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Standard Guide for Microbially Induced Corrosion of Concrete Products[1]

This standard is issued under the fixed designation C1894; 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 guide discusses microbially induced corrosion (MIC) of concrete products and laboratory test methods for determining the resistance of concrete to MIC. Although the guide is intended for concrete products, it also covers cementitious mortar and paste that are used in specialized applications or laboratory investigations.

1.2 While this guide discusses concrete materials and admixtures, the document is not intended to specifically address field exposure conditions or sewage pipe, concrete tank, or concrete riser network design.

1.3 This guide does not cover live trial tests where concrete coupons or other specimens are monitored in sewers.

1.4 This guide does not cover concrete deterioration due to chemical sulfate attack, which is caused by the reaction of sulfate compounds that exist in wastewater with the hydration products of cement. Test methods for assessing sulfate attack are provided by Test Methods C452 and C1012/C1012M.

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 The text of this guide references notes and footnotes that provide explanatory material. These notes and footnotes (excluding those in tables and figures) shall not be considered as requirements of the standard.

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

1.8 *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:*[2]

C31/C31M Practice for Making and Curing Concrete Test Specimens in the Field
C33/C33M Specification for Concrete Aggregates
C42/C42M Test Method for Obtaining and Testing Drilled Cores and Sawed Beams of Concrete

[1] This test method is under the jurisdiction of ASTM Committee C13 on Concrete Pipe and is the direct responsibility of Subcommittee C13.03 on Determining the Effects of Biogenic Sulfuric Acid on Concrete Pipe and Structures.

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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.

C125 Terminology Relating to Concrete and Concrete Aggregates
C150/C150M Specification for Portland Cement
C192/C192M Practice for Making and Curing Concrete Test Specimens in the Laboratory
C260/C260M Specification for Air-Entraining Admixtures for Concrete
~~C267 Test Methods for Chemical Resistance of Mortars, Grouts, and Monolithic Surfacings and Polymer Concretes~~
C294 Descriptive Nomenclature for Constituents of Concrete Aggregates
C452 Test Method for Potential Expansion of Portland-Cement Mortars Exposed to Sulfate
C494/C494M Specification for Chemical Admixtures for Concrete
C497 Test Methods for Concrete Pipe, Concrete Box Sections, Manhole Sections, or Tile
C595/C595M Specification for Blended Hydraulic Cements
C618 Specification for Coal Fly Ash and Raw or Calcined Natural Pozzolan for Use in Concrete
C822 Terminology Relating to Concrete Pipe and Related Products
C989/C989M Specification for Slag Cement for Use in Concrete and Mortars
C1012/C1012M Test Method for Length Change of Hydraulic-Cement Mortars Exposed to a Sulfate Solution
C1017/C1017M Specification for Chemical Admixtures for Use in Producing Flowing Concrete (Withdrawn 2022)[3]
C1240 Specification for Silica Fume Used in Cementitious Mixtures
C1600/C1600M Specification for Rapid Hardening Hydraulic Cement
C1898 Test Methods for Determining the Chemical Resistance of Concrete Products to Acid Attack
C1904 Test Methods for Determination of the Effects of Biogenic Acidification on Concrete Antimicrobial Additives and/or Concrete Products
D4262 Test Method for pH of Chemically Cleaned or Etched Concrete Surfaces
D4783 Test Methods for Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi
G21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

2.2 *Other Standards:*[4]

ISO 22196 Measurement of antibacterial activity on plastics and other non-porous surfaces

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this practice, refer to Terminology standards C125 and C822.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *antimicrobial admixture, n*—EPA registered chemical admixture that is intended to inhibit microorganism growth (-static effect) or kill microorganisms (-cidal effect). Antimicrobial admixtures are registered according to the organisms they are effective against and typically, due to their chemical nature for industrial use, have broad spectrum effectiveness against many organism types, including bacteria, fungi and algae.

