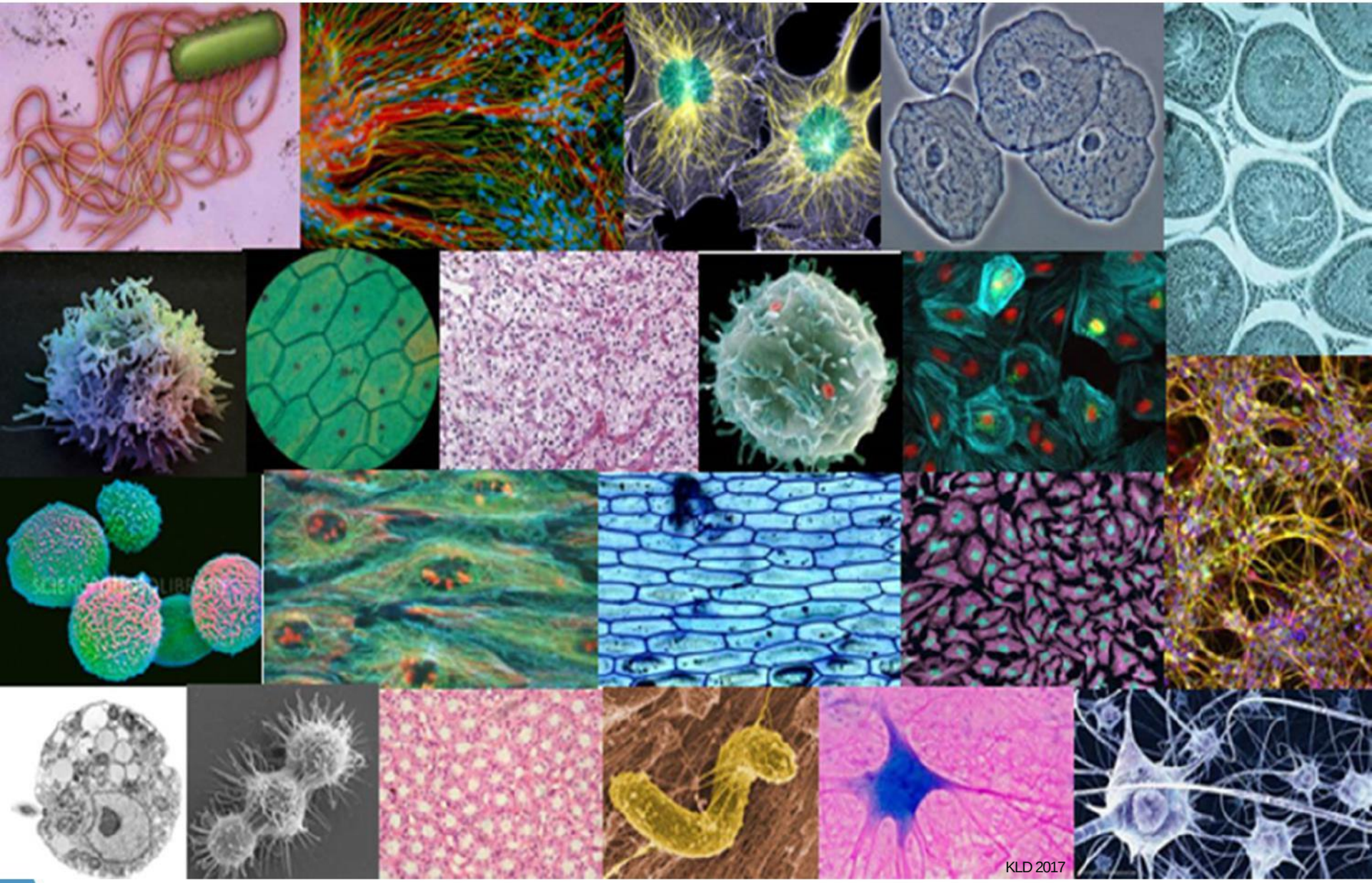
INSTITUTE OF CORROSION

Corrosion Awareness Day 2023

Oilfield Microbiology and Microbiologically Influenced Corrosion – Analysis & Monitoring

Dr Carol Devine

22nd August 2023


KLD 2017

Dr Carol Devine, Consultant Microbiologist, NCIMB Ltd

BSc Microbiology University of Aberdeen

PhD Subsurface molecular ecology University of Aberdeen

Commercial Microbiology / Intertek 11 Years

NECE/ICR

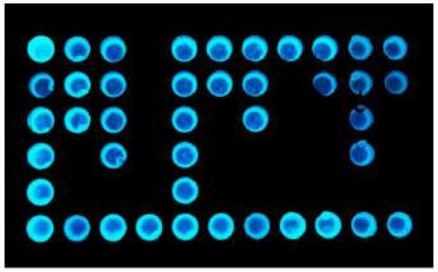
- 10 years

NCIMB

3 years

We are all about managing microorganisms.....

NCIMB

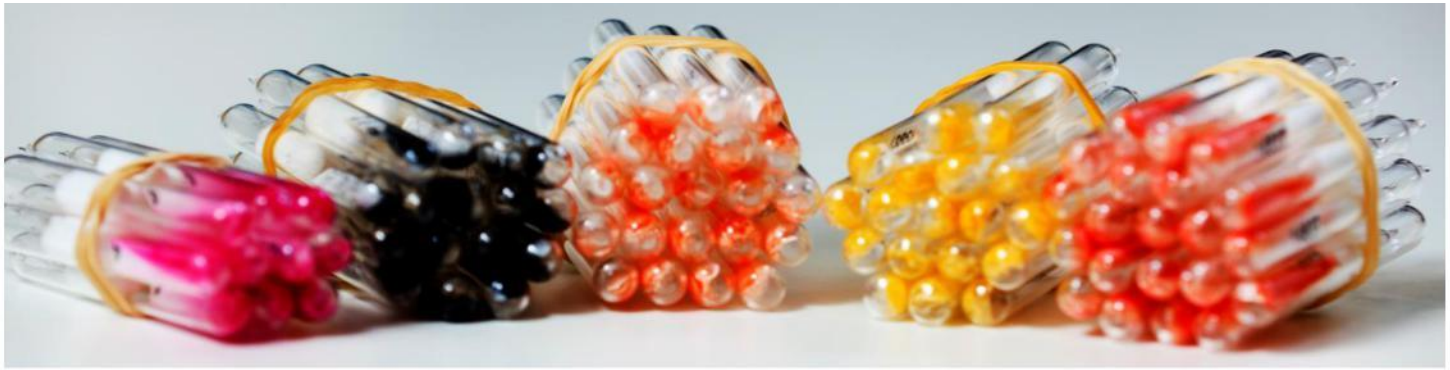


Microbiology | Identification | Biological Storage

Preserve, store and supply: The culture collection

NCIMB

- Largest collection of industrial, marine and food bacteria in the UK
- We have approx. 10,000 strains; 300 genera; 2000 species - and growing!
- Mainly bacteria, plus yeasts, plasmids & bacteriophage
- Sold to companies and universities globally
- Industrial applications



Introduction to Microbiology

- Oilfield Microbiology

Microbiological Issues

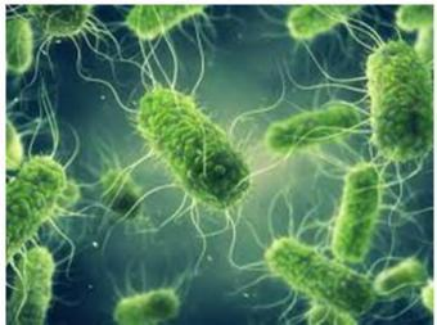
- Where do the microbes come from?
- What are the consequences?

