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Standard Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals[1]

This standard is issued under the fixed designation E 1196; 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 screening procedures for determining the anaerobic biodegradability of organic chemicals. Laboratory procedures for determining the conversion of organic substances into methane (CH_4) and carbon dioxide (CO_2) are described.

1.2 The procedures are designed to be applicable to all organic compounds which, at the concentration used in the test method, are not inhibitory to bacteria.

1.3 *This standard does not purport to address all of the safety problems, 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.* Specific hazard statements are given in Section 7.

2. Referenced Document

2.1 *ASTM Standard:*
D 1193 Specification for Reagent Water[2]

3. Summary of Test Method

3.1 A chemically defined anaerobic medium, containing resazurin as an oxidation/reduction indicator and 10 % (v/v) primary anaerobic digestor sludge from treatment of primary clarifier sludge at a waste treatment plant, is dispensed in 100-mL portions into 160-mL capacity serum bottles. Selected bottles are supplemented with test substance at a concentration equivalent to 50 mg/L as organic carbon. Gas production is measured with a pressure transducer or syringe. The extent of biodegradation is determined by comparing gas production from blank (control) bottles containing no test material and bottles containing the test substance.

3.2 The average cumulative gas production ($CH_4 + CO_2$), in millilitres, is reported for blank controls, solvent controls, test substances, and any reference compounds. Also reported are the percent of theoretical anaerobic biodegradation at test completion or 56 days, whichever comes first, and the standard deviation between replicate bottles.

4. Significance and Use

4.1 Biodegradation is an important process for the transformation of many chemical substances in anaerobic environments. In many cases the ultimate fate of organic substances in such environments is dissimilation to methane (CH_4) and carbon dioxide (CO_2), a process referred to as anaerobic digestion or methanogenesis.

4.2 This test method has been developed to screen organic chemicals for anaerobic biodegradation potential. A high-biodegradability result in this test method is good evidence that the test substance will be biodegradable in waste treatment plant anaerobic digestors and in many natural anaerobic environments. Conversely, a low-biodegradation result may have causes other than poor biodegradability of the test substance. Inhibition of the microbial inoculum by the test substance may have occurred at the concentrations tested, or conditions in the test may have been inappropriate for the development of an acclimated microbial population. In such cases, further work is needed to assess the anaerobic biodegradation potential of the chemical, and to determine if toxic effects are a factor. An estimate of the expected environmental concentration will help to put any observed toxic effects into perspective.

5. Apparatus

5.1 *Pressure Transducer*, for measuring gas production, consisting, for example, of a 20-gage syringe needle attached by an inert capillary tube to a three-way valve, fitted to a pressure transducer, and an appropriate ohmmeter. If preferred, gas production may be measured using only a syringe (see 5.2).

5.2 *Syringe*, for measuring gas production, such as a 20-mL capacity freely moving gas-tight or water-lubricated glass syringe fitted with a 20 to 25-gage syringe needle.

5.3 *Gas Chromatograph*, or other apparatus, equipped with a suitable detector and column(s), if methane and carbon dioxide are quantified using an analytical procedure specific for methane and carbon dioxide.

5.4 *Incubator*, sufficient to store the test bottles at 35 ± 2°C in the dark for the duration of the test.

5.5 *Medium Handling Apparatus*, suitable for the maintenance of anaerobic conditions during medium preparation and inoculation, **(1)**[3] (See Fig. 1).

5.6 *Serum Bottles*, 160-mL capacity, with butyl rubber stoppers and crimp caps to hold the rubber stoppers.

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade chemicals shall be

[1] This test method is under the jurisdiction of ASTM Committee E-47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.06 on Environmental Fate of Chemical Substances.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] The boldface numbers in parentheses refer to the list of references at the end of this test method.

