microbialinsights

Integrating Molecular Microbiological Methods into Industrial Corrosion Management Programs

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Integrating Molecular Microbiological Methods into Industrial Corrosion Management Programs

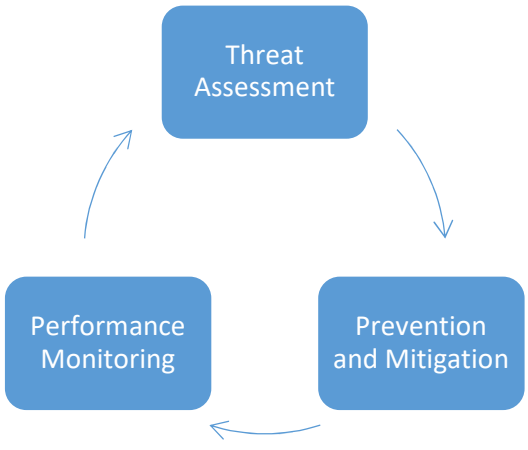
1.0 Industrial Microbiologically Influenced Corrosion

Microbiologically influenced corrosion (MIC) impacts nearly all industries and can exact a severe toll in terms of loss of production, O&M costs, equipment deterioration and potential consequences for health, safety, and the environment due corrosion-related failures. MIC includes a variety of complex processes that are affected, either directly or indirectly, by the actions and interactions of diverse microbial communities. As such, MIC can occur via several different corrosion mechanisms, including under deposit corrosion, pitting, crevice corrosion, electrical MIC, and metabolite MIC.

Although notable investments have been made in MIC research related to medical implants and the oil and gas industry, other industries are increasingly recognizing the importance of devoting resources to diagnose, mitigate, and monitor MIC to protect their system operations and assets. MIC has been identified in industrial sectors as diverse as pulp and paper, automotive, semiconductors, microchips, military, food and beverage, marine, oil and gas, energy and power, aviation, and more.

1.1 Stages of MIC management

Corrosion management involves three major stages: threat assessment, prevention and mitigation, and performance monitoring. As shown in the following diagram, each stage informs the next. During the threat assessment stage, sampling is performed to determine if there is a current corrosion issue or the potential for one to occur. Results from molecular microbiological methods (MMMs) are correlated with chemical conditions, corrosion severity, and operating conditions to determine if MIC is an issue.



Based on the data collected during threat assessment, a prevention or mitigation strategy is developed. Efforts to manage MIC threats are tailored to system processes and material characteristics of the affected assets. Strategies for controlling MIC can include chemical and physical measures such as

biocides, protective coatings, cathodic protection, corrosion inhibitors, scale control, physical cleaning, and UV treatment.

After a remedy has been implemented, performance monitoring is conducted to evaluate and optimize its efficacy. For MIC mitigation, monitoring necessitates collecting multiple lines of evidence to identify changes in the microbial community as well as corrosion rates. Results from MMMs demonstrating a decrease in microorganisms in areas of concern can be a useful early indicator of the success of a MIC control program while a longer time frame may be needed to document a treatment's effects on corrosion rates. Continuing to maintain a routine monitoring program can show when MIC indicators are increasing and trigger a more in-depth threat assessment, beginning the cycle again.

1.2 A multiple lines of evidence approach for assessing MIC

Due to its complexity, adequately addressing MIC requires interdisciplinary expertise from operators, corrosion engineers, chemists, microbiologists, and materials scientists. Unfortunately, there is not a single test that can definitively confirm MIC. Instead, a multiple lines of evidence approach is needed. Inferring the corrosion potential from chemical and mineralogical data alone can underestimate the role of microorganisms and can lead to selecting treatment methods that don't adequately address MIC.