Microbiologically Influenced Corrosion (MIC)

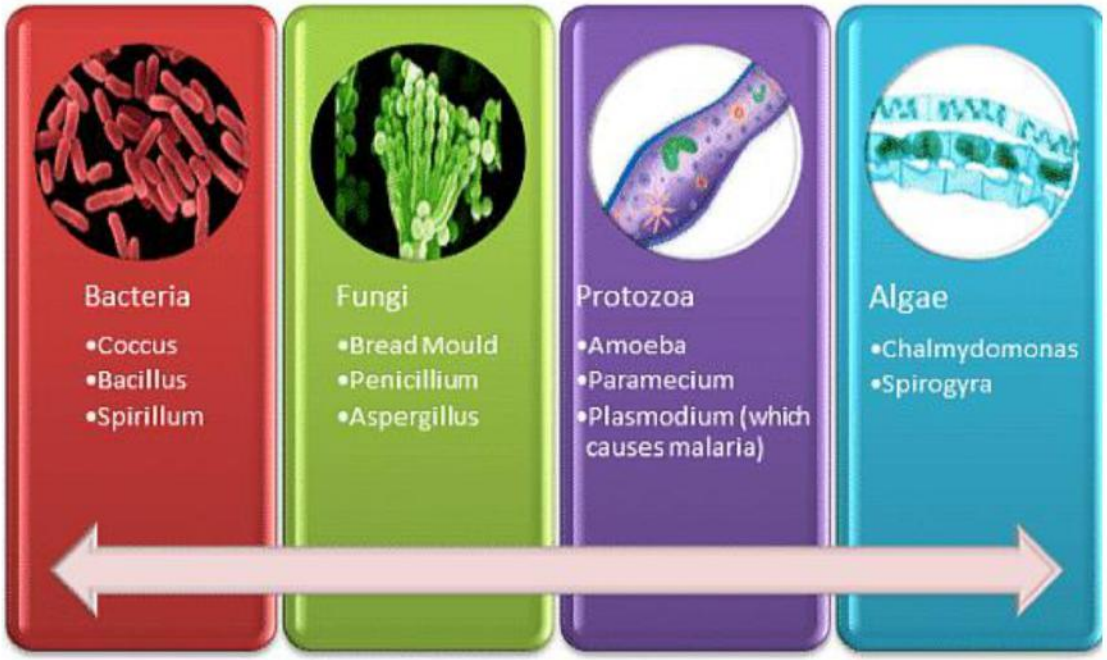
- Systems
- Samples

Monitoring & Management

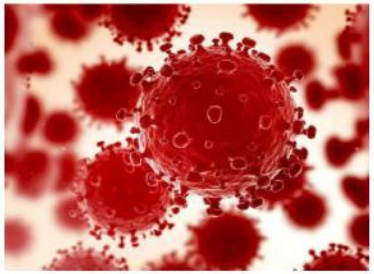
- Analytical Techniques
- Data Trending



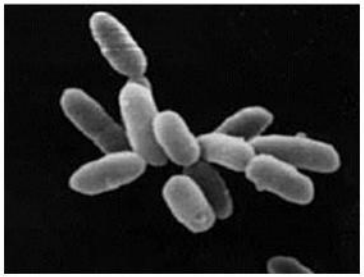
Microbes



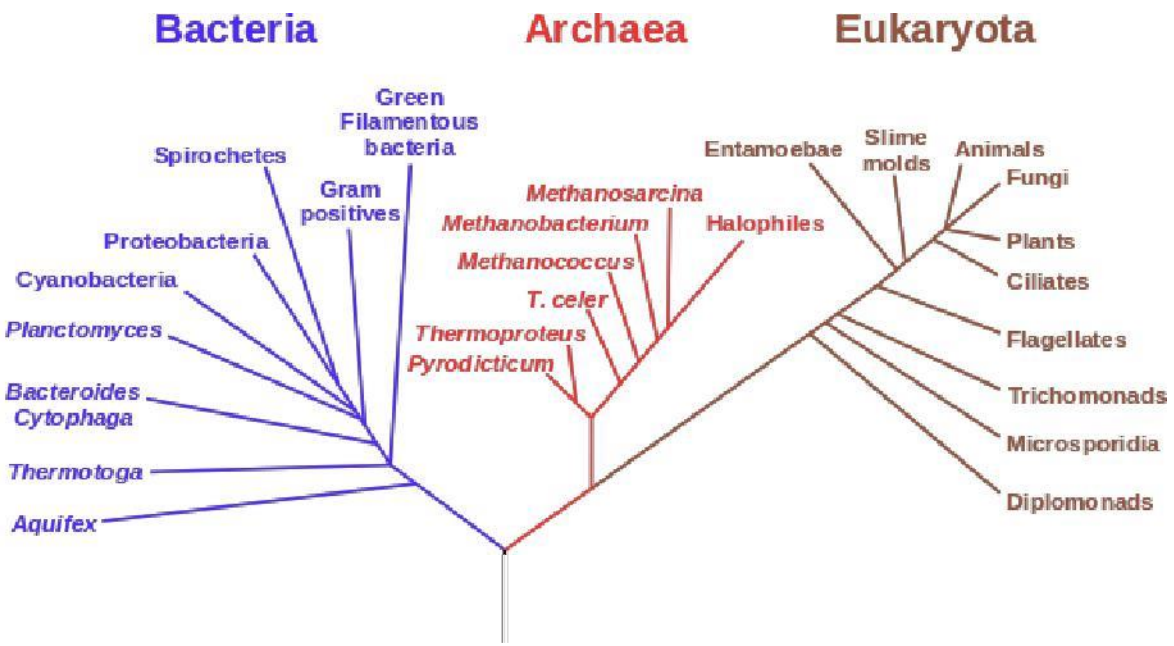
Plus
- Viruses

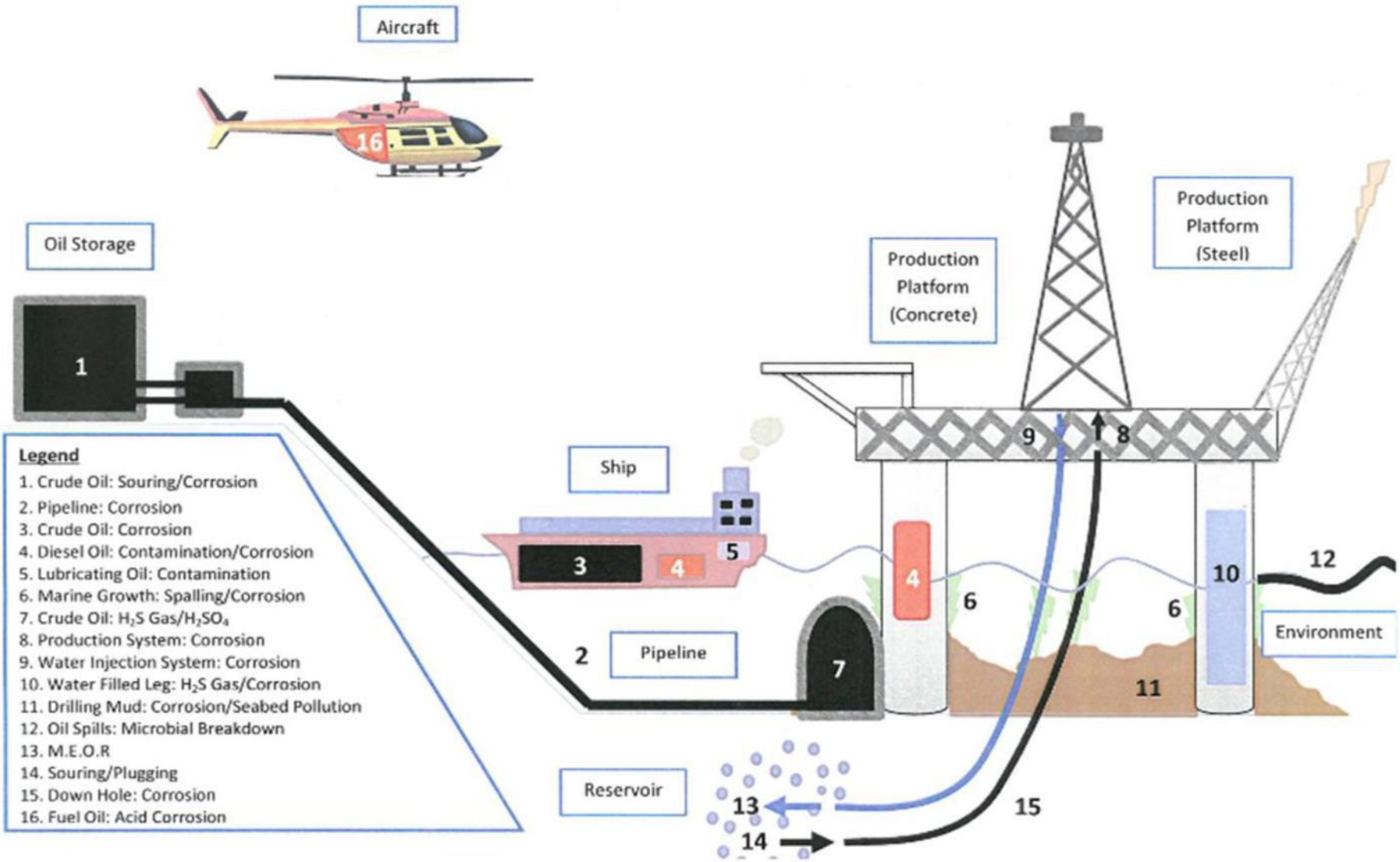


Plus
- Archaebacteria



Tree of Life


Bacteria
Archaea
Eukaryota
Green Filamentous bacteria
Spirochetes
Gram positives
Proteobacteria
Cyanobacteria
Planctomyces
Bacteroides
Cytophaga
Thermotoga
Aquifex
Methanosarcina
Methanobacterium
Methanococcus
T. celer
Thermoproteus
Pyrodicticum
Halophiles
Entamoebae
Slime molds
Animals
Fungi
Plants
Ciliates
Flagellates
Trichomonads
Microsporidia
Diplomonads



