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.

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Our analytical section, in particular R. A. Greff, provided technical assistance and obtained much of the data presented.

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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-

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degradability standards are applicable only to those anionic surfactants having an alkyl side chain, a benzene ring and a sulfonate group.

For purposes of measuring biodegradability in this cooperative study, the methylene-blue procedure described in "Standard Methods for the Examination of Water and Wastewater," 11th Edition, was specified. However, cooperating laboratories were authorized to use other procedures if the results obtained were comparable to those achieved by the standard method.

Cooperative studies included testing of branched-chain alkyl benzene sulfonate and six samples of linear alkylate sulfonate. The considerable data collected on these seven materials were subjected to rigorous statistical analyses (Appendix B) to determine the reproducibility of the test and to establish biodegradability under the test conditions. A summary of the results obtained will be discussed later. The seven materials evaluated are described in Table I.

Based on the experience gained by the cooperating laboratories during this study, a two-step procedure seemed best suited to meet the needs of a useful biodegradability test method. The method would consist of a presumptive and a confirming step. This approach has been used in microbiological testing, particularly in the water and waste treatment field. A similar concept is presently used in testing for the presence of coliform organisms—a basic test for evaluating the bacterial safety of drinking water, bathing areas, etc.

Furthermore, the two-step approach effectively combined the elements of simplicity with a thorough evaluation of the biodegradability of the surfactant under test.

Essentially two of the previously described test methods were combined in a single procedure. The relatively simple shake flask method is used as the screening or presumptive step, while the more complex and time-consuming semicontinuous activated sludge procedure serves as the confirming phase.

Results Obtained

A summary of the results obtained for the seven surfactants tested is presented in Table II.

TABLE I

ABS/LAS Materials Evaluated in Cooperative Evaluation

Material	Composition	%
(1) Dodecene-1 Derived Reference LAS	Dodecene-1 LAS	90.29
	Sodium sulfate	7.86
	Free oil	0.69
	Water	1.16
(2) LAS #3S (an early LAS composite of pilot plant production)	LAS	41.0
	Sodium tripolyphosphate	10.0
	Sodium sulfate	44.5
	Water	5.5
	Equiv. wt.	346
(3) LAS Composite Lot #1-1 (a blend of several available materials from early commercial production)	LAS	60.8
	Sodium sulfate	36.1
	Free oil	0.4
	Water	2.7
	Equiv. wt.	348
(4) ABS-Lot #3	ABS (tetrapropylene derived)	54.8
	Sodium sulfate	40.3
	Free oil	0.5
	Sodium hydroxide	1.3
	Sodium carbonate	0.7
	Water	2.6
	pH (1% solu.)	11.0
	Equiv. wt.	348
(5) Unknown A	LAS	37.0
(6) Unknown B	LAS	40.0
(7) Unknown C	LAS	94.8
(Matrials 5–7 are three linear alkylate sulfonates of varying biodegradability supplied by different manufacturers)		

To give some indication of the scope of these investigations, it is perhaps of interest to report on the magnitude of the study itself. Seventeen laboratories took part in this cooperative study which consisted of some 1,300 individual laboratory runs. Of this total, 600 runs involved use of the shake flask method; 250 were on the semicontinuous activated sludge method; the river die-away test was tested in 400 instances; and 50 runs were made on a modified continuous activated sludge procedure. This work required the analysis of approximately 30,000 samples and took over 9,000 man-hours.

Both methods easily differentiate ABS and LAS as is observed from the means. Also presented in Table II are the lower tolerance limits calculated for each surfactant. These values are the lower limits above which 95% of individual determinations are expected to fall. Due to the variability of any test procedure, individual results will be distributed about the average value of many determinations.

Biodegradability Standards

Biodegradability standards were based on three independent factors.

1. The statistical evaluation of data collected during the cooperative testing program,
2. The existence of commercial materials which would routinely meet standards established under the procedure and
3. The understanding that materials meeting these standards would be removed to essentially the same extent as other soluble organic constituents of sewage when subjected to activated sludge type sewage treatment.

