COST Action CA20130

Work Group 5 – Standardisation

Proposed Pilot Study of a Laboratory-Based Microbiologically Influenced Corrosion (MIC) Test Using Microbiological Consortia Sampled from the Field

Draft Version 2.2

Prepared By:

Scott Wade, Swinburne University of Technology

Julian Wharton, University of Southampton

Rick Eckert, Microbial Corrosion Consulting, LLC

Table of Contents

1 Introduction

This document describes guidelines for conducting laboratory-based tests of microbiologically influenced corrosion (MIC) using microbiological consortia sampled from the field.

There is general consensus from experts that verification of MIC requires multiple lines of evidence (MLOE) that includes information on relevant metallurgical, microbiological and environmental/chemical aspects (Little, 2006; Eckert and Skovhus, 2021). Most standards related to MIC (e.g. AMPP TM0106, TM0194) are focussed on providing guidance on gathering such evidence. While this is current best practice, all of the information obtained is indirect (i.e. yes some MIC related microorganisms were present, yes increased localised corrosion rates were observed, etc.) and conclusions are drawn after the fact. The testing proposed in the current document is focussed on a laboratory-based test providing direct confirmation that the consortia of microorganisms sampled from the field location of interest are capable of and responsible for increasing/changing corrosion.

There are a number of examples of previous tests where microbiological samples (bulk and/or fluids) have been sampled from the field and used in laboratory-based MIC tests. The test procedures used however vary widely and there is currently no general guidance information available. This document provides examples of testing procedures that could be used to examine the use of microbiological consortia sampled from the field for laboratory-based MIC tests. The tests are not intended to be strictly prescriptive and are not the only methods that could be used. They do however attempt to take into account factors that could affect the test outcome, and they could provide either a starting point or a baseline procedure for those looking to develop a better understanding of MIC in the field.

The document includes:

- Information on how to set-up a basic laboratory-based MIC test with field sampled microorganisms, and,
- Guidance on associated analysis methods.

The outcomes of the tests described could potentially be used as:

- An extra test to validate observed corrosion has been influenced by microorganisms,
- A method for predicting the potential for MIC occurring in the future, to allow planning for building or maintaining infrastructure, and/or,
- Another method/procedure for testing the ability of new materials or mitigation methods to prevent MIC.

The current pilot study is an exercise to assess interested and capable laboratories, and to evaluate various combinations of available technologies for MIC characterisation. It is planned that the outcomes of the pilot study will be used to inform future development of a guidance document for MIC testing.

2 Aim of the Pilot Study

The aim of the work is to develop and establish a relatively simple protocol to test and characterise MIC in the laboratory using environmental samples and analytical methods. The

combination of analytical methods used must be capable of distinguishing between MIC and abiotic corrosion on the test specimen, hence the essential use of controls.

It is important to note this test procedure is not exhaustive and attempts to strike a balance between practicality and critical requirements. All details of the testing procedure should be carefully documented, especially any variations from the recommended methods. Seemingly minor/unintentional changes in testing parameters have the potential to affect the testing outcome.

There are a number of steps that may involve hazardous chemicals or procedures, it is the responsibility of the personnel undertaking the tests to check all associated health and safety matters. Hydrogen sulfide (H_2S), a by-product of sulfate reducing bacteria, is particularly dangerous. Always think safety first as a priority during these tests.

3 Materials and Equipment

Inoculum – obtain samples of sediment, orange corrosion products (rust tubercles), biofilm or other product believed to be associated with the microbial corrosion (suggested total mass ~100-200 g). Samples should be obtained as close to testing date as possible and stored in cool conditions (around 4 °C) between sampling and testing. Details of the location, date/time and methods of sampling should be recorded.

Base fluid – obtain ~20 L of fluid from the location associated with the microbial corrosion. For example, this could be seawater, river water, aqueous process fluid or other liquid of interest. Details of the geographical location, date/time and methods of sampling should be recorded.

Nutrients – this will depend largely on what microorganisms are of interest, e.g. sodium lactate (3.5 g/L) and yeast extract (1 g/L) have been used where sulfate reducing bacteria were of interest (based on the composition of modified Baar's media, also known as ATCC Medium 1249).

Carbon steel test coupons – obtain 40× carbon steel coupons (e.g. AISI 1010/UNS G10100), dimensions ~25×25 mm (thickness between 2 and 6 mm), and surface finish prepared using ~P1200 (ANSI 600) grit. Samples should have hole drilled near one edge to allow them to be hung in test bottles. Samples should be stored in a cool, dry atmosphere (e.g. desiccator or in sealed bags with silica gels) before testing. It can be helpful if individual coupons have a way of being identified (e.g. a stamped code). See Figure 1.

Test bottles – recommended 500 mL glass bottle (e.g. Schott bottles) that have a cap and can be sterilised (discussed in more detail later). Caps need to have a small hole (~2 mm diameter) drilled in the centre of the top to pass the nylon string to hold the coupons.

Nylon string – to suspend coupons in the bottles/fluids during the test (e.g. fishing line).