P. Sanders *et al.*

Systems affected by the presence and activity of micro-organisms:

- Production • Seawater Cooling
- Water Injection • Cooling/heating
- Produced Water • Firewater

  Reinjection (PWRI) • Diesel storage &
- Ballast water distribution

The aim of the oilfield microbiologist is to generate useful and appropriate data in order to:

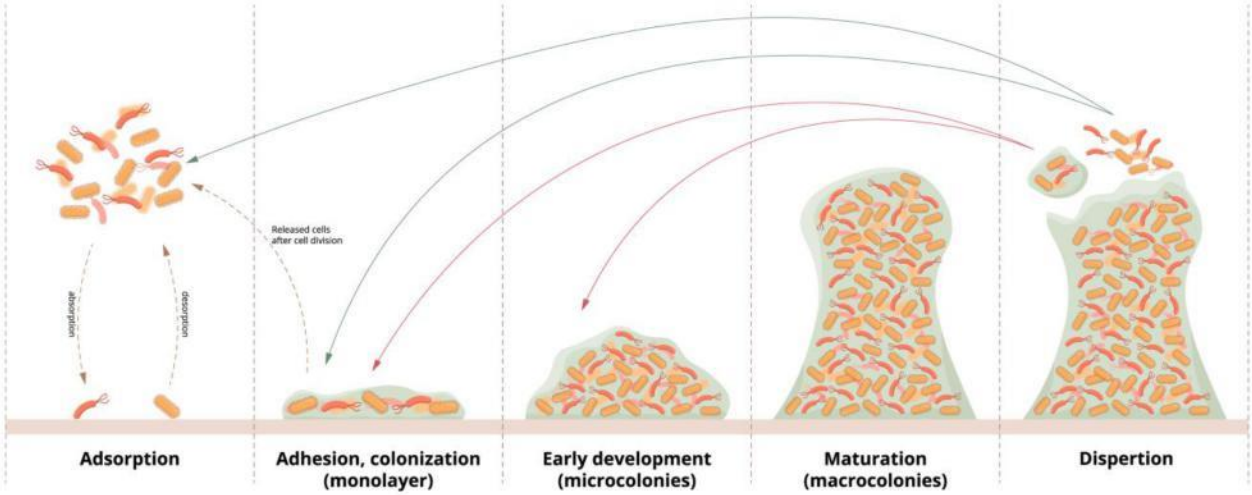
- **Predict** which particular systems, vessels, pipelines, locations are under threat from microbiologically influenced corrosion (MIC)

- **Prioritise** areas for treatment according to budget and time available

- **Apply** and monitor appropriate strategies to mitigate against the effects of MIC or biofouling

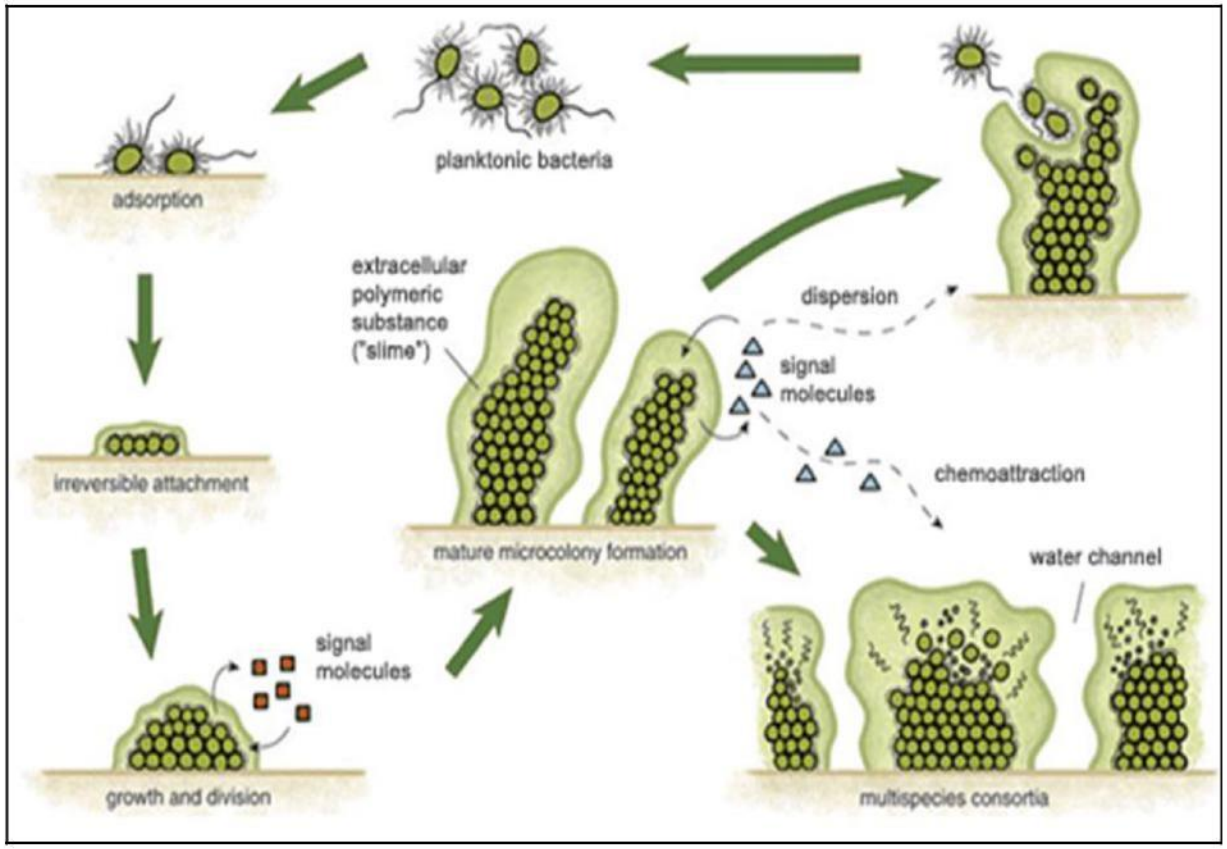
Planktonic – bulk phase from water, crude, diesel, cooling medium

Sessile – biofilm from coupons, bio-sidestreams and/or other intrusive devices



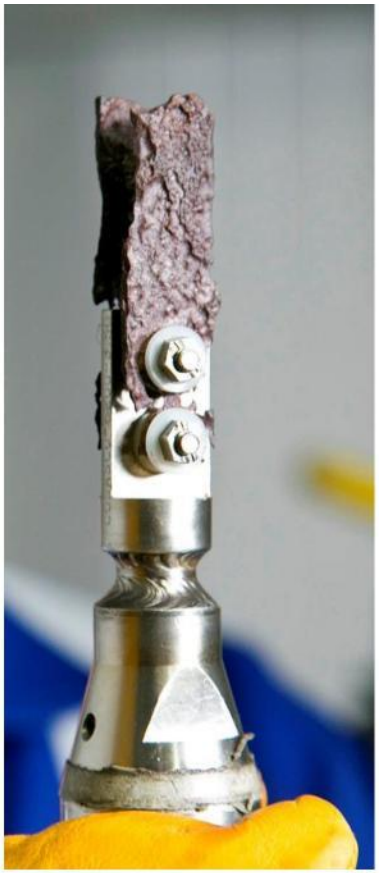
Biofilm lifecycle

Sessile – biofilm from coupons, bio-sidestreams and/or other intrusive devices



Monitoring biofilm formation using corrosion coupons

Direct system exposure



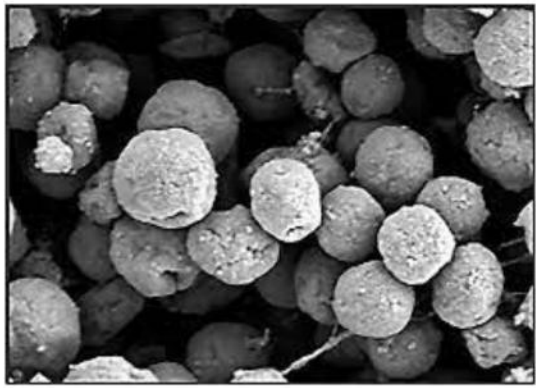
Sessile microbial samples and weight loss analysis

Monitoring and Management – analytical techniques

Choice of analytical technique depends on what you are looking for – and on the budget!

