New Accelerated Test for Rapid Measurement of Detergent Biodegradability

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Abstract

A new accelerated biodegradation test has been developed in order to determine rapidly the biodegradability of detergents. Natural river water is fortified with additional microorganisms isolated from sewage effluent by continuous centrifugation to remove residual detergent. This increases the reproducibility between different samples of river water and decreases seasonal effects. Using detergents of different chemical structures, this accelerated procedure gave the same biodegradable results as obtained with the usual river die-away test. However, such data can now be obtained in as little as ten days or less.

Multiple test results are presented showing the rate and degree of biodegradation of representative test detergents and the reproducibility between different series of tests with the same compound. Examples are given showing that the extent and completeness of biodegradability of the test compounds depends on the degree of branching.

Introduction

THE INCREASED USE OF synthetic detergents throughout the world has caused considerable concern due to problems associated with their lack of complete biodegradability. This has led to the search and development of new products for detergents which are completely biodegradable. One problem which has hindered this search is the lack of a standard biodegradable test which is easy and rapid to perform.

Some of the methods and tests which have been proposed to measure compound biodegradability are the activated sludge, trickling filter, anaerobic septic tank, Warburg and the river die-away tests. Each of these methods shows the extent of biodegradability under selected test conditions and stresses some particular aspect of biodegradability. However, all are subject to problems, either in regard to inconvenience of procedure, time, equipment or lack of reproducibility. Although each test could and possibly should be used in a complete testing program, none seems suitable as a routine screening method for many compounds by different laboratories. The need for such a simple reproducible method which can screen many compounds rapidly governed our decision to develop and employ an accelerated die-away test.

Some of the initial studies and observations using natural waters to measure detergent decay were made by Hammerton (1) and Sawyer et al. (2). These tests measured the disappearance of a low concn of the detergent in a natural river water over a period of time. In more recent tests with different detergents and waters, several difficulties have arisen in conducting the tests and in the interpretation of the results. Some of these difficulties are long duration of the test, lack of reproducibility between different river waters, manpower requirements and changes in the river water due to seasonal and environmental effects. However, the river die-away test is simple to run, requiring a min of equipment, and is relatively realistic as to conditions encountered in nature. The following proposed procedure retains the basic characteristics of the river die-away test while eliminating or significantly reducing the difficulties associated with it.

Procedure

In the normal river die-away test, the test actives are added to freshly sampled river water after a "blank" of the river water has been analyzed. This check is to make certain that sufficient alkylbenzene sulfonate (ABS) is not present in the water sample to alter the test results. Most river waters do not contain enough ABS to cause concern in the test, but indicated blank levels of 1 ppm or more should be considered in the addition of the test active and resultant analyzed values. The normal microflora in the natural river water are considered the inoculum.

The river die-away procedure as outlined by Procter and Gamble (3) has been used as the present normal river die-away test. In this procedure a new, one-half gallon Mason jar with screw cap is rinsed with distilled water and then 980 ml freshly-sampled river water is added. The test active (20 mg) is added as 20 ml of a 1000 ppm stock solution, resulting in a 20 mg/l or 20 ppm solution. A magnetic bar is immediately placed in the jar and the solution stirred for one min. A sample is withdrawn while the solution is still being stirred and analyzed by the methylene blue method (as given in Standard Methods for Examination of Water and Wastewater, 11th Edition (4)). The jar remains quiescent at normal room temp until the next sampling the following day or at convenient intervals. As many test actives as desired, or as can be handled, may be run with the same basic river water, each test active requiring a separate jar.

In the new Accelerated Biodegradation Test the same materials and procedures are employed except that the natural river water is fortified with added microorganisms. These added microorganisms are obtained by continuous centrifugation of water from a municipal sewage plant effluent, although other sources such as the river water itself could be used. In this technique enough river water of low ABS level is obtained the morning of the test in polyethylene carboys to supply each test active with 980 ml. At the same time two liters of sewage effluent is obtained in similar containers for each test active or for each 980 ml river water. The sewage effluent is taken from immediately upstream of sewage plant discharge to the river. The waters are returned to the laboratory and continuous centrifugation begun on the sewage effluent. The continuous centrifugation can be done in any type continuous centrifuge, but we have used the Szent-Gyorgi and Blum, 8-tube continuous flow system for the type RC-2 Servall automatic, super-speed, refrigerated centrifuge. The centrifuge is operated at 17,000 rpm at a temp of 30F with the highest flow rate which will remove and precipitate the microorganisms. By this technique approx 40 liters sewage effluent can be centrifuged in 5 hr, which amt will be sufficient to perform 20 tests. After centrifugation, the tubes are removed from the centrifuge, the supernatant decanted and the precipitate resuspended in one-liter of the test river water.

