Oil dispersion characterization of microorganisms producing biosurfactants

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Abstract. The article is dedicated to studying the ability of crude oil emulsification and displacement by bacteria producing low-molecular-weight biosurfactants. Six hydrocarbon-oxidizing cultures of microorganisms were used in this study: 3 strains of Pseudomonas aeruginosa (T1, T4, D2) and 3 Bacillus cultures: Bacillus licheniformis A3, Bacillus subtilis subsp. subtilis A12, Bacillus subtilis A9 from the collection of the Biotechnology Department of Al-Farabi Kazakh National University.

The research findings indicate that cells of P. aeruginosa T1 exhibited maximum emulsifying and displacing properties, with an E24 value of 76.4% and an oil displacement ability of 5.1 cm. Among the three genes responsible for the production of low-molecular-weight biosurfactants, the presence of one gene, rhlA, responsible for the synthesis of rhamnolipid biosurfactants, has been confirmed. It was demonstrated that strains P. aeruginosa T1, P. aeruginosa T4, B. subtilis A9, and B. subtilis subsp. subtilis A12 showed high emulsifying activity, making them potentially effective for application in biotechnological processes aimed at enhancing oil recovery from mature reservoirs and in bioremediation processes.

1 Introduction

Oil in the reservoir is in a dispersed system consisting of rock and formation water. A dispersed system is a system consisting of two or more substances, with one of them in the form of very small particles uniformly distributed in the volume of the other. The oil disperse characteristic of microorganisms includes oil emulsifying and oil displacing properties of microorganisms due to the synthesis of various biosurfactants. Biosurfactants are natural surface-active biomolecules that are secondary metabolites of various microorganisms, mainly representatives of the genera Pseudomonas, Bacillus, Rhodococcus, Corynebacterium, Candida and others [1-5]. Depending on their molecular weight, they are classified into low-molecular weight ones, such as glycolipids, lipopeptides, fatty acids, neutral lipids and phospholipids, with the ability to reduce surface and interfacial tension in liquids, as well as high-molecular weight ones (polysaccharides, liposans, emulsans and protein complexes), which provide the formation of stable emulsions [6].

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Biosurfactants have various applications in the petroleum industry, such as enhancing oil recovery, cleaning contaminated tanks, and facilitating pipeline transportation of heavy crude oil. Biosurfactants can reduce surface and interfacial tension by imparting properties such as emulsification, foaming and dispersing. They can be used as emulsifying/demulsifying agents, suitable for oil mobility enhancement, anti-corrosion agents, biocides and other innovative applications in the petroleum industry [7-9].

Biosurfactants have such advantages as biodegradability, stability in a wide range of pH and temperatures, low toxicity, surface and emulsifying activity compared to their synthetic analogs [10]. In addition, the interest in utilizing inexpensive carbon sources (molasses, whey, vegetable oils, petroleum waste, etc.) makes the production of biosurfactants economically viable, giving them utility in various applications [11].

The aim of the present study is to evaluate the oil emulsifying and oil displacing properties of bacteria producing low molecular weight biosurfactants.

2 Materials and Methods

Six hydrocarbon-degrading microbial cultures were used as the objects of the study: 3 strains of Pseudomonas aeruginosa (T1, T4, D2); and 3 Bacillus cultures - Bacillus licheniformis A3, Bacillus subtilis subsp. subtilis A12, Bacillus subtilis A9 from the collection of the Department of Biotechnology, Al-Farabi Kazakh National University. Previously, the authors have determined the presence of lchAA, rhlA, srfA genes responsible for the formation of biosurfactants associated with microbial oil emulsification and the ability of bacteria to reduce interfacial tension [12, 13].

The following nutrient media were used for the research: Nutrient agar (NA), Nutrient Broth (NB), and Mineral Salt Solution (MSS). NA and NB were used as the main media for the storage, activation, purity check, and biomass production of microorganisms. The synthetic MSS medium served as a mineral background with the following composition (g/L): NaCl - 10.0; NH_4NO_3 - 2.0; Na_2HPO_4 - 5.0; KH_2PO_4 - 2.0; $MgSO_4 \cdot 7H_2O$ - 0.2, with glycerol (10%) used as the sole carbon source [14].

