Designation: E875 – 15

Standard Practice for Evaluation of Fungal Control Agents as Preservatives for Aqueous-Based Products Used in the Paper Industry[1]

This standard is issued under the fixed designation E875; 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 laboratory practice is used to determine the efficacy of a fungal control agent to prevent spoilage of in-process aqueous-based products used in the paper industry.

1.2 For information on bacterial control agents, see Test Method E723.

1.3 It is the responsibility of the investigator to determine whether good laboratory practices (GLP) are required and to follow them when appropriate (see 40 CFR 160).

1.4 A knowledge of microbiological techniques is required for these procedures.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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:*[2]

D1193 Specification for Reagent Water

E723 Practice for Evaluation of Antimicrobials as Preservatives for Aqueous-Based Products Used in the Paper Industry (Bacterial Spoilage)

E1839 Test Method for Efficacy of Slimicides for the Paper Industry—Bacterial and Fungal Slime

2.2 *Federal Standard:*

40 CFR 160 Good Laboratory Practice Standards[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *fungal control agent, n*—an agent that either kills or prevents growth of fungi and either kills or prevents the germination of fungal spores. This term is applied to chemical biocidal or biostatic agents.

3.1.2 *preservative, n*—chemical agent used to prevent microbial spoilage of products due to microbial growth.

4. Summary of Practice

4.1 Aqueous material to be preserved is inoculated with an appropriate fungal innoculum followed by addition of a concentration of fungal control agent that will kill the fungi or prevent their growth for a desired period of time, or both. In addition, the agent will also prevent fungal spore germination. Fungal growth is determined by visible signs of deterioration in the test sample, and by obtaining fungal numbers and comparing them to a sample without any fungal control agent. The proper level of fungal control agent is one that prevents product deterioration and reduces and keeps the organisms to an acceptable level in the test material, as determined by the tester or user.

5. Significance and Use

5.1 This practice should be used to determine if a fungal control agent is effective to preserve pigment suspensions, dye solutions, pulp slurries, starch solutions, polymers, sizing agents, latex emulsions, and other specific aqueous-based materials used in the paper industry. Separate evaluations should be made on a representative type for each specific class of product to be preserved.

NOTE 1—Control of bacterial spoilage of similar products can be evaluated by Test Method E723.

NOTE 2—Slimicides for control of fungal or bacterial slime can be evaluated by Test Method E1839.

[1] This practice is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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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.

[3] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

6. Apparatus

6.1 *Two Balances*— One should be sensitive to 0.1 g at a load of 200 g with a platform to accommodate bottles being used in the test. The second balance (analytical) should be sensitive to 0.1 mg and used for weighing test chemicals.

6.2 *Clean Sample Containers,* Containers (120 mL) with screw- cap lids are ideal for test aliquots. Other suitable containers include milk dilution bottle, 4 oz glass bottles, or sterile sampling bags.

6.3 *Flaming Equipment*—An alcohol lamp, bunsen burner, or electric device may be used to flame inoculating needles and other equipment.

6.4 *Incubators*—Incubators that control the temperature of the test ± 2°C. Temperatures for test should be temperature at which the product will be stored.

6.5 *Petri Dishes,* 100 by 15-mm, plastic or borosilicate glass, sterile.

6.6 *pH Measurement*—Any pH meter is suitable to standardize the pH of the culture medium or to determine pH of samples. Nonbleeding test strips may be used for determining pH of test aliquots.

6.7 *Pipets*—1.0-mL graduated in 0.01 mL and 10-mL graduated in 0.1 mL. Serological pipets should not be used for highly viscous materials. Automatic pipettors may be used.

6.8 *Pipetting Aid*—rubber bulb or other device to eliminate mouth pipetting.

6.9 *Sterilizers*—pressurized steam sterilizer (121°C at 15 psi) or hot air oven capable of reaching 180 ± 2°C for 2 ± 0.2h.

6.10 *Swabs*—Sterile swabs (cotton or other appropriate fabric type) for aiding in removal of fungal spores from slants.

6.11 *Sterile Funnel*—Funnel with sterile glass wool for filtration of spore suspension.

6.12 *Sterile Glass Beads*—Glass beads (3-5 mm).

6.13 *Tubes*—Tubes for preparation of slanted media.

6.14 *Milk Dilution Bottles, (100 mL).*

7. Reagent and Materials

7.1 *Purity of Water*—Unless otherwise indicated, water shall be understood to mean distilled water or water of equal purity, as defined in Specification D1193, Type 3.

7.2 Freshly prepared test solutions of the fungal control agent shall be used in all tests. Some preservatives can be added with a micropipet.

7.3 *Test Materials*—Freshly prepared pigment slurries, adhesives, dye rosin, polymer, sizing solutions, and other classes of aqueous-based materials to be preserved should be used as the substrate.

7.4 *Culture Medium*—Dehydrated Sabouraud's Agar (maltose or dextrose) is recommended for fungi. A more selective medium may be used provided it is used in addition to Sabouraud. Results should indicate the data obtained with each medium.

7.4.1 *Spore Suspending Medium and Container*—Milk dilution bottles containing 100 mL Butterfield Buffer[4] with solid glass beads, for preparing sterile spore suspensions.

7.4.2 *Culture Media,* slants of the selected agar.

