Designation: E 1798 – 96 (Reapproved 2008)

Standard Test Method for Assessing Treatability or Biodegradability, or Both, of Organic Chemicals in Porous Pots[1]

This standard is issued under the fixed designation E 1798; 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 simulating the activated sludge sewage treatment process and therefore gives a measure of the extent of biodegradation or removal likely to occur during sewage treatment.

1.2 Assessment of treatability or biodegradability, or both, of water soluble organic compounds in the porous pot test requires dissolved organic carbon (DOC) measurements or specific chemical analysis.

1.2.1 Dissolved organic carbon (DOC) measurements, relative to the controls, can be used to calculate the removal of the test chemical or water soluble residues by the porous pot treatment (see 12.3). The DOC measurements do not identify water soluble chemicals. Specific chemical analysis, on the other hand, can be used to identify and quantify the parent test chemical or (if standards are available) any water soluble residues formed by the porous pot treatment. A specific chemical analytical method must have a limit of detection (LOD) ≤0.1 mg/L in water or ≤0.1 mg/Kg in solids.

1.3 The feature that distinguishes this test from other activated sludge simulation tests is the retention of the activated sludge in a porous liner, that eliminates the need for a secondary clarifier and facilitates control of the critical parameter, the sludge retention time (SRT).

1.4 Porous pots can be completely sealed and tests using ^{14}C-labeled test compounds are then possible. Carbon dioxide in the exhaust gas and bicarbonate in the effluent can be used together to assess the extent of mineralization, and levels of radiolabel in the sludge and in the aqueous phase may also be determined.

1.5 By simultaneously measuring the efficiency of the pots in removing DOC, it is also possible to determine whether the test compound has any adverse effect on normal sewage treatment processes.

1.6 The SI units given in parentheses are for information only.

1.7 *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 6.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

E 178 Practice for Dealing With Outlying Observations

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *aeration chamber*—the interior volume of the porous liner or candle that holds the activated sludge.

3.1.2 *activated sludge (mixed liquor)*—a heterogeneous mixture, suspended in sewage influent, consisting of a variety of microorganisms (primarily bacteria) formed into flocculent particles, that is cultured for the purpose of removing organic substrates and certain inorganic constituents from wastewaters by metabolic uptake and growth on these substrates. Normal operating concentrations of activated sludge in aeration units range from 2000 to 5000 mg/L **(1)**.[3]

3.1.3 *biochemical oxygen demand (BOD)*—the biochemical oxygen demand, measured as the amount of oxygen used for respiration during the aerobic metabolism of an energy source by acclimated microorganisms. Carbonaceous BOD is a measure of the amount of oxygen used during the metabolism of an organic substrate and represents the amount of COD that has been oxidized biologically at any time. Nitrogenous BOD is a measure of the amount of oxygen required for the biological oxidation of inorganic nitrogen compounds (nitrification). BOD_5 is the biochemical oxygen demand after five days of incubation **(1)**.

3.1.4 *biodegradation*—destruction of chemical compounds by the biological action of living organisms **(2)**.

[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.

Current edition approved Feb. 1, 2008. Published April 2008. Originally approved in 1996. Last previous edition approved in 2001 as E 1789–96(2001).

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

3.1.5 *chemical oxygen demand (COD)*—the amount of oxygen required to oxidize the organic matter in a given sample under the best possible analytical conditions for maximum oxidation of the organic matter to carbon dioxide and water. The theoretical COD (COD_{th}) is the COD that can be calculated from a balanced equation for total oxidation of the organic matter to carbon dioxide and water; for this, the empirical formula for the organic matter must be known (**1**).

3.1.6 *effluent—as used in this standard*, treated and clarified wastewater leaving an activated sludge treatment system.

3.1.7 *hydraulic retention time (HRT)—as used in this standard*, average liquid through-put time. Mathematically equal to reactor volume/liquid flow rate.

3.1.8 *mineralization*—conversion of organic compounds in a wastewater to CO_2, H_2O and simple salts by microbiological oxidation (**1**).

3.1.9 *mixed liquor volatile suspended solids (MLVSS)—as used in this standard*, that portion of the activated sludge which is lost by ignition at 550°C for 15 min. It corresponds to the biological and organic fraction of the solids.

3.1.10 *OECD*—Organization for Economic Cooperation and Development (**3**).

3.1.11 *primary biodegradation*—oxidation or alteration of a molecule by bacterial action to such an extent that characteristic properties of the chemical are no longer evident or when it no longer responds to analytical procedures specific for detecting the original compound (**2**).

3.1.12 *primary treatment*—the removal of separable materials from wastewaters by sedimentation (**1**).

3.1.13 *secondary clarifier*—a settling tank used to separate the solids from the liquids in activated sludge mixed liquor (**1**).

3.1.14 *secondary treatment*—the removal of colloidal and soluble organic material from wastewaters. Settleable material is usually removed prior to secondary treatment. Secondary treatment processes are usually biological in nature, for example, activated sludge or trickling filtration, but may be chemical and physical in nature. The term secondary treatment is sometimes used to indicate a certain level of removal of biochemical oxygen-demanding materials (**1**).

3.1.15 *settled domestic sewage—as used in this standard*, raw domestic sewage which has been allowed to settle for at least 2 h.

3.1.16 *sludge retention time (SRT)—as used in this standard*, average time (usually measured in days) that activated sludge is retained in the aeration or treatment chamber. Mathematically equal to the total solids in the system/solids wasted per day.

3.1.17 *treatability*—removal of a compound from wastewater by a particular sewage treatment process whether by biodegradation or by some other means (**2**).

3.1.18 *ultimate biodegradation*—complete conversion of a molecule to carbon dioxide, water, inorganic salts and products associated with the normal metabolic processes of bacteria (**2**).

4. Summary of Test Method

4.1 This test method is designed to simulate the activated sludge sewage treatment process and is performed using a porous pot-type laboratory-scale activated sludge apparatus, based on an original design developed by the United Kingdom Water Research Centre (**4,5**). The original design was modified (see Fig. 1) (**6**) and has been utilized in determining the effects of temperature and growth media components, for example, phosphate, on the growth of activated sludge and the toxicity of treated effluents (**7,8**). It has also been used in the environmental safety evaluation of a new product (**9**). The modified test facilitates control of the SRT, and the effect of this fundamental parameter on the efficiency of removal of surfactants in porous pots has been described (**10**).

4.2 The test and control pots are filled with mixed liquor from an activated sludge plant treating predominately domestic sewage and then operated as continuous-flow systems with primary effluent or settled domestic sewage as background feed.

4.3 A solution or suspension of the test compound is dosed into the test pot by means of a suitable micro-metering pump. The concentration of the test compound in the influent sewage is 10 to 20 mg C/L since the practical lower detection limit of the DOC analyzer is 1 to 3 mg C/L. A lower concentration of the test compound may be used if a highly sensitive analytical method is available or if radiolabeled compound is used. The total flow to the pot (sewage + test compound dosing solution) is controlled to give the required hydraulic retention time.

