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Diagnosing Microbiologically Influenced Corrosion: A State-of-the-Art Review

B.J. Little, J.S. Lee,‡,* and R.I. Ray**

ABSTRACT

Diagnosing microbiologically influenced corrosion (MIC) after it has occurred requires a combination of microbiological, metallurgical, and chemical analyses. MIC investigations have typically attempted to 1) identify causative microorganisms in the bulk medium or associated with the corrosion products, 2) identify a pit morphology consistent with an MIC mechanism, and 3) identify a corrosion product chemistry that is consistent with the causative organisms. The following sections provide a discussion of available techniques, their advantages and disadvantages, and, most importantly, their limitations.

KEY WORDS: detection, methods, microbiologically influenced corrosion

IDENTIFICATION OF CAUSATIVE ORGANISMS

For many years the first step in identifying corrosion as microbiologically influenced corrosion (MIC) was to determine the presence of specific groups of bacteria in the bulk medium (planktonic cells) or associated with corrosion products (sessile cells). There are four approaches:

—culture the organisms on solid or in liquid media
—extract and quantify a particular cell constituent
—demonstrate/measure some cellular activity
—demonstrate a spatial relationship between microbial cells and corrosion products using microscopy

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Culture Techniques

The method most often used for detecting and enumerating groups of bacteria is the serial dilution to extinction method using selective culture media. To culture microorganisms, a small amount of liquid or a suspension of a solid (the inoculum) is added to a solution or solid that contains nutrients (culture medium). There are three considerations when growing microorganisms: type of culture medium, incubation temperature, and length of incubation. The present trend in culture techniques is to attempt to culture several physiological groups including aerobic, heterotrophic bacteria; facultative anaerobic bacteria; sulfate-reducing bacteria (SRB), and acid-producing bacteria (APB). Growth is detected as turbidity or a chemical reaction within the culture medium. Traditional SRB media contain sodium lactate as the carbon source.[1-2] When SRB are present in the sample, sulfate is reduced to sulfide, which reacts with iron (either in solution or solid) to produce black ferrous sulfide. Culture media are typically incubated over several days (30 days may be required for the growth of SRB). There have been several attempts to improve culture media and to grow higher numbers of bacteria or to shorten the time required for some indication of growth. A complex SRB medium was developed containing multiple carbon sources that can be degraded to both acetate and lactate. In comparison tests, the complex medium produced higher counts of SRB from waters and surface deposits among five commercially available media.[3] Jhobalia, et al., developed an agar-based culture medium for accelerating the growth of SRB.[4] The authors noted that over the range from

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1.93 g/L to 6.50 g/L SRB grew best at the lowest sulfate concentration. Cowan developed a rapid culture technique for SRB based on rehydration of dried nutrients with water from the system under investigation.[5] The author claimed that using system water reduced the acclimation period for microorganisms by ensuring that the culture medium had the same salinity as the system water used to prepare the inoculum. The author reported quantification of SRB within one to seven days.

The distinct advantage of culturing techniques to detect specific microorganisms is that low numbers of cells grow to easily detectable higher numbers in the proper culture medium. However, there are numerous limitations for the detection and enumeration of cells by culturing techniques. Several investigators have followed the changes in microflora as a function of water storage. Zobell and Anderson[6] and Lloyd[7] demonstrated that when water is stored in glass bottles, the bacterial numbers fall within the first few hours followed by an increase in the total bacterial population with a reduction in the number of species. If results from culturing techniques are to be related to the natural populations, culture media should be inoculated within hours of collection and the sample should be chilled during the interim. Under all circumstances culture techniques underestimate the organisms in a natural population.[8,9] Kaeberlein, et al., suggest that 99% of microorganisms from the environment resist cultivation in the laboratory.[10] One major problem in assessing microorganisms in natural environments is that viable microorganisms can enter into a nonculturable state.[11] Another problem is that culture media cannot approximate the complexity of a natural environment. Growth media tend to be strain-specific. For example, lactate-based media sustain the growth of lactate-oxidizers, but not acetate-oxidizing bacteria. Incubating at one temperature is further selective. The type of medium used to culture microorganisms determines, to a large extent, the numbers and types of microorganisms that grow. Zhu, et al., demonstrated dramatic differences in the microbial population from a gas pipeline depending on the enumeration techniques.[12] For example, using culture techniques SRB dominated the microflora in most pipeline samples. However, using culture-independent genetic techniques they found that methanogens were more abundant in most pipeline samples than denitrifying bacteria and that SRB were the least abundant bacteria. Similarly, Romero, et al., used genetic monitoring to identify bacterial populations in a seawater injection system.[13] They found that some bacteria present in small amounts in the original waters were enriched in the culture process.

Biochemical Assays

Biochemical assays have been developed for the detection of specific microorganisms associated with MIC. Unlike culturing techniques, biochemical assays for detecting and quantifying bacteria do not require growth of the bacteria. Instead, biochemical assays measure constitutive properties including adenosine triphosphate (ATP),[14] phospholipid fatty acids (PLFA),[15] cell-bound antibodies,[16] and DNA.[17] Adenosine-5′-phosphosulfate (APS) reductase[18] and hydrogenase[19] have been used to estimate SRB populations.

Since ATP is a compound found in all living matter, ATP assays estimate the total number of viable organisms in a sample. ATP assays are based on the luciferin-luciferase reaction where ATP provides the energy for the oxidation of luciferin by the enzyme luciferase. The procedure requires that a water sample be filtered to remove solids and salts that might interfere. A reagent is added to the filtered sample to lyse the cells and release the ATP. The reaction is sensitive to sulfide, some metals, and some types of biocides. Emitted light is measured with a photometer and the amount of light released during the reaction is directly related to the amount of ATP in the sample.

