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Changes in Interfacial Properties of the Fluid/Fluid/Rock Affected by Activity of *Bacillus licheniformis* and *Pseudomonas putida* Bacteria during Growth in Different Carbon

Mansure Farid[1], Shahriar Osfouri[1]*, Reza Azin[2] and Seyed Ali Jafari[1]

1. Department of Chemical Engineering, Faculty of Petroleum, Gas and Petrochemical Engineering, Persian Gulf University, Bushehr, Iran

2. Department of Petroleum Engineering, Faculty of Petroleum, Gas and Petrochemical Engineering, Persian Gulf University, Bushehr, Iran

osfouri@pgu.ac.ir

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Abstract

The most important mechanism of microbially enhanced oil recovery processes is changes in interfacial properties of fluid/fluid/rock affected by bacterial cells and their metabolites. In this study, two bacteria *B.licheniformis* and *P.putida* and three different sources of carbon were used. Results showed that due to the effect of carbon source on bacterial growth and also type and value of produced metabolites, changes in interfacial properties of fluid/fluid/rock is affected by used carbon source. The better growth of both bacteria occurred in a culture medium containing glucose. Pendant drop tensiometry, electrophoretic mobility measurement, interfacial dilational rheology and contact angle test were used to investigate the effect of cell structure and culture medium composition on interfacial tension reduction, emulsion stability, and wettability alteration mechanisms. The results of this section showed more potential of *P.putida* bacteria in surfactant production. Also, results showed that glucose is a better culture medium for *B.licheniformis* bacteria. Produced biosurfactants and bacterial culture of B.licheniformis bacteria in medium with glucose carbon source reduced surface tension and interfacial tension by 29.07% and 43.87%, respectively. Submerging thin section in glucose culture medium of *B.licheniformis* bacteria reduced the contact angle of water drop from 109.081 to 44.898°. In the case of *P.putida* bacteria, the oily and glucose culture media are respectively the effective media for influencing the fluid-fluid and fluid-rock interface. The produced biosurfactants and bacterial culture of *P.putida* bacteria in medium with olive oil carbon source reduced surface tension and interfacial tension by 36.4% and 27.1%, respectively. The contact angle of drop water was reduced from 109.081 to 54.050° in glucose culture medium of *P.putida* bacteria.

Keywords: *B.licheniformis*, *P.putida*, Interfacial Tension Reduction, Wettability Alteration, Interfacial Rheology, Hydrophobicity

INTRODUCTION

The most important mechanism of MEOR processes is the alteration of oil/water/rock interfacial properties. In the case of fluid-fluid interface modification, interfacial tension (IFT) reduction and rheological properties modification of water-oil interface are the most important governing mechanisms [1]. In addition to biosurfactant production, adhering bacteria and biofilm formation on the interface can cause IFT reduction by altering rheological properties of the interface [2]. Three groups of factors including the characteristics of microbial cells, oil surface properties, and environmental factors are effective in adhering bacteria to an interface [1, 2]. Investigation of bacterial adsorption by interfacial rheometer and pendant drop tensiometer showed biofilm elasticity magnitude was in harmony with the order of measured IFT reduction [2]. In this study, Bacillus *licheniformis* and *Pseudomonas* putida bacteria with different cell structure were investigated to compare their effects on MEOR mechanisms.

EXPERIMENTAL

BACTERIA, CRUDE OIL, THIN SECTION AND MEDIA

The candidate bacteria chosen for this study were Bacillus *licheniformis* and *Pseudomonas* putida obtained from IBRC[1]. The crude oil was a relatively light oil with API= 33.9 from NISOC[2]. The used thin sections were taken from a carbonate reservoir in the southern Iran. Two types of media were used to culture bacteria. The first medium used to enhance the growth bacteria was Luria Bertani (LB) broth (Merck, Germany) and the second medium was minimal salt medium optimized by Joshi et al. [3]. Olive oil was used as the hydrophobic substrate.

1. Iranian Biological Resource Center
2. National Iranian South Oil Company

METHODS

Experimental measurements were carried out on the three minimal medium with glucose, olive oil and equal mixture of them as carbon sources. Optical density (OD) was measured using a spectrophotometer (Lambda 25, Perkin Elmer) at 600nm every 4h for a period of 48h and then every 12h until 72h. The bacterial supernatant was prepared by centrifuging bacterial suspension at 5500 rpm for 10min. For measuring the bacterial cell hydrophobicity, solutions of 24, 30 and 34h incubated bacteria in the 10X phosphate buffer with pH=7 were prepared. We measured the OD of the sample before and after mixing with normal hexadecane (with a ratio of 6). Then wecalculated bacterial adhesion (BATH). For contact-angle experiments, oil-wet thin sections were soaked into bacterial solutions for 48 hours, and finally, contact angles were measured by placing the water drop on the surfaces. The oil-wet thin section before soaking it into bacterial solutions was used as the control sample.