8. Test Organisms

8.1 The test organisms selected may vary with the purpose of the test. If evaluating the basic effectiveness of a fungal control agent, the use of standard fungal cultures is recommended (see 8.2). If attempting to qualify a fungal control agent for a particularly difficult, or highly specific preservation application, the use of fungal spoiled product or selected fungal organisms isolated from the problem system, or similar systems, may be appropriate (see 8.3 and 8.4).

8.2 Standard fungal cultures suitable for this procedure include the following:

8.2.1 *Aspergillus niger:* ATCC 6275.

8.2.2 *Penicillium pinophalum:* ATCC 9644.

8.2.3 *Trichoderma virens:* ATCC 9645.

8.2.4 *Candida albicans:* ATCC 10231.

8.2.5 *Saccharomyces cerevisiae:* ATCC 4111.

8.3 To verify that a spoiled sample contains fungal organisms, the spoiled sample should be streaked onto plates of Sabouraud Maltose Agar (or other media selected). When fungal contamination is verified, if sample is large enough, it may be used directly as the inoculum (see 10.1). If sample is too small for use as inoculum, add the spoiled sample to a larger sample of unprotected material and incubate for 7 to 14 days at an appropriate temperature (based on use conditions) until spoiled, then proceed with testing (see 10.1).

8.4 If specific or combinations of fungal isolates from spoiled material are to be used, proceed with testing as for standard fungal organisms. See 9.1.

9. Inoculum Preparation

9.1 *Standard Fungi and Fungal Isolates*—Organisms should be grown as slant cultures on the culture medium selected. Grow for 7 to 14 days at 25 to 30°C, until cultures sporulate. Add 10 mL of sterile Butterfields buffer to the slants. Gently remove the spores from the surface of the agar by rubbing with a sterile swab. Add the resulting suspension to a bottle containing glass beads and buffer. Cap the bottle tightly, and then shake vigorously to liberate spores from fruiting bodies and to break up spore clumps. Filter the resulting suspension through sterile glass wool in a sterile funnel into a sterile container to remove mycelial fragments. This filtrate is the spore suspension to be used as an inoculum for test samples.

10. Procedure

10.1 To provide a uniform inoculated substrate, the inoculum of spoilage organisms or fungal spore suspension should be added to the entire quantity of the test substrate at one time, mixed thoroughly, then divided into test aliquots.

[4] Butterfields Buffered Phosphate Diluent, Official Methods of Analysis of the Association of Official Analytical Chemists, K. Helrich, 15th ed., 1990, p. 429.

10.1.1 Streak-out the test substrate (aqueous-based products) before and after fungal inoculation to and determine the fungal contamination. Incubate plates at 25 to 30°C for seven days or until the control plates show sufficient growth for rating. Some test materials may not support growth of fungi. These materials most likely do not require a preservative to prevent fungal growth. If the test material only supports slow growth of the fungi in question, then incubation periods must be sufficiently long to allow those fungi to grow.

10.1.2 Evaluate the level of growth with either a growth/no-growth rating, or a rating scale (such as 0 to 4, with 0 being no-growth and 4 being heavy-growth).

10.2 Disperse 50-g aliquots, or any other suitable quantity, of the inoculated test substrate aseptically into the containers selected. Set up controls (no biocide added) in duplicate.

10.2.1 Aseptically add the stock solution of the test fungal control agent to each bottle to give the desired concentration in parts per million (mg/mL) or percent. Shake vigorously using 20 complete cycles in a vertical motion or other method to ensure complete mixing. Stock solution of the fungal control agent should be of such strength that the volume of the solution added is no more than 1 % of the total volume of sample in each bottle. Do not add a fungal control agent to the untreated controls. Include a minimum of five concentrations of the fungal control agent in each test. The test concentration range should include at least one concentration that is ineffective and one that is effective. Record the initial pH of all samples and other physical characteristics such as color, odor, and viscosity.

10.2.2 Incubate all samples at 25 to 30°C, (or other temperature that the product would encounter either in use or storage situations), with the bottles loosely capped. At weekly or other suitable time intervals, mix each sample thoroughly and immediately streak on Sabouraud's Agar according to standard streaking techniques and incubate at 25-30°C for seven days. In addition, observe each sample bottle for the evidence of spoilage such as visual growth of fungi, or changes in color, odor, and pH. Record the growth of the fungi for the streaked samples using rating system in 10.2.1.

10.2.3 Rechallenge all treated samples showing no visible growth with 1 mL of inoculum obtained from one of the duplicate fungal controls samples (no biocide added). Incubate for at least one more week.

10.2.4 Base success for each preservation experiment upon the expected time of usage of the material to be preserved. Continue the test for at least the average length of time that the specific type of product would need protection, generally six weeks, unless the material under test is preserved for a shorter period of time, for example, one week. Record the nature and extent of spoilage based upon the criteria used (10.1.2 and 10.2.1).

11. Results

11.1 At each sampling time and at the end of the test, record all results from observations of the sample bottles and the streak plates. Visual deterioration or other signs of degradation in the bottles, such as changes in pH color, odor, and loss of viscosity, should also be used to judge the degree of preservation obtained. The study is not valid unless fungal spoilage is evident in the control, based on visual fungal growth, obvious changes in physical characteristics, or other reliable criteria.

12. Precision and Bias

12.1 A precision and bias statement cannot be made for this practice.

13. Keywords

13.1 aqueous-based products; fungi; fungal control agent; fungal spores; paper; preservative