Note: more details on variations of test equipment and materials, including the rationale for selection, can be found in the Appendix A.

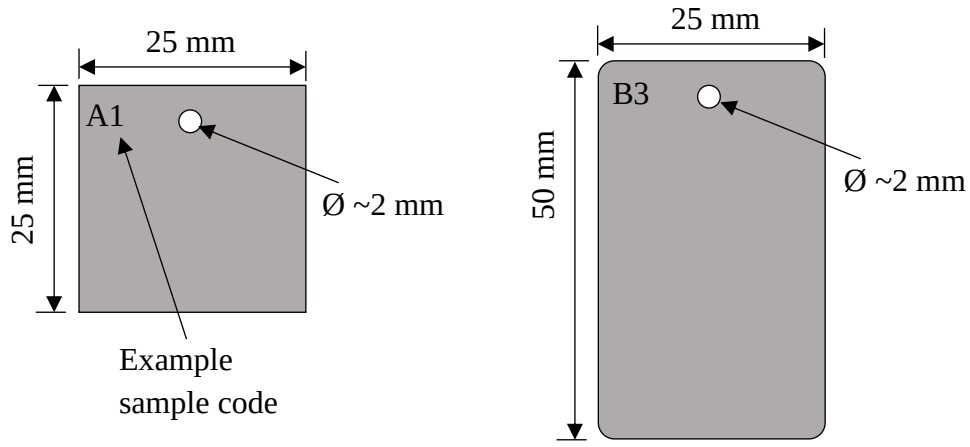

Figure 1: Some examples of metal coupons for use in the MIC trial.

4 Generic Test Procedure Set-up

Test conditions

There are four suggested test conditions recommended to determine the effect of the presence of microorganisms on the corrosion of the test coupons. Two include the microbial inoculum and the other two act as *essential* controls required for comparison:

1. Microbial inoculum, filtered test fluid, nutrients
2. Microbial inoculum, filtered test fluid, no nutrients
3. Filtered test fluid, nutrients (control)
4. Filtered test fluid, no nutrients (control)

It is suggested that at least three steel coupon replicates are tested separately for each of the above test conditions. More may be necessary depending on the chosen methods of analysis.

Analysis of base test fluid

If possible, it is recommended to analyse the physicochemical properties of the base test fluid being used. The exact parameters to be recorded will depend on the situation, but may include parameters like pH, chloride levels, conductivity, sulfate levels, indications of pollutants (chemical and biological) and hardness. Some of these parameters (e.g. dissolved oxygen, temperature, etc.) may need to be measured directly in the test bottles.

Preparing, surface analysis and weighing metal coupons

Metal coupons need to be prepared so that they are free of any surface corrosion products and the surfaces are smooth enough to be able to distinguish the morphology of any corrosion that takes place during the testing. Stepwise grinding to a finish of P1200 is recommended. Make sure samples are cleaned appropriately, e.g. ultrasonic bath and/or isopropyl alcohol rinse, following grinding. Additional samples should be prepared using the same methods to be used to analyse initial surface properties (e.g. roughness, SEM images) for comparison with the samples subjected to the corrosion tests.

Cleaned metal coupons (see above) should be weighed individually (multiple measurements for each coupon) with an accurate analytical mass balance, preferably with relatively high resolution (e.g. readability 0.001 g or better). Coupons should be cleaned and sterilised prior to weighing to remove any contaminants (samples should be free of any obvious corrosion products on the surface. This process should preferably be undertaken less than 24 hours before the start of the test. Make sure samples are stored and handled correctly (use gloves) at all times when not being tested to avoid the possibility of any flash rusting.

Disinfection/Sterilisation

Metal coupons and nylon string should be cleaned and disinfected shortly prior to testing. This could be done for example by immersing the components in isopropyl alcohol (70%) and if possible, subsequent exposure to a UV light source.

Glassware and bottle caps used for tests should be cleaned and sterilised prior to use. Steam/autoclave sterilisation is preferable, although other techniques such as isopropyl alcohol (70%) and UV exposure can be used if an autoclave is not available, or bottles could be purchased pre-sterilised.

These procedures should be done as close to the start of the test as possible.

Test fluid preparation (including sterilisation)

(i) Test fluid containing nutrients

Add nutrients to base test fluid, combine by gentle agitation, and then filter sterilise (use 0.2 μm filter if possible) into clean bottle to be used for testing. Leave 10-20% unfilled headspace in bottles (see Figure 2). Attach bottle cap loosely to help maintain sterility. If possible then pasteurise solution by placing in 70 °C oven for 2 hrs.

(ii) Test fluid without nutrients

Follow steps above without the addition of any nutrients.

An extra amount of the test fluid is recommended to be prepared and used for analysis of the initial properties of the test fluid. See discussion in the Analysis Methods section in relation to some of the different types of analysis that could be considered.

Adding inoculum and metal coupons

Add inoculum (approx. 5 g wet weight) to test solution and gently mix into solution by rotating the bottle.

Attach disinfected nylon string to cleaned and disinfected metal coupon, with enough length so that it can hang roughly in the middle of the height of the test fluid and there is plenty of string to tie off.