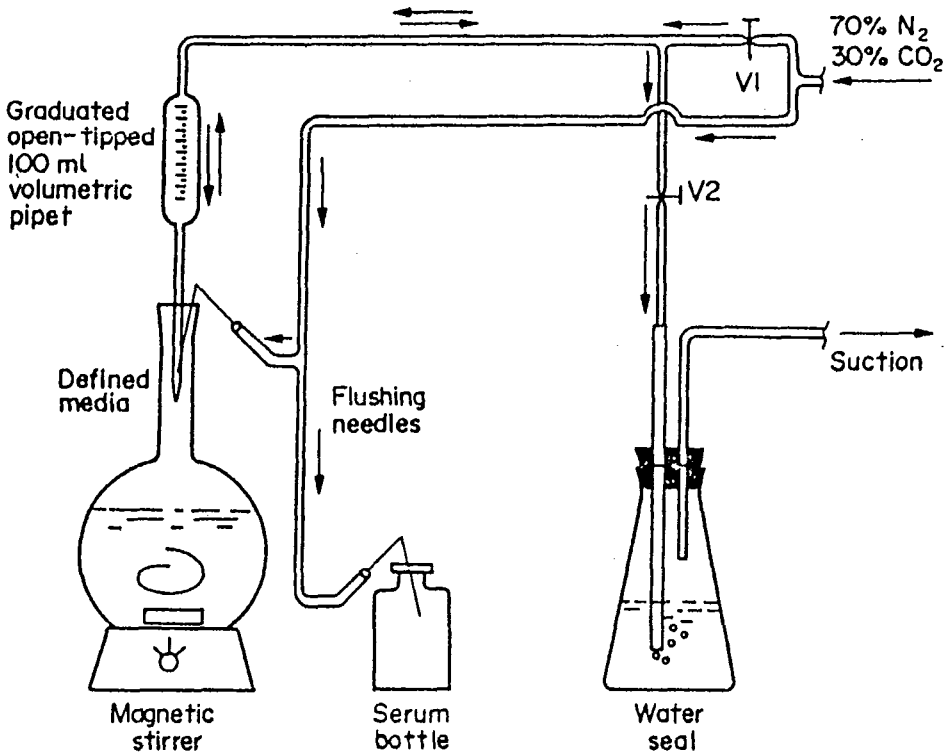

FIG. 1 Schematic Diagram of Apparatus Suitable for Maintenance of Anaerobic Conditions During Medium Preparation and Inoculation (Printed with permission by *Water Research*, 13:487 (1979)).

used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[4] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type IV of Specification D 1193.

6.3 Stock solutions are prepared as shown in Table 1 using dechlorinated tap water, Type IV reagent water, or better. Trace metal concentrations are based on Parkin et al (2).

6.4 Up to 1 mL of concentrated HCl may be added to stock solution S-3 to improve the solubility of the salts. This solution should be well shaken before use in order to distribute any undissolved material throughout the solution.

7. Hazards

7.1 This test method includes the use of hazardous chemicals. Avoid contact with the chemicals and follow manufacturer's instructions and Material Safety Data Sheets.

7.2 **Precaution**—This test method involves the use of sludge from a waste treatment plant. Avoid contact with the sludge by using gloves and other appropriate protective equipment. Use good personal hygiene to minimize exposure to potentially harmful microbiological agents.

8. Inoculum

8.1 The inoculum should consist of sludge from a well-operating anaerobic sludge digestor with a total organic solids level of at least from 1 to 2 %. It is recommended that well-mixed sludge from a digester with a solids retention time from 15 to 30 days be used. Experience may dictate that at the time of collection the sludge should be sieved through a 2-mm mesh screen or one layer of cheese cloth.

8.2 If necessary, most sludges can be stored for 2 weeks at 4°C, but it is recommended that fresh sludge be used. Care should be taken to minimize exposure of the sludge to oxygen during collection, handling, and storage.

9. Procedure

9.1 *Inoculum Medium:*

9.1.1 Prepare the prereduced medium using the stock solutions (see Table 1). Add 8 mL of stock solution S-1, 8 mL of S-2, and 40 mL of S-3 to approximately 3500 mL of dechlorinated tap water, Type IV water, or better in a 4-L Florence or Erlenmeyer flask. Heat this to a boil, while stirring with a magnetic stir bar and sparging with a 30 % carbon dioxide and 70 % nitrogen mixture. This gas mixture may be directly purchased or prepared by mixing nitrogen and carbon dioxide using calibrated flow meters on each gas or a commercial mass flow controller with appropriate calibration. Readily available commercial nitrogen containing less than 10 ppm oxygen may be used mixed with commercial carbon dioxide containing less than 200 ppm oxygen.

[4] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, D.C. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, N.Y., and the "United States Pharmacopeia."