The standards of biodegradability established for the method are as follows:

If, under the provisions of the Presumptive Test, ABS/LAS reduction equals or exceeds 90%, the surfactant is considered to be adequately biodegradable and no further testing is required. If surfactant reduction falls between 80 and 90%, its biodegradability *must* be determined by the Confirming Test. If percent reduction falls below 80% in the Presumptive Test, the material is considered to be not adequately biodegradable and no further testing is justified. For a material to be considered adequately biodegradable in the Confirming Test, reduction of at least 90% is required. If a surfactant falls below this value, it is not considered to be adequately biodegradable.

The procedure and standards are designed to be applicable only to anionic surfactants of the ABS and LAS type and not to total detergent formulations containing varying quantities of these surfactants. Obviously it is much more desirable to control biodegradability on the raw material rather than on the many different finished products using this same raw material. Also it is possible that certain finished product formulations— such as those of a low surfactant-high inorganic salt content or those containing bacteriostats or bactericides—would have side effects on the microbial population which would make invalid the measurement of biodegradability of the surfactant portion of the finished product under the laboratory test conditions.

It must be emphasized that the biodegradability standards are established for the two-step procedure described. In any microbiological test procedure, differences may be observed in results obtained due

TABLE II

Surfactant Removed, Percent

Sample	Shake flask test					Semicontinuous test				
	Mean	95% Conf. limits	Lower tolerance limit[a]	Number labs.	Number reps.	Mean	95% Conf. limits	Lower tolerance limit[a]	Number labs.	Number reps.
1. Dodecene-1 derived LAS	99.5	99.3 to 99.7	98.0	17	113	99.6	99.2 to 99.9	97.1	11	43
2. LAS Composite 1-1	93.5	92.1 to 94.8	86.8	11	52	97.4	95.9 to 98.6	92.3	7	27
3. LAS 3S	95.6	94.5 to 96.5	89.7	15	86	98.3	97.1 to 99.2	93.9	11	43
4. ABS Lot 3	21.5	14.0 to 29.0	<0	13	43	58.2	46.5 to 69.9	9.4	12	12
Unknowns										
5. A	94.5	92.2 to 96.5	88.2	7	23	97.5	95.6 to 98.8	92.5	4	11
6. B	90.0	87.2 to 92.5	82.0	8	25	94.5	92.8 to 96.0	87.8	5	15
7. C	94.0	91.3 to 96.1	87.4	7	25	97.4	95.0 to 99.1	92.4	4	10

[a] 95% of individual results will fall above this value (95% confidence).

to the inherent variability of living matter. The standards established for the two-step procedure provide for these differences by permitting confirmation of the biodegradability of a material which may initially fall into the marginal category. Therefore, it is imperative that the two-step procedure be maintained in its entirety in order to assure the accurate appraisal of a material's biodegradability.

The biodegradability of LAS has been well established by many workers using a variety of microbiological and biochemical techniques, and has been demonstrated in several field tests that have shown LAS to be as degradable as other soluble organics in sewage (3,4,11,13–15). Through this research in depth, it is possible to correlate biodegradability at specified conditions with chemical specifications of a given commercial mixture. Thus manufacturers of finished detergents can communicate their needs to raw material suppliers by the use of chemical specifications. However, as a continued check of this correlation, and as an evaluation of the biodegradability of new surfactants, or surfactants of unknown composition, biological methods are also needed.

The development of this two-step biodegradability test procedure, as well as the applicable biodegradability standards should prove quite useful in standardizing techniques in industrial and private research laboratories. For the first time a rational analysis, statistically documented, has been completed on this controversial subject. It is hoped that future Committee work will permit the broadening of the scope of the method to include other types and classes of surfactants.

Abstract

A two-step procedure for determining the biodegradibility of alkyl benzene sulfonate (ABS) and linear alkylate sulfonate (LAS) surfactants has been described. Basically, it involves the sequential use of two commonly accepted microbiological techniques. The shake flask technique is used as the presumptive step in the procedure and each surfactant must be tested by this method. If a surfactant is 90% or more degraded in the presumptive step, no further testing is needed. If it is not degraded at least 80%, it is considered to be not adequately biodegradable. However, if its biodegradability falls between 80% and 90% by the Presumptive Test, its biodegradability must be determined by the Confirming Test. The confirming step is the semicontinuous activated sludge test which more closely simulates sewage treatment plant operation. A material must be degraded at least 90% under this procedure to be considered adequately biodegradable.