Immerse the metal coupon in the fluid in the test bottle and close the bottle cap tightly. Label the test bottle with a code to identify test conditions.

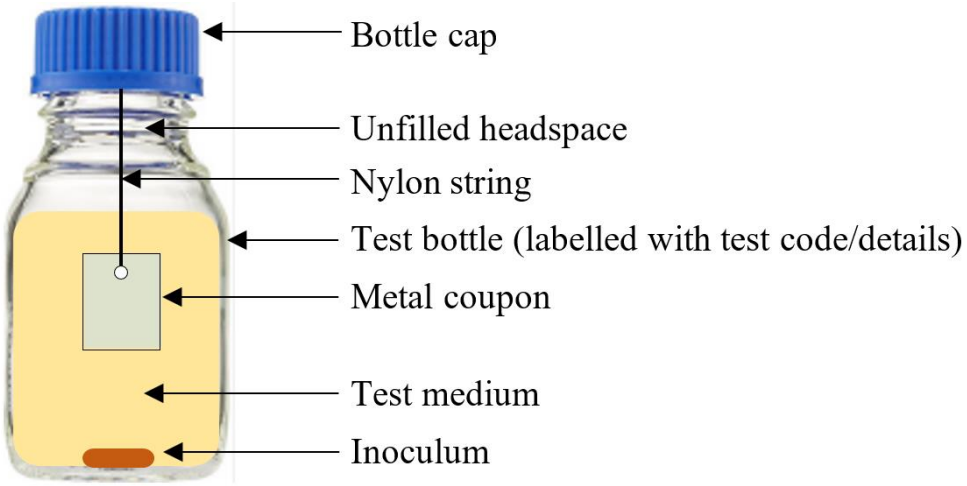

Figure 2: Diagram showing example test set-up of microbial corrosion test including microbial inoculum.

Where at all possible perform the above steps using aseptic techniques to help minimise contamination. Wear appropriate gloves and perform relevant steps in a laminar flow hood or with a Bunsen burner.

5 Running the Test

It is recommended that the metal samples undergo an immersion period of a minimum of 30 days before removal for inspection. Where possible, additional samples could be tested for longer times, e.g. 60 days, 90 days or even longer. The longer test durations can provide more meaningful data; however, issues with contamination can become increasingly problematic.

The test bottles should be stored in relatively clean conditions that mimic, within reason, the environment that the trial is aiming to simulate/test. Considerations may include, for example, exposure (or not) to sunlight and the ambient temperature of interest. Another example could be if the location is with “running water”, where gentle stirring or shaking might be needed to mimic the fluid flow. If possible, avoid storage locations where there is lots of movement of people or work being undertaken that may lead to the bottles being moved, bumped or broken. Bottles can be stored with a spill container underneath just in case. It is important to record the environmental conditions which the test bottles are exposed to during the trial (e.g. use a simple temperature logger).

Important*:* The tests should be performed in a well-ventilated location to help minimise any problems/dangers due to gaseous by-products (e.g. H_2S) that may be produced by microorganisms in the test solutions. A risk assessment should be performed to make sure that the tests can be undertaken safely and that they comply with any local occupational health and safety requirements.

Regular (e.g. daily) observations (including photos) should be made of the test bottles and metal coupons during the testing period, with an emphasis on the early stages of testing (i.e.

the first week). Note any changes in colour of test solutions, or to the surfaces of the metal coupons.

6 End of Test

Ideally coupons and the test fluid should be analysed shortly (i.e. directly) after removal of the coupon from the test fluid. Avoid removing coupons and storing for extended periods prior to analysing (i.e. greater than a few days after removal).

Take photos of each of the coupons (hint: add written code to identify coupon/test conditions in the photo) straight after removal from the test fluid.

Sampling the test fluid/biofilm/surface deposits

Please note that the nature and type of samples collected will be based upon the analysis methods chosen for use. All sampling should be performed immediately after the end of each test period (on the last day of the test) and not delayed. In some cases, this may need to be done with a high priority, for example prior to any movement mixing of the fluid and/or before metal coupon removal (e.g. for dissolved oxygen). See the Section 7: Analysis Methods section for discussion about different types of analysis that can be performed.

Sampling of liquid and biofilms

Collection of biofilm and corrosion products from the coupons must be performed as soon as the coupons are removed from the test medium. Similarly, chemical and microbiological sampling/testing of the test medium should be performed immediately after each sample container is opened. The timing and sampling methods used need to be recorded.

Biofilm and surface deposit samples may be collected on sterile swabs so as not to cause any damage to the coupon surface. The use of scrapers or other hard instruments to collect solid samples is not recommended and can affect the validity of the weight loss/pitting results. If solids on the coupon surface contain iron sulfides keep in mind that these will quickly oxidize and change once the coupon is exposed to air, unless efforts are made to keep the coupon under anoxic conditions after testing.

Cleaning the metal coupons

Please note that some analysis methods may involve testing the corrosion/biofilm products that form on the surface of the metal coupons. Any of those tests need to be performed prior to cleaning the coupons.

