SYMPOSIUM: BIODEGRADABLE DETERGENTS. PART II

conducted by The American Oil Chemists' Society
at its 55th Annual Meeting, New Orleans, Louisiana
April 19-22, 1964

ERIC JUNGERMANN, PRESIDING
H. P. DUPUY, PROGRAM CHAIRMAN

A Comparison by Warburg Respirometry and Die-Away Studies of the Degradability of Select Nonionic Surface-Active Agents

L. J. GARRISON and R. D. MATSON, Jefferson Chemical Company, Inc., Houston, Texas

Abstract

The relative biodegradability of several classes of nonionic surfactants have been determined by Shake Flask, Die-Away and Warburg Respirometer tests. Analytical techniques used to follow the degradation processes involved the measurement of loss of surfactant properties (surface tension or foamability), colorimetric determinations and oxygen uptake studies. Nonionic products prepared from naturally occurring or synthesized straight chain hydrophobes were shown to exhibit a higher degree of biodegradability than products based upon branched chain materials. A good correlation of data by the various analytical techniques was obtained on the straight chain based products.

Introduction

RECENT EVENTS HAVE WITNESSED vast changes in the manufacture, formulation and evaluation of detergent products and their chemical intermediates. The industry's declared switch from alkylbenzene sulfonate (ABS) to linear alkylate sulfonate (LAS) is well known. Equally significant changes are occurring in the field of nonionic products. These changes result from increased attention focused on the biologically hard industry work horse—the branched chain alkylphenol ethylene oxide adduct family (1).

Each month during the past year, new and differing reports were issued on the degree of biological softness or hardness of nonionic detergents prepared from straight or branched chain hydrophobes. This was not unexpected since different yardsticks were applied to measure degradability—as determined by surface tension or foaming characteristics—in a river die-away test. Within the past few months at least one reference has appeared to an analytical procedure for the direct determination of ethylene oxide based nonionics (2).

TABLE I

Products Studied—Arranged by Class

1. Alcohol ethoxylates
   a. Ziegler (straight chain) $C_{12\ 15}$ alcohol + 7–9 EO
   b. Oxo (branched chain) C_{13} alcohol + 9 EO
   c. Natural (straight chain) C_{12} alcohol + 3 EO
2. Amides
   a. C_{12} diethanolamide
   b. C_{12-18} amide ethoxylate
3. Alkylphenol-phenol ethoxylates
   a. Phenol + 9 EO
   b. C_9 (straight chain alkylphenol) + 9–10 EO
   c. C_9 (branched chain alkylphenol) + 9–10 EO
4. Anionic
   a. Alkylbenzene sulfonate, Na salt (branched)

TABLE II

Shake Flask Tests
Composition of Basal Medium

Ammonium chloride	3.00 g
Dipotassium phosphate	1.00 g
Magnesium sulfate	0.25 g
Potassium chloride	0.25 g
Ferrous sulfate	0.002 g (Trace)
Yeast extract	0.30 g
Distilled water	1.0 liter

INDEX

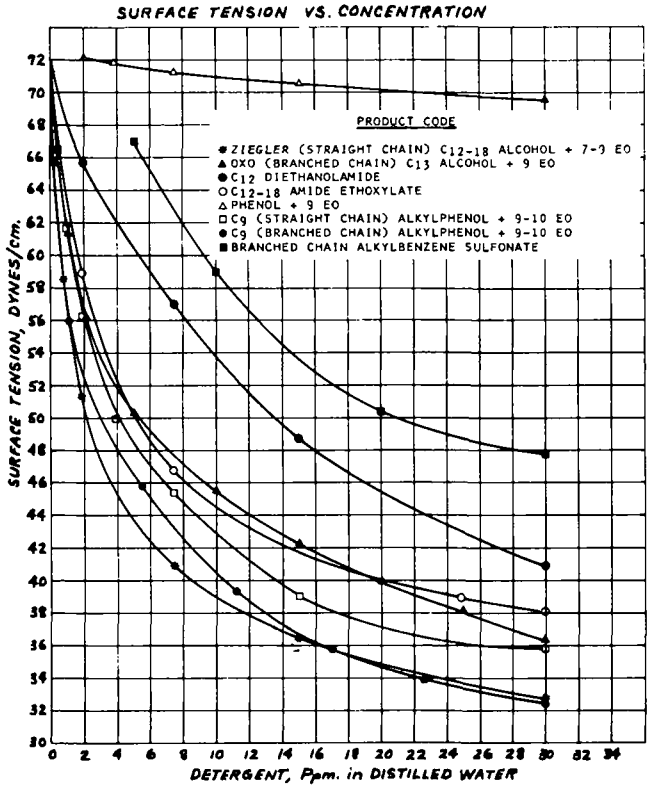

FIG. 1.

