Designation: D 2667 – 95 (Reapproved 2001)

Standard Test Method for Biodegradability of Alkylbenzene Sulfonates[1]

This standard is issued under the fixed designation D 2667; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method[2] covers the determination of the degree of biodegradability of alkylbenzene sulfonates. It serves as an index of the suitability of the sulfonate for general use as a surfactant.

1.2 In general, this test method distinguishes between sulfonates in which the alkyl side chains are linear and those in which they are branched, since the former are more readily biodegradable. If the alkylbenzene sulfonate in fully formulated products is to be examined, it must be extracted using the method noted in Annex A1. (See Appendix X1 for data.)

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Material Safety Data Sheets are available for reagents and materials. Review them for hazards prior to usage.

2. Referenced Documents

2.1 *ASTM Standards:* [3]

D 1293 Test Methods for pH of Water

D 2330 Test Method for Methylene Blue Active Substances

E 1625 Test Method for Determining Biodegradability of Organic Chemicals in Semi-Continuous Activated Sludge (SCAS)

3. Summary of Test Method

3.1 The sample is first subjected to a presumptive test based on shake culture. When necessary, the sample may be subjected to a confirming test based on semicontinuous treatment with activated sludge.

3.2 In the presumptive test, microorganisms are inoculated into a flask that contains a chemically defined microbial growth medium (basal medium) and the surfactant to be tested. 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.

3.3 In the confirming test, activated sludge obtained from a sewage treatment plant is used. 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 h, allowed to settle, and the supernatant material 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.

4. Significance and Use

4.1 This test method is designed to determine whether the sulfonate tested will be removed sufficiently by usual methods of sewage treatment for the effluent to be safely discharged to the environment without further treatment.

4.2 If the surfactant reduction in the presumptive test equals or exceeds 90 %, the material is considered to be adequately biodegradable without further testing.

4.3 If the surfactant reduction in the presumptive test is between 80 and 90 %, the material should be subjected to the confirming test.

4.4 If the surfactant reduction in the presumptive test is below 80 %, the material is considered inadequately biodegradable.

4.5 If it is necessary to run the confirming test, the surfactant reduction in this test must be at least 90 % for the material to be considered adequately biodegradable.

4.6 An example of data from both the presumptive and confirming test can be found in Appendix X4.

PRESUMPTIVE TEST (SHAKE CULTURE)

5. Apparatus

5.1 *Shaking Machine*—A reciprocating shaker operating at about 128 strokes of 51 to 101.6 mm (2 to 4 in.)/min or a gyrator shaker operating at 225 to 250 r/min with an amplitude

[1] This test method is under the jurisdiction of ASTM Committee E-47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.04 on Environmental Fate of Chemical Substances.

Current edition approved Oct 10, 1995. Published December 1995. Originally published as D 2667 – 67 T. Last previous edition D 2667 – 89.

[2] This test method is based on "A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate" by the Committee on Biodegradation Test Methods of the Soap and the Detergent Association, *Journal of the Americal Oil Chemists' Society,* Vol 42, 1965, p. 986.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

of 25 to 51 mm (1 to 2 in.) should be used. (Other shakers may be used if equivalent aeration can be demonstrated.)

6. Reagents and Materials

6.1 *Purity of Water*—Either distilled or deionized water may be used in this test. It must be free of bacteriostatic materials. Water derived from steam condensate in many cases will contain amines which are inhibitory to microbial growth.

6.2 *Basal Medium*:

6.2.1 The composition of the basal medium shall be 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	1.0 L

6.2.2 The basal medium may be prepared by sequentially dissolving the NH_4Cl, K_2HPO_4, KCl, and $FeSO_4$ in approximately 800 mL water and adjusting the pH to 7.2 ± 0.2 with a dilute solution of hydrochloric acid or sodium hydroxide. The yeast extract and $MgSO_4$ dissolved in 200 mL water are then added with stirring to the former solution. Alternatively, the medium may be prepared using suitable stock solutions of the salts, but the pH must be adjusted before the $MgSO_4$ is added. In either case, the yeast extract must be added in dry form immediately before use. It is important to use the basal medium immediately after preparation to avoid bacterial growth. The basal medium shall be dispensed into one of the following Erlenmeyer flasks: 500 mL in a 1-L flask, 1000 mL in a 2-L flask, and 1500 mL in a 4-L flask.

NOTE 1—The 1-L and 2-L flasks are best suited for a gyratory shaker and the 4-L flask for a reciprocating shaker.

NOTE 2—The pH of the medium should be checked before use and adjusted to pH 6.8 to 7.2 if necessary.