ACKNOWEDGMENT

J. E. Thompson, Procter & Gamble Company, and G. Leigh, Colgate-Palmolive Company, designed the cooperative testing scheme and carried out the statistical analysis of the results.

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APPENDIX A

SDA Procedure for the Determination of ABS/LAS Biodegradability

A. Presumptive Test (Shake Culture)

A1. *Definition of Biodegradability*

The Presumptive Test will be used first in determining surfactant biodegradability. The following rules will apply in determining whether the surfactant meets minimum biodegradability standards:

a. If surfactant reduction equals or exceeds 90%, the material is considered to be adeqately biodegradable and no further testing is required.

b. If surfactant reduction falls between 80% and 90%, the material must be evaluated under the Confirming Test.

c. If surfactant reduction is below 80%, the material is considered to be not adequately biodegradable.

A2. *Procedure*

Microorganisms are inoculated into flasks which contain a chemically defined microbial growth medium, i.e., the basal medium, and test surfactant(s). Aeration is accomplished by continuous shaking of the flask. Following two adaptive transfers, biodegradation is determined by measuring the reduction in surfactant content during the test period.

A2.1 *Basal Medium*

The composition of the basal medium is as follows:

NH_4Cl	3.0 g
K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.25 g
KCl	0.25 g
$FeSO_4 \cdot 7H_2O$	0.002 g
Yeast Extract	0.30 g
Water (distilled or deionized)[1]	1.0 liter

This medium may be prepared by sequentially dissolving the dry ingredients in the water, or by adding stock solutions of the salts. The yeast extract should be added in dry form immediately before use; or alternately, solutions containing yeast extract must be sterilized if to be held more than 8 hr before beginning the test.

The basal medium is dispensed into one of the following standard Erlenmeyer flask sizes: 500 ml/1 liter flask; 1000 ml/2 liter flask; 1500 ml/4 liter flask.

(The former two are best suited for a gyratory shaker and the latter for a reciprocating shaker).

The flasks are stoppered with cotton plugs or equivalent to reduce evaporation and contamination.

A2.2 *Microbial Culture*

a. *Source*

The microbial inoculum may be obtained from any of the following:

1. Natural sources (soil, water, sewage, activated sludge, etc.)
2. Laboratory cultures (activated sludge, river die-away, etc.)
3. Culture obtained from:
   Leberco Laboratories, Inc.
   123 Hawthorne Street
   Roselle Park, New Jersey 07204
   Phone: 201-245-1933

b. *Maintenance*

If desired, the culture may be maintained as a shake flask culture by weekly transfers in the basal medium plus 30 mg/liter dodecene-1 derived LAS (note 2). For each weekly transfer use 1 ml of 7-day culture for each 100 ml of fresh medium.

[1] Water derived from steam condensate will in many cases contain amines which are inhibitory to microbial growth. Water for use in this test should be free of bacteriostatic materials.

A2.3 *Operation*

a. *Addition of Surfactant to Basal Medium*

Add 30 mg/liter surfactant (active basis) to the flasks containing basal medium. If surfactant stock solutions are used, stability during storage must be confirmed.

Use one flask for each surfactant being tested, plus one control flask for dodecene-1 derived LAS (note 2), additional controls if desired (note 3), and one blank flask containing no surfactant.

b. *Inoculation*

Using the culture described in Section A2.2, inoculate the flasks. Use the same culture for all flasks including control and blank. Use 1 ml inoculum for each 100 ml of medium in the flask.

c. *Incubation*

Place flasks containing basal medium, surfactant, and inoculum on a shaking machine for aeration.

A reciprocating shaker operating at about 128 two–four inch strokes/minute or a gyratory shaker operating at 225–250 one–two inch revolutions/minute should be used (other shakers may be used if equivalent aeration can be demonstrated).