This paper compares and discusses biodegradation results of ethoxylated (3,4) alcohol, amide and alkylphenol types of nonionic detergents and of one ABS anionic detergent by shake flask, rapid die-away and Warburg Respirometer techniques. The products studied in these four classes are shown in Table I.

Experimental Procedures and Data

Shake Flask Tests

Microbial cultures were grown and adapted for our studies by transfers in a medium containing a total of 30 ppm of mixed detergent "feed." The original culture obtained from the Soap and Detergent Assoc. was adapted to a medium containing 30 ppm of LAS prepared from dodecene-1. This culture was modified by growth and adaptation in a medium containing mixed detergent feed consisting of 15 ppm of dodecene-1 derived LAS and 15 ppm of C_9 (branched chain alkylphenol) + 9–10 EO or 15 ppm of Ziegler (straight chain) C_{12-18} alcohol + 7–9 EO, all on an active basis. These mixed detergent adaptations served a dual purpose in developing cultures for studies with either anionic or nonionic detergents and with "hard" or "soft" nonionic detergents.

The basal medium composition for all adaptive growth transfers and for detergent biodegradation studies is shown in Table II. This medium and 30 ppm of the test detergent were added to a 2-liter Erlenmeyer flask and sterilized in an autoclave at 15 lb pressure for 15 min. To develop and maintain adapted cultures, the basal medium and 30 ppm of the mixed detergent (LAS/alcohol ethoxylate or LAS/alkylphenol ethoxylate) were treated in the same manner. The flasks were cooled and inoculated with 10 ml of microbial culture. Flasks were then incubated on a Eberbach rotary shaker at 220–240 rpm and room temp (24–26C).

In the above manner, growth cultures were maintained by weekly transfers. Adapted cultures for biodegradation studies were transferred at two 72-hr periods and the test was started at the following 72-hr interval. Blank samples were run in a similar manner except that no detergent was added to the inoculated medium.

Samples for analytical determinations were taken immediately after inoculation and at 24-hr intervals thereafter during the 7-day test period.

TABLE III

Comparison of Foaming Tendency with Surface Tension and Colorimetric Values

Product and procedure	Percent surfactant remaining		
	Foam dilution[a]	Surface tension	Colorimetric
Alcohol ethoxylates			
Ziegler (straight chain) C_{12-18} alcohol + 7–9 EO			
A[b]	0	1.2	0
B	1.5	1.2	0.15
C	3.0	1.0	0
Oxo (branched chain) C_{13} alcohol + 9 EO			
A	....	....	.....
B	100	64	89
C	....	....	.....
Amides			
C_{12} diethanolamide			
A	0	0	0
B	0	0	0
C	0	0	0
C_{12-18} amide ethoxylate			
A	3	1.7	0
B	3	1.9	0.63
C	12.5	5.4	0.35
Alkylphenol ethoxylates			
Phenol + 9 EO			
A	0	78	6.4
B	0	91	68.5
C	....	....	.....
C_9 (straight chain alkylphenol) + 9–10 EO			
A	25	34	43
B	100	43	38
C	50	37	37
C_9 (branched chain alkylphenol) + 9–10 EO			
A	100	68	67
B	100	80	89
C	100	83	91
Anionic			
Alkylbenzene sulfonate, Na salt (branched)			
A	50	112	131
B	100	...	100
C	100	....	100

[a] Foam threshold assumed to be zero concentration.
[b] Code: A = Die away test.
B = Shake flask test surface tension.
C = Shake flask test—colorimetric.