4.3.1 A similar flow of sewage and a dosing solution of a suitable reference compound such as sodium benzoate are added to the control pots. Benzoate biodegrades easily and completely in this test system, and is added at such a concentration as to ensure that the total organic carbon load and the total sewage flow are the same in both control and test pots. Reference compounds may also have other uses (see 11.5).

4.4 Air is supplied to the pots through a diffuser stone to ensure adequate oxygen transfer to the mixed liquor, and an additional flow through a 5 mm open tube is provided to ensure complete mixing of the system. The air flow should be sufficient to maintain and thoroughly mix the solids in suspension and keep the concentration of dissolved oxygen above 2 mg/L at all times. In order to maintain an adequate dissolved oxygen (DO) concentration it will be necessary to maintain an air to wastewater flow ratio of 5 to 10/1 on a volume to volume basis.

4.5 Sludge is wasted directly from the aeration chamber through the base of the pot by means of a suitable peristaltic pump. To avoid problems caused by the low flow rates required, the pump is fitted with a timer and operated intermittently.

4.6 The levels of biodegradable materials remaining in the unit effluents are dependent on the SRT and the growth kinetics of those organisms that are involved in the metabolism of the compound under consideration. The test is therefore, in effect, a kinetic study and consequently should be conducted at a constant temperature. Further, by making measurements at two or more temperatures, the biodegradability of the test compound under summer and winter operating conditions may be established.

4.7 The removal of test compounds is determined by analysis of effluents and comparison of the results obtained from pots containing test compound to those from control pots