Increasing cost and complexity of a monitoring program is a natural concern for site managers. However, microbial samples are easy to collect and can provide a key line of evidence needed to increase confidence in MIC management decisions—from diagnosing MIC threats to selecting appropriate mitigation measures and monitoring their effectiveness. Keep in mind, MIC costs companies hundreds of billions of dollars annually. Investing in thorough monitoring will pay significant dividends when it comes to protecting assets.

The following chart provides examples of operational, chemical, material, mineralogical, and microbial lines of evidence to consider when assessing MIC. The MMMs recommended for collecting microbial data will be discussed in Section 2.0.

Design and Operation

- Material grade, dimensions, and wall thickness
- Fabrication procedures, welding
- Operational history
- Operational parameters: temperature, pressure, flow rate
- System inputs and outputs
- Down time and dead legs
- Previous corrosion mitigation efforts

Chemistry

- Surface and bulk phase chemical compositions
- pH
- Redox potential
- Salinity
- Electron acceptors
- Dissolved gases

Material Surfaces and Corrosion Products

- Location(s) and severity of corrosion
- Physical characteristics of corroded surface
- Mass loss and corrosion rate from a test coupon
- Mineralogical and elemental composition of corrosion products

Microbiology

- Culture-based methods (e.g., plate counts, most probable number, test kits for microbial groups)
- ATP
- Molecular microbiological methods (e.g., CENSUS® qPCR, QuantArray®-MIC, Next Generation Sequencing)

2.0 Molecular Microbiological Methods

2.1 Molecular versus culture-based microbiological methods

Microbial data from a system can provide important information for MIC management programs. In the past, and sometimes still today, plate counts, most probable number (MPN) tests, and other culture-based techniques were used to evaluate the microbial community. However, it is very difficult to replicate conditions of the original environment when growing microorganisms in the laboratory. Also, less than 1% of microorganisms are capable of being cultured, so culture-based methods can underestimate these populations. Culture methods may also overestimate microorganisms that weren't dominant in the original sample but thrived under the laboratory conditions. Lastly, culture-based methods provide little to no functional or taxonomic information on the microorganisms present.

Analysis of adenosine triphosphate (or ATP) is another common monitoring method. ATP analysis provides a rough estimate of cell activity in a sample. However, similar to culture-based methods, ATP should be used in context and with caution. The analysis is unable to differentiate which bacteria are present, only that there is activity. There are many more microorganisms that do not influence corrosion than those that do, and so additional microbial data is necessary to truly understand risk. Molecular methods are capable of filling in key information gaps that ATP is unable to answer.

MMMs have gradually replaced culture-based methods and supplemented ATP analysis for MIC investigations as molecular techniques have matured and become more cost effective. MMMs directly analyze DNA or RNA extracted from a sample, and taxonomic and functional genes of interest can be targeted. These methods remove the need to grow microorganisms, eliminating the biases associated with traditional culture-based approaches. Quantitative polymerase chain reaction (qPCR) and next generation sequencing are two MMMs that have proven to be beneficial to MIC management.

2.2 Measuring microorganisms and functional genes associated with MIC

CENSUS® qPCR and QuantArray® are DNA-based MMMs used to accurately target and quantify microorganisms and functional genes. For MIC applications, QuantArray®-MIC analysis is recommended as it provides simultaneous quantification of over 20 different gene targets associated with MIC, including sulfate reducing bacteria and archaea, methanogens, acid producing bacteria, iron oxidizing bacteria, slime formers, and sulfur oxidizing bacteria among others.

There are several different strategies for interpreting CENSUS® qPCR and QuantArray® data. For baseline sampling events during the threat assessment stage, compare results from areas where corrosion is observed to an uncorroded background location. Higher concentrations of MIC-associated microorganisms and functional genes in areas with corrosion indicate an increased potential for MIC. For an example of this application, see the case study in Section 3.1.

Don't rule out the potential for MIC based on operating conditions alone. Microorganisms have been detected everywhere from pulp and paper bleaching systems to cleanroom environments where microchips are fabricated. Verify the presence or absence of microorganisms using CENSUS® qPCR or QuantArray®. These are sensitive analyses with practical detection limits as low as 10 cells per sample and a dynamic range over seven orders of magnitude.

