Designation: E 1839 – 96 (Reapproved 2002)

Standard Test Method for Efficacy of Slimicides for the Paper Industry—Bacterial and Fungal Slime[1]

This standard is issued under the fixed designation E 1839; 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 presents a procedure to evaluate the efficacy of slimicides for the control of bacterial and fungal slimes in paper mill systems and their counterparts.

1.2 It is the responsibility of the investigator to determine whether Good Laboratory Practices (GLP) are required and to follow them where appropriate (40 CFR 160).

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.*

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 1193 Specification for Reagent Water

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfection, Sanitizer, Antiseptic, or Preserved Products

2.2 *TAPPI Standard:*

T 205 Forming Handsheets for Physical Tests of Pulp[3]

2.3 *CFR Standard:*

Title 40, Code of Federal Regulations (CFR), Part 160, Good Laboratory Practice Standards[4]

3. Terminology

3.1 *Definitions:*

3.1.1 *furnish*, *n*—pulp slurry fed to a paper machine. The type of pulp (sulfite, Kraft, mechanical), the source of fiber (virgin, recycled including pre- or post-consumer waste paper), and the pH are used to designate a specific type of furnish.

3.1.2 *pulp*, *n*—wood separated by chemical or mechanical means into their fibrous components. The pulp is used to make paper, paper board, or pulp sheets after specific treatments. Hardwood pulp is made from trees, such as maples or oaks, and softwood pulp is produced from trees, such as pines.

3.1.3 *pulp slurry*, *n*—an aqueous combination of cellulosic fibers, fillers, and other additives used for specific grades of paper.

3.1.4 *slimicides*, *n*—chemicals added during pulp and paper processing to control the growth of slime-forming microorganisms.

4. Summary of Test Method

4.1 Bacterial cells or fungal spores are added to acid or alkaline pulp slurries, or both, treated with slimicides to achieve final concentrations of 2×10^6 to 1×10^7 bacteria/mL or 10^5 to 10^6 fungal spores/mL, and incubated at appropriate temperature for determined time periods. Aliquots of the test suspension are then neutralized, plated onto bacterial or fungal medium, and observed for growth. Results with biocide are compared to results without biocide (control).

4.2 As a performance standard, an effective slimicide is one that shows a continued reduction in bacterial and fungal counts relative to the control over the duration of the test.

5. Significance and Use

5.1 This test method is to be used to determine if a slime control agent has application in the paper industry for control of bacterial or fungal slime.

5.2 This test method is run in acid, alkaline, or acid and alkaline conditions to determine the efficacy of the slime control agent.

5.3 The test conditions may be modified to reflect intended use patterns in typical paper mill systems, including use of actual paper mill furnish.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antibacterial 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] Forming Handsheets for Physical Tests of Pulp, TAPPI Test Method T 205 on-88, 1994–1995, TAPPI, Atlanta, GA, 30348.

[4] Available from U.S. Government Printing Office, Superintendent of Documents, Mail Stop: SSOL, Washington, DC 20402-9328.

6. Apparatus

6.1 *Balance*:

6.1.1 *Plant Balance*, sensitive to 0.1 g and used to weight furnish.

6.1.2 *Analytical Balance*, sensitive to 0.1 mg and employed to weigh the candidate slime control agent to be used in the preparation of the stock solutions.

6.2 *Sample Containers (Sterile)*, 120-mL plastic specimen containers with screw-cap lids are ideal for holding test materials. Other suitable containers include 150/160-mL milk dilution bottles or WHIRL-PAKS.

6.3 *Culture Containers*, Petri plates, tissue culture bottles or glass tubes (15 × 125 mm or 18 × 150 mm without lip, preferably of borosilicate glass).

6.4 *Closures*, for tubes and containers.

6.5 *Disintegrators*[5]

6.6 *Flaming Equipment*—Depending upon circumstances, either an alcohol lamp, a bunsen burner, or electric incinerator may be used to flame inoculating needles and other equipment.

6.7 *Reliable incubators* that control at the temperature required, ± 2°C. Temperatures used should be consistent with the temperatures of the systems.