Biofilm community structure can be analyzed using cluster analysis of PLFA profiles.[15] Phospholipids are found in the membranes of all cells. Under the conditions in natural communities, bacteria contain a relatively constant proportion of their biomass as phospholipids. Phospholipids are not found in storage lipids and have a relatively rapid turnover so that their assay gives a measure of the viable cellular biomass. The phosphate of the phospholipids or the glycerol-phosphate and acid-labile glycerol from phosphatidyl glycerol-like lipids can be assayed to increase the specificity and sensitivity of the phospholipids assay. The ester-linked fatty acids in the phospholipids are both the most sensitive and useful chemical measures of microbial biomass and community structure. PLFA profiles for natural biofilms have been shown to be more complex than profiles for laboratory biofilms. None of the laboratory profiles clustered closely with profiles from natural biofilms. Also, the PLFA profiles for attached bacteria clustered separately from profiles of the same bacteria in the bulk phase, suggesting that either the community or the physiology of attached bacteria differs from that of bulk phase bacteria. Despite the fact the PLFA analysis cannot provide an exact description of each species in a given environment, the analysis does provide a quantitative description of the microbiota in a particular environment. Analysis of other components of the phospholipids fraction give insight into community structure. For example, SRB contain lipids that can be used to identify at least a portion of the class. Dowling, et al., identified unusual fatty acids as biomarkers for two SRB: iso 17:1w7c and branched monoenoics for a hydrogen-oxidizing *Desulfovibrio* sp. and 10 methyl 16:0 for an acetate oxidizing *Desulfobacter* sp.[20]

Both APS reductase, an intracellular enzyme found in all SRB, and hydrogenase, an enzyme pres-

ent in some SRB (hydrogenase-positive), can be extracted from liquids or solids, including corrosion products and sludge. In a procedure to quantify APS reductase, cells are lysed to release the enzyme, added to an antibody reagent and exposed to a color-developing solution. In the presence of APS reductase a blue color appears whose intensity and development rate is proportional to the amount of enzyme and roughly to the number of cells from which the enzyme was extracted. Similarly, hydrogenase activity may be measured in a procedure where the enzyme is extracted from cells and exposed to hydrogen anerobically.[19] The rationale for relating hydrogenase to MIC is that during corrosion in anaerobic environments, molecular hydrogen is produced at the cathode. Some, but not all SRB, are hydrogenase positive, meaning that they possess the enzyme required to oxidize molecular hydrogen. In the assay, hydrogenase reacts with hydrogen and reduces an indicator dye in solution. The activity of hydrogenase is established by the development and intensification of a blue color proportional to the rate of hydrogen uptake by the enzyme. The technique does not attempt to estimate specific numbers of SRB. Bryant, et al., suggested that hydrogenase levels were better indicators of MIC than numbers of SRB.[21] Mara and Williams reported that hydrogenase was more important when the environment contained low concentrations of ferrous ions, but was less important in the presence of sufficient ferrous ions to precipitate the sulfide produced by SRB.[22] Other investigators found no relationship between levels of hydrogenase enzyme and the rate or extent of corrosion.[23]

Cell Activity

Roszak and Colwell reviewed techniques commonly used to detect microbial activities in natural environments, including transformations of radiolabeled metabolic precursors.[11] Phelps, et al.,[24] and Mittelman, et al.,[25] used uptake or transformation of ^{14}C-labeled metabolic precursors to examine activities of sessile bacteria in natural environments and in laboratory models. Phelps, et al., used a variety of ^{14}C-labeled compounds to quantify catabolic and anabolic bacterial activities associated with corrosion tubercles in steel natural gas transmission pipelines.[24] They demonstrated that organic acid was produced from hydrogen and carbon dioxide in natural gas by acetogenic bacteria, and that acidification could lead to enhanced corrosion of the steel. Mittelman, et al., used measurement of lipid biosynthesis from ^{14}C-acetate, in conjunction with measurements of microbial biomass and extracellular polymer, to study effects of differential fluid shear on physiology and metabolism of *Alteromonas* (formerly *Pseudomonas*) *atlantica*.[25] Increasing shear force increased the rate of total lipid biosynthesis, but decreased per cell biosynthesis. Increasing fluid shear also increased cellular biomass and greatly increased the ratio of extracellular polymer to cellular protein. Maxwell developed a radiorespirometric technique for measuring SRB activity on metal surfaces that involved two distinct steps: incubation of the sample with ^{35}S sulfate and trapping the released sulfide.[26]

Techniques for analyzing microbial metabolic activity at localized sites have been developed. Franklin, et al., incubated microbial biofilms with ^{14}C-metabolic precursors and autoradiographed the biofilms to localize biosynthetic activity on corroding metal surfaces.[27] The localized uptake of labeled compounds was related to localized electrochemical activities associated with corrosion reactions.

Reporter genes can signal when the activity of a specific metabolic pathway is induced. King, et al., engineered the incorporation of a promotorless cassette of lux genes into specific operons of *Pseudomonas* to induce bioluminescence during degradation of naphthalene.[28] Using reporter genes, Marshall demonstrated that bacteria immobilized at surfaces exhibit physiological properties not found in the same organisms in the aqueous phase.[29] Some genes are turned on at solid surfaces despite not being expressed in liquid or on solid media. It is also likely that other genes are turned off at surfaces. They further demonstrated gene transfers within biofilms even in the absence of imposed selection pressure.

Genetic Techniques

Genetic techniques using ribosomal RNA (rRNA) or their genes (rDNA) have been used to identify and quantify microbial populations in natural environments.[30-32] These techniques involve amplification of 16S rRNA gene sequences by polymerase chain reaction (PCR) amplification of extracted and purified nucleic acids. The PRC products can be evaluated using community fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE). Each DGGE band is representative of a specific bacterial population and the number of distinctive bands is indicative of microbial diversity. The PCR products can also be sequenced, and the sequences are compared to the sequences in the Genbank database,[11] which allows the identity of the species within an environmental sample. Horn, et al., identified the constituents of the microbial community within a proposed nuclear waste repository using the following two techniques:[33]

—isolation of DNA from growth culture and subsequent identification by 16S rDNA genes
—isolation of DNA directly from environmental samples followed by subsequent identification of the amplified 16S rDNA genes (Table 1 and Figure 1)

[11] GenBank, National Center for Biotechnology Information, National Library of Medicine, 38A, 8N805, 8600 Rockville Pike, Bethesda, MD 20894.

TABLE 1

Organisms Isolated after Growth in Various Yucca Mountain Simulated Groundwaters and 16S rDNA Sequence Divergence from Reference Organisms[33]

	Growth Medium from Which Organism Isolated	
	1×J13 Synthetic with Glucose	1×J13 Synthetic without Glucose
Organism[A]	% Divergence from Database[B]	
Ralstonia pickettii		
Ralstonia eutrophus	4.5/MS	6.9/MS
Burkholderia cepacia		
Blastobacter natatorius		
Sphingomonas paucimobilis		2.0/GB
Methylobacterium mesophilicum		
Caulobacter subvibroidies		
Uncultured bacterium oxSCC-6[C]	4.0/GB	
Pseudomonas (Janth) *mephitica*	6.06/MS	
Microbacterium barkeri	4.55/MS	
Microbacterium keratanolyticum	4.15/MS	4.15/MS
Microbacterium chocolatum	5.16/MS	
Arthrobacter sp. SMCC G964[D]	0.0/GB	
Pseudomonas stutzeri	3.93/MS	1.0/GB
Afipia genosp. 14		4.0/GB

[A] Closest relative in 16S rDNA sequence comparisons to three separate databases (i.e., MS, GB, RDP, below).