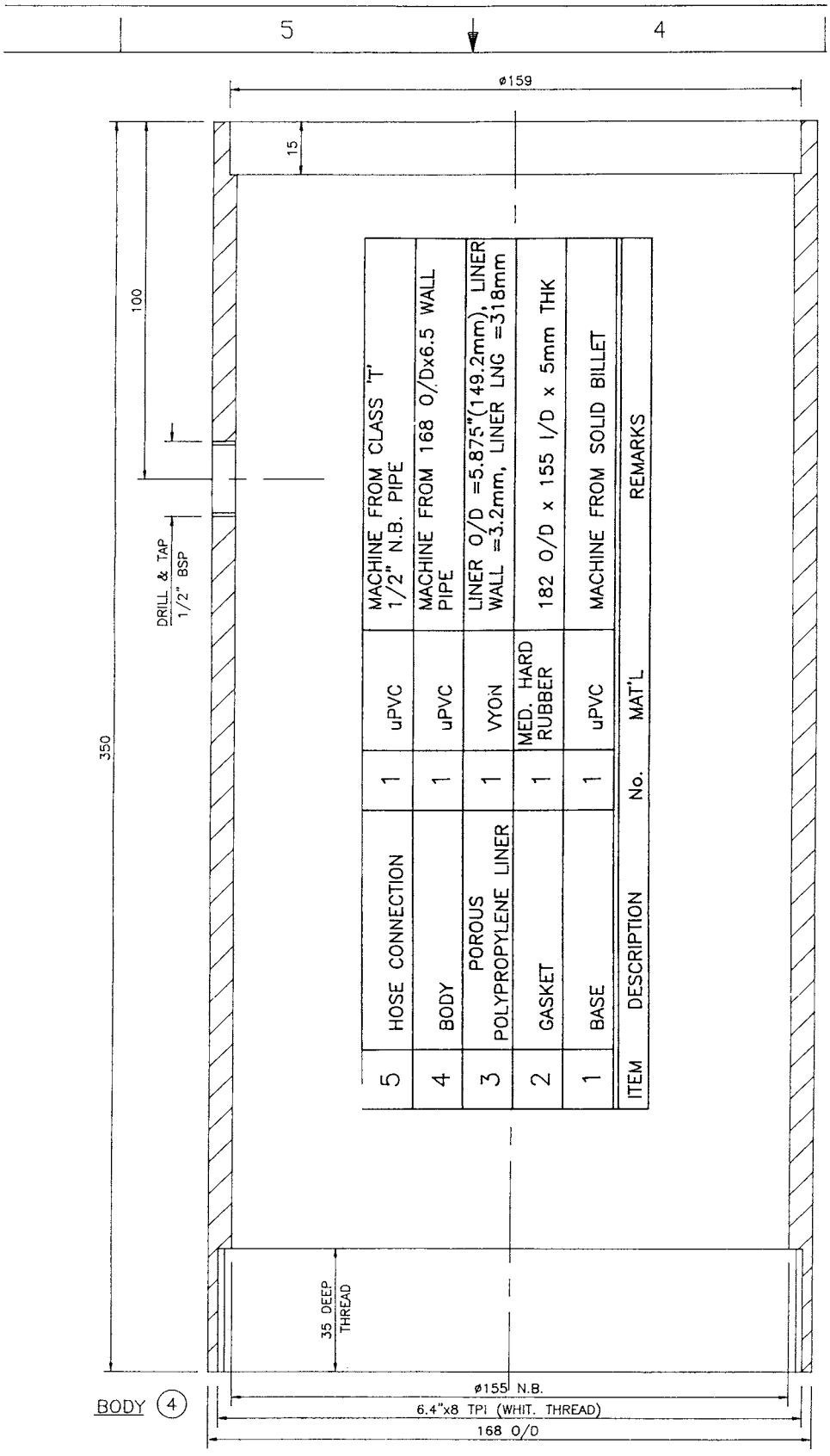

FIG. 1 Porous Pot Unit

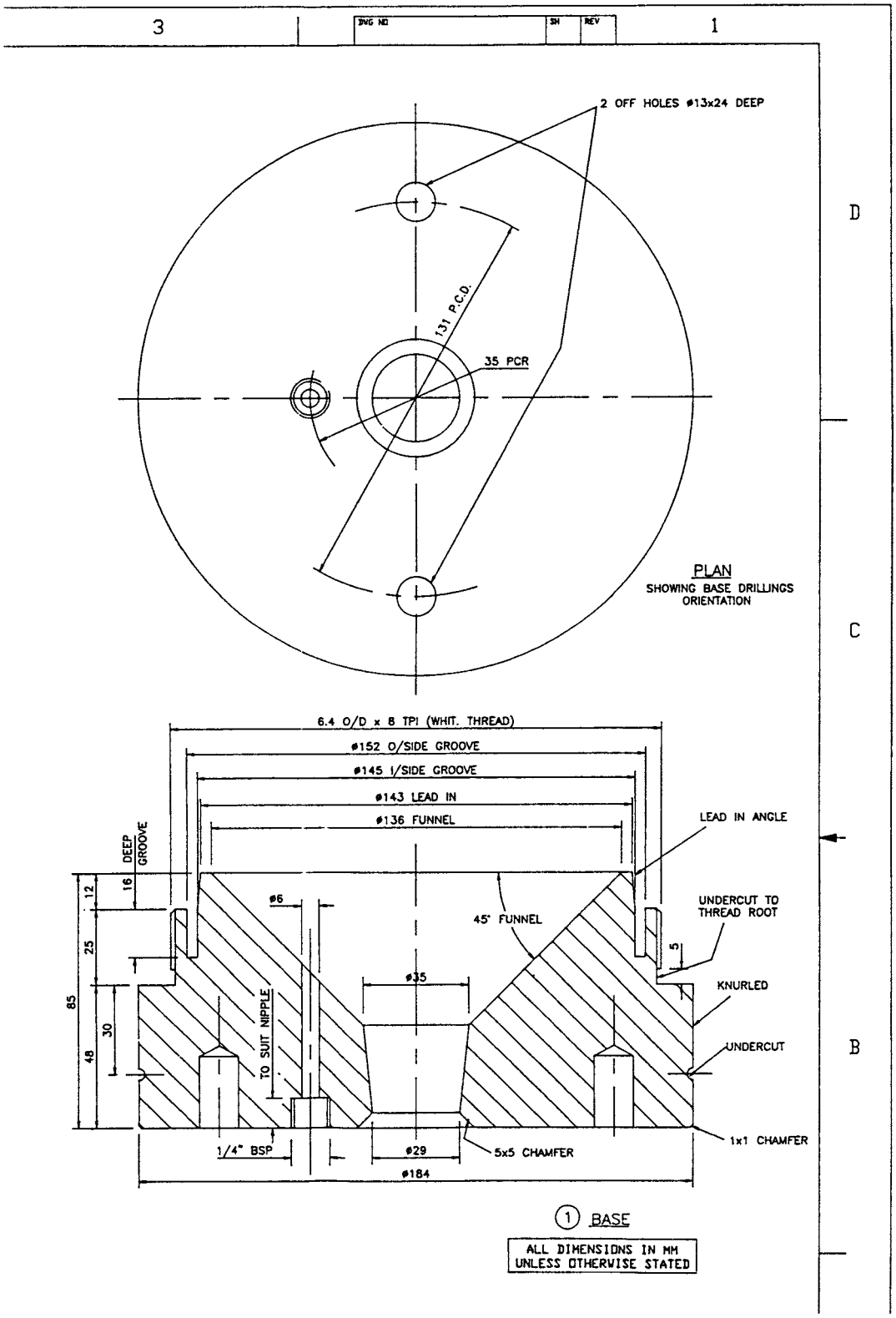

FIG. 1 *(continued)*

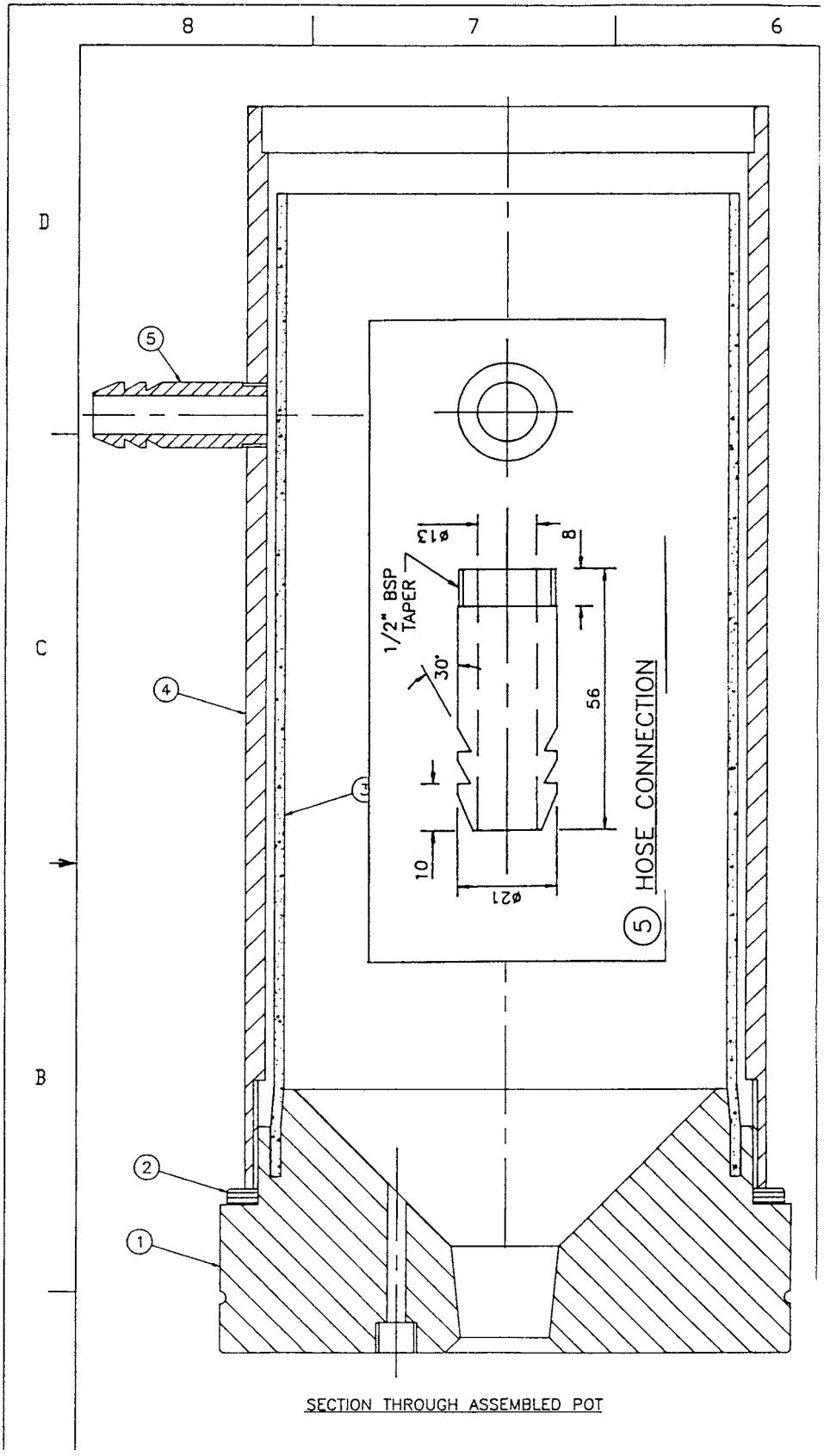

FIG. 1 *(continued)*

treating only settled sewage and benzoate. Primary biodegradation is assessed by specific analysis of the test compounds in effluents after correction for volatilization and adsorption of the parent compounds onto activated sludge. Further, analysis of DOC in effluents provides a measure of ultimate biodegradation after corrections have been applied for volatilization and adsorption of parent compounds and biodegradation intermediates onto sludge.

4.7.1 For materials that are insoluble or are absorbed or precipitated onto the activated sludge, additional information will be required to distinguish between biodegradation and removal by these other processes. The additional information may be obtained by analysis of the sewage sludge or by using ^{14}C-labeled test compounds.

