. ch. alkyl NI	100	15	0	46	70
Br. ch. alkyl ϕ NI	97	15	7	48	51
St. ch. C_9 alkyl ϕ NI	99	19	6	39	57
St. ch. C_{12} alkyl ϕ NI	90	16	7	44	52
Medium blank		2	0	70	71

TABLE IV

Biodegradation Residue in the River Die-Away and Semicontinuous Systems

Chloroform extractions from acidified samples minus blank

	mg/liter			
	St. ch. alkyl NI	Br. ch. alkyl ϕNI	St. ch. C_9 alkyl ϕNI	St. ch. C_{12} alkyl ϕNI
Semicontinuous				
Initial	20	20	20	20
After biodeg. (25th day)	1.5	12	8	19.1
River die-away				
Initial	10	10	10	10
After biodeg. (20th day)	0.95	4.7	5.4	7.5

TABLE V

Alteration of the Polyethoxy Chain During Biodegradation Infrared and NMR Spectroscopic Data

Product—moles of EO		Moles EO remaining after degradation: Die-away (25th day)	Moles EO remaining after degradation: Semi-continuous (20th day)
St. ch. alkyl NI	6	~ 0	~0
Isooctyl alkyl ϕ NI	9	~ 4	~4
St. ch. nonyl ϕ NI	9	~ 5	~3
St. ch. dodecyl ϕ NI	12	~11	~7

TABLE VI

Nonionic Biodegradation Three Systems—Three Analytical Techniques

	% Biodegradation			
	St. ch. alkyl NI	Br. ch. alkyl ϕ NI	St. ch. C_9 alkyl ϕ NI	St. ch. C_{12} alkyl ϕ NI
Shake culture				
Color	100	8	42	58
S.T.	100	<10	40–50	50–70
Foam	100	<25	10–30	20–40
River die-away				
Color	100	95	94	93
S.T.	100	20	80	80
Foam	100	80	>90	70
Wt. loss	91	53	46	25
Semicontinuous				
Color	100	97	99	90
S.T.	>95	50	85	65
Foam	100	75	75	50
Wt. loss	93	40	60	5

NOTE: S.T. and foam values computed from standard concentration curves of unaltered NI.

the definition of a biodegradable detergent. Where the cobaltothiocyanate colorimetric test is used to evaluate biodegradability of nonionics, in particular the alkyl phenol structures, the results should be accompanied by adequate surface tension and foam data.

ACKNOWLEDGMENT

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A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate

The Subcommittee on Biodegradation Test Methods of The Soap and Detergent Association [1]

Introduction

DURING THE EARLY 1950's the soap and detergent industry first became aware of a possible relationship between the residues of its products and foaming in some locations. When these incidents did occur they were most often observed in activated sludge aeration tanks of sewage plants although foaming did occasionally take place in surface and ground waters as well. It is important to note that foaming may be caused by natural surfactants as well as by detergent surfactants; nonetheless, the industry proceeded to develop new detergent surfactants which would biodegrade more rapidly than those in use at the time, thus reducing the potential for such foaming incidents.

This ten-year industry effort came to completion on the first of July, 1965, when linear alkylate sulfonate (LAS) totally replaced tetrapropylene derived alkyl benzene sulfonate (ABS) as the principal surfactant in U.S. detergent production.

Soon after work began in the development of the more biodegradable surfactants, it became apparent that standardization of methodology would be necessary to assure a uniform evaluation of the many materials under test. Several biodegradability test methods had been used by individual companies but no single method had received general industry acceptance. Once standard methodology was established, there was a need to define biodegradability so that performance goals and achievements would be meaningful.

In 1961 the Technical Advisory Committee of The Soap and Detergent Association established a Subcommittee on Biodegradation Test Methods and charged this new group with two major assignments: 1) to review and evaluate existing procedures; 2) to develop, where necessary, new methods and standards to meet the needs of the industry in this country.

This committee was made up of representatives of most of the principal detergent raw materials suppliers and product formulators. These companies were interested in developing methods which would not only be useful as a scientific tool but which would also have practical application in such areas as surfactant screening and quality control as well. To meet these goals it was agreed that any method accepted would have to: 1) be as relatively simple as possible; 2) be as economical as possible; 3) be reproducible, and 4) report results in terms which would be relatable to field sewage treatment experience.

Review of Methods Considered by the Subcommittee

The range of experimentation varied from the simple and inexpensive river die-away test (1–9) to the complex and costly continuous activated sludge procedure, which in one form is specified in the West German Detergent Law (10).

Methods of intermediate complexity which were also evaluated were the shake flask (11) and semicontinuous activated sludge procedures (12).

Development of the Test Procedure

After extensive cooperative investigation, two methods—the shake flask and the semicontinuous activated sludge—seemed to meet equally the requirements set forth for a suitable biodegradability test. It was agreed by the Committee to concentrate their efforts on these methods, and a plan was developed for cooperative evaluation.

Since surfactants of the ABS/LAS type predominate in American detergent production, it was of primary importance to concentrate the initial evaluation to this class of surfactants. Existing analytical procedures lent themselves to an accurate appraisal of the biodegradability of these materials. (Additional work, currently underway, concerns itself with other anionic and nonionic surfactants). Thus, the described procedure and the related bio-

[1] C. M. Snow, Phillips Petroleum Company, Chairman; R. Allred, Continental Oil Company; K. A. Booman, Rohm and Haas Company; J. W. Compton, Wyandotte Chemicals Corporation; R. A. Conway, Union Carbide Corporation; M. Ettinger, U.S. Public Health Service (*ex officio*); N. Franke, Gulf Oil Corporation; L. J. Garrison, Jefferson Chemical Company, Inc.; M. A. Goldberg, Lever Brothers Company; R. House, Chevron Research Company; C. A. Houston, Shell Chemical Company; E. Jungermann, Armour and Company; C. M. Knowles, General Aniline and Film Corporation; F. J. Lowes, Dow Chemical Company; M. Mausner, Ultra Chemical Company; G. Orgel, Allied Chemical Corporation; F. A. Rupp, Colgate-Palmolive Company; J. E. Shewmaker, Esso Research and Engineering Company; A. Shultz, Allied Chemical Corporation; R. D. Swisher, Monsanto Company; R. Taylor, Atlantic Refining Company; P. J. Weaver, The Procter & Gamble Company; T. E. Brenner, The Soap and Detergent Association.