MIC is a highly complex microbial process which is thought to involve:

- sulfate-reducing bacteria
- sulfate-reducing archaea
- methanogens
- acid-producing general heterotrophic bacteria (APGHB or APB)
- Iron-utilising bacteria
- (general heterotrophic bacteria)



Testing for : sulfate-reducing bacteria (SRB)
general heterotrophs
acid-producing bacteria
nitrite-reducing bacteria
bacteria and fungi in diesels
nitrate-reducing bacteria

sulfate-reducing bacteria
sulfate-reducing archaebacteria (SRA)
methanogens
Total archaebacteria

Techniques: microscopy (DAPI)
traditional viable counts (MPNs)
molecular techniques – qPCR, FISH, DAPI, ATP

Chemistry: pH, sulfide, bisulfite, volatile fatty acids (VFAs),
chlorine residuals, total iron, nitrite, nitrate, sulfate etc

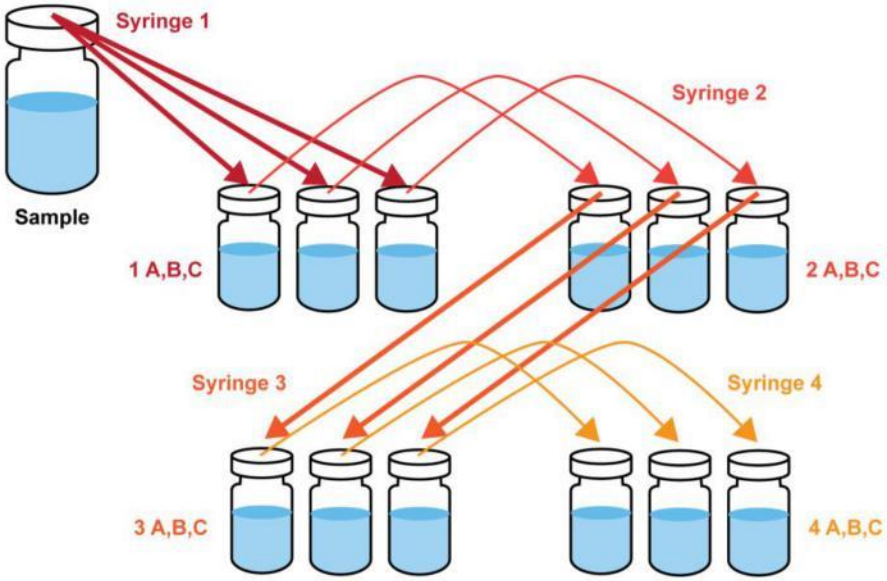
1. Triplicate MPNs

- Can detect a variety of active microbes
- Can be performed in field by trained personnel
- Vast body of historical data

• Media must match environmental conditions the sample comes from i.e., temperature and salinity
• Detects less than 5% of the total community

- SRB incubation period is 28 days

(according to TMO194)

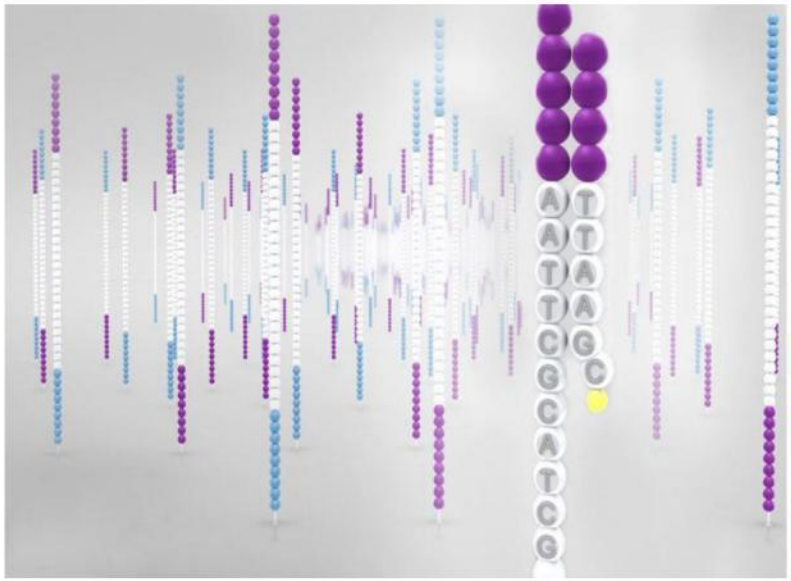


2. qPCR

- A laboratory technique based on the polymerase chain reaction
- Used to amplify and simultaneously quantify a targeted DNA molecule
- The cells are lysed, and a chemical reaction set up where the DNA is amplified exponentially
- A DNA-binding dye binds to all double-stranded (ds)DNA in the PCR reaction, causing fluorescence of the dye
- An increase in DNA product therefore leads to an increase in fluorescence
- Allows DNA concentrations to be quantified and the number of cells present in the original sample to be estimated.

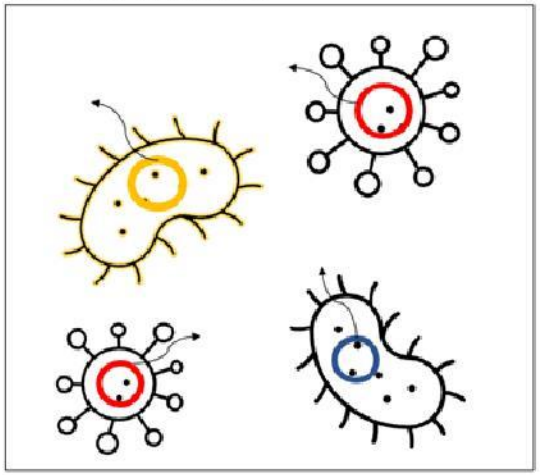
3. Metagenomic Analysis (NGS)

- Application of New Generation Sequencing to characterise the total microbial community
- Identifies all micro-organisms present

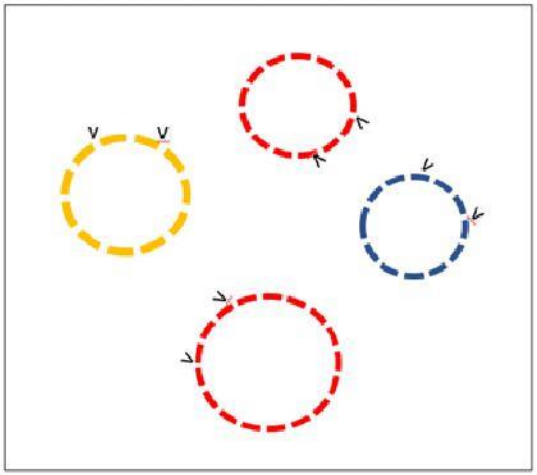


Sequencing

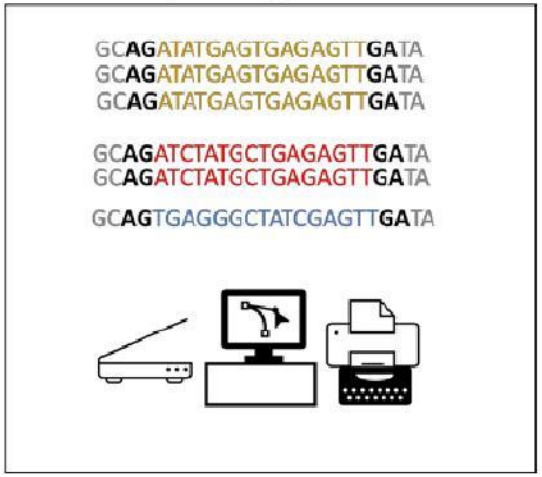
1. DNA Extraction



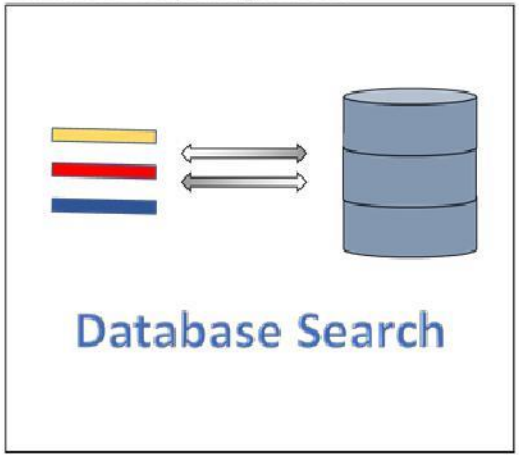
2. Gene Amplification (PCR)