3.2.2 *aerobic bacteria, n*—bacteria that have a metabolic requirement for the presence of available oxygen to grow and thrive.

3.2.3 *anaerobic bacteria, n*—bacteria that do not live or grow when oxygen is present.

3.2.4 *biofilm, n*—a complex mixture of established microorganisms, microorganism components (extra-cellular matrix) and environmental detritus.

3.2.5 *biogenic (biotic) acidification, n*—process of production of mixture of inorganic and organic acids from respiring organisms resulting in acidification of the microbial environment.

3.2.6 *chemical (abiotic) acidification, n*—when compounds like ammonia, nitrogen oxides and sulphur dioxides are converted in a chemical reaction into acidic substances.

3.2.7 *Desulfovibrio desulfuricans, n*—anaerobic dissimilatory sulfate-reducing bacterium.

3.2.8 *dissolved oxygen (DO) content, n*—oxygen (O_2) molecules available for respiration to aquatic organisms.

[3] The last approved version of this historical standard is referenced on www.astm.org.
[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

3.2.9 *hydrogen sulfide (H_2S), n*—a colorless poisonous gas made by the action of acids on sulfides. At low concentrations, H_2S has the odor of rotten eggs, but at higher, lethal concentrations, it is odorless.

3.2.10 *microbially induced corrosion (MIC) of concrete, n*—a multi-stage deterioration process influenced by the presence and activities of bacteria within wastewater collection, storage and treatment infrastructure. Also referred to as biogenic sulfuric acid (BSA) corrosion, and biological corrosion of concrete, hydrogen sulfide corrosion, microbial corrosion of concrete.

3.2.11 *chemical oxidation, n*—chemical reaction in which the atoms in a molecule lose electrons and the net valence of the molecule is correspondingly increased, commonly associated with addition of molecular oxygen to the chemical composition of an 'oxidized' material.

3.2.12 *sulfate oxidizing bacteria (SOB), n*—bacteria that can convert hydrogen sulfide (H_2S) into elemental sulfur (S) by partial oxidation, or sulfate (SO_4^{2-}).

3.2.13 *sulfate reducing bacteria (SRB), n*—bacteria that can obtain energy by oxidizing organic compounds or molecular hydrogen while reducing sulfate to hydrogen sulfide. Most sulfate reducing bacteria can also reduce other oxidized inorganic sulfur compounds, such as sulfite, thiosulfate/elemental sulfur. A common mechanism for anaerobic bacterial for respiration in the absence of oxygen.

3.2.14 *Thiobacillus species (for example, Thiobacillus thioparus, Starkeya novella, Halothiobacillus neapolitanus, Thiomonas intermedia and Acidithiobacillus thiooxidans.), n*—a genus of gram negative bacteria, known for using sulfur and sulfur compounds as part of their respiration cycle (sulfur a.k.a. thio-).

3.2.15 *turbulence, n*—violent or unsteady movement of air or water, or of some other fluid.

4. Microbially Induced Corrosion (MIC) of Concrete

4.1 The MIC of concrete is considered to be a three-stage process (**1-3**)[5] with the reduction in pH (Stage I) (for example, 12.5 > pH > 9-10) (**4, 5**), the establishment of biofilms which further lowers the pH (Stage II) (for example, 9-10 > pH > 4-6) (**1, 4, 6, 7**) and eventual deterioration due to biogenic acid exposure (Stage III) (for example, < ~4 pH) (**7-11**).Fig. 1 illustrates these

