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Standard Guide for Selecting Test Methods to Determine the Effectiveness of Antimicrobial Agents and Other Chemicals for the Prevention, Inactivation and Removal of Biofilm[1]

This standard is issued under the fixed designation E 1427; 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.

ε[1] NOTE—Sections 1.1 and 1.4 were editorially updated in June 2001.

1. Scope

1.1 Microorganisms attach to surfaces and grow, forming communities that are called biofilms. In addition to microorganisms, biofilms may contain the by-products of microbial growth (that is, polysaccharides, enzymes, etc.), inorganic ions (that is, Mg, Ca, Fe, etc.) and organic materials (that is, oil, exudates from plants or animals, etc.). Biofilms may be found in many places, including on cooling system equipment (that is, cooling towers, heat exchangers, etc.), water and oil pipelines, food and pharmaceutical processing surfaces and lines, dental water unit lines and medical prosthetic devices.

1.2 Biofilm formation may lead to reduced heat transfer in cooling towers, decreased fluid flow in pipelines, corrosion of metal surfaces, spoilage of food and pharmaceutical products, and infection in humans. The adverse impact of biofilm growth has led to the need for chemical or physical treatments for controlling them. This may involve preventing biofilm formation, inactivating microbes in biofilms and removing biofilms.

1.3 Since biofilms may form in many different types of systems, no one method can be presented that evaluates all the factors affecting biofilm control; therefore, many methods are presented for forming biofilms. Detecting and measuring biofilms and microorganisms within biofilms are important in evaluating control procedures. Many procedures are listed and referenced for measurement of microorganisms in biofilms and biofilm mass and activity.

1.4 The purpose of this guide is to inform the investigator of methods that can be used for biofilm formation and measurement, allowing development of test procedures for determining the effectiveness of chemical treatments for prevention, inactivation, and removal of unwanted biofilm. This guide is a teaching tool that will help the researcher in planning studies for controlling biofilms. This guide is not an exhaustive survey of biofilm methods. It is recommended that the researcher consult the latest information on biofilm methods from the published scientific literature and from appropriate internet sites, using biofilm as the keyword.[2]

1.5 Discussions of various methods for evaluating efficacy of potential control materials against microorganisms in solution are available.[3]

2. Referenced Documents

2.1 This guide lists methods that can be used in forming and measuring biofilms, which allows development of test methods for determining the effectiveness of chemical and physical treatments for prevention, inactivation, and removal of unwanted biofilm. Published procedures for biofilm formation and measurement (Sections 4 and 5) are referenced.

3. Significance and Use

3.1 This guide should be used by individuals responsible for the following:

3.1.1 The maintenance of systems in which fluids come in contact with surfaces, which adversely could be effected by the presence of biofilm.

3.1.2 The development of methods, that is, chemicals, to prevent, inactivate, or remove biofilm from various systems.

3.1.3 The verification of specific claims for chemicals to prevent, inactivate, or remove biofilm from specific systems.

3.2 The systems considered include, but are not limited to, those designed for drinking water distribution, food processing, industrial process fluids, and treated or untreated body fluids.

3.2.1 The adverse effects of biofilm in these systems include product spoilage, loss of production, corrosion, reduced heat transfer, increased morbidity and mortality of the general population, and outbreaks of hospital-acquired infections. Since many different published methods, which have not undergone the rigors of ASTM Interlaboratory Testing, are

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[2] Suggested internet sites are PubMed at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the American Society for Microbiology (www.journals.asm.org). Utilizing this technology the researcher may obtain the latest information on biofilms, and tailor their search for the specific information they need.

[3] ASTM Standards on Materials and Environmental Microbiology, 2nd Edition, 1993.

referenced, it is the responsibility of the investigator to verify the validity of the methods selected or developed for the intended application.

3.3 The information presented in Section 4 is a limited listing of test procedures, with references, for biofilm formation. These procedures are a guide to the many ways that are used to form biofilms. Selection of specific test parameters enables simulation of applicable field conditions. Among the parameters that should be considered are nutrients, miscellaneous nonnutrients organics, inorganic salts and ions, corrosion products, temperature, pH, redox potential, aerobic conditions, flow rate, shear, time, substratum (type and texture), and microorganism types and their interactions **(1,2)**.[4] Methods that can be used to measure biofilm formation are outlined in Section 5. These are a limited number of referenced methods and are intended only as a guide. Methods selected by investigators depend on which criteria are most important in the system, that is, microbial population densities, biomasses accumulation, or metabolic activities, or a combination thereof. In any case, these methods should be used by individuals familiar with microbiological techniques.

