ASSESSING THE BIODEGRADATION OF WATER POLLUTING OILS BY *Marinobacter aquaeolei* AND *Marinobacter lipolyticus*: APPLICATION IN THE BIOLOGICAL TREATMENT OF PRODUCED WATER

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ABSTRACT

Marinobacter aquaeolei (also known as *Marinobacter hydrocarbonoclasticus*) and *Marinobacter lipolyticus* are facultative and halotolerant microorganisms primarily found in oily environments. They possess lipolytic activity capable of degrading hydrocarbon compounds, making them potentially valuable for biological treatment, especially of produced water (a byproduct of the oil industry). In this study, these microorganisms were introduced into brine containing representative hydrocarbons commonly found in produced water and tested with real effluent to assess their degradation capacity. The results showed that both *M. aquaeolei* and *M. lipolyticus* can degrade hydrocarbons, with the first being better at degrading n-hexadecane and the second at degrading cyclohexanecarboxylic acid. However, when used together, they competed, leading to lower degradation of oily compounds compared to when used separately. Nonetheless, *M. aquaeolei* alone could remove 70% of the oil content in produced water. Thus, the study underscores the potential technological application of *M. aquaeolei* in treating water contaminated with specific types of oil compounds in offshore environments.

Keywords: Produced water. Hydrocarbon pollutant. *Marinobacter aquaeolei*. *Marinobacter lipolyticus*. Wastewater.

1 INTRODUCTION

Produced water, a byproduct of oil exploration, lacks value and is typically managed by either reinjecting it into oil wells to boost productivity or disposing of it in the environment[1]. Regardless of its destination, the water's composition must be monitored and its parameters adjusted to prevent environmental harm. Total oil and grease (TOG) levels in the water are particularly important, since oil can obstruct fish gills, impairing respiration and leading to mortality[2]. In most cases, produced water contains high levels of hydrocarbons due to prolonged contact with oil layers. The oil in produced water can exist in various forms: free, dispersed, emulsified, or solubilized. In offshore environments, technologies capable of efficiently removing free and dispersed fractions are already used[3]. However, eliminate soluble and emulsified fractions remains a challenge. Recirculating the water through the treatment process can help reduce oil and grease levels, but some organic compounds are stubborn and may still cause TOG levels to exceed legal limits. Water deemed unsuitable for disposal is stored in slop tanks and transported by boats for treatment on land[4].

Biological treatments offer effective solutions for removing hydrocarbons, particularly the soluble ones. Microorganisms utilize these pollutants to sustain their metabolism, breaking them down into harmless byproducts[5]. Developing products for use in storage tanks could lead to significant cost savings in waste management. Local application is advantageous as it requires minimal changes into platform layouts and doesn't generate additional waste requiring further treatment. Produced water, mainly composed of seawater, typically has salinity levels exceeding 30 g/L[1]. These high salinity levels can cause enzyme denaturation, cell membrane disruption, and organism dehydration. Additionally, slop tanks, which can hold up to 2000,000 m^3 of produced water, create low oxygen zones[6]. Furthermore, microbial growth in conditions of low oxygenation has low energy consumption and produces fewer amounts of sludge, which would not require operations to dispose this waste. Thus, organisms capable to degrade oil hydrocarbons is of great interest to be applicated in offshore environments[7]. Given these conditions, biological treatment is best carried out by halotolerant organisms capable of facultative respiration.

Species of bacteria from the genus *Marinobacter sp.* are commonly found in sites contaminated by oil. *Marinobacter aquaeolei* has been identified as an organism proficient in degrading pentadecane, hexadecane, and pristane under aerobic conditions. Flourishing in marine habitats, this microorganism exhibits resilience in saline environments (20%) and displays facultative respiration[8]. These attributes suggest its potential suitability for remediating oil-contaminated water. However, the existing literature lacks comprehensive information about its capabilities. Hence, this study aims to investigate its performance concerning other classes of hydrocarbons and under anaerobic conditions. Another bacterial species sharing similar traits is *Marinobacter lipolyticus*. This organism has genes that enables this microorganism to emulsify and degrade oils and greases in special phenols and benzoates[9]. Similar to *M. aquaeolei*, *M. lipolyticus* warrants further exploration regarding its efficacy in degrading specific hydrocarbon types. Additionally, assessing potential synergies between species is imperative for developing biotechnological solutions leveraging these organisms. Identifying microorganisms proficient in degrading oils and greases in produced water can pave the way for eco-friendly biotechnological products focus in application at offshore environments, minimizing operational disruptions to existing plants.