[B] MS, MicroSeq database (Applied Biosystems, Foster City, California); GB, GenBank database (National Center for Biotechnology Information, Bethesda, Maryland); RDP, Ribosomal Database Project (Michigan State University, East Lansing, Michigan).

[C] H. Lüdemann, I. Arth, W. Liesack, Appl. Environ. Microbiol. 66 (2000): p. 754-762.

[D] L.G. VanWaasbergen, D.L. Balkwill, F.H. Crocker, B.N. Bjornstad, and R.V. Miller, Appl. Environ. Microbiol. 66 (2000): p. 3,454-3,463.

Comparison of the data from the two techniques demonstrates that culture-dependent approaches underestimated the complexity of microbial communities. Zhu, et al., used genetic techniques to characterize the types and abundance of bacterial species in gas pipeline samples and made similar observations.[34-35] Another example of genetic techniques is the fluorescent in situ hybridization (FISH), which uses the specific fluorescent dye-labeled oligonucleotide probes to selectively identify and visualize SRB both in established and developing multispecies biofilms.[32]

Microscopy

Light Microscopy — Using light microscopy and proper staining, investigators have demonstrated a relationship between an unusual variety of copper corrosion and gelatinous, polysaccharide-containing biofilms.[36-37] Blue water (also called copper by-product release or cuprosolvency) is observed in copper tubing, primarily in soft waters after a stagnation period of several hours to days and is typically associated with copper concentrations of 2 mg/L to 20 mg/L.

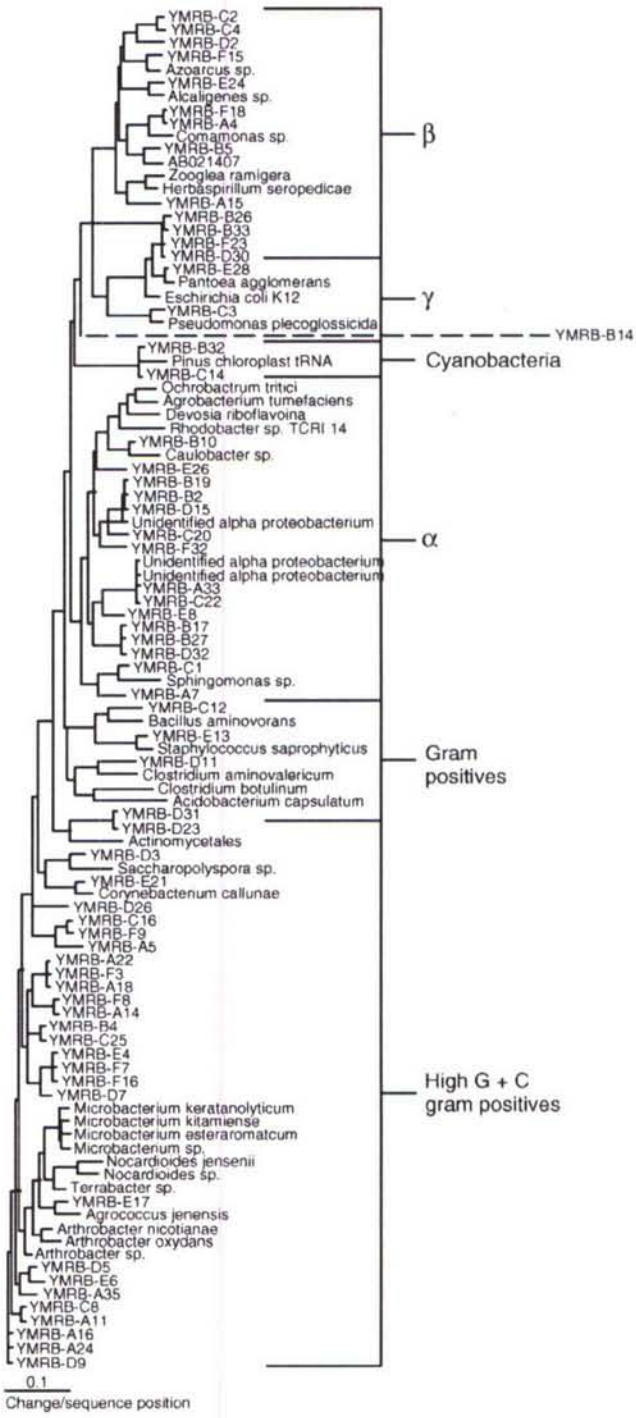


FIGURE 1. *Phylogenetic tree of Yucca Mountain (YM) bacterial community as identified by 16S rDNA analysis of DNA extracted from YM rock.*[33]

This phenomenon is distinct from other types of copper corrosion in that it does not significantly compromise the integrity of the tube, but instead leads to copper contamination and coloring of the water.

Epifluorescence Microscopy — Immunofluorescence techniques have been developed for the

identification of specific bacteria in biofilms.[38-39] Epifluorescence cell surface antibody (ECSA) methods for detecting SRB are based on the binding between SRB-specific antibodies and the SRB cells, and subsequent detection of SRB-specific antibodies with a secondary antibody through two approaches. First, the secondary antibodies can be linked to a fluorochrome that enables bacterial cells marked with the secondary antibody to be viewed with an epifluorescence microscope. Second, the secondary antibodies can be conjugated with an enzyme (alkaline phosphatase) that then can be reacted with a colorless substrate to produce a visible color proportional to the quantity of SRB present. Detection limits for the field test are 10,000 SRB mm^{-2} filter area. The color reagent used for field tests is unstable at room temperature and tends to bind nonspecifically with antibodies adsorbed directly at active sites on the filter, creating a false positive that may interfere with the detection of SRB at levels below 10,000 cells mm^{-2}. Antigenic structures of marine and terrestrial strains are distinctly different and therefore antibodies to either strain did not react with the other.

