Integrated molecular approaches for microbial monitoring in the **Oil and Gas Sector**

NCIMB's oilfield microbiology services include an innovative approach that integrates cutting-edge molecular methodologies to bring an unparalleled insight and depth of understanding to the management and mitigation of microbially influenced corrosion.

Benefits:

- Improved biomonitoring methods that allow detailed, data-driven assessments of MIC risk.
- Clearer data reporting and the ability to set improved key performance indicators (KPI).

Meeting the microbial monitoring challenge

Microbiologically influenced corrosion (MIC) is a complex and difficult to predict process. Effective management to prevent the costly and damaging failures that are associated with MIC therefore relies on regular monitoring of the microbial populations present.

Microbial enumeration

The most probable number method (MPN) has been commonly used for microbial enumeration in the oil and gas industry and is described in NACE standard TMO194-2014. This process involves serial dilution of a sample in selective growth media followed by incubation at an optimal temperature for the microbes of interest. The "most probable number" present in the original sample is estimated from the dilution at which no growth occurs.

Leveraging molecular technologies

Advances in molecular biology have provided additional techniques for assaying microbes, revolutionizing environmental microbiology in terms of both sensitivity and specificity of results. These analytical methods can be applied to the oilfield environment. There is substantial, industry-wide interest in the use of DNA-based detection and enumeration techniques to gain a deeper functional understanding of the microbial populations.

qPCR

Quantitative polymerase chain reaction (qPCR) is used extensively by the oil and gas industry, and continues to grow in popularity. It is a flexible tool which can be used to rapidly obtain quantitative data about microorganisms e.g. total bacteria or total archaea, a narrower group of particular interest e.g. sulphate reducing bacteria, or even a single species.

Results are achieved more quickly as qPCR has no requirement for microbial growth, whereas the MPN method can require up to four weeks incubation. Another benefit of qPCR in comparison to MPN is that it can detect and quantify microbes that are present and active in the environment but cannot be cultured under laboratory conditions.

A qPCR assay is based on the selection of DNA primers that replicate a specific DNA sequence from the microbes of interest, along with a dye that fluoresces when bound to the double stranded DNA. Measurement of fluorescence is used to quantify the amount of DNA present, which can be linked directly to the number of microbes present in the initial sample. Selection of appropriate primers is therefore key to the specificity and accuracy of this technique.

Metagenomic analysis

A more recent molecular approach is 16S metagenomic analysis (also known as metabarcoding), and this is gaining traction in the oil and gas industry. Recent technology developments have rapidly decreased the costs and increased the accuracy of this approach, meaning it is now more feasible as a commercial assay.

16S metagenomics is based on sequencing part of the variable regions of the 16S rRNA gene. As it does not rely on the use of selective media or restrictive DNA primers, it offers a more unbiased look at microbial composition than either qPCR or MPN, effectively assaying and identifying every group of bacteria and archaea present to offer a game-changing insight into the microbial diversity of an environmental sample, in a single analysis.

Similarly, to qPCR, metagenomic analysis has no requirement for an incubation phase for microbial growth, but the data produced is semi-quantitative, rather than quantitative, as 16S metagenomics analysis describes the relative abundances of all the different taxonomic groups within the microbial community sampled.

Data Integration

The different microbial monitoring methods available all have their strengths and limitations. However, pairing the use of qPCR for quantification of total bacteria and archaea, with metagenomic analysis for determination of the groups of microbes present, results in a comprehensive and powerful analytical tool for the detection and management of problem microbes. It provides a more meaningful understanding of the microbiological burden of the system under study than either qPCR or metagenomic analysis alone, and allows

Providing more meaningful data

Combining 16S metagenomics with qPCR gives greater quantitative power and provides a more meaningful understanding of the microbiological burden of the system under study.

us to more accurately quantify the levels, and taxonomic distribution of MIC relevant archaea and bacteria. While qPCR alone can be used to quantify groups of microbes, a published case study (Lomans *et al.*, 2016) concluded the integrated approach gave more accurate results in terms of predicting pipeline failure. A worked example of the data integration is shown in Figure 1.

Microbial Community Functional Analysis

The data generated by this integrated approach has further benefits over and above understanding the prevalence and composition of the groups of microbes in a system, such as SRB, SRA and methanogens. Additional value can be leveraged. For example, the oil and gas industry does not currently benefit from information about what the microbial community present is doing, its functional dynamics and metabolic potential. At NCIMB we can use the data generated to establish enhanced reporting with additional insight down to the level of individual biochemical pathways, such as sulphur reduction, and nitrate utilisation. This data can be used to further develop and refine the assessment of MIC risk.

Sampling requirements

We recommend that sampling includes both planktonic and sessile samples from the production and water injection systems.

Both bacteria and archaea are known to be associated with MIC, and therefore, for qPCR, in order to achieve the most reliable data, we recommend separate analysis of total bacteria and total archaea. The minimum requirement is analysis of total prokaryotes to be used in conjunction with the 16S metagenomic data.

