

**Identification and Characterization of Planktonic and Sessile Consortium
Associated with Microbiologically Influenced Corrosion (MIC) in the Oil and Gas
Industry**

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ABSTRACT

In Oil and Gas (O&G) facilities, it is estimated until a 40% of failures are due to corrosion induced by microorganisms (MIC). These microorganisms remain in planktonic or sessile form and a high diversity of metabolisms can be found. In the present study, planktonic and sessile microbial consortia were isolated and characterized from production waters of unconventional and conventional process. The water production showed major presence of the genus *Halanaerobium* (94.2%) and *Arcobacter* (73.6%) in sample of unconventional and conventional processes respectively. In the culture media formulated, *Halanaerobium* (68%) and genera *Desulfovibrio* (33.8%), *Geotoga* (33.3%), *Proteiniphilum* (30.89%) were found in a higher proportion for unconventional and conventional processes respectively. All these microbial genera have been reported as related to MIC. On the other hand, the biofilm formation was characterized by SEM-EDS and electrochemical techniques, using its ability of MIC in carbon steel; showing that the unconventional microbial consortium slightly higher corrosion capacity (2.35 mm/y) than the conventional microbial consortium (1.25 mm/y). These results represent the synergy of the different metabolisms that interact in the MIC-related communities, such as thiosulfate and sulfate reduction, iron oxidation/reduction and acid production microorganisms in unconventional and conventional processes.

Key words: Microbiologically-Influenced Corrosion, planktonic and sessile microbial consortia, SEM, electrochemical techniques.

INTRODUCTION

Corrosion causes an annual financial loss of US\$4 trillion worldwide, half of that cost is in corrosion prevention and control, the other half in damages and lost productivity¹. It is estimated that 20% to 40% of the failures caused by corrosion is produced by MIC²⁻⁴. MIC is a type of localized corrosion caused by microbial activities. This includes a variety of processes by which microbes contribute to corrosion directly or indirectly and severely affect the integrity of facilities in various industrial sectors, especially in the O&G industry^{2,5,6}. Biofilms are widely believed to be responsible for MIC, in most cases^{7,8}.

This corrosion mechanism can occur in aqueous environment and is a common problem in O&G facilities due to the ubiquitous nature of microorganisms and corrosives in the pipes. Some facilities that can suffer MIC are water injection lines, storage tanks, sewage treatment systems, filtration systems, pipes, reverse osmosis membranes, and potable water distribution systems⁴. Secondary recovery processes in mature fields have water cuts greater than 90%, which creates the right conditions for the development of microbial populations due to their abundant sources of carbon and energy^{9,10}. MIC has also been reported on crude oil and refined products⁷.

Many microorganisms, including bacteria, archaea, and fungi, contribute to increasing the corrosion rate¹. Therefore, it is important to understand the impact of microbial communities and characterize the biofilms formed on the surfaces of facilities. Sulfur species metabolizing bacteria, such as sulfate-reducing bacteria (SRB), thiosulfate-reducing bacteria (TSRB), and sulfur-oxidizing bacteria (SOB), have been widely investigated as the main microorganisms causing MIC. These bacteria can use sulfate, bisulfite, thiosulfate, and elemental sulfur as terminal electron acceptors^{3,11–13}. Under anoxic conditions, these bacteria obtain energy by reducing sulfur compounds to form hydrogen sulfide (H₂S), a corrosive substance that accelerates the corrosion rate of metal surfaces¹³.

Methanogens, iron-oxidizing bacteria (IOB), acid-producing bacteria (APB), and fungal species have also been associated with MIC^{3,11}. Therefore, it is important to identify the various microbes that cause MIC and to understand their different mechanisms. Historically, the O&G industry has based MIC diagnostics using serial dilution methods (MPN, most probable number method). However, in the last decade, molecular microbiological methods (MMM) have been replacing them^{4,7}. MMMs provide information about the microbiological environment, including the species present, their abundance, specific functions, and level of activity⁷. In this study, we established a strategy to obtain MIC-associated microbial consortia from O&G facilities of conventional and unconventional processes in Argentina. Consortia of sessile and planktonic native microbes were identified and characterized by culture, MMM and electrochemical techniques (carbon steel MIC). The composition of the microbial community was determined by metagenomic analysis and biofilm formation was characterized by microscopic and electrochemical techniques.