Confocal Laser Scanning Microscopy — Confocal laser scanning microscopy (CLSM) permits one to create three-dimensional images, determine surface contour in minute detail, and accurately measure critical dimensions by mechanically scanning the object with laser light. A sharply focused image of a single horizontal plane within a specimen is formed while light from out-of-focus areas is repressed from view. The process is repeated again and again at precise intervals on horizontal planes and the visual data from all images are compiled to create a single, multidimensional view of the subject. Geesey, et al., used CLSM to produce three-dimensional images of bacteria within scratches, milling lines, and grain boundaries.[40]

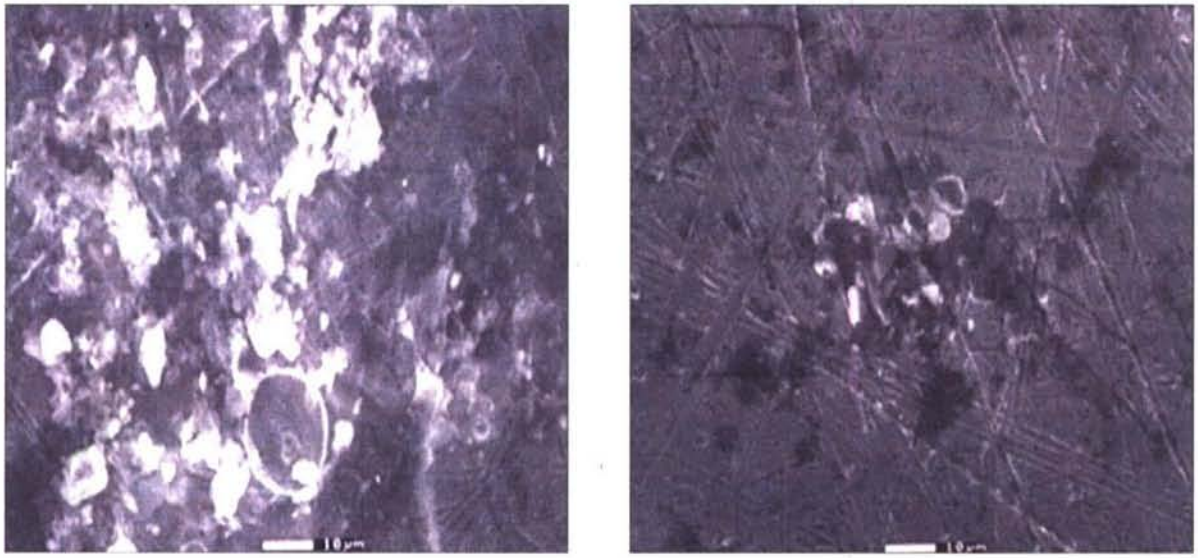
Atomic Force Microscopy — Atomic force microscopy (AFM) uses a microprobe mounted on a flexible cantilever to detect surface topography by scanning at a subnanometer scale. Repulsion by electrons overlapping at the tip of the microprobe cause deflections of the cantilever that can be detected with a laser beam. The signal is read by a feedback loop to maintain a constant tip displacement by varying voltage to a piezoelectric control. The variations in the voltage mimic the topography of the sample and, together with the movement of the microprobe in the horizontal plane, are converted to an image. Telegdi, et al., imaged microorganisms associated with corrosion on several substrata.[41]

Electron Microscopy — Many of the conclusions about biofilm development, composition, distribution, and relationship to substratum/corrosion products have been derived from traditional scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM has been used to image SRB from corrosion products on Type 904L (UNS N08904),[42,(2)] microorganisms in corroding gas pipelines,[35] and iron-oxidizing *Gallionella* in water distribution systems.[43] TEM has been used to demonstrate that bacteria are intimately associated with sulfide minerals and that on copper-containing surfaces the bacteria were found between alternate layers of corrosion products and attached to the base metal.[44]

In traditional SEM, nonconducting samples including biofilms associated with corrosion products must be dehydrated and coated with a conductive film of metal before the specimen can be viewed. Traditional TEM methods for imaging biofilms require fixation of biological material, embedding in a resin and thin-sectioning to achieve a section that can transmit an electron beam. Environmental electron microscopy includes both scanning (ESEM) and transmission (ETEM) techniques for the examination of biological materials with a minimum of manipulation, i.e., fixation and dehydration. Little, et al., used ESEM to study marine biofilms on stainless steel surfaces.[45] They observed a gelatinous layer in which bacteria and microalgae were embedded. Traditional SEM images of the same areas demonstrated a loss of cellular and extracellular material (Figure 2). Little and coworkers used ESEM to demonstrate sulfide-encrusted SRB in corrosion layers on copper alloys (Figure 3) and iron-depositing bacteria in tubercles on stainless steels (Figure 4).[46-47] Little, et al., used ETEM to image *P. putida* on corroding iron filings and to demonstrate that the organisms were not directly in contact with the metal.[48] Instead, the cells were attached to the substratum with extracellular material (Figure 5). Design and operation of the ESEM and ETEM have been described elsewhere.[46]

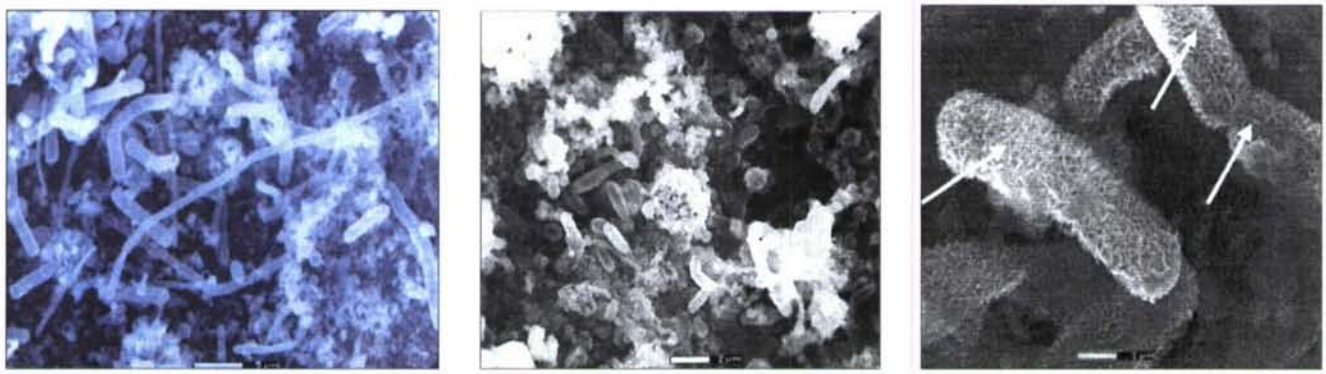
There are fundamental problems in attempting to diagnose MIC by establishing a spatial relationship between numbers and types of microorganisms in the bulk medium and those associated with corrosion products using any of the techniques previously described. Zintel, et al., established that there were no relationships between the presence, type, or levels of planktonic or sessile bacteria and the occurrence of pits.[49] Because microorganisms are ubiquitous, the presence of bacteria or other microorganisms does not necessarily indicate a causal relationship with corrosion. In fact, microorganisms can nearly always be cultured from natural environments. Little, et al.,[50] reported that electrochemical polarization could influence the number and types of bacteria associated with the surface.[50] Artificial crevices created in Type 304 (UNS S30400) stainless steel in abiotic seawater were associated with large numbers of bacteria after 5-day exposures to natural seawater. Bacteria did not cause the crevice; instead, bacteria were attracted to

[2] UNS numbers are listed in *Metals and Alloys in the Unified Numbering System*, published by the Society of Automotive Engineers (SAE International) and cosponsored by ASTM International.