RESULTS AND DISCUSSIONS

BACTERIAL GROWTH

Figure 1 shows the improved growth of *B.licheniformis* in comparison with *P.putida.* The results indicated that high concentration of glucose in the culture medium does not lead to better growth of the bacterium. Surface activity: The results of surface tension (ST) measurements in Table 1 showed that the maximum ST reduction percentage of *B.licheniformis* and *P.putida* supernatant were related to the culture mediums with glucose and olive oil as carbon source respectively.

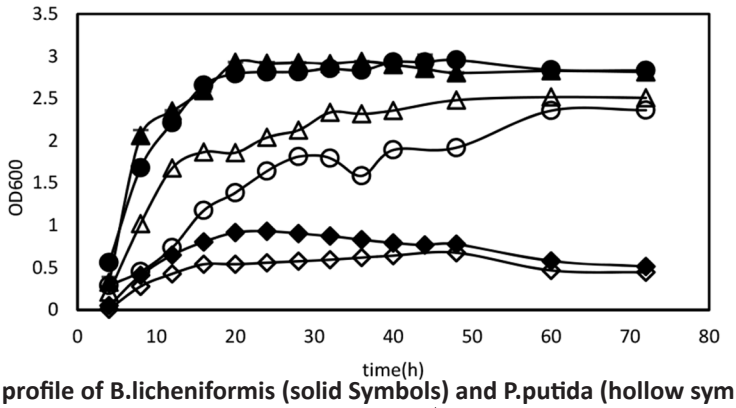

Figure 1: Time-course profile of B.licheniformis (solid Symbols) and P.putida (hollow symbols) growth in three media containing carbon glucose (○), glucose-oil (Δ) and oil (◆).

Table 1: The ST reduction percentages of supernatant solutions of B.licheniformis and P. putida bacteria

bacteria	Batch culture carbon source		
	glucose	Olive oil	Glucose + olive oil
P.putida	30.19	36.4	28.26
B.licheniformis	29.07	27.87	23.96

Comparison of the two bacteria in biosurfactant production showed that *P.putida* bacteria is more potent.

Dynamic IFT in the water-oil interface between 48h incubated bacterial culture as water phase and n-hexadecane as oil phase by pendant drop tensiometry method were measured. The drop size was fixed for 900 s. In the case of *B.licheniformis*, glucose culture medium due to more surfactant has minimum equilibrium IFT and range of IFT changes. In the case of *P.putida* all three cultures of P.putida have an approximately equivalent range of IFT changes.

HYDROPHOBICITY

To compare the tendency of the two bacteria to adsorb at a hydrophobic interface, BATH test and bacterial electrophoretic mobility were measured. Results showed that P.putida is more hydrophobic than *B.licheniformis* due to its less negative charge (-6.17 mV as compared to -19.7 mV) and higher BATH (%) value. In addition, bacterial culture with olive oil carbon source had the highest hydrophobicity values.

INTEFACIAL RHEOLOGY

The loss and storage modules were determined from Fourier transform of sinusoidal oscillation data. Moreover, these modules were measured to determine the effect of bacteria and their metabolites on the film at the interface and the stability of the formed emulsions. The results of Figure 2, show in the case of *B.licheniformis* when aqueous phase is culture medium with olive oil, the formed biofilm is more viscoelastic. Comparison of the formed biofilms by *B.licheniformis* and *P.putida* show P.putida culture media form more viscoelastic biofilms. The results of measuring the contact angle of the water drop in the gas/water/rock system showed the wettability alteration toward more water-wet condition when thin sections were soaked in culture solution with glucose carbon source.

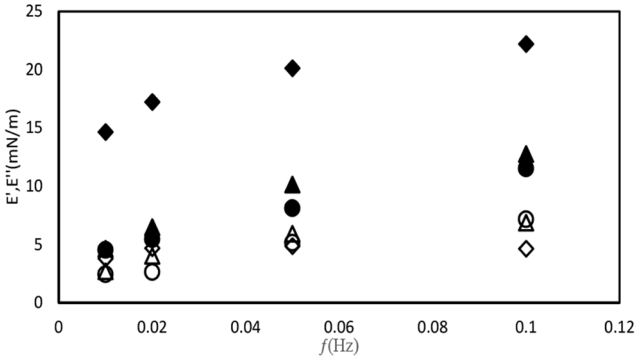

Figure 2: Interfacial elastic E' (solid symbols) and viscous E''(hollow symbols) moduli for B.licheniformis cultures with glucose (○), glucose with oil (Δ) and oil (◆) carbon source.

CONCLUSIONS

In this research, the effect of culture medium on most important mechanisms in MEOR processes namely IFT reduction and wettability alteration was studied. The results of rheological studies on the formation of a stable film at water-oil interface showed the positive role of bacterial cells and the inhibitory role of surfactants in increasing network points. The results of the experiments showed that *B.licheniformis* bacteria with a less hydrophobicity forms a hydrophilic film that has the ability to accumulate at water-carbonate rock interface. On the other hand, the P.putida bacterium forms a hydrophobic film that has an accumulation potential at the water-oil interface.

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