To allow weight loss measurements and any observations of corrosion morphology the corrosion/biofilm products that form on the surface of the metal coupons need to be removed. There are a number of methods to do this and care needs to be taken that any cleaning method does not adversely affect the test coupon, while removing all deposits from the surface. Clarke's solution cleaning using fresh solution, limited immersion time and careful post cleaning with distilled water and isopropyl alcohol is one such method (see more information in the References section and take note of associated health and safety matters). This can be

combined with initial ultrasonic cleaning in distilled water to initially remove bulk products. Electrolytic cleaning is another cleaning process that could be used.

Weighing for mass loss measurements (e.g. ASTM G1) should be performed shortly after cleaning. Test samples should be stored in a cool, dry atmosphere (e.g. desiccator or in sealed bags with silica gels) after cleaning.

Disposal of fluids

Where possible, it is recommended to disinfect the test fluid prior to disposal. This can be done for example using a diluted bleach solution. Follow all local occupational health and safety requirements

7 Analysis Methods

Objective of Selected Methods

The principal objective of the analysis methods selected is to provide discrimination between the inoculated tests and un-inoculated "abiotic" controls such that the influence of the microorganisms on any corrosion that forms can be determined. Regardless of which methods are selected, the combination of methods used should be able to provide the ability to determine whether or not MIC has occurred.

The methods used for taking samples and storage prior to testing can significantly influence the results obtained, so please take time to investigate the optimal methods for these and to document how it was done.

Multiple lines of evidence (see Figure 3) are required to be used in the analysis, including tests on microbiological conditions, metallurgical conditions and media and surface chemistry. The exact methods used will depend upon various factors such as what methods are available, costs and any specific information that might be related to particular corrosion processes of interest. In general, the more techniques used the better (assuming that they are performed and analysed correctly) but at the very least they should cover microbiological, metallurgical and media chemistry aspects.

Suggestions for some tests that would be of high value include:

- Molecular microbiological testing of biofilms removed from the coupon surfaces
- ATP testing of the test medium and biofilms
- Elemental and mineralogical analysis of corrosion products recovered from (or present on) the coupons
- Liquid chemistry parameters at the start and end of the test, such as pH, organic acid analysis, presence of sulfide ion, cations/anions (particularly sulfate)
- High quality photo documentation of coupon conditions before and after cleaning
- Measurements of mass change of coupons from before and after testing
- Microscopic/profilometric examination of coupon surface condition, particularly the size, number and depth of pits

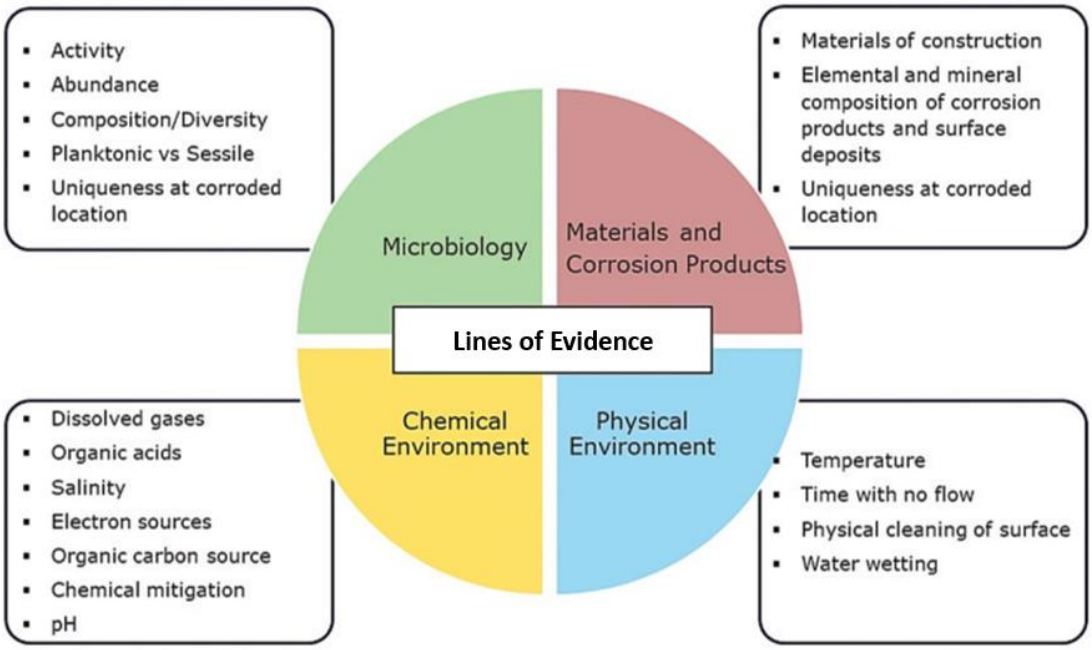

Figure 3: Illustration of multiple lines of evidence that can be used to characterise MIC and abiotic corrosion. (Kotu and Eckert, 2019).

Care should be taken to make sure that the number of measurements is sufficient, both in the number of individual measurement replicates and that different areas and coupons are tested where possible. Increased numbers of measurements help to improve statistical accuracy. Details of any test equipment used, and the associated measurement resolutions, should be recorded.