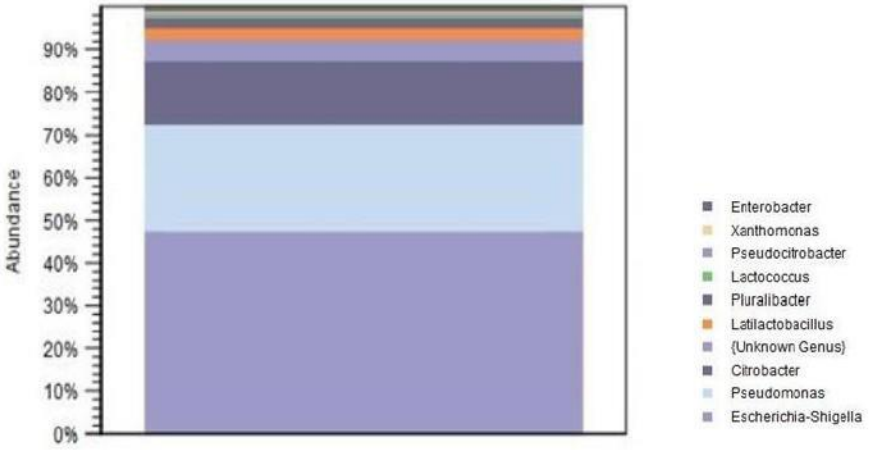
3. DNA Sequencing



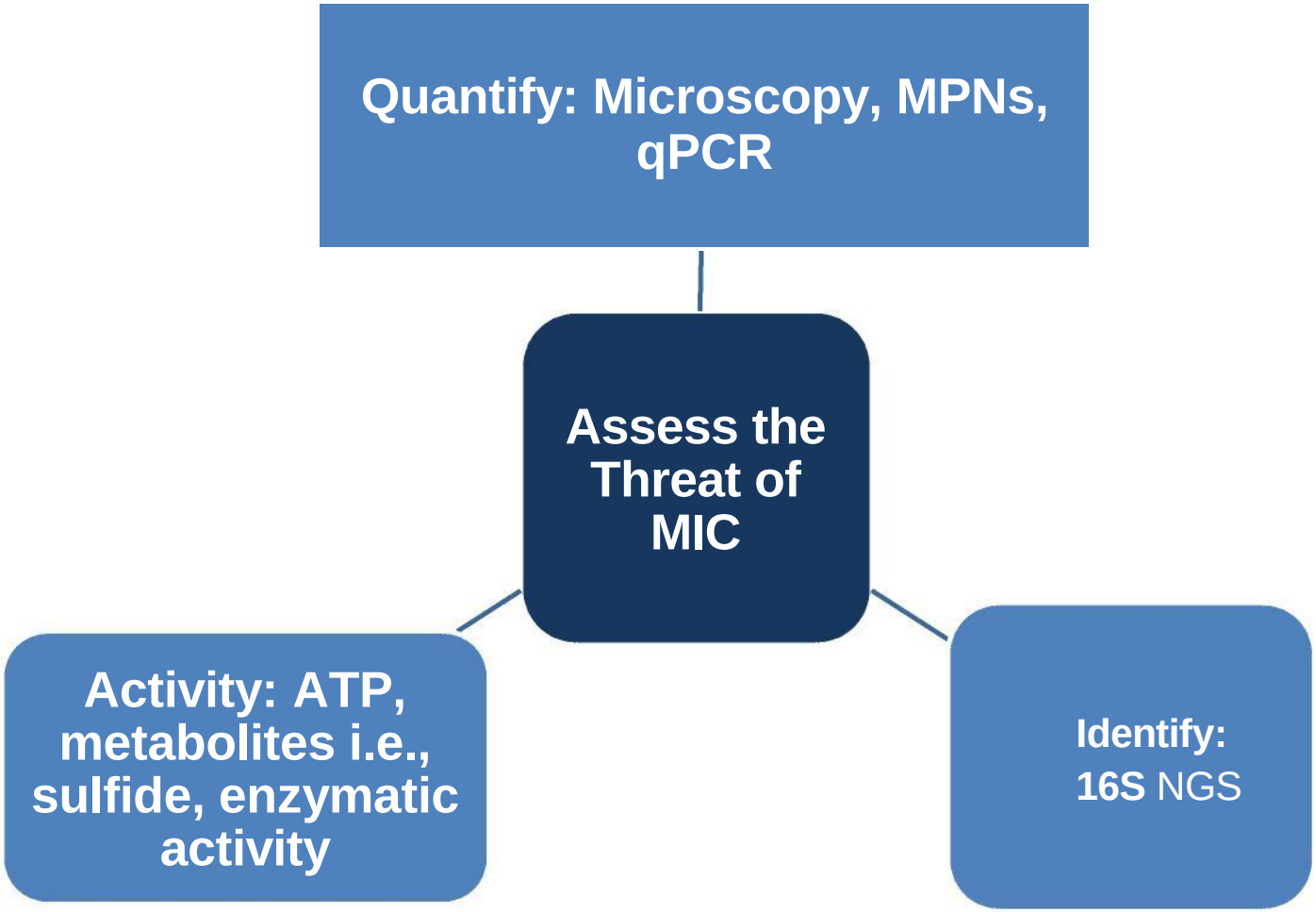
4. Taxonomy assignment



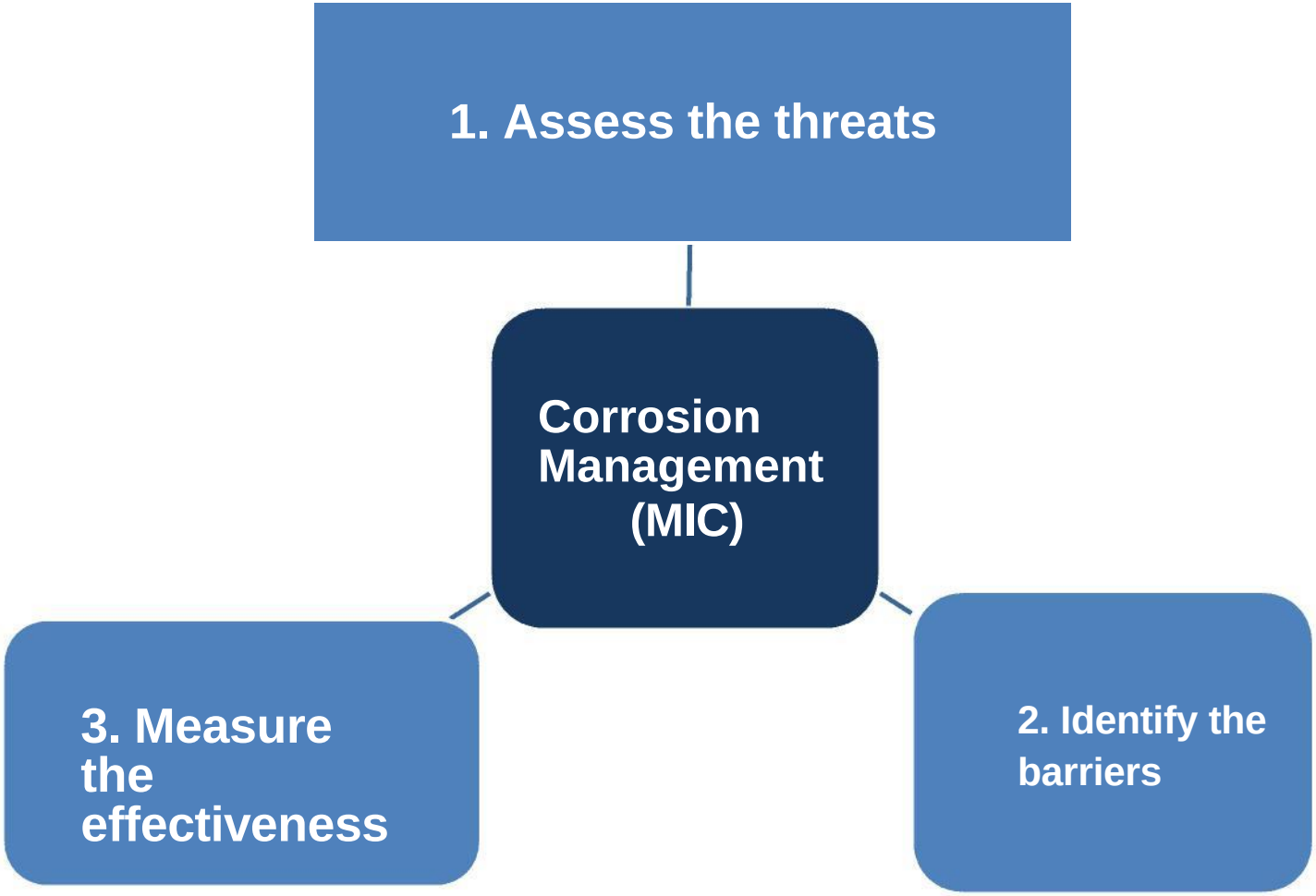
5. Analysis and visualization



How do we apply these techniques to give us data that we can use to manage our systems?



