



Designation: E 1279 – 89 (Reapproved 1995)

Standard Test Method for Biodegradation By a Shake-Flask Die-Away Method¹

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1. Scope

1.1 This test method describes procedures for assessing the biodegradation of chemicals in natural surface water samples.

1.2 This test method provides an opportunity to evaluate rates of biodegradation in the presence and absence of natural sediment materials. It also may provide limited information on the abiotic degradation rate, and sorption to sediment and vessel walls.

1.3 This test method allows for the development of a first-order rate constant, based on the disappearance of the test compound with time, and a second-order rate constant, normalized for changes in microbial biomass.

1.4 This test method requires a chemical specific analytical method and the concentrations of test substance employed are dependent on the sensitivity of the analytical method.

1.5 This test method is designed to be applicable to compounds that are not inhibitory to bacteria at the concentrations used in the test method, which do not rapidly volatilize from water, that are soluble at the initial test concentration and that do not degrade rapidly by abiotic processes, such as hydrolysis.

1.6 *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.*

2. Referenced Documents

2.1 ASTM Standards:

D 1193 Specification for Reagent Water²

D 4129 Test Method for Total and Organic Carbon in Water by Oxidation and Coulometric Detection³

E 895 Practice for Determination of Hydrolysis Rate Constants of Organic Chemicals in Aqueous Solutions⁴

E 896 Test Method for Conducting Aqueous Direct Photolysis Tests⁴

E 1194 Test Method for Vapor Pressure⁴

E 1195 Test Method for Determining a Sorption Constant (K_{OC}) for an Organic Chemical in Soil and Sediments⁴

3. Summary of Test Method

3.1 The shake-flask die-away biodegradation method is similar to river water die-away tests described by many authors, including Degens et al (1),⁵ Eichelberger and Lichtenberg (2), Saeger and Tucker (3), Paris et al (4), and Cripe et al (5). It differs from most die-away methods by providing for an evaluation of the effects of natural sediments on the transformation of the test compound and by the use of shaking to ensure a dissolved oxygen supply. Each test compound (substrate) is dissolved in water collected from a field site, with and without added natural sediment and with and without sterilization. Initial substrate concentrations typically are relatively low ($\mu\text{g/L}$), analytical capabilities permitting. Loss of test compound with time is followed by an appropriate, chemical-specific analytical technique. Changes in microbial biomass also may be followed by the use of an appropriate technique such as bacterial plate counts. Data obtained during use of the test method are used to provide the following information: (a) the abiotic degradation rate in the presence and absence of sediment and (b) the combined biotic and abiotic degradation rate in the presence and absence of sediment.

4. Significance and Use

4.1 Most of the simpler methods used to screen chemicals for biodegradation potential employ measurements that are not specific to the test substance, such as loss of dissolved organic carbon, evolution of respiratory carbon dioxide, or uptake of dissolved oxygen. Such methods generally are used to evaluate the transformation of the test substance to carbon dioxide, water, oxides or mineral salts of other elements, or products associated with the normal metabolic processes of microorganisms (ultimate biodegradability), or both. These methods require the use of relatively high initial concentrations of the test substance, generally 10 mg/L or higher, unless the tests are conducted using ¹⁴C-radiolabeled test compounds. Biodegradation tests measuring ¹⁴C-CO₂ evolution, for example, can be conducted using initial concentration of test compound at parts per billion. These tests, however, require specialized equipment and the custom preparation of appropriately labeled compound is often very expensive.

4.2 Die-away biodegradation methods are simple simulation

¹ This specification is under the jurisdiction of 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 Annual Book of ASTM Standards, Vol 11.02.

⁴ Annual Book of ASTM Standards, Vol 11.05.

⁵ The boldface numbers in parentheses refer to the list of references at the end of this test method.