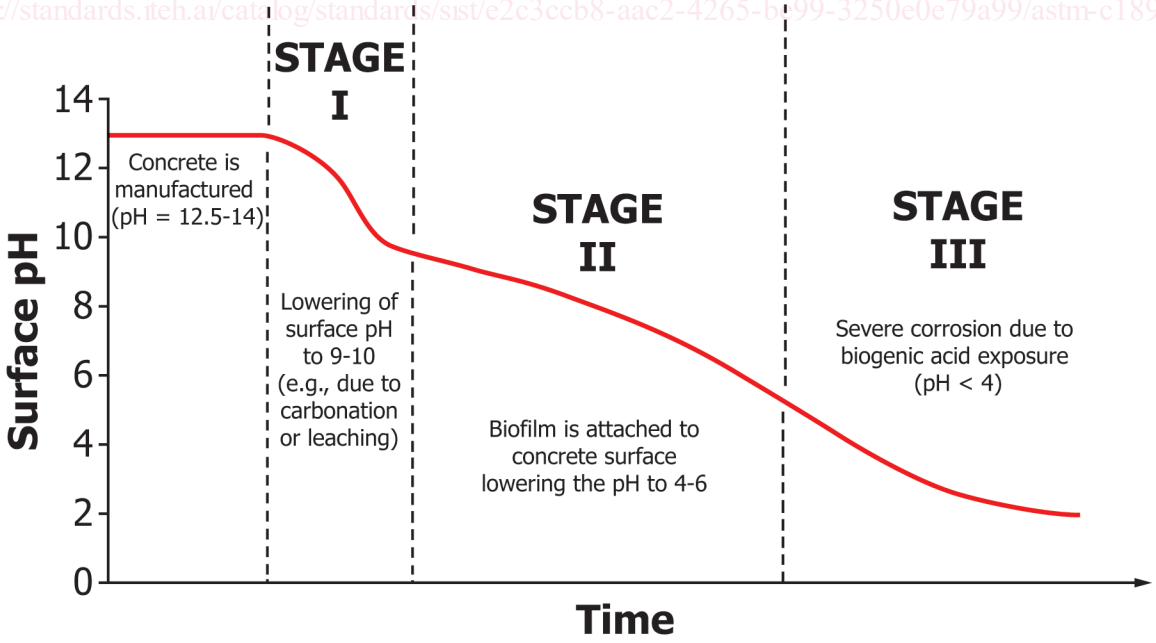

FIG. 1 Three-Stage Process of MIC of Concrete

[5] The boldface numbers in parentheses refer to a list of references at the end of this standard.

stages that have been observed in laboratory testing. Testing procedures are described that simulate all three stages or individual stages. This document clarifies the stages where each test applies.

4.2 This section provides a brief summary of the commonly accepted chain of events that lead to the initiation and propagation of MIC in wastewater collection networks. Additional details are provided in 4.3.

4.2.1 Abiotic lowering of the concrete surface pH takes place before colonization by bacteria can occur. Carbonation, the process by which atmospheric carbon dioxide reacts with calcium hydroxide and water within the cement microstructure, is typically credited with the initial reduction in surface pH of the concrete. Leaching of calcium hydroxide through contact with wastewater may also lead to a reduction in pH near the concrete surface (**12, 13**). It is also claimed that H_2S undergoes inorganic chemical reaction to lower the initial pH of concrete from pH ~12.5 to ~9 (**14**). However, H_2S is not needed for abiotic lowering of the concrete surface pH.

4.2.2 Sulfates in the waste stream are converted to aqueous hydrogen sulfide (H_2S) through the biological activity of anaerobic sulfate reducing bacteria (SRB) residing in biofilms below the water line (**15**).

4.2.3 H_2S is released into gas phase under influence of several factors including turbulence (**16, 17**).

4.2.4 H_2S partitions into the moisture layer present on surfaces above the water line where it is converted to sulfuric acid by aerobic sulfur oxidizing bacteria (SOB) (**6, 16-19**).

4.2.5 Sulfuric acid attacks the cementitious paste portion of the concrete matrix through dissolution of calcium hydroxide by the hydrogen ion and the formation of the expansive corrosion products gypsum and ettringite from the reaction of sulfate and calcium hydroxide (**6, 12, 18, 19**).

4.2.6 The surface area susceptible to attack increases as coarse aggregate is dislodged and the thickness of concrete members is reduced as the attack proceeds into the structure.