(a) (b)

FIGURE 2. *(a) ESEM image of wet estuarine biofilm on Type 304 stainless steel surface. (b) ESEM image of estuarine biofilm on Type 304 stainless steel surface after treatment with acetone/xylene.*[45]



(a) (b) (c)

FIGURE 3. *ESEM images of bacteria within corrosion layers on copper foils (a, b markers = 5 μm; c marker = 1 μm). Arrows indicate sulfide-encrusted cells in 3c.*[46]

the anodic site. Several other investigators have made similar observations. For example, de Sánchez and Schiffrin demonstrated that Cu(II) and titanium ions were strong attractants for *Pseudomonas*.[51] Detection or demonstration of bacteria associated with corrosion is not diagnostic for MIC.

PIT MORPHOLOGY

Pope completed a study of gas pipelines to determine the relationship between the extent of MIC and the levels/activities of SRB.[52] He concluded that there was no relationship. Instead he found large numbers of APB and organic acids, particularly lactic acid, and identified the following metallurgical features in carbon steel:

—large craters from 5 cm to 8 cm or greater in diameter surrounded by uncorroded metal (Figure 6)
—cup-type hemispherical pits on the pipe surface or in the craters (Figure 7)
—striations or contour lines in the pits or craters running parallel to the longitudinal pipe axis (rolling direction) (Figure 8)
—tunnels at the ends of the craters also running parallel to the longitudinal axis of the pipe (Figure 9)

Pope reported that these metallurgical features were "fairly definitive for MIC."[52] However, the author did not advocate diagnosis of MIC based solely on pit morphology. Subsequent research has demonstrated that these features can be produced by abiotic reac-

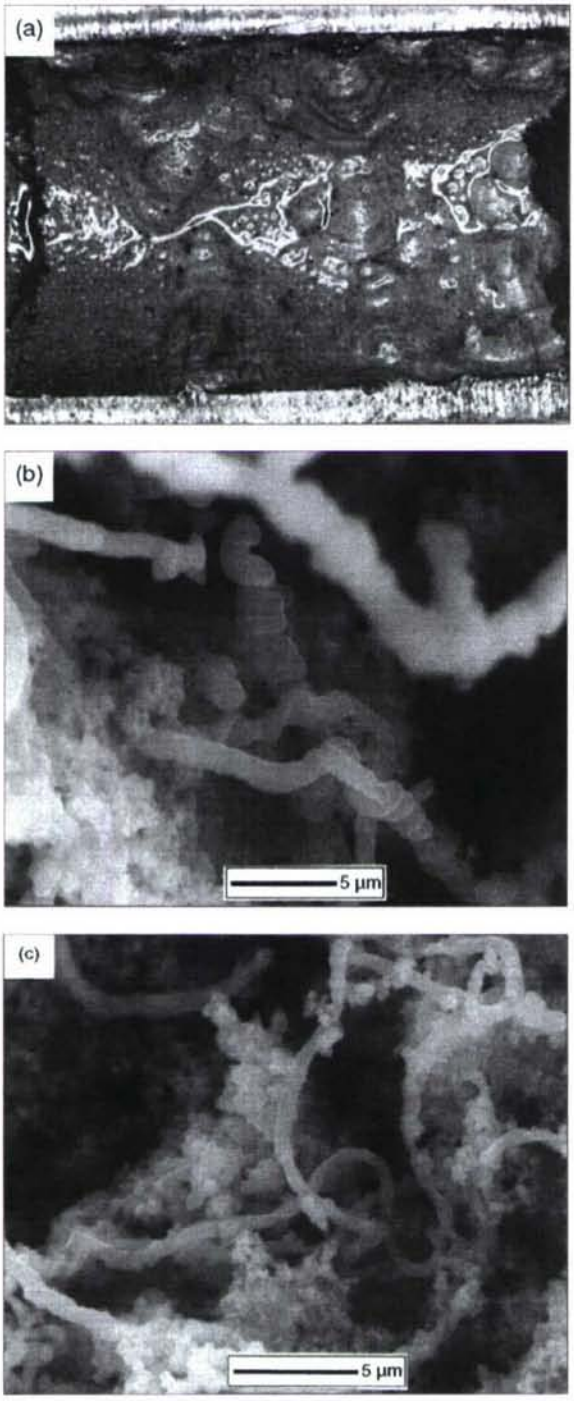


FIGURE 4. *(a) Tubercles associated with pitting in galvanized steel pipe from water distribution system (2X); (b) and (c) ESEM views of* Gallionella *filaments observed in tubercles. Horizontal field width: (b) 57 µm and (c) 29 µm.*[47]

tions[53] and cannot be used to independently diagnose MIC.

Other investigators described ink bottle-shaped pits in 300 series stainless steel that were supposed to be diagnostic of MIC (Figure 10). Borenstein and Lindsay reported that dendritic corrosion attack at

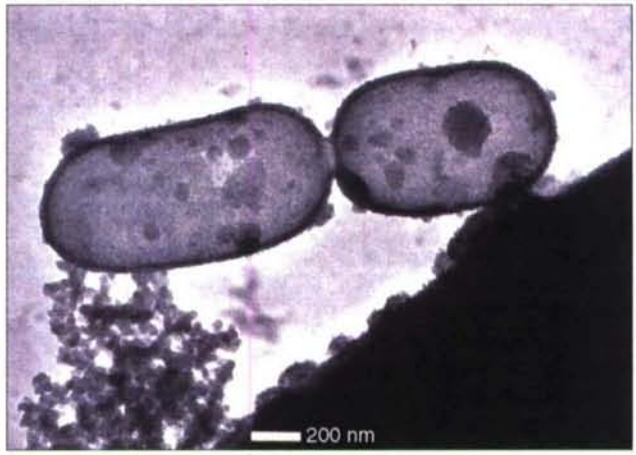


FIGURE 5. *Hydrated* Pesudomonas putida *after removal of excess moisture by circulation of air through the environmental cell.*[48]

welds was "characteristic of MIC."[54,55] Hoffman suggested that pit morphology was a "metallurgical fingerprint...definitive proof of the presence of MIC."[56] Chung and Thomas compared MIC pit morphology with non-MIC chloride-induced pitting in Types 304/304L (UNS S30400/UNS S30403) and Type 308 (UNS S30800) stainless steel base metals and welds.[57] A faceted appearance was common to both types of pits in Types 304 and 304L base metals (Figures 11[a] and [b]). Facets were located in the dendritic skeletons in MIC and non-MIC cavities of the Type 308 weld metal. They concluded that there were no unique morphological characteristics for MIC pits in these materials. The problem that has resulted from the assumption that pits can be independently interpreted as MIC is that MIC is often misdiagnosed. For example, Welz and Tverberg reported leaks at welds in a stainless steel (Type 316L [UNS S31603]) hot water system in a brewery after six weeks in operation were a result of MIC.[58] The original diagnosis was based on the circumstantial evidence of attack at welds and the pitting morphology-scalloped pits within pits. However, after a thorough investigation, MIC was dismissed. There were no bacteria associated with the corrosion sites; deposits were too uniform to have been produced by bacteria. The hemispherical pits had been produced when carbon dioxide gas (CO_2) was liberated and low-pH bubbles nucleated at surface discontinuities.

