Biodegradability of Nonionic Surfactants: Screening Test for Predicting Rate and Ultimate Biodegradation

R.N. STURM, Environmental Water Quality Research Department,
Procter and Gamble Company, Cincinnati, Ohio 45217

ABSTRACT

Environmental water quality evaluations of raw materials in consumer products occupy a position of critical importance in many industries throughout the world. The rapid growth and diversity of the household detergent market requires continuous consideration of new materials needed to meet the demands of new, improved and modified products. As household cleaning products are normally disposed of as a component of domestic sewage, surface active compounds, including nonionic surfactants, would reach surface waters only as a part of a sewage effluent and would be subject to the same degree of biological treatment as the balance of the waste. For this reason, evaluations of such new materials include an environmental assessment in which biodegradability testing of organic materials is an important first step. Biodegradability characteristics of nonionic surfactants, as a class, are generally more difficult to ascertain because of wide structural diversity and a usual lack of functional groups. Such determinations usually involve intricate and laborious test methods which necessitate development of analytical techniques for each degradation product of a given material. A method has been developed, modified and used in our laboratory, that provides, after reasonable opportunity for biological acclimation, a measure of the rate and degree of ultimate biodegradation (conversion to CO_2 and H_2O). This method, which uses simple equipment, has been used to assess the biodegradability of a wide variety of nonionic surfactants, without necessitating the development of specific analytical methods for each surfactant under consideration. Additionally, this method can be adapted to measure degradation under conditions of anaerobiosis or low temperature.

INTRODUCTION

Interest in biodegradability testing is not new. The chemical industry began looking toward biodegradability as early as the late 1950's, when a link was established between detergent surfactant residues and foaming incidents in sewage treatment plants. The subsequent search for a more easily biodegradable surfactant resulted in an industry-wide changeover to a material designed to end foaming problems. It also signaled the beginning of a period in which the property known as "biodegradability" has become as important as the effective performance of materials used in consumer products. Environmental water quality evaluations of raw materials for use in consumer products now occupy a position of increasing importance in many industries throughout the world. The rapid growth and wide diversity of the household detergent market requires continuous consideration of new materials for new, improved and modified products. Household cleaning products are normally disposed of as a component of domestic sewage and should be as amenable to treatment as the balance of the waste. Evaluations of a major new organic material should therefore include biodegradability testing as an important first step in its environmental assessment.

Biodegradability has been defined in many different contexts, the definition depending primarily upon the parameter being utilized for measuring biodegradability. The state of biodegradability studies has reached a point where more precise definitions are now possible. Swisher (27) has indicated definitions for two degrees of biodegradation: (a) "*Primary degradation* occurs when the molecule has been oxidized, or otherwise altered by bacterial action to such an extent that its characteristic properties are no longer evident or when it no longer responds to analytical procedures more or less specific for detecting the original surfactant; and (b) *ultimate degradation* is defined as the complete conversion of the surfactant molecule to carbon dioxide, inorganic salts, and products associated with the normal metabolic processes of the bacteria."

In general, surfactants may be conveniently divided into four major categories, which include: (a) anionics, such as LAS and ABS; (b) cationics, such as quaternary ammonium derivatives; (c) amphoterics, such as alkyl ammonio propane sulfonates; and (d) nonionics, which include polyoxyethylene and polyoxypropylene derivatives.

In 1965, the Soap and Detergent Association's Subcommittee on Biodegradation Test Methods published a paper describing a two part method for determining the biodegradability of LAS and ABS (26). The criterion for biodegradability was, in this case, a loss of colorimetric response to the Methylene Blue Active Substance (MBAS) analysis. This was a measure of *primary degradation.* Since that time, additional work has shown LAS to undergo ring cleavage and *ultimate biodegradation.* The biodegradability characteristics of nonionic surfactants, as a class, are generally more difficult to ascertain because of a wide structural diversity and a usual lack of common functional groups. The class of nonionic surfactants (Fig. 1) includes not only primary and secondary linear alcohol ethoxylates, but also alkyl phenol ethoxylates, sugar-fatty acid esters, alkyl amine oxides, alkyl alkanolamides and various polyoxypropylene derivatives. The principal difficulty in establishing a test procedure applicable to this broad class of compounds lies not with the biological system but with the analytical methodology.

PREVIOUS WORK

Relatively simple procedures have been developed that are designed to quantitate nonionic surfactants by colorim-

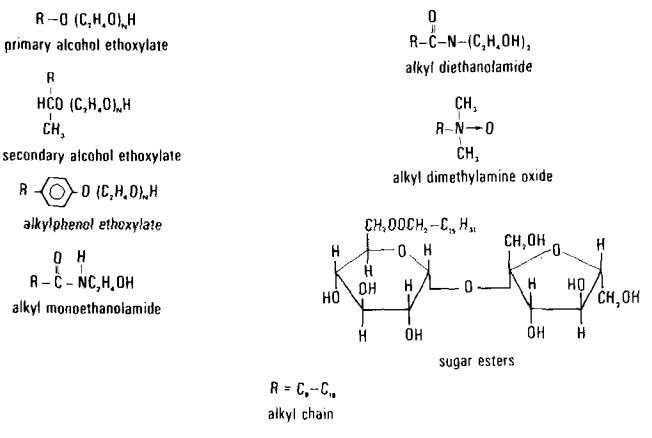

FIG. 1. Typical nonionic surfactants: examples of diverse chemical structures within the class of nonionic surfactants.