4.3 *Formation of Aqueous Hydrogen Sulfide*—The presence of aqueous (dissolved) sulfides in the waste stream is required for the formation of $H_2S_{(g)}$, a component necessary to initiate MIC in sewer networks. Although sulfides may be present in wastewater as a result of industrial processes, the formation of aqueous $H_2S_{(aq)}$ is most commonly attributed to the activity of anaerobic sulfate reducing bacteria (SRB) such as *Desulfovibrio desulfuricans*, which is an obligate anaerobe that relies on the availability of organic substances for a food supply (electron donor) and utilizes sulfate as an oxygen source (electron acceptor). The presence of both organic substances and sulfates is therefore necessary for the biological production of sulfides. Eq 1 describes the formation of hydrogen sulfide through the reduction of sulfates by SRB where *C* represents organic matter (**15-17, 20**):

$$SO_{4\,(aq)}^{2-} + 2C + H_2O \rightarrow 2HCO_{(aq)}^{3-} + H_2S_{(aq)} \quad (1)$$

4.3.1 The majority of sulfate reduction takes place in anaerobic biofilm layers present on surfaces below the water line. The thickness of the biofilm differs depending on the structure and local conditions. For example, the thickness of the biofilm present in concrete sewer pipes is typically between 0.3 and 1.0 mm, but it can also be several millimeters, depending on the velocity of flow and frequency of abrasion by solids in the waste stream (**11**). In the case of a waste stream with an appreciable dissolved oxygen (DO) content, the biofilm will contain aerobic SOB at the liquid/biofilm interface. As oxygen diffuses into the biofilm it is consumed by the SOB, resulting in a gradient of DO that approaches zero near the structure wall. Beyond the highly aerobic zone is a SRB population that proliferates in the oxygen deficient conditions. Nearest to the concrete surface resides a layer of inert anaerobic bacteria whose activity is limited by the diffusion of organic food substances into the biofilm. Sulfates from the waste stream diffuse into the biofilm towards the anaerobic zone where they can be reduced to sulfide as described in Eq 1. Under conditions with sufficient DO, sulfides will be partially or completely oxidized by SOB as they diffuse back towards the waste stream. Any sulfides that escape the biofilm will undergo chemical or biological oxidation in the aqueous phase before release to the gas phase is possible. Under anoxic conditions, sulfides will diffuse out the biofilm unimpeded and partition into the waste stream.

4.4 *Partition of Aqueous H_2S into the Gas Phase*—The biological oxidation of $H_2S_{(g)}$ to sulfuric acid on concrete surfaces is reliant on the availability of $H_2S_{(g)}$ in the sewer headspace. Oxygen is also needed in the headspace to enable thiobacillus bacteria to thrive and produce sulfuric acid. Once present in the waste stream, the release of $H_2S_{(aq)}$ into the gas phase will be heavily influenced by the pH of the wastewater, the equilibrium conditions between gas and liquid phases, temperature, and the turbulence of the flow. Ventilation conditions above the water line will influence the sustained concentration of $H_2S_{(g)}$ in the headspace.

4.5 *Oxidation of $H_2S(g)$ to Sulfuric Acid*—Once present in the sewer headspace, $H_2S_{(g)}$ is free to partition into moisture films present on surfaces above the water line. Back in solution, $H_2S_{(aq)}$ is subject to both biological and chemical conversion to multiple oxidation states, ultimately leading to the production of sulfuric acid and the corrosion of the cement paste portion of the concrete matrix. Sulfur oxidation states are dependent on the local pH and the type and activity of SOB present. Several *Thiobacillus* species have been identified as contributors to MIC in concrete wastewater networks. Multiple species may be present on sewer walls at pH values of 3.0-8.0. Thiobacillus thioparus makes use of sulfides, elemental sulfur and thiosulfate in the production of sulfuric acid. *Thiomonas intermedia* and *Starkeya novella* are the next species to colonize the surface, relying mainly on thiosulfate as a substrate. As pH is reduced to below 7, *Halothiobacillus neapolitanus* becomes prevalent until surface pH is reduced to around 3. Being highly acidophilic, *Acidithiobacillus thiooxidans* thrives at pH values below 3 where it oxidizes both sulfides and elemental sulfur to sulfuric acid. *Acidithiobacillus thiooxidans* continue to lower the surface pH until acid production becomes self-inhibitory at pH values from 0.5 to 1.0. Preferred substrates and pH ranges for SOB involved in MIC in concrete sewers are given in Table 1.

