

## Standard Test Method for Determining Biodegradability of Organic Chemicals in Semi-Continuous Activated Sludge (SCAS)<sup>1</sup>

This standard is issued under the fixed designation E 1625; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

### 1. Scope

1.1 This test method covers procedures for the determination of the biodegradability or removability, or both, of nonvolatile organic chemicals (Henry's Constant  $<10^{-3}$  atm/m<sup>3</sup>/day) using a laboratory bench scale test and activated sludge from a domestic wastewater treatment plant.

1.2 This test method is derived from a procedure developed for surfactants by the Soap and Detergent Association (**1**, **2**),<sup>2</sup> one developed for alkylbenzene sulfonates by ASTM (see Test Method D 2667) and one developed by the Organization for Economic Cooperation and Development (OECD) for assessing inherent biodegradation (**3**) and also codified in the Toxic Substances Control Act Test Guidelines (**4**). For assessment of variability, replicate test systems (three or more) should be employed. It is recommended that the tests be used for chemical compounds that can be well characterized with respect to chemical and physical properties. Testing of mixtures or fully formulated products can lead to serious problems in data interpretations.

1.3 The procedures involve the exposure of the test chemical(s) to activated sludge mixed liquor microorganisms over a finite time cycle in specially designed aeration chambers. Biodegradability is determined from dissolved organic carbon (DOC) measurements, from radiochemical analyses, or from measurements of test chemical concentration using a specific analytical method. Based on DOC analyses alone, biodegradation can only be claimed if other removal mechanisms (for example, adsorption, volatility, or chemical transformation) are discounted by means of specific testing or knowledge of physical chemical properties of the test chemical. Modifications of this test method for water insoluble and moderately volatile chemicals are presented in this test method and principles are described in somewhat more detail elsewhere (see **5**, **6**).

1.4 These procedures may also be used as a means of acclimating microorganisms to an organic chemical over an extended period. The acclimated microorganisms may be used as an inoculum source for other biodegradation tests.

1.5 *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.* For specific hazard statements see Section 7 and in Note 1.

### 2. Referenced Documents

#### 2.1 ASTM Standards:

- D 1129 Terminology Relating to Water<sup>3</sup>
- D 1193 Specification for Reagent Water<sup>3</sup>
- D 1293 Test Methods for pH of Water<sup>3</sup>
- D 2579 Test Method for Total Carbon and Organic Carbon in Water<sup>4</sup>
- D 2667 Test Method for Biodegradability of Alkylbenzene Sulfonates<sup>5</sup>
- D 2777 Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water<sup>3</sup>
- D 4375 Terminology for Basic Statistics in Committee D-19 on Water<sup>2</sup>
- E 178 Practice for Dealing with Outlying Observations<sup>6</sup>

### 3. Summary of Test Method

3.1 Biodegradation testing of an organic chemical is carried out using a laboratory scale test using activated sludge from a domestic wastewater treatment plant.

3.2 The chemical is exposed to activated sludge mixed liquor (obtained from a local domestic sewage treatment plant) in an aerated chamber operated in a semi-continuous (fill-and-draw) basis.

3.3 The mixed liquor is dosed with the test chemical at the beginning of each cycle. The normal cycle length is 24 h, although 72-h weekend-cycle may be used. The mixed liquor is aerated continuously except for the last 30 to 60 min of the cycle. During this latter period, the aeration is suspended, the mixed liquor solids are settled and supernate removed leaving one third of the original mixed liquor volume. The test compound and synthetic or natural sewage (see section 7.7) are added to bring the volume to its original volume and the aeration is restarted.

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.06 on Environmental Fate of Chemical Substances.

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<sup>2</sup> The boldface numbers given in parentheses refer to a list of references at the end of the text.

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 11.01.

<sup>4</sup> *Annual Book of ASTM Standards*, Vol 11.02.

<sup>5</sup> *Annual Book of ASTM Standards*, Vol 15.04.

<sup>6</sup> *Annual Book of ASTM Standards*, Vol 14.02.

3.4 The extent of biodegradation of a non-sorbing, water-soluble test chemical or chemical mixture is determined by comparison of DOC concentration of the influent feed or dosing solution and the effluents from the test and control units.