4.8 The capabilities of the porous pots to efficiently remove soluble organic carbon and ammoniacal nitrogen from sewage feed is done by measuring the loss of DOC and ammoniacal nitrogen during porous pot treatment. However, the loss of ammonia can only be used when the SRT is sufficiently long for viable populations of nitrifying bacteria to become established in the sludge.

5. Significance and Use

5.1 Secondary wastewater treatment using activated sludge is one of the most important biological treatment processes in use today. The porous pot simulates the activated sludge sewage treatment process in the laboratory and provides data that can be used to predict the fate of organic compounds in full scale plants.

5.2 A good correlation between the laboratory test and full scale plants is achieved by the use of primary effluent or settled domestic sewage and controlling key parameters in ranges typical of such treatment process. These parameters include temperature, pH, DO concentration, hydraulic residence time (HRT) and sludge retention time (SRT).

6. Apparatus

6.1 *Porous Pot Aeration Vessel (Engineering Drawing of URPSL Design (see Fig. 1))*—The porous pot vessel liner is constructed from porous high density polyethylene sheets. The thickness ranges from 3.2 to 13.6 mm and pore sizes are from 65 to 90 µm. The retention of the liner is about 20 µm and all particles above this size are retained in the system. The outer vessel can be constructed of glass or an impermeable plastic such as acrylonitrile butadiene syrene copolymer (ABS).

6.2 *Oil-Free Compressor*, for supplying compressed air to the aeration vessel.

6.3 Suitable pumps are required to dose porous pots with test substance solutions and sewage at the required rates (0 to 1.0 mL/min for test substance solutions, 5 to 20 mL/min for sewage). If the URPSL apparatus is used, an additional pump is required to waste sludge from the pot.

6.3.1 Low rates of sludge wastage are attained using a pump set at a high flow rate but operating intermittently. The actual flow is calculated as follows: pump throw (mL/min) by pumping time (s)/timer cycle (s); for example, when the pump in operating for 10 s each minute and the pump throw is 3 mL/min, the wastage rate would be 0.5 mL/min.

6.4 *Sample Bottles*, 1 L, to hold test substance dosing solutions.

6.5 *Silicone Rubber Tubing*, bore, 0.5 mm inside diameter (ID).

6.6 *Polypropylene Transmission Tubing*.

6.7 *Tube Connectors*.

6.8 *Diffuser Stones*.

6.9 *Measuring Cylinders*, 25-mL.

6.10 *Graduated Pipettes*, 1-mL.

6.11 *Stopwatch*.

6.12 *Sample Bottles*, 40-mL, for collection of samples for waste sludge and mixed liquor suspended solids determinations.

6.13 *Thermometer*, 0 to 50°C.

6.14 *Measuring Cylinders* 1 and 2 L, for each pot to collect waste sludge.

6.15 *Timer*, for sludge wastage pump allowing intermittent operation.

6.16 *Right-Angled Plastic Tube*, to fit on one end of the air line to ensure complete mixing of activated sludge.

7. Reagents and Materials

7.1 *Activated Sludge Mixed Liquor*, collected from aeration basin or oxidation ditch of domestic wastewater treatment plant.

7.2 *Natural Sewage Feed*—Primary effluent or settled domestic sewage from a domestic wastewater treatment plant. Supplementation with synthetic sewage stock (see 7.3) to obtain at least 200 mg DOC/L is recommended, but not required.

7.3 *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 mL of this stock solution is added to each liter of tap water to form the synthetic sewage **(12)**.

7.4 *Compressed Air*, (filtered for oil and water) for aeration of porous pots.

7.5 *Test and Reference Compounds*, of known carbon content (for DOC analyses) or composition (for specific analyses).

7.6 *Extraction Apparatus*, and solvent for hydrophobic test compounds.

7.7 *Deionized or Distilled Water*, for preparation of test/reference compound stock solutions.

7.8 *Glycerol*, for lubricating the rollers of the peristaltic pumps.

7.9 *Sodium Hypochlorite Solution*.

7.10 *Stock Solutions of Test and Reference Compounds*:

7.10.1 For compounds that are sufficiently soluble and chemically stable, a stock solution ten times the strength of the dosing solution may be prepared and diluted to the required strength each day.

7.10.2 If chemically unstable materials are being tested, it may be necessary to prepare stock/dosing solutions immediately before use.

NOTE 1—For insoluble materials a suitable stable dispersion is required.

7.11 *Dosing Solutions of Test/Reference Compound*:

7.11.1 To avoid biodegradation of the test/reference compound before it is introduced into the test system, which might occur if the test/reference compound and sewage are premixed, the test solution and the sewage are dosed into the porous pot separately.

7.11.2 The total flow rate into the pot [sewage (mL/min) + test/reference compound solution (mL/min)] is calculated as follows:

$$\textit{total flow}\ (\text{mL/min}) = \frac{\textit{volume of porous pot}\ (\text{mL})}{\textit{required sewage retention time}\ (\text{h}) \times 60\ (\text{min/h})} \quad (1)$$

7.11.3 For a pot volume of 3 to 6 L, it is convenient to dose with a solution of the test/reference compound at about 0.5 mL/min.

7.11.4 If the total flow, as calculated above, is F (mL/min) and the required concentration in the influent sewage is C (mg/L), then the concentration of the solution to be dosed into the pot (at a rate of 0.5 mL/min) is given as follows:

$$\textit{concentration of test substance dosing solution} = \frac{F\ (\text{mL/min}) \times C\ (\text{mg/L})}{0.5\ (\text{mL/min})} \quad (2)$$

7.11.5 The dosing solution is usually prepared daily by diluting a suitable stock solution.

8. Hazards

8.1 This procedure involves the use of mixed liquor and natural sewage from a domestic wastewater treatment plant. Consequently, individuals performing this test may be exposed to microbial agents that are dangerous to human health. It is recommended that porous pots be operated in a separate room and the exhaust air vented outside the building.

8.2 Personnel that work with sewage organisms may choose to keep current with pertinent immunizations such as typhoid, polio, hepatitis B, and tetanus.

8.3 Effluent from the porous pots is treated with a chemical disinfectant (chlorine bleach—5 %) or autoclaved prior to disposal. Safety glasses and protective gloves should be worn when using sodium hypochlorite to clean pot liners.

8.4 Unless shown to be non-toxic, all test compounds should be treated as potentially harmful.

9. Sampling and Analytical Procedures

9.1 *Stabilization Period*—Over the early period of the test, take influent sewage and effluent samples and analyze for DOC and ammoniacal nitrogen to monitor the overall performance of the units. Specific analysis for test compound or degradation products may also be performed on these samples. These results are not used to assess either the biodegradability or treatability of the test compound, but to establish that the units have reached steady state, are operating properly and are acclimated to the test substance. In certain instances, such as when information is desired on treatability of test compounds that are released only intermittently to wastewater treatment systems, data gathered during the stabilization period may be useful for assessing treatability. In order to establish that the acclimation is complete, it is necessary to measure the concentrations on sludge of an absorptive test substance. The stabilization period should be at least three times the sludge retention time (SRT). A similar period should be allowed (see 10.19) following any major change in the operating conditions before sampling is re-started. When all measured parameters are consistent, the calculation period can commence and data for assessing the treatability of the test material collected.