To evaluate the effectiveness of MIC mitigation efforts, compare results from baseline and post-treatment sampling events. Figure 1 provides an example of using qPCR data for performance monitoring after a biocide treatment. The graph shows that concentrations of sulfate reducing bacteria decreased by several orders of magnitude after a biocide was introduced into the system. Over time, however, the population of sulfate reducing bacteria began to rebound, reaching concentrations near baseline levels by times t4 and t5, suggesting that another biocide application is needed. Incorporating CENSUS® qPCR or QuantArray®-MIC into routine monitoring lets facility managers track temporal trends in the microbial data. Increases in microorganisms associated with MIC can serve as an early warning sign, allowing responsible parties to investigate and intervene sooner.

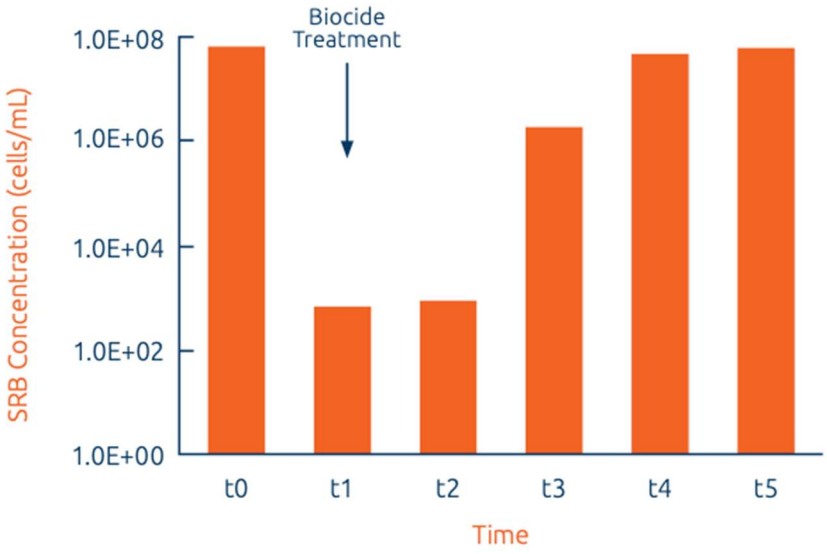


Figure 1. Concentrations of sulfate reducing bacteria before and after a biocide treatment.

2.3 Microbial community composition

Next generation sequencing (NGS) is a DNA-based technique used to identify the dominant bacteria or archaea in a sample down to the genus level, requiring no prior knowledge of the microbial community composition. The NGS profile of the microbial community can provide insight into the microbial processes, such as sulfate reduction, that may be occurring in a system. While not quantitative, NGS reports include the relative proportions of the microorganisms identified in the sample based on number of classified sequence reads as well as brief descriptions of the top genera identified, which highlight the metabolic capabilities commonly associated with each genus. For an example of how NGS results can be incorporated into MIC threat assessment and mitigation strategy selection, see the case study in Section 3.2.

Since no prior knowledge of the microbial community is needed for NGS, this analysis can identify microorganisms even if they currently can't be detected by CENSUS® qPCR and QuantArray®. If a microorganism is detected by NGS and is highly correlated with other MIC indicators in a system, Microbial Insights can use this information to design a custom CENSUS® qPCR assay for more targeted monitoring.

3.0 Case Studies

3.1 Using QuantArray®-MIC as a key line of evidence for investigating MIC

In this study, operators needed to conclusively determine whether MIC was a contributing factor to observed localized corrosion. QuantArray®-MIC was performed on swab samples of internally pitted and adjacent non-pitted areas of piping components. As part of a multiple lines of evidence approach, QuantArray®-MIC was used to quantify the total bacteria, total archaea, and a suite of key microorganisms commonly implicated in MIC. A portion of the results is shown in Figure 2 below.