6.2.3 The flasks shall be stoppered with cotton plugs or the equivalent to reduce contamination and evaporation.

6.3 *Microbial Culture*:

6.3.1 *Source*—The microbial inoculum may be obtained from any of the following sources:

6.3.1.1 Natural sources (soil, river/lake water, sewage, activated sludge, secondary effluent, and so forth).

6.3.1.2 Laboratory cultures (activated sludge, river die-away, and so forth).

6.3.2 *Maintenance of Culture*—If desired, the culture may be maintained as a shake flask culture by weekly transfers in the basal medium plus 10 mg/L linear alkyl sulfonate (LAS).[4] For each weekly transfer use 1 mL of 7-day culture for each 100 mL of fresh medium.

7. Standardization

7.1 As a control on the culture and test conditions used, the total run is invalid if the result with a suitable reference sample of linear alkyl sulfonate[4] is less than 90 % removal as measured by methylene blue active substance (MBAS) loss.

[4] LAS may be obtained through the EPA, Quality Assurance Branch of the Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

8. Procedure

8.1 *Addition of Surfactant to Basal Medium*:

8.1.1 Add 10 mg/L of surfactant[5] (active basis) to the flasks containing basal medium. If surfactant stock solutions are used, stability during storage must be confirmed.

8.1.2 Use one flask for each surfactant being tested, plus one control flask for LAS,[4] additional controls if desired (see Note 3), and one blank flask containing all basal medium components but with no surfactant.

NOTE 3—A reference LAS sample that meets the standards of biodegradability of both the presumptive and confirming tests is available through the Environmental Protection Agency (EPA). 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. Biodegradation values for the EPA LAS standard are noted in the accompanying data from the EPA. A more complete and recent analysis may be found in Appendix X2.

NOTE 4—Failure to repeatedly attain prescribed biodegradation values for the surfactant control (LAS) indicates that conditions are unfavorable for normal microbial activity or that an analytical problem exists. Such problems should be investigated by an experienced microbiologist or an analytical chemist.

8.2 *Inoculation*—Using the culture described in 6.3, inoculate the flasks. Use the same culture for all flasks including control and blank. Use 1 mL of inoculum for each 100 mL of basal medium in the flask.

8.3 *Incubation*—Place flasks containing basal medium, surfactant, and inoculum on a shaking machine that will produce acceptable aeration and mixing for biodegradation. Maintain the temperature of the flask contents at 25 ± 3°C, and measure, and, if necessary, adjust the pH of the media at the start of each incubation period to pH 6 to 8.

8.4 *Adaptation (acclimation)*—Prior to beginning the biodegradation test, make two 72-h acclimation transfers from the flasks from 8.3 according to the following illustrative drawing describing the sequence comprising 8.2, 8.3, and 8.4.

8.5 *Analysis* (see Test Method D 2330):

NOTE 5—It is important to follow Test Method D 2330 exactly since it is known to eliminate the effects of interfering ions that might be present.

8.5.1 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 contents) and on the seventh and eighth days. Samples at zero time of the two adaptive transfers are desirable to ensure proper initial concentration. Unless analyses are run immediately, the addition of 1 mL of formaldehyde/100 mL of sample should be used for preservation for any sample (0 time or 7 or 8 days). When preservative is used, add to all samples including blank, and store the samples at 4°C.

[5] Supporting data suggest that concentrations in excess of 10 mg/L may be inhibitory to the microorganisms in the shake culture. Data are available from ASTM Headquarters.

8.5.2 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.

9. Calculation

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

9.2 Calculate the percentage removal from the reduction in surfactant concentration as follows:

$$\text{Percentage removal (Day } x) = [((S_0 - B_0) - (S_x - B_x))/(S_0 - B_0)] \times 100 \quad (1)$$

where:

S_0 and S_x = analyses of test surfactant cultures, and
B_0 and B_x = analyses of blank cultures, on Days 0 and x, expressed as concentrations of MBAS, mg/L.

9.3 The result of the test shall be calculated as the average of the seventh and eighth day percentage removals.

10. Precision and Bias

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

10.2 *Statistical Approach Used*:

10.2.1 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: laboratory-to-laboratory, run-to-run within laboratories, unit-to-unit within runs, and analysis-to-analysis within units.

10.2.2 Since all the 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; for example, the grand mean is the average of laboratory means rather than the average of individual runs or unit means. It is believed that 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.

TABLE 1 Sources of Variability

Source of Variation	Shake Flask		Semicontinuous	
	Variance	Degrees of Freedom	Variance	Degrees of Freedom
Laboratory-to-laboratory	0.1928	14	0.2045	10
Run-to-run	0.0585	66	0.0425	39
Unit-to-unit	0.0120	97	0.0033	36
Total for single determination	0.2633	31[A]	0.2503	20[A]

[A] Harmonic mean.

10.2.3 It was observed from the first set of data that variaility increased as the percentage removal decreased, and that the distribution of results was skewed toward the lower percentage removal values. As a variance stabilizing step, the square root transformation attributed to Yates and discussed by Bartlett[6] was applied to the data prior to analysis. The transformation used was:

$$X = (100 - Y + Z) \quad (2)$$

where:

Y = observed percentage removal value, and
Z = 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.