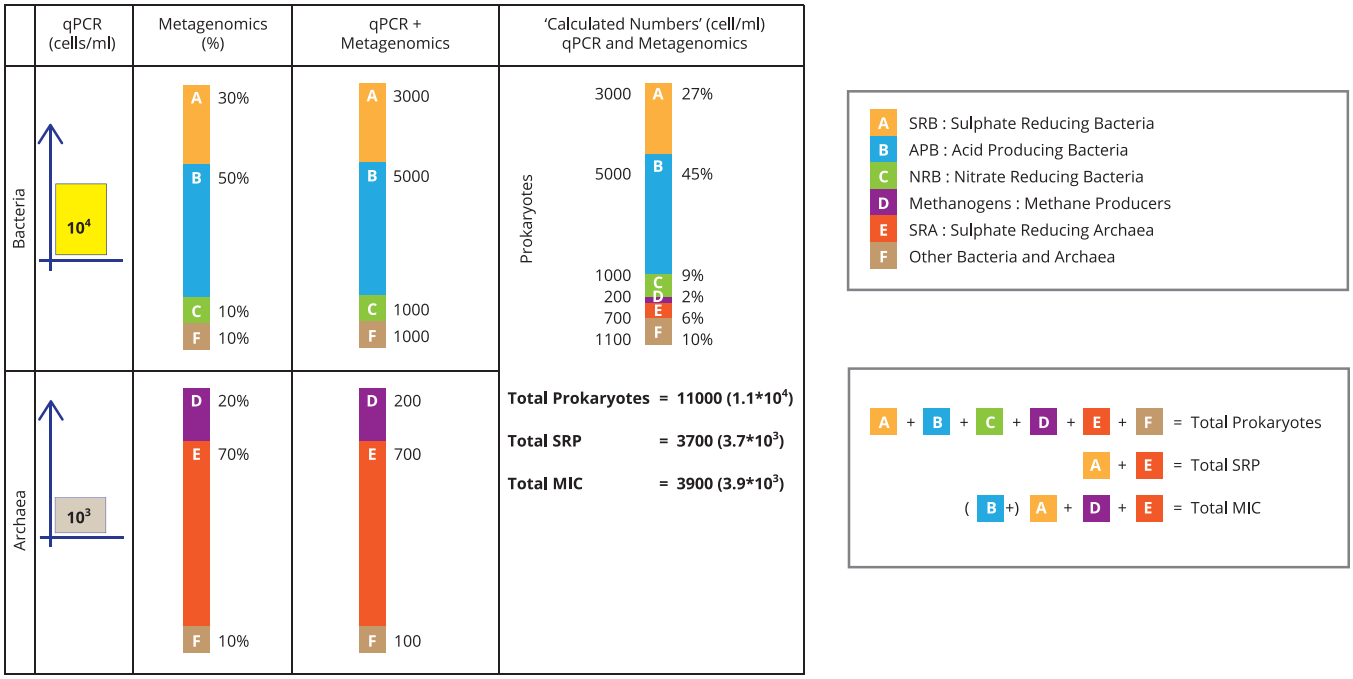

Figure 1: *Worked example of data integration: Total bacteria count (1 x 10^4 cell/ml) are multiplied with the relative abundances of bacterial species (%). This is repeated for archaea. Total bacteria + total archaea = size of prokaryotic community. Species that are known to contribute to MIC are combined to generate 'calculated numbers' for total MIC. Reproduced from Lomans et al., 2016.*

Integrated molecular approaches for microbial monitoring in the **Oil and Gas Sector**

Benefits of an integrated approach

Integrating qPCR and 16S metagenomic analysis delivers an improved method of biomonitoring for the purpose of determining the risk of MIC. It offers accurate detection, identification and enumeration of the microbial community present in each sample for:

- Identification of production areas with high potential of MIC allowing earlier effective remediation.
- Monitoring and trend analysis of changes in microbial communities over time and in response to interventions.
- Improved visual data reporting
- The ability to set more effective Key Performance Indicators (KPIs) for monitoring and management.
- Allowing improved selection of effective biocides based on analysis of the microbial community.

References

Lomans, B, de Paula, R, and Geissier, B (2016). Proposal of improved biomonitoring standard for purpose of microbiologically influenced corrosion risk assessment. SPE-179919-MS

NACE Standard TMO194 - 2014 – SG. Field monitoring of bacterial growth in oil and gas systems. Publication date: 2014, NACE International.

For more information contact

Dr Carol Devine,
Consultant Microbiologist, NCIMB
Michelle Robertson,
Analytical Services Manager, NCIMB

Additional services for the Oil and Gas Sector include:

Analytical services

We offer a suite of tests that are commonly required by the oil and gas industry, including total hydrocarbon content, COD, BOD, pH, total suspended solids, sulphide/sulphate, and chlorine/chloride. We accept a range of solid and liquid samples.

Ecotoxicity testing

NCIMB provides whole effluent assessment as part of a risk-based approach to produced water management. Our multispecies microbial toxicity tests MARA and LumiMARA were recommended as part of the preferred approach for companies operating in the UKCS.

Fuel testing

NCIMB tests diesel and marine gas oil for the presence of bacteria and fungi, particulate contamination and water content. We carry out investigations to problem solve contamination issues, and can test for the presence of a suspected contaminant.

Contact us for a quote or more information

NCIMB Limited
Wellheads Place
Aberdeen AB21 7GB
United Kingdom

t. +44 (0) 1224 009333
e. enquiries@ncimb.com
w. www.ncimb.com

@ncimb
ncimb-ltd
ncimbltd

Achilles FPAL MEMBER

bmtrada ISO 9001 CERTIFICATION SYSTEM CERTIFICATION
UKAS MANAGEMENT SYSTEMS 0012

January 2023