Die-Away Tests

Activated sludge was obtained from the Austin, Texas Sewage Treatment Plant and filtered. This sludge is from a predominantly domestic source. A stock solution of the filtered solids was prepared containing 10 g of solids/liter of distilled water. This stock solution was used in preparing die-away tests in 2-quart Mason jars having a detergent concn of 30 ppm. The composition is as follows:

Distilled water	870 ml
Detergent stock solution*	30 ml
Sludge stock solution	100 ml

* 1000 ppm test surfactant on an active basis in distilled water.

The die-away test solutions were vigorously agitated for mixing and aeration for one min using a Teflon coated stirring bar and a magnetic stirrer. After samples had been taken for analytical determinations, the jars were capped and stored in boxes at room temp (24–26C). The stirring and sampling procedures were repeated at 24-hr intervals for the duration of the 9-day test period. Blank samples containing distilled water (900 ml) and sludge stock solution

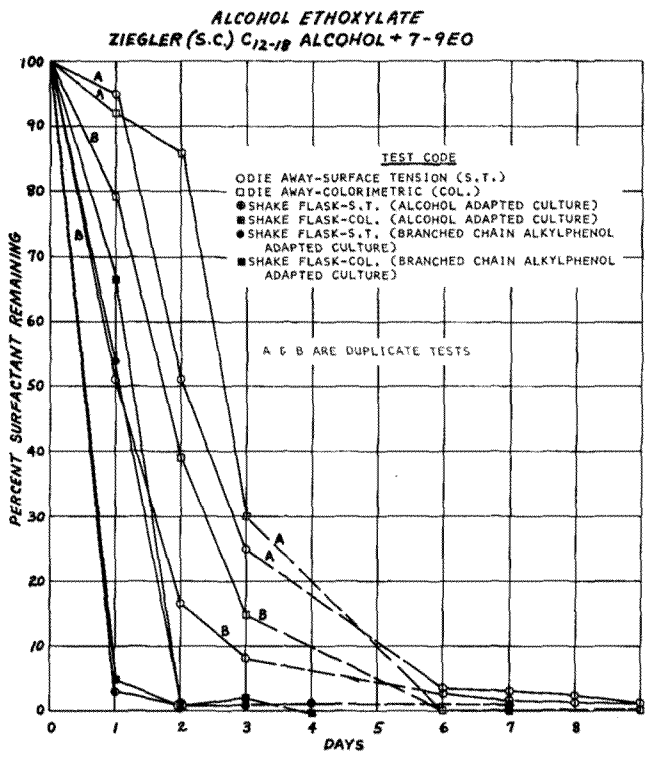

FIG. 2.

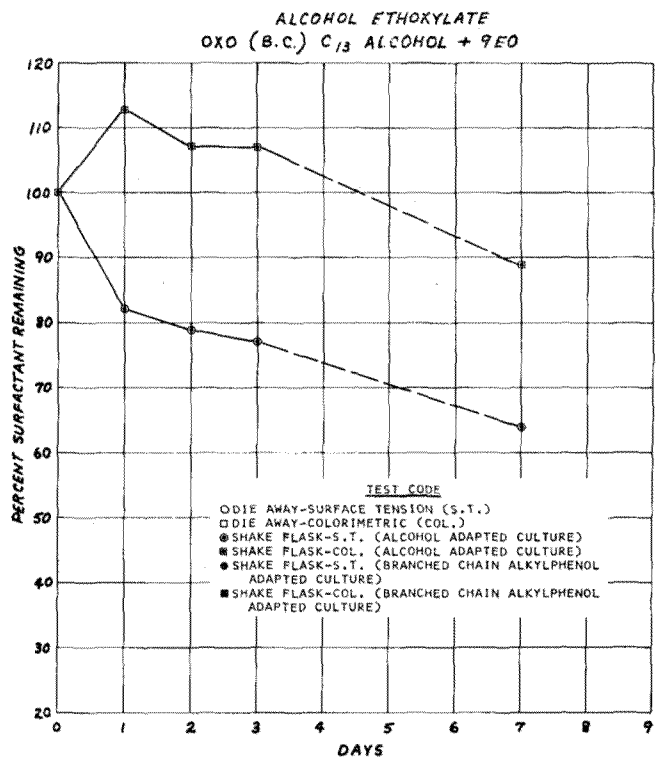

FIG. 3.

(100 ml) were prepared and sampled in the same manner.

Analytical Tests

The progress of detergent biodegradation in shake flask and die-away studies was evaluated by three analytical procedures.