Corrosion Management



Adapted From Skovhus & Eckert

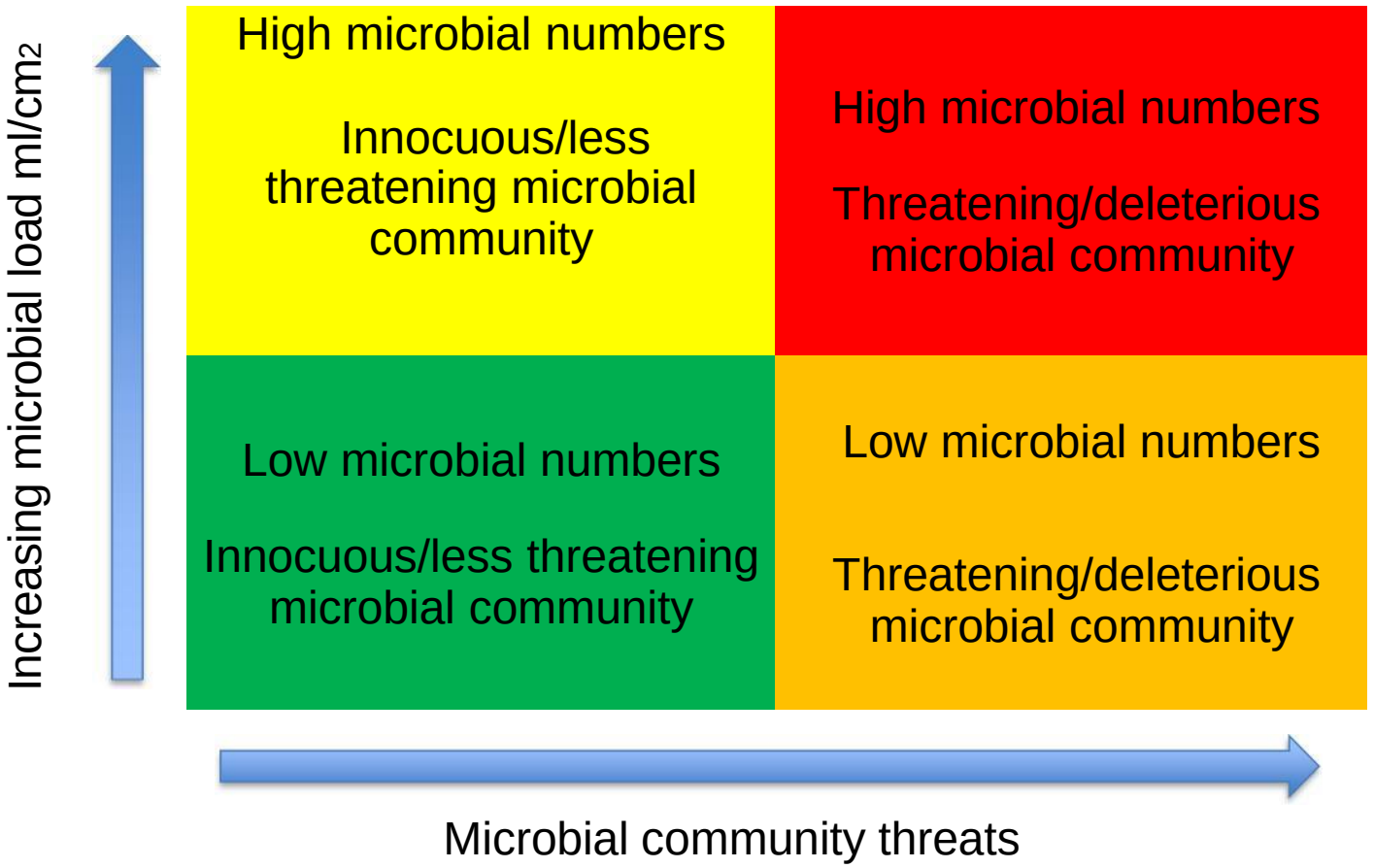
Assessing the Threat of MIC

The threat of MIC is defined as the product of microbial load (cell abundance or bioburden) and the community composition i.e.,

- a low threat is defined as a low bioburden with innocuous genera of microorganisms present.
- a high threat is defined as a high bioburden (high qPCR data or high MPN cell counts) plus the presence of detrimental/deleterious microorganisms associated with MIC, souring and biofouling.

Therefore, both enumeration and identification are required (preferably from the same sample) for an assessment of the threat of MIC to be performed, or to determine if MIC was a contributory mechanism when anomalies are found.

Assessing the Threat of MIC



From NACE TMO194 (currently under review)

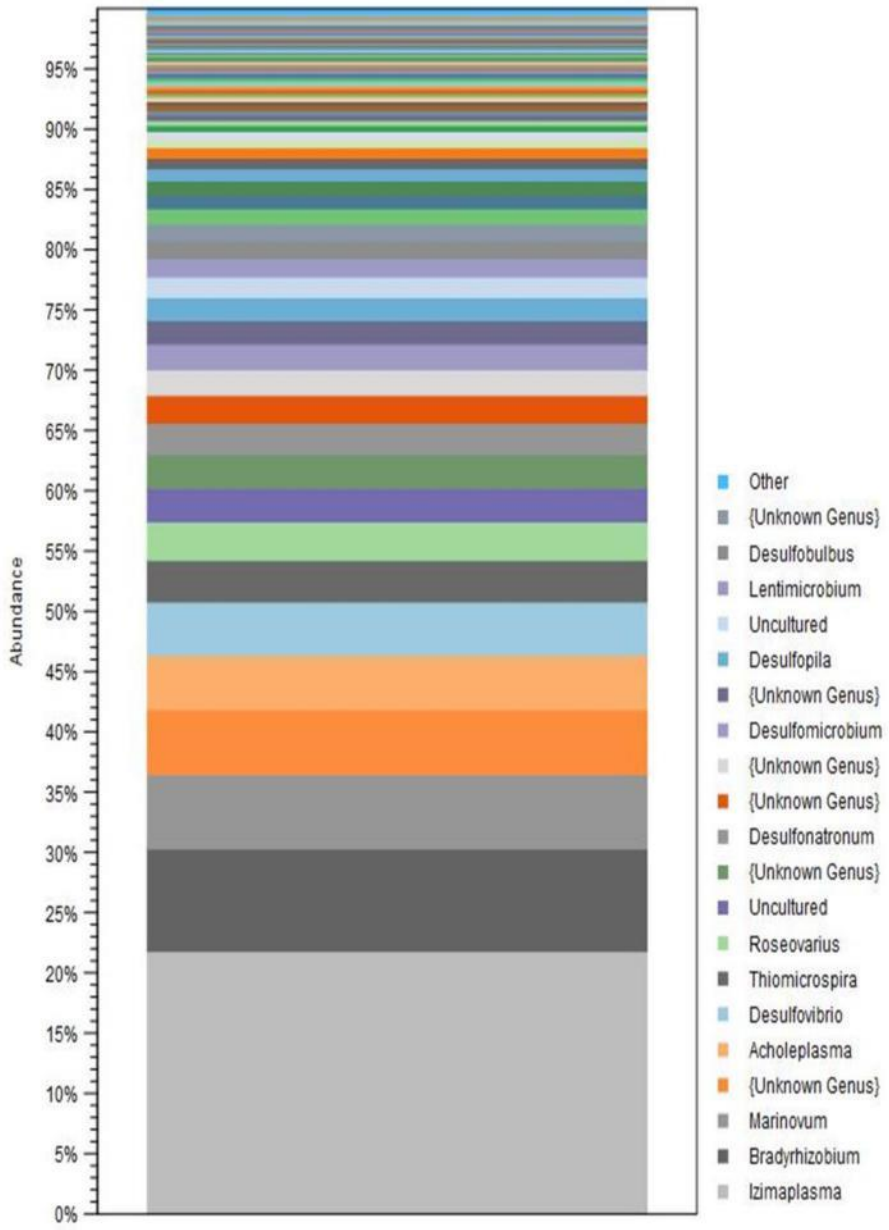
qPCR Data - Quantitative

Sample 1	Total Bacteria	SRB	SRA	Total Methanogens
1. TB - Control Sample (Intact area)	6.4×10^3	<LDL	<LDL	<LDL
1. TB Swab from Pit	3.0×10^5	<LDL	<LDL	2.2×10^3
1. TB Pit Solids	2.6×10^5	<LDL	<LDL	<LDL
Sample 2				
1. EB - Control Sample	5.2×10^7	7.2×10^4	1.6×10^3	1.3×10^4
1. EB Swab from Pit	2.9×10^6	9.4×10^3	<LDL	8.3×10^2
1. EB Pit Solids	4.6×10^6	<LDL	<LDL	3.3×10^4