10.2.4 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 percentage removal and lower tolerance limits for individual results were calculated.

10.3 *Results*:

10.3.1 *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, laboratory-to-laboratory variations were significantly greater than variation between runs in the same laboratory. Table 1 summarizes the relative importance of the sources of variability. These data are pooled variances from five LAS materials.

10.3.2 *Confidence and Tolerance Limits*—Table 2 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).

CONFIRMING TEST (SEMICONTINUOUS ACTIVATED SLUDGE)

11. Apparatus

11.1 *Aeration Chambers* (see Fig. 1):

11.1.1 *Construction*—Use methyl methacrylate tubing 83 mm (3¼ in.) in inside diameter. Taper the lower end 30° from the vertical to a 13-mm (½-in.) hemisphere at the bottom. Locate the bottom of a 25.4-mm (1-in.) diameter opening for insertion of the air delivery tube 25.4 mm above the joint of the vertical and tapered wall. 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. Units are left open to the atmosphere. Glass can be used as an alternative to methyl methacrylate.

11.1.2 *Mounting*—Mount the units perpendicularly.

11.1.3 *Sampling*—Sample optionally, by siphon, through the top of the unit or by a drain tube at the 500-mL level.

[6] Barlett, M. S., "The Use of Transformation," *Biometrics,* Vol 3, No. 1, March 1947, pp. 39–52.

TABLE 2 Precision and Bias of Surfactant Removed, Percent

Sample	Shake Flask Test					Semicontinuous Test				
	Mean	95 % Confidence Limits	Lower Tolerance Limits[A]	Number of Laboratories	Number of Reps	Mean	95% Confidence Limits	Lower Tolerance, Limits[A]	Number of Laboratories	Number of Reps
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
LAS 3S	95.6	94.5 to 96.5	89.7	15	86	98.3	97.1 to 99.2	93.9	11	43
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:										
A	94.5	92.2 to 96.5	88.2	7	23	97.5	95.6 to 98.8	92.5	4	11
B	90.0	87.2 to 92.5	82.0	8	25	94.5	92.8 to 96.0	87.8	5	15
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).

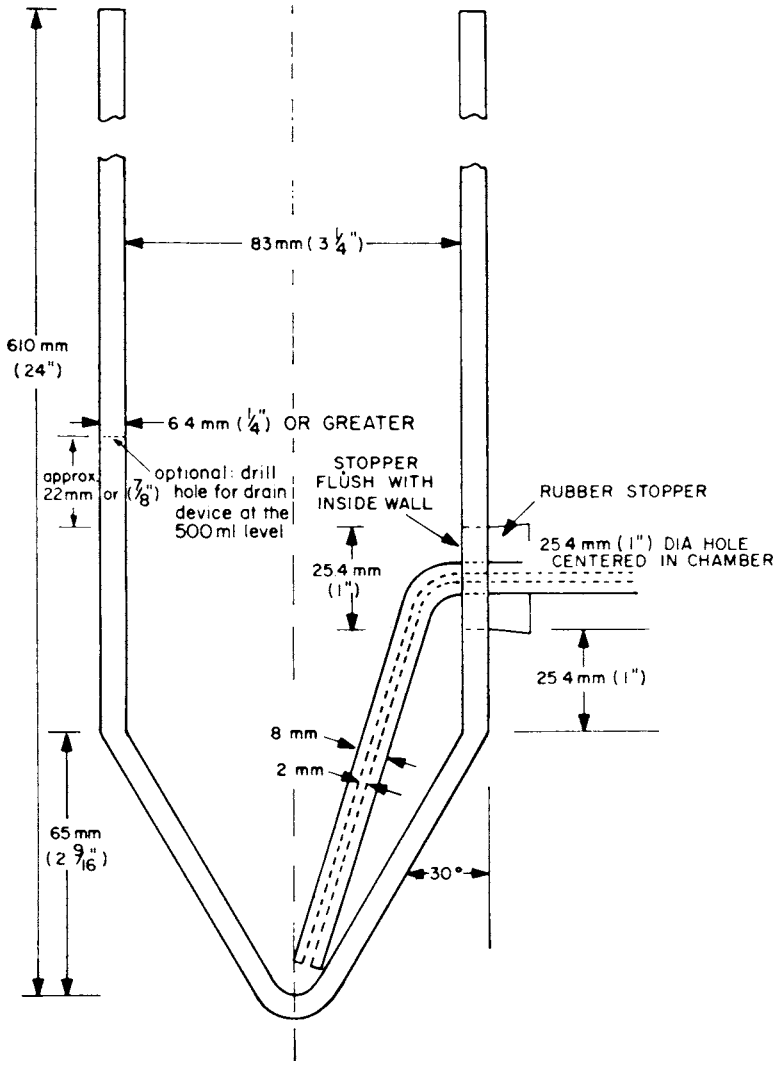
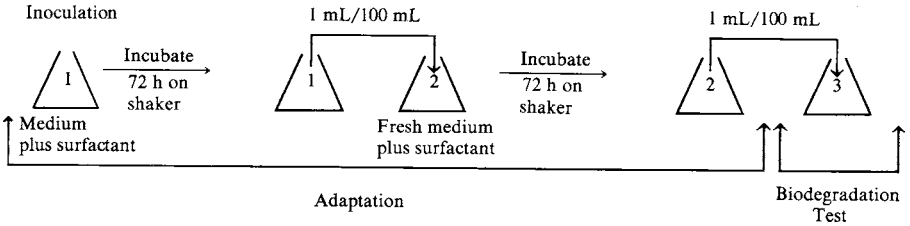