More recently, several investigators have demonstrated that the initial stages of pit formation due to certain types of bacteria do have unique characteristics. Geiser, et al., found that pits in Type 316L stainless steel due to the manganese-oxidizing bacterium *Leptothrix discophora* had different morphologies than pits initiated by anodic polarization.[59] Pits initiated by these organisms in a solution of sodium chloride were approximately 10 times longer than they were wide (Figures 12[a] and [b]).[59] Pits produced by micro-

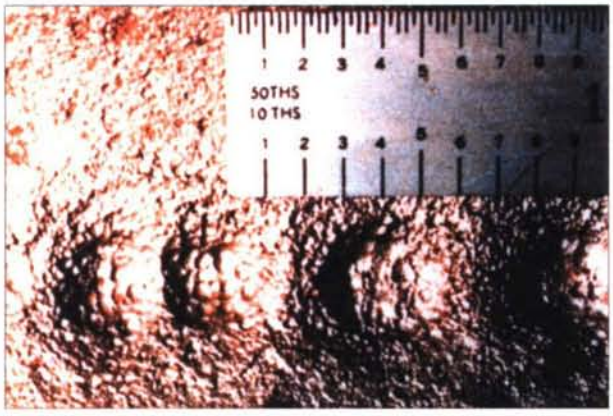


FIGURE 6. *Cup-type scooped out hemispherical pits on flat surfaces with craters in pits.[52] Reproduced with permission from The Gas Technology Institute.*

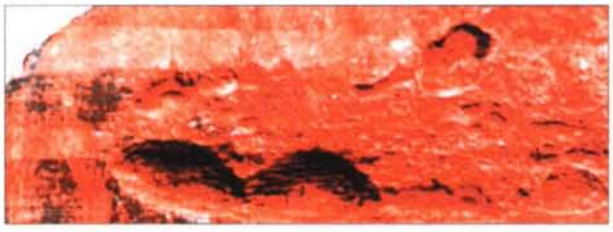


FIGURE 8. *Corrosion pits with striations.[52] Reproduced with permission from The Gas Technology Institute.*

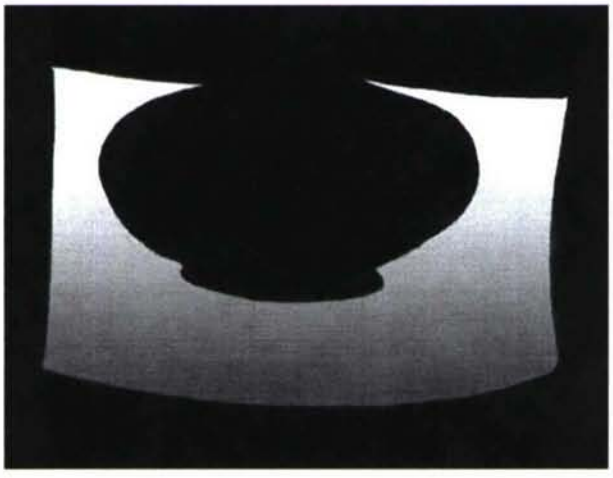


FIGURE 10. *An illustration of an ink bottle-type pit noted in many cases of MIC and commonly found in the Type 904L tubes from failed heat exchangers.*

organisms were much smaller than, and not nearly as deep as, pits produced in the same solution by electrochemical means. Pits had almost identical sizes and aspect ratios as the sizes and aspect ratios of the manganese-oxidizing bacteria. The similarity between the dimensions of the bacterial cells attached to the surface and the dimensions of corrosion pits indicate

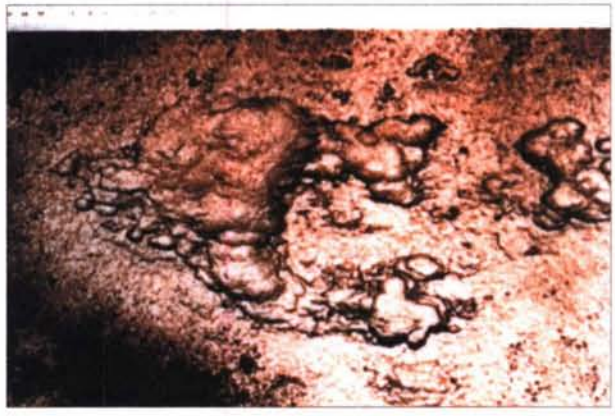


FIGURE 7. *Close-up of sand-blasted surface showing MIC pattern.[52] Reproduced with permission from The Gas Technology Institute.*