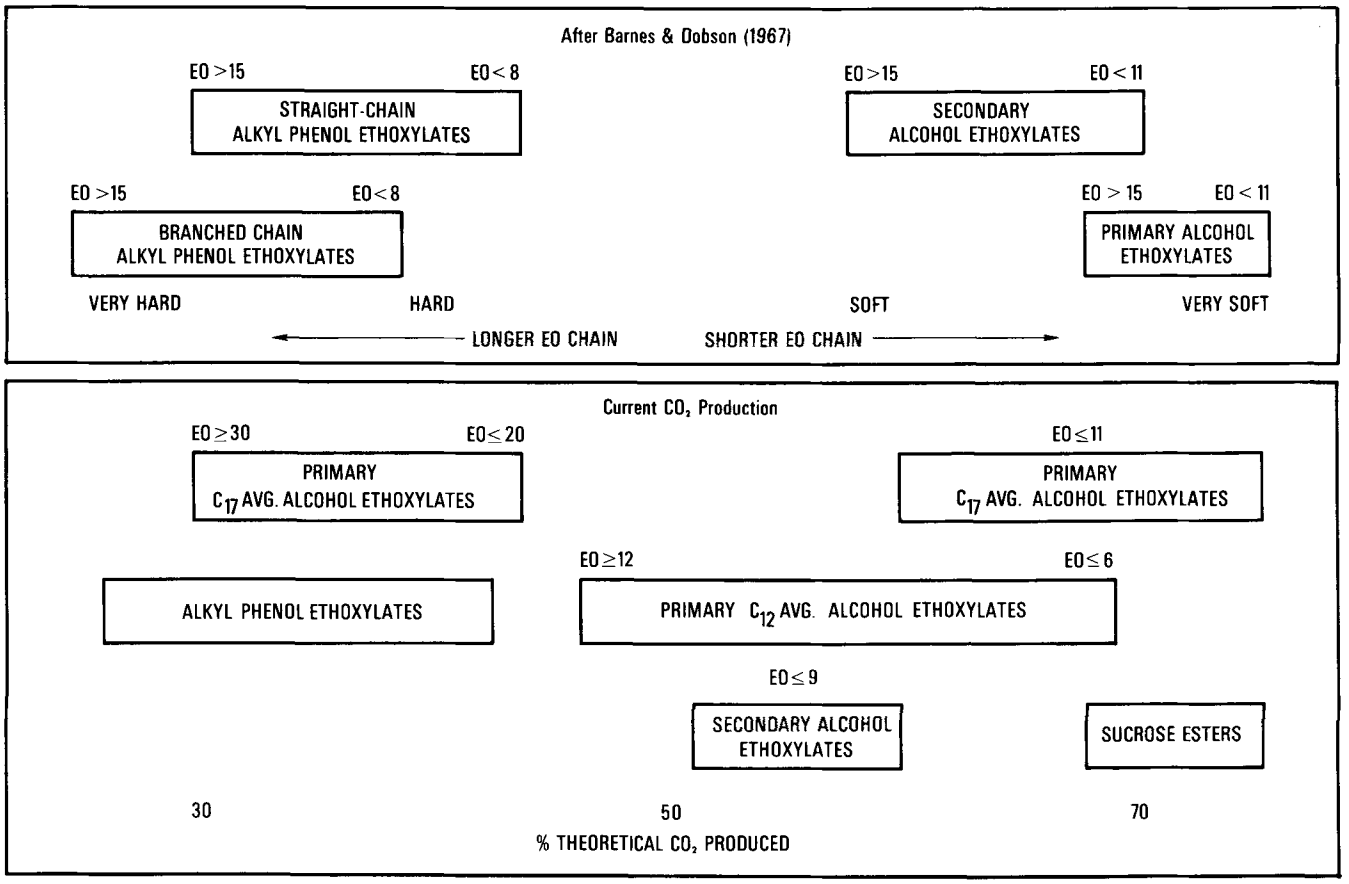

FIG. 2. Comparative biodegradabilities of some nonionic surface active agents. (a) Effects of chemical structure on previous biodegradability testing results of nonionic surfactants (Barnes and Dobson [2]). (b) Effect of chemical structure results obtained using CO_2 production as a criterion of biodegradability.

etry (5,10), surface tension reduction (18) and foaming (18), in a variety of biological systems. Much of the early laboratory work involving degradation studies of nonionic surfactants has utilized one or more of these measures.

Of particular interest to those persons involved in new product formulations where estimates of biodegradability must be made are studies that relate chemical structure to biodegradability. Borstlap and Kortland (3) examined this relationship for a variety of nonionic surfactants by determining the nonvolatile organic matter after a fixed degradation time. In general, benzene rings, branched alkyl chains and long ethylene oxide chains were found to adversely affect biodegradation. Barnes and Dobson (2), in a 1967 review paper, divided nonionic surfactants into four categories (very hard, hard, soft and very soft). As indicated in Figure 2, branched chain and straight chain alkyl phenol ethoxylates were categorized as very hard and hard, respectively, while secondary alcohol ethoxylates and primary alcohol ethoxylates were judged to be soft and very soft, respectively. Conway and Waggy (7) examined degra-

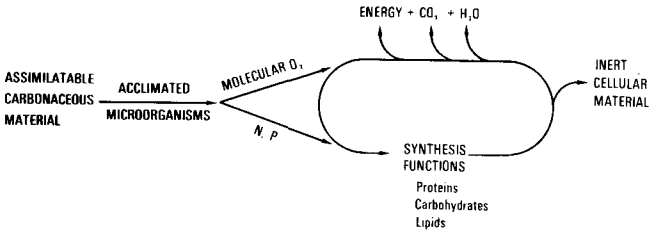

FIG. 3. Aerobic biodegradation: general biological pathway for metabolism of carbonaceous materials (Vath [29]).

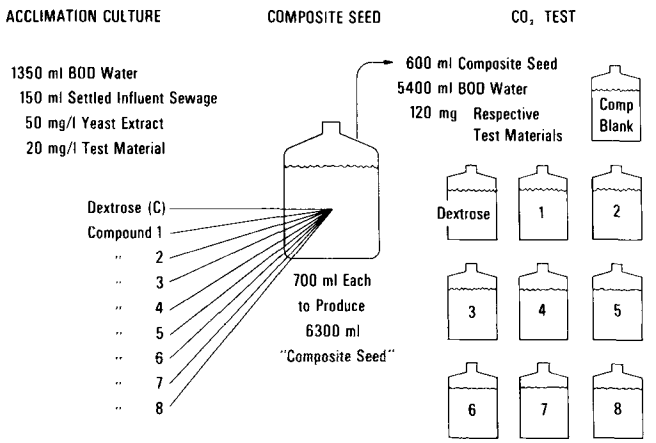

FIG. 4. General protocol for a 10 unit CO_2 production test.

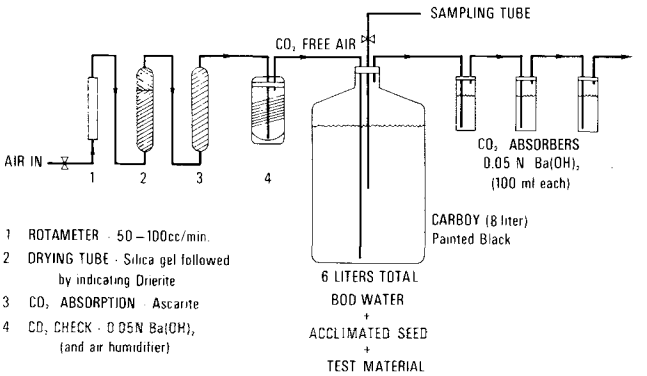

FIG. 5. Diagram of individual CO_2 production test unit. Air supply treated for entire 10 unit set rather than for each individual unit.