For determining the oil-emulsifying and oil-displacing properties of microorganisms, crude viscous oil from the Ariskum field was used, with the following characteristics: density - 854 kg/m³, sulfur content up to 0.46%, paraffin content 9.7-27.2%, silica gel asphaltenes and resins up to 16.65%, condensate containing paraffin within 0.01-1.3% and sulfur within 0.01-0.06% [15].

Traditional microbiological methods, such as the determination of cell content using the Koch method, and physicochemical methods including the Cooper method (for determining the oil emulsification index) [16] and the Morikawa method (for determining oil displacement), were utilized in this work [17].

The obtained data were processed by the method of variation-statistical analysis, taking the criterion of probability $P < 0.05$ [18]. The tabular processor Excel 2016 was used for statistical calculations.

3 Results and Discussion

The emulsifying and oil-displacing properties of microorganisms are valuable for biotechnologies used in the petroleum industry, particularly in areas such as bioremediation of soil and water, well stimulation for enhanced oil recovery, and cleaning of well equipment [1-3]. These properties are based on the ability of microorganisms to produce various biosurfactants that facilitate the dispersion and displacement of oil. Specifically, the ability of microorganisms to emulsify oil is often based on the production of high-

molecular-weight biosurfactants that form tiny oil emulsions, which enhances the efficiency of bacterial contact with the oil, while the ability to reduce interfacial and surface tensions is associated with low-molecular-weight biosurfactants [4-5, 8].

To determine microbial emulsification and oil displacement, heavy, crude oil from the Ariskum field was used. It is known that the formation of various target metabolites in microorganisms is related to specific genes, such as the synthesis of biosurfactants linked to microbial oil emulsification and the ability of bacteria to reduce interfacial tension is governed by the presence of genes lchAA, rhlA, srfA [12, 19].

The results of studying microbial emulsification, oil displacement, and the presence of genes responsible for the synthesis of biosurfactants associated with these properties are presented in Table 1.

Table 1. Determination of microbial emulsifying index, oil displacement and presence of genes responsible for biosurfactant synthesis.

Microorganisms	Emulsifying index (E_{24}), %	Oil displacement (O_s), cm	Biosurfactant genes		
			rhlA	*srfA*	*lchAA*
Control	0	0			
P.aeruginosa D2	51±2,4	2,5±0,2	+	+	-
P.aeruginosa T1	76,4±3,2	5,1±0,3	+	-	-
P.aeruginosa T4	66±3,2	4,2±0,3	+	+	-
B. subtilis ssp.subtilis A12	66,5±3,2	3,3±0,2	-	+	-
B. licheniformis A3	50,5±1,8	2,2±0,2	-	-	+
B. subtilis A9	60,1±3,1	3,7±0,2	-	+	-

As shown, for all 6 cultures, the emulsification index (E24) of crude oil was above 50%, indicating the potential of bacteria as producers of biosurfactants used in the petroleum industry [20]. It is worth noting that the maximum value of E24 is observed for Pseudomonas (P. aeruginosa T1) - 76.4%, and for Bacillus (B. subtilis ssp. subtilis A12) - 66.5%.

In Figure 1, a picture of microbial oil emulsification with the formation of small dispersed particles is presented, accompanied by a color change and looseness of the oil monolayer at the boundary with the aqueous suspension, as a control variant - a non-microbial «oil-water» system.

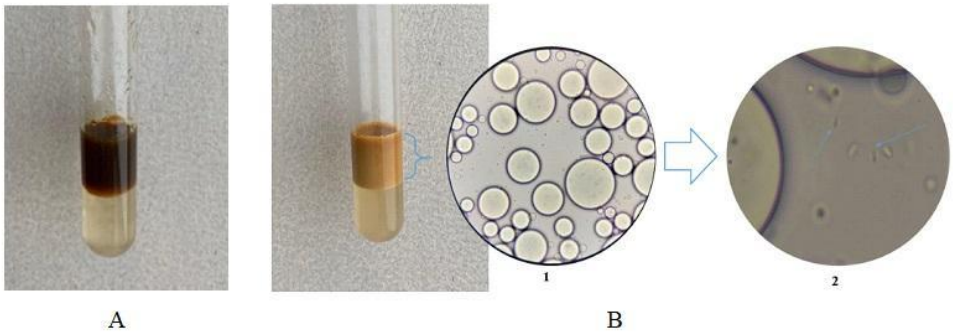

Fig. 1. Control sample (A). Emulsification of oil with B. subtilis A9 (B), emulsion microscopy at x40 (1) and x100 (2). Arrows indicate bacteria.