Tables 1 and 2 provide some further details of different types of analysis that can be performed.

Microbiological Tests

The purpose of the microbiological testing is to identify differences in activity, numbers and microbial community composition between the control and inoculated test samples.

Examples include:

- Microbiological testing of biofilms removed from the coupon surfaces and the test medium using:
  - Quantitative polymerase chain reaction (qPCR) using primers for functional groups relevant to MIC
  - Planktonic and sessile culturable bacteria counts (e.g. plate counting, MPN)
  - Adenosine triphosphate (ATP)
  - High throughput/next-generation (16S rRNA gene sequencing)

Metallurgical/Corrosion Damage Characterisation

The purpose of this testing is to characterise differences in corrosion damage (e.g. morphology, severity) between the control and test samples.

Examples are:

- Weight loss, pitting information, corrosion attack morphology using microscopy or surface profiling
- High quality photo documentation of coupon conditions before and after cleaning
- Microscopic/profilometric examination of coupon surface condition, particularly the size, number and depth of pits

Note: while electrochemical test methods can provide important information for MIC studies, for the sake of simplicity they have not been included in this pilot.

Media/surface deposit chemistry

The purpose of this testing is to characterise differences in the chemical composition of the test medium and surface deposits between the control and test samples.

Examples of tests that could be used for media chemistry:

- Changes in pH
- Cations/anions
- Organic acids
- Sulfate/sulfide ions
- Total iron
- Dissolved oxygen level

Examples of tests that could be used for surface or deposit chemistry:

- Energy dispersive X-ray spectroscopy (EDS) of coupon with solids or collected solids
- X-ray diffraction (XRD) of collected solids
- X-ray photoelectron spectroscopy (XPS) of coupon with solids or collected solids

See Tables 1 and 2 for lists of analysis methods, their advantages and limitations.

Table 1. Examples of analytical techniques that can be used to obtain microbiological information from the MIC tests

Method	**Pros**	**Cons**
Plate counts	Low cost. By using selective medium, types of microorganisms can be isolated.	<1% of microorganisms can be cultured; very limited information on taxonomy. Takes several days to get results. Molecular approaches needed to confirm results.
Most Probable Numbers (MPN)	Low cost. Easy to use.	Fails to recognize activity. Many MIC related microorganisms are non-culturable, cannot be detected by MPN.
Test kits to enumerate MIC related microorganisms	Easy to use. No trained personnel are needed.	Low specificity. Takes days to get results. False results occur. Use of circumstances has to be carefully determined.
Light microscopy	Easy to use. Low cost.	Detection of microorganisms is not diagnostic for MIC.
Epifluorescence Microscopy	Useful for testing effectiveness of biocide killing or to show the presence of microorganisms at the site of corrosion.	Sample needs to be fixed and stained. Often difficult to differentiate.
Confocal Laser Scanning Microscopy (CLSM)	By using specific staining, the biofilm on the surface can be visualised.	Skilled personnel are needed.
Electron Microscopy (EM)	Visualisation of microorganisms/biofilm at the site of corrosion. Direct contact of microorganism with metal can be shown.	Skilled personnel needed. During SEM analysis, the original structure of biofilm can be disturbed. Very limited ability to determine types of microorganisms present.
Atomic Force Microscopy (AFM)	Higher resolution than SEM.	Skilled personnel needed. Typically used to scan smaller areas.
Hybridization methods, e.g. FISH, DNA microarrays	Quantitative. Selected genes (groups of microorganisms, e.g. SRB, SRA, Bacteria, Archaea) can be visualised.	Skilled personnel needed. Prior knowledge of the microorganisms to be detected is needed. Trained personnel needed.

Method	Pros	Cons
Polymerase chain reaction (PCR) / quantitative PCR	Relatively cheap and fast. Can provide results in a few hours.	Genes have to be selected for amplification, unknown species remain unidentified. Skilled personnel required, or samples need to be sent to a commercial accredited laboratory.
Amplicon sequencing	Specific for bacteria or fungi at genus or species level. Less costly than shot-gun metagenomics.	Contamination is amplified (amplification bias). Less taxonomic resolution. Functional profiling is not possible. Skilled personnel required, or samples need to be sent to a commercial accredited laboratory. Data analysis/interpretation requires skilled personnel.
Shot-gun metagenomics	High specificity. High taxonomy resolution. Functional profiling possible. Covers viruses, bacteria, archaea and eukarya at species or strain level.	Does not differentiate between active or passive cells. Can be costly, though getting cheaper. Samples have to be sent to specialised laboratories for analysis. Data analysis/interpretation requires skilled personnel.
Metatranscriptomics	Provides valuable information about gene activity.	Larger sample size is needed to perform analyses. Samples are more sensitive to degradation. Can be costly and specialised laboratory is needed. Data analysis/interpretation requires skilled personnel.
Metabolomics	Identification and quantification of metabolites related to MIC	Can be costly and specialised laboratory needed. Multiple analytical methods may be needed. Care needed in sample handling. Data analysis/interpretation requires skilled personnel.
ATP assay method	Easy to use, fast measure to provide estimation of microbial inhibition, metabolic state of microorganism, and total biomass.	Does not provide information about the composition of the biomass.