4.5.1 Fresh concrete is highly alkaline, often exhibiting pH between 12.5 and 14 **(13)**. Abiotic lowering of the concrete surface pH is therefore necessary before colonization by *Thiobacillus* can occur. Carbonation, the process by which atmospheric carbon dioxide reacts with calcium hydroxide and water within the cement microstructure, is typically credited with the initial reduction in surface pH of the concrete. Leaching of calcium hydroxide through contact with wastewater **(12, 13)** or inorganic reaction of H_2S on the concrete surface **(14)** may also lead to a reduction in pH near the concrete surface. Once the concrete surface reaches a pH value of 9-10, colonization by SOB can potentially begin. After SOB are established, abiotic lowering of the concrete pH is no longer relevant as biological production of sulfuric acid governs the surface pH. Initial SOB colonization is followed by a successive establishment of more acidophilic species of *Thiobacillus*.

4.6 *Acid Degradation of Cementitious Systems:*

4.6.1 The end product of the oxidation of H_2S by SOB is sulfuric acid. The chemical composition of hydrated portland cement makes concrete susceptible to degradation when exposed to acidic conditions. The volume occupied by hydrated cement paste is generally composed of the following proportions of four solid products: 50-60 % calcium silica hydrate (C-S-H), 20-25 % calcium hydroxide (CH), 15-20 % calcium sulfoaluminates, and varying amounts of unhydrated cement grains. Exposure to acid results in the decalcification of these hydrated products, beginning with CH, and the eventual breakdown of the microstructure resulting in increased porosity and decrease in mechanical properties. After decalcification, calcium ions either diffuse out of the microstructure or combine with the salt of the acid to form insoluble calcium salts of little structural value. The presence of these products results in the formation of a porous layer on the concrete surface. Degradation continues as hydrated products become more unstable with decreasing alkalinity in the system. The degradation mechanisms and severity of acid attack on concrete are dependent on the type of attack, strength and type of acid. The ability of concrete to resist acid attack is related to acid neutralization capacity, composition of hydrated products, and porosity.

4.6.2 The following reactions summarize the decalcification of C-S-H gel (Eq 2) and the dissolution of calcium hydroxide by sulfuric acid (Eq 3) to form gypsum, as well as the formation of ettringite (Eq 4 and 5) **(21)**:

$$3CaO{\cdot}2SiO_2{\cdot}3H_2O + 3H_2SO_4 \rightarrow 3(CaSO_4 \cdot 2H_2O) + 2SiO_2 \quad (2)$$

$$Ca(OH)_2 + H_2SO_4 \rightarrow CaSO_4{\cdot}2H_2O \quad (3)$$

$$3(CaSO_4 \cdot 2H_2O) + 3CaO{\cdot}Al_2O_3 + 26H_2O \rightarrow 3CaO{\cdot}Al_2O_3{\cdot}3CaSO_4{\cdot}32H_2O \quad (4)$$

$$2(CaSO_4 \cdot 2H_2O) + 3CaO{\cdot}Al_2O_3{\cdot}CaSO_4{\cdot}12H_2O + 16H_2O \rightarrow 3CaO{\cdot}Al_2O_3{\cdot}3CaSO_4{\cdot}32H_2O \quad (5)$$

TABLE 1 Preferred Substrates and pH Ranges for SOB Involved with MIC in Concrete Sewers

