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.

Designation: E2196 – 17

Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with Medium Shear and Continuous Flow Using Rotating Disk Reactor[1]

This standard is issued under the fixed designation E2196; 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 is used for growing a reproducible (**1**)[2] *Pseudomonas aeruginosa* biofilm in a continuously stirred tank reactor (CSTR) under medium shear conditions. In addition, the test method describes how to sample and analyze biofilm for viable cells.

1.2 Although this test method was created to mimic conditions within a toilet bowl, it can be adapted for the growth and characterization of varying species of biofilm (rotating disk reactor—repeatability and relevance (**2**)).

1.3 This test method describes how to sample and analyze biofilm for viable cells. Biofilm population density is recorded as $\log_{10}$ colony forming units per surface area (rotating disk reactor—efficacy test method (**3**)).

1.4 Basic microbiology training is required to perform this test method.

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.*

1.7 *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. Referenced Documents

2.1 *ASTM Standards:*[3]

D5465 Practices for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods

2.2 *Other Standards:*

Method 9050 C.1.a Buffered Dilution Water Preparation (**4**)

3. Terminology

3.1 *biofilm, n*— microorganisms living in a self-organized community attached to surfaces, interfaces, or each other, embedded in a matrix of extracellular polymeric substances of microbial origin, while exhibiting altered phenotypes with respect to growth rate and gene transcription.

3.1.1 *Discussion*—Biofilms may be comprised of bacteria, fungi, algae, protozoa, viruses, or infinite combinations of these microorganisms. The qualitative characteristics of a biofilm, including, but not limited to, population density, taxonomic diversity, thickness, chemical gradients, chemical composition, consistency, and other materials in the matrix that are not produced by the biofilm microorganisms, are controlled by the physiochemical environment in which it exists.

3.2 *coupon, n*—biofilm sample surface.

4. Summary of Test Method

4.1 This test method is used for growing a reproducible *Pseudomonas aeruginosa* biofilm in a rotating disk reactor. The biofilm is established by operating the reactor in batch mode (no flow) for 24 h. Steady state growth (attachment is equal to detachment) is reached while the reactor operates for an additional 24 h with continuous flow of the nutrients. The residence time of the nutrients in the reactor is set to select for biofilm growth, and is species and reactor parameter specific. During the entire 48 h, the biofilm is exposed to continuous fluid shear from the rotation of the disk. At the end of the 48 h, biofilm accumulation is quantified by removing coupons from

[1] This test method 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] The boldface numbers in parentheses refer to a list of references at the end of this standard.

[3] 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.

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