Maintain temperature of the flask contents at 25 ± 3C.

d. *Adaptation*

Make two 72-hr adaptive transfers prior to the 8-day test. Transfer 1 ml of the 72-hr culture into each 100 ml of fresh medium plus surfactant. Transfer from control to control, blank to blank, test surfactant I to test surfactant I, etc.

e. *Analysis* (Note 1)

To follow the course of biodegradation, remove samples from the shake flasks for analysis.

Samples must be taken during the 8-day test at zero time (immediately after inoculation and mixing of the flask) and on the 7th and 8th days. Samples at zero time of the two adaptive transfers are desirable to insure proper initial concentration.

Unless analyses are run immediately the addition of one ml of formaldehyde per 100 ml of sample should be used for preservation. When preservative is used, add to all samples including blank.

Since the analytical result from the blank sample is used to correct the results from the other flasks, use the same sample size (or dilution factor) for the blank as is used for the other samples.

A2.4 *Results*

a. *Calculation*

Calculate net surfactant concentration by subtracting the analyzed blank value from the analyzed values for the other flasks.

The percent removal is calculated from the reduction in surfactant concentration:

% removal (day X) =

$$\frac{(S_o - B_o) - (S_x - B_x)}{S_o - B_o} \times 100$$

where S_o, S_x are analyses of test surfactant culture and B_o, B_x are analyses of blank culture, on days O and X.

The result of the test is the average of 7th and 8th day percent removals.

b. *Validation*
As a control on the culture and test conditions used, the total run is invalid if the result for dodecene-1 derived LAS is less than 97.5% removal.

B. Confirming Test (Semicontinuous Activated Sludge)

B1. *Definition of Biodegradability*
If under the provisions of Section A.1 (Definition of Biodegradability—Presumptive Test), surfactant biodegradability must be confirmed by the Confirming Test, the following rules will apply:

a. If the surfactant reduction is at least 90%, the material is considered to be adequately biodegradable.
b. If surfactant reduction is less than 90%, the surfactant is considered to be not adequately biodegradable.

B2. *Procedure*
Activated sludge obtained from a sewage treatment plant is used in this test. The sludge, the surfactant to be tested and a synthetic sewage used as an energy source for the sludge microorganisms are all placed in a specially designed aeration chamber. The mixture is aerated for 23 hr, allowed to settle, and the supernatant removed. The sludge remaining in the aeration chamber is then brought back to volume with fresh surfactant and synthetic sewage and the cycle repeated. Biodegradation is determined by the reduction in surfactant content during each cycle.

B2.1 *Aeration Chambers* (Figure 1)

a. *Construction*—Use Plexiglas tubing 83 mm (3¼ in.) I.D. Taper the lower end thirty degrees from the vertical to a 13 mm (½ in.) hemisphere at the bottom. 25.4 mm (1 in.) above the joint of the vertical and tapered wall, locate the bottom of a 25.4 mm (1 in.) diameter opening for insertion of the air delivery tube. The total length of the aeration chamber should be at least 600 mm (24 in.). An optional draining hole may be located at the 500 ml level to facilitate sampling (Figure 1). Units are left open to the atmosphere.
b. *Operating Liquid Volume*—1500 ml.
c. *Effluent and Feed Volume*—1000 ml daily (500 ml of settled sludge and liquid remains in unit after effluent is removed).
d. *Mounting*—Mount the units perpendicularly.
e. *Sampling*—Optionally by siphon through top of unit, or by a drain tube at the 500 ml level.