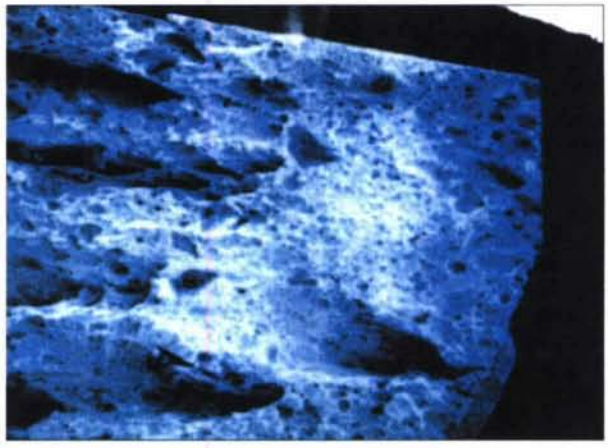
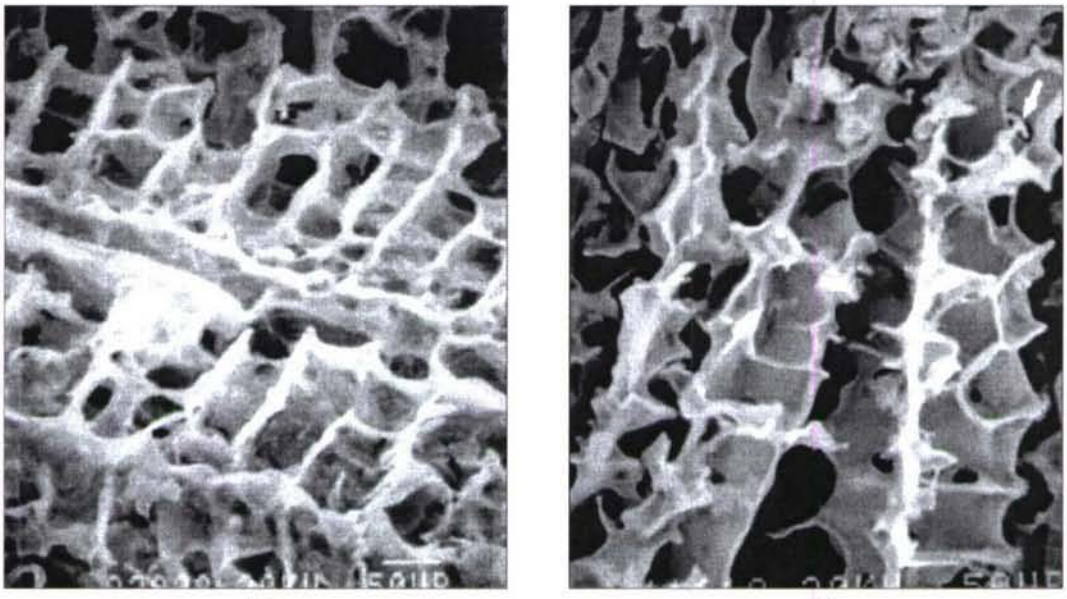


FIGURE 9. *Close-up view of tunnels (100X).[52] Reproduced with permission from The Gas Technology Institute.*

a possibility that the pits were initiated at the sites where the microbes were attached. Eckert used API 5L steel to demonstrate micro-morphological characteristics that could be used to identify MIC initiation.[60] Coupons were installed at various points in a pipeline system and were examined by SEM at 1,000X and 2,000X. They demonstrated that pit initiation and bacterial colonization were correlated and that pit locations physically matched the locations of cells. Telegdi, et al., used AFM to image biofilm formation, extracellular polymer production, and subsequent corrosion.[41] Pits produced by *Thiobacillus intermedius* had the same shape as the bacteria. None of these investigators claim that these unique features can be detected with the unaided eye or that the features will be preserved as pits grow, propagate, and merge.

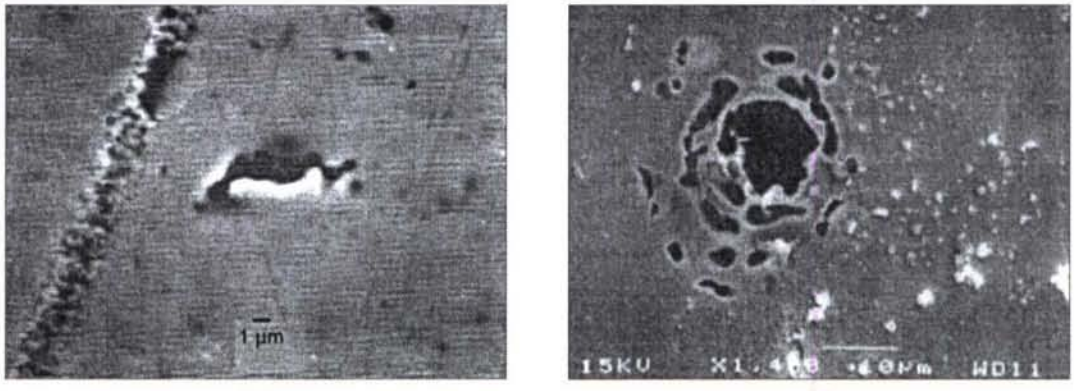
CHEMICAL TESTING

Analyses for corrosion product chemistry can range from simple field tests to mineralogy and isotope fractionation. Field tests for solids and corrosion



(a) (b)

FIGURE 11. *(a) SEM of dendritic skeletons in MIC cavities in E308 stainless steel weld (1,000X). (b) Micrograph of the non-MIC chloride-induced corrosion pits in E308 weld root (300X).*[57]



(a) (b)

FIGURE 12. *(a) SEM image showing a heavy line on the left indicating square etching by iron milling. The indention in the center was detected after the coupon was microbially ennobled. It was not there before microbial colonization. (b) SEM image of a typical pit initiated in Type 316L stainless steel using anodic polarization.*[57]

products typically include pH and a qualitative analysis for the presence of sulfides and carbonates. A drop of dilute hydrochloric acid placed on a small portion of the corrosion product will indicate the presence of carbonates if noticeable bubbling occurs. If a rotten egg smell is present following acid treatment, sulfides are present in the corrosion product. Sulfides can be verified by exposing a piece of lead acetate paper to the acidified corrosion product and watching for a color change from white to brown.

Elemental Composition

Elements in corrosion deposits can provide information as to the cause of corrosion. Energy-dispersive x-ray analysis (EDS) coupled with SEM can be used to determine the elemental composition of corrosion deposits. Because all living organisms contain ATP, a phosphorus peak in an EDS spectrum can be related to cells associated with the corrosion products. Other potential sources of phosphorus, e.g., phosphate water treatments, must be eliminated. The activities of SRB and manganese-oxidizing bacteria produce surface-bound sulfur and manganese, respectively. Chloride is typically found in crevices and pits and cannot be directly related to MIC. There are several limitations for EDS surface chemical analyses. Samples for EDS cannot be evaluated after heavy metal coating; therefore, EDS spectra must be collected

prior to heavy metal coating. It is difficult or impossible to match spectra with exact locations on images. This is not a problem with the ESEM because nonconducting samples can be imaged directly, meaning that EDS spectra can be collected of an area that is being imaged by ESEM. Little, et al., documented the changes in surface chemistry as a result of solvent extraction of water, a requirement for SEM (Table 2).[45] Other shortcomings of SEM/EDS include peak overlap. Peaks for sulfur overlap peaks for molybdenum and the characteristic peak for manganese coincides with the secondary peak for chromium. Wavelength dispersive spectroscopy can be used to resolve overlapping EDS peaks. Peak heights cannot be used to determine the concentration of elements. It is also impossible to determine the form of an element with EDS. For example, a high-sulfur peak may indicate sulfide, sulfate, or elemental sulfur.