3.5 For test chemicals that are hydrophobic and sorbed to the bacterial floc, the extent of biodegradation may be determined from analysis of mixed liquor samples (activated sludge plus supernate) taken at the beginning and end of a cycle. Solvent-extraction and concentration steps are carried out prior to the specific chemical analysis. The tendency of a chemical to adsorb to the activated sludge can also be determined by measuring the DOC removal at 5 to 15 min into the 24-h cycle on the first day.

3.6 For highly polar organic chemicals that are sorbed to the bacterial floc, the use of a radiolabeled material or an alternate analytical method may be necessary to establish biodegradability.

3.7 The duration of a SCAS test is typically three months, but may range from a few weeks to twelve months depending on the time required for acclimation and achievement of steady-state conditions, inhibitory effects, analytical requirements, and the use of the mixed liquor as an inoculum source for other biodegradation tests.

#### 4. Significance and Use

4.1 Secondary wastewater treatment using activated sludge is one of the most important biological treatment processes in use today. The semi-continuous activated sludge (SCAS) test employs activated sludge from a domestic activated sludge plant to assess biodegradation of organic compounds.

4.2 The SCAS system provides a high potential for biodegradation because of the high biomass to chemical substrate ratio, the regular reinoculation with a variety of microorganisms from the natural sewage, the possibility of co-metabolism because of the variety of organic substrates present in sewage or synthetic feed, the opportunity for slow-growing microorganisms to be retained due to the high sludge age, and a long hydraulic retention time to increase selection pressure.

#### 5. Apparatus

5.1 *Borosilicate Glass Aeration Chambers*—Two types are shown in Figs. 1 and 2. Miniaturized versions, such as the 270-mL units shown in Fig. 3, have also been used successfully and are acceptable.

5.2 *Magnetic Stirrer*,<sup>7</sup> for use with 1500-mL chamber shown in Fig. 1.

5.3 *Flowmeter*, suitable for 0.1 to 2.0-ft<sup>3</sup>/h airflow.

5.4 *Manifold*, for filtered compressed air.

5.5 *Refrigeration*, for storage of sewage or synthetic feed.

5.6 *Gooch Number 04 Crucibles and Glass Microfibre Filters*,<sup>8</sup> for suspended solids determinations.

5.7 *pH Meter*.

5.8 *Disposable Syringes*, or glass syringes for hydrophobic chemicals—60 mL for use with 1500-mL SCAS units and

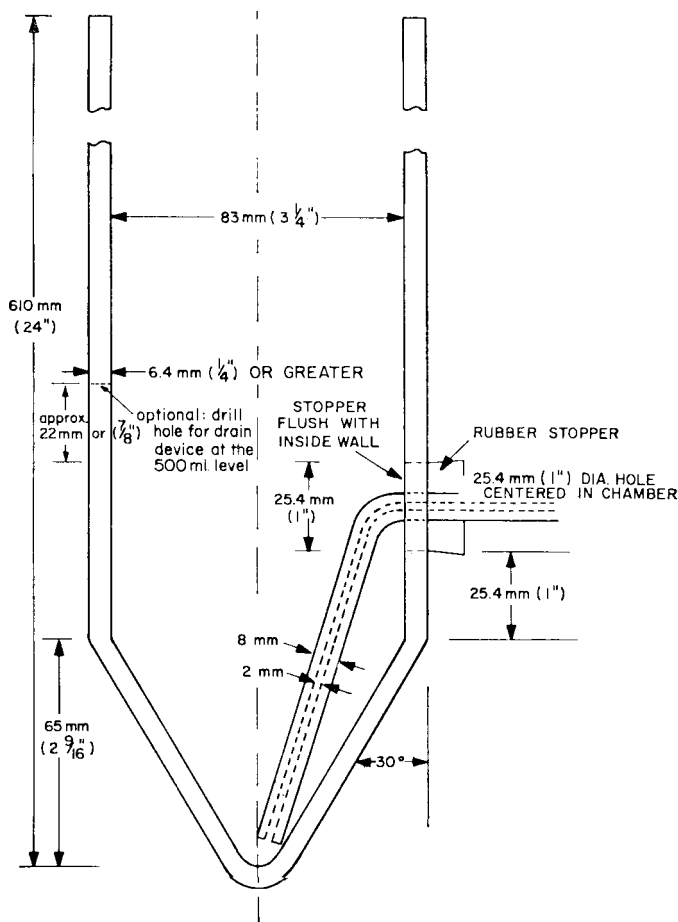


FIG. 1 1500-mL SCAS Unit Original Soap and Detergent Design

smaller sizes for miniature SCAS units.

