

The 1000's of Microbial Genera Found in Argentina's O&G Fields: Their Impact on Microbially Induced Corrosion and Integrity of Facilities

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ABSTRACT

MIC assessment and management in O&G facilities depends on the accurate and early evaluation of microbial communities involved in material degradation. Traditional monitoring by culture-based methods allow the detection only 0.1% of the microorganisms in the sample, underestimating the real bacterial concentration in the samples, and in some cases, their potentiality for corrosion. However, novel molecular-biology based strategies may overcome these issues offering more reliable and sensitive tools for the study of microbial communities.

The novel methodologies have been useful for the study and understanding of microbial populations in diverse environments, including general environmental samples, water reservoirs, ocean samples and the human microbiome as well. In this context, we aimed to gain a general understanding of microbiomes and their correlation with microbially induced corrosion processes in O&G production systems in Argentina.

To achieve our goal, we studied more the 500 samples collected from production and injection wells, and water- and crude-treatment facilities. The sampling strategy included obtaining samples of crude oil, water and solid residues collected from filters and scrapers. The samples collected are

representative of conventional and non-conventional production processes and were distributed along the Argentinean productive basins.

Amplicon sequencing based on the 16S rRNA gene, also known as 16S metagenomics, allowed the identification of the 1000 most relevant microbial taxa native to the production facilities and their correlation to MIC. Overall, our results allowed the identification and classification of microbial taxa native to O&G facilities. This microbial map allowed a better understanding of the microbial distribution and the individual contribution of each microbiome to distinct mitigation strategies. From this integral study, we also highlight the potential impact of individual species to the corrosion process, and collectively demonstrate that sulfate-reducing bacteria are just the tip of the iceberg in an integral process that includes many others biological process beyond just sulfate reduction.

Key words: Microbiologically Influenced Corrosion (MIC), Molecular Microbiology Methods (MMM), Amplicon sequencing based on the 16S rRNA gene.

INTRODUCTION

Microbiologically influenced corrosion (MIC) is a phenomenon that negatively impacts O&G facilities. Different sources estimate that countries invest 2 to 5% of their GDP each year to cope with corrosion,¹ and MIC phenomenon represents the 20% of those costs.^{2,3} The strategies to control these effects are complex because they require a complete assessment of the whole microbiome to choose an effective mitigation treatment and select the appropriate monitoring strategies.

In Argentina, it is estimated that at least 20% of the costs of chemical treatments correspond to the application of biocides to mitigate the MIC phenomenon, thus it is an important aspect that increases the OPEX for O&G production.

In this context, the first step of an integrity analyses is the assessment of the microbiome native to the facilities. During last few years, assessment strategies based on molecular biology have gained considerable relevance in the O&G field.^{4,5,6,7} Among the most effective strategies for microbial assessment are protocols based on next-generation sequencing such as 16S rRNA gene sequencing which is based on a targeted amplicon sequencing strategy.

In response to this problem, we have developed an integral assessment based on amplicon sequencing of the 16S rRNA gene to characterize bacteria and archaea with the main goal of determining microbial communities native to Argentinean O&G facilities.

To fulfill our quest, we collected a total of 500 samples from conventional and non-conventional systems, production and flowback water, crude oil, solid deposits, and water samples from different sites in water circuits. For the processing of the samples we optimized DNA preparation protocols, analyzed DNA quality and conducted microbial community analysis based on the 16S rRNA gene.

The collected data allowed the identification of more than 1000 different microorganisms present in two of the most relevant basins for oil production in Argentina.

Our research yielded a microbial catalog describing the criticality of the microbial populations for MIC phenomena and their synergy among in different sample. This dataset is of great interest to design and develop more effective mitigation strategies and microbial monitoring in O&G fields.

EXPERIMENTAL PROCEDURE

Sample collection

Production water and crude samples were collected in clean plastic containers with no headspace after filling with the samples. Sessile samples were obtained from scraped sludge during a cleaning program in the pipeline and corrosion coupon installed in the facilities, which were aseptically placed in sterile containers.