1. *Surface Tension.* Surface tension calibration curves were constructed from measurements of known concn of the individual test detergents in distilled water. Subsequently, these calibration curves were used to estimate concn of detergents in biodegradation studies. All measurements were made at 25C using a Du Noüy direct reading tensiometer. Standard curves showing surface tension values for each of the products studied are given in Figure 1.
2. *Colorimetric Procedures.* Both anionic and nonionic detergents were determined by colorimetric procedures. The APHA Methylene Blue Method was followed for anionics (5). For nonionic detergents an ammonium cobaltothiocyanate complex was compared with that of a standard complex of the same nonionic at approx 320 mμ. From this relationship the concn of detergent remaining was calculated (6).
3. *Foam Thresholds.* Foam thresholds were determined for test detergents by a series of successive dilutions of 50 ml of solution of known concn in distilled water. After each dilution, 50 ml of the solution was agitated in a 100-ml graduated cylinder by inverting 10 times. Foaming characteristics were evaluated. This dilution technique was repeated until the foam threshold was reached. This threshold was not always as easily recognized, and operators frequently had difficulty in reproducing their results. Detergent concn at the threshold was determined from the known starting concn and the dilution factor to reach the threshold. Dilution factors for detergents in biodegradation studies were evaluated in a similar way and compared with those for the unaltered detergents.

Warburg Respirometry Tests

Manometric techniques to determine and follow oxygen uptake during product biodegradation are well known. Details concerning test procedures will not be given here. Surfactant concn used was 30 ppm as in the previously described tests. The disadvantages and advantages of Warburg testing are recognized and are discussed by Swisher (7).

Results and Discussion

Shake Flask and Die-Away Tests

Foam dilution, surface tension and colorimetric data for biodegradation studies by rapid die-away and shake flask tests are presented in Table III. In general, fairly good correlation was obtained between surface tension and colorimetric data. The poorest correlation was found during early and late stages in the biodegradation processes. At the high detergent concn studied, small differences are not detected by surface tension. On the contrary, very low concn of detergents exert definite surface tension lowering. The biodegradation system may be further complicated by the presence of surface-active metabolic fragments that have an effect on surface tension. The foam dilution technique was found to be a less useful method for following the biodegradation process. In most cases, poor correlation was obtained between the foam threshold and the surface tension or colorimetric methods. However, the foam technique readily demonstrated differences between high and low detergent concn within the 1–30 ppm range.

Alcohol ethoxylates. As shown in Figures 2 and 3, Ziegler (straight chain) C_{12-18} alcohol + 7–9 EO was readily biodegraded in either shake flask or rapid die-away tests while the oxo (branched chain)

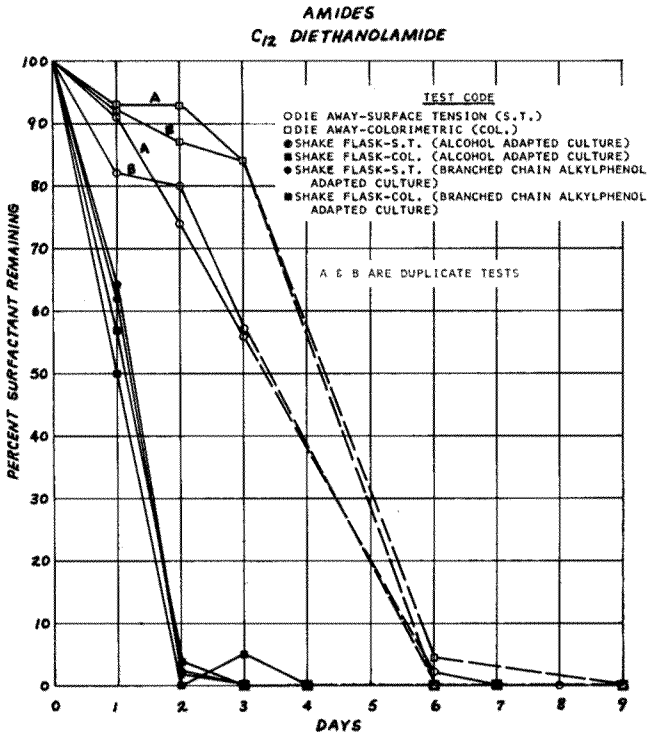

FIG. 4.

