Designation: ~~G29 – 16~~ G29 – 21

Standard Practice for Determining ~~Algal~~Cyanobacterial Resistance of Polymeric Films[1]

This standard is issued under the fixed designation G29; 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.

This standard has been approved for use by agencies of the U.S. Department of Defense.

1. Scope

1.1 This practice covers the determination of the susceptibility of polymeric films to the attachment and proliferation of surface-growing ~~algae.~~cyanobacteria.

1.2 *Units*—The values stated in SI units are to be regarded as ~~the~~ standard. The ~~inch-pound units~~ values given in parentheses ~~are for information only.~~ after SI units are provided for information only and are not considered standard.

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~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Summary of Practice

2.1 In this practice, test strips of polymeric film are suspended in glass jars maintained at room temperature. The test strips are exposed to fluorescent light and in direct contact with a standardized inoculum of the filamentous blue-green ~~alga~~cyanobacterium *Oscillatoria* in culture medium. The sample test jars are re-inoculated with fresh ~~alga~~cyanobacteria every second or third day. A control using untreated polymeric film is used as a basis of comparison. The inoculum is prepared with the help of a propagation apparatus made from a small fish tank. The test is terminated at the end of two weeks, or whenever the untreated control shows dense ~~algal~~cyanobacterial growth.

3. Significance and Use

3.1 Bodies of water, such as swimming pools, artificial ponds, and irrigation ditches often are lined with polymeric films. ~~Algae~~Cyanobacteria tend to grow in such bodies of water under the proper atmospheric conditions, and they can produce slimy and unsightly deposits on the film. The method described herein is useful in evaluating the degree and permanency of protection against surface growth of ~~algae~~cyanobacteria afforded by various additives incorporated in the film.

4. Apparatus

4.1 *Propagation Tank:*

[1] This practice is under the jurisdiction of ASTM Committee G03 on Weathering and Durability and is the direct responsibility of Subcommittee G03.04 on Biological Deterioration.

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4.1.1 A small fish tank (10 gal) is used to contain ~~an algae~~a cyanobacteria propagation system where culture medium is recirculated through a polymeric tube with holes punched in the bottom over the top of a polymeric mesh screen inside of the tank. This design was developed in order to provide ideal conditions for propagation of the ~~algae~~cyanobacteria that serve as inocula for each test. The polymeric mesh is supported in such a way that water cascades over the top from a distributor tube above. A small, fully immersed recirculating pump rests on the bottom of the tank and operates continuously to deliver the tank contents to the distributor tube. The light required for ~~algal~~cyanobacterial propagation is provided by a ~~100-W~~100 W bulb placed ~~300 mm~~ 300 mm (12 in.) away from the polymeric mesh. A timing device turns the light on for the desired light cycle each day.

4.1.2 The propagation tank that is used as the permanent source of inoculum is filled to approximately one-third capacity with the culture medium. Heavy growth of *Oscillatoria* rapidly develops on the polymeric mesh screen and, at different phases, this growth appears light green, dark green, or black.

NOTE 1—Culture medium in the propagation tank is discarded monthly and replaced with fresh media.

4.2 *Test Chambers:*

4.2.1 ~~One-litre (1-qt)~~One litre (1 qt) wide-mouth glass jars, ~~170 mm~~ 170 mm (6¾ in.) high by ~~76 mm (3 in.)~~ 76 mm (3 in.) in inside diameter, or equivalent, serve as test chambers wherein water containing an inoculum of the ~~algal~~cyanobacterial organisms and strips of the polymeric film are maintained in contact.

4.2.2 The jars in 4.2.1 are placed in a suitable glass container, such as a ~~38-L (10-gal)~~38 L (10 gal) fish tank that is illuminated by four ~~20-W~~20 W "cool white" fluorescent bulbs, arranged two on each long side of the tank, at the level of the growing ~~algae~~cyanobacteria in the jars. The lamps are mounted on a bracket that holds the outer surface of the bulbs 25 mm (1 in.) from the wall of the tank. The tank is filled with water to within 25 mm (1 in.) of the top of the exposure jars in order to create uniform temperature conditions for all jars.

4.3 *Homogenizer*—Any suitable commercial homogenizer for preparing the ~~algal~~cyanobacterial inocula.

5. Reagents and Materials

5.1 *Purity of Reagents*—Reagent grade chemicals shall be 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.[2] 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.