EXPERIMENTAL PROCEDURES

Sample collection

It was used early production facility flowback waters from unconventional process, and polymer injection well waters from a conventional tertiary recovery process, located in the Neuquén and Chubut region, Argentina, respectively. A sample was taken from the aqueous phase of the production fluids from several wells that entered the early production facility. Production water was collected in clean plastic containers with no headspace after filling with the samples.

Microbial Consortium Culture

MIC planktonic and sessile microbial consortia were obtained from production waters of conventional and unconventional process. Microbial consortia were isolated in a modified Postgate B culture medium¹⁴, glucose and lactate were used as a carbon source for isolated consortia of unconventional and conventional waters, respectively. Salinity was adjusted according to the production water physicochemical; and the addition of Na₂S₂O₃, Na₂SO₃, and Na₂SO₄ as sulfur sources. 10 mL vials with septum and aluminum cap were used to ensure anaerobiosis. A carbon steel coupon (SAE 1040) was added to each vial for the growth of sessile cells. The media were inoculated with the production waters, nitrogen was added to generate an anaerobic atmosphere and they were incubated at 38°C and 150 rpm (revolutions per minute). Four replicates were made for each culture medium. Salinity according to the physicochemical analysis of

each production water (g L^{-1}): CaCl_2 16.6; MgCl_2 50.7; Na_2SO_4 1.5; NaHCO_3 1.2; NaCl 131.8. Conventional (g L^{-1}): CaCl_2 0.1; MgCl_2 0.3; NaHCO_3 2.1; NaCl 8.3. Before inoculation and after incubation, the pH of the microbiological medium was measured. The pH did not change over time and was approximately 7.

Metagenomic characterization

The characterization of the native microorganisms of the production waters and the planktonic and sessile microbial consortiums was carried out through the metagenomic analysis of the 16S gene using the Ion-Torrent/Illumina technology (Illumina, USA)[†]. DNA extraction was performed using the NucleoSpin Soil Kit (Macherey-Nagel)[†]. The conserved and variable regions (V2 and V6) of 16S were sequenced using universal primers for bacteria and ad hoc designed primers for archaea.

Biofilms formation characterization

Biofilm formation on carbon steel coupons was monitored for one week. As a blank, the surface of carbon steel coupons without microbial inoculum was immersed in the same culture medium, under the same incubation conditions, and was monitored over time. Coupons were sampled daily and visualized by Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM/EDS). The cells were fixed in 2.5% v/v glutaraldehyde for 90 min at 4°C. Dehydration was then carried out in a series of ethanol solutions (30, 50, 70, 90, 95 and 100% v/v, 15 min of incubation each). Samples were sputter-coated with gold using, and later, the samples were viewed using a FEI ESEM Quanta 200 microscope[†] microscope with the "high vacuum mode". Coupons were not scraped before viewing to evaluate the biofilm and its full corrosion products

Surface morphology analysis of the corrosive process on the coupon

Analysis of surface corrosion was carried out with a stereoscopic magnifying glass. The corrosion products formed on the coupon were cleaned using an ASTM G1-03 solution: 250 mL of HCl, 5 mL of SbO_3 , and 12.5 mL of SnCl_2 for 25 min. A coupon incubated under the same conditions but without microbial inoculum was evaluated as a control.

Electrochemical characterization

Electrochemical impedance spectroscopy (EIS) and open circuit potential (OCP) measurements were performed to characterize the corrosive capacity of microbial consortia obtained for conventional and unconventional processes. Both measurements were performed simultaneously under biotic (inoculated with microbial consortia) and abiotic (control without inoculation) conditions for five consecutive days every 24 h.