FIG. 1 Semicontinuous Activated Sludge Aeration Chamber



11.1.4 *Air Delivery*—Use an 8-mm outside diameter, 2-mm inside diameter capillary tube. Locate the end of the capillary 7 mm (¼ in.) from the bottom of the aeration chamber.

12. Reagents and Materials

12.1 *Activated Sludge*—For initial tests, collect activated sludge from a sewage plant that treats principally domestic wastes. Adjust the suspended solids by dilution with city tap water to 2500 mg/L to start the test. If desired, laboratoryacclimated sludge (that is, acclimated to the synthetic sewage and the feeding schedule) may be used. Maintain the mixed liquor suspended solids at 2500 ± 500 mg/L by discarding solids as necessary throughout the test. If mixed liquor suspended solids fall below 2500 mg/L, sludge thickening may be required. (See Test Method E 1625 for thickening activated sludge suspended solids.)

12.2 *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 1 L with city tap water; dissolve by heating to just below the boiling point. Store in a refrigerator at less than 7°C. Discard stock solution if evidence of biological growth appears (turbidity to the eye—confirm with a microscope if desired).

12.3 *Silicone Defoamant.*[7]

12.4 *Compressed Air*—Filter through glass wool or other suitable medium to remove contamination (oil, and so forth).

13. Calibration and Standardization

13.1 *Blank Controls*—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.)

13.2 *Internal Control Surfactant*—With each run, include one unit-fed LAS[4] as a control on sludge suitability and operating conditions.

13.3 *Validation*:

13.3.1 For each surfactant, the result is invalid if the conditions of level operation are not met (see 15.2).

13.3.2 As a control on the sludge and operating conditions, results of the total run are invalid if the result for LAS (Note 3) is not met.

14. Procedure

14.1 *Aeration Chamber*:

14.1.1 *Operating Liquid Volume*—1500 mL.

14.1.2 *Effluent and Feed Volume*—1000 mL daily (500 mL of settle sludge and liquid remains in the unit after effluent is removed).

14.1.3 *Air Rate*—Maintain at 500 mL/min (1 ft^3/h).

14.1.4 *Temperature*—Maintain at 25 ± 3°C.

14.2 *Aeration and Settling*—The aeration period must average as a 23 h/day with individual deviations of no more than 1 h. The settling period must be at least ½ h.

14.3 *Defoamant*—If excessive foaming occurs, use a minimum amount of silicone defoamant to keep foam within the unit.

14.4 *Chamber Care*—In order to prevent the accumulation of solids and surfactant above the liquid, the walls of the unit should be cleaned periodically. Maintain a separate scraper or brush for each unit to reduce cross contamination. Just after feeding, scrape and rinse down the residual solids which cling to the chamber walls; and scrape later as necessary, but not during the last 8 h of the cycle.

14.5 *Initial Feeding of Test Surfactants to Fresh Sludge*—If the sludge is not acclimated to the test surfactant, use the following incremental surfactant feed schedule:

Day 0 Feed 4 mg/L surfactant
Day 1 Feed 8 mg/L surfactant
Day 2 Feed 12 mg/L surfactant
Day 3 Feed 16 mg/L surfactant
Day 4 Feed 20 mg/L surfactant and continue daily throughout the test

14.6 *Daily Routine*:

14.6.1 If necessary, remove sufficient mixed liquor or thicken sludge to maintain suspended solids between 2000 and 3000 mg/L. (See 12.1.)

14.6.2 Stop aeration to allow settling for 30 min.

14.6.3 Read 30-min settled sludge volume (see 14.10). This step is optional.

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

14.6.5 Resume aeration.

14.6.6 Add 1000 mL feed to chamber; target composition of the feed is:

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

14.6.7 When influent analysis is needed (see 14.7), combine the following:

10 mL of synthetic sewage stock solution (12.2)
20 mg of surfactant (if stock solution is used, stability during storage must be confirmed)
Tap water to bring to volume (1000 mL total).