Mineralogical Fingerprints

McNeil, et al., used mineralogical data determined by x-ray crystallography, thermodynamic stability diagrams (Pourbaix), and the simplexity principle for precipitation reactions to evaluate corrosion product mineralogy.[61] They concluded that many sulfides under near-surface natural environmental conditions could only be produced by microbiological action on specific precursor metals. They reported that djurleite, spinonkopite, and the high-temperature polymorph of chalcocite were mineralogical fingerprints for the SRB-induced corrosion of copper-nickel alloys. They also reported that the stability or tenacity of sulfide corrosion products determined their influence on corrosion.

Jack, et al., maintained that the mineralogy of corrosion products on pipelines could provide insight into the conditions under which the corrosion took place.[62] For example, under anaerobic conditions in the absence of SRB an iron(II) carbonate (siderite [$FeCO_3$]) was identified in water trapped under defective coatings. Introduction of air caused a rapid discoloration of the white corrosion product to orange iron(III) oxides. In the presence of SRB, indicator minerals are siderite and iron(II) sulfide in a ratio of 3:1 or more (Figure 13). Mackinawite (FeS), the first formed crystalline sulfide, converts to gregite (Fe_3S_4) in a time- and pH-dependent manner. Pyrrhotite ($Fe_{1-x}S$) may form after nine months. At aerobic corrosion sites, the minerals are iron(III) oxides: magnetite (Fe_3O_4), hematite (Fe_2O_3), lepidocrocite (γ-FeO[OH]), and goethite (α-FeO[OH]) (Figure 14).

Isotope Fractionation

The stable isotopes of sulfur (^{32}S and ^{34}S), naturally present in any sulfate source, are selectively metabolized during sulfate reduction by SRB and the resulting sulfide is enriched in ^{32}S.[63] The ^{34}S accumulates in the starting sulfate as the ^{32}S is removed

TABLE 2

Weight Percent of Elements Found on Commercially Pure Copper Surfaces After Exposure to Estuarine Water for 4 Months and Sequential Treatment with Acetone and Xylene[45]

Element	Base Metal	After Exposure to Estuarine Water	After Acetone	After Xylene
Al		9.49	1.22	0.74
Si		21.38	1.89	1.27
Cl		0.93	15.9	15.93
Cu	99.9	59.62	80.99	82.06
Mg		1.96	0	0
P		0.98	0	0
S		0.95	0	0
Ca		0.49	0	0
K		0.67	0	0
Fe		3.52	0	0

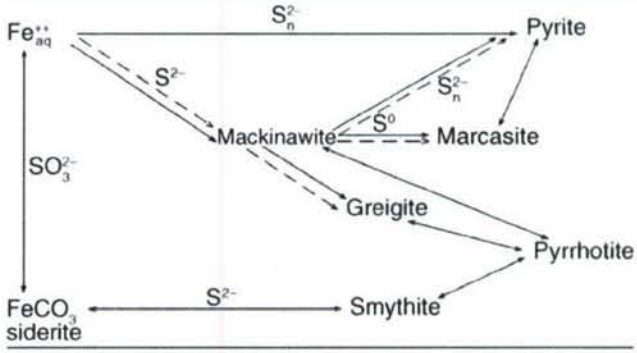
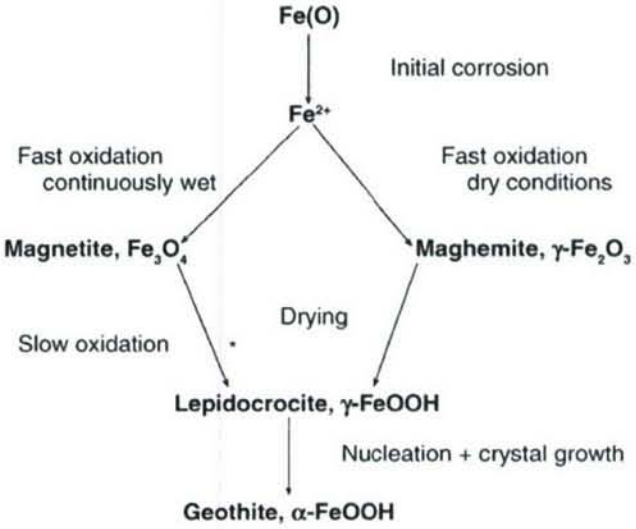


FIGURE 13. *Transformations of iron(II) sulfides formed at pipeline corrosion sites (dashes, biological processes, solid lines, abiological processes).*[62]



* Hematite may form from magnetite as an intermediate.

FIGURE 14. *Transformation of iron(III) oxides formed at pipeline corrosion sites.*[62]

and becomes concentrated in the sulfide. Little, et al., demonstrated sulfur isotope fractionation in sulfide corrosion deposits resulting from activities of SRB within biofilms on copper surfaces.[64] ^{32}S accumulated in sulfide-rich corrosion products and ^{34}S was concentrated in the residual sulfate in the culture medium. Accumulation of the lighter isotope was related to surface derivatization or corrosion as measured by weight loss. Use of this technique to identify SRB-related corrosion requires sophisticated laboratory procedures.

CONCLUSIONS

❖ The following are required for an accurate diagnosis of MIC: a sample of the corrosion product or affected surface that has not been altered by collection or storage, identification of a corrosion mechanism, identification of microorganisms capable of growth and maintenance of the corrosion mechanism in the particular environment, and demonstration of an association of the microorganisms with the observed corrosion. Three types of evidence are used to diagnosis MIC: metallurgical, chemical, and biological. The objective is to have three independent types of measurements that are consistent with a mechanism for MIC.

❖ It is essential in diagnosing MIC to demonstrate a spatial relationship between the causative microorganisms and the corrosion phenomena. However, that relationship cannot be independently interpreted as MIC. Pitting caused by MIC can initiate as small pits that have the same size and characteristics of the causative organisms. These features are not obvious to the unaided eye and are most often observed with an electron or atomic force microscope. MIC does not produce a macroscopic, unique, metallographic feature. Metallurgical features previously thought to be unique to MIC, e.g., hemispherical pits in 300 series stainless steel localized at weld or tunneling in carbon steel, are consistent with some mechanisms for MIC, but cannot be interpreted independently. Bacteria do produce corrosion products that could not be produced abiotically in near-surface environments, resulting in isotope fractionation and mineralogical fingerprints. The result is corrosion where none could be anticipated based on the composition of the bulk medium, e.g., low-chloride waters, and corrosion rates that are exceptionally fast. Development of sophisticated genetic and imaging techniques has made it possible to more accurately characterize microorganisms and their spatial relationships to corrosion products and localized corrosion. However, the requirements for diagnosing MIC have not changed.

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