Designation: E1625 – 94(Reapproved 2008)

# Standard Test Method for Determining Biodegradability of Organic Chemicals in Semi-Continuous Activated Sludge (SCAS)[1]

This standard is issued under the fixed designation E1625; 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 covers procedures for the determination of the biodegradability or removability, or both, of nonvolatile organic chemicals (Henry's Constant $<10^{-3}$ atm/$m^3$/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**),[2] one developed for alkylbenzene sulfonates by ASTM (see Test Method D2667) 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:*[3]

D2667 Test Method for Biodegradability of Alkylbenzene Sulfonates

## 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, Safety Precautions) are added to bring the volume to its original volume and the aeration is restarted.

[1] This test method is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.04 on Environmental Fate and Transport of Biologicals and Chemicals.

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[2] The boldface numbers given in parentheses refer to a list of references at the end of the text.

[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.

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,* for use with 1500-mL chamber shown in Fig. 1.

5.3 *Flowmeter,* suitable for 0.1 to 2.0-$ft^3$/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,* 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 smaller sizes for miniature SCAS units.

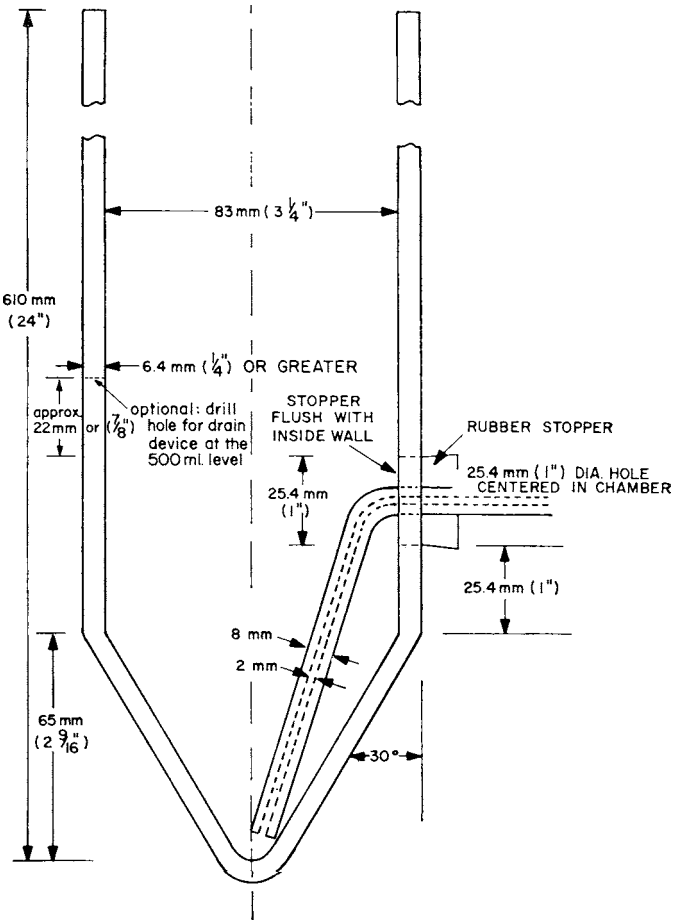


**FIG. 1 1500-mL SCAS Unit Original Soap and Detergent Design**

5.9 *Syringe Filters,* 1.2 µm.

5.10 *Total Organic Carbon (TOC) Analyzer,* 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 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.

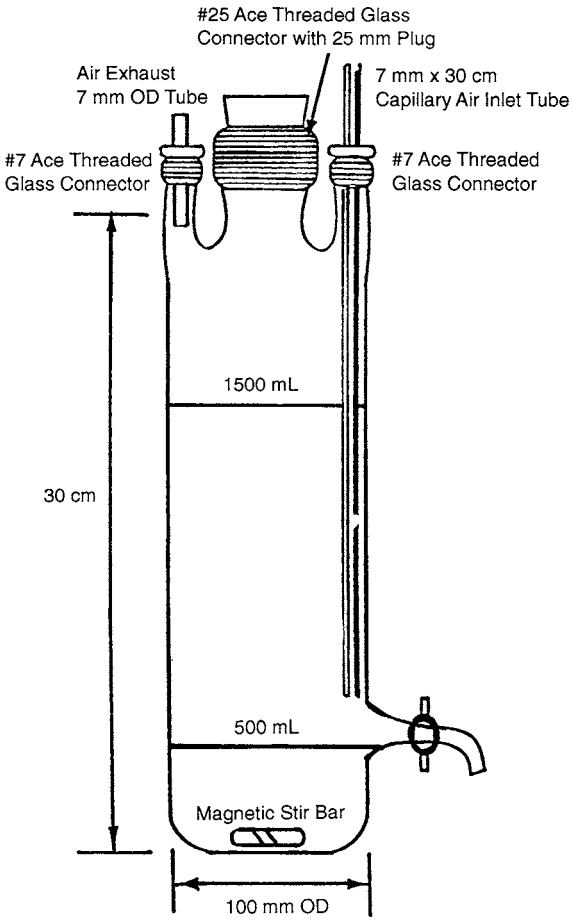


**FIG. 2 1500-mL SCAS Unit Alternative Design**

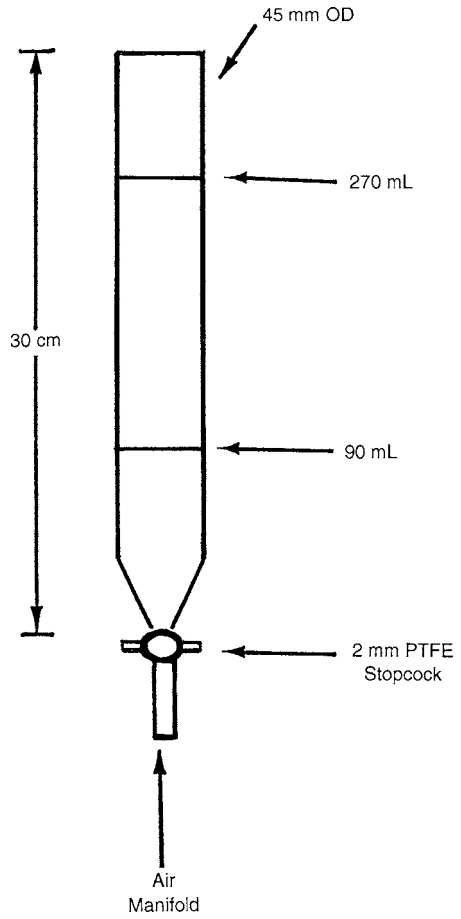


**FIG. 3 270-mL SCAS Unit Miniature Design**

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-µm 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.

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.