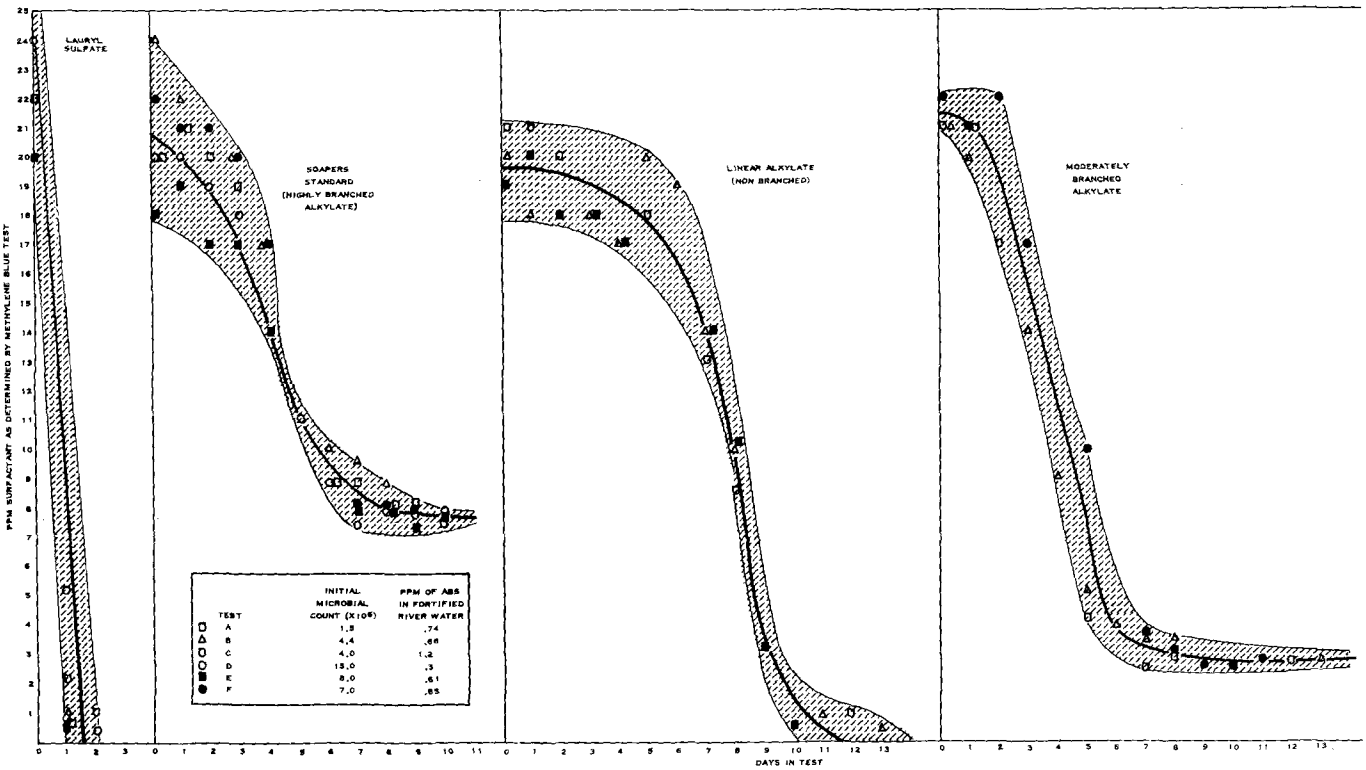

FIG. 1. Comparison of the biodegradability of four compounds as tested by the Accelerated Biodegradation Test.

This suspension of the packed cells is then blended (Waring Blender) at high speed for one min or until a uniform suspension of cells is obtained. The suspension is then added to the total amt of river water required to run the test actives in that series and shaken vigorously. This fortified river water is then used to run a standard die-away test. An additional step may be incorporated in the procedure in which the fortified river water is diluted to a standard optical density in order to initiate all tests in different series with the same number of cells. This added step has not been found necessary for tests using the same river water and sewage effluent in order to obtain adequate reproducible results, but may be necessary to achieve reproducibility between laboratories. In other cases it could be desirable to centrifuge down a large amt of cells and then by freezing to maintain the cells until needed for other tests or for comparative purposes.

Results and Discussion

When using the normal river die-away test procedures it is found that the local river waters took an extremely long time to cause biodegradability. The water was not heavily polluted and the microbial count was low. In addition, seasonal changes, water levels and other phenomena caused the microbial count to fluctuate greatly. In attempts to rectify this problem, it was found that tests and compounds varied in their time response to waters from different rivers. These problems caused a lack of reproducibility between tests and between laboratories, but especially it resulted in tests of long duration with resultant increases in manpower requirements and costs. In spite of these difficulties, it was decided that the river die-away test procedure offered the most promise as a simple test to screen large numbers of compounds because of its simple equipment and procedure.

The problem resolved itself to one of retaining the natural river water as unchanged as possible by adding to it enough organisms of a type characteristic of the water to produce a rapid test. The most convenient source of additional organisms was to be found in the sewage effluent. However, the effluent from the local sewage plant was too high (approx 10 ppm) in ABS which precluded its being used directly in the test or as a supplement to the river water. For this reason the organisms were removed by centrifugation from the sewage effluent while leaving behind the water containing the high concn of ABS. The addition of these organisms to the natural river water did not measurably increase its ABS content. Thus the technique permits the population level in the natural water to be adjusted to any desired level with typical organisms while not increasing its ABS level.