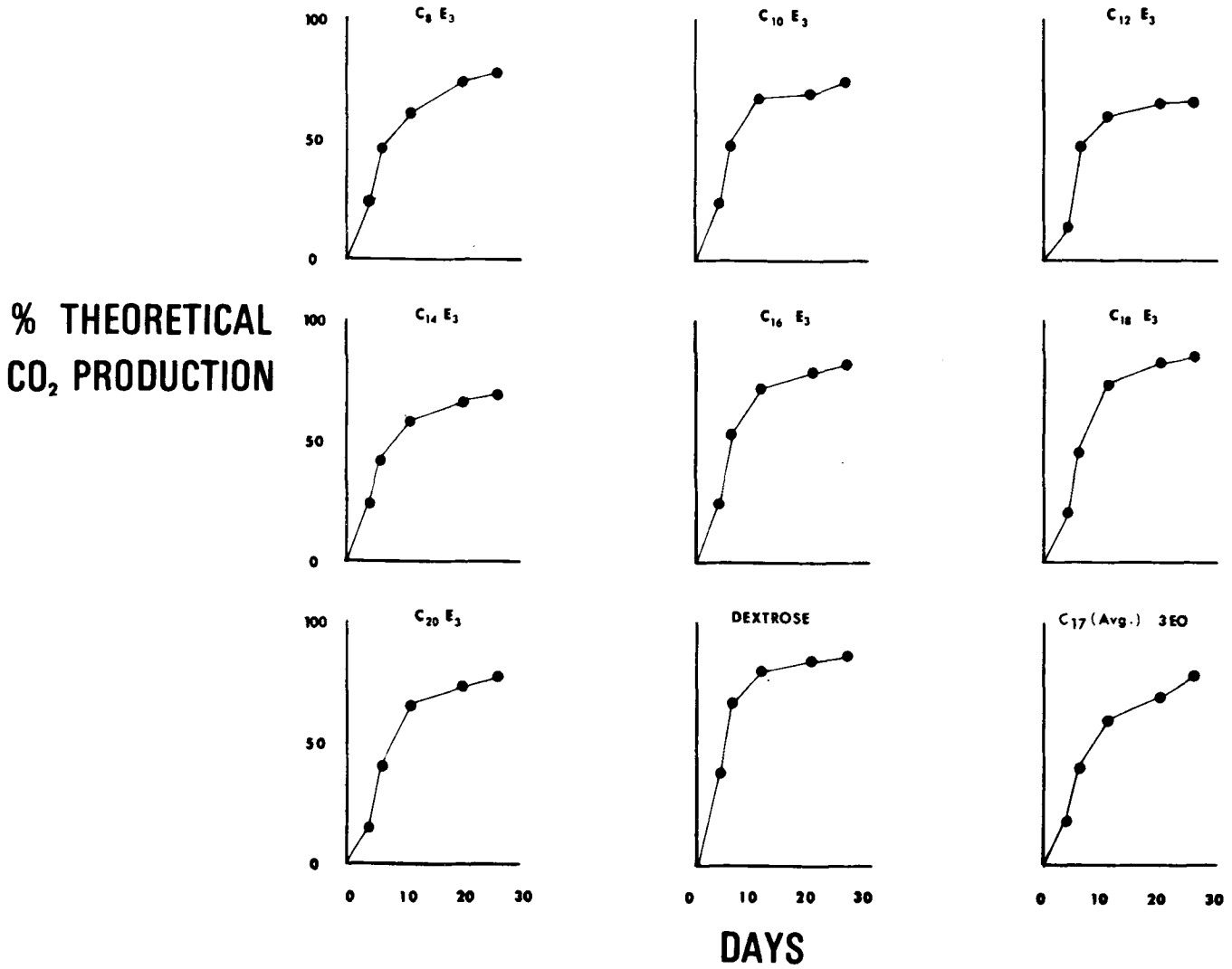

FIG. 6. Biodegradation-CO_2 production: effect of hydrophobe chain length upon biodegradability of a linear alcohol ethoxylate (3EO).

dation of nonionic surfactants in a variety of laboratory systems. The authors concluded that linear primary and secondary alcohol ethoxylates are efficiently metabolized by activated sludge microorganisms. This was not the case with the alkyl phenol ethoxylates tested. Their data further suggested that the rate of BOD exertion was dependent upon ethylene oxide content. Huddleston (12) tested nonionic surfactants for biodegradation in Mississippi River water and obtained results in agreement with those of Conway and Waggy. Additionally, in a continuous activated sludge system, the primary straight chain alcohol nonionic was found to be as biodegradable as LAS, while straight and branched chain alkylphenols were found to be somewhat resistant. Allred and Huddleston (1) concluded that (a) branched chain structures are less susceptible to biological attack than straight chain materials and (b) the presence of a phenol group in the molecule will significantly retard degradation. Steinle et al. (25) reported that straight chain primary and secondary alcohol ethoxylates containing ethylene oxide chains up to 10 units undergo complete loss of surfactant properties, while straight chain alkylphenols were not as rapidly or completely degraded.

The question of an inverse relationship between ethylene oxide content and degradability was attacked directly by Fincher and Payne (8) in work that utilized growth of a soil bacterium as an indication of degradation of a variety of polyethylene glycols (PEG's) of increasing chain length. Growth was observed on PEG's up to a molecular weight of 400 (EO = 9-10), which correlates well with the work on nonionic surfactants.

More specific analytical techniques have also been brought to bear on the question of nonionic surfactant biodegradability, particularly in the area of degradation mechanisms. Patterson et al. (20,21), using a thin layer chromatographic (TLC) technique, found alcohol polyethoxylates more rapidly degradable than alkyl phenol ethoxylates. In an extension of this work (22), TLC was used to investigate the initial mechanism of degradation of the surfactants.

Frazee et al. (9) have applied IR spectroscopy to studies of the degradation of alcohol ethoxylates and alkyl phenol ethoxylates. An alcohol ethoxylate (tetradecyl $AE_{8.3}$) showed complete disappearance after 5 days in river water, whereas nonyl phenol ethoxylate (EO = 9.0) required 34 days. Osborn and Benedict (19), also using IR, found degradation of the ether chain only when the chain contains 10 or less units of ethylene oxide, and suggested that degradation of the ether chain occurred by a hydrolysis mechanism.

These more sophisticated methods, while very valuable for in-depth research investigations, are less useful in a screening program because of analytical development work needed for each new material. A method has been developed, modified and used in our laboratory which provides, after reasonable opportunity for biological acclimation, a measure of the rate and degree of ultimate degradation (conversion to CO_2 and H_2O) of a given compound. This method, which uses simple equipment, has been used to assess the biodegradability of a wide variety of nonionic surfactants, without necessitating the develop-

