



Standard Test Method for Assessing the Microbial Detoxification of Chemically Contaminated Water and Soil Using a Toxicity Test with a Luminescent Marine Bacterium¹

This standard is issued under the fixed designation D 5660; 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 (1)² covers a procedure for the rapid evaluation of the toxicity³ of wastewaters and aqueous extracts from contaminated soils and sediments, to the luminescent marine bacterium *Photobacterium phosphoreum*,⁴ prior to and following biological treatment. This test method is meant for use as a means to assess samples resulting from biotreatability studies. Sensitivity data for *P. phosphoreum* to over 1300 chemicals have been reported in the literature (2). Some of the publications are very relevant to this test method (3). The data obtained from this test method, when combined with respirometry, total organic carbon (TOC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), or spectrophotometric data, can assist in the determination of the degree of biodegradability of a contaminant in water, soil, or sediment (3). The percentage difference between the IC₂₀ of treated and untreated sample is used to assess the progress of detoxification.

1.2 This test method is applicable to the evaluation of the toxicity (to a specific microbe) and its implication on the biodegradation of aqueous samples from laboratory research bio-reactors (liquid or soil), pilot-plant biological treatment systems, full-scale biological treatment systems, and land application processes (see Notes 1 and 2).

NOTE 1—If the biologically treated material is to be discharged in such

a manner as to potentially impact surface waters and ground water, or both, then the user must consult appropriate regulatory guidance documents to determine the proper test species for evaluating potential environmental impact (4). Correlations between data concerning reduction in toxicity produced by this test method and by procedures for acute or short-term chronic toxicity tests, or both, utilizing invertebrates and fish (see Guides E 729 and E 1192), should be established, wherever possible.

NOTE 2—Color (especially red and brown), turbidity, and suspended solids interfere with this test method by absorbing or reflecting light. In these situations data are corrected for these effects by use of an absorbance correction procedure included in this test method (see 5.3, 6.1, and 6.2).⁵

1.3 The results of this test method are reported in terms of an inhibitory concentration (IC), which is the calculated concentration of sample required to produce a specific quantitative and qualitative inhibition. The inhibition measured is the quantitative reduction in light output of luminescent marine bacteria (that is, IC₂₀ represents the calculated concentration of sample that would produce a 20 % reduction in the light output of exposed bacteria over a specified time).

1.4 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

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.* Specific hazard statements are given in Section 9.

2. Referenced Documents

2.1 ASTM Standards:⁶

D 888 Test Methods for Dissolved Oxygen in Water

¹ This test method is under the jurisdiction of ASTM Committee D34 on Waste Management and is the direct responsibility of Subcommittee D34.07 on Municipal Solid Waste.

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² The boldface numbers in parentheses refer to the list of references at the end of this standard.

³ Toxicity measured as toxic inhibition of bacterial light output.

⁴ Microbics Corp. is currently the only known supplier of the reagents (test organism *Photobacterium phosphoreum* strain NRRL B-11177) specific to this test method. There are two known manufacturers of analyzers that can be used to measure bioluminescence under temperature control: Microbics Corp., 2232 Ruthertford Road, Carlsbad, CA 92008 (Microtox Model 500 and Model 2055 Analyzers), and Pharmacia LKB, 9319 Gaither Road, Gaithersburg, MD 20877 (LKB Wallac Model 1250 and Model 1251 Luminometers). Other instruments would be considered when they become available. Please notify ASTM Subcommittee D34.09 if you are aware of any additional systems or instruments capable of performing this testing.

⁵ At present (1993) use of the color correction scheme described in this procedure is known to be effective only with the Microbics Corporation's toxicity analyzers, due to the fact that the correction mathematics involve the detailed geometry of both the ACC and the light meter. Please notify ASTM Subcommittee D34.09 if you are aware of any other source of equipment capable of providing color or turbidity correction, or both, for the *P. phosphoreum* test. Data validating the absorbance correction procedure are available from Microbics Corp.

⁶ 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.