Electrochemical measurements were performed using a Gamry potentiostat (Interface 1000 model)[†]. A typical electrochemical cell was used in a three-electrode configuration. Carbon steel (C1018) was used as working electrode (WE), a graphite rod (99.999% purity) (Sigma Aldrich, USA)[†] was used as a counter electrode (CE) and saturated Calomel electrode was the reference electrode (RE). The RE was immersed in a Luggin capillary. The working electrode was coupled to a rotating cylinder system (RCE) to simulate flow, with a rotational speed of 500 rpm. As the electrolytic solution, the culture media described above was used, both for the consortium obtained in conventional and unconventional processes. It was left to work for five days under abiotic and biotic conditions. For biotic condition, an inoculum for a final nominal density of 1×10^7 cells/mL was transferred to a 70 mL of unconventional and conventional culture media inside the electrochemical cell (in separate trials), where the three electrodes (WE, CE, and RE) were attached. The impedance spectra were obtained considering the frequency range from 10^5 to 10^{-2} Hz and the amplitude of ± 10 mV. The spectra that met

[†]Trade name

the requirement of being in a stationary state were fitted to an equivalent electrical circuit (eec), the EIS spectra were fitted and analyzed using the ZView program (Scribner Associates Inc)[†].

RESULTS AND DISCUSSION

Metagenomic characterization of production water samples

The characterization of the native planktonic microorganisms of production waters (unconventional and conventional) was carried out using metagenomics.

Unconventional process samples: It was found that 47.3% of the population of native planktonic microorganisms was related to MIC and 51.3% not (Figure 1). The genus found in the highest proportion related to MIC was *Halanaerobium*, the reducing-thiosulfate bacteria (TSRB) and those not related to MIC was *Modicisalibacter*, a nitrate-reducing bacteria (NRB).

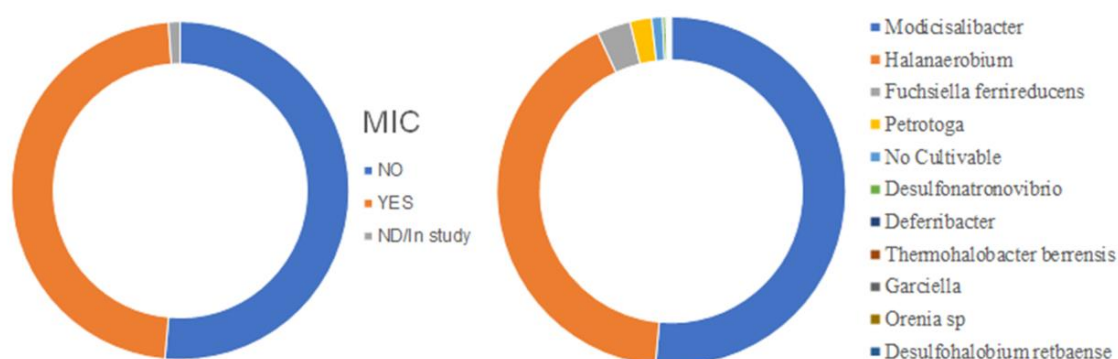


Figure 1: Characterization of the native planktonic microorganisms of the unconventional production waters.

Conventional process samples: From this analysis, it was found that 73.6% of the population of native planktonic microorganisms was related to MIC (Figure 2). *Arcobacter*, sulfur-oxidizing bacteria (SOB) and *Pseudomonas*, biofilm-forming microaerophilic bacteria were found in a higher proportion.

Arcobacter is a SOB that has been reported to be part of MIC-associated microbial communities in production waters together with bacteroides, SRB as *Desulfovibrio*, and APB as *Firmicutes*¹⁵. It has been suggested that the combination of partially oxygenated water with sulfide-containing production water formulated in ideal conditions for the growth of *Arcobacter* and other SOB, as this genus of microorganisms oxidizes sulfide in the presence of nitrate or oxygen¹⁵. The predominance of SOB and SRB in production waters may be indicative that sulfur is prevalent in the system and MIC may be involved in this environment.