5.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean distilled water or water of equal purity.

5.3 *Culture Medium for Propagation Apparatus*—Prepare this medium by dissolving in ~~15 L~~ 15 L of water the designated amounts of the following reagents:

~~Arnon's trace metal solution (see Note 2)~~	~~15 mL~~
~~Ethylenediamine tetraacetic acid disodium salt~~	~~0.04 g~~
~~Ferric ammonium citrate~~	~~0.85 g~~
~~Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)~~	~~10.0 g~~
~~Potassium acid phosphate (K_2HPO_4)~~	~~12.3 g~~
~~Potassium nitrate (KNO_3)~~	~~12.1 g~~
~~Sodium citrate~~	~~2.0 g~~
~~Sulfuric acid, 10 %~~	~~10 mL~~

[2] *Reagent Chemicals, American Chemical Society Specifications* American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

Arnon's trace metal solution (see Note 2)	15 mL
Ethylenediamine tetraacetic acid disodium salt	0.04 g
Ferric ammonium citrate	0.85 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	10.0 g
Potassium acid phosphate (K_2HPO_4)	12.3 g
Potassium nitrate (KNO_3)	12.1 g
Sodium citrate	2.0 g
Sulfuric acid, 10 %	10 mL

NOTE 2—Prepare this solution by combining the following reagents in the order given in the amounts designated in ~~1 L~~ 1 L of water:

~~Boric acid~~	~~2.86 g~~
~~Copper sulfate ($CuSO_4 \cdot 5H_2O$)~~	~~0.079 g~~
~~Manganese chloride ($MnCl_2 \cdot 4H_2O$)~~	~~1.18 g~~
~~Molybdic oxide (MoO_3)~~	~~0.018 g~~
~~Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)~~	~~0.22 g~~
Boric acid	2.86 g
Copper sulfate ($CuSO_4 \cdot 5H_2O$)	0.079 g
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Molybdic oxide (MoO_3)	0.018 g
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.22 g

5.3.1 BG 11 Medium for Cyanobacteria:

	stock solution [g/100 ml]	nutrient solution [ml]
$NaNO_3$	15	10
$K_2HPO_4 \cdot 3H_2O$	0.4	10
$MgSO_4 \cdot 7H_2O$	0.75	10
$CaCl2 \cdot 2H_2O$	0.36	10
citric acid	0.06	10
ferric ammonium citrate	0.06	10
EDTA (dinatrium-salt)	0.01	10
Na_2CO_3	0.2	10
Trace mineral solution[A]		1
de-ionized or distilled water		919

[A]Composition of the Trace minerals solution (from Kuhl and Lorenzen 1964): ~~Add to 1000 ml of de-ionized or distilled water:~~

~~H_3BO_3~~	~~61.0 mg~~
~~$MnSO_4 \cdot H_2O$~~	~~169.0 mg~~
~~$ZnSO_4 \cdot 7H_2O$~~	~~287.0 mg~~
~~$CuSO_4 \cdot 5 H_2O$~~	~~2.5 mg~~
~~$(NH_4)6Mo7O_{24} \cdot 4H_2O$~~	~~12.5 mg~~
Trace Minerals Solution:	
H_3BO_3	61.0 mg
$MnSO_4 \cdot H_2O$	169.0 mg
$ZnSO_4 \cdot 7H_2O$	287.0 mg
$CuSO_4 \cdot 5 H_2O$	2.5 mg
$(NH_4)6Mo7O_{24} \cdot 4H_2O$	12.5 mg

Note—Add the above minerals to 1000 mL of de-ionized or distilled water.

5.3.2 BG 11 Medium without sodium nitrate (BG 11–$NaNO_3$):
Prepare BG 11 medium without sodium nitrate ($NaNO_3$) and add ~~929 mL~~ 929 mL instead of ~~919 mL~~ 919 mL of water.

5.3.3 Adjust to 7.2 to 7.5 pH range if necessary. Filter sterilization is recommended.

5.4 *Culture Medium for Test Vessels*—Dilute ~~50 mL~~ 50 mL of the culture medium in 5.3 with ~~950 mL~~ 950 mL of deionized or distilled water and place this medium in the test vessels.

6. Test Specimens

6.1 Select at random from the sample sufficient film to prepare the required number of test specimens ~~25 mm~~ 25 mm by 65 mm (1 in. by 2½ ~~in.).~~ in.).