Establishment of Microbiologically Influenced Corrosion Research and Teaching Laboratory Projects

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Abstract – The phenomenon of microbiologically influenced corrosion (MIC) is a topic in need of additional research efforts. Even though there is significant literature about this subject and recognized causative microorganisms for MIC, there is considered to be a poor understanding of how metal surfaces and bacteria colonies interact. Due to the gaps in knowledge on the subject and the wide variety of experiments that can be conducted, preparing to conduct MIC experiments for research or teaching purposes is difficult. Much of the needed knowledge to conduct experiments is widely dispersed through literature. The objective of this paper is to compile a variety of details about MIC experimental procedures both from literature and experience, inform on the often unforeseen complications, and to offer possible solutions.

Keywords: microbiologically, influenced, induced, corrosion, research

RECOMMENDATIONS AND REVIEW OF LITERATURE

Microbiologically influenced corrosion (MIC) is corrosion affected by the presence and/or activity of microorganisms. MIC can occur in "seawater, freshwater, distilled and demineralized water, process chemicals, foodstuffs, soil, oil, gasoline and aircraft fuels, human plasma, and sewage" as well as in various industrial and power generation systems. MIC can affect a vast range of materials including different types of metals, polymers, and ceramics [Little, 23]. This paper concentrates on microbiologically influenced corrosion of metals and MIC corrosion experiments pertaining to metals.

Microorganism Overview

The microorganisms responsible for microbiologically influenced corrosion do not cause corrosion. Corrosion is a natural process that occurs over time. The corrosion by-products of MIC would be found in nature with or without microorganisms [Dexter, 12]. The microorganisms' involvement is influencing the rate of the corrosion by increasing or inhibiting the rate. This is why the proper term for MIC is influenced corrosion and not induced corrosion. Figure 1 illustrates the different effects of the bacteria's influence on corrosion.

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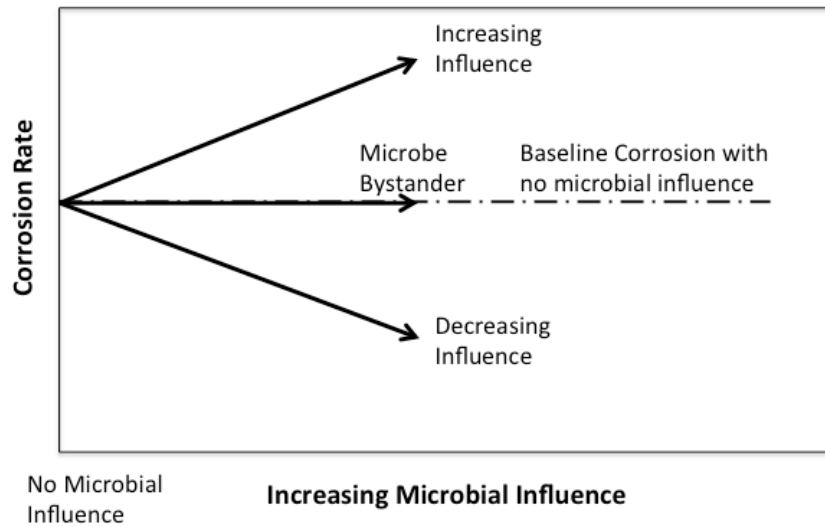
Figure 1: Illustration of the microbial influence on corrosion rates. [Dexter, 12]

Iron bacteria are bacteria that use the oxidation of iron in metabolism; they were among the earliest bacteria [Johnson, 18] and the first microorganisms involved in MIC to be discovered [Emerson, 14]. The scope of MIC microorganisms has been expanded past iron bacteria to include a wide range of aerobic and anaerobic microorganisms and fungi [Ashassi-Sorkhabi, 1]. Though several of the causative organisms are known, see Table 1, the actual scope of MIC microorganisms is unknown due to a lack of research in the field. Recent discoveries include the issue that liquid culturing techniques do not provide an accurate assessment of the numbers or types of microorganism from natural environments [Little, 23]. Such issues led to one author stating, "The effect of attached bacteria on corrosion processes, and the nature at the bacterial-mineral interface, are always poorly understood" [Pisapia, 32]. Due to these issues, the microorganisms that are known to influence corrosion represent a small percentage of those present in nature [Johnson, 18]. This is why microbiologically influenced corrosion rates in the environment and in natural mixed cultures of microorganism result in faster corrosion rates than in laboratory cultures of single species of corroding microorganisms [Little, 23].