B2.2 *Activated Sludge*
For initial tests, collect activated sludge sample from a sewage plant that treats principally domestic wastes. Adjust the suspended solids by dilution with city tap water to 2500 mg/

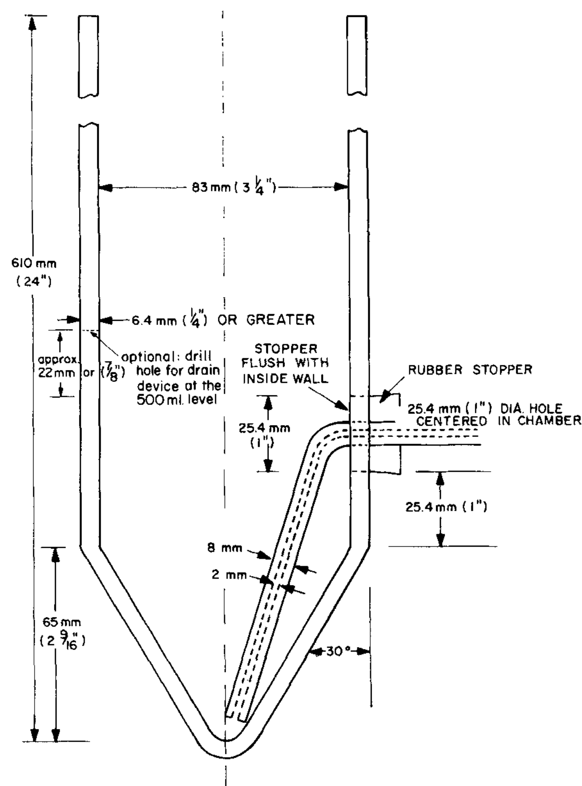

FIG. 1. Semicontinuous activated sludge aeration chamber.

liter to start the test. Maintain the mixed liquor suspended solids at 2500 ± 500 Hg/liter by discarding solids as necessary throughout the test.

If desired, laboratory acclimated sludge (i.e., acclimated to the synthetic sewage and the feeding schedule) may be used.

B2.3 *Aeration and Mixing*

a. *Compressed Air*—Filter through glass wool or other suitable medium to remove contamination (oil, etc.).
b. *Air Rate*—Maintain at 500 ml/minute (1 ft^3/hour).
c. *Air Delivery*—(Figure 1) via an 8 mm O.D., 2 mm I.D. capillary tube. Locate the end of the capillary 7 mm (¼ in.) from the bottom of the aeration chamber.
d. *Temperature*—Maintain temperature at 25 ± 3C.

B2.4 *Aeration—Settling*
Aeration period must average 23 hr per day with individual deviations of no more than 1 hr. Settling period must be at least ½ hr.

B2.5 *Defoamant*
If excessive foaming occurs use a minimum amount of silicone defoamant to keep foam within the unit. (SAG 470 Union Carbide or equivalent).

B2.6 *Chamber Care*
In order to prevent the accumulation of solids and surfactant above the liquid the walls of the unit should be periodically cleaned.

Maintain a separate scraper or brush for each unit to reduce cross contamination. Just after

feeding, scrape and rinse down residual solids which cling to the chamber walls; and scrape later as necessary, but not during the last 8 hr of the cycle.

B2.7 *Synthetic Sewage Stock Solution*

Glucose	13.0 g
Nutrient broth	13.0 g
Beef extract	13.0 g
Dipotassium hydrogen phosphate	13.0 g
Ammonium sulfate	2.5 g

Make up to one liter with city tap water; dissolve by heating to just below the boiling point. Store in refrigerator at less than 7C. Discard stock solution if evidence of biological growth appears.

B2.8 *Initial Feeding of Test Surfactants to Fresh Sludge*

If sludge is not acclimated to the test surfactant use the following incremental surfactant feed schedule:

Day 0	Feed 4 mg/liter surfactant
Day 1	Feed 8 mg/liter surfactant
Day 2	Feed 12 mg/liter surfactant
Day 3	Feed 16 mg/liter surfactant
Day 4—Finish	Feed 20 mg/liter surfactant

B2.9 *Controls*

a. *Blank*—With each run, maintain one blank unit on feed as for the other test units but without surfactant. (The surfactant analyses on influents and effluents of this unit are subtracted from those of the test units.)

b. *Internal Control Surfactant*—With each run, include one unit fed dodecene-1 derived LAS (Note 2) as a control on sludge suitability and operating conditions. Additional controls are desirable (Note 3).