Table 2. Examples of analytical techniques for the study of metal surfaces and corrosion by-products in MIC tests

Analysis techniques	**Description**	**Pros**	**Cons**
Mass loss	Measuring the change of mass of a test coupon provides an indication of corrosion rate.	Relatively simple	Assumes general / uniform corrosion; does not measure localised corrosion. Localised corrosion often more important for MIC.
Localised corrosion (pitting) rate	Calculating localised corrosion penetration rate based on microscopic or profilometric observations/measurements.	Relatively simple; important metric for MIC, which is often localised	Can be challenging to measure the depth of very small diameter, deep pits.
Surface profiling	Provides information on localised corrosion rates and morphology. Various methods (e.g. AFM, 2D and 3D scanners).	Localised corrosion attack common for MIC	Analysis can be time consuming and/or expensive equipment required.
Scanning electron microscopy (SEM)	Provides information on localised corrosion morphology.	Quick, widely available, may require sample to be vacuum compatible	Morphology cannot necessarily be directly related to MIC attack.
Energy-dispersive X-ray spectroscopy (EDS)	Often combined with SEM. Provides spatially resolved elemental analysis, useful for determining corrosion products.	Quick, widely available	Semi quantitative, numerous elemental peaks overlap, less information than other surface analysis methods.
X-ray diffraction spectroscopy (XRD)	Technique for characterising crystalline materials, useful for determining corrosion products.	Minimal sample preparation. Can identify key MIC by-products	Cannot identify amorphous materials
X-ray photoelectron spectroscopy (XPS)	Based on the photoelectric effect, identifies elements present on the surface of a sample, useful for determining corrosion products.	Provides detailed chemical state information, quantitative analysis	Not commonly available, sample must be ultra-high vacuum compatible.

Analysis techniques	Description	Pros	Cons
X-ray fluorescence spectroscopy (XRF)	Uses the emission of characteristic "secondary" (or fluorescent) X-rays to non-destructively determine the chemical composition of a base material.	Rapid method to determine elemental composition	Does not provide crystallographic information.
FTIR (ATR-FTIR)	Allows identification of chemical functional groups, able to differentiate organic and or inorganic materials and microorganisms present on the surface of a sample, can identify corrosion products, bacterial presence or even monitor bacterial-surface interactions.	Quick, non-destructive, widely available, minimal sample preparation to obtain chemical complex information	It can only be used in a laboratory setting and is sensitive to water, which can mask and disturb the obtained spectra. There is no distinction between viable and dead cells.

Notes:

1. Where possible avoid performing analysis close to the edges, near the hole drilled in the coupon or close to any stamped codes.

Appendix A – Details on Materials, Equipment and Sample Sources

Materials/Equipment

The following text provides some more details of the justification for and types of materials/equipment recommended for the testing and the rationale in some cases.

Inoculum – the aim of the inoculum is to provide a source of microorganisms that might be involved in MIC. This pilot study is aimed at trying to mimic/test real-world relevant conditions with the inoculum being either a solid product that may be a source of microorganisms (e.g. sediment) or a sample of a biofilm/corrosion product associated with MIC.

If possible, analysis of the microbial communities present in the inoculum (e.g. via high throughput/next-generation (16S rRNA gene sequencing) would be helpful.

Base fluid – the aim should be to provide a base fluid which is identical or closely matches the fluid in the location for which MIC is being investigated. This should provide the same chemical conditions and nutrient supply required for both the microorganisms and the corrosion processes taking place. Where possible this based fluid sample should be taken from the field test site of interest (from which the inoculum was collected).

Nutrients – the provision of nutrients is aimed at providing a kick start to accelerate any microbial processes that may result in MIC, to help cover the depletion of the limited supply of nutrients provided in the base test fluid and to provide some required nutrients, which may only periodically be present in the base fluid (e.g. pollutants) and may not have been present (or elevated) at the time the base test fluid was sampled. The exact nutrients to be used will depend on the microorganisms and microbial processes of specific interest. The nutrients suggested in this document are based on MIC associated with sulfate-reducing bacteria.

Carbon steel test coupons – the aim of using carbon steel for these tests is to limit one of the key variables of the trial, and steel was chosen as it is one of the most common materials used in key infrastructure and is also susceptible to MIC. Where possible, a standard grade of carbon steel (e.g. AISI 1010/UNS G10100) should be used for consistency in the pilot study trial. If this is not available a structural grade steel could be used. If so, details of the exact grade, composition and microstructure should be sought/determined and documented.

Some methods used to cut coupons to size can heat the steel close to the cutting edge, which can affect the local microstructure. Where possible use/specify low temperature cutting methods (e.g. waterjet cutting or water cooled abrasive sectioning).

The corrosion that can occur in the tests can obscure the code used to identify a test coupon. Hence stamping is recommended rather than engraving. However, in some cases even a stamped code can become obscured. Additional care should be taken to keep track of which unique coupon sample is in each test bottle.