9.2 *Calculation Period*:

9.2.1 When the pots have achieved steady state, the removal of the test compound is determined by specific compound analysis, measurement of DOC, or both. A porous pot is considered to be in a steady state if over a seven day period of operation at a set SRT, the coefficient of variation (standard deviation/mean) of the DOC of its effluents is less than 20 %.

9.2.2 Assess the treatability of the test compound by measurement of DOC removal, removal of ammonia, sludge production and sludge activity. Of these parameters, DOC and NH_3-N removal are the most important. Note that when pots are being operated at short SRT or reduced temperature, ammonia removal may be less than complete and will then be a less reliable indicator of efficiency. However, the critical assessment of any adverse effect of the test compound on the process is always based on the absence of any significant difference between the test compound and control pots rather than the actual values of the observed parameters.

9.3 *DOC Analysis*:

9.3.1 DOC analysis for monitoring the porous pot test is generally employed only for test compounds whose water solubility exceeds the test concentration; for example, a concentration equivalent to about 10 mg C/L.

9.3.2 Since precipitation as salts or sorption onto the sludge floc may occur even with water-soluble test compounds, DOC removal does not necessarily indicate biodegradation in all cases.

9.3.3 DOC analyses are carried out on supernatant samples of influents and effluents from the pots. Samples can either be filtered using 0.45 µm pore-size filters or centrifuged at 3500 × g for 10 min (**13**).

NOTE 2—**Precaution:** An aliquot of the dosing solution should be evaluated for adsorption of test compound to the filter or elution of DOC from the filter itself.

9.3.4 The DOC concentration of aqueous samples is determined using a suitable organic carbon analyzer.

9.4 *Specific Compound Analysis*:

9.4.1 For the assessment of primary biodegradability, the porous pot method applies to water-soluble compounds provided that a suitable method of specific analysis is available.

9.4.2 Insoluble compounds or compounds that adsorb strongly onto the activated sludge may also be examined by this procedure, but it will then be necessary to determine the level of the test compound associated with the activated sludge.

9.4.3 For nonpolar hydrophobic test compounds, the compound is usually isolated from the sludge matrix by extraction with an immiscible solvent, such as methylene chloride or hexane. The extract is dried, concentrated, and analyzed by an appropriate instrumental method; for example, GC, HPLC, GC-MS, or UV/visible spectroscopy.

9.4.4 Highly polar extractible or nonextractible test compounds 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 radiolabeled materials and special apparatus. However, the porous pot operating system may be used if appropriate mass balances can be obtained.

9.4.5 The porous pot test is not recommended for volatile compounds (Henry's law constant $>10^{-3}$ atm-m^3/mol); however, it can be used for compounds that are not completely volatilized. For compounds of moderate volatility, volatilization losses during testing may be evaluated by scrubbing aeration off-gases through a solvent train (usually three consecutive traps containing acetone, methylene chloride, or hexane) or polymeric traps. Specific compound analysis of each solvent trap or polymeric trap is then carried out.

10. Procedure

10.1 Maintain the temperature of the mixed liquor at the required working temperature (±2°C) throughout the test. When setting up the test pots, test/reference compound and sludge wastage rates may initially be set. Start the test only after conditions are adjusted to the values defined in the study plan and the pots have been operating for some time under these conditions.

10.2 Set up the number of pots required by the study plan. Each test shall have at least two control pots (pots fed settled sewage and benzoate or other easily degradable reference compound) and it is recommended (but not required) that each test compound be tested in duplicate.

10.3 Fill the aeration vessel with mixed liquor to the level of the effluent overflow. The volume is 3.8 L for a URPSL porous pot. The initial MLVSS should be 1500 to 3000 mg/L. If the DOC in the feed is maintained at about 200 mg/L DOC, then it will be possible to maintain the MLVSS in the range from 1500 to 3000 mg/L. If the feed is not supplemented, then the MLVSS might fall below 1500 mg/L during periods of dilute feed such as may occur during rainfall events.

10.4 Start aeration and set the air flow. The air flow should be sufficient to maintain and thoroughly mix the solids in suspension and keep the concentration of dissolved oxygen above 2 mg/L at all times. In order to maintain an adequate DO concentration it will be necessary to maintain an air to wastewater flow ratio of 5 to 10/1 on a volume to volume basis.

10.5 Place 1 L of test/reference compound dosing solution in the dosing vessel.

10.6 Start the dosing pumps, lubricating the tubes with a small amount of glycerol.

10.7 Start the sludge wastage pump at the required rate to give the desired SRT. The required flow rate is given by:

$$F\ (\text{mL/min}) = \textit{aeration chamber volume}\ (\text{litres})/[\textit{SRT}(\textit{days})\ 1.44] \quad (3)$$

Since the pump tube will tend to block at the low flow rates required, the wastage pump is operated intermittently. For example, if the required flow rate is 0.25 mL/min the flow is set to 2.5 mL/min and the pump operated for 6 s/min.

10.8 Set the sewage dosing rate to give the required HRT and the test/reference compound dosing rate at about 0.5 ± 0.05 mL/min.

10.9 Daily measurements of sewage flow rates should be made using a 25-cm^3 measuring buret and a stopwatch. The flow rates should be adjusted to within ±0.05 mL/min of the required flow.

10.10 Dosing solution flow rates should be calculated from measuring the volume left after 24 h of dosing.

10.11 The dosing rates should be recorded and corrected to the nominal value given in the study plan. The sewage flow should be adjusted if the measured flow differs by more than 0.5 mL/min from the nominal value.

10.12 Return sludge that gathers around the rim of the porous liner to the mixed liquor at least once per day by scraping with a large spatula. This should always be done before taking a sample of mixed liquor for MLVSS determination.

10.13 Measure the temperature, pH, and dissolved oxygen concentration of the mixed liquor at least every other day.

10.14 Periodically remove a 40-mL sample of mixed liquor from the aeration vessel for MLVSS determination. Three times weekly is usually sufficient.

10.15 Measure and record the volume of mixed liquor wasted from the porous pot daily. At least once per week remove a representative 40-mL sample from the sludge wastage bottle and determine the MLVSS level.

10.16 Change the porous pot liner at the first sign of blocking of the pores; that is, when the mixed liquor rises above the effluent overflow. To change the liner proceed as follows: syphon the mixed liquor into a suitable container and remove any solids from the inner surface of the outer vessel. Place a fresh liner in the outer vessel. Return the mixed liquor to the aeration vessel. Scrape off and transfer any sludge adhering to the sides of the blocked liner. Thoroughly clean the blocked liner before reuse by immersing in a 20 % solution of hypochlorite bleach for several hours. Thoroughly mix the liners in clean tap and deionized water before re-use.