Table 1 shows that the oil displacement activity of the studied cultures ranged from 2.2 to 5.1 cm, indicating their ability to actively reduce interfacial tension. In the literature we reviewed, a microorganism culture with an oil displacement ability of 2.6 cm is recognized as a promising producer of low molecular weight biosurfactants [2].

Figure 2 illustrates the results of microbial oil displacement, forming a zone of purification where the hydrophobic layer is displaced. This is due to the presence of biosurfactant in the bacterial suspension with the property of reducing interfacial tension. A non-microbial version was used as a control.

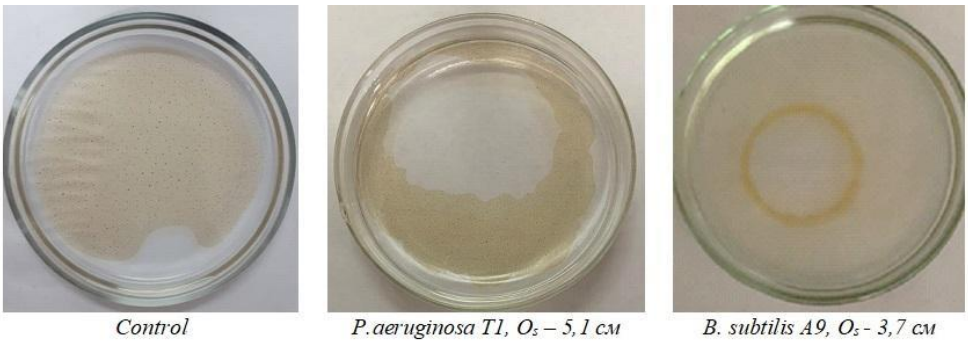

Fig. 2. Microbial oil displacement.

Earlier, the genes responsible for the production of low-molecular-weight biosurfactants, which contribute to oil displacement and emulsification, were identified in these bacteria [12, 13]. It is known that in the bacteria of the Pseudomonas and Bacillus genera, the genes rhlA, srfA, lchAA are responsible for the ability to displace oil, based on the synthesis of low-molecular-weight biosurfactants that effectively reduce surface and interfacial tension [21, 22]. It should be noted that out of the 6 cultures studied, for 5 bacteria, the E24 values correlate with the oil displacement ability. The maximum emulsifying index for P. aeruginosa T1 was 76.4%, and this culture also demonstrated the maximum ability to displace oil by 5.1 cm.

Thus, as a result of studying microbial emulsification and displacement of crude oil by three strains of P. aeruginosa (T1, T4, D2) and 3 Bacillus cultures, it has been established that the cells of P. aeruginosa T1 possess the maximum emulsifying and displacing properties. The E24 value was 76.4%, and the ability to displace oil was 5.1 cm. Moreover, out of the three genes responsible for the production of low-molecular-weight biosurfactants, the presence of one gene, rhlA, responsible for the synthesis of rhamnolipid

biosurfactants, has been confirmed. It is known that most rhamnolipids have dual functions, being capable of both emulsifying and displacing oil [23].

4 Conclusion

The conducted research has demonstrated that the strains P. aeruginosa T1, P. aeruginosa T4, B. subtilis A9, and B. subtilis subsp. subtilis A12 have high emulsifying activity, making them potentially effective for use in biotechnological processes aimed at enhancing oil recovery from mature fields and in bioremediation based on microbial oil degradation. The strain P. aeruginosa T1 demonstrated the best results in oil emulsification at 76.4±3.2%. Furthermore, our study confirmed that low-molecular-weight biosurfactants are capable not only of reducing surface and interfacial tension but also of actively emulsifying oil.

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