4. Substratum and Laboratory Methods for Biofilm Formation, Either Static or Dynamic Models (Continuous or Batch) (1-9)

4.1 Coupons overlayed with microbial suspension **(10, 11)**.

4.2 Coupons (metals, plastic, glass, etc.) in beakers or fouling loops **12-14)**.

4.3 Coupons overlayed with hydrogel **(15)**.

4.4 Polycarbonate membranes overlain with microbial suspension **(16)**.

4.5 Plexiglass, reactor **(17)**.

4.6 Glass beakers **(18)**.

4.7 Powders or small beads in column or beaker **(19-22)**.

4.8 Hydroxyapatite beads or discs **(23, 24)**.

4.9 Alginate beads **(25, 26)**.

4.10 Tubing or pipe sections filled with or immersed in microorganism suspension **(27)**.

4.11 Tubing/ or pipes in lab biofouling loop **(28, 29)**.

4.12 Prescored sample sections **(30)**.

4.13 Stainless steel rings **(14)**.

4.14 Microtiter plates **(31-33)**.

4.15 Plugs (Robbin's device), discs in rubber strips **(5, 34)**.

4.16 Rototorque (annular reactor) **(35-37)**.

4.17 Constant depth film fermentor **(38)**.

4.18 Rotating Biological Reactor **(39)**.

4.19 Rotating Disc Reactor Method **(29, 40)**.

4.20 Model cooling tower **(41, 42)**.

4.21 Parallel plate flow chamber or cell **(43-46)**.

4.22 Capillary tubes (flowcells) **(9, 47, 48)**.

5. Measurements of Biofilm

5.1 *Population Viable Cell Density*:

5.1.1 Microscopic methods (Brightfield, Epifluorescence, Scanning Confocal).

5.1.1.1 Growth response to nalidixic acid **(52-54)**.

5.1.2 *Vital Dyes*:

5.1.2.1 Viablue 2 **(59)**.

5.1.2.2 Fluorescein diacetate **(56-58)**.

5.1.2.3 Rhodamine 123 **(59)**.

5.1.2.4 Tetrazolium salts **(60-65)**.

5.1.3 *Molecular Probes*:

5.1.3.1 r-RNA **(66)**.

5.1.3.2 Immunologic probes **(66, 67)**.

5.1.4 Colony forming units or most probable number methods:

5.1.4.1 Scraping and plating **(68, 69)**.

5.1.4.2 Swabbing and plating **(70)**.

5.1.4.3 Sonicating and plating **(71)**.

5.1.4.4 Agar contact method **(72, 73)**.

5.1.4.5 Squeegee and rinse **(74)**.

5.1.4.6 Alginate or hydrogel/dissolve/plate **(15, 75)**.

5.1.4.7 Biofilm growth in microtiter plates **(31)**.

5.1.5 *Radiolabelling to determine population density:*

5.1.5.1 Microautoradiography **(76, 77)**.

5.1.5.2 Radiolabelled cells **(78)**.

5.2 *Metabolic Activity*—Gross activity of biofilm:

5.2.1 Bioluminance **(76)**,

5.2.1.1 ATP **(77)**

5.2.1.2 Lux gene **(78, 79)**

5.2.1.3 Tryptophan **(80)**

5.2.2 Radiolabelled substrate uptake or metabolism of substrate with release of radioactive compound **(54, 84-86)**.

5.2.3 Enzymatic **(80, 87-90)**.

5.2.4 Impedance **(91-93)**.

5.2.5 Respirometry **(94–95)**.

5.2.6 Microcalorimetry **(96)**.

5.2.7 Nuclear magnetic Resonance **(97)**.

5.2.8 Attenuated-total-reflection (ATR) Fourier-transform-infrared-spectroscopy (FTIR) **(98)**.

5.3 *Biomass*—Total Viable and nonviable cells with associated biofilm material:

5.3.1 *Microscopy* **(99)**:

5.3.1.1 Brightfield.

5.3.1.2 Phase contrast.

5.3.1.3 Epifluorescence **(100-104)**.

5.3.1.4 Scanning Electron Microscope **(105–106)**.

5.3.1.5 Interference reflection and light section **(107)**.

5.3.1.6 Differential interference contrast microscopy **(108)**.

5.3.1.7 Electron microscope **(109, 110)**.

5.3.1.8 Confocal microscope **(111)**.

5.3.2 *Spectroscopic*:

5.3.2.1 Bacteria on translucent surface **(13, 112)**.

5.3.2.2 DNA absorption (260 nm/280 nm).

5.3.3 Components of microorganisms (organic nitrogen, carbon, chlorophyll, lipopolysaccharide lipid, protein, carbohydrate, fatty acid analysis, glycocalyx **(4, 20, 88, 101, 113-116)**.

5.3.4 Weight (dry at 103°C, volatile 550°C) **(117, 118)**.

5.3.5 Thickness of biofilm **(119)**.

5.3.6 Biofilm mass **(120)**.

5.3.7 Heat transfer resistance **(17)**.

5.3.8 Pressure gradients/friction resistance **(15, 120, 121)**.

[4] The boldface numbers in parentheses refers to the list of references at the end of this standard.

6. Keywords

6.1 biofilm; biomass; formation; inactivation; microbial activity; population density; prevention; removal; sessile population

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