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NGS: Characterizing Microbial Communities and Assessing MIC Threats

PROJECT SUMMARY

- Next generation sequencing (NGS) was performed on liquid samples from key locations with different operating conditions.
- NGS revealed diverse microbial communities and the presence of microbial groups commonly implicated in MIC including sulfate reducing bacteria (SRB), methanogens, and acetogens.
- In conjunction with a review of operating conditions, corrosion monitoring, and inspections, the NGS results added a key line of evidence indicating a threat of microbiologically influenced corrosion (MIC).

PROJECT CHALLENGE

Correct diagnosis of MIC is critical for making the best chemical treatment and corrosion control decisions. However, MIC can result from the actions of a broad spectrum of microorganisms that cannot be identified using culture methods alone. 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 locations.

SAMPLING AND ANALYSIS

NGS provides comprehensive identification of microorganisms present in a sample down to the genus and even species level. While not quantitative, the relative proportions of the microorganisms identified can provide insight into potential microbial processes and identify shifts in microbial communities that can occur after long-term chemical treatment. Often NGS is performed periodically for an overall profile of the microbial community while QuantArray or CENSUS qPCR is used to routinely quantify Total Bacteria and specific groups like SRB that are commonly implicated in MIC. For easy-to-follow sampling protocols, please visit www.microbe.com.

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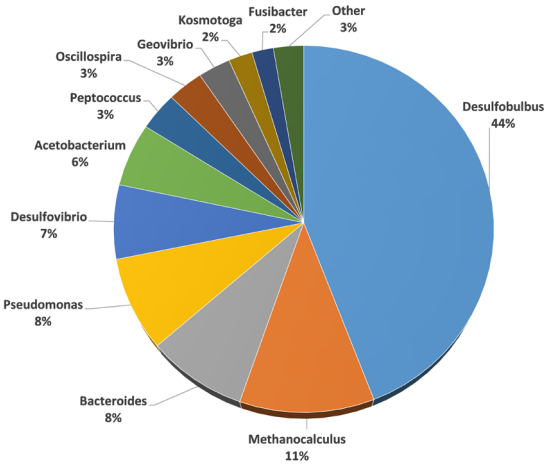
TOP GENERA

Along with statistical analyses, NGS reports include pie graphs and tables of the top genera detected in each sample along with brief descriptions highlighting the common characteristics of each genus as shown below for one of the samples.



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 but some species are capable of nitrate reduction or complete denitrification.
Desulfovibrio	61,255	7%	Genus of sulfate reducing bacteria. Sulfate and other sulfur compouds 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 CO2 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 chemoortganotroph. 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.

- *Desulfobulbus*, the most frequently identified genus, and *Desulfovibrio* are SRB, indicating that the potential for H_2S production should be considered. In addition, several of the other genera including *Peptococcus*, *Geovibrio*, *Kosmotoga*, and *Fusibacter* are capable of reducing 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 SRB.
- 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 a carbon and energy sources.
- *Pseudomonas* are well known slime forming bacteria which contribute to biofilm formation.

Decision: Since the potential for MIC appears to be elevated, additional monitoring using coupons and inspection for localized corrosion can be implemented to determine the extent of the MIC threat rather than monitoring using electrical resistance probes, for example, that would not be helpful for localized corrosion.

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KEY BENEFITS

NGS gave an overall profile of the microbial community and a more comprehensive assessment of potential MIC threats that would simply not be possible with traditional methods.

- **Comprehensive:** Who is there? NGS provided comprehensive identification of the microorganisms present in a sample down to the genus level.
- **Descriptive:** Brief descriptions of the top genera provided insight into what microbial groups and processes could be contributing to corrosion.
- **Inclusive:** Further, many genera such as acetogens and methanogens cannot be measured using common media.
- **Compatible:** NGS analysis used to characterize the microbial community clearly highlighted important microbial groups that should be quantified by CENSUS qPCR or QuantArray-MIC in routine sampling. The electron donors/acceptors and energy sources known to be present based on liquid composition analysis further supporting the potential for MIC.

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