C_{13} alcohol + 9 EO was comparatively hard. Biodegradation rates for the straight chain product were somewhat more rapid in shake flask tests than in die-away tests. However, similar amt of biodegradation were indicated to have occurred by the end of the respective test periods.

Amides. Both the C_{12} diethanolamide and the C_{12-18} amide ethoxylate studied in this series of tests were biodegraded at rapid rates. Results of these tests are summarized in Figures 4 and 5. Again, as

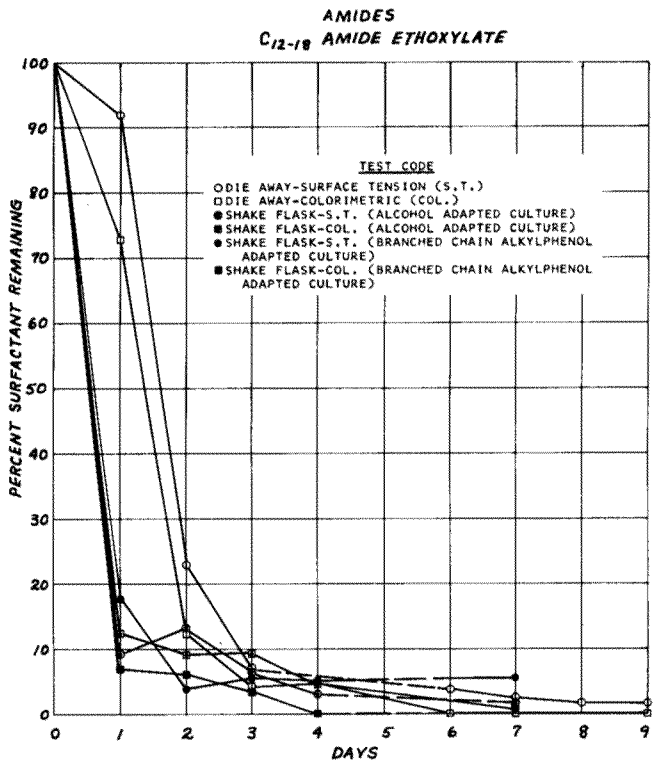

FIG. 5.

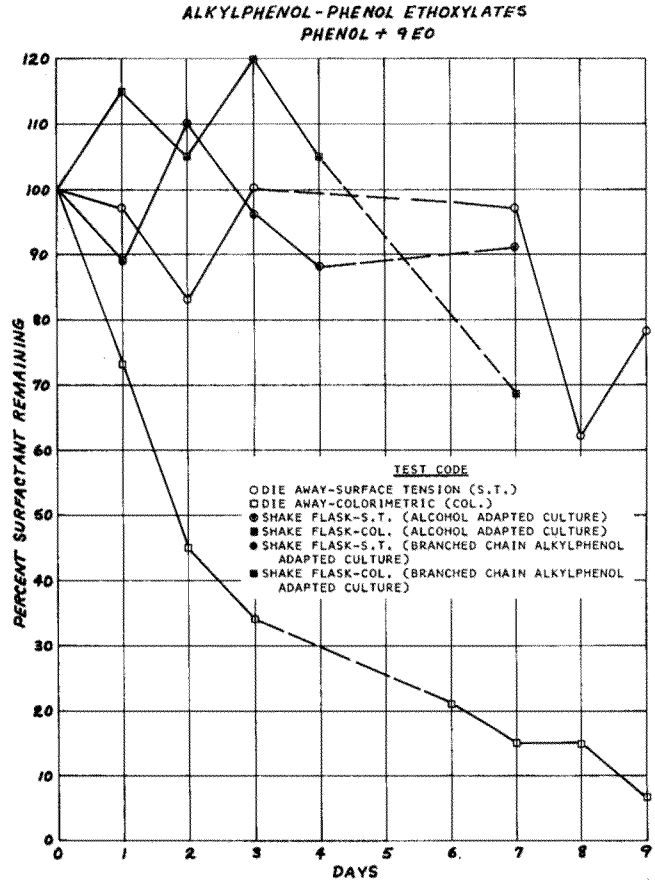

FIG. 6.

in the case with the alcohol ethoxylates, the biodegradation occurred more rapidly in shake flask tests than in die-away tests. Similar low levels of detergent residue concn were indicated by the end of the test periods. No significant differences were noted between results obtained using cultures adapted to either type of mixed detergent feed. Acclimatization times varied for the two products tested with slower rates of biodegradation indicated for C_{12} diethanolamide during the early stages of either test procedure. Once acclimated, though, this product was degraded at a rapid rate to a very low concn.

