Bacteria in the produced water and wastewater samples from the oil industry

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Abstract. Today, studying the diversity of microbial communities associated with samples of highly mineralized oil industry waters is expanding our knowledge of the ecology of polyextremophilic microorganisms. During this work, samples of produced water and wastewater from the oil industry were thoroughly analyzed. The analyzed waters were characterized by very high concentrations of Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), and Cl\(^-\) ions. Furthermore, enriched and pure bacterial cultures from oilfield waters were obtained. Additionally, enriched cultures were analyzed using high-throughput sequencing of the bacterial 16S rRNA gene on the Illumina platform. Among the representatives of the studied bacterial communities, members of the genera Halomonas, Marinobacter, Modicisalibacter, Bacillus, Clostridium, Prauserella, and Rubrobacter were identified. They can be considered for various biotechnological applications.

1 Introduction

Today, the petroleum industry is a leading industry where a lot of research is carried out related to production, refining, transportation, and corrosion control. Monitoring of microorganisms, both living in the subsurface and introduced during the operation of oil reservoirs, is one of the main tasks of the oil industry, since it is necessary to control and reduce the proportion of microorganisms that synthesize aggressive products for pipelines. Corrosion is a result of chemical or electrochemical reactions between materials and substances in their environment, and this process affects several sectors, including the petroleum industry [1]. Corrosion caused by microorganisms (biocorrosion or microbial corrosion) causes millions of dollars in losses every year [2] and is a serious problem in a wide range of industries [3]. Biocorrosion is affected by the presence and activity of various microorganisms on the surface of the corroding material [4,5]. Despite the relatively long history of microbial corrosion research, there are still many unresolved problems in this area. The list of microorganisms that researchers have linked to corrosion is certainly growing, but the gaps in biocorrosion are not narrowing, especially in relation to non-sulfate-reducing microorganisms. The effective operation of metal structures and product pipelines (water, gas, and oil pipelines) directly depends on the identification of microbial agents that cause corrosion.

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2 Materials and methods

2.1 Sample collection

2.2 Characterization of water samples
2.3 Cultivation of bacteria

The detection and quantification of reducing bacteria were evaluated by MPN technique. The bacteria were cultivated in stainless steel plates, purged with nitrogen, and incubated at room temperature. The colonies were counted and identified using a Qubit 2.0 fluorometer. The concentration of sodium, potassium, calcium, magnesium, and aluminum was measured using the Hanna Instruments..

2.4 Sequence analysis of the 16S rRNA gene of bacterial isolates

The 16S rRNA gene sequences were amplified using Bakt_341F (5′-CCT ACG GGO YTM TTR AGT TACT-3′) and Univ1492r (5′-TAC GGG CTC AGG AGA GTT-3′) primers. The amplicons were submitted to the DNA sequencing analysis of the 16S rRNA genes of the bacterial isolates from each main cluster were partially sequenced. The sequences were deposited in GenBank database with accession numbers.

2.5 High-throughput sequencing of bacterial 16S rRNA gene

High-throughput sequencing was performed using the Illumina MiSeq platform. The library was constructed using the QIIME software pipeline. The sequencing was performed using the MiSeq Reagent Kit v3. The results were analyzed using the BLAST search and reference at the NCBI database. The 16S rRNA gene fragments were sequenced and assigned to operational taxonomy units with 97% sequence similarity. The results were analyzed using the phylogenetic tree and operational taxonomy units.
3 Results and discussion

3.1 Characteristics of samples

Table 1. Characteristics of samples

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<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>$\text{NH}_4^+$ (g L$^{-1}$)</th>
<th>$\text{Na}^+$ (g L$^{-1}$)</th>
<th>$\text{Ca}^{2+}$ (g L$^{-1}$)</th>
<th>$\text{Mg}^{2+}$ (g L$^{-1}$)</th>
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3.2 Cultivable bacteria

Halomonas, Marinobacter, Bacillus, Micrococcus, Kocuria, Proteobacteria, and Halomonas were detected in the oil field water samples.
3.3 Biodiversity of enriched bacterial communities based on high-throughput sequencing

![Pie charts showing bacterial community composition in different water samples.](image)

**Fig. 1.** The relative abundance of bacterial phyla found in produced and recycled water from the oil industry.

**Fig. 2.** The relative abundance of bacterial families found in produced and recycled water from the oil industry.
Bacterial communities associated with metal surfaces. In this study, samples were collected from different environments, including water bodies, industrial sites, and natural habitats. The bacterial communities were characterized using 16S rRNA gene sequencing, followed by bioinformatic analysis.

4 Conclusions

The bacterial communities isolated from metal surfaces showed high diversity and richness, with significant contributions from the phyla Proteobacteria and Firmicutes. The genera exhibiting high abundance included Bacillus, Moraxella, Alloprevotella, Acinetobacter, Sphingomonas, Finegoldia, Prausnerella, Rubrobacter, and other closely related genera. These bacteria were found to be tolerant to harsh conditions, including high salinity and extreme temperatures, indicating their potential role in metal corrosion.

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References