14.6.8 When influent analysis is not needed, add the following directly to the chamber:

10 mL of synthetic stock sewage solution (see 12.2)
20 mg of surfactant
Tap water to bring to volume (1000 mL total)

14.6.9 Clean walls of aeration chamber (see 14.4).

14.6.10 Take sample, if required, for suspended solids (see 14.9) 2 to 3 h after feeding.

14.7 *Surfactant Analysis (MBAS)* (see Test Method D 2330):

14.7.1 *Samples*:

14.7.1.1 Influent for each unit including blank (see 14.6.7).

14.7.1.2 Unfiltered effluent from each unit including blank (14.6.4).

14.7.2 *Frequency*:

14.7.2.1 *Influent*—On each of 5 days, not including the incremental surfactant build-up period (see 14.5). At least three of the influent samples should fall within the "level operation" period (15.2).

[7] Union Carbide's SAG 470 has been found satisfactory.

14.7.2.2 *Effluent*—Daily.

14.7.3 *Sample Preservation*—Preserve samples with 1 mL of 37 % formaldehyde solution per 100 mL of samples, and store at 4°C unless analyses are run immediately after sampling.

14.7.4 *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.

14.8 *Effluent pH Analysis (Optional)*— (See Appendix X3) Determine pH on unfiltered effluent.

14.9 *Suspended Solids Analysis* (see Appendix X3):

14.9.1 Sample mixed liquor 2 to 3 h after feeding. Scrape walls within 30 min prior to sampling. To remove possible stratification of sludge, temporarily increase air flow 2 to 5 min prior to sampling.

14.9.2 Sample at 3 to 4-day intervals.

14.10 *Sludge Volume Index Determination (Optional)* (see Appendix X3):

14.10.1 Determine on the same days as for settled solids.

14.10.2 Observe settled sludge volume on the unit after 30-min settling time.

14.11 *Test Duration*—The minimum time required for testing a new surfactant is 15 days, as follows:

5 days for incremental surfactant buildup (see 14.5)
3 days equilibration at 20-mg/L surfactant
7 days level operation as defined below (see 15.2)

15. Calculation

15.1 *Surfactant Removal*:

15.1.1 Calculate daily percentage surfactant removals starting with the fourth day on which the surfactant feed is 20 mg/L:

$$\text{Removal (Day } x\text{), \%} = [(S_i - S_e)/S_i] \times 100 \quad (3)$$

where:

S_i = average of five influent analyses corrected by subtracting blank influent analyses, and

S_e = effluent analyses minus the blank effluent analyses for that day.

15.1.2 The result of the test is the average percentage removal over a 7-day period of level operation as defined in 15.2.

15.2 *Level Operation*—Level operation is determined separately for each unit and is defined as a 7-day period during which the difference in percentage removal on any two consecutive days is no more than 5 % and the difference in average percentage removal for the first 3 days and the average for the last 3 days is no more than 3 %. Unless analyses are run immediately, the addition of 1 mL of formaldehyde/100 mL of sample should be used for preservation for any sample. When preservative is used, add to all samples including the blank.

15.3 *Sludge Volume Index* (Note 6):

Sludge volume index = settled volume in mL after 30 min/suspended solids in mg/L × 667 (4)

NOTE 6—The factor 667 is used since the total volume being settled is 1500 mL. This calculation gives the same result as the method given in Appendix X3.

16. Precision and Bias

16.1 See Section 10.

17. Keywords

17.1 alkylbenzene sulfonate; biodegradability; confirming test; presumptive test; semicontinuous activated sludge; shake culture

ANNEXES

(Mandatory Information)

A1. EXTRACTION OF ALKYLBENZENE SULPHONATE (ABS) FROM DETERGENT PRODUCTS

A1.1 The method recommended for extraction of the surfactant from the product is based on the Organisation for Economic Cooperation and Development (OECD) publication "Pollution by Detergents—Determination of the Biodegradability of Anionic Synthetic Surface Active Agents."[8] A wide range of conditions are specified, depending on the product involved, but the ratio of product:water:isopropanol is not critical, provided that the aqueous phase contains at least 70 g anhydrous potassium carbonate per 100 mL throughout the extraction procedure. This ensures that the "salting out" of the isopropanol and the ABS from the aqueous phase is complete.

A1.2 Recovery of the surfactant from the product must exceed 90 % w/w, and it is necessary to determine the anionic surfactant content of the product if this is unknown. This value and the concentration of the stock solution of surfactant used in the biodegradability tests can be determined by titration with a standard solution of the cationic surfactant Hyamine.

A1.3 The quantities of product, water, and isopropanol used in the extraction vary with both product type and surfactant content and may need to be established in each case, but the general procedure is given below.