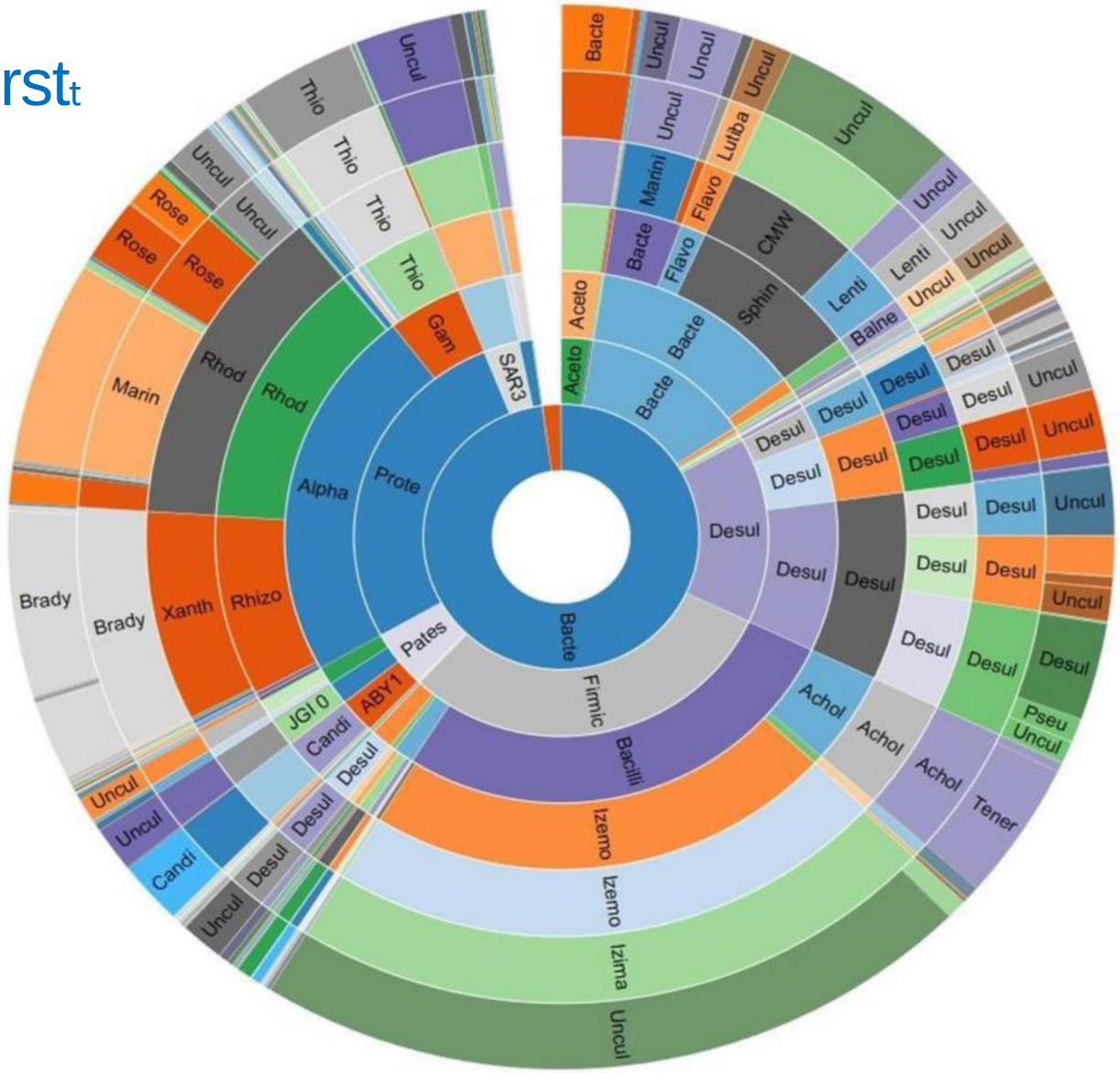
Metagenomics (16S NGS)

Use of New Generation Sequencing (NGS) (16S Metagenomics) to characterise the total microbial community

- Identifies all micro-organisms
- Typical Bar Chart



Sunburst chart



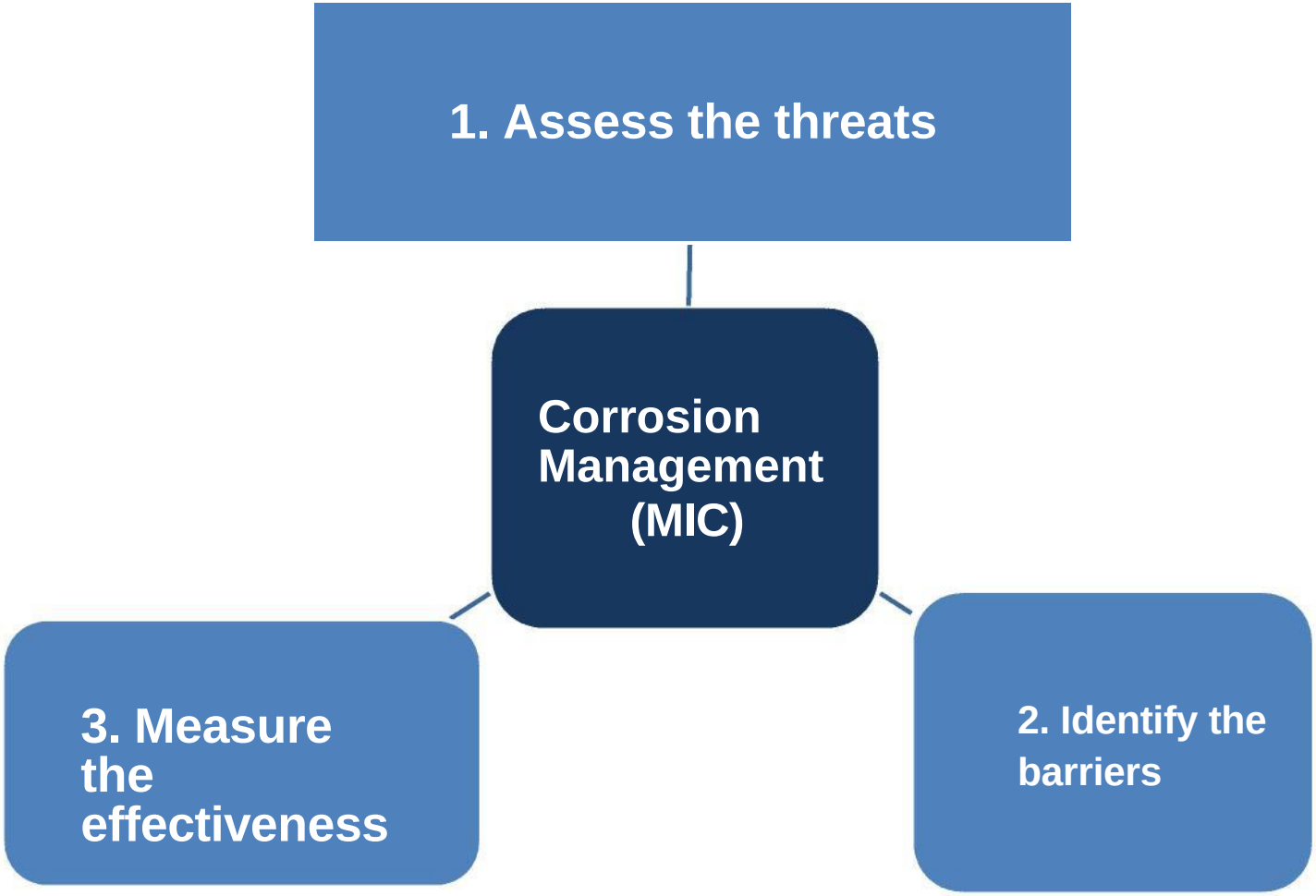
We are looking for 'deleterious organisms' – i.e., those involved in MIC