Species	Preferred Substrate	Preferred pH Growth Range
Thiobacillus thioparus	H_2S, S^0, $S_2O_3^{2-}$	5-9
Starkeya novella	$S_2O_3^{2-}$	2.5-8
Thiomonas intermedia	$S_2O_3^{2-}$	2.5-8
Halothiobacillus neopolitanus	S^0, $S_2O_3^{2-}$	3-7
Acidithiobacillus thiooxidans	H_2S, SO	0.5-3

5. Test Methods for Evaluating Concrete Resistance to MIC

5.1 *Laboratory Investigations:*

5.1.1 *General*—Reproducing MIC in the laboratory is one way to investigate specific mechanisms of attack or evaluate the corrosion resistance of cementitious materials; however, the complex nature of MIC makes laboratory reproduction and the design of straightforward testing techniques difficult. The use of microorganisms requires knowledge of microbiology and introduces a level of variability that makes repeatability of experimental conditions difficult to achieve.

5.1.2 *Materials:*

5.1.2.1 Materials that are described here are intended to be used with both chemical and biogenic acidification tests. Modifications and exceptions to these sections are provided under each test method.

5.1.2.2 *Concrete*—Unless otherwise specified by a specific test method, concrete can be prepared following Practice C192/C192M, Practice C31/C31M, or obtained from existing structures following Test Method C42/C42M, Test Methods C497. Other methods of concrete production or extraction from existing structures are possible, as long as these procedures and applicable standards are specified as part of the reporting process. Mixture proportions and curing methodology shall also be documented if procedures described in the cited standards are not followed.

5.1.2.3 *Cementitious Materials*—Concrete may contain ASTM C150/C150M portland cements (non-air entrained), ASTM C595/C595M blended portland cements, and ASTM C1600/C1600M rapid hardening hydraulic cements. Additionally, supplementary cementitious materials (SCM) may be added to non-air entrained portland cements following Specification C150/C150M. These SCM include ASTM C618 coal fly ash and raw and calcined natural pozzolans for use in concrete, ASTM C989/C989M slag cement for use in concrete and mortars, and ASTM C1240 silica fume used in cementitious mixtures. Material specification reports for all cements and SCM shall be part of the reporting process.

5.1.2.4 *Admixtures*—Concrete may contain admixtures including those that are intended to increase the resistance of concrete to MIC. The type, dosage and application procedure must be specified as part of the reporting process (Specifications C260/C260M, C494/C494M, C1017/C1017M). Available material specification reports for the admixtures should be provided.

5.1.2.5 *Antimicrobial Admixtures (integrally or topically applied)*—Concrete may contain antimicrobial admixtures that are added in concrete during mixing or topically applied on the surface of concrete after hardening to increase the resistance of concrete to MIC (**22**, **23**). The type, dosage and application procedure must be specified as part of the reporting process. Available material specification reports for the antimicrobial admixtures should be provided.

5.1.2.6 *Aggregate*—The composition of aggregate is thought to have an effect on the sulfuric acid resistance of concrete. Calcareous limestone aggregates are soluble in acid due to their high calcium carbonate ($CaCO_3$) content whereas siliceous aggregates are highly resistant to acid degradation. Calcareous aggregates are thought to have a local neutralization effect near the surface of the concrete that increases the surface area of the acid attack, hence, slows down the thinning of the concrete wall thickness. Due to these confounding factors, the type of the aggregate used in concrete must be specified as part of the reporting process (Specification C33/C33M, Terminology C125, Descriptive Nomenclature C294).

5.1.3 *Specimens:*

5.1.3.1 Specimens for each test will be prepared following the procedures described in respective test methods.

5.1.3.2 All specimens will be conditioned in environments as described in respective test methods before exposed to the acidification conditions.

5.1.3.3 Mass of each specimen will be recorded with a balance accurate to 0.1 %.

5.1.3.4 Volume of each specimen will be recorded using a needle point caliper capable of accounting for surface roughness created by exposed aggregates, or other techniques, such as immersion in water, as long as the measurement process will not affect subsequent measurements or test procedures.