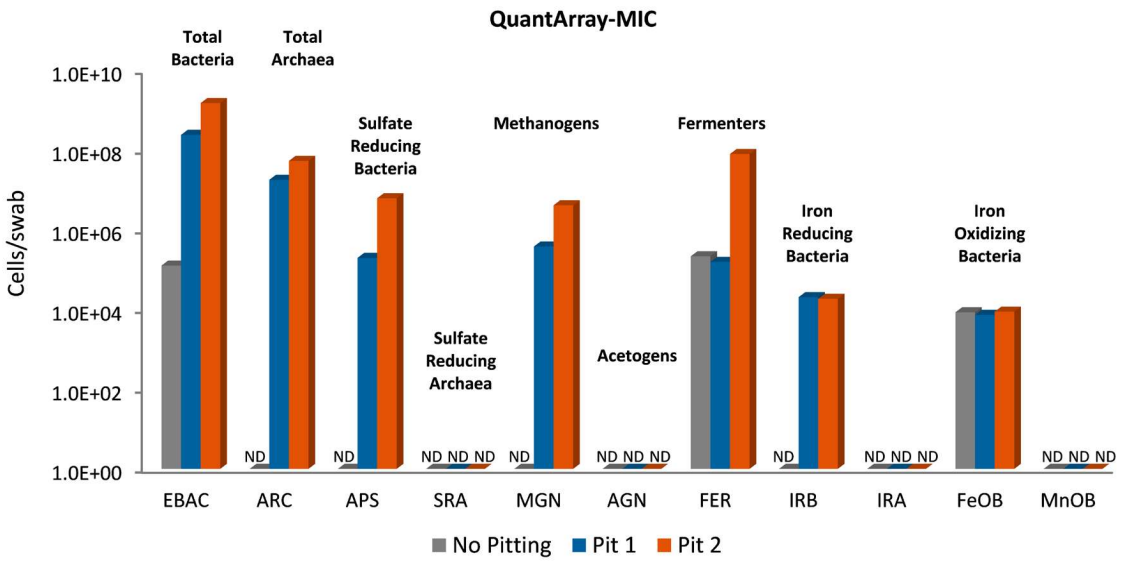


Figure 2. Comparison of QuantArray®-MIC results from pitted and non-pitted areas

- Concentrations of the Total Bacteria (EBAC) on swabs from Pit 1 (blue bars) and Pit 2 (orange bars) were more than three orders of magnitude greater than on the swab of the area with No Pitting (gray bars). Furthermore, the concentration of Total Archaea (ARC) was on the order of 10^7 cells/swab in Pit 1 and Pit 2 but was below the detection limit in the area with No Pitting.
- Concentrations of sulfate reducing bacteria (APS) and methanogens (MGN) were on the order of 10^5 to 10^6 cells/swab in the Pit 1 and Pit 2 swabs. Neither sulfate reducing bacteria nor methanogens were detected in the sample from the area with no pitting.
- Another important group of anaerobes, iron reducing bacteria (IRB), were also detected in Pit samples but not in the area with no pitting. Fermenters were detected at a high concentration in Pit 2. Fermenters are known to produce organic acids, including acetate, and support growth of other anaerobes, such as sulfate reducers.

Overall, concentrations of the total bacteria and archaea as well as sulfate reducing bacteria, methanogens, and other microorganisms commonly associated with MIC were substantially greater in the Pit samples compared to the background sample collected from the area with no pitting.

Decision

Comparing concentrations of total and specific microbial groups between pits and non-pitted areas quantitatively demonstrated growth of MIC-associated microorganisms. Combined with a review of operating conditions, chemistry, corrosion products and morphology, QuantArray®-MIC results indicating high concentrations of total bacteria, sulfate reducers, and methanogens suggested there was a substantial MIC threat. The mitigation program was then optimized to address that specific threat, using a biocide for MIC.