On the other hand, *Pseudomonas* is a Gram-negative mobile rod bacterium widely distributed in nature and has been widely associated with MIC processes^{8,16,17}. The genus *Pseudomonas* is highly involved in corrosion processes and has been recognized as the pioneer colonizer in the biofilm formation process. *P. aeruginosa* can generate extracellular polymeric substances (EPS) that creates a differential aeration gradient for SRB proliferation that leads to MIC¹⁸. *P. aeruginosa* has been shown to have the propensity to increase the mild steels and alloys corrosion rates in aquatic environments^{8,19,20}. *Pseudomonas* sp. can act in synergy with sulfur-reducing microorganisms and other strict anaerobic microbial species associated with MIC, since being biofilm-forming allows the creation of oxygen and nutrient gradients, where there is a higher concentration at the interface between the biofilm and the surrounding

environment and a lower concentration in the center of the microcolonies^{21,22}. These gradients create numerous micro-niches within the biofilm that can aid the establishment of microorganisms with different metabolisms²².

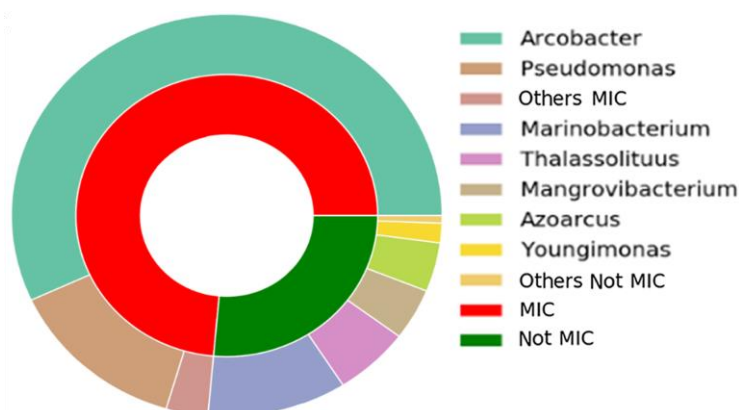


Figure 2: Characterization of the native planktonic microorganisms of the conventional production waters.

Metagenomic characterization of MIC consortia isolated

MIC planktonic and sessile microbial consortia were obtained from production waters of conventional and unconventional process.

Unconventional process: In the planktonic consortia, 99.5% of the microorganism genera were related to MIC, with major presence of the genera *Halanaerobium* (94.2%) and *Arhodomonas* (5.17%), both TSR bacteria (Figure 3).

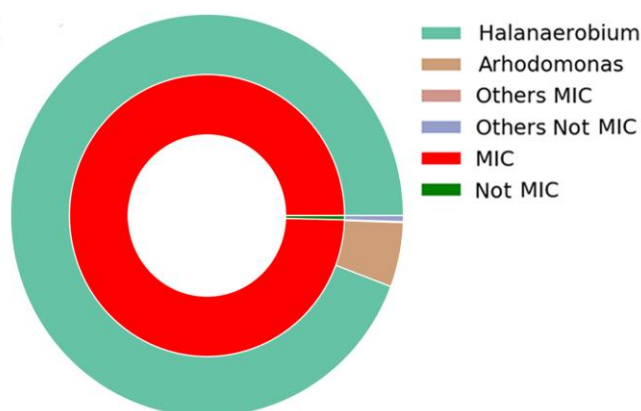


Figure 3: Planktonic consortium obtained from production waters from the unconventional process.

Sessile microbial consortia were obtained on the surface of carbon steel coupons. As found in the planktonic consortium, most of the microbial genera were related to MIC (93.2%), but, in comparison, a higher microbial diversity was found (Figure 4). The main genera were *Halanaerobium* (68%) and *Arhodomonas* (4.4%) (both TSRB), then *Desulfomicrobium* (SRB) and APB. The microbial genera found within this sessile microbial consortium represent the synergy of the different metabolisms that interact in MIC-related communities, such as TSRB, SRB, and APB^{23,24}.