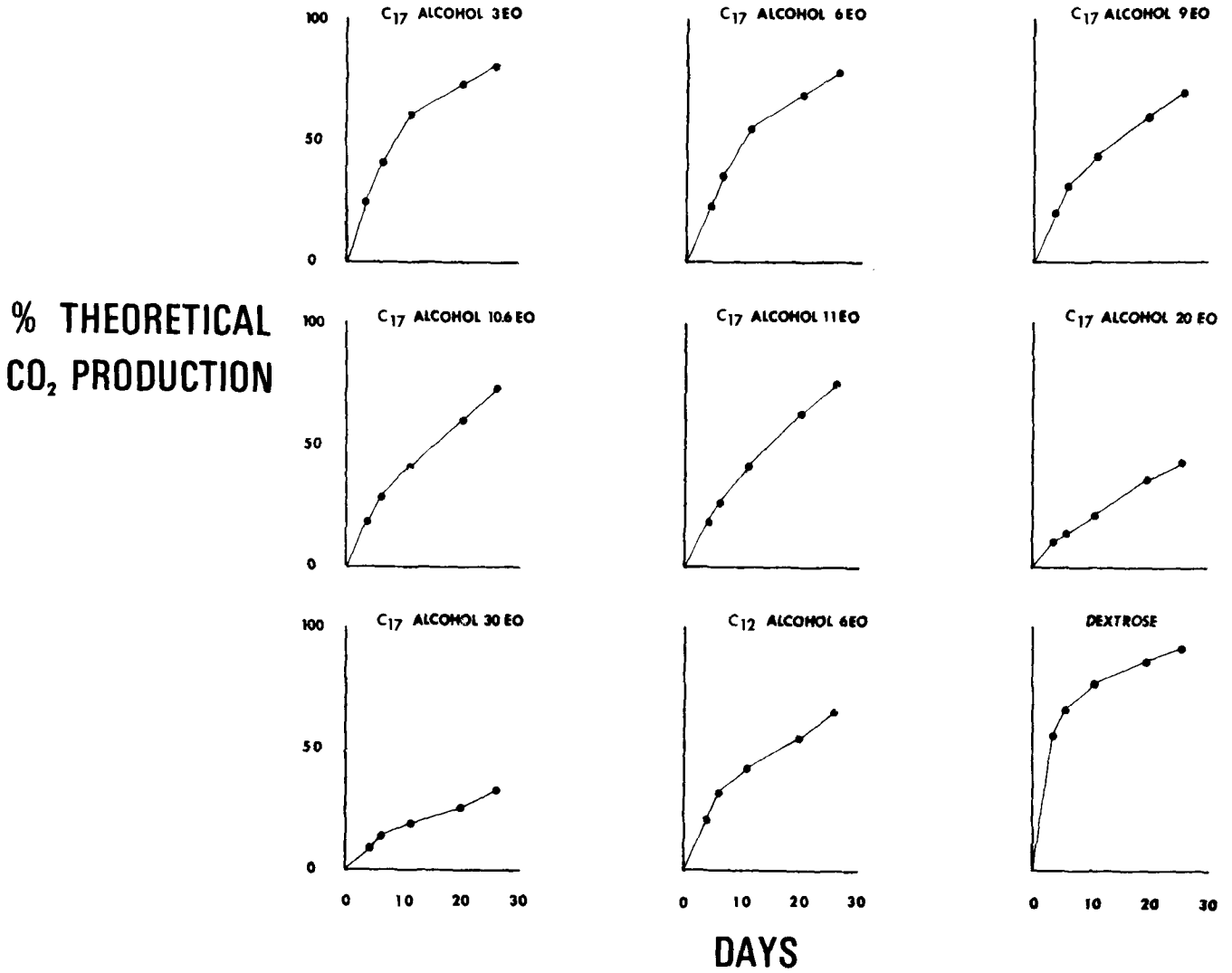

FIG. 7. Biodegradation-CO_2 production: effect of ethoxylate chain length on biodegradability of C_{17} (average) alcohol ethoxylates.

ment of specific analytical procedures for each surfactant under consideration.

EXPERIMENTAL PROCEDURES

Ludzack et al. (14) investigated the biochemical oxidation of some organic cyanides in river water, using CO_2 production as the primary index of biodegradability. This work was extended to a study (15) of organic chemicals isolated from rivers, in which biodegradability data were expressed in terms of cumulative CO_2 recovered as a per cent of theory. Ludzack's method was modified in two major areas by Thompson and Duthie (28) during their study of NTA biodegradation. The use of BOD dilution water as a basal medium in place of river water was considered appropriate, since it would be readily available as a standard diluent in any laboratory that would perform such a test. In addition, the protocol of the Bunch-Chambers die-away (4) with no transfer was used for a 14 day acclimation period of sewage-derived bacteria and a seed source for the CO_2 production test. The Screening Test Method, described in more detail in the Appendix, is based on the assumption that, as a carbonaceous material is acted upon by bacteria capable of utilizing it as a carbon and energy source (Fig. 3), the molecule will be converted in the presence of molecular O_2 to cellular material (through synthesis functions), and to CO_2 and water (in the production of energy). The biodegradability of a given material may then be estimated by measuring the amount of CO_2 produced or the amount of O_2 consumed by acclimated bacteria during a given time period, and relating these figures to calculated theoretical yields based on the structure and molecular weight of the material under investigation. Where a structure is unknown, CO_2 production calculations may be made on the basis of total organic carbon or COD analyses.

The present screening test consists of the Thompson-Duthie CO_2 test, which has been scaled down from 20 to 6 liters, and a biochemical oxygen demand test (Appendix) performed with acclimated sewage-derived microorganisms. This paper is concerned exclusively with the application of the CO_2 production method to the problem of nonionic surfactant degradation. While the BOD test generally will provide comparable results, the CO_2 test, which utilizes higher test material concentrations and measures the actual production of byproducts of degradation (CO_2), is thought to be more reliable. Hence results of BOD determinations, generally performed only as a back-up test, are not discussed in this paper.

The CO_2 test apparatus has been arranged in such a manner that eight materials and a positive control may be tested simultaneously (Fig. 4). Nine individual acclimation cultures—each combining settled raw sewage as a source of microorganisms, yeast extract as an easily assimilatible nutrient source, BOD water as a diluent and source of inorganic nutrients, and a test material—are permitted to sit quiescently in the dark for 14 days. At the end of that time, equal aliquots from each of these cultures are used to make a composite seed for use in the CO_2 test. The final

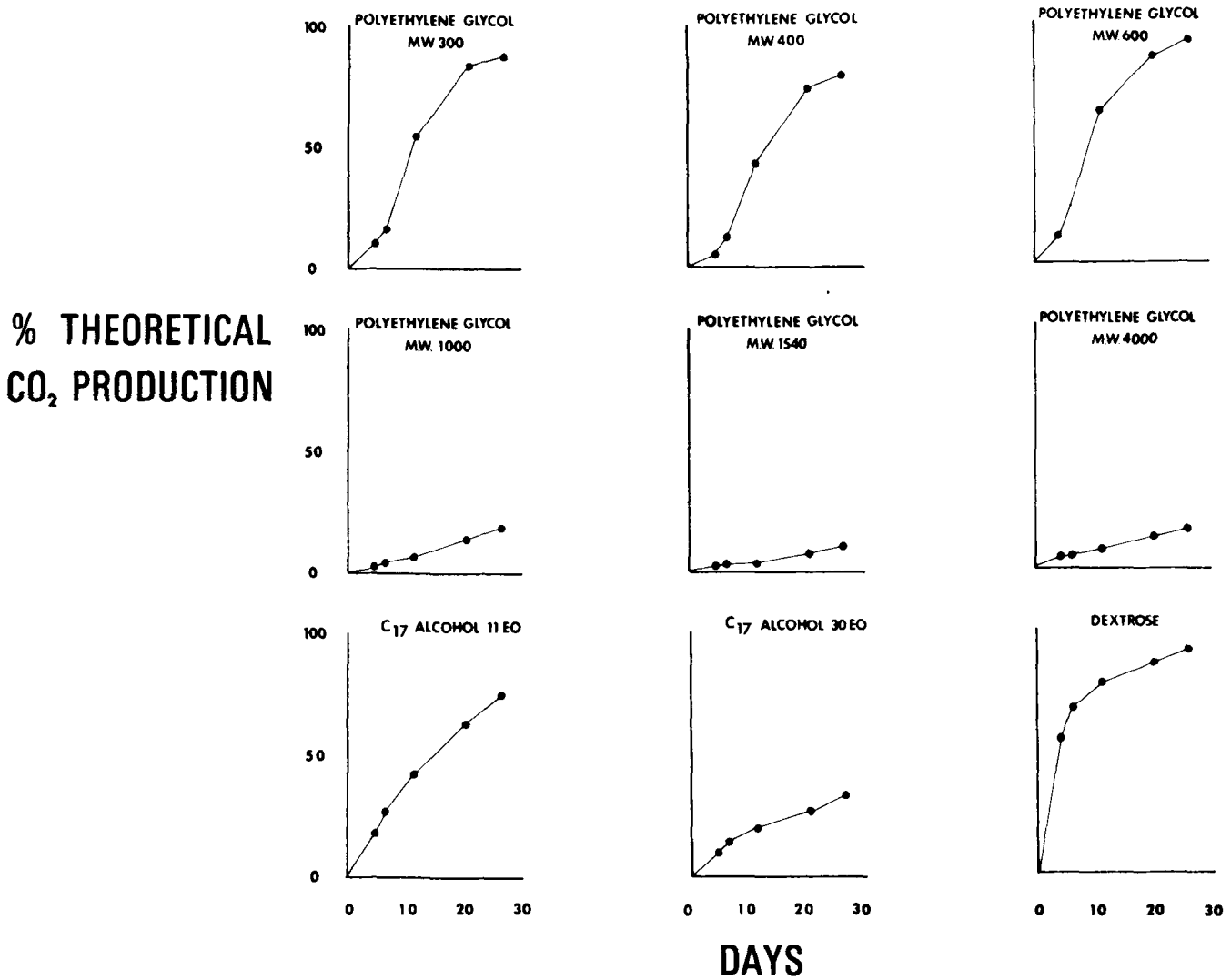

FIG. 8. Biodegradation-CO_2 production: effect of molecular weight on biodegradability of polyethylene glycol.