Classification	Activity	Issues	Genera
Sulfate reducers	Convert sulfate to H_2S	• Cause souring • chemical corrosion • reduction in quality of oil phase	*Desulfovibrio; Desulfomicrobium* *Desulfohalobium; Nitrospirae* *Thermodesulfovibrio; Archaeoglobus*
Sulfur and thiosulfate reducers	Convert elemental sulfur or thiosulfate to H_2S	• Associated with severe MIC • cause souring • chemical corrosion • reduction in quality of oil phase	*Anaerobaculum; Haloanerobium* *Fervidobacterium; Proteus;* *Thermotoga; Desulfuromonas*
Nitrate-reducers	Convert nitrate to nitrite or nitrogen gas (depending on temperature	• Associated with MIC, • pit formation under biofilms • production of ammonia or nitrite	Many genera, *Pseudomonas* are NRB
Methanogenic archaea	Convert CO2 to methane	• Utilise H_2 • Can induce MIC	*Methanosarcinales;* *Methanobacteriales*
Sulfide and sulfur oxidisers	Oxidise H_2S back to elemental sulfur (or sulfate)	• Associated with MIC • Create elemental sulfur • Can occur due to oxygen scavenger dosing (bisulfite)	*Sulfurospirillum; Sulfurovum* *Thiomicrospira; Sulfurimonas*
'Acid-producers'	Fermentative, therefore excrete organic acids	• Associated with MIC via acidification • Produced substrates for sulfate reducers	Many genera *Clostridia* are acid-producers

qPCR Data - Quantitative

Sample 1	Total Bacteria	SRB	SRA	Total Methanogens	Most Abundant Genera (%)
1. TB - Control Sample (Intact area)	6.4×10^3	<LDL	<LDL	<LDL	*Sediminibacterium* 28% *Aquabacterium* 14%
1. TB Swab from Pit	3.0×10^5	<LDL	<LDL	2.2×10^3	*Sediminibacterium* 22% *Acidovorax* 20%
1. TB Pit Solids	2.6×10^5	<LDL	<LDL	<LDL	NA
Sample 2					
1. EB – Control Sample	5.2×10^7	7.2×10^4	1.6×10^3	1.3×10^4	*Pseudomonas* 28% *Halanaerobium* 21%
1. EB Swab from Pit	2.9×10^6	9.4×10^3	<LDL	8.3×10^2	*Salinisphaera* 56% *Sediminibacterium* 19% *Desulfoplanes* 14% *etc*
1. EB Pit Solids	4.6×10^6	<LDL	<LDL	3.3×10^4	NA

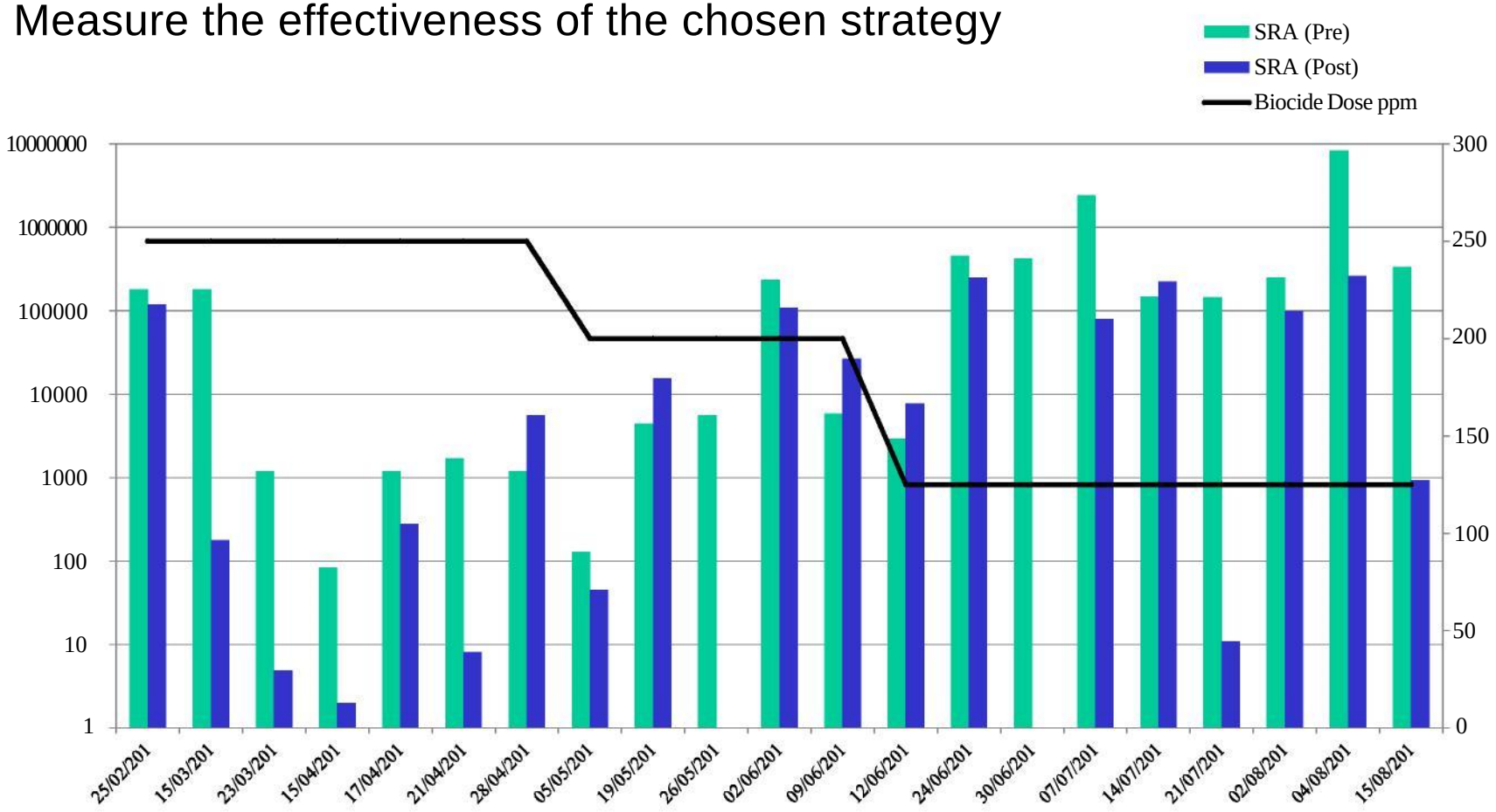
Corrosion Management



Adapted From Skovhus & Eckert

Monitoring

Measure the effectiveness of the chosen strategy



Document type	Document Title	Contents
AMPP NACE Standard	TM0212-2018, Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion on Internal Surfaces of Pipelines	Standard test method for MIC sampling and testing on internal surfaces of pipelines
AMPP NACE Standard	TM0194-2014, Field Monitoring of Bacterial Growth in Oil and Gas Systems (Under Review, publication end of 2023)	Describes field methods with an emphasis on culture-based testing for identifying microorganisms and NOT for MIC assessment
AMPP NACE Standard	TM21465, Molecular Microbiological Methods – Sample Handling and Laboratory Processing (UPCOMING)	Standard procedures for sample collection, sample processing, and laboratory analysis (includes gene targets and primer information) of microbiological samples.
AMPP NACE Standard	TM21495, Laboratory Evaluation of the Effect of Biocides on Biofilms (UPCOMING)	Test method for the testing of biofilms in the laboratory using field samples for selecting biocide for biofilm mitigation.
Energy Institute Guideline	Guidance on the use of Biocides in the Oil Industry, 2022	Technical review of biocides, factors affecting biocide effectiveness, advantages and limitations of biocide use, biocide application methods, compatibility issues, safety, environmental impact, and regulatory requirements.
Energy Institute Guideline	Guidelines on managing microbiologically influenced corrosion (MIC) in water injection systems, 2022	Identification of MIC threat for water injection systems and monitoring the effectiveness of biocide.

Thank you!
Any questions?

Carol Devinec.devine@ncimb.com

Tel: 01224 009333

Mob: 07458 077932

Thank you for your attention...

Q & A