Alkylphenol-phenol ethoxylates. Figures 6, 7 and 8 present results for tests with alkylphenol-phenol ethoxylates. As might be expected, C_9 (straight chain alkylphenol) + 9–10 EO was found to be more biodegradable in either shake flask or die-away tests than was the corresponding branched chain product. With C_9 (branched chain alkylphenol) + 9–10 EO a somewhat greater amount of degradation was indicated in die-away tests than in shake flask tests. This difference was not observed with the straight chain alkylphenol product where comparable final degradation results were obtained in both tests. Somewhat erratic results were found with phenol + EO. It was felt that neither the surface tension or colorimetric methods were adequate for following this degradation process.

The acclimation of cultures to the mixture of detergents containing branched chain nonylphenol ethoxylate or Ziegler straight chain alcohol ethoxylate did not appear to have a significant effect on results obtained in shake flask tests with either product.

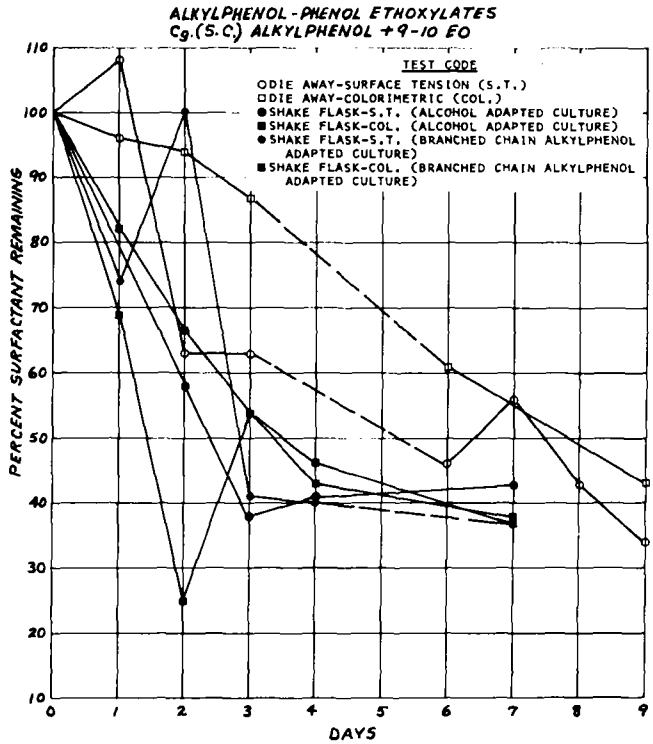

FIG. 7.

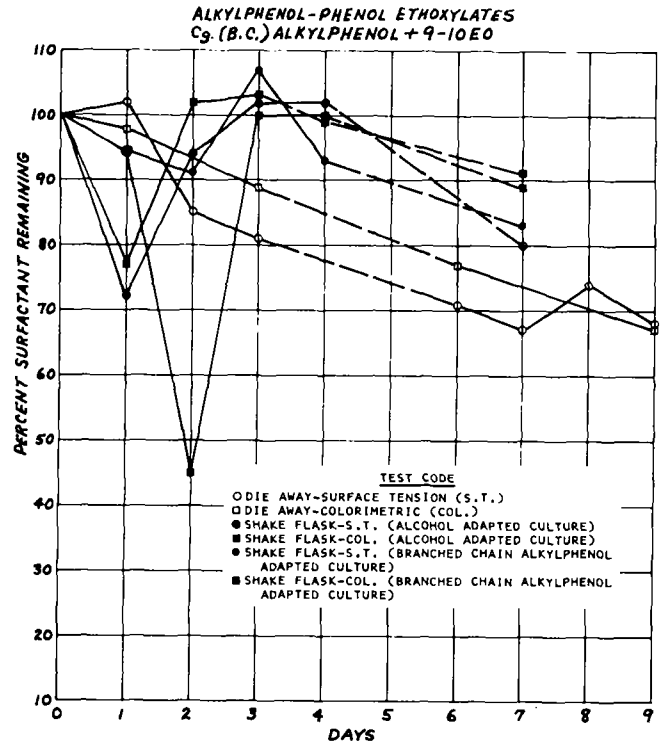

FIG. 8.