10.17 Take sewage, dosing solution, and effluent samples at least twice weekly during the stabilization ("running in") period for organic carbon analysis and specific compounds analysis if required. If necessary, ammoniacal nitrogen, nitrate, nitrite, COD, and BOD_5 may also be determined.

10.18 When the pots have attained steady state, the sewage, dosing solution, and effluents are analyzed periodically to determine the extent of biodegradation/removal of the test compound during sewage treatment.

10.19 If information on the effects of various operating conditions on removal is required; for example, temperature, SRT or HRT, etc., any changes should be made gradually. Operate the unit for a period of at least three SRT under the new conditions before data are collected to determine the effect of the new condition(s) on treatability.

11. Interpretation

11.1 Because the porous pot test system is a simulation of activated sludge wastewater treatment rather than a test to measure "ready" or "inherent" biodegradability, there are no pass or fail criteria. The levels of removal observed in the porous pot test should approximate levels of removal expected in full-scale activated sludge treatment systems.

11.2 Information on the physical/chemical properties of the test compound will be useful for interpretation of results and in the selection of appropriate test compound concentrations. These properties include structure, composition, purity, molecular weight, water solubility, organic carbon content, vapor pressure, octanol/water partition coefficient, adsorption isotherm, surface tension, and Henry's Law constant.

11.3 Information on the toxicity of the test compound or potential toxic transformation products to activated sludge microorganisms may be useful to the interpretation of low biodegradation results and in the selection of appropriate test compound concentrations. The OECD Respiration Inhibition Test (**11**) can be used to indicate such toxicity. Furthermore, chemical substances in solution or in the air that may negatively affect the growth or metabolism of sludge microorganisms, for example, organic solvents, toxic metals, strong alkalis, and bactericides—may result in low removals and should be avoided.

11.4 Use of synthetic versus natural sewage is an important consideration. It is sometimes assumed that use of synthetic sewage leads to more reproducible results; however, the microbial population that develops differs from that which is present in full-scale activated sludge plants. Generally, the most rapidly growing microorganisms will dominate the more slowly growing populations that are present in full-scale treatment plants. Natural domestic sewage varies from source to source and in nutrient content. However, it provides both the nutrients needed to support the natural microbial population and a continuous supply of fresh microorganisms to the test system.

11.4.1 On some occasions, particularly during periods of heavy rainfall, the strength of the primary effluent or settled domestic sewage from the treatment plant may be too low to sustain a typical biomass concentration in the porous pot unit, that is, 1500 to 3000 mg/L. A background feed blend made by supplementation of natural sewage with synthetic sewage to achieve a DOC level of at least 200 mg/L and an approximate 100:12:2 ratio of C:N:P is recommended but not required.

11.5 Reference compounds may be useful in establishing the activity of the activated sludge and in comparing results from different laboratories. While specific reference compounds cannot be recommended for these purposes, data are available for several chemicals (**6,10**).

12. Interpretation of Results

12.1 The data to be analyzed from this test method are measurements of chemical concentration in the influent and effluent waters passing through different experimental units. Because of the variability in influent wastewater composition, data from units with test chemicals must be compared with simultaneous control unit data, so a paired-sample approach is preferable. Data are typically collected for several sequential days from each experimental unit after a period of acclimation. Because the wastewater retention time in these systems is much less than 24 h (typically 6 h), data from successive days are treated as independent data, not repeated measures of the same system. This is consistent with observations that upsets in a unit will persist less than a day.

12.2 *Outlier Detection*—Data that do not appear to be in conformance with the substantial majority are often referred to as outliers, and might be due to random variation or to clerical or experimental errors. Statistical outlier detection procedures are screening procedures that indicate whether a datum is extreme enough to be excluded. Barnett and Lewis (**14**) describe many outlier detection procedures and Feder and Collins (**15**) illustrate their use. Dixon's test (**16**) has been frequently recommended for use with this procedure. Further information is provided in Practice E 178. If outliers can be shown to be due to clerical or experimental error (for example, pump failure or clogging), they should either be corrected or deleted from the data prior to analysis. If outliers are not known to be erroneous values, the question of how to deal with them is a matter of judgment. It is often desirable to analyze the data both with and without questionable values in order to assess their importance, because one or a few extreme outliers can sometimes greatly affect the outcome of an analysis.

12.3 *Primary Biodegradation/Removal*—The percentage removals for the test compound during the observation period are calculated to the nearest 0.1 % using the following equation:

$$\%\ removal = [1 - (C_E/C_I)] \times 100\ \% \quad (4)$$

where:

C_I = mean concentration of test compound in the influent (mg/L), and

C_E = mean concentration of test compound in the effluent (mg/L).

12.4 *Ultimate Biodegradation/Removal*—Ultimate removal is calculated using DOC data from the observation period, calculated to the nearest 0.1 % using the following equation:

$$\%\ removal = [1 - \Delta D_E/D_I)] \times 100\ \% \quad (5)$$

where:

D_I = mean DOC in the influent (mg of organic C/L), and

ΔD_E = mean difference in DOC between the effluent of control units and test chemical unit (mg of organic C/L) (see 12.4.2.1).

12.4.1 *Control Unit DOC*—In order to evaluate whether the difference between two control units is significant, a paired sample hypothesis test is conducted on the differences in DOC of the control units. For each sampling event (day), the difference is calculated as:

$$\Delta D_{Ci} = (DOC\ in\ control\ unit\ A) - (DOC\ in\ control\ unit\ B) \quad (6)$$

where:

ΔD_{Ci} = the difference between controls for the i^{th} of n samples.

12.4.1.1 The mean and standard deviation of all n values of$\Delta D_{Ci}$are calculated and used to calculate a Student's t value using the equation:

$$t = [Mean\ (\Delta D_{Ci}) \times \sqrt{n}]\ /SD\ (\Delta D_{Ci}) \quad (7)$$

12.4.1.2 This t value is compared with the critical t statistic for a two-sided test, with $p = 0.95$ and $n-1$ degrees of freedom. If the calculated t is less than the critical t statistic, then the control units are inferred to be equivalent, that is, their difference is not statistically different from zero. To proceed, the mean control values (D_{Ci}) should be calculated for each sample event i.

12.4.1.3 If the calculated t is greater than the critical t, a difference between control systems is inferred. In such a case, the cause of the difference must be addressed before attempting to evaluate the data further.

12.4.2 *Test Unit DOC*—In order to evaluate whether a test unit is significantly different from the control units, a paired sample hypothesis test is conducted on the differences in DOC. The paired sample approach reflects a belief that there is some type of correlation between the experimental units, that is, it reflects the recognition that influent wastewater is highly variable, so the effluent of different experimental units at each sampling time will reflect the particular wastewater influent at that time. If there is no correlation and no reason for pairing the experimental units, treating the data as a two-sample problem will provide slightly greater statistical power (**15**), (**17**). However, if the sample size is about ten or greater, the difference is small (**15**).