composition in each carboy will include 600 ml of composite seed and 120 mg of a test material brought to a total of 6 liters with BOD water. The use of a common composite seed has two advantages: (a) it allows using a single blank for eight test units, and (b) it provides a bacterial population acclimated to a variety of materials, including the test compound.

During the test CO_2-free air is bubbled through the test units (Fig. 5), and the effluent gas is passed through a series of CO_2 absorbers containing barium hydroxide. Periodically, the proximal absorber is removed for titration. The remaining two absorbers are each moved one place closer to the carboy and a new absorber placed at the distal end of the series. A positive control is tested along with each group of test materials as a means of measuring the variability of the tests, which may be attributable to the "strength" of the raw sewage seed. CO_2 production from dextrose should exceed 80% of theoretical in a valid test. While it may be possible to normalize results from a CO_2 test in which the positive control yields less than 80% of theoretical CO_2 production, we have found more confidence in repeating the entire series. The test is continued for a period of 26 days. The 26 day time period is based mainly on the loss of usefulness of the positive control after this point due to a plateau of CO_2 production, as well as a possible build-up of inhibitory metabolites in the static system. Generally the greatest portion of the CO_2 production occurs within the initial 14 days of the test followed by a plateau at 20-22 days into the test. At day 25, the test vessels are acidulated to pH 3.0 in order to release CO_2 that may be trapped in the medium. This release will, of course, show an apparent continuing CO_2 production on some test materials.

RESULTS AND DISCUSSION

A wide range of nonionic surfactants have been screened for biodegradability by this method, and the results, in general, correlate well with those of other investigators (1-3,7,8,25). In order to examine the effect of hydrophobe chain length on biodegradability, a series of specially prepared alcohol ethoxylates, in which the average ethoxylate chain length was held constant at 3 EO while the hydrophobe chain length was increased in increments of two carbons from C_8 to C_{20}, was screened for biodegradability using CO_2 production as a measure of degradation. As may be seen in Figure 6, increasing the hydrophobe chain length from C_8 to C_{10} has no significant effect on biodegradability. While subsequent increases to C_{12}, C_{14}, C_{16}, C_{18} and C_{20} show some variability in CO_2 production, all yield in excess of 65% of theoretical CO_2. It should be noted that LAS, a material which has been used without difficulty and shown to be readily biodegradable, will yield ca. 65-70% of theoretical CO_2 under conditions of this test. Overall, when compared with dextrose, which in a series of tests should yield in excess of 80% of theoretical CO_2, these materials appear to be very soft. The specially prepared materials were compared with a commercial C_{17} (average) alcohol ethoxylate. The results correlate well, since the commercial material shows a theoretical CO_2 yield of 83%, while the specially prepared $C_{16}AE_3$ and

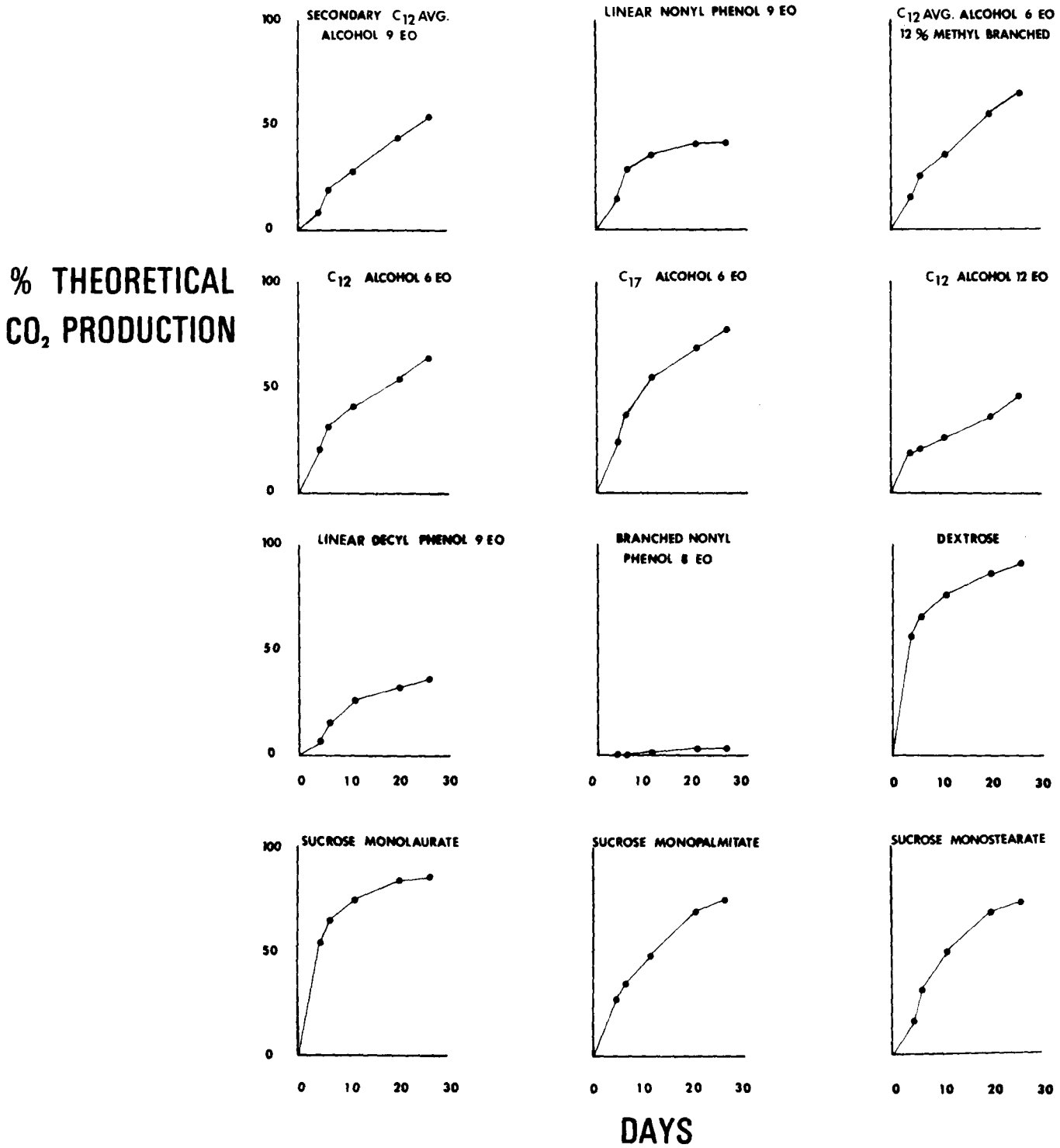

FIG. 9. Biodegradation-CO_2 production: comparisons of biodegradability properties of ethoxylated nonionic surfactants of various hydrophobe structures and sugar-fatty acid esters.