5.9 *Syringe Filters*, 1.2 µm.

5.10 *Total Organic Carbon (TOC) Analyzer*,<sup>9</sup> with sensitivity in the 0 to 25-mg/L range.

#### 6. Reagents and Materials

6.1 *Activated Sludge Mixed Liquor*, collected from aeration basin or oxidation ditch of domestic wastewater treatment plant (DWTP).

6.2 *Natural Sewage Feed*—Primary effluent from domestic wastewater treatment plant, (sewage should contain at least 50 mg/L DOC). Supplementation with the synthetic sewage stock (see 6.3) to achieve 150 to 200-mg/L DOC is recommended.

6.3 *Synthetic Sewage Stock Solution*:

Glucose	130 g
Nutrient broth	130 g
Beef extract	130 g
Dipotassium hydrogen phosphate	130 g
Ammonium sulfate	25 g
Tap water	1 L

Dissolve by heating to just below the boiling point and store in the refrigerator below 7°C. Discard if any visual evidence of

<sup>7</sup> Available from Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219-4785, Fisher Catalog No. 14-511-1A, or equivalent, has been found suitable for this purpose.

<sup>8</sup> Available from Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219-4785, Whatman 934-AH2.1 cm, or equivalent, has been found suitable for this purpose.

<sup>9</sup> Available from OI Corporation Model 700, P.O. Box 2980 College Station, TX 77841-2980, or equivalent, has been found suitable for this purpose.