B2.10 *Daily Routine*

a. If necessary remove sufficient mixed liquor to maintain suspended solids between 2000–3000 mg/liter.

b. Stop aeration to allow settling for 30 minutes.

c. Read 30 min. settled sludge volume (B2.14). This step is optional.

d. Remove upper 1000 ml (effluent) for subsequent analyses, leaving 500 ml settled sludge and liquor in aeration chamber.

e. Resume aeration.

f. Add 1000 ml feed to chamber; target composition of feed is:

Glucose, nutrient broth, beef extract, and phosphate—130 mg/liter each
Ammonium sulfate—25 mg/liter
Surfactant—20 mg/liter (or zero for blank)

1. When influent analysis is needed (B2.11):
   (a) Combine the following:
   10 ml synthetic sewage stock solution (B2.7)
   20 mg surfactant (If stock solution is used, stability during storage must be confirmed).
   Tap water to make 1000 ml
   (b) Mix well, sample for surfactant analysis, and add to chamber.
2. When influent analysis is *not* needed, add directly to chamber:
   a. 10 ml synthetic sewage stock solution (B2.7)
   b. 20 mg surfactant
   c. Tap water to bring to volume (1500 ml total).

g. Clean walls of aeration chamber (B2.6).

h. Take sample, if required, for suspended solids (B2.13) 2–3 hr after feeding.

B2.11 *Surfactant Analysis* (Note 1)

a. *Sample*
   1. Influent for each unit including blank (B2.10-f-1).
   2. Effluent—Unfiltered effluent of each unit including blank (B2.10-d).

b. *Frequency*
   1. Influent—On each of five days, not including the incremental surfactant build-up period (B2.8). At least three of the influent samples should fall within the "level operation" period (B2.15-c).
   2. Effluent—Daily.

c. *Sample Preservation*—Preserve samples with one milliter 37% formaldehyde solution per 100 ml sample unless analyses are run immediately after sampling.

d. *Blank Analysis*—Since the analytical result of the blank unit is used to convert the results of the other units, use the same sample size (or dilution factor) for the blank as is used for the other samples.

B2.12 *Effluent pH Analysis (Optional) (Note 1)*

Determine pH on unfiltered effluent.

B2.13 *Suspended Solids Analysis (Note 1)*

a. *Sample*—Mixed liquor 2–3 hr after feeding. Scrape walls within 30 min prior to sampling. To remove possible stratification of sludge, temporarily increase air flow 2–5 min prior to sampling.

b. *Frequency*—Three or four day intervals.

B2.14 *Sludge Volume Index Determination (Optional) (Note 1)*

a. *Frequency*—same days as for suspended solids.

b. Observe settled sludge volume in the unit after 30 minutes settling time.

c. Calculate sludge volume index as:

$$SVI = \frac{\text{Settled Volume after 30 min (ml)} \times 667\,*}{\text{Suspended Solids mg/liter}}$$

B2.15 *Results*

a. *Test Duration*
   1. The minimum time required for testing a new surfactant is 15 days:
      (a) Five days for incremental surfactant build-up (B2.8);
      (b) Three days equilibration at 20 mg/liter surfactant;
      (c) Seven days level operation as defined below (B2.15c)

* The Factor 667 is used since the total volume being settled is 1500 ml. This calculation gives the same result as *Standard Methods*.

b. *Calculation*
   1. Calculate daily percent surfactant removals starting with the 4th day on which surfactant feed is 20 mg/liter:

$$\% \text{ removal (day X)} = \frac{S_i - S_e}{S_i} \times 100$$

   where S_i is average of 5 influent analyses corrected by subtracting blank influent analyses, and S_e is effluent analyses minus the blank effluent analyses for that day.
   2. The result of the test is the average percent removal over a 7-day period of level operation as defined below (B2.15c).

c. *Level Operation*
   Level operation is determined separately for each unit and is defined as a 7-day period during which:
   1. Difference in percent removal on any two consecutive days is no more than 5%.
   2. Difference in average percent removal for the first three days and average for the last three days is no more than 3%.

d. *Validation*
   1. For each surfactant, the result is invalid if the conditions of level operation are not met.
   2. As a control on the sludge and operating conditions, results of the total run are invalid if the result for dodecene-1 derived (LAS) (note 2) is less than 97.5%.