3.2 Characterizing microbial communities to evaluate MIC threats

As part of the MIC threat assessment, corrosion engineers wanted to generate an overall profile of the microbial communities and identify the microorganisms present at different points of the system. NGS was performed on liquid samples from key locations with different operating conditions.

Along with statistical analyses, the NGS reports from Microbial Insights included pie charts and tables of the top genera detected in each sample along with brief descriptions highlighting the common characteristics of each genus. The results from one of the samples is shown below in Table 1 and Figure 3.

Table 1. Top genera detected in Water Sample 1

Genus	Reads	Percent	Description
Desulfobulbus	414,443	44%	Strictly anaerobic genus of sulfate reducing bacteria. Sulfate and other sulfur compounds serve as electron acceptors and are reduced to hydrogen sulfide. Metabolism can be respiratory or fermentative. A limited number of organic acids and alcohols are utilized as carbon sources and electron donors which are oxidized incompletely to acetate.
Methanocalculus	104,197	11%	Genus of hydrogenotrophic methanogens. Require acetate as a carbon source.
Bacteroides	77,893	8%	Anaerobic genus of carbohydrate utilizing (saccharolytic) chemoorganotrophs. Acetic and succinic acids are fermentation products.
Pseudomonas	77,181	8%	Genus of metabolically versatile bacteria. Generally aerobic but some species are capable of nitrate reduction or complete denitrification.
Desulfovibrio	61,255	7%	Genus of sulfate reducing bacteria. Sulfate and other sulfur compounds reduced to sulfide. Some species utilize hydrogen as an electron donor. Also capable of fermentation producing acetate.
Acetobacterium	52,629	6%	Genus of acetogenic bacteria. Hydrogen and CO_2 are utilized producing acetate.
Peptococcus	31,988	3%	Anaerobic genus of chemoorganotrophs. Amino acids are fermented to acetic and other organic acids. Sulfite, thiosulfate, and elemental sulfur can be used as electron acceptors producing hydrogen sulfide.
Oscillospira	29,456	3%	Anaerobic bacteria suspected to produce butyric acid.
Geovibrio	26,681	3%	Strictly anaerobic genus of chemoorganotroph. Ferric iron and elemental sulfur are utilized as electron acceptors. Acetate, hydrogen, and some volatile fatty acids serve as electron donors.
Kosmotoga	19,688	3%	*Kosmotoga* are anaerobic chemoorganotrophs capable of fermenting carbohydrates and peptides. Sulfur compounds including elemental sulfur and thiosulfate are reduced enhancing growth.

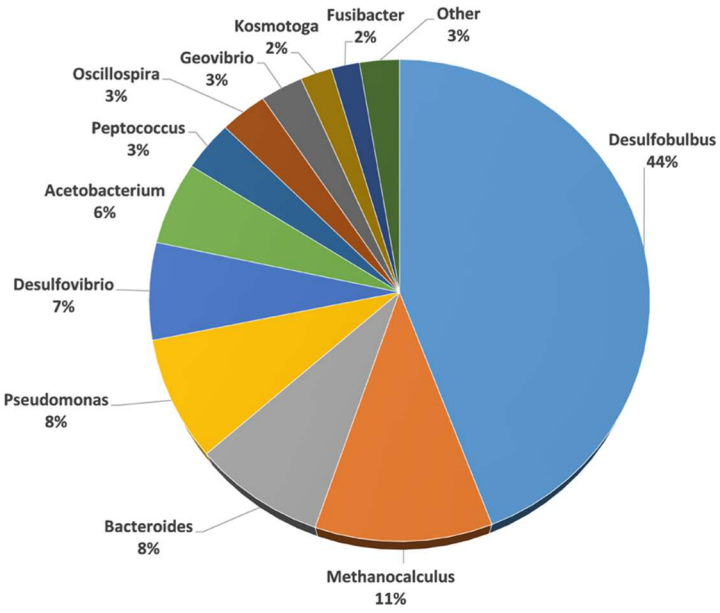


Figure 3. Pie chart of genera detected in Water Sample 1

The NGS results from Water Sample 1 indicated a diverse microbial community that included microbial groups commonly implicated in MIC, including sulfate reducing bacteria, methanogens, and acetogens.