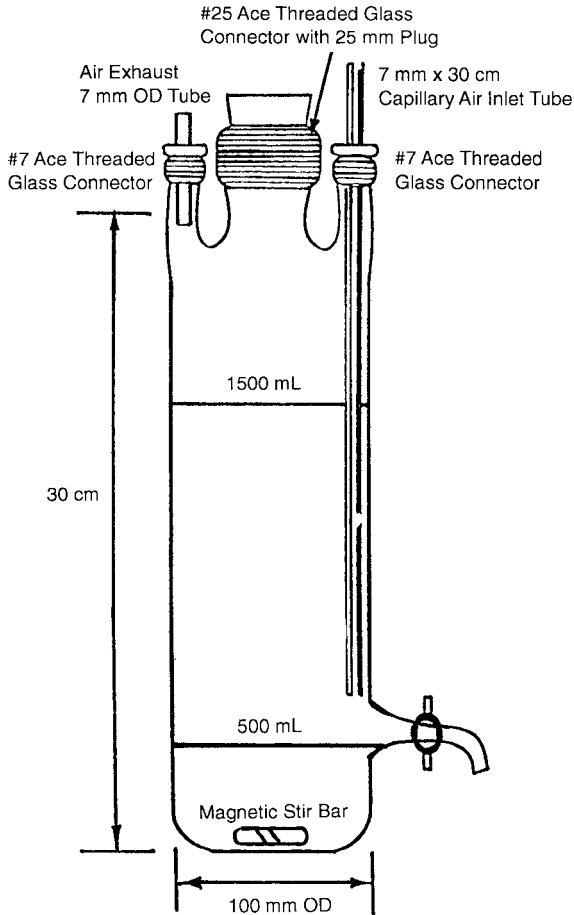


FIG. 2 1500-mL SCAS Unit Alternative Design

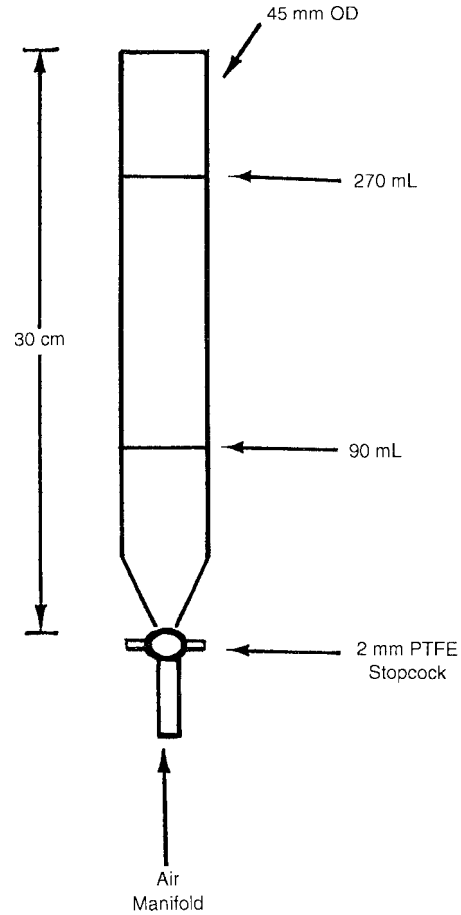


FIG. 3 270-mL SCAS Unit Miniature Design

biological growth (turbidity) is observed. One millilitre of this stock is added to each litre of tap water to form the synthetic sewage. Other synthetic sewages, (see Refs 1-4), may be employed.

6.4 *Compressed Air*, for aeration of SCAS chambers.

6.5 *Test and Reference Chemicals of Known Carbon Content*, (for DOC analyses) or composition (for specific analyses).

6.6 *Extraction Apparatus and Solvent*, for hydrophobic test chemicals.

**7. Safety Precautions**

7.1 This test method involves the use of mixed liquor and natural sewage from a domestic wastewater treatment plant. Consequently, individuals performing this test method may be exposed to microbiological agents that are dangerous to human health. It is recommended that SCAS units be operated in a separate room and ventilated to building exhaust air. Glass apparatus should be sterilized after use.

7.2 Those that work with the sewage organisms may opt to keep current with pertinent immunizations such as typhoid, polio, Hepatitis B, and tetanus.

7.3 Effluents from the SCAS units are treated with a chemical disinfectant (chlorine bleach—5 %) or autoclaved prior to disposal.

**8. Sampling and Analytical Procedure**

8.1 *Dissolved Organic Carbon (DOC) Analysis:*

8.1.1 DOC analysis for monitoring the SCAS test is generally employed only for test chemicals whose water solubility exceeds the test concentration, for example, concentration equivalent to 10 mg C/L.

8.1.2 Since precipitation as salts or sorption on the sludge floc may occur for water-soluble test chemicals, DOC removal does not in all cases indicate biodegradation. DOC analysis of mixed liquor supernate sampled 5 to 15 min after addition of test chemical provides an indication whether removal may be due to sorption or precipitation.

8.1.3 Carry out DOC analyses on supernate samples removed at the end of each cycle from the test chemical unit and a control unit that received no test chemical. Filter samples (about 12 mL) into 16 by 100-mm autosampler tubes using disposable syringes with either 0.2 or 0.45- $\mu$ m filters.

NOTE 1—**Precaution:** An aliquot of the dosing solution should be evaluated for adsorption of test chemical to the filter or elution of DOC from the filter itself.

8.1.4 Determine the DOC concentration of aqueous samples with a suitable organic carbon analyzer (for example, OI Corporation Model 700 TOC Analyzer<sup>9</sup> equipped with an autosampler.

8.2 *Specific Chemical Analysis:*

8.2.1 Specific chemical analysis may also be utilized for test chemicals that remain dissolved in the aqueous phase, but are required for chemicals that are sorbed to the activated sludge floc if the removal process is to be quantified.

8.2.2 Unlike soluble test chemicals whose steady-state concentration cannot exceed the initial level due to daily draining of the supernate, floc-associated test chemicals may build up indefinitely. A steady-state condition in which addition is equivalent to losses by all processes may take a very long time to achieve. Loss processes may include biodegradation, chemical degradation, and volatilization. In order to determine the extent of removal during a cycle, samples of mixed liquor must be analyzed at the beginning and end of a cycle. Because mixed liquor sampling causes depletion of solids, the 1500-mL SCAS units are normally employed in such tests.