Table 1: Examples of known MIC causative microorganisms. [Little, 23], [Dexter, 12]

Genus or Species	pH	Temperature (F)	Oxygen Requirements
Desulfovibrio	4-8	50-105	Anaerobic
Desulfotomaculum	6-8	50-105 (some 115-165)	Anaerobic
Desulfomonas	6-8	50-105	Anaerobic
Acidithiobacillus thiooxidans	0.5-8	50-105	Aerobic
Acidithiobacillu ferrooxidans	1-7	50-105	Aerobic
Gallionella	7-10	70-105	Aerobic
Siderocapsa	Unknown	Unknown	Microaerophilic
Leptothrix	6.5-9	50-95	Aerobic
Sphaerotilus	7-10	70-105	Aerobic
Sphaerotilus	Unknown	Unknown	Unknown
Pseudomonas	4-9	70-105	Aerobic
P. aeruginosa	4-8	70-105	Aerobic
Cladosporium resinae (Fungi)	3-7	50-115 (best 85-95)	Aerobic
Hormoconis resinae (Fungi)	3-7	50-115(best 85-95)	Aerobic

Interactions Between the Microorganisms and their Environment

The MIC caused by mixed species populations of bacteria occurs under biofilms. As mentioned before, culturing all causative bacteria of MIC makes knowing the true diversity of these biofilms difficult and usually undetermined. Despite the relationship between biofilms and corrosion, not all bacteria found in corroding biofilms affect corrosion and are simply bystanders. Also, some of the non-corroding bacteria in biofilms can create local environments that are conducive to corroding bacteria that otherwise could not survive the bulk environment [9, 12, 15, 16, 23, 31].

For example, anaerobic bacteria, such as sulfate-reducing bacteria, which are known to be the organisms most notorious for MIC, can flourish in aerobic bulk medium due to aerobic bacteria in the outer perimeter of the biofilm creating an anaerobic environment inside of the biofilm [10, 12, 16, 18, 23, 28]. The microorganisms sometimes influence the corrosion by altering the local environments around the metal. Changes in the local environment include: pH, oxidizing power, velocity of flow, and concentration of chemical species at the metal surface [Dexter, 12]. Due the complexity and abundance of information on the subjects of biofilms, the types of bacteria involved, and MIC corrosion mechanisms, the complete details exceed the scope of this paper. For a thorough explanation refer to references [11, 12, 13, 18, 23, 24].

MIC occurs as localized corrosion attacks, which include pitting corrosion, dealloying, enhanced erosion corrosion, enhanced galvanic corrosion, stress corrosion cracking, and hydrogen embrittlement [Little, 23]. Due to MIC occurring in a variety of corrosion attacks and its ability to affect many different surfaces, MIC is a problem for industrial systems. A few of the dilemmas MIC can cause in industrial systems include: clogged pipes and equipment, poor water quality, and the degradation of equipment [Starosvestsky, 38]. For a review of industrial and municipal issues and failures involving MIC, the ASM Handbook volume 13A suggests referring to references [3, 10,21,28,34, 35, 39, 42].