12.4.2.1 For each test chemical at each sampling time, the difference in DOC in the test unit from the mean control value is calculated using the equation:

$$\Delta D_{Ti} = (DOC\ in\ \ test\ \ unit\ for\ sample\ i) - D_{Ci} \quad (8)$$

where:

D_{Ci} is the mean control DOC for the i^{th} sample.

The mean and standard deviation for ΔD_{Ti} should be calculated using all available samples. If data are missing for either the test unit or controls, no difference can be calculated for that sampling event. The mean value is used as the ΔD_o for calculating the percentage removal in 12.4.

12.4.2.2 A Student's t value is calculated for the differences using the equation:

$$t = [Mean\ \Delta D_{Ti}) \times \sqrt{n}]\ /SD\ (\Delta D_{Ti}) \quad (9)$$

This t value is compared with the critical t statistic for a one-sided test, with $p = 0.95$ and $n-1$ degrees of freedom. If the calculated t is less than the critical t statistic, then the test chemical is inferred to be equivalent to the control, that is, their difference is not statistically different from zero.

12.4.2.3 The comparison of other test chemicals is completed by repeating the sequence in 12.4.2.1 and 12.4.2.2.

12.4.3 *Confidence Intervals for Percent Removal*—The mean, standard deviation, n and critical Student's t are used to calculate the confidence interval for the percentage removal (see section 12.4). The 95 % upper confidence limit (UCL) is calculated as:

$$5\ \%\ UCL\ of\ \%\ Removal = \{I - [\Delta D_o - (SD \times t_{95\ \%}/\sqrt{n})]/D_I\} \times 100\ \% \quad (10)$$

where:

ΔD_o = the mean difference in DOC between the test chemical unit and the control units, as calculated in 12.4.2.1 (mg of organic C/L),

SD = the standard deviation (n – 1 degrees of freedom) for differences in DOC between the test chemical unit and the control units, as calculated in 12.4.2.1 (mg of organic C/L).

$t_{95\ \%}$ = the critical t value for n – 1 degrees of freedom for a two-tailed test,

n = the number of data pairs, and

D_I = the mean DOC in the influent (mg of organic C/L).

12.4.3.1 The 95 % lower confidence limit (LCL) is calculated as:

$$95\ \%\ LCL\ of\ \%\ Removal = \{I - [\Delta D_o + (SD \times t_{95\ \%}/\sqrt{n})]/D_I\} \times 100\ \% \quad (11)$$

12.4.4 *Example*—A typical data set for two control pots and three test plots is given in Table 1. The first step is to establish that the two control pots are operating in parallel, that is, that their difference is not statistically different from zero, using the procedure of 12.4.1. As shown in Table 1, the mean difference in DOC between the two control pots ($Mean\ (\Delta D_{Ci})$) is 0.21, the standard deviation ($SD\ (\Delta D_{Ci})$) is 0.53 and sample size (n) is 17. The resulting Student's t value is:

$$t = 0.21 \times \sqrt{17}/0.53 = 1.63 \quad (12)$$

12.4.4.1 The critical t-value at the 0.05 significance level for a two-tailed test and 16 df is 2.12, and since this is not exceeded by the calculated value, the difference between the controls is not significantly different from zero.

12.4.4.2 Note that a two-tailed test is used because there is no preconception as to which control pot will have the higher effluent DOC concentration.

12.4.4.3 Having accepted that the two controls are operating in parallel, their mean is calculated and used in subsequent comparisons with test chemical units.

12.4.4.4 For Test Chemical 1, the mean difference between the test chemical pot and the controls is – 0.29, the standard deviation is 1.69 and the number of paired observations is 17. The resulting Student's t value is:

$$t = -0.29 \times \sqrt{17}/1.69 = 0.71 \quad (13)$$

12.4.4.5 The critical t-value at the 0.05 significance level for a one-tailed test and 16 df is 1.75, and since this is not exceeded by the calculated value, the difference between Test Chemical 1 and the controls is not significantly different from zero. A one-tailed test is used since it is only necessary to establish if the test pot effluent has a significantly higher DOC than the control pot, that is, their difference is greater than zero. The converse is not important for this type of test and is not normally observed.

TABLE 1 Test Data

Effluent DOC (mg/L)								
Control 1	Control 2	Control Mean	Test 1	Test 1—Control Mean	Test 2	Test 2—Control Mean	Test 3	Test 3—Control Mean
9.6	9.2	9.40	10.9	1.50	11.2	1.80	11.8	2.40
9.5	10.1	9.80	9.8	0.00	11.1	1.30	12.1	2.30
9.2	10.2	9.70	11.9	2.20	11.7	2.00	12.5	2.80
7.8	7.4	7.60	8.4	0.80	8.6	1.00	10.1	2.50
7.5	8.1	7.80	9.1	1.30	9.2	1.40	11.0	3.20
7.3	7.2	7.25	9.2	1.95	8.4	1.15	8.9	1.65
6.7	7.1	6.90	7.9	1.00	...	...	...	...
11.8	13.2	12.50	13.2	0.70	12.2	–0.30	14.9	2.40
12.6	12.8	12.70	11.6	–1.10	11.8	–0.90	14.5	1.80
18.0	17.7	17.85	13.8	–4.05	14.9	–2.95	16.5	–1.35
11.1	11.1	11.10	9.2	–1.90	11.5	0.40	14.2	3.10
8.8	9.3	9.05	7.5	–1.55	8.4	–0.65	10.5	1.45
8.5	8.3	8.40	7.6	–0.80	9.1	0.70	9.6	1.20
10.8	10.5	10.65	9.8	–0.85	11.0	0.35	11.9	1.25
12.0	11.9	11.95	9.7	–2.25	...	...	...	...
11.1	11.9	11.50	10.0	–1.50	11.8	0.30	12.9	1.40
14.2	14.1	14.15	13.7	–0.45	14.7	0.55	16.3	2.15
	Mean[A]	10.49	10.19	–0.29	11.04	0.41	12.51	1.88
	Standard Deviation[B]	2.83	2.03	1.69	2.04	1.25	2.36	1.10

[A] Mean of (Control 2 – Control 1) = 0.21.
[B] Standard deviation of (Control 2 – Control 1) = 0.53.

12.4.4.6 Repeating the procedure for Test Chemicals 2 and 3 obtains the results shown in Table 2. Only for Test Chemical 3 is there evidence for a non-zero difference, that is, that the DOC in Test Chemical 3 unit is significantly greater than in the controls.

13. Quality Assurance

13.1 To ensure the integrity of data developed using this method and to comply with current regulatory requirements, a quality assurance program meeting EPA (**18**) or FDA (**19**) good laboratory practices (GLP) guidelines should be followed. This may require replicates (three or more) for GLP compliance and assessment of variability.