8.2.3 Take samples of mixed liquor, for example, 10 to 50 mL, 5 to 15 min after dosing and prior to maintenance at the end of the cycle with either a graduated cylinder using the side port in the SCAS unit or a disposable pipet through the top of the unit. In order to minimize sludge depletion and analytical cost, analysis is not carried out for every cycle. For 50-mL samples, analysis is generally done on a one cycle per week basis.

8.2.4 For chemicals that partition to mixed liquor suspended solids (MLSS), the extent of adsorption can be estimated on the first day of test material introduction by sampling SCAS MLSS just before test compound addition and then again 15 min afterward. The “before” and “after” samples are centrifuged at  $2000 \times g$  for 15 min and the supernatants are analyzed for DOC or test material. The difference between the two values provides a measure of the extent of adsorption of test material to MLSS. Comparison of the 15-min value to the 24-h value also may provide supporting evidence that the 15-min value was due to adsorption and not very rapid biodegradation.

8.2.5 For non-polar hydrophobic test chemicals, the chemical is usually isolated from the sludge matrix by use of an immiscible extraction solvent, for example, methylene chloride or hexane. The extract is dried, concentrated, and analyzed by an appropriate instrumental method, for example, gas chromatography (GC), high performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS), or ultra violet (UV)/visible spectroscopy.

8.2.6 The SCAS test is not recommended for volatile test chemicals (Henry’s constant  $>10^{-3}$  atm/m<sup>3</sup>/day); however, it has been used for chemicals that are not completely volatilized between additions to the SCAS system (5, 7). For chemicals of moderate volatility, volatilization losses during a cycle may be evaluated by scrubbing of aeration off-gases through a solvent train (usually three consecutive traps containing acetone, methylene chloride, or hexane) or polymeric trap (for example, Tenax<sup>10</sup> or SEP-PAK<sup>11</sup>). Specific chemical analysis of each solvent trap or polymeric trap is then carried out.

8.2.7 Highly polar non-extractable test chemicals that are associated with the mixed liquor solids require specialized testing and analytical procedures that cannot be fully documented in this test method, for example, use of <sup>14</sup>C-labeled materials and special apparatus. However, the basic SCAS

operating system can be employed if appropriate mass balances can be obtained.

## 9. Procedure

9.1 The activated sludge mixed liquor suspended solids (MLSS) are generally obtained from the treatment plant several weeks prior to the start of the SCAS test. Source of MLSS is either the aeration basin or return stream from the secondary clarifier.

9.2 Filter MLSS through a 20-mesh stainless steel screen to remove extraneous particulate matter and concentrated by settling and decanting of supernate to a suspended solids content of 4000 to 6000 mg/L. This concentration is typically found in the activated sludge return line from the secondary clarifier.

9.3 Mixed liquor suspended solids (MLSS) from 9.1 (4000 to 6000 mg/L) are then charged to a 10-L reservoir where they are aerated prior to test initiation. Maintain the reservoir in the same manner as the smaller SCAS test chambers using 24 and 72-h weekend time cycles. At the end of the time-cycle, aeration is halted, solids are allowed to settle, and supernate drained to one third of the original mixed liquor suspended solids volume. The reservoir is then charged with natural or synthetic sewage to the original volume and aeration, at a rate to maintain vigorous agitation of MLSS, is resumed. Determine stabilization of the activated sludge by monitoring DOC removal until steady-state removal is achieved.

9.4 When natural sewage (obtained either before or after primary clarification) is used to maintain the reservoir and SCAS units, it is obtained generally on a weekly basis from the DWTP. Filter the sewage through glass wool to remove large particulates and stored in a refrigerator at 5°C until use.

9.5 The SCAS units are charged with MLSS from the reservoir generally one week prior to the start of a test.

9.5.1 The MLSS of the reservoir is determined on a weekly basis using a 5-mL aliquot of the mixed liquor. Collect solids on a weighed glass-fiber filter contained in a Gooch crucible and dry in an oven at 105°C.