Applying and Adapting Corrosion Standards to MIC Experiments

The extreme variety of microbiological environments where engineered components are found means there is no unique test available for MIC. In 2006, there existed no published standards for specific evaluation of MIC [Dexter, 11]. At the time of this paper's publication, the ANSI standards store (including ASTM, ISO, JIS, and others) was searched and no standards were found for the evaluation specifically of MIC. A variety of standards have been published on general corrosion testing which should apply to any MIC research. Some of the most important of these are:

ASTM G1 - 03 Standard Practice for Preparing, Cleaning, and Evaluating Corrosion Test Specimens

ISO 8407:2009 Corrosion of Metals and Alloys - Removal of Corrosion Products from Corrosion Test Specimen

ASTM G46 – 94 (2013) Standard Guide for Examination and Evaluation of Pitting Corrosion

Standards such as *ASTM G78 – 01 (2012) Standard Guide for Crevice Corrosion Testing of Iron-Base and Nickel-Base Stainless Alloys in Seawater and Other Chloride-Containing Aqueous Environments* may be of use in determining experimental procedures even if the alloy under investigation is not the alloy referred to in the standard.

Since the previously mentioned standards do not specialize in the needs of MIC experiments, some adaption is needed before a university laboratory experiment is prepared or a research project established. First, sample size should be considered. The size of a metal sample should depend on the type of results that are wanted at the end of the experiment. If mass loss is to be taken into account, small samples are preferred because the mass comparison between the beginning and ending mass will have a more noticeable difference. This concept also applies to volume and weight.

After choosing the size and cutting the metal samples, the samples should undergo surface finishing. If the metal tested is supposed to reflect MIC on an industrial surface, the metal should be finished to resemble the attributes of the industrial surface as much as possible. If test metal is not meant to reflect an industrial surface, the metal should be polished using metallurgical paper to remove burs, smooth the samples' surfaces, and make the different test samples more similar, in general. Better sample consistency should lead to less scatter in resulting data. The best procedure to be used during the smoothing process is to start with a low-grit metallurgical paper to smooth the surface and gradually build up to a high-grit paper [Bailey, 2]. Abrading the surface is a standard practice and can affect the bacteria corrosion. MIC occurs as localized attacks; therefore, inconsistencies in the surface of the experiment samples will affect MIC and bacteria dispersal. The testing of MIC on polished surfaces is best because differences in the corrosion rates between MIC samples and controls can be more noticeable. Polished surfaces are less acceptable to general corrosion, making their natural corrosion rates slower; therefore, the corrosion caused by MIC is a greater percentage of the total corrosion rate and more easily distinguished. [12,13]

If using more than one sample, then the individual samples need to be identified and kept separate. The ASTM standard G1-03 lists several common practices for metallic sample identification in general corrosion testing [Bailey, 2]. Altering the sample surfaces physically or chemically should be avoided due to the bacteria's relation to surface adhesion and because physical alterations such as engraving provide pre-existing pits that will increase pitting corrosion. ASTM G1-03 also states that dimensions should be measured to the third significant figure and the mass weighed to the fifth significant figure or, if available, more significant figures. For precise measurement of the sample dimensions and more significant figures, a caliper is recommended. At the time of this paper, a decent vernier or digital caliper may be purchased online for an engineering laboratory for approximately thirty to fifty dollars depending on accuracy.

Before starting an experiment, samples need to be degreased and disinfected. ASTM Standard G31 gives several options of degreasing solvents, for example, scour the surface with a non-bleach powder and then rinse with water and a solvent such as acetone [Bailey, 3]. There are several ways mentioned in the literature to disinfect the metal samples. The samples can be autoclaved in an acidic solution or in a fresh medium followed by UV exposure [Pisapia, 32]. A simpler method is to clean the samples with 75% ethanol [Li, 22] or soak the samples for 2 hours in 70% ethanol [Ashassi-Sorkhabi, 1]. Rinse the samples with distilled water after degreasing, disinfecting, and before adding the samples to the test solution. Before using a disinfecting or degreasing technique, a control should be run to guarantee that the techniques do not damage the metal specimens. Bleach is an example of a powerful disinfectant that can damage samples. Samples placed in bleach will be completely dissolved within twenty-four hours.