13.2 This type of result can be informative about the potential to form water-soluble residues during the wastewater treatment. For Test Chemical 3, there is a significant amount of DOC in excess of the controls, evidence that water soluble residues are likely to be present. For Test Chemicals 1 and 2, the results do not prove that residues are not formed, but should be interpreted as indicating that no evidence for formation of residues was provided by this test method.

13.3 The DOC data provide estimates of the percentage (ultimate) removal for each test chemical, as shown in Table 1, using the equation in 4.3, with the additional information that mean DOC in the influent (D_I) was 10 mg organic C/L. The 95 % upper and lower confidence limits are calculated using the equations in 12.4.3.

14. Report

14.1 Prepare a protocol giving a general overview of the study goals and procedures before the study is initiated. If a substantive modification of this test method is deemed necessary for the test compound, document deviation from this test method in the protocol.

14.2 Document final results of this study in a final report. Include the following in the final report:

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

14.2.2 A brief description of the test compound including its log number, chemical name(s), composition, and other appropriate information,

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

14.2.4 Summary of specific analytical methods, if employed,

14.2.5 If applicable, tabular and graphical presentation of DOC removal data as a function of time after test initiation. Data are expressed as % DOC removal (weekly mean for 24-h periods),

14.2.6 If applicable, tabular and graphical presentation of specific compound analysis data as a function of time after test initiation. Data are expressed as steady state concentrations of test compound in influents and effluents, or primary biodegradation during 24-h periods,

TABLE 2 Analysis of Test Data

	Test 1	Test 2	Test 3
Mean difference [test – mean control]	–0.29	0.41	1.88
Standard deviation	1.69	1.25	1.1
Number of paired observations	17	15	15
Calculated Student's *t* value	0.71	1.27	6.62
Critical Student's *t* value (one-tailed)	1.75	1.76	1.76
Critical Student's *t* value (two-tailed)	2.12	2.145	2.145
% Removal (C_o = 10 mg/L)	102.9	95.9	81.2
95 % Confidence interval	94.2 to 111.6	89.0 to 102.8	75.1 to 87.3

14.2.7 A listing of relevant references including all notebook pages containing raw data from this study, and

14.2.8 The following raw data should be recorded:

14.2.8.1 Date,

14.2.8.2 Pot number,

14.2.8.3 Sewage flow,

14.2.8.4 Temperature of mixed liquor,

14.2.8.5 Volume of test compound dosing solution remaining,

14.2.8.6 Time of replacement of dosing solution,

14.2.8.7 pH of mixed liquor,

14.2.8.8 Dissolved oxygen concentration of mixed liquor,

14.2.8.9 Weight of test compound used to prepare concentrated stock solutions,

14.2.8.10 Volume of activated sludge mixed liquor wasted by continuous wastage (URPSL design),

14.2.8.11 Date of start of calculation period,

14.2.8.12 Change of porous pot liners,

14.2.8.13 Faults with tubing, pumps, sewage or air supply,

14.2.8.14 Signature of operator,

14.2.8.15 Study number,

14.2.8.16 Calibration of pH meter, and

14.2.8.17 Calibration of DO meter.

REFERENCES

(1) Gaudy, A. F., Jr., and Gaudy, E. T., *Microbiology for Environmental Scientists and Engineers*, McGraw-Hill, New York, 1980.

(2) Swisher, R. D., *Surfactant Biodegradation*, Marcel Dekker, Inc., New York and Basel, 1987.

(3) Buttress, F. A., *World Guide to Abbreviations of Organizations*, 9th Edition by H. J. Heaney, Gale Research Inc., Detroit and New York, 1991.

(4) Hopwood, A. P., and Downing, A. L., "Factors Affecting the Rate of Production and Properties of Activated Sludge in Plants Treating Domestic Sewage," *Journal and Proceedings of the Institute of Sewage Purification 64*, 1965, pp. 435–452.

(5) Department of the Environment, Standing Committee of Analysts, National Water Council, *Assessment of Biodegradability 1981. Methods for the Examination of Waters and Associated Minerals. Continuous Simulation (Activated Sludge) Test for the Assessment of Biodegradability*, Volume 1981, Her Majesty's Stationery Office, London, U.K., 1983.

(6) Painter, H. A., and King, E. F., *WRC Porous Pot Method for Assessing Biodegradability*, WRC Technical Report TR70, 1978.

(7) Painter, H. A., and King, E. F., "The Effect of Phosphate and Temperature on the Growth of Activated Sludge and on the Biodegradation of Surfactants," *Water Research 12*, 1978, pp. 909–915.

(8) Watson, H. M., "Important Considerations in Choosing a Synthetic Feed for Laboratory-Scale Wastewater Treatment Systems," *Environmental Toxicology and Risk Assessment: 2nd Volume, STP 1216*, J. W. Gorsuch, F. J. Dwyer, C. G. Ingersoll, and T. W. La Point, Eds., ASTM, Philadelphia, PA, 1993, pp. 228–239.

(9) Waters, J., Kleiser, H. H., How, M. J., Barrat, M. D., Birch, R. R., Fletcher, R. J., Haigh, S. D., Hales, S. G., Marshall, S. J., and Pestell, T. C., "A New Rinse Conditioner Active with Improved Environmental Properties," *Tenside Surfactants Detergents 28(6)*, 1991, pp. 460–468.

(10) Birch, R. R., "Prediction of the Fate of Detergent Chemicals During Sewage Treatment," *Journal of Chemical Technology and Biotechnology 50*, 1991, pp. 411–422.

(11) Organization for Economic Cooperation and Development (OECD), *Activated Sludge Respiration Inhibition Test*, OECD 209, 1981, OECD, Paris.

(12) The Subcommittee on Biodegradation Test Methods of the Soap and Detergent Association, "A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate," *Journal of the American Oil Chemists' 42*, 1965, pp. 986–993.

(13) American Public Health Association, "Method 5310. Total Organic Carbon (TOC)," *Standard Methods for the Examination of Water and Wastewater*, 19th Ed., Mary A. H. Franson, Editor, American Public Health Association, Washington, DC, 1995.

(14) Barnett, V., and Lewis, F., *Outliers in Statistical Data*, Wiley, New York, NY, 1978.

(15) Dixon, W. J., and Massey, F. J., Jr., *Introduction to Statistical Analysis*, McGraw-Hill, New York, NY, 1969.

(16) British Standard Institution, British Standard BS 5497. *Precision of Test Results, Part 1*, 1987.

(17) Zar, J. H., *Biostatistical Analysis*, Second Edition. Prentice-Hall, Englewood Cliffs, NJ, 1984.

(18) Code of Federal Regulations, *40. Part 792—Good Laboratory Practice Standards*, 1993.

(19) Code of Federal Regulations, *21. Part 58—Good Laboratory Practice for Nonclinical Laboratory Studies*, 1995.