Anionic. Comparisons of die-away and shake flask results for alkylbenzene sulfonate, Na salt (branched) are shown in Figure 9. All data indicated this to be a biologically "hard" product. Again, the acclimation of cultures did not appear to have a significant effect on the rate or degree of degradation.

Warburg Respirometry Tests

Interpretation of respirometer data can be made in several ways (8,9). Discussion of these results will be presented in terms of a five (5) day biological oxygen demand (BOD). Table IV summarizes the results.

These 5-day figures were obtained at the completion of three transfers of the activated sludge seed which had been exposed to the test surfactant for a minimum of 15 days prior to each transfer. According to established procedure, products with 5-day BOD values in excess of 0.5 are considered substantially degraded. These test results do not indicate that any of the phenol or alkylphenol—straight or branched chain ethoxylates—were significantly degraded although some attack did occur. Other degradation processes and detection techniques discussed in this paper indicate degradation of alkylphenol type products to a greater extent than shown here.

At the time this Warburg work was initiated, there was no known, accurate chemical method for the direct determination of ethylene oxide containing nonionics in the ppm conen range. An attempt was made using gas liquid chromatography techniques to identify residue fragments after test in the Warburg apparatus. Our efforts in this direction ceased when it was established that the original 30 ppm surfactant solutions would not respond to the chromatographic conditions employed.

TABLE IV

Warburg Respirometer Test Results

	Grams oxygen consumed/g product tested
1. Alcohol ethoxylates	
a. Ziegler (straight chain) C_{12-18} alcohol + 7–9 EO	0.87
b. Oxo (branched chain) C_{13} alcohol + 9 EO	Not run
c. Natural (straight chain) C_{12} alcohol + 3 EO	1.53
2. Amides	
a. C_{12} diethanolamide	1.73
b. C_{12-18} amide ethoxylate	0.73
3. Alkylphenol-phenol ethoxylates	
a. Phenol + 9 EO	Nil
b. C_9 (branched chain alkylphenol) + 9–10 EO	0.1
c. C_9 (branched chain alkylphenol) + 9–10 EO	Nil
4. Anionic	
a. Alkylbenzene sulfonate, Na salt (branched)	0.07

Conclusions

Products based upon naturally occurring materials

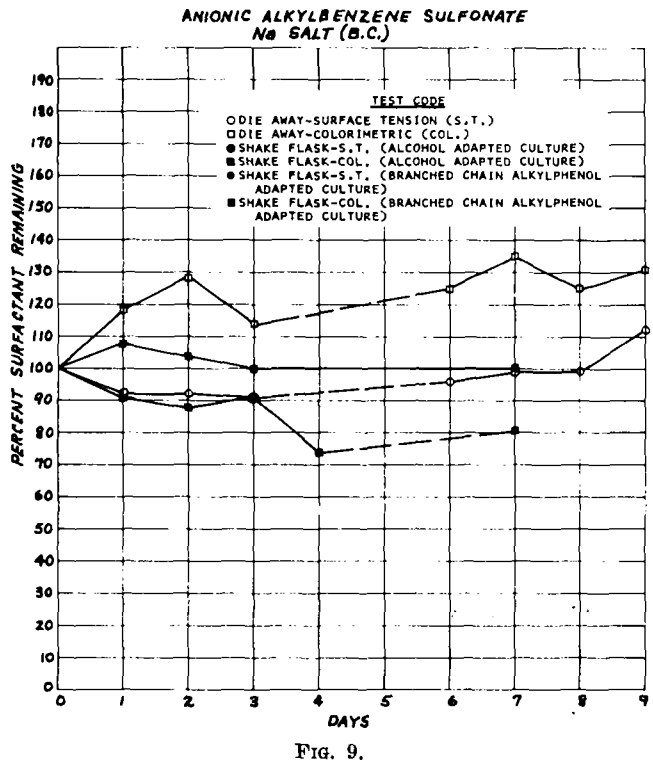

FIG. 9.

or based upon synthesized straight chain materials exhibit the greatest degree of biodegradability in all tests run. Varying degrees of indicated degradation can be obtained from specific tests on products possessing unknown or doubtful degradation structures. Branched chain materials continue to show greatest resistance to degradation.