Bacteria Preparation and Handling

As previously mentioned, the fastest MIC occurs in natural mixed cultures of bacteria, including aerobic and anaerobic bacteria, because creating mixed cultures in the laboratory as diverse as in nature is difficult and sometimes impossible due to the discovery that not all related bacteria can be cultured. Because of this difficulty and the need for further research on the mechanisms of specific causative bacteria, most experiments are conducted on the known MIC causative bacteria listed in Table 1. For details on culturing and sustaining specific bacteria refer to references [6,20,27, 36,37,43]. At many universities, biology lab courses concentrate on culturing and sustaining microorganisms, and some biology students conduct research experiments specifically on this subject. This presents an opportunity to conduct cross-disciplinary labs and research between microorganism culturing and sustaining in biology and MIC corrosion tracking experiments in material sciences.

A method for simulating a mixed culture from the environment is to collect dirt and water from the area where MIC has been observed. Keep this mixed culture alive by adding nutrients from its natural environment, for example, disinfect dirt and organic matter from the infected area and use as nutrient additions. To insure this method works, a mixed culture was created in a container of the volume of sixty-six cubic inches and the nutrients included approximately twelve cubic centimeters of disinfected dirt and organic matter from a location where MIC microorganisms were found. This culture was successful. Trials on smaller cultures are being conducted.

The majority of MIC research is performed with water-borne microorganisms. The reason for this is simply because of the prevalence of engineered products encountering water for some time during service life. The water used in experiments tends to be low in oxygen. This is primarily because oxygen is not a necessary feature of distilled water and lab water remains stagnant for long periods of time, which reduces the oxygen content. There are two ways of dealing with oxygen needs. One is to aerate the experiment medium with air prior to the beginning of the experiment [Panchanadikar, 30]. Thorough oxidation at the beginning of an experiment can provide enough oxygen for the duration of the trial, even if the containers are capped. Another way of providing oxygen is to pump air through the culture during the experiment, but this should be done carefully because the pumping of air is considered disturbance. Disturbance is an added variable because water velocity has been connected to bacteria growth with low velocities reducing growth and moderate velocities encouraging it [Ashassi-Sorkhabi, 1].

Water evaporation can be an issue for experiments exposed to the atmosphere. For experiments run at a universal temperature, excess water can be added at the beginning of the experiment or to all samples at the same time when water is needed. During experiments involving different fluid medium temperatures, evaporation becomes more noticeable and uneven. One easy solution is to cap the trail containers to cancel exposure to the atmosphere, but this is not always the desired action. If the experiment needs atmospheric exposure, lids that have a small opening can be

used to slow evaporation. Another option is a gas permeable membrane or hydrophobic gas-permeable plastic film [Pisapia, 32]. A downfall to this solution is that these membranes can be expensive. If there are time and resources, a side experiment on the effect of disturbance due to water addition on the corrosion rate of the particular bacteria culture being used can be conducted. If the effect of disturbance from water addition is negligibly small, then the variable can be ignored and water can be added as needed.

During the experiment, the primary bacteria population might change or die off due to nutrient depletion or other factors. Therefore, before ending the experiment and beginning a corrosion removal process, it is advisable to take a sample of the corrosion medium(s) (such as soil and water) in order to test for surviving bacteria, bacteria count, and/or identifying the surviving bacteria.

Experimentation Methodology

A variety of experimental techniques have successfully been applied to study MIC. Many engineering departments have access to at least one of these. Among these are optical microscopy, electron microscopy, X-ray Diffraction (XRD) of corrosion products, chemical testing, pit morphology, and a variety of electrochemical techniques that often include potentiodynamic polarization and electrochemical impedance spectroscopy (EIS). A summary of the effectiveness and detailed procedures involved in using each of these methods can be found in references [7, 12, 19, 23, 30, 40, 41].