$C_{18}AE_3$ averaged 84% of theoretical CO_2 production. The results compare well with those of Barnes and Dobson (2), who found biodegradation of primary alcohol ethoxylates to be unaffected by variations in alkyl chain length.

Ethoxylate chain length, however, does affect biodegradability. Studies by Bortslap and Kortland (3), Patterson et al. (20,22) and Steinle et al. (25) have indicated ethoxylate content of nonionic surfactants to affect their biodegradability properties. The terms C_{17} and C_{12} are used to denote alcohols with approximate average alkyl chain lengths of 17 and 12, made up with distributions consisting of $C_{14,16,18}$ and $C_{10,12,14}$, respectively. Seven commercially prepared C_{17} alcohol ethoxylates, with average ethoxylate chain lengths ranging from 3 to 30, were tested for biodegradability (Fig. 7). Results for $C_{17}AE_3$, $C_{17}AE_6$, $C_{17}AE_9$, $C_{17}AE_{10.6}$ and $C_{17}AE_{11}$ suggest that ethoxylate chain length up to 11 EO units has no significant effect on the biodegradability of the C_{17} (average) alcohol-based nonionic surfactant. However ethoxylate content greater than 20 EO's appears to effect a marked reduction in the biodegradability of these materials.

The role of ethoxylate chain length was examined further by testing a series of polyethylene glycols (PEG's) ranging in molecular weight from 300 to 4000. Figure 8 illustrates the results of these tests. PEG 300, 400 and 600 appear to be readily biodegradable, yielding CO_2 production figures of 87, 80 and 90% of theoretical, respectively. PEG's of higher molecular weight (1000, 1540, and 4000)

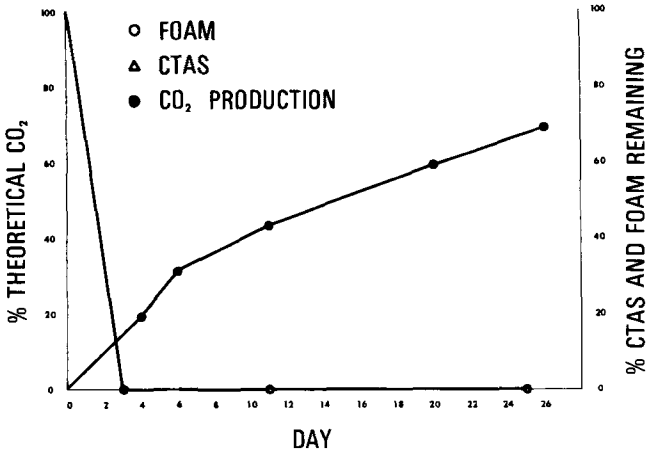

FIG. 10. Comparison of per cent of theoretical CO_2 production, foam reduction and CTAS removal for tallow alcohol ethoxylate 9EO (TAE_9).

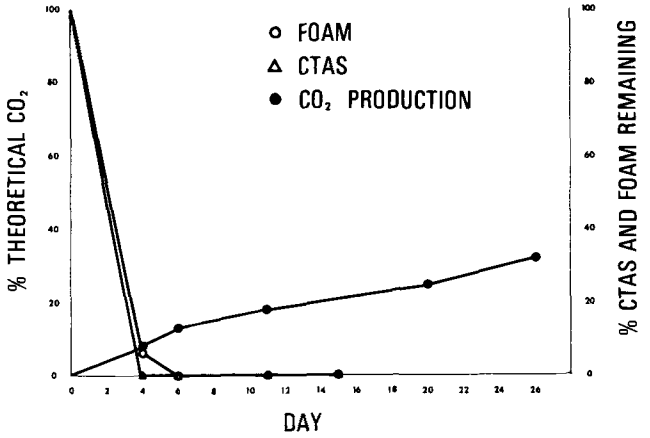

FIG. 11. Comparison of per cent of theoretical CO_2 production, foam reduction, and CTAS removal for tallow alcohol ethoxylate 30 EO (TAE_{30}).

appear to be resistant to biodegradation. This compares well with CO_2 production results exhibited by alcohol ethoxylates with ethylene oxide chains of comparable molecular weights. $C_{17}AE_{11}$, which possesses an ethoxylate group of ca. 500 mol wt, degrades easily; while $C_{17}AE_{30}$, with an ethoxylate chain of ca. 1300 mol wt, exhibits some resistance to degradation.

Comparisons of the effect of hydrophobe structure on biodegradability were made between primary and secondary alcohol ethoxylates, straight and partially branched alkyl chain nonionics, and alkyl phenol ethoxylates (Fig. 9). In general, primary alcohol ethoxylates are slightly more degradable than secondary alcohol ethoxylates, and slight (~12%) methyl branching in a primary alcohol ethoxylate appears to have no significant effect. The presence of a phenolic group in the molecule, however, appears to reduce the biodegradability of those nonionics tested (linear nonyl and linear decyl phenol ethoxylate), and the presence of a branched alkyl chain in a nonyl phenol ethoxylate reduces

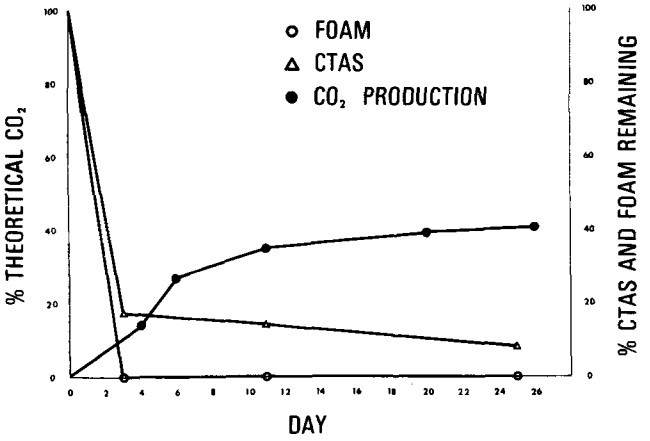

FIG. 12. Comparison of per cent of theoretical CO_2 production, foam reduction and CTAS removal for a straight chain alkylphenol ethoxylate 9 EO ($LAPE_9$).