Note 1: All routine analytical procedures shall be in accordance with the most recent edition of *Standard Methods for the Examination of of Water and Wastewater*, published by the American Public Health Association, 1790 Broadway, New York, New York 10019.
   a. ABS/LAS-Methylene Blue Method: 11th Edition, p. 246.
   b. Suspended Solids: 11th Edition, p. 430.
   c. pH: 11th Edition, p. 194.
   d. Sludge Volume Index: 11th Edition, p. 431.

   Other analytical procedures may be used if they are shown to yield equivalent results.

Note 2: Dodocene-1 derived LAS samples may be obtained through The Soap and Detergent Association, 40 East 41st Street, New York, New York 10017.

Note 3: A reference LAS sample which meets the standards of biodegradability of both the presumptive and confirming tests is available through The Soap & Detergent Association. This sample is a composite of several commercially available products, believed to be typical (from a biodegradability standpoint) of LAS surfactants in commercial use. It is suggested that a control test should be conducted using this material, whenever surfactant biodegradability determinations are undertaken.

APPENDIX B

Statistical Analysis

Statistical analyses were employed to determine the reproducibility of the methods, and the best estimate of the true percent removal. Using these statistics for each surfactant, confidence limits around the true percent removal and lower tolerance limits for individual results were calculated.

Statistical Approach Used

Three cooperative experiments were conducted during a 15-month period. Each experiment was designed to provide for replicate units within each run and replicate runs for each laboratory. Additionally, in the first experiment, replicate analyses for each unit were obtained. Thus, four levels or sources of variability were investigated: 1) laboratory-to-laboratory; 2) run-to-run within laboratories; 3) unit-to-unit within runs; 4) analysis-to-analysis within units.

Since all participating laboratories did not have the facilities to conduct the entire testing scheme, the statistical analysis was performed recognizing the varying number of degrees of freedom in the experimental design. Test results at each level of variability were averaged to yield the average for the next higher level; e.g., the grand mean is the average of laboratory means rather than the average of individual runs or unit means. It is believed than any slight loss in precision of the confidence limits is of less importance than unduly biasing the results when a few laboratories submit a larger proportion of the determinations.

It was observed from the first set of data that variability increased as the percent removal decreased, and that the distribution of results was skewed toward the lower percent removal values. As a variance stabilizing step, the square root transformation attributed to Yates and discussed by Bartlett [1] was applied to the data prior to analysis. The transformation used was:

$$X = \sqrt{(100 - Y) + Z}$$

where Y is the observed percent removal value and Z is a small value. As all calculations were done by computer, a range of Z values from 0 to 2.0 was explored. It was found that Z = 0.1 successfully stabilized the variance. In the transformed state the population was found to approach normality.

After transformation, means were determined and an analysis of variance performed to estimate the components of variance for the sources listed above. Using these statistics, confidence limits around the true percent removal and lower tolerance limits for individual results were calculated.

Results

Data from the cooperative experiments were screened to cull out data not meeting the requirements of the test procedures, i.e., 97.5 minimum percent removal of dodecene-1 derived LAS and level operation.

Components of Variance. During the early work, analyses of the components of variance indicated no need for duplicate analyses and only single analyses were run for the remainder of the study. Considering the other sources of variability, lab-to-lab variations were significantly greater than variation between runs in the same lab. The following table summarizes the relative importance of the sources of variability. These data are pooled variances from five LAS materials (dodecene-1 derived LAS excluded because of its significantly smaller variance).

[1] Bartlett, M. S., "The Use of Transformation," Biometrics 3, 1 (March 1947), pp. 39–52.

Source of variation	Shake flask Variance	Degrees of freedom	Semicontinuous Variance	Degrees of freedom
Lab-to-lab	.1928	14	.2045	10
Run-to-run	.0585	66	.0425	39
Unit-to-unit	.0120	97	.0033	36
Total for single determination	.2633	31 [a]	.2503	20 [a]

[a] Harmonic mean.