While removing corrosion, there should be a control specimen that goes through the exact same procedures as the experiment samples. The control is to be used to check that only corrosion products are removed and none of the base metal. The control losing no mass or base metal does not guarantee that the corroded samples have lost no base metal. Fresh polished surfaces are less susceptible to corrosion than previously corroded surfaces. This means that some cleaning techniques will affect the control and corroded samples differently. Therefore, the corrosion removal process should be conducted with care.

Removing corrosion products at the end of an experiment is a two-stage process. The first step is mechanical cleaning, and the second step is chemical corrosion removal. ASTM G1-03 offers directions and further information on the different types of mechanical, chemical, and electrolytic corrosion removal options. These methods do not need altering since MIC produces the same types of corrosion by-products as corrosion without microorganism involvement. Note that mechanical removal offers the opportunity to collect samples of the corrosion products for testing such as X-ray diffraction. Even if testing is not planned it is a good practice to collect some of the corrosion products.

The mechanical and chemical procedures should be conducted gradually, instead of all at once. Trying to remove all of the corrosion at once increases the chance of removing base metal. While conducting the corrosion removal, keep the mass loss recorded. Record the mass each time anything is done to the sample. ASTM G1-03 suggests that a graph of the mass loss should be created and kept updated. The main goal is to have no mass loss over several corrosion removal attempts or to get the slope of the graph as flat as possible. When the slope is essentially zero, the removal of corrosion is complete [Bailey, 2].

Since corrosion is a form of material failure, information collection considerations from the field of failure analysis apply. Images and video should be taken when possible and should include a form of dimensional reference such as a ruler. Experimental samples, that initial data collection is completed on, can be placed into an environment protected by a desiccant. Doing so protects the samples from general atmospheric corrosion in case further analysis needs to be conducted. In experimental work, as with failure analysis, attempts should be made to not overlook or hastily discard observations. A laboratory notebook is highly advised to keep detailed records. The following list is information that may be obtainable in an MIC study. Portions of the list are based on suggestions from [Schweitzer, 41].

- length of the experiment and timing/interval of data collection
- description of experimental setup and any specimen holding or test racks
- composition, pH, color, pressure, aeration, flow rate, turbulence, and temperature of any electrolyte or other solutions used

- specimen material, dimensions, fabrication history (cold work, heat treatment, welding, etc.), preparation methods (cutting methods, surface cleaning, etc.), microstructure, weight, color, chemical composition, impurities, and any chemical or microstructural segregation of note
- any coatings, their thickness and variation thereof, and the same information as for specimens in general
- weight, color, surface texture, chemical composition, dimensions, odor, and time of appearance of any corrosion products and microorganism by-products (Procedures for sampling of corrosion products and microorganism by-products discussed in [Little, 25] and [Little, 26])
- microorganism types, sources, culturing techniques, distribution along samples, and population (Some procedures for sampling and identifying bacteria associated with MIC are in [Little, 25])
- details of any methods used to disinfect specimens, electrolytes or other solutions, and test equipment
- laboratory conditions such as humidity, lighting, temperature, and any variations in such.

In some experiments, non-destructive testing such as dye penetration or ultrasonic testing of specimens before and after experimentation may provide additional information on pit formation, thickness loss, or corrosion underneath coatings.

CONCLUSION

The phenomenon of microbiologically influenced corrosion (MIC) is a topic in need of additional research efforts. Recent discoveries indicate that the microorganisms known to influence corrosion only represent a small proportion of the microorganisms capable of influencing corrosion in the environment [Johnson, 18]. Adding to complications with MIC studies is the fact that MIC rates in the environment and in cultures of mixed microorganisms result in faster corrosion rates than in cultures of a singular species, which is what most published literature focuses on [Little, 23]. The knowledge needed to conduct these experiments is widely dispersed in literature and includes but is not limited to culturing, corrosion tracking, and disinfecting methods. Also, many of the issues with conducting a MIC experiment are not addressed in literature and only arise while developing procedures for the experiments. This paper should be a beginning stepping stone to conducting MIC research and experiments in undergraduate labs.

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