A1.4 The quantity of the powder sample used should be sufficient to give approximately 1 g of surfactant. It is weighed into a dry 250-mL beaker and the appropriate volume of reagent grade water added to produce a thin paste. A magnetic stirrer was added to the sample that was placed on a stir plate. The stirrer speed is adjusted so that the liquid is stirred without

[8] Publications de L' OCDE, 2 rue Andre-Pascal, Paris-16e, No. 29.651, Depot Legal 2296, 1972.

splashing. The required mass of anhydrous potassium carbonate is weighed into a dry 50-mL beaker and added gradually to the stirred liquid. The mixture is stirred for 10 min, and then an appropriate quantity of isopropanol was added. The mixture tends to thicken at this stage, and it may be necessary to increase the stirrer speed to avoid separation of the organic phase. The viscosity of the mixture falls again after some minutes, and at this point it is necessary to reduce the stirrer speed to prevent splashing. The mixture is stirred for a total period of at least 30 min, starting at the time when the isopropanol is added. The mixture is filtered through a Whatman No. 541 filter using a Büchner funnel and washed with a further portion of isopropanol. The filtrate is transferred carefully to a 250-mL separating funnel washing the Büchner flask with small quantities of isopropanol.

A1.5 The phases are separated, and the alcoholic extract is transferred into a preweighed 100-mL beaker. The separating funnel is washed with isopropanol and the washings added to the extract. The extract is evaporated to dryness on a steam bath by passing a stream of nitrogen gently over the surface of the liquid. The extract is dried to constant weight, that is, until two successive weighings differ by less than 0.1 g.

A1.6 The ABS content of the extract is determined by diphasic titration with standard Hyamine solution using dimidium bromide/disulphine blue mixed indicator (see Annex A2), and the weight of surfactant extracted from the product is calculated. This should be greater than 90 % to ensure that the extracted material is representative of the surfactant in the product.

A1.7 The stock solution of surfactant used in the biodegradability tests is prepared by dissolving a suitable weight of extract in 1 L of reagent grade water, and the surfactant content is also determined by titration with Hyamine.

A2. DETERMINATION OF ABS CONTENT OF THE PRODUCT AND ABS CONCENTRATION OF THE STOCK SOLUTION

A2.1 The titration procedure used to determine the ABS content of the product and the concentration of the ABS in the stock solution is a standard method used for the determination of anionic surfactants in aqueous solutions.[9] Anionic surfactants are determined by titration with standardized solutions of the cationic surfactant, Hyamine 1622. A two-phase (chloroform/water) titration and a mixed indicator system (that is, dimidium bromide/disulphine blue) are used.

[9] Reid, V. W., Longman, G. F., and Heinerth, E., "The Determination of Anionic Active Detergents by Two Phase Mixed Indicator Titration," Tenside, January 1967.

APPENDIXES

(Nonmandatory Information)

X1. EXTRACTION DATA

TABLE X1.1 Extraction of LAS from Formulated Products—OECD Isopropanol Extraction Method[A,B]

Product	A	B	C[C]	D	E	F
Weight of product, g	300	300	350	300	300	200
Volume of water, mL	1250	1250	...	1250	1250	1000
Weight of K_2CO_3, g	1050	1050	315	1050	1050	700
Volume of isopropanol 1st extraction, mL	2250	2250	525	2250	2250	1500
Volume of isopropanol 2nd extraction, mL	750	750	175	750	750	500
Calculated weight of LAS, g	39.0	53.7	34.7	48.6	60.0	48.0
Weight of LAS recovered, g	39.4	54.1	34.5	48.9	56.7	47.2
LAS recovered, %	101.0	100.7	99.4	100.6	94.5	98.3

[A] OECD Environment Directorate, Proposed Method for the Determination of the Biodegradability of Surfactants Used in Synthetic Detergents, Paris 1976, Section 5.1.2, pp. 20–23.
[B] Results obtained with two extractions with IPA. Testing Laboratory—Water Research Centre, Medmenham, United Kingdom.
[C] Liquid product.

TABLE X1.2 Extraction of LAS from Formulated Products—OECD Isopropanol Extraction Method[A,B]

Product	A[C]	B[C]	C[C]	D	E	G
Weight of product, g	20	20	20	10	10	10
Volume of water, mL	...	...	...	50	50	50
Weight of K_2CO_3, g	14	14	14	35	35	35
Volume of isopropanol 1st extraction, mL	30	30	30	50	75	50
Volume of isopropanol 2nd extraction, mL	...	...	...	...	...	...
Calculated weight of LAS, g	2.02	2.01	2.00	1.93	2.24	1.98
Weight of LAS recovered, g	1.83	1.81	1.83	1.91	2.35	2.15
LAS recovered, %	90.5	90.1	91.4	98.8	105.2	108.4

[A] OECD Environment Directorate, Proposed Method for the Determination of the Biodegradability of Surfactants Used in Synthetic Detergents, Paris 1976, Section 5.1.2, pp. 20–23.
[B] Results obtained with single extraction with IPA. Testing Laboratory—Unilever Research Port Sunlight Laboratory, Merseyside, United Kingdom.
[C] Liquid product.