2 MATERIAL & METHODS

The brine utilized in the experiments was formulated to mimic the average concentrations of salts typically found in produced water (PW). Its composition consisted of 441 mg/L KCl, 708 mg/L $CaCl_2$, 2,626 mg/L $MgCl_2$, 29,250 mg/L NaCl, 49 mg/L Na_2SO_4, and 382 mg/L NH4Cl. Subsequently, the solution was enriched with 100 mg/L of cyclohexanecarboxylic acid for testing both cells, 220 mg/L of n-hexadecane for test *Marinobacter aquaeolei* or 120 mg/L of n-hexadecane for test *Marinobacter lipolyticus*. The incorporation of these hydrocarbons was achieved using an ultra-turrax disperser (T18, IKA) at 22,000 rpm for 15 minutes. To maintain the pH of the synthetic solution close to neutrality, 0.150 g/L of sodium bicarbonate ($NaHCO_3$) was added. Nutrients were also introduced to ensure the proper metabolic function of the cells, including 0.044 g/L of monopotassium phosphate (KH_2PO_4), 0.056 g/L of dipotassium phosphate (K_2HPO_4), 0.729 g/L of sodium nitrate ($NaNO_3$), and 0.382 g/L of ammonium chloride (NH4Cl). Additionally, a 0.5 mL/L solution of micronutrients containing some metals necessary to the metabolism was also added[10].

M. aquaeolei and *M. lipolyticus* were cultivated in marine broth 2216 (Sigma-Aldrich). The grown cells were separated by centrifugation at 8,000 rpm for 10 minutes (ThermoFisher ST16R), and then 100 mg/L were inoculated into the brine containing the naphthenic acids. The system was maintained for 14 days at room temperature under nitrogen bubbling for deplete oxygen.

The degradation of naphthenic acids was assessed in terms of total oil and grease (TOG), following standard procedures. Nitrate concentration and chemical oxygen demand (COD) were also measured to determine the microorganisms' consumption of organic matter. The determination of COD followed standard procedures. Samples were collected at the beginning and end of the experiment and then filtered through 0.45 µm membranes before assessing certain physicochemical parameters. Nitrate was analyzed using the brucine colorimetric method[11].

3 RESULTS & DISCUSSION

The experiments conducted aimed to address gaps in understanding microorganism behavior in high-salinity environments exceeding 30 g/L and in anaerobic conditions, which are not well-studied in existing literature. Initial tests utilized Marinobacter aquaeolei in brine containing hexadecane, a compound commonly found in produced water. This choice was made because hexadecane represents saturated open-chain aliphatic hydrocarbons present in such water. M. lipolyticus was also tested under the same conditions. Results indicated that both microorganisms could degrade hexadecane, with M. aquaeolei showing higher removal (62%) compared to M. lipolyticus (41%).

Another class of hydrocarbons found in produced water is naphthenic acids, represented by cyclohexanecarboxylic acid due to its high solubility in water. Both microorganisms significantly reduced total oil and grease (TOG) content. However, while M. aquaeolei showed higher removal of hexadecane, M. lipolyticus exhibited greater removal of hydrocarbons (64% vs. 45%) in the case of cyclohexanecarboxylic acid. Further tests were conducted to evaluate the synergy of both species in the same system. However, no degradation of cyclohexanecarboxylic acid was observed after 14 days, suggesting competition between the species for nutrients, hindering the assimilation of the carbon source.

Water from oil platforms was also used to assess microorganism biodegradation capacity. M. aquaeolei reduced TOG by 72%, while M. lipolyticus showed no significant reduction. This difference may be attributed to variations in hydrocarbon types present in the water used, as different oil wells can contain varying organic compounds. When both cells were used simultaneously, TOG removal was only 10%, indicating lower performance compared to individual treatments.

Table 1 Initial and final values of TOG and percentage of removal for the bacteria M. aquaeolei and M. lipolyticus in different oily solutions.

Hydrocarbon	Microrgaganism	TOG (mg/L)		Removal (%)
		Initial	Final	
n-Hexadecane	*M. aquaeolei*	213 ± 4	81 ± 2	62
	M. lipolyticus	90 ± 4	53 ± 1	41
Cyclohexanecarboxylic acid	*M. aquaeolei*	22 ± 1	12 ± 2	45
	M. lipolyticus	22 ± 1	8 ± 1	64
	M. aquaeolei & M. lipolyticus	31 ± 4	31 ± 5	0
Produced water	*M. aquaeolei*	95 ± 8	27 ± 10	72
	M. lipolyticus	43 ± 3	43 ± 2	0
	M. aquaeolei & M. lipolyticus	61 ± 2	55 ± 3	10

4 CONCLUSION

Marinobacter sp. are known to possess genes enabling the degradation of hydrocarbons. However, their capability hasn't been thoroughly assessed in environments with salinity levels exceeding 30 g/L and under anaerobic conditions. Results under such conditions demonstrated the biodegradation potential of these microorganisms for various hydrocarbons. In dispersed and dissolved brine, *M. aquaeolei* degraded 62% of n-hexadecane, while *M. lipolyticus* performed less efficiently, degrading only 41% of this oil. Conversely, when the hydrocarbon was cyclohexanecarboxylic acid, M. lipolyticus degraded approximately 19% more than *M. aquaeolei*. Their application in treating produced water contaminated with polluting oils was also assessed. When applied separately, *M. aquaeolei* degraded 72% of the total oil and grease (TOG) present, whereas *M. lipolyticus* couldn't consume the oil. The difference in performance can be understood due to the composition of the solution. Both species were also introduced simultaneously, their performance was lower compared to individual applications. Together, they failed to degrade cyclohexanecarboxylic acid and only reduced 10% of the TOG content in the water produced from the platform. These findings underscore the technological potential of using *M. aquaeolei* in treating TOG in produced water.

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