5.1.3.5 Laser profile measurements might be used to characterize surface loss.

5.2 *Test Methods:*

5.2.1 *Biogenic Acidification Tests*—In these tests, the acidification of the media is achieved by the bacterial activity; therefore, they represent field conditions more realistically. They are intended to simulate all three stages of MIC; that is, reduction in pH (Stage I), the attachment of biofilms with a lowering of the pH (Stage II), and the rapid deterioration process at low pH (Stage III).

5.2.1.1 *Accelerated Chamber Tests*—Some biogenic acidification tests emulate field conditions and the main stages of MIC in controlled breeding chambers, in which H_2S is produced by bacterial activity and acidification is the result of the conversion of this H_2S to sulfuric acid. Because $H_2S_{(g)}$, the precursor to production of sulfuric acid by SOB, is highly toxic, it presents an immediate risk to human health even at low concentrations. Safety concerns are therefore an important consideration in design of these experiments. The desire to accelerate corrosion conditions requires the control of temperature, adequate nutrients, and humidity. With these considerations combined, laboratory systems designed to simulate MIC in breeding chambers are inherently custom built, cumbersome, and time intensive to operate.

(1) The results obtained by these test methods should serve as a guide in, but not as the sole basis for, selection of a MIC-resistant material for a particular application. No attempt has been made to incorporate into these test methods all the various factors that may affect the performance of a material when subjected to actual service.

(2) The breeding chambers can be constructed in different sizes and configurations as long as they can provide the required conditions for the growth of biogenic acidification conditions and satisfy the safety requirements related to the production, use and purging of $H_2S_{(g)}$ and associated toxic and hazardous conditions. This, however, is not recommended for standardized evaluation due to issues associated with testing repeatability, cost, and safety.

(3) The exact simulation of MIC in laboratory conditions is extremely difficult due to complex deterioration and microbiological processes (**20**). The use of H_2S gas requires extra precautions and special permissions and as such should be avoided. The construction of an environmental chamber can be very expensive and complex for standard labs. These tests can require many months or years to perform (**22**).

(4) Testing protocols for the accelerated chamber tests are described in (**22**).

5.2.1.2 *Benchtop Biogenic Immersion ~~Tests~~—Tests:* ~~In these tests, the biogenic acidification is achieved by SOB which can convert elemental sulfur or thiosulfate to sulfuric acid without the use of H_2S gas. The tests can be performed in simulated exposure solutions containing well-controlled bacterial strains that are grown in the laboratory (23) or bacterial cultures (for example, activated sludge samples) obtained from sewers (24). The cementitious samples are immersed in (23) or exposed to (24) the media where biogenic acidification occurs. The bacteria are applied to the sample surface, not in the solution, to stimulate bacterial attachment (23, 24). Because H_2S is not used, the tests are safer and easier to operate than breeding chamber tests.~~

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(2) The test method for a benchtop biogenic immersion test is described in ASTM C1904.

(3) Biogenic immersion tests enable the user to simulate MIC relatively easily and safely without the use of H_2S gas and sophisticated chambers used for research purpose. A biogenic immersion test is more realistic than an acid immersion test because acidification is achieved by the action of bacteria. It is also more practical and safer than H_2S chamber tests. The methods involve the use of bacteria that can consume elemental sulfur or thiosulfate, instead of H_2S, to biogenically acidify the exposure environment for concrete. These tests do not require an environmental chamber, and can be completed within weeks. Biogenic immersion tests that use bacterial strains that are grown in the laboratory are suitable for simulation of Stage II and III because the pH range of the solution can be controlled within the ranges of each stage (**23**). When bacterial strains are grown in the laboratory, biosafety Level I laboratory conditions can be achieved.

(4) Testing protocols for Stage II and Stage III benchtop biogenic acidification using bacterial strains that are produced in the laboratory are described in (**23**).

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