TABLE X1.3 Extraction of LAS from Formulated Products—OECD Isopropanol Extraction Method[A,B]

Product	A[C]	B	C	D
Weight of product, g	350	300	200	200
Volume of water, mL	...	1500	1000	1000
Weight of K_2CO_3, g	315	1050	700	700
Volume of isopropanol 1st extraction, mL	525	2250	1500	1500
Volume of isopropanol 2nd extraction, mL	175	750	500	500
Calculated weight of LAS, g	35.2	36.3	44.8	39.7
Weight of LAS recovered, g	35.2	34.7	44.5	38.9
LAS recovered, %	100.0	95.6	99.3	98.0

[A] OECD Environment Directorate, Proposed Method for the Determination of the Biodegradability of Surfactants Used in Synthetic Detergents, Paris 1976, Section 5.1.2, pp. 20–23.
[B] Results obtained with two extractions with IPA. Testing Laboratory—Unilever Research Port Sunlight Laboratory, Merseyside, United Kingdom.
[C] Liquid product.

X2. BIODEGRADATION AND ANALYTICAL DATA ON EPA LAS REFERENCE SOLUTION

X2.1 See for biodegradation[10] and analytical[11] data.

TABLE X2.1 Results of This Test Method (Presumptive Test—Shake Culture) on EPA LAS Reference Sample Batch 0990, Average Molecular Weight 342, % Active 6.03

Test Substance	t_o Measured Concentration, mg active/L	Removal Day 7, %	Removal Day 8, %	Removal Average, %
EPA LAS reference solution	25.46	97.3	96.3	96.8

TABLE X2.2 Results of This Test Method (Confirming Test—Semi-Continuous Activated Sludge) on EPA LAS Reference Sample Batch 0990, Average Molecular Weight 342, % Active 6.03

Test Substance	% MBAS Removed
EPA LAS reference solution	99.6

[10] Conducted by Roy F. Weston Inc. Fate and Effects Laboratory, 254 Welsh Pool Road, Lionville, PA 19341-1345.

[11] Conducted by Industrial Testing Laboratories, Inc., 2350 S. Seventh St., St. Louis, MO 63104-4296.

TABLE X2.3 Analytical Results

RFW No.:	9403F140-009
Sample description:	EPA Standard (0990)
ITL Laboratory No.:	244088
Collection date:	March 8, 1994
Date received (laboratory):	March 9, 1994
Sample matrix:	analytical standard
Preparation (desulfonation) date:	March 15, 1994
Analysis date:	May 13, 1994
Sample weight/volume:	1.7196 g
Extract volume:	100 mL
Dilution factor:	1
Aliquot volume for desulfonation:	1 mL
Final extract volume:	0.5 mL
Injection volume:	1 µL
Total organics (1):	N.A.
Sulfur trioxide, before hydrolysis (2):	1.46
Sulfur trioxide, after hydrolysis (2):	1.46
Active content (3):	6.19
Isomer Distribution:	
C_{10}, %	15.68
C_{11}, %	38.62
C_{12}, %	38.81
C_{13}, %	6.89
C_{14}, %	0.00
Average chain length:	11.37
Average molecular weight:	339.2

TABLE X2.3 *Continued*

Report No: 94-03-00993 Client: Weston		
ITL Number	244088	
RFW No.	9403F140-009	
Sample description	EPA standard	
Desulfonation data	3/15/94	
Date of analysis (month/day/year)	5/13/94	
Run Number	19	
Chromatogram number	12	
Isomer Distribution, %		retention time (min)
C10-5, area	3.352	24.65
C10-4, area	3.003	25.07
C10-3, area	3.375	25.97
C10-2, area	4.937	27.76
C10-total, %	15.68	
C11-6, area	3.595	29.47
C11-5, area	8.112	29.62
C11-4, area	6.620	30.10
C11-3, area	7.594	31.10
C11-2, area	10.207	32.87
C11-total, %	38.62	
C12-6, area	7.110	34.29
C12-5, area	7.320	34.51
C12-4, area	5.796	35.07
C12-3, area	7.494	36.06
C12-2, area	8.580	37.83
C12-total, %	38.81	
C13-7,6, area	1.632	38.97
C13-5, area	1.291	39.28
C13-4, area	1.082	39.88
C13-3, area	1.407	41.02
C13-2, area	1.033	43.18
C13-total, %	6.89	
C14-7, area	0.000	
C14-6, area	0.000	
C14-5, area	0.000	
C14-4, area	0.000	
C14-3, area	0.000	
C14-2, area	0.000	
C14-total, %	0.000	
Average chain length	11.37	
Molecular weight	339.2	
Total area	93.54	