	AEROBIC DEGRADATION	ANAEROBIC DEGRADATION	LOW OXYGEN DEGRADATION	LOW TEMPERATURE	DEGRADATION PATHWAY
SOURCE OF MICROORGANISMS	Raw Sewage, River Water	Raw Sewage, Digester Sludge	Raw Sewage, River Water	→	
MEDIUM	BOD Water	→	→	→	→
ACCLIMATION	2 Weeks, Open Vessel	2 Weeks, Sealed Vessel	2 Weeks, Sealed Vessel	2 Weeks, Open Vessel	→
GAS SUPPLY	CO_2 Free Air	O_2, CO_2 Free Argon	N_2/O_2 Gas Mixture	CO_2 Free Air	→
METHOD OF MEASUREMENT	CO_2 Trapped in $Ba(OH)_2$	Combustion of Effluent Gases, CO_2 Trapped in $Ba(OH)_2$	→	CO_2 Trapped in $Ba(OH)_2$	→
LENGTH OF TEST (DAYS)	26	26+	26+	26	→
pH	7.1 Initial	→	→	→	→
TEMP. (°C)	22-24	35-40	22-24	5-15	22-24
SUBSTRATE	Com. or Lab Prep.	→	→	→	^{14}C Tagged Material
TEST VESSEL	7 Liter Carboy	→	→	→	→
D.O. (mg/l)	6-8	0	0.1, 0.5, 1.0, 2.0, 4.0	6-8	→

FIG. 13. Special purpose modifications of basic CO_2 production test apparatus.

biodegradability even further. Comparative tests of sugar-fatty acid esters (Fig. 9) indicate that each of these materials may be classified as "biologically soft."

Since a large amount of previous biodegradability data on nonionic surfactants has been reported as "per cent of foam reduction" or "per cent of CTAS removal," studies were performed to correlate CO_2 production with these two parameters. These studies included two C_{17} alcohol ethoxylates (9 EO and 30 EO), and a linear nonyl phenol ethoxylate (9 EO). In each case, significant reduction in foam and CTAS resulted at a point where less than 20% of the theoretical CO_2 yield had been evolved. A comparison of CTAS, foam and CO_2 production as measures of biodegradability indicates that, following rapid reduction of CTAS and foam, the molecule continues to undergo further degradation, resulting in additional removal of trace organic materials. However the extent of this additional degradation is independent of the rate of foam and CTAS removal. As may be seen in Figure 10, CTAS and foam removal for $C_{17}AE_9$, a readily biodegradable material, is essentially complete at day 3 of the test—a point where CO_2 production is less than 20% of theoretical, while CO_2 production approaches 70% of theoretical at 26 days. A similar result is seen in Figure 11 with $C_{17}AE_{30}$, a material that is somewhat less biodegradable than $C_{17}AE_9$. CTAS and foam response disappears at 4 and 6 days, respectively, at times when CO_2 production is less than 20% of theoretical. In this case, however, total CO_2 production is approximately half that of $C_{17}AE_9$. As may be seen in Figure 12, foaming and CTAS from an alkyl phenol ethoxylate disappear rather rapidly, as was the case for both $C_{17}AE_9$ and $C_{17}AE_{30}$. However, as was seen with only $C_{17}AE_{30}$, CO_2 evolution is slow, with a plateau near 40% of theoretical.

Mausner et al. (18) pointed to the diversity of structures within the class of nonionic surfactants and the problems of residue analysis as being among the more serious obstacles in the development of a standard laboratory technique for measuring nonionic surfactant biodegradability. These difficulties still exist in the area of treatability studies where it may be necessary to recover the material from among the other components of domestic wastewater. The concept of using CO_2 production as a means for measuring biodegradability is useful in that it (a) allows a rapid screening of a large number of organic materials without necessitating the development of specific analytical techniques, and (b) uses rate and degree of ultimate biodegradation, rather than primary biodegradation, as the basis for a preliminary judgment. This test is not intended as a substitute for the advanced studies that major materials should undergo, but as a preliminary screening test that may be easily set up in any laboratory. The system described in this paper is a comparatively weak degradation system, in that the CTAS and foam removal, which takes place over a 3-4 day time period in this test, may occur in just a few hours in an actual sewage treatment situation. The design of this test, however, is such that it will permit one to distinguish differences between surfactants, which may not be detectable by measuring only primary degradation. This is pointed out quite clearly in experiments comparing foam reduction and CTAS removal with CO_2 production data for several surfactants.

Overall, the judgments based on the results of the CO_2 production test may compare reasonably well with previous work (Fig. 2) which measured only primary degradation. As is indicated in Figure 13, this method should lend itself quite readily to modifications for measuring degradation of nonionic surfactants under a wide variety of special conditions. These modifications of the basic test, which include anaerobiosis, low temperature, limited oxygen and the use of ^{14}C-tagged materials for determining the mechanisms of degradation, are currently under development in our laboratories.

Our work demonstrates that using foaming or CTAS as a measure of biodegradability for guidance in selection of nonionics may prevent foaming problems, but that the CO_2 test, by measuring the degree and rate of ultimate biodegradation, provides more complete assurance of continuing degradation and reduction of organic residues.

REFERENCES

1. Allred, R.C., and R.L. Huddleston, Presented at the Kentucky-Tennessee Branch ASM Meeting, Lexington, Ky., 1965.
2. Barnes, W.V., and S. Dobson, J. Soc. Dyers Colourists 83(8):313 (1967).
3. Borstlap, C., and C. Kortland, FSA 69:736 (1967).
4. Bunch, R.C., and C.W. Chambers, J. Water Pollut. Contr. Fed. 39(2):181 (1967).
5. Burttschell, R.H., JAOCS 43:366 (1966).
6. Conway, R.A., C.A. Vath and C.E. Renn, Water Works Wastes Eng. 2(1):28 (1965).
7. Conway, R.A., and C.T. Waggy, Amer. Dyestuff Reporter 1966 (Aug. 1):33.
8. Fincher, E.L., and W.J. Payne, Appl. Microbiol. 10(6):542 (1962).
9. Frazee, C.D., Q.W. Osborn and R.O. Crisler, JAOCS 41:808 (1964).
10. Greff, R.A., E.A. Setzkorn and W.D. Leslie, Ibid. 42(3):180 (1965).
11. Hammerton, C., J. Appl. Chem. 5:517 (1955).
12. Huddleston, R.L., Amer. Dyestuff Reporter 55(2):42 (1966).
13. Lashen, E.S., F.A. Blankenship, K.A. Booman and J. Dupre, JAOCS 43:371 (1966).
14. Ludzack, F.J., R.B. Schaffer, R.N. Bloomhuff and M.B. Ettinger, 13th Annual Industrial Waste Conference, Purdue University, 1958.
15. Ludzack, F.J., and M.B. Ettinger, 18th Industrial Waste Conference, Purdue University, 1963.
16. Mann, A.H., and V.W. Reid, JAOCS 48:794 (1971).
17. Mann, A.H., and V.W. Reid, Ibid. 48:798 (1971).
18. Mausner, M., J.H. Benedict, K.A. Booman, T.E. Brenner, R.A. Conway, J.R. Duthie, L.J. Garrison, C.D. Hendrix and J.E. Shewmaker, Ibid. 46:432 (1969).
19. Osborn, Q.W., and J.H. Benedict, Ibid. 43(3):141 (1966).
20. Patterson, S.J., C.C. Scott and K.B.E. Tucker, Ibid. 44:407 (1967).
21. Patterson, S.J., C.C. Scott and K.B.E. Tucker, Ibid. 45:528 (1968).
22. Patterson, S.J., C.C. Scott and K.B.E. Tucker, Ibid. 47:37 (1970).
23. Patterson, S.J., K.B.E. Tucker and C.C. Scott, Third International Conference on Water Pollution, Research Paper 6 (1966) in "Advances in Water Pollution Research," Vol. 2, WPCF, Washington, p. 103, 1967.
24. "Nonionic Surfactants," Surfactant Science Series, Vol. I, Edited by M.J. Schick, Marcel Dekker, Inc., New York, 1967.
25. Steinle, E.C., R.C. Myerly and C.A. Vath, JAOCS 11(12):804 (1964).
26. Subcommittee on Biodegradation Test Methods, Ibid. 42:986 (1965).
27. Swisher, R.D., "Surfactant Biodegradation," Surfactant Science Series, Vol. III, Marcel Dekker, Inc., New York, 1970.
28. Thompson, J.E., and J.R. Duthie, J. Water Pollut. Contr. Fed. 40:306 (1968).
29. Vath, C.A., Soap Chem. Spec. 40(2):56,182 (1964).