Confidence and Tolerance Limits. Table II presents the means and limits obtained. The lower tolerance limit is that value above which 95.0% of the results of single determinations are expected to fall (*with 95% confidence*). For dodecene-1 derived LAS and ABS lot #3, the lower tolerance limits are derived from the individual variances since the variance of these materials was found to be significantly smaller and greater, respectively, than the variance for the other materials. The tolerance limits for the other materials are derived from a pooled variance weighted by the degrees of freedom for each surfactant.

Soil Redeposition Versus Deposition Tests in Evaluation of Laundry Detergents

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Abstract

Whiteness retention results obtained with a soil "deposition" type test, in which soil material as such is added to the detergent bath, are found to be in contradiction to those obtained with soil "redeposition" tests, in which clean and soiled cloth are washed together. A carbon soil deposition test shows polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) to be superior to sodium carboxymethyl cellulose (CMC), and a polyethylene glycol (PEG) equal to CMC in improving whiteness retention results with a built anionic detergent, with pronounced synergistic effects for PVA-CMC and PEG-CMC combination. In contrast, the redeposition tests, employing either carbon black or tagged clay soil, show only the CMC to be effective, the nonionic polymers being ineffective alone and in combinations with CMC. Further, in evaluating the effect of tripolyphosphate builder with an alkylbenzene sulfonate, the deposition and redeposition tests give quite contradictory results. The observed contradictions cast considerable doubt on the validity of the usual carbon soil deposition tests, and emphasize the need for further study of whiteness retention test methods.

Introduction

IN CONSIDERING the performance of a laundry detergent, we tend to think primarily in terms of its ability to remove soil. The reverse phenomenon of soil redeposition, however, can be equally important. It has been suggested that, in home laundering, poor performance of a detergent may be due more often to excessive soil redeposition, or poor whiteness retention, than to inadequate soil removal (1). The need for soil redeposition measurements in connection with laundry detergency evaluations has been generally recognized, and the literature on the subject is fairly extensive. The approaches to this problem, and test methods developed by various investigators, have been well covered in reviews and bibliographies (2–5).

Perhaps the obvious approach to a soil redeposition test is to simulate practice, washing clean cloth along with soiled, and determining soil redeposition on the clean cloth, usually by reflectance measurement. This is a true "redeposition" type test in that soil is washed from cloth and redeposited. In such a test, however, the amount of soil redeposited depends on the amount of available soil in the wash liquor, which in turn depends on the soil-removing ability of the detergent. This complicates the comparison of soil redeposition results for two detergents of widely different soil removal abilities. Further, in such tests simulating practice, the soil redeposited in one wash is likely to be too slight for accurate determinations. Time-consuming multiple-wash tests often are required in order to build up the redeposited soil so as to bring out differences among detergents. In order to bypass these complications, most detergency workers have turned to "deposition" type tests, in which the soil material is added as such to the detergent bath (generally as aqueous carbon black dispersion) and soil pick-up by clean cloth determined. This approach permits accurate control of the soil loading in the detergent bath, and if the total soil loading is high in comparison to that deposited, the free soil loading in the bath is always essentially constant. The heavy soil loading results in a high, readily measured soil deposition. Because of these experimental advantages, deposition-type tests generally have been preferred over redeposition types, and most published work has been based on the former. In the absence of contradictory evidence, it has been generally assumed that deposition and redeposition type measurements give essentially equivalent results, at least on a qualitative basis (4).

Along with others concerned with detergency testing, our laboratories developed a whiteness retention test based on carbon soil deposition (6) and employed it for a number of years for basic studies and routine evaluations. Concurrently, we developed detergency test methods employing various radioactive tagged soils (7,8). With the use of these soils, it was found to be convenient to determine both soil removal and redeposition in a single test. The extreme sensitivity of the radiotracer method permitted accurate measurements of redeposited soil after a single wash. Also, it was found to be feasible to correct soil redeposition results in such a way as to compensate for differences in soil removal, permitting what we consider to be reasonably valid whiteness retention comparisons at different soil removal levels.

During the course of many evaluations with a tagged clay soil, it was observed that whiteness retention values often contradicted those given by the