TABLE X2.3 *Continued*

Report No: 94-03-00993 Client: Weston		
ITL Number	244088-2 (duplicate)	
RFW No.	9403F140-009	
Sample description	EPA standard	
Desulfonation data	3/15/94	
Date of analysis (month/day/year)	5/13/94	
Run Number	18	
Chromatogram Number	13	
Isomer Distribution, %		retention time (min)
C10-5, area	3.197	24.65
C10-4, area	2.873	25.08
C10-3, area	3.191	25.98
C10-2, area	4.796	27.76
C10-total, %	14.81	
C11-6, area	3.585	29.46
C11-5, area	8.009	29.62
C11-4, area	6.614	30.10
C11-3, area	7.622	31.10
C11-2, area	10.411	32.87
C11-total, %	38.17	
C12-6, area	7.340	34.29
C12-5, area	7.474	34.50
C12-4, area	5.773	35.07
C12-3, area	7.764	36.06
C12-2, area	9.184	37.83
C12-total, %	39.53	
C13-7,6, area	1.789	38.97
C13-5, area	1.412	39.29
C13-4, area	1.202	39.89
C13-3, area	1.537	41.03
C13-2, area	1.174	43.18
C13-total, %	7.49	
C14-7, area	0.000	
C14-6, area	0.000	
C14-5, area	0.000	
C14-4, area	0.000	
C14-3, area	0.000	
C14-2, area	0.000	
C14-total, %	0.000	
Average chain length	11.40	
Molecular weight	339.6	
Total area	94.95	

X3. ANALYTICAL METHODS

X3.1 *Suspended Solids*[12]

X3.1.1 *Applicability*— This method shall be used for samples from the confirming test.

X3.1.2 *Apparatus*:

X3.1.2.1 *Aluminum Dish* with a perforated bottom, similar to a Büchner funnel, with an inside diameter of 92 mm and a height of 25 mm.

X3.1.2.2 *Filter Paper*, 90-mm diameter, rapid, qualitative.

X3.1.2.3 *Sponge Rubber Ring*, 93-mm outside diameter, 75-mm inside diameter, approximately 3 mm thick.

X3.1.2.4 *Büchner Funnel*, No. 2A, inside diameter at bottom 93 mm.

X3.1.2.5 *Filter Flask*, 1-L size with side tube.

X3.1.3 *Procedure*—Fit the filter paper in the aluminum dish and dry both in an oven at 103 to 105°C. Cool in a desiccator and weigh. Wet the filter paper. Place the dish on the rubber ring in the Büchner funnel and apply about 51 cm (20 in.) Hg of vacuum to the flask. Immediately add to thedish 20 to 100 mL of sample, which should yield 0.1 to 0.4 g of dry solids. After the water has been extracted, dry the dish and contents for about 30 min at 103 to 105°C. Cool in desiccator and weigh.

X3.1.4 *Calculation*:

$$\text{Suspended solids, mg/L} = [(W_1 - W_0)/\text{ mL of sample}] \times 1000 \quad \text{(X3.1)}$$

where:

W_1 = dry weight of dish and contents after filtration, and
W_0 = dry weight of dish with filter paper.

X3.1.5 *Standard Deviation*—0.6 mg on a 100-g sample.

X3.2 *pH (Optional):*

X3.2.1 pH may be determined colorimetrically or by Test Methods D 1293, Tests for pH of Water, if greater accuracy is desired.

[12] The methods for suspended solids and sludge volume index are adapted from those given in *Standard Methods for the Examination of Water and Waste Water* (12th Ed.), published by the American Public Health Association, 1790 Broadway, New York, NY 10019.

X3.3 *Sludge Volume Index*[12] *(Optional):*

X3.3.1 *Definition—sludge volume index (SVI)*—the volume occupied by 1 g of activated sludge after settling the aerated liquor for 30 mm.

X3.3.2 *Procedure*—Take a 1-L sample from the aeration chamber; settle 30 min in a 1000-mL graduated cylinder; and read the volume occupied by the sludge in millilitres.

X3.3.3 *Calculation*:

$$\text{SVI} = \text{mL settled sludge} \times 1000/\text{mg/L of suspended solids} \quad \text{(X3.2)}$$

X4. BIODEGRADATION DATA FOR A TYPICAL LAS SAMPLE

TABLE X4.1 LAS Biodegradation in Presumptive Test[A]

Initial concentration	9.4 mg/L (MBAS)
Biodegradation (Day 7), %	93.6
	94.7
	97.9
Biodegradation (Day 8), %	96.8
	97.9
	96.8

[A] Examples are taken from supporting data available from ASTM Headquarters.

TABLE X4.2 LAS Biodegradation in Confirming Test[A]

Test Sample	Biodegradation, Mean %
LAS paste	97.0

[A] Examples are taken from supporting data available from ASTM Headquarters.