APPENDIX: BIODEGRADABILITY SCREENING TEST

I. Theory

This screening test is based on the proposition that, as a carbonaceous material is acted upon by aerobic bacteria capable of utilizing it as a carbon and energy source, the molecule will be converted to cellular material, carbon dioxide and water. The ultimate biodegradability of a given material may then be estimated by measuring the amount of CO_2 produced or the amount of O_2 consumed by acclimated bacteria during a given time, and relating these figures to calculated theoretical yield based on the structure and molecular weight of the compound under investigation.

II. Scope

The details include preparation of cultures, set-up and

operation of the CO_2 test and calculation of its results. The BOD test is discussed briefly. A more detailed discussion of this procedure may be found in *Standard Methods* (1).

III. Testing Procedures

A. Acclimatization culture

The biodegradability screening test (CO_2 production and BOD) is preceded by a 14 day acclimation period during which microorganisms contained in the sewage are given an opportunity for adaptation to metabolism of a specific test material. Such cultures consist of a 10% solution of settled raw sewage in 1500 ml BOD water (1) as a source of bacteria in a basal medium, 50 mg/liter yeast extract as a readily metabolizable substrate to increase the bacterial count, and 20 mg/liter of test compound. As indicated in Figure 5, eight test material cultures, plus a culture for a dextrose control, are required for a 10 unit test. The acclimatization cultures, contained in 2000 ml wide mouth capacity flasks, are incubated quiescently in the dark for 14 days at 22-24 C.

B. Performance of CO_2 production test

Upon completion of the 14 day acclimation period, 700 ml aliquots from each of the nine cultures are blended to form a "composite seed." A 10% solution of this "composite seed" is distributed among the 10 test units to a final volume of 6 liters. All carboys are then aerated for 24 hr with CO_2-free air. At the end of this period, each test material is added to its respective 8 liter carboy to a concentration of 20 mg/liter in the 6 liters of seed solution and the test is begun.

The CO_2 test (Fig. 6) is based on the utilization of CO_2 production as a criterion for biodegradability. CO_2-free air is bubbled through a solution containing bacterial seed and the test material in BOD water. Effluent air from the unit is passed through three CO_2 absorbers, each containing 100 ml of 0.05 N $Ba(OH)_2$. Periodically, the CO_2 absorber most proximal to the carboy is removed for titration. The remaining two absorbers are each moved one place closer to the carboy, and a new absorber placed at the distal end of the series.

CO_2 produced during the test is trapped as barium carbonate, which may be quantitated by titrating the remaining $Ba(OH)_2$ with 0.1 N HCl. Twenty-five milliliters of the used $Ba(OH)_2$ solution is titrated to the phenolphthalein end point with 0.1 N HCl. One milliliter of titrant is equivalent to CO_2 production of 8.8 mg. (Details of the calculations are discussed in the calculations section.) CO_2 absorbers are titrated daily for the first 12-14 days and every other day thereafter for a total of 26 days.

Of the 10 carboys used in the current apparatus, one serves as a "seed blank" to compensate for CO_2 which may be produced as a result of organic material remaining in the "seed" at the end of the 14 day acclimatization culture. As the measurement of CO_2 consists of titration of the remaining $Ba(OH)_2$, the amount of CO_2 produced by a particular material as determined by the difference (in ml of titrant) between the experimental and blank carboy.

EXAMPLE: Blank 6.1 ml
Experimental 5.7
.4 ml = 3.52 mg CO_2 (see Calculations section)

C. Biochemical Oxygen Demand (BOD) determination

"Bacterial seed" for the BOD tests are taken from the same acclimatization cultures used for the CO_2 test. However individual rather than composite seeds are used. A test for a single test material is set up as follows: Five liters of a 10% solution of acclimatized seed in aerated BOD water is needed for the BOD determination for each material. Using this seed, 14 standard BOD bottles are prepared, which include: four seed blanks, two control (Dextrose at 3 ppm), four test material at 2 ppm, and four test material at 5 ppm. Two concentrations of test material are used to reveal any concentration-related inhibitory properties which may be present. Following set-up, the bottles are incubated at 20 C for 5 or 20 days. At the 5 day time point, dissolved oxygen titrations (azide modification of the Winkler method) (1) are carried out on the following bottles: two seed blanks, two Dextrose, two test material at 2 ppm, two test material at 5 ppm.

D. Calculations

1. Theoretical CO_2 production (TCO_2)
   Calculate weight of CO_2 produced by 1 mg of test material:
   a. Calculate number of carbons contained in a molecule of test material, e.g., dextrose = 6
   b. Formula: mg CO_2/mg test material
   $$= \frac{\text{no. of carbons x mol wt of } CO_2}{\text{mol wt of test material}}$$
   $$= \frac{6 \times 44}{180}$$
   $$= 1.46 \text{ mg } CO_2\text{/mg dextrose}$$

2. Per cent theoretical CO_2 production
   At any time point during the test, it is possible to determine the per cent theoretical CO_2 production by the following formula:
   $$\% \, TCO_2 = \frac{\text{mg } CO_2 \text{ produced}}{TCO_2}$$

3. Amount (mg) of CO_2 trapped in one absorber
   a. $$\text{mg } CO_2 \text{ produced} = \frac{44 \times \text{meq } Ba(OH)_2 \text{ consumed} \times 4}{2}$$
   meq = (ml HCl) (normality)
   Example:
   b. (HCl [ml] needed to titrate 25 ml of absorber) (4) (.1) = meq $Ba(OH)_2$ in 100 ml
   HCl titrant
   blank 6.1
   dextrose 5.7
   .4 x .1 N = .04 meq $Ba(OH)_2$ consumed
   as in a.
   1) $\frac{44 \times .04}{2}$ = .88 mg CO_2
   2) multiply above by 4 to correct results to 100 ml of $Ba(OH)_2$ absorber
   3) .88 x 4 = 3.52 mg CO_2

4. Total oxygen demand (TOD)
   Calculate weight of O_2 (g) needed to completely oxidize 1 g of test material to CO_2 and H_2O. For example: glucose = $C_6H_{12}O_6$
   a. Number of oxygens needed to take 6 carbons to CO_2 = 12
   Number of oxygens needed to take 12 hydrogens to H_2O = 6
   18
   Number of oxygens contained in the molecule = -6
   12 0
   b. Formula:
   $$\text{g } O_2 = \frac{\text{no. of oxygen x atomic wt of oxygen}}{\text{mol wt of compound}}$$

REFERENCE

1. "Standard Methods for the Examination of Water and Wastewater," 13th Edition, American Public Health Association, New York, 1971.

[Revised manuscript received February 5, 1973]