Biomass isolation

Microbial cells from water samples were concentrated by filtration through 0.22 µm pore size filters. Solid samples were recovered directly from the sludge and from coupon surface. In the case of crude samples, a procedure for bacterial recovery was developed. The microbial community dispersed in the oil fraction was recovered with 0.005% Tween 20 solution using three consecutive washes, followed by centrifugation at 1000 rpm during 5 min to allow organic and aqueous phases separation. Aqueous extracted microorganisms were collected after filtration on 0.22 µm pore size filters.

DNA extraction

DNA was extracted from the microbial pellets (resulting filters or deposits) using a commercial DNA isolation kit for soil samples according to the manufacturer's specifications. The quality and integrity of DNA were determined by gel electrophoresis on 0.8% agarose and stained with syber gold solution. Concentration was assayed by spectrophotometric analysis.

16S rDNA-based microbiome assessment

Samples sequencing was performed after 16S rDNA PCR amplification using primers for V3-V4 regions⁸:

Forward Primer

(TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and

Reverse Primer

(GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC).

Amplicons were further processed for library preparation using Nextera XT[†] library preparation kit (Illumina, USA). Samples were pyrosequenced at Universidad de Quilmes (Argentina). Trimming of adaptor sequences and sequence analysis was conducted according to the following pipeline:

- Quality control with FastQC v0.11.5 and multiQC
- Stitch pair end reads with PEAR software
- Filter stitched reads by score with the programs: FASTQX-Toolkit and BBDMap
- Chimeras filter with the software VSEARCH using as reference the Ribosome Database Project
- Identify OTUs (Operational Taxonomic Units) with QIIME2
- Remove low confidence OTU's with reads below 0.1%
- Rarefaction
- Alpha diversity
- Beta diversity

Sequences were clustered as operational taxonomic units (OTU) at 3% dissimilarity using the furthest neighbor algorithm and classified using a naïve Bayesian classifier and the Ribosomal Database Project training set. Representative sequences for each OTU were taxonomically classified using Greengenes, SILVA, and NCBI GenBank.

Data Analysis

The results of 16S rRNA gene-based microbiome analysis from each sample were classified according to system type, critical control point (CCP) where sampling was carried out, metabolisms, and MIC potential. For each system type, the 10 most relevant genera were analyzed determining: appearance frequencies, the relative abundance of each metabolism and the microbial community in each CCP to follow their distribution in the production process.

[†] Trade name

RESULTS

Non- Conventional Facilities

The microbiomes present in non-conventional facilities, namely shale fields, were studied in 34 samples and resulted in 155 different microbial genera. In Table 1, the most relevant genera are presented including the frequency at which they appear in the studied samples. The same table also includes the characteristic metabolism of each genera in relation to the MIC process (Figure 1). Overall, these results highlight the importance of thiosulfate, metal reduction, and biofilm forming bacteria as the likely main causes of under deposit corrosion⁹.

Figure 2 shows the microbial community in different sites of the production circuit from the water collecting point up to the crude processing plant (Figure 3). The results describe a rather limited microbial community with an important contribution of *Halanaerobium* sp. and *Orenia* sp.

Table 1. Ten most representative genera in non-conventional production samples

Genera	Metabolism	Known to be involved in MIC?	Frequency
<i>Halanaerobium</i> sp.	TSRB	YES	32
<i>Orenia</i> sp.	Reduces metal oxides	YES	15
<i>Marinobacter</i> sp.	NRB	NO	11
<i>Arcobacter</i> sp.	SOB	YES	8
<i>Modicisalibacter</i> sp.	NRB	NO	8
<i>Pseudomonas</i> sp.	Biofilm former	YES	8
<i>Flavobacterium</i> sp.	IRB	YES	8
<i>Limnohabitans</i> sp.	Typical of fresh water	NO	8
<i>Petrotoga</i> sp.	Production of H ₂ S	YES	7
<i>Marinobacterium</i> sp.	NRB	NO	7

TSRB: Thiosulfate-reducing bacteria

NRB: Nitrate-reducing bacteria

SOB: Sulfur-oxidizing bacteria

IRB: Iron-reducing bacteria