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Standard Test Method for Determining Anaerobic Biodegradation Potential of Organic Chemicals Under Methanogenic Conditions[1]

This standard is issued under the fixed designation E2170; 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 the determining the biodegradation potential of organic chemicals under methanogenic conditions using an inoculum from anaerobic sewage treatment. The conditions described in this test method are not necessarily optimal for biodegradation, since it involves a dilute inoculum and a relatively high concentration of test chemical.

1.2 The test method is applicable to most organic chemicals, assuming that they can be accurately dosed into the test systems. The test is not applicable to chemicals that are inhibitory at the concentration used in the test or those whose volatility generates significant head pressure in the test vessels.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazard statements are given in Section 8.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

D5904 Test Method for Total Carbon, Inorganic Carbon, and Organic Carbon in Water by Ultraviolet, Persulfate Oxidation, and Membrane Conductivity Detection

3. Terminology

3.1 *Definitions:*

3.1.1 *biodegradation potential*—the ability of an organic chemical to undergo partial or complete biodegradation under the conditions of the test. In the context of this test, biodegradation is evidenced by the production of methane and carbon dioxide, which is measured as an increased pressure in the headspace and increased dissolved inorganic carbon in the medium.

3.1.2 *digester sludge*—sludge which is obtained from an anaerobic reactor used to reduce the solids recovered and produced during the treatment of wastewater. Such reactors are generally operated at 35°C with a typical retention time of 25 to 30 days.

4. Summary of Test Method

4.1 The test chemical and an anaerobic digester sludge inoculum are suspended in a defined anaerobic medium and incubated in a sealed vessel at 35°C. The increase in headspace pressure resulting from the production of carbon dioxide and methane is measured at various time intervals using a pressure measuring device. At the termination of the test, the level of dissolved carbon dioxide is assayed by measuring dissolved inorganic carbon in the medium. The amount of total gas produced from the test chemical is determined by comparing total gas production in the experimental treatments and the blanks.

5. Significance and Use

5.1 Biodegradation is an important process for the removal of many chemical substances in anaerobic environments. In methanogenic environments, this process involves dissimilation to carbon dioxide and methane.

5.2 This test method has been developed to screen organic chemicals for anaerobic biodegradation potential under methanogenic conditions, where there is no ingress of oxygen. A high-biodegradability result in this test method is good evidence that the test substance will be biodegradable in waste treatment plant anaerobic digesters and in many natural 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. Toxicity should be suspected when the gas production in the blanks exceeds that in the test

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

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[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.

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vessels. In such cases, further work is needed to assess the anaerobic biodegradation potential of the chemical. An estimate of the expected environmental concentration will help to put any observed toxic effects into perspective.

6. Apparatus

6.1 *Incubator,* water or sand bath, thermostatically controlled at 35 ± 2°C.

6.2 *Pressure-Resistant Glass Test Vessels,* each fitted with a gas-tight septum, capable of withstanding about 2 bar. From a practical point of view, the use of 160 mL serum bottles, which are often described in catalogs as 125 mL serum bottles, sealed with butyl rubber serum stoppers with crimped aluminum caps is recommended.

6.3 *Pressure Measuring Device*—An example of such a device would be a pressure transducer attached to a syringe needle, which may include a three-way gas-tight valve for releasing excess pressure. The device should be calibrated under conditions relevant to the test setup.

6.4 *Carbon Analyzer,* suitable for the direct determination of organic and inorganic carbon in the test vessels, consistent with specification in Test Method D5904.

6.5 System for preparing and manipulating media, inocula and samples under anaerobic conditions. The system could be an anaerobic chamber or gas sparging system.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. 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.

7.2 *Purity of the Water*—Unless otherwise indicated, reference to water shall be understood to mean reagent water as defined by Type IV of Specification D1193.

7.3 *Test Medium*—The test medium consists of the following constituents in water.

Anyhdrous potassium dihydrogen phosphate (KH_2PO_4)	0.27 g/L
Disodium hydrogen phosphate dodecahydrate ($NaHPO_4$- 12 H_2O)	1.12 g/L
Ammonium chloride (NH_4Cl)	0.53 g/L
Calcium chloride dihydrate ($CaCl_2$- 2 H_2O)	0.075 g/L
Magnesium chloride hexahydrate ($MgCl_2$- 6 H_2O)	0.10 g/L
Iron (II) chloride tetrahydrate ($FeCl_2$- 4 H_2O)	0.02 g/L
Resazurin (oxygen indicator)	0.001 g/L
Sodium sulfide nonahydrate (Na_2S - 9 H_2O)	0.1 g/L

7.4 With the exception of sodium sulfide, concentrated (100 ×) stock solutions can be prepared for each constituent.

8. Hazards

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

8.2 This test involves the use of digester sludge from a wastewater treatment plant. Avoid contact with sludge by using gloves and other appropriate protective equipment. Use good personal hygiene to minimize exposure to potentially harmful microbiological agents.

8.3 Since pressure can build-up in the test vessels, select inoculum levels, test substance concentrations and head space volumes that will avoid headspace pressures that could exceed safe pressure specifications established for the test vessels.

9. Preparation of the Test Medium

9.1 All constituents with the exception of the sodium sulfide are added to the water. The constituents can be added neat or as stock solutions. The use of stock solutions generally improves accuracy and convenience. The solution is autoclaved or alternatively heated to a boil while being mixed with a magnetic stirrer and being sparged with oxygen-free nitrogen. After being autoclave or reaching a boil, the medium is cooled in an anaerobic chamber or with continued nitrogen sparging. Once the medium has cooled to approximately 35°C, the sodium sulfide is added to the medium at which point, the resazurin in the medium should be colorless.

10. Inoculum

10.1 Collect anaerobic sludge from a well functioning anaerobic digester at a sewage treatment plant receiving predominantly domestic wastewater. Sludge must be sampled and transported in a manner that preserves its anaerobic integrity. One approach is to collect sludge in a wide necked polyethylene bottle filled to within 1 cm of the top and sealed tightly. Since pressure can build up in the bottle, the use of glass should be strictly avoided, and excess pressure must be periodically released during transport and storage.

10.2 If the sludge contains large particulates, it may be appropriate to sieve it through a 2-mm mesh screen or a single layer of cheese cloth prior to use. To reduce background gas production, it also may be appropriate to allow the sludge to digest in the laboratory without addition of nutrients or substrates for up to 10 days. Work by ECETOC (**1**)[3] suggests that 5 days gives an optimum decrease in background gas production without an unacceptable increase in lag or incubation period. Some laboratories monitor gas production and use the inoculum once gas production has reached a plateau. All procedures must be conducted under conditions that preserve the anaerobic integrity of the sludge (that is, in an anaerobic chamber).

10.3 Immediately prior to use, it may be appropriate to wash the sludge inoculum with anaerobic test medium to reduce levels of inorganic and organic carbon. This is accomplished by centrifuging sludge samples at a low speed (that is, 3000 g) and decanting the supernatant. The supernatant is discarded, and the pellet resuspended in an equal volume of the test medium. This process can be repeated as needed to reduce background DIC below 10 mg DIC/L (**2**), which is usually accomplished with two washings. It is critical that all washing

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

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