9.5.2 Add sufficient MLSS to each SCAS unit so that the MLSS level is maintained in the 2000 to 3000-mg/L range. Use synthetic or natural sewage for dilution, if necessary.

9.5.3 Check the MLSS of the individual SCAS units using a single 10 to 20-mL mixed liquor aliquot prior to initial dosing and on a once per week basis thereafter. The nutrient level of the natural sewage should also be checked on a weekly basis. If the MLSS level should fall below 2000 mg/L, the addition of non-acclimated MLSS from the reservoir may be advisable if the study is to continue for any extended period of time. If MLSS concentrations builds to >4000 mg/L, remove activated sludge to lower the concentration to the 2000 to 3000-mg/L range.

9.6 Connect the SCAS units to a compressed air manifold and aerate each unit at a flow rate sufficient to maintain dissolved oxygen (DO) levels of at least 2 mg/L (about 0.3 mL/min/mL liquid). Reduced flow may be necessary for volatile chemicals. Removal by way of volatility or adsorption, or both, can also be assessed in a separate SCAS unit metabolically poisoned, for example, with 1 % mercuric chloride. Additional mixing for the 1500-mL SCAS units can be

<sup>10</sup> Tenax®, available from Alltech Association Inc., 2051 Waukegan Road, Deerfield, IL 60015-1899, has been found suitable for this purpose.

<sup>11</sup> SEP-PAK®, available from Water Chemical Products Department, Milford, MA 01757, has been found suitable for this purpose.

provided by magnetic stirring as shown in Fig. 2. Maintain temperature at  $22 \pm 3^\circ\text{C}$ .

9.7 The basic time cycle used in this test is 24 h (optional 72-h cycle on weekends is permitted).

9.7.1 At the beginning of each cycle, stop aeration or stirring, or both, (if used) and allow the sludge solids to settle until they are less than one third of the liquid volume, for example, 500-mL for 1500-mL units. Settling time is usually 30 min to 1 h.

9.7.2 Remove aqueous supernate amounting to two thirds of the liquid volume, for example, 1000 mL for 1500- mL units, from the unit by means of the stopcock or by application of vacuum. Then resume aeration and stirring. Then fill units with fresh sewage (natural or synthetic) feed. Retain a sample of sewage feed for DOC and pH analysis. Retain aqueous supernate samples and analyze for dissolved DOC and pH. If desired, also determine the concentration of test chemical in the supernatant or on the sludge by means of specific analytical procedures previously developed.

9.7.3 Then dose the test chemical to the unit as an aqueous or nonaqueous (for water-insoluble chemicals) stock solution.

9.7.3.1 For water-soluble test chemicals, the standard dose level is a concentration equivalent to 10 mg carbon/L (C/L) based on the volume of wastewater replaced. The normal dosing volume is 5 mL equating to stock solution concentrations of 2 mg C/mL (1500-mL units). An incremental buildup to 10 mg C, for example, Day 0 = 2 mg; Day 1 = 4 mg; Day 2 = 6 mg; Day 3 = 8 mg; Day 4 = 10 mg, may be employed if inhibitory effects are anticipated. This approach, however, may cause difficulties in determination of adsorption. At the end of the 24 or 72-h cycle, repeat the maintenance operations. It is also acceptable to test at higher test chemical concentrations if they are not inhibitory to the activated sludge.

9.7.3.2 For water-insoluble hydrophobic chemicals, lower test chemical levels are normally employed, for example, 1 to 3 mg/cycle and specific analytical procedures to measure the concentration of chemical bound to sludge are required. Hydrophobic chemicals are generally sorbed to the mixed liquor solids and, if not biodegradable may not be removed to any significant degree when supernate is drained. Consequently, with repetitive additions of test chemical, there will be buildup in the concentration of the chemical on the solids until a steady state is achieved, that is, amount degraded or removed during a cycle equals the input level. If degradation or removal is complete during a cycle, no buildup occurs. If initial data indicate higher test chemical levels can be tolerated, testing at higher levels may be subsequently carried out. Water-insoluble chemicals are generally added in 200  $\mu\text{L}$  of acetone stock solution, for example, 1 mg/200  $\mu\text{L}$ . If acetone solubility is inadequate, other solvents such as methylene chloride may be employed.