Attempts to develop acclimated microorganisms to specific structures (i.e., branched chain detergents) did not significantly change biodegradation rates. The acclimation time using two 72-hr transfers may not have been sufficient.

Ethylene oxide derivatives of amides and straight chain alcohols biodegraded to a substantial degree in all tests.

ACKNOWLEDGMENT

Warburg Respirometry tests were performed under contract by the Dept. of Environmental Science, Rutgers The State University, New Brunswick, N.J.

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[Received June 17, 1964—Accepted October 19, 1964]

Surfactants Containing Ethylene Oxide: Relationship of Structure to Biodegradability

E. C. STEINLE, R. C. MYERLY and C. A. VATH, Union Carbide Corporation, Chemicals Division, Research and Development Department, South Charleston, West Virginia

Abstract

Anionic ethoxy sulfate and nonionic ethoxylate surfactants were prepared from the following straight-chain hydrophobes: fatty or Ziegler primary alcohols, oxo alcohols derived from straight-chain olefins, secondary straight-chain alcohols and straight-chain alkylphenols. These were studied to relate biodegradability to the following elements of structure: the nature of the connecting link, its position of attachment to the hydrophobe, the chain length of the hydrophobe, and the length of the ethylene oxide chain used. Previously described methods were used to estimate both rate and completeness of degradation in river water as well as activated sludge environments.

Data are presented to support the following conclusions.

1) All surfactants derived from straight-chain primary and secondary alcohols are rapidly and completely degraded with loss of surfactant properties. The length of the ethylene oxide chain from zero up to ten units has no effect on the rate or the completeness of degradation. In such surfactants, the ethylene oxide chain is completely degraded.

2) In contrast, surfactants from straight-chain alkylphenols are not as rapidly or as completely degraded as those described above. The position of attachment of the phenol ring to the straight-chain has a large effect on degradability; normal or primary attachment leads to a faster rate of disappearance than secondary attachment. Nonionic surfactants from straight-chain alkylphenols containing ten to twelve moles of ethylene oxide are not completely degradable.

Introduction

WHEN THIS PROJECT was started several years ago, it appeared evident that surfactants which were not biodegradable were in for a siege of bad publicity and poor reputation, deserved or not. The manufacturers of detergent products through the Soap and Detergent Assoc. had produced evidence that detergents were not a major pollution problem (1). Yet the sweep of bad publicity sustained by the presence of tell-tale foam has carried the country along the road toward restrictive legislation. After much effort, the manufacturers of alkylbenzene sulfonate are now able to offer a wide range of linear alkylate sulfonate (LAS) products at competitive prices to meet the needs of this pollution or foam problem.

Long Term Goal: Complete Biodegradability. During these years, suggested screening and legislative standards for biodegradability have often coned more on the elimination of foam than on proof of the complete biodegradability of the new products. It was a relatively simple matter to demonstrate the rate of disappearance of the LAS molecules and related foam and other surface active properties in river water dieaway tests. It has taken more sophisticated methods to demonstrate that the molecule is completely biodegradable, for which we are indebted to the work of Swisher (2), Allred (3) and many others.

This progress with LAS biodegradability is encouraging. The long term goal of complete biodegradability for other new surfactants cannot be realized until these new products can also meet tests for completeness of biodegradation as well as tests to demonstrate rate of disappearance of the initial molecules.

Short Term Goal: Loss of Surface Activity. Initial prospects for the preparation of completely biodegradable products did not appear favorable for the manufacturers of ethoxylated surfactants. The limited screening data available at that time indicated that the ethylene oxide chain itself was not biodegradable (4). Thus the manufacturer had to look not only for a biodegradable hydrophobe but also for a biodegradable water-solubilizing nonionic group to replace the versatile and inexpensive ethylene oxide chain.

The thought did occur, however, that a short-term goal for biodegradability might be readily obtained. Such a goal would be satisfied with degradation of