- *Desulfobulbus*, the most frequently identified genus, and *Desulfovibrio* are sulfate reducers, indicating that the potential for hydrogen sulfide (H_2S) production should be considered. In addition, several of the other genera including *Peptococcus*, *Geovibrio*, *Kosmotoga*, and *Fusibacter* can reduce thiosulfate and/or elemental sulfur further indicating the potential for H_2S production.
- *Methanocalculus*, a group of hydrogenotrophic methanogens, was the second most frequently detected genus in the sample. Methanogens utilize hydrogen for growth and can contribute to MIC.
- Acetogens like *Acetobacterium* are strict anaerobes that produce acetate from hydrogen and CO_2. Acetogenesis has been demonstrated in high pressure natural gas pipelines and acetic acid is known to exacerbate carbon dioxide corrosion of carbon steel. Acetate can also support growth of other MIC associated microorganisms such as sulfate reducers.
- The metabolism of fermentative bacteria including *Bacteroides*, *Peptococcus*, *Kosmotoga* produces organic acids (acetate, lactate, etc.) which can dramatically reduce pH within a biofilm and can potentially re-supply protons consumed by corrosion. In addition, acetate and other volatile fatty acids are used by other anaerobes as carbon and energy sources.
- *Pseudomonas* is a well-known slime forming bacteria that contributes to biofilm formation.

Decision

NGS provided an overall profile of the microbial community and a more comprehensive assessment of potential MIC threats that would not be possible with culture-based methods. In conjunction with a review of operating conditions, corrosion monitoring, and inspections, the NGS results added a key line of evidence that indicated a threat of MIC. Since the potential for MIC appeared to be elevated, additional monitoring using

coupons and inspection for localized corrosion was implemented to determine the extent of the MIC threat rather than monitoring using electrical resistance probes, which would not be helpful for localized corrosion.

4.0 QuantArray®-MIC and NGS Sample Collection Procedures

QuantArray®-MIC and NGS analyses can be performed on nearly any sample type including water, coupons, pipe samples, corrosion product scrapings, pig run solids, and swabs of biofilms. Whenever practical, it is recommended to collect samples as close to the corrosion issue as possible as the microbial community nearest to a corroded surface can vary widely from the microbial community found in the bulk fluid. As a point of comparison to aid in interpretation, collecting a control sample from an area with similar conditions that is not experiencing corrosion is also recommended. For more detailed information on sample collection, complete protocols are available on the sampling page of the Microbial Insights website (https://microbe.com/us-sampling-protocols/).

Below are guidelines to follow when collecting samples for any DNA-based analysis.

1. Use clean latex (or similar) gloves when collecting and handling samples.
2. Keep samples cold (~4°C) to minimize changes in the microbial community.
    a. Place samples on ice or freezer packs in a cooler after collection.
    b. As soon as possible (preferably overnight), ship samples to the laboratory.
    c. Include enough ice/freezer packs to ensure that samples remain cold during shipment.

Microbial Insights has been receiving field samples for DNA-based analyses for over two decades and has performed extensive in-house testing of sample preservation and shipping requirements. Overnight shipment at 4°C combined with immediate DNA extraction upon sample receipt at the laboratory minimizes changes to the microbial community. If the sampling location is remote and overnight shipping to the lab isn't possible, on-site DNA extraction using Bio-Extract™ kits can be beneficial. Extracted DNA is more stable compared to a standard sample, and this increased preservation eliminates the need for daily overnight shipments.