9.8 The duration of the SCAS test is dependent on a number of factors, for example, the time required for acclimation or achieving steady-state conditions, number of concentrations, consistency of removal data, inhibitory or toxic effects, depletion of sludge solids due to sampling, and use of mixed liquor as inoculum for other tests.

9.9 In order to prevent accumulation of solids on the walls of the units, periodic cleaning with a scraper or brush should be done just after feeding. Use separate brushes for each unit to prevent cross contamination. Do not scrape the walls during the last 8 h of the cycle.

## 10. Calculation

### 10.1 Dissolved Organic Carbon (DOC):

10.1.1 Calculate the percent DOC loss or removal for the test chemical during a cycle from the following equation:

$$\% \text{ DOC removal} = 100 - \frac{(\text{EffDOC}_{TC} - \text{EffDOC}_{CNT})}{\text{concentration}_{TC}} \times 100 \quad (1)$$

where:

$\text{EffDOC}_{TC}$  = dissolved organic carbon (mg/L) in effluent from test chemical SCAS unit,

$\text{EffDOC}_{CNT}$  = dissolved organic carbon (mg/L) in effluent from control SCAS unit, and

$\text{Concentration}_{TC}$  = mg test chemical organic carbon added to SCAS unit per litre of wastewater influent.

10.1.2  $\text{Concentration}_{TC}$  can be calculated from the following equation:

$$\text{Concentration}_{TC} = \frac{\text{concentration stock concentration, mg/mL} \times \text{volume, mL} \times \% \text{C}/100}{\text{influent wastewater volume, mL}/1000} \quad (2)$$

where:

$\% \text{C}$  = percent carbon content of test chemical calculated from DOC analysis of stock dilution (see Note 2), and

$\text{influent wastewater volume}$  = volume of natural sewage added to SCAS unit at beginning of cycle, for example, 180 or 1000 mL.

NOTE 2—It is highly advisable to determine the DOC of the test chemical solution.

### 10.2 Specific Chemical Analysis:

10.2.1 Calculate the percent loss or removal for the test chemical during a cycle from the following equation:

$$\% \text{ removal} = \frac{C_o - C_n}{C_o} \times 100 \quad (3)$$

where:

$C_o$  = concentration of test chemical after addition, mg/L, and

$C_n$  = concentration of test chemical at end of cycle, mg/L.

NOTE 3—If control unit has a background concentration of test chemical, this value should be subtracted from  $C_n$ .



## 11. Interpretation

11.1 Organic chemicals are considered ultimately biodegradable if DOC removal equals or exceeds 70 % and inherently biodegradable if DOC removal equals or exceeds 20 % (6). As previously indicated, because of the tendency of some chemicals to partition to activated sludge, one must be careful to distinguish biodegradation from removal by means of adsorption, volatility, or chemical transformation (hydrolysis, oxidation, etc.). However, the 70 % value has been challenged and a more thorough discussion is presented by Painter (7). Depending on molecular structure, DOC removal values of up to 95 % have been suggested.

11.2 Information on the toxicity of the test chemical or potential toxic transformation products to activated sludge macroorganisms may be useful to the interpretation of low biodegradation results and in selection of appropriate test chemical concentrations. Indications of toxicity can be gained from observations of significant elevation of effluent DOC over the control SCAS unit or from standard tests such as the Organization for Economic Cooperation and Development (OECD) 209 respiration inhibition test.

11.3 Information on the physical-chemical properties of the test chemical may be useful for interpretation of results and in the selection of appropriate test chemical concentrations. These properties include molecular weight, vapor pressure, octanol-water partition coefficient, adsorption isotherm, surface tension, water solubility, and Henry's constant. Knowledge of hydrolytic or oxidative properties of the test chemical is also important for differentiation of biodegradation from chemical transformation processes.