6.8 *pH Meter*—Any reliable pH meter is suitable to standardize the pH of the culture.

6.9 *Pipets*—1.1-mL milk dilution type, 1.0 mL graduated in 0.01 mL, and 10 mL graduated in 0.1 mL. Pipetters may be used, but not for highly viscous materials.

6.9.1 *Pipetting Aid*—Rubber bulb or other device to accomplish the transfer of liquid.

6.10 *Sterilizers*, steam sterilizer (121°C) or hot-air oven (180 ± 2°C for 2 h), or both.

6.11 *Filter Apparatus for Filter Sterilizing*, Disposable filter units, appropriate volume, 0.22-µm pore size.

6.12 *Sterile Funnel*, with sterile glass wool or sterile cotton gauze for filtration of spores.

6.13 *Colony counter*, manual, such as the Quebec, Buck, Wolffhuegel, or equivalent; or a colony image analyzer (electronic/scanner type) are suitable for counting plates after incubation. A hand tally for recording of bacteria count is recommended.

6.14 *Swabs*, sterile, for aiding in removal of fungal spores from agar surface.

6.15 *Hemacytometer*, for counting spore suspension.

6.16 *Microscope*, that provides a magnification of 400 to 1000× and is complete with a suitable light source. Phase contrast or dark field capability is desirable.

6.17 *Constant Temperature Shaker*—A reliable constant-temperature shaker (water bath or incubator type), shall be used to provide mixing and aeration and to maintain a selected temperature (± 2°C) during the contact period.

6.18 *Mechanical Stirrer*—Magnetic or propeller-type stirrers or any other suitable device.

[5] Forming Handsheets for Physical Tests of Pulp. Appendix A: Specifications and Care of Apparatus (Disintegrator), TAPPI Test Method T 205 on-88. 1994–1995. TAPPI, Atlanta, GA 30348.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent 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.[6] 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 Water*—Unless otherwise indicated, references to water shall be understood to mean distilled water or water of equal purity (see Specification D 1193, Type III).

7.3 *Buffer for Suspending Spores and for Dilutions*, sample containers having 100-mL phosphate buffer dilution water, sterile, for spore suspension have solid, sterile glass beads in container.

7.3.1 *0.25 M Phosphate Buffer Stock Solution*—Dissolve 34 g of reagent grace KH_2PO_4 in 500 mL of distilled water and mix. Adjust to pH 7.2 with 1 *N* NaOH and dilute to 1 l.

7.3.2 Phosphate buffer dilution water. Add 1.25 mL of 0.25 *M* phosphate buffer stock solution to 1 L of distilled water and mix. Dispense to sample container and sterilize.

7.4 *Aluminum Sulfate (Alum)* $[Al_2(SO_4)_3\ 18H_2O]$—Prepare a 0.4 % solution of the hydrated aluminum in distilled water and sterilize in an autoclave. Any loss of water during sterilization is made up by adding sterile distilled water. Alternately, the solution may be filter sterilized.

7.5 *Acid and Base for pH Adjustment to Make Acid and Alkaline Furnish*:

7.5.1 Prepare a 2 *N* solution of sulfuric acid in water. Sterilize by filtration.

7.5.2 Prepare a 2.0 *N* solution of sodium hydroxide in water. Sterilize by filtration.

7.6 *Pulp*—A two-third hardwood and one-third softwood pulp, typical of current production techniques, and that has been produced without slimicide is needed.[7] Disintegrate the sheet in distilled water until free of fiber clots and undispersed fiber bundles.[5] Avoid methods which involve extensive cutting of fibers. The concentration of the pulp in water should be 1 %.

7.7 *Bacterial and Fungal Culture Medium*:

7.7.1 *Bacteria*—Standard dehydrated tryptone glucose extract agar or equivalent is recommended. Adjust pH of culture medium to pH of the test system.

7.7.2 *Fungi*—Sabouraud Dextrose Agar or Potato Dextrose Agar are recommended for enumeration. Adjust pH of culture medium to pH of the test system.

[6] *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.

[7] The sole source of supply of the apparatus known to the committee at this time is Zellerbach, 808 Rhodes Ave., Columbus, OH 43205. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.