The surface finish can affect the outcome of MIC tests and the ability to visualise corrosion attack, particularly using optical microscopy, profilometry and scanning electron microscopy. A surface finish using ~P1200 (ANSI 600) grit is recommended for consistency across the trial

and also this finish has been found suitable to allow localised corrosion of the levels expected to be observed. If profilometry will be used in the analysis, coupons must be sufficiently flat.

Verify the neck size of the test bottles that will be used before the coupons are made, as the metal coupons will need to be small enough to fit into the bottle.

Test bottles – the tests are recommended to be performed in clear containers that can be sterilised. Glass or plastic can be used.

Nylon string – this just needs to be something that can be sterilised and is strong enough to hold the weight of the metal coupons being tested.

Possible Sources of Materials

Inoculum – the inoculum being either a solid product, which may be a source of microorganisms (e.g. sediment), or a sample of a biofilm/corrosion product associated with MIC.

If a source of inoculum is not available from a suspected MIC location or area suspected of being associated with MIC, other sources of relevant microorganisms may be possible for the purposes of this trial. All details of how such samples were obtained (e.g. date/time, location, etc.) and any information about the microbial communities present (e.g. via high throughput/next-generation (16S rRNA gene sequencing) would be helpful. Other sources of microbial communities could be marine sediment, or mud/sediment from wetlands or rivers, or slime or tubercles from wells or plumbing systems. Black sediments and systems with a sulfur/rotten egg smell can be an indication of the presence of sulfate-reducing bacteria (SRB).

Safety Note

Hydrogen sulfide (H_2S) is a by-product of SRB, and is a toxic and flammable gas (www.osha.gov/hydrogen-sulfide/hazards). SRB are present in many cases of MIC and may be present in environmental samples taken for use as an inoculum in this trial. There is a possibility that the conditions used in the pilot study may select for and enhance the growth of SRB. As such, it is strongly recommended that appropriate information about H_2S is obtained from an appropriate local source and a risk-assessment is undertaken to ensure that the tests can be performed safely. It is recommended that a H_2S detector (e.g. portable devices are available from a wide range of companies) be incorporated into the testing program.

Appendix B – Pilot Study: Test Form

Purpose: The purpose of this form is to collect information from potential participating labs regarding their envisioned experimental set-up and analyses to be performed after the test.

The pilot study is not a round robin test program; its intent is to lay the groundwork for a future MIC round robin procedure. The experiences shared by labs participating in the pilot study will help promote a common understanding of how MIC and abiotic corrosion can be differentiated from one another.

Institution Name:…

Project Leader name and contact information:…

Does your lab have previous experience working with biofilms or MIC? If so, please briefly describe.

Estimated length of time (weeks) needed to set-up and conduct tests, and perform analysis after exposure?

Materials and Equipment

Please describe your proposed experimental setup regarding the following details:

Inoculum Source:

Base Fluid Source/Type:

How will the inoculum be collected?

Does your lab have experience working with this inoculum?

Does your lab have experience maintaining sterile controls?

Nutrients:

Coupon Material Type:

Coupon Surface Finish:

Coupon Dimensions:

Type/Volume of Test Bottles:

Means of supporting coupon in bottle:

Please list any other relevant details, particularly for deviations from the pilot test procedure.

Test Procedures

Will your experiment include each of the 4 test conditions below?

1. Microbial inoculum, filtered test fluid, nutrients
2. Microbial inoculum, filtered test fluid, no nutrients
3. Filtered test fluid, nutrients (control)
4. Filtered test fluid, no nutrients (control)

If not, or other tests are planned, please explain how they would be different and the reasoning:

Number of replicates to be used for each test condition:

List all proposed tests to document physicochemical properties of the base test fluid prior to the test:

Chemical Tests:

Microbiological Tests:

Other Tests:

Will these same tests be applied to the test medium after the experiments?

Please indicate any variations from the test procedure in section 4 of the pilot RR.

Coupon Preparation:

Disinfection/Sterilization:

Test Fluid Preparation:

Adding Inoculum and coupon:

Running the Test

Proposed duration of test(s):

Where will the bottles be stored during the test?

What is the range of temperatures expected in the storage location?

Have considerations for hydrogen sulfide safety been addressed? Please describe.

How will the conditions in the test bottles be documented over the course of the test?

Will any other monitoring be performed during the test? Please describe.

End of Test

Will samples be collected immediately after the end of the test?

What liquid sampling will be performed? How will samples be handled?

How will biofilms and surface deposits be sampled and preserved for analysis?

After samples are collected, how will the coupons be cleaned?

How will the coupons be protected from further corrosion after the test?

Analysis Methods

Multiple lines of evidence are required to be used in the analysis, including tests on microbiological conditions, metallurgical conditions and media and surface chemistry. Please provide details for the following tests that will be used.

Microbiological tests of liquids, solids and swabs:

Chemical Analysis of Deposits:

Chemical Analysis of Liquids:

Which methods will be used for characterization of corrosion damage on the coupons?