11.4 Use of synthetic versus natural sewage is an important consideration. Synthetic sewage was thought to lead to more reproducible results; however, the microbial population that develops differs from that which is present in domestic activated sludge plants. Generally, the most rapidly growing microorganisms evolve and the more slowly growing populations that are present in domestic activated sludge plants decline. Natural domestic sewage varies from source to source and in nutrient content. However, it provides the natural nutrients that support the natural microbial population and a semi-continuous supply of fresh microorganisms to the test system. DOC values in many plants, however, can be as low as 20 ppm and may be insufficient to maintain the 2000 to 3000 mg/L biomass required for the test. A blend made by supplementation of natural sewage with synthetic sewage to achieve a DOC level in the 150 to 200 mg/L and a 100:12:2 ratio of C:N:P may be desirable.

11.5 Reference chemicals may also be useful in monitoring the performance of the activated sludge and in comparing results between laboratories. While specific reference chemicals cannot be recommended, data are available for several chemicals. Table 1 summarizes data from intralaboratory tests

**TABLE 1 Results from SCAS Tests on Various Chemicals Used in the OECD/EEC Ring Test (5)<sup>A</sup>**

Test Chemical	% Biodegradation or Bioelimination
4-Acetylamino benzene sulfonate	85
Tetrapropylene benzene sulfonate	51
4-Nitrophenol	95
Diethylene glycol	99
Aniline	96
Cyclopentane tetracarboxylate	81

<sup>A</sup> Duration of test was 40 days, except 120 days for cyclopentane tetracarboxylate.

so that calibration may be determined from time to time. Other reference chemicals may also be appropriate, especially if there are historical data.

## 12. Quality Assurance

12.1 To ensure the integrity of data developed using this test method and to comply with current regulatory requirements, a quality assurance program meeting EPA, FDA, or OECD guidelines should be followed. This may require replicates (three or more) to be run for Good Laboratory Practice (GLP) compliance and assessment of variability.

## 13. Report

13.1 A protocol giving a general overview of the study goals and procedures must be prepared before the study is initiated. If a substantive modification of this test method is deemed necessary for the test chemical, deviation from this test method should be documented in the protocol.

13.2 Document final results of this study in a final report. Report the following information in the final report:

13.2.1 Names of study, investigator(s), and laboratory,

13.2.2 A brief description of the test material including its log number, chemical name(s), composition, and other appropriate parameters,

13.2.3 Summary of test method including deviations from the written method,

13.2.4 Summary of specific analytical methods, if employed,

13.2.5 Tabular and graphical presentation of DOC removal data (if determined) as a function of time after test initiation. Data are expressed as % DOC removal (weekly mean for 24-h cycles and individual values for 72-h weekend cycles),

13.2.6 Tabular and graphical presentation of specific chemical analysis data (if determined) as a function of time (cycle number) after test initiation. Data are expressed as concentration of test chemical at beginning and end of the cycle and percent removal or primary biodegradation during the cycle, and

13.2.7 A listing of relevant references including all notebook pages containing raw data from this study.

## REFERENCES

- (1) Soap and Detergent Association Subcommittee on Biodegradation Test Methods, "A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate," *Journal of the American Oil Chemists' Society*, Vol 42: 1965, pp. 986–993.
- (2) Soap and Detergent Association Subcommittee on Biodegradation Testing, "The Status of Biodegradability Testing of Nonionic Surfactants," *Journal of the American Oil Chemists' Society*, Vol 46: 1969, pp. 432–438.
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- (4) Environmental Protection Agency, *Toxic Substances Control Act Test Guidelines; Final Rules*, 40 CFR Parts 796, 797, and 798. Federal Register Vol 50, No. 188, Sept. 27, 1985, pp. 39506–8.
- (5) Environmental Protection Agency, *Inherent Biodegradability: Modified SCAS Test for Chemical Substances That are Water Insoluble or Water Insoluble and Volatile*, 40 CFR (7-1-90 ed.) Subpart B, Provisional Chemical Fate Guidelines, 1990, pp. 46–49.
- (6) Organization for Economic Cooperation and Development (OECD), Chemicals Testing Programme, Degradation/Accumulation Group, Ring Test Programme 1978/79, *Detecting Degradable Chemicals in Water*, GSF, MBH Munich.
- (7) Painter, H. A., *Detailed Review Paper on Biodegradability Testing*, Manuscript prepared for the OECD Guidelines Programme.

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