In every series of compounds run by this technique there are at least two controls: a rapidly biodegradable compound, lauryl sulfate; and a standard ABS compound, the Soapers Standard (Standard alkyl benzene sulfonate obtained from Soap and Detergent Assoc., New York). These compounds serve as controls both as to the activity of the organisms in one test series and to the reproducibility between different test series. Results with these compounds (each run six times) show (Fig. 1) that in the Accelerated Biodegradation Test the lauryl sulfate will be completely degraded within 48 hr. This is not true with Soapers Standard which degrades to approx the 60% level and then shows no further change except for a minor downward trend with extended incubation.

Other compounds in a test series can be compared to these two standards and among themselves as to rate and extent of degradability. For example, a nonbranched alkyl structure will show complete biodegradability (down to the level of residual ABS in the river water) while compounds having moderate branching will degrade to points in between. Although there may be a spread of initial values, it can be seen (Fig. 1) that the tests are reproducible within usable limits. Microbial plate counts made throughout the test period indicate that counts increase rap-

idly and that adequate numbers of microorganisms are present in all samples to cause biodegradability. The initial inoculum is not critical as long as it is adequate to cause the lauryl sulfate to degrade in two days. Increasing the inoculum beyond this point does not seem to cause an appreciable increase in the rate of degradation and it does not cause a more complete degradation. Final levels of degradation are the same as in the standard river die-away test, with the accelerated test simply compressing the test time to less than one-half.

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Determination of the Glyceride Structure of Fats; Analysis of 14 Animal and Vegetable Fats[1]

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Abstract

The glyceride compositions of seven animals and seven vegetable fats have been determined by GLC analysis of the oxidized esterified glycerides as described in an earlier paper in this series. The compositions determined are compared with those calculated from lipase hydrolysis data according to the method of VanderWal. Good agreement was found between the calculated and determined compositions for the majority of the 14 fats. The exceptions were human fat and the more saturated vegetable fats, palm oil and cocoa butter, where some discrepancies occurred.

Introduction

THE GLC ANALYSIS of oxidized esterified glycerides (1) provides a rapid and accurate means of determining the fatty acid distribution in natural fats. Initial investigations on a few fats showed good agreement between the determined glyceride composition and that calculated from lipase hydrolysis data according to the method of VanderWal (2). The present work extends this type of analysis to 14 animal and vegetable fats. The results obtained are compared with those calculated from lipase hydrolysis data.

Experimental

The vegetable oils studied were commercial, refined and bleached samples. The animal fats were cold-extracted with chloroform from fresh adipose tissue. Twenty mg of each fat was oxidized with permanganate-periodate as described earlier (1). The resulting azelao-glycerides were methylated with diazomethane and the oxidized esterified glycerides analysed by GLC. Conditions for GLC analysis and methods of calculation were as previously described (1).

The original fatty acid compositions of the fats were determined by GLC of their methyl esters on an *o*-phthalic-ethylene glycol column. The method of Youngs (3) was used for lipase hydrolysis in which the composition of the liberated fatty acids was determined rather than that of the unhydrolysed monoglycerides. All results are reported as mole percentages.

Results and Discussion

Tables I and II give the glyceride compositions as determined and as calculated from lipase hydrolysis data. Since the separation of the oxidized esterified glycerides by GLC is dependent on their effective carbon number, those glycerides giving rise to the same carbon number emerge together and are determined as a group as indicated in the tables. The individual glycerides can be calculated from lipase hydrolysis data and this has been done. Comparisons are then made on the basis of the sum of the calculated glycerides in a group where two or more glycerides have the same carbon number. Since myristic acid is a minor component in the fats investigated, the calculated proportion of myristic-containing glycerides is small. Glycerides containing more than one myristic acid are less than 0.1%.

Tables III and IV give the original fatty acid compositions of the fats, as determined by GLC of their methyl esters, and those calculated from the glyceride compositions obtained. The agreement between these compositions for the individual fats serves as a check on the glyceride analysis. The fatty acid composition in the 1–3 positions is also given in the Tables. These latter figures were used in calculating the glyceride compositions on the basis of VanderWal's theory.

Good agreement between the determined and calculated compositions was found for linseed, corn, olive, cottonseed and soybean oils. For the more saturated fats, cocoa butter and palm oil, the proportion of disaturated glycerides found was somewhat higher than that calculated, with the remaining glycerides being lower than calculated. In view of the good agreement obtained for the other vegetable fats, this suggests that the actual glyceride distribution for the more saturated vegetable fats may be slightly different than that predicted from lipase hydrolysis data. This however, requires further investigation.

Agreement between the calculated and determined glyceride compositions for the animal fats was generally good with the exception of human fat. In the latter case considerably more monosaturated glycerides were found than would be expected from lipase hydrolysis data, with a corresponding drop in the proportions of the fully unsaturated glycerides and more saturated glycerides. A similar pattern was found for two other samples of human fat. Since humans undoubtedly receive a much higher proportion of dietary fat than the other animals tested, this discrepancy may represent the effect of combined endogenous and exogenous fats.

In general it appears that glyceride composition calculated on the basis of lipase hydrolysis data provides a good estimate for the majority of natural

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