How will localized corrosion (pitting) on the coupons be characterized?

Will all the testing be performed at your institution? If not, which of the tests are performed by others?

Reporting and Management

The EUROMIC COST Action does not directly fund research activity, rather, COST supports collaboration and dissemination.

Will your lab be seeking grant money to perform the testing? If so, who are the funding entities you will approach?

Do you have a rough cost estimate for the work performed by your institution for this experiment?

At the end of the experiment, the collected information should be integrated and analyzed to develop the conclusions. A report be required from any institution performing the pilot study, so the information can be combined and shared with others. Is your institution agreeable to this?

Do you anticipate publishing the results of your experiment outside of participation in the EUROMIC program?

Is there a particular discipline area where you would welcome support from other experts involved in EUROMIC?

Useful References

AMPP TM0106-2016-SG, (2016) Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion (MIC) on External Surfaces of Buried Pipelines.

AMPP TM0194-2014-SG, (2014) Field Monitoring of Bacterial Growth in Oil and Gas Systems.

ASTM G1 (2017) Standard Practice for Preparing, Cleaning, and Evaluating Corrosion Test Specimens, ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA, 19428-2959 USA.

ASTM G46 Standard Guide for Examination and Evaluation of Pitting Corrosion, ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA, 19428-2959 USA.

ATTC 1249, Modified Baar's Medium For Sulfate Reducers, www.atcc.org

Abilio AA, Wolodko J, Eckert RB, and Skovhus TL, (2021) Review and Gap Analysis of MIC Failure Investigation Methods in Alberta's Oil and Gas Sector, in Failure analysis of microbiologically influenced corrosion, 1st ed., R.B. Eckert and T.L. Skovhus, Eds. CRC Press, pp. 25–66. http://dx.doi.org/10.1201/9780429355479-3

Chaves IA, Jeffrey R, Melchers RE, (2015) Rust removal from steel coupons after short-term marine immersion, *Corrosion* **71**(7), p. 811–818. https://doi.org/10.5006/1649

Eckert RB and Skovhus TL, (2018) Advances in the application of molecular microbiological methods in the oil and gas industry and links to microbiologically influenced corrosion, *International Biodeterioration and Biodegradation* **126**, pp. 169-176. https://doi.org/10.1016/j.ibiod.2016.11.019

Eckert RB, and Skovhus TL (Eds.). (2021). Failure Analysis of Microbiologically Influenced Corrosion (1st ed.). Chapter 3 A Practical Approach to Corrosion Failure Analysis CRC Press. https://doi.org/10.1201/9780429355479

Lahme S, Mand J, Longwell J, Smith R, and Enning D, (2021) Severe corrosion of carbon steel in oil field produced water can be linked to methanogenic archaea containing a special type of [NiFe] hydrogenase, *Applied Environmental Microbiology* **87**(3), e01819-20. https://doi.org/10.1128/aem.01819-20

Little B, Gerchakov S, Udey L, (1987) A method for sterilization of natural seawater, *J. Microbiol. Meth.* **7**(4–5), p. 193–200. https://doi.org/10.1016/0167-7012(87)90040-6

Little BJ, Lee JS, Ray RI, (2006) Diagnosing Microbiologically Influenced Corrosion: A State-of-the-Art Review, *Corrosion* 62(11), p. 1006–1017. http://dx.doi.org/10.5006/1.3278228

Kotu SP and Eckert R, (2019) A framework for conducting analysis of microbiologically influenced corrosion failures, *Inspectioneering Journal*, **25**, Issue 4. Failure Analysis of Microbiologically Influenced Corrosion (MIC) (inspectioneering.com)

Okpala GN, Eresia-Eke R and Gieg L, (2021) Molecular methods for assessing microbial corrosion and souring potential in oilfield operations, Chapter 9 in Microbial Bioinformatics in the Oil and Gas Industry: Applications to Reservoirs and Processes, edited by Kenneth Wunch, et al., Taylor & Francis Group, 2021. https://doi.org/10.1201/9781003023395

Sharma M, Liu H, Tsesmetzis N, Handy J, Place T, Gieg LM, (2022) Diagnosing microbiologically influenced corrosion at a crude oil pipeline facility leak site – A multiple lines of evidence approach, *International Biodeterioration and Biodegradation* **172**, 105438. https://doi.org/10.1016/j.ibiod.2022.105438

Wade SA and Lizama Y, (2015) Clarke's solution cleaning used for corrosion product removal: Effects on carbon steel substrate, Conference Corrosion & Prevention, Adelaide, Australia, Paper 050.

Wade SA, Javed MA, Palombo, EA McArthur SL and Stoddart PR, (2017) On the need for more realistic experimental conditions in laboratory-based microbiologically influenced corrosion testing, *International Biodeterioration and Biodegradation* **121**, pp. 97-106. https://doi.org/10.1016/j.ibiod.2017.03.027

Wade SA and Blackall LL, (2018) Development of a laboratory test for microbial involvement in accelerated low water corrosion, Microbiology Australia **39**(3